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MULTIFREQUENT WORK RATE FORCINGS IN THE ASSESSMENT OF OXYGEN UPTAKE KINETICS

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DECLARATION

I declare that the work for this thesis was undertaken as part of my Ph.D. studentship. I was the principle contributor to all sections, except were indicated in the text.

David R. Jarvis

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SECTION II

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ABSTRACT

During dynamic exercise, the response of the cardiorespiratory system is structured to maintain homeostasis at the cellular level. The rate at which homeostasis is established is largely dependent on the system's structural and physiological integrity. Evidence suggests that any impairment in the functioning of the system might be reflected in a determination of oxygen uptake ($\dot{V}O_2$) kinetics.

The kinetics of $\dot{V}O_2$ have been quantified in response to step, impulse, ramp and sinusoidal changes in work rate (WR). An alternative approach uses a technique in which the WR is perturbed according to a pseudorandom binary signal. Pseudorandom binary sequence (PRBS) WR forcings have the advantage of being able to provide a determination of $\dot{V}O_2$ kinetics from a single test session of ~30 min duration.

The assessment of $\dot{V}O_2$ kinetics using PRBS WR forcings demands that the controlling process behaves in a linear manner. To minimise the contribution of non-linear influences, changes in work intensity must be constrained to the sub-lactate threshold domain. When examining clinical, untrained or young subjects, the necessary reduction in the upper work limit of a PRBS forcing can effect a fall in the distribution of power across the bandwidth of the sequence. If the distribution of power should fall below a critical level, then it can become difficult to elicit discernible responses from the forcing. To resolve this problem, this thesis investigated the potential for developing a multifrequent WR forcing altered to enhance identification of the underlying $\dot{V}O_2$ response.

The multifrequent WR forcing developed for use in this thesis took the form of a binary sequence. Binary transitions were determined according to a specially constructed multifrequent signal. Signal construction involved redistributing the available signal power to specific harmonics in a chosen range of frequencies. To validate estimates of $\dot{V}O_2$ kinetics derived from the multifrequent binary sequence (MFBS) WR forcing, comparisons were made with the data obtained from an established PRBS forcing.

When comparing physiological data, it is necessary to consider the amount of variability between trials. Therefore, prior to assessing the agreement between data obtained from the MFBS and PRBS methods, this thesis sought to establish the degree of variability in estimates of $\dot{V}O_2$ kinetics derived from PRBS exercise tests.

The results presented in this thesis show estimates of the mean response time (MRT) of $\dot{V}O_2$ derived from the MFBS method to be 46.8 (4.2) s (mean (standard deviation) seconds), compared with 45.2 (5.0) s for the PRBS method. This suggests that the two methods yield comparable determinations of $\dot{V}O_2$ kinetics. Supporting evidence is provided by the limits of agreement. These indicate that the maximum difference likely to occur between the MRT obtained from the two methods (-6.5 to +9.6 s) is less than that expected due to variability in the MRT derived from PRBS forcings (-11.6 to +8.0 s). However, the limits also reveal the poor repeatability of $\dot{V}O_2$ response data obtained from the PRBS used in the thesis. Consequently, the use of this data to assess the validity of the MRT derived from MFBS forcings is not recommended.

In addition to poor repeatability, the possibility exists that assessments of $\dot{V}O_2$ kinetics derived from MFBS WR forcings will also depend on the distribution of power across the harmonic content of the sequence. Therefore, whilst MFBS WR forcings may be suited to the assessment of $\dot{V}O_2$ kinetics in subjects with a reduced tolerance to exercise, there remain doubts concerning both the validity of the response data and applicability of the method. Until these issues have been resolved, care would need to be taken when using estimates of $\dot{V}O_2$ kinetics derived from MFBS WR forcings to determine the functional state of the cardiorespiratory system.

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~	Approximately
α	Amplitude ratio
τ	Exponential time constant
ϕ	Phase angle
φ	Phase shift
Δ	The change in
ω	Unit duration
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
[ATP]	Concentration of adenosine diphosphate
Cr	Free creatine
[Cr]	Concentration of free creatine
PCr	Phosphocreatine
[PCr]	Concentration of phosphocreatine
CaO_2	Oxygen content of arterial blood
CvO_2	Oxygen content of venous blood
$C(a\text{-}v)O_2$	Arteriovenous oxygen content difference
$C\bar{v}\ O_2$	Oxygen content of mixed venous blood
$C(a\text{-}\bar{v})O_2$	Pulmonary arteriovenous oxygen content difference
CO_2	Carbon dioxide
O_2	Oxygen
PCO_2	Partial pressure of carbon dioxide
PO_2	Partial pressure of oxygen
$\dot{Q}$	Blood flow
$\dot{Q}Leg$	Leg blood flow
$\dot{Q}M$	Muscle blood flow
$\dot{Q}NW$	Non-working tissue blood flow
$\dot{Q}P$	Pulmonary blood flow
$\dot{Q}T$	Cardiac output

$\dot{Q}O_2$	Muscle oxygen utilisation
$\dot{Q}O_2Leg$	Leg muscle oxygen utilisation
$\dot{V}E$	Expired minute ventilation
$\dot{V}I$	Inspired minute ventilation
$\dot{V}O_2$	Oxygen uptake
$\dot{V}O_2A$	Alveolar oxygen uptake
$\dot{V}O_2Leg$	Leg oxygen uptake
$\dot{V}O_2M$	Muscle oxygen uptake
$\dot{V}O_{2max}$	Maximal oxygen uptake
$\dot{V}O_2M_{est}$	Estimate of muscle oxygen uptake
$\dot{V}O_2P$	Pulmonary oxygen uptake
$\dot{V}O_{2ss}$	Steady state oxygen uptake
$\dot{V}O_2T$	Tissue oxygen uptake
A	Amplitude
ACF	Auto-correlation function
ANOVA	Analysis of variance
β-blockade	Beta-adrenergic receptor blockade
CCF	Cross-correlation function
CPSD	Cross power spectral density
FRC	Functional residual capacity
HR	Heart rate
HTR	Heart transplant recipients
k	Harmonic number
LAT	Lactate threshold
LBNP	Lower body negative pressure
LBPP	Lower body positive pressure
M	Magnitude
MFBS	Multifrequent binary sequence
MRT	Mean response time
NLV	Nominal lung volume
NMR	Nuclear magnetic resonance
PRBS	Pseudorandom binary sequence

PSD	Power spectral density
STPD	Standard temperature and pressure, dry (0°C, 760 mm Hg)
SV	Stroke volume
t	Time
$t_{½}$	Half-time
T	Time period
TD	Time delay
TLT	Total lag time
WR	Work rate
Z	Maximum number of units

C	Centigrade
°	degree
Hg	mercury
Hz	cycles per second
l	litre
ln	natural logarithm
ml	millilitre
mm	millimetre
mmol	millimole
ms	millisecond
min	minute
's	second
W	watt

1.0 INTRODUCTION

During muscular exercise, activity within the cardiorespiratory system intensifies to meet the increased metabolic rate and gas exchange of the working muscles. The ability to minimise the stress imposed by the exercise is determined by the system's structural and physiological integrity (Wasserman et al. 1991).

Following a period of prolonged inactivity, improper training or exposure to disease, the response of one or more of the physiological components that constitute the cardiorespiratory system can become impaired. The most likely sources of impairment are in those components involved with the transport and utilisation of oxygen (O_2) (Whipp 1994a). If the impairment leads to an inability to meet the energy demands of a given task through aerobic metabolism, then anaerobic metabolism must accelerate to meet the additional requirements. The accompanying elevations in tissue and circulating lactate, carbon dioxide (CO_2) production and minute ventilation can be sufficient to induce exercise intolerance (Tjahja et al. 1994). At one level, this intolerance may manifest itself in decreased athletic performance. At another, it may be evident in a subject's inability to complete mild domiciliary tasks (Whipp 1994a).

Cardiorespiratory exercise testing offers the possibility of studying the behaviour of the cardiorespiratory system during the stress of a carefully controlled exercise modality. The approach is recognised as a non-invasive method of evaluating the integrity of the cardiorespiratory system, and is a vital source of information for both physicians and exercise physiologists.

In a clinical environment, the administration of cardiorespiratory exercise testing can

help to:

i) assist in identifying the underlying mechanism(s) of exercise intolerance in patients with exertional dyspnea;

ii) aid the diagnosis of previously unsuspected diseases;

iii) establish the severity of functional impairment in patients with cardiorespiratory system (and other) diseases;

iv) track the progression of underlying disease processes;

v) assist in general prognosis;

vi) assess the efficacy of intervention programs aimed at improving functional impairment in patients, using drug and rehabilitation therapies or surgical procedures;

vii) assess a patient's suitability to undergo rehabilitative therapies and surgical procedures or to perform occupational tasks (Lamarra and Whipp 1995; Oren et al. 1987; Whipp 1994a).

For the exercise physiologist, data obtained from cardiorespiratory exercise testing provides the necessary information to support physical performance through:

i) development of individual physical profiles;

ii) monitoring of health status;

iii) objective evaluation of new training methods or strategies;

iv) optimisation of training regimes;

v) monitoring of progress during rehabilitation;

vi) prediction of current and future physical potential;

vii) judgement of an individual's suitability to undertake training or meet specific goals (MacDougall and Wenger 1991; Whipp 1994a).

The most common method of assessing the integrity of the cardiorespiratory system involves the determination of maximal oxygen uptake ($\dot{V}O_{2max}$). This parameter indicates the limit of a subject's ability to utilise atmospheric O_2 for cellular energetics (Taylor et al. 1955). In exercise physiology, $\dot{V}O_{2max}$ is generally accepted as the main

criterion for assessing endurance performance potential (Sutton 1992; Noakes 1988). However, as $\dot{V}O_{2max}$ is normally measured using incremental exercise tests to exhaustion, the evaluation of clinical, untrained or young subjects can prove difficult because of limitations such as physical discomfort (Koike et al. 1994).

As an alternative to tests requiring maximal exercise effort, submaximal measurements of aerobic function have been shown to provide valuable information concerning the behaviour of the cardiorespiratory system. Such measurements can be obtained during steady state (Casaburi et al. 1992; Weltman and Katch 1976) or non-steady state (Linnarsson 1974; Whipp et al. 1982) exercise. Of these two approaches, the kinetic response data derived from non-steady state exercise have proved to be of considerable benefit in both clinical and physical assessment (Babcock et al. 1994a; Hamar 1991; Koike et al. 1994; Nery et al. 1982).

In physiological terms, kinetics describes the rates at which processes within the body adapt to changing metabolic demands in order to maintain homeostasis at the cellular level (Cooper et al. 1985). During muscular exercise, the ability to maintain cellular homeostasis is largely dependent on the body's capacity to transport and metabolise O_2. As O_2 stores in the body are very small, relative to metabolic demand, in healthy subjects oxygen uptake ($\dot{V}O_2$) determined at the mouth is closely coupled in time to cellular requirements (Barstow et al. 1994; Grassi et al. 1996). Consequently, any functional impairment of the cardiorespiratory system is likely to be reflected in slower $\dot{V}O_2$ kinetics.

Oxygen uptake kinetics can be determined in the transition between a wide range of work intensities. However, the majority of investigations have evaluated kinetic data collected in the transition from rest or light intensity work to work of a moderate intensity (Babcock et al. 1994b; Cerretelli et al. 1977; Convertino et al. 1984a; Hughson

and Smyth 1983; Karlsson et al. 1975; Whipp et al. 1982). Light intensity work, such as that which might be encountered during zero-load pedalling, should not generate any discernable increase in blood lactate. For work of a moderate intensity, blood lactate may increase transiently shortly after the onset of work, before falling to resting values as a new steady state in $\dot{V}O_2$ is approached. If the work provokes an initial rise in blood lactate proceeded by either a fall to levels above those observed at rest or a continued increase throughout exercise, then the intensity may be considered heavy or extreme (Wasserman and Whipp 1975).

Previously, assessments of $\dot{V}O_2$ kinetics in the transition from rest or light intensity work to work of a moderate intensity have been shown to be sensitive to the effects of cardiovascular and pulmonary diseases (Auchincloss et al. 1974; Cooper et al. 1992; Koike et al. 1994; Nery et al. 1982; Sietsema et al. 1986; Sietsema et al. 1994; Spiro 1977). Several investigators (Cerretelli et al. 1979; Eßfeld et al. 1987; Powers et al. 1985; Stegemann et al. 1985) have also observed a correlation between aerobic capacity and the dynamic response in $\dot{V}O_2$.

The kinetics of $\dot{V}O_2$ can be quantified in response to step (Whipp et al. 1982), impulse (Miyamoto et al. 1983) and ramp (Whipp et al. 1981) changes in work rate (WR). Such tests are generally repeated several times in each subject so that the resulting $\dot{V}O_2$ response data can be averaged before being fitted with linear mathematical models. The parameters of the model may then be used to provide a description of the subject's $\dot{V}O_2$ kinetics. A practical disadvantage of this approach is that it is relatively time-consuming and can require tests on different days (Cunningham et al. 1993; Hoffmann et al. 1994a).

An alternative approach to obtaining a description of $\dot{V}O_2$ kinetics uses sinusoidal (Bakker et al. 1980; Casaburi et al. 1977) or multifrequent (Eßfeld et al. 1987; Hughson

et al. 1990b) WR forcings. With the multifrequent approach, WR is normally switched between two levels within the aerobic range according to a pre-determined pseudorandom binary sequence (PRBS). The resultant data can be analysed in either the frequency domain (Stegemann et al. 1985) or the time domain (Hughson et al. 1991a). The main attraction of the PRBS method is that an estimate of $\dot{V}O_2$ kinetics can be derived from a single test session of ~30 min duration (Hughson et al. 1990d; Hughson et al. 1991a).

Hughson et al. (1991a) have reported that mean estimates of $\dot{V}O_2$ kinetics obtained from PRBS tests are not significantly different to those acquired using step changes in WR. This suggests that PRBS tests offer a valid alternative to more established methods of determining $\dot{V}O_2$ kinetics.

When examining clinical, untrained or young subjects, it can prove necessary to reduce the overall magnitude of a PRBS WR forcing. Due to the pre-determined nature of a PRBS, such a reduction will effect a fall in the power distributed across the bandwidth of the sequence. If the power should fall below a certain level, then it may not be possible to elicit discernible responses from the forcing (Eßfeld et al. 1987). To resolve this problem, WR would need to be perturbed according to a multifrequent signal tailored to enhance identification of the underlying $\dot{V}O_2$ response (Bakker et al. 1980; Eßfeld et al. 1987). Although specially adapted multifrequent forcings have been employed in the investigation of engineering based systems, no evidence can be found of their application to the assessment of $\dot{V}O_2$ kinetics. Accordingly, the main aim of this thesis will be to investigate the development of a multifrequent WR forcing for use with populations in which exercise intolerance is a feature.

To validate estimates of $\dot{V}O_2$ kinetics derived from a modified multifrequent forcing, kinetic parameters might be compared with those obtained using the established PRBS

method. If, however, the PRBS method has poor repeatability, then even a new method that is perfect will not agree well with it (Bland and Altman 1995). Prior to performing investigations, this thesis will therefore seek to establish the degree of variability in estimates of $\dot{V}O_2$ kinetics derived from PRBS exercise tests.

To facilitate understanding, the opening chapters of the thesis will review the literature dealing with the study and measurement of $\dot{V}O_2$ kinetics. Special consideration will be given to the examination of $\dot{V}O_2$ kinetics using PRBS WR forcings and the theory underpinning this approach.

2.0 THE STUDY OF OXYGEN UPTAKE KINETICS

Since the early work of Hill and Lupton (1923), the study of the $\dot{V}O_2$ response to dynamic exercise has provided a basis for evaluating the structural and physiological integrity of the cardiorespiratory system. More recently, the integration of advanced technology has helped yield detailed information on the response characteristics of $\dot{V}O_2$ and the underlying mechanisms of control. The resultant increase in knowledge has contributed to a significant improvement in the understanding of cardiorespiratory system impairment.

The aim of this chapter is to summarise the extensive material concerning $\dot{V}O_2$ kinetics and to establish a current position of thinking. To provide the necessary understanding for later work, emphasis is given to the $\dot{V}O_2$ response to work intensities that do not provoke a sustained increase in blood and muscle lactate concentration. To simplify the interpretation of the characteristics and mechanisms of the response, only the kinetics of the on-transient will be considered.

2.1 THE COUPLING OF EXTERNAL TO CELLULAR OXYGEN UPTAKE

At the beginning of light to moderate intensity exercise, energy-yielding reactions increase almost immediately to the level required to maintain contractile activity. Whilst initial energy demands can be met by adenosine triphosphate (ATP) already present in the cell, for the continuation of activity it is essential that the rate of adenosine diphosphate (ADP) re-phosphorylation accelerates to the same rate as ATP utilisation. However, the oxidative processes that provide energy for the re-synthesis of ATP do not proceed at a rate that meets demands instantaneously. Rather, these processes follow a slower time course before reaching an asymptotic value commensurate with demands (di Prampero 1981).

The multitude of intervening factors that contribute to the rate at which O_2 is made available for the re-phosphorylation of ADP can be divided into three categories: i) O_2 delivery from environmental air to mitochondria; ii) blood and tissue O_2 stores; and iii) the biochemical rate control system that links ATP re-synthesis to the terminal reduction of molecular oxygen (Cerretelli et al. 1980; Karlsson et al. 1975; Kushmerick and Meyer 1985). To understand the influence these factors have on the kinetics of oxidative metabolism, it is necessary to examine the characteristic response in pulmonary $\dot{V}O_2$ ($\dot{V}O_2P$) as determined from measurements at the mouth.

2.2 OXYGEN UPTAKE FOLLOWING THE ONSET OF MODERATE INTENSITY CONSTANT-RATE EXERCISE

In 1923, Hill and Lupton reported that, following the onset of constant-rate exercise, the rise in $\dot{V}O_2$ measured at the mouth followed an approximately exponential time course. Subsequent studies have shown that, for light to moderate intensity exercise, the rise in $\dot{V}O_2$ may be separated into three temporal phases (Linnarsson 1974; Whipp et al. 1982). These phases are most evident when data recorded during several rest-to-work transitions are averaged. An idealised description of the three phases appears in Figure 2.1 (overleaf).

2.2.1 PHASE I

At the onset of exercise, there is a small abrupt increase in $\dot{V}O_2$. This increase is followed by an interval of between ~10 and 20 s (Barstow and Molé 1987; Hughson et al. 1988; Whipp et al. 1982) during which $\dot{V}O_2$ remains relatively constant (Sietsema et al. 1989). This period of the response has been termed *phase I*, and is generally initiated within the first breath following the commencement of exercise.

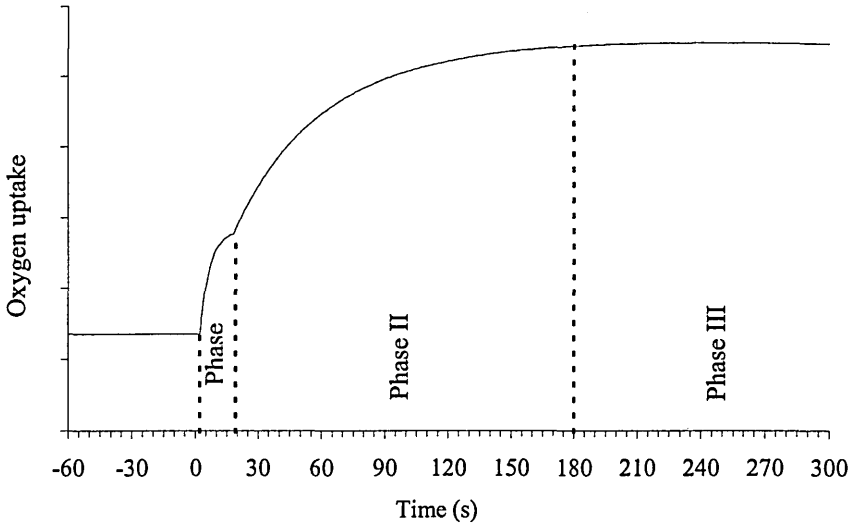


Figure 2.1. Idealised representation of the three phases in oxygen uptake following the transition from rest to moderate intensity constant-rate exercise. Exercise onset is at 0 seconds. The figure is adapted from the diagram appearing in work by Sietsema et al. (1989)

Prior to *phase I*, that is, in the resting steady state, $\dot{V}O_2$ at the lungs is largely determined by the amount and degree of reduced haemoglobin entering the pulmonary capillary bed (Whipp et al. 1982). Thus, steady state $\dot{V}O_2$ ($\dot{V}O_{2ss}$) is dependent upon pulmonary blood flow ($\dot{Q}P$) and the mixed venous O_2 content ($C\bar{v}O_2$). After the start of exercise, changes in the venous O_2 content (CvO_2) and partial pressure of oxygen (PO_2) resulting from muscle oxygen utilisation ($\dot{Q}O_2$) will not be expressed in $\dot{V}O_2P$ straight away. Rather, $\dot{V}O_2P$ will be dissociated from $\dot{Q}O_2$ by circulatory transit delays from the working muscle to the lung (Whipp et al. 1980; Whipp et al. 1982). The increase in $\dot{V}O_2$ during *phase I* should therefore reflect the greater $\dot{Q}P$ following the instantaneous adjustment in cardiac output ($\dot{Q}T$) at the onset of exercise (Krogh and Lindhard 1913; Weissman et al. 1982; Weiler-Ravell et al. 1983; Whipp and Mahler 1980). The adjustment in $\dot{Q}T$ is the combined result of changes in both heart rate (HR), due to vagal withdrawal, and stroke volume (SV) (Linnarsson 1974; Shoemaker et al. 1994).

That *phase I* $\dot{V}O_2$ is both initiated by and reflects the cardiovascular response to exercise is supported by several independent findings (Whipp and Ward 1990):

i) *phase I* is still evident even when ventilation ($\dot{V}E$) during exercise is kept at resting levels by controlled breathing (Weissman et al. 1982);

ii) the rise in $\dot{Q}P$ measured by impedance techniques following the onset of exercise is directly proportional to the increase in $\dot{V}O_2$ during *phase I* (Miyamoto et al. 1982);

iii) the rate of change in $\dot{V}O_2$ during *phase I* is slowed when the rapid adjustment in SV at the start of exercise is attenuated by initiating the exercise from a lower level of prior work rather than rest (Karlsson et al. 1975; Whipp et al. 1982);

iv) $\dot{V}O_2$ can be seen to increase when $\dot{Q}T$ is raised without perceptual cues during rest in patients with implanted cardiac pacemakers (Jones et al. 1981);

v) the magnitude of the $\dot{V}O_2$ response during *phase I* is reduced in conditions such as chronic obstructive pulmonary disease, suggesting an attenuation of $\dot{Q}P$ during *phase I* (Nery et al. 1982).

Although the rise in $\dot{V}O_2$ during *phase I* may be primarily attributed to altered $\dot{Q}P$, it has also been demonstrated that $C\bar{v}O_2$ may decrease within the same time frame, that is, prior to any influence that $\dot{Q}O_2$ may have (Casaburi et al. 1989b; Cochrane et al. 1990; Edwards et al. 1972). Indications are that the fall in $C\bar{v}O_2$ is the result of a redistribution of blood from regions with a high resting O_2 extraction (Sietsema et al. 1989; Whipp and Ward 1990). Sietsema et al. (1989) have also suggested that $C\bar{v}O_2$ may be influenced by a decrease in the venous return from vascular beds with relatively high venous saturations. Therefore, whilst altered $\dot{Q}P$ may be the predominant originator of the rise in $\dot{V}O_2$ during *phase I*, it should not be considered as the only determinant.

2.2.2 PHASE II

At the end of *phase I*, $\dot{V}O_2P$ begins to increase more rapidly as the influence of the widened arteriovenous O_2 content difference ($C(a\text{-}v)O_2$) across the contracting muscle (initially dissociated from $\dot{V}O_2P$ by the transient delay from the sites of increased metabolism) becomes evident at the lungs (Whipp and Ward 1990). This subsequent period of the $\dot{V}O_2$ response is known as *phase II*, and may also reflect any residual component of the increase in $\dot{Q}T$ that extends beyond *phase I* (Whipp 1987).

The transition between *phases I* and *II* is marked by an increase in the PCO_2 and decrease in the PO_2 in mixed venous blood entering the pulmonary capillary bed (Whipp 1987). Following the transition, $\dot{V}O_2$ increases toward a new steady state as a simple first-order function, that is, the response can be characterised by a monoexponential function of time (Linnarsson 1974; Miyamoto et al. 1982; Whipp et al. 1982).

The time constant (τ) for the *phase II* response in $\dot{V}O_2$ has been reported as ~45 s, corresponding to a reaction half-time ($t_{½}$) of ~30 s ($t_{½} = \tau \cdot \ln 2$) (Whipp and Mahler 1980). Although it has been reported that τ does not differ for work rates of light to moderate intensity (Barstow et al. 1993; Linnarsson 1974; Whipp and Mahler 1980; Whipp and Ward 1993), the findings of Casaburi et al. (1989a) and Hughson et al. (1988) do not agree with this assumption. It is widely accepted, however, that τ may vary according to levels of physical conditioning (Berry and Moritani 1985; Hagberg et al. 1978b; Hagberg et al. 1980; Hickson et al. 1978; Powers et al. 1985), age (Babcock et al. 1994b; Chilibeck et al. 1996; Cunningham et al. 1993), disease states (Nery et al. 1982; Sietsema et al. 1986; Sietsema et al. 1994) and the muscle groups involved in exercise (Casaburi et al. 1992; Cerretelli et al. 1977).

2.2.3 PHASE III

For light to moderate intensity exercise, *phase II* extends toward a new $\dot{V}O_{2ss}$ known as *phase III*. This period of the response is normally reached within 3 min of the start of exercise (Cerretelli et al. 1979; Wasserman et al. 1967). During *phase III*, energy requirements are met by aerobic metabolism using atmospheric O_2 (Bock et al. 1928). Therefore, if the intensity of the work remains constant, $\dot{V}O_2P$ should reflect cellular respiration.

The steady state of $\dot{V}O_2$ during *phase III* is generally assumed to increase as a linear function of increasing WR (Whipp 1987). Hansen et al. (1984) have reported that the increase in $\dot{V}O_2$ per increase in WR is ~10 ml·min·W^{-1} during cycle ergometry.

2.3 MUSCLE OXYGEN UPTAKE KINETICS AS INFERRED FROM THE RESPONSE IN PULMONARY OXYGEN UPTAKE

In early attempts to determine the kinetics of $\dot{Q}O_2$ in humans, various inferences were made from measurements of the kinetic time course of $\dot{V}O_2P$. To use $\dot{V}O_2P$ kinetics as a means of inferring $\dot{Q}O_2$ kinetics, it is necessary to take into account: (i) the utilisation of O_2 stored in the muscles, lungs and vascular compartments (Inman et al. 1987); and (ii) the temporal dissociation between $\dot{V}O_2P$ and $\dot{Q}O_2$ resulting from the circulatory transit delay between the working muscle and the lung (Whipp and Ward 1990). Furthermore, since the $C(a-\bar{v})O_2$ established at the contracting muscle will be associated with a higher blood flow ($\dot{Q}$) at the lung due to increasing $\dot{Q}T$ during the transit delay from the muscle, consideration must also be given to the different rate of change in $\dot{V}O_2P$ (Whipp and Ward 1990). Of these factors, evidence suggests that O_2 stored in arterial blood (Bjurstedt and Wigertz 1971) and myoglobin (Whipp 1994a) need not be accounted for, since the contribution to $\dot{Q}O_2$ will be negligible unless local PO_2 falls below 5 Torr

(Whipp 1994a). To make amendments for O_2 stored in the lungs, $\dot{V}O_2$ at the alveolar level ($\dot{V}O_2A$) can be estimated from measurements at the mouth (see Beaver et al. 1981). Of the O_2 stored in venous blood, computer simulations by Barstow et al. (1990) have shown that any contribution to $\dot{Q}O_2$ kinetics is likely to occur during *phase I.* By removing this period of the response from determinations, the influence of venous O_2 stores can therefore be disregarded. Since exclusion of the *phase I* component will also account for the initial dissociation between $\dot{V}O_2P$ and $\dot{Q}O_2$, consideration need only be given to the effect of the transit delay during *phase II.* As the increase in $\dot{Q}T$ during this period has been shown to have little influence on the $C(a-\bar{v})O_2$ established at the muscle (Barstow et al. 1990), any dissociation between $\dot{V}O_2P$ and $\dot{Q}O_2$ should remain relatively uniform. Therefore, the time constant of the rise in $\dot{Q}O_2$ ($\tau\dot{Q}O_2$) following the onset of exercise should closely match $\tau\dot{V}O_2P$ (as inferred from $\tau\dot{V}O_2A$) during *phase II.* This implies that the rise in $\dot{Q}O_2$ is not only monoexponential, but also occurs with no, or relatively little delay. That $\dot{Q}O_2$ might follow such a pattern of response was originally supported by research into the time course of the change in $\dot{Q}O_2$ in animal muscle (Mahler 1978 and 1985). As the experiments conducted by Mahler (1978 and 1985) were not repeated using human muscle, establishing whether $\tau\dot{V}O_2P$ (as inferred from $\tau\dot{V}O_2A$) during *phase II* could be used to estimate $\tau\dot{Q}O_2$ was not possible. However, as $\dot{Q}O_2$ is both preceded and controlled by the energy transfer reactions of intramuscular high-energy phosphates (Whipp and Ward 1990), any rise in $\dot{Q}O_2$ should be matched by a proportional decrease in muscle phosphocreatine (PCr) concentration. That is, the kinetics of PCr hydrolysis should mirror those of $\dot{Q}O_2$ (at least in conditions of sufficient O_2). Support for this theory comes from evidence of a direct proportionality between the products of PCr breakdown and $\dot{Q}O_2$ in animals (Mahler 1985; Meyer 1988; Piiper et al.

1968). Therefore, the kinetics of PCr hydrolysis in humans should provide an indirect means of confirming the dynamic response characteristics of $\dot{Q}O_2$.

Although it is possible to determine changes in human muscle PCr concentration ([PCr]) using needle biopsy techniques (for example Bergström 1967), this approach is not only invasive, but lacks the necessary time resolution to allow accurate sampling in the non-steady state. To overcome these problems, several authors (Barstow et al. 1994; Binzoni et al. 1992; McCreary et al. 1996; Molé et al. 1985; Yoshida and Watari 1993) have employed phosphorus-31 nuclear magnetic resonance (^{31}P-NMR) spectroscopy as a means of establishing the changes in a variety of phosphate compounds during dynamic exercise. Although the majority of data resulting from the use of this technique suggest that PCr breakdown is monoexponential and starts immediately after the onset of exercise, Molé et al. (1985) have reported findings that are not consistent with this theory. These authors noted that ATP concentration ([ATP]) changed biphasically following the start of exercise. As this implies a similar, though unobserved, pattern of change in [PCr], it may be that the kinetics of $\dot{Q}O_2$ in humans are also biphasic.

Since the study of Molé et al. (1985), no further evidence has been reported of biphasic responses in a variety of phosphate compounds (Barstow et al. 1994; Binzoni et al. 1992; McCreary et al. 1996; Yoshida and Watari 1993). Indeed, the similarity of the time constants for the change in muscle phosphate and *phase II* $\dot{V}O_2P$ recorded by Barstow et al. (1994), led these authors to conclude that the kinetics of $\dot{V}O_2P$ during *phase II* were a good approximation of those of $\dot{Q}O_2$.

More recently, Grassi et al. (1996) have used a modified constant-infusion thermodilution technique to measure leg blood flow ($\dot{Q}$Leg) during conditions of dynamic exercise. By frequent measurement of the $C(a\text{-}v)O_2$ across the leg, these authors were able to determine the actual leg $\dot{V}O_2$ ($\dot{V}O_2$Leg) at 3 to 4 s intervals

throughout the rapid transient phase of the on-kinetics. The data obtained by Grassi and his co-workers revealed two distinct phases in $\dot{V}O_2$Leg after the onset of exercise. This finding is therefore in agreement with the work of Molé et al. (1985). Grassi and his colleagues reported that the two phases corresponded temporally with those of $\dot{V}O_2$A. However, $\dot{V}O_2$Leg during the initial phase increased only slightly and with a markedly different rate of change compared to the rise in *phase I* $\dot{V}O_2$A. During the second phase, which occurred after a delay of 10 to 15 s, $\dot{V}O_2$Leg was observed to increase as a monoexponential function of time. It was reported that the time constant for the rise in leg muscle O_2 utilisation ($\dot{Q}O_2$Leg), as inferred from $\tau\dot{V}O_2$Leg, during this period closely reflected *phase II* $\tau\dot{V}O_2$A. This was despite the interposition of O_2 stores, changes in $\dot{Q}$M, and circulatory transit delays. Nevertheless, Grassi and his co-workers did not rule out the possibility that the time course of $\dot{V}O_2$Leg may have been subject to methodological limitations and therefore not necessarily a true reflection of $\dot{Q}O_2$Leg. Particular concern was expressed regarding the potential effect of blood flow heterogeneity. If the flow past non-active muscle fibres was faster than that past active fibres, then early venous effluent may largely have come from non-active muscle fibre. The outcome would be a transient reduction, or diminished increase, in the C(a-v)O_2 across the leg (Tschakovsky and Hughson 1999). Additional concern regarding the time delay between O_2 utilisation and measurement was also noted, though efforts were made to address this problem by including an estimate of the delay in calculations.

Presently, some uncertainty still exists regarding the exact response characteristics of $\dot{Q}O_2$ following the onset of exercise in humans. Until technological advances allow the precise nature of the dynamic response in $\dot{Q}O_2$ to be examined, it must be assumed that

the kinetics of $\dot{V}O_2P$ (as inferred from $\dot{V}O_2A$) during *phase II* can be used as a reasonable estimator of $\dot{Q}O_2$ kinetics.

2.4 THE OXYGEN DEFICIT

Evidence shows that the oxidative processes that provide energy for the re-synthesis of ATP do not match demands until ~3 min after the onset of exercise (Cerretelli et al. 1979; Wasserman et al. 1967). During this time, the energy difference must be supplied from sources other than those that utilise atmospheric O_2. These include: i) anaerobic alactic metabolism, that is, PCr splitting; ii) the utilisation of O_2 dissolved in blood and stored in oxyhaemoglobin and myoglobin (assuming a fall in local PO_2 below 5 Torr); and iii) the possible early onset of anaerobic glycolysis (Cerretelli et al. 1979; Connett et al. 1985; di Prampero et al. 1989).

The difference between the actual O_2 consumed and the total O_2 that would have been consumed had a new steady state in aerobic metabolism been reached immediately is known as the oxygen deficit.

Since the increment in $\dot{V}O_{2ss}$ is similar (~10 $ml \cdot min \cdot W^{-1}$) for individuals of 'standard' body mass, that is, non-obese subjects (Hansen et al. 1984), the magnitude of the O_2 deficit will be smallest in those individuals with the most rapid kinetics of $\dot{V}O_2$. This concept is explained schematically in Figure 2.2 (overleaf). It should be noted that determination of the O_2 deficit requires that *phase I* be included in the computation (Whipp et al. 1982).

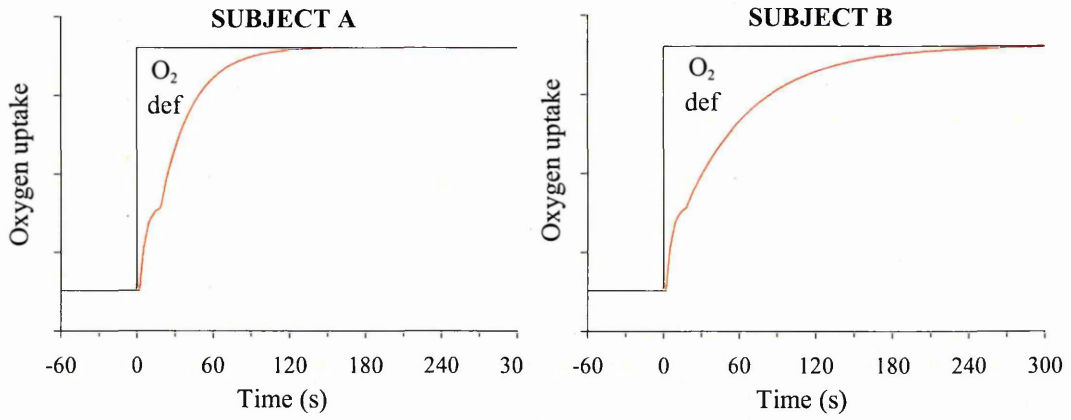


Figure 2.2. Comparison of the oxygen deficit (O_2 def) in two subjects following the onset of constant-rate moderate intensity exercise. The fastest oxygen uptake kinetics and thus smallest oxygen deficit are evident in Subject A. The figure is adapted from an illustration published by Whipp and Wasserman (1972).

It can be deduced from Figure 2.2 that those individuals with the fastest $\dot{V}O_2$ kinetics will be less likely to deplete their high-energy phosphate and O_2 stores to critical levels at lower work rates. The need to use anaerobiosis to supplement energy requirements will therefore be reduced (Hamar 1991; Whipp and Wasserman 1986).

2.5 Oxygen uptake kinetics during heavy exercise

At work intensities above the lactate threshold (LAT), that is, those that provoke a significant and sustained increase in blood lactate, $\dot{V}O_2$ kinetics become more complex.

Following the onset of constant-rate heavy exercise, the primary increase in $\dot{V}O_2$ tends to match the rise that occurs in low or medium intensity work (Casaburi et al. 1989a; Barstow et al. 1993). Before reaching a steady state, however, an additional 'slow component' of $\dot{V}O_2$ becomes evident. It has been demonstrated that the slow component is statistically better described as beginning ~100 s into exercise (Barstow and Molé 1991; Barstow et al. 1993) (Figure 2.3 overleaf).

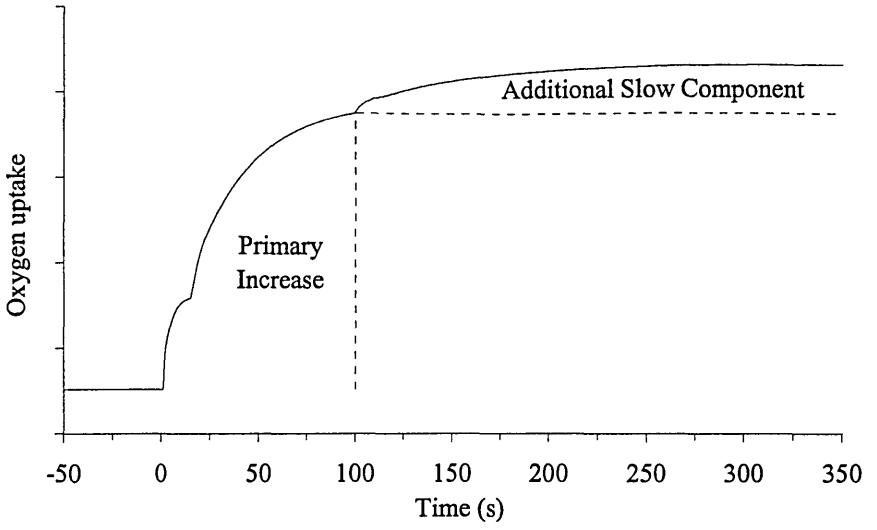


Figure 2.3. Schematic of the oxygen uptake response to the onset of heavy exercise at 0 seconds. The additional slow component in oxygen uptake is shown to begin approximately 100 seconds following the start of exercise. The figure is an adaptation of the diagram presented by Barstow and Molé (1991).

Increases in blood lactate during heavy exercise are assumed to reflect the cytosolic production of ATP from anaerobic glycolysis (Barstow et al. 1993). For WRs accompanied by a modest increase in lactate (2 to 4 mmol·l^{-1}), $\dot{V}O_2$ may reach a delayed steady state before the subject becomes too fatigued to continue (Roston et al. 1987). Barstow and Molé (1991) have shown that if $\dot{V}O_{2ss}$ is achieved, then the increase in $\dot{V}O_2$ per increase in WR will be ~12 to 13 ml·min·W^{-1}. This exceeds by ~2 to 3 ml·min·W^{-1} the $\dot{V}O_{2ss}$ versus WR relationship reported by Hansen et al. (1984) for exercise below the LAT (Figure 2.4 overleaf).

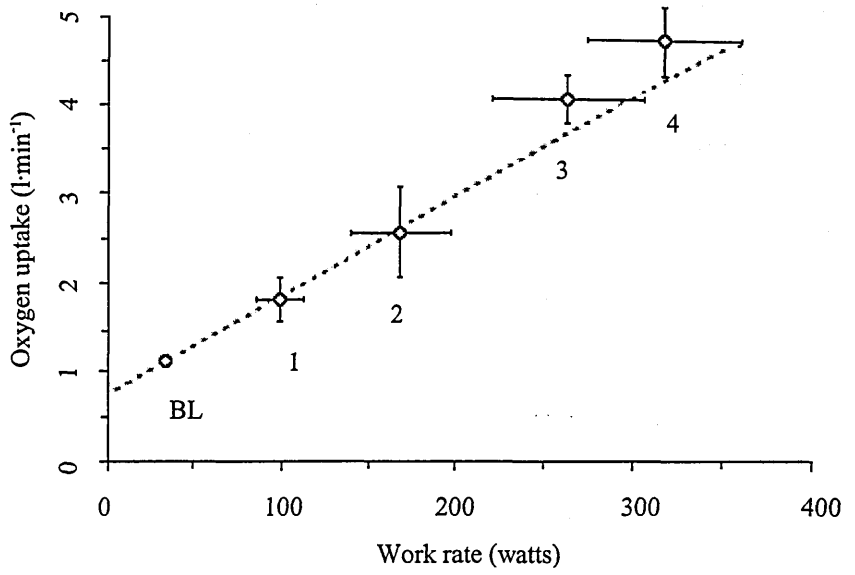


Figure 2.4. Group mean end-exercise oxygen uptake as a function of work rate. Values are mean (standard deviation). The work rates comprise baseline (33 watt, BL); 35% (1) and 55% (2) of work at maximum oxygen uptake (representing work below the lactate threshold); and 85% (3) and 100% (4) of work at maximum oxygen uptake (representing work above the lactate threshold). The dashed line is the linear regression of oxygen uptake at BL, and work rates 1 and 2. The illustration is taken from Barstow and Molé (1991).

At WR intensities that provoke an increase in lactate above 4 mmol·l^{-1}, $\dot{V}O_2$ usually increases progressively until the exercise is terminated or the subject is unable to continue due to exhaustion (Roston et al. 1987).

For dynamic exercise in the supra-LAT domain, there is a slowing in $\dot{V}O_2$ kinetics that correlates with increasing work intensity (Casaburi et al. 1989a). It has been shown that the slowing in $\dot{V}O_2$ kinetics is due to the emergence of the slow component, rather than any influence of the higher WR on the primary rise in $\dot{V}O_2$ (Casaburi et al. 1989a; Barstow et al. 1993).

Currently, there is a great deal of uncertainty regarding the physiological mechanism(s) responsible for the slow component rise in $\dot{V}O_2$ during heavy exercise. Several investigators have identified a linear relationship between the magnitude of the $\dot{V}O_2$

slow component and blood lactate concentration (Capelli et al. 1993; Roston et al. 1987; Whipp and Wasserman 1986; Zhang et al. 1993). Yet, neither the injection of lactate into working animal muscle (Poole 1994; Poole et al. 1994b), nor elevation of blood lactate levels during epinephrine infusion in human subjects (Gaesser et al. 1994) have been shown to influence $\dot{V}O_2$ kinetics. It would therefore seem unlikely that lactate is the (sole) mediator of the slow component rise in $\dot{V}O_2$. In other studies, data have been reported that would suggest the $\dot{V}O_2$ slow component might be related to an increase in serum levels of catecholamines (Galbo 1983), a rise in body temperature (Brooks et al. 1971; Hagberg et al.1978a) or the extra work performed by the respiratory muscles (Aaron et al. 1992; Hagberg et al.1978a). However, later findings discount such hypotheses (Casaburi et al. 1987; Gaesser et al. 1992; Poole et al. 1988; Poole et al. 1991). More recently, it has been proposed that the relative contribution of the slow component to the overall $\dot{V}O_2$ response during heavy exercise might be related to muscle fibre type and/or the pattern of fibre type recruitment (Barstow et al. 1996; Barstow and Molé 1991; Poole et al. 1994a). Unfortunately, further elucidation of this proposal is not within the scope of this thesis.

2.6 THE CONTROL OF OXYGEN UPTAKE KINETICS DURING EXERCISE BELOW THE LACTATE THRESHOLD

After the onset of moderate intensity constant-rate exercise, it has been shown that the *phase II* rise in $\dot{V}O_2$ can be described by first-order exponential kinetics (Linnarsson 1974; Miyamoto et al. 1982; Whipp et al. 1982). Such a simple response suggests that a single process controls the kinetics of $\dot{V}O_2$ during this period (Hughson 1990; Mahler 1980; Walsh 1992; Whipp and Mahler 1980). Although this concept is well supported, the site of the rate-limiting process is the subject of controversy. Several investigators

have argued that the rise in $\dot{V}O_2$ is determined by the rate of O_2 delivery to the mitochondria of the exercising muscle (Convertino et al. 1984b; Hughson et al. 1993; Hughson and Morrissey 1982; Hughson and Smyth 1983). Others support the theory that $\dot{V}O_2$ kinetics are regulated by biochemical factors controlling the rate of O_2 utilisation (Mahler 1980 and 1985; Pendergast et al. 1980; Whipp and Mahler 1980). The following sections draw on the main sources of evidence to provide an overview of each argument.

2.6.1 OXYGEN UTILISATION AND THE CONTROL OF OXYGEN UPTAKE KINETICS

Given sufficient O_2, muscle cells should be capable of maintaining a steady-flux of ATP from mitochondrial oxidative phosphorylation to the cytosolic adenosinetriphosphatases to produce energy (Balaban 1990). To establish whether $\dot{V}O_2$ kinetics are likely to be limited by O_2 supply, the intra- and extra-cellular PO_2 necessary to sustain a required ATP flux can be determined. In tests on various cell suspensions, the flux of ATP has been shown to remain unaffected as long as intra-cellular PO_2 is above 1 to 2 mmHg (Chance 1957 cited by Whipp and Mahler 1980; Hill 1948). Evidence suggests that this condition is satisfied both at rest and for a wide range of steady-state exercise intensities (Gayeski et al. 1987; Keul et al. 1972). For extra-cellular O_2 supplies to become limiting, capillary PO_2 must fall below 15 to 20 mmHg (Wittenberg and Wittenberg 1989). Doll et al. (1968) have shown that, even during maximal work, venous PO_2 is rarely less than 20 mmHg. Whilst both intra- and extra-cellular PO_2 may be subject to heterogeneity of intra-muscular blood flow during dynamic exercise, should these findings be taken to imply that O_2 supply mechanisms do not limit $\dot{V}O_2$ kinetics, then regulation could be ascribed to one of several O_2 utilisation processes involved in the production of energy (Balaban 1990; Mahler 1980).

Following much investigation, it has been hypothesised that the creatine shuttle (Bessman and Geiger 1981) is the most likely regulator of the dynamic response in $\dot{V}O_2$ (Whipp and Mahler 1980). If it is assumed that there are no putative barriers to O_2 diffusion within the cell, and that pH, total phosphate and adenylate pool concentrations remain constant, then the hypothesis proposes that changes in the concentration gradients of PCr and free creatine (Cr) combine to drive the rate of ATP production. A more detailed description of the hypothesis is provided by Walsh (1992). Walsh explains how, following the onset of exercise, [PCr] at the actin-myosin locus will fall almost immediately. The resultant rise in free creatine concentration ([Cr]) will cause an increase in the flux of Cr to the mitochondrion. In turn, an abundance of Cr at the mitochondrion will enhance the rate of high-energy phosphate generation, thus increasing O_2 utilisation. This increase in O_2 utilisation will reduce PO_2 at the mitochondrion, bringing about an increase in the O_2 pressure gradient between the blood and the mitochondrion. As the pressure gradient rises, O_2 flux into the cell will be increased. At the same time, the reduction in [PCr] at the actin-myosin locus should create a pressure gradient that results in PCr being shuttled from the mitochondrion. As a consequence of these processes, the error signal between the [Cr], [PCr] and O_2 concentration gradients will become less as steady state is approached. Since the rate of change in the concentration gradients should be proportional to the error signal, the time course of the change will be exponential in character. This would therefore explain the similar pattern of response observed in *phase II* $\dot{V}O_2$.

Although the Cr shuttle hypothesis implies that Cr is the limiting factor in $\dot{V}O_2$ kinetics, mitochondrial [Cr] is determined by the rate of PCr hydrolysis at the actin-myosin locus. As PCr hydrolysis is closely associated with the mitochondrial enzyme, creatine kinase,

it is possible that $\dot{V}O_2$ kinetics are in fact controlled by the activities of this enzyme (Mahler 1985).

If $\dot{V}O_2$ kinetics are dependent on creatine kinase, then the creatine shuttle hypothesis suggests that the kinetics of PCr hydrolysis should mirror those of $\dot{V}O_2$ (Connett and Honig 1989). Support for such a relationship was initially derived from studies of animal muscle preparations (Hultman 1973; Kushmerick and Paul 1976; Mahler 1985; Piiper and Spiller 1970). In these studies, measurements were made of the change in [PCr] (Δ[PCr]) following a period of artificially induced tetanus. From the resultant data, the time course of Δ[PCr] during recovery from the tetanus was shown to be mono-exponential and directly proportional to the time course of $\dot{Q}O_2$ in well-oxygenated animal muscle (Connett and Honig 1989; Mahler 1978, 1980 and 1985; Meyer 1988; Piiper et al. 1968). Marconi et al. (1982) have since shown that $\tau\dot{Q}O_2$ under these conditions is similar to $\tau\dot{V}O_2$ in intact animals.

More recently, NMR spectroscopy has allowed studies of PCr hydrolysis to be performed on humans *in vivo*. Investigations by Barstow et al. (1994), McCann et al. (1995) and Yoshida and Watari (1993) have revealed that the time course of PCr hydrolysis during plantar (Barstow et al. 1994), wrist (McCann et al. 1995) and femoral (Yoshida and Watari 1993) flexion agrees well with that of $\dot{V}O_2$ recorded during leg ergometry (Barstow et al. 1994; di Prampero et al. 1970; Linnarsson 1974; Whipp et al. 1982). Although comparing data from different muscle groups exercising at different metabolic rates may be considered ill-advised, in support of these findings, a within-subject study by McCreary et al. (1996) has shown that, during supine plantar flexion, the time constant for Δ[PCr] is not significantly different to that of $\dot{V}O_2$. It should be noted, however, that the difference in intensity of plantar flexion employed by these authors only generated a small change in $\dot{V}O_{2ss}$. As the confidence limits of kinetic

parameter estimation are highly dependent on the $\dot{V}O_{2ss}$ increment attained during dynamic exercise (Lamarra et al. 1987), such small changes will reduce confidence in the time constant of $\dot{V}O_2$.

In addition to reflecting the dynamic response in $\dot{V}O_2$, the creatine shuttle hypothesis implies that changes in [PCr] should also be linearly related to changes in $\dot{V}O_2$ (Hughson 1990; Walsh 1992). In the examination of $\dot{V}O_2$ kinetics, linearity denotes both static and dynamic linearities. Underlying the concept of linearity is Boltzmann's principle of superposition (Fujihara et al. 1973a & 1973b). The principle states that:

> (i) in a system that demonstrates static linearity, a change in the magnitude of a stimulus will lead to a directly proportional change in the magnitude of any resulting response.
>
> (ii) in a system that demonstrates dynamic linearity, the time course of the response will be independent of both the form and magnitude of the stimulus (Casaburi et al. 1989a; Hughson 1990).

Dynamic linearity implies static linearity, though not vice versa (Hoffmann et al. 1992).

The assumption that the process that governs $\dot{V}O_2$ kinetics might exhibit linear behaviour was first made by Stegemann in 1958 (cited by Fujihara et al. 1973a). Rather than demonstrating unconstrained linearity, however, Stegemann suggested that the linear characteristics of the process would only be evident under certain conditions. It has since been shown that, for WR intensities associated with a sustained increase in blood and muscle lactate concentration, non-linear influences become apparent in the kinetic parameters of the $\dot{V}O_2$ response (Barstow 1993; Casaburi et al. 1987; Casaburi et al. 1989a; Whipp 1994b; Whipp and Wasserman 1986).

For exercise intensities that do not lead to a sustained increase in lactate, data from animal muscle (Altschuld and Brierley 1977; Connett and Honig 1989; Mahler 1985;

Meyer 1988) indicate that the kinetics of $\dot{Q}O_2$ are linearly related to the kinetics of PCr. Furthermore, in experiments on isolated skeletal muscle, it has been noted that $\tau\dot{Q}O_2$ in the recovery from a period of tetanus does not vary over a range of intensities if O_2 supply is plentiful (di Prampero and Margaria 1968; Mahler 1978 and 1985). Piiper et al. (1968) have also shown that, when *in situ* animal muscle performs brief isotonic contractions at a constant rate, $\tau\dot{V}O_2$ remains independent of the change in $\dot{V}O_{2ss}$.

In humans, a linear relationship between $\dot{V}O_2$ kinetics and [PCr] kinetics would suggest that a simple mono-exponential model should characterise the time course of PCr hydrolysis after the onset of exercise. In investigations by Marsh et al. (1993a), changes in [PCr] and inorganic phosphate concentration were recorded during dynamic wrist exercise. Data were then plotted as a function of time and fitted with a single-exponential model and a double-exponential model. Analysis revealed that the second-order model did not provide a significantly better fit to the data than the first-order model. Marsh and his co-authors (1993a) therefore concluded that first-order proportionality existed between PCr hydrolysis and O_2 utilisation.

Whilst the findings of Marsh et al. (1993a) lend support to the role of the creatine shuttle in the regulation of $\dot{V}O_2$ kinetics, first-order proportionality should not be taken to signify the existence of only one controlling process. Rather, it implies that one component of the control system dominates the dynamic response (Cunningham et al. 1993). Certainly, given the complex nature of the mechanisms underlying the $\dot{V}O_2$ response, it would seem unlikely that a single process could be rate limiting under all conditions. In support of this assumption, data have been reported that suggest other processes influence the dynamic response in $\dot{V}O_2$ during moderate intensity exercise (Casaburi et al. 1989a; Hagberg et al. 1978b; Hughson et al. 1988). These data reveal how $\dot{V}O_2$ kinetics slow for increasing step changes in WR when the intensity of work is

below the LAT. Casaburi et al. (1992) have speculated that this slowing might reflect transient glycolytic ATP production, as evidenced by slight and temporary elevations in blood lactate (Cerretelli et al. 1979; Cerretelli et al. 1980). ATP generated from this source would spare early O_2 utilisation at a rate proportional to the step increase in exercise intensity. Such sparing of O_2 would manifest itself in a gradual slowing in $\dot{V}O_2$ kinetics (Casaburi et al. 1992). The work of Binzoni et al. (1992) appears to be in agreement with this proposal. These authors noted that, whilst the kinetics of PCr in human subjects matched those of $\dot{Q}O_2$ in isolated perfused animal muscle (Piiper et al. 1968), when considering $\dot{V}O_2$ recorded at the mouth, such equivalence would be found only in the absence of early lactate and changes in muscle O_2 stores.

Prior to the study of Binzoni et al. (1992), Hughson et al. (1988) compared estimates of $\dot{V}O_2$ kinetics derived from step and impulse WR forcings. Although the total work performed during each type of forcing was approximately equal, significant differences were observed in the kinetic parameters of the $\dot{V}O_2$ response. As these findings are not consistent with the principle of superposition, Hughson and his co-authors proposed that $\dot{V}O_2$ kinetics must be controlled by a non-linear process. Such a proposal is in agreement with investigations that have shown $\dot{V}O_2$ kinetics below the LAT to be dependent on the magnitude of a step change in WR (Casaburi et al. 1989a; Hagberg et al. 1978b; Hughson et al. 1988).

The non-linear characteristics reported by Casaburi et al. (1989a), Hagberg et al. (1978b) and Hughson et al. (1988) contradict the findings of earlier investigators (di Prampero and Margaria 1968; Mahler 1978 and 1985; Marsh et al. 1993a; Piiper et al. 1968). If $\dot{V}O_2$ kinetics are dictated by the rate of PCr hydrolysis at the mitochondrion, then the data reported by Casaburi et al. (1989a), Hagberg et al. (1978b) and Hughson et al. (1988) suggest that Δ[PCr] following the onset of exercise is not a

simple linear first-order process (Hughson 1990). Although previous studies have reported PCr kinetics that are linear and first-order, these studies have been restricted to animal muscle preparations (Mahler 1985; Meyer 1988) and to tests of relatively small muscle mass in humans (Barstow et al. 1994; Marsh 1993a; McCreary et al. 1996). Consequently, when considering exercise in humans involving large muscle groups, it is possible that PCr kinetics are more complex than has been previously speculated. Alternatively, the findings of Hughson et al. (1988) might reflect the influence on $\dot{V}O_2$ kinetics of other sub-dominant processes (Cunningham et al. 1993), or the regulation of $\dot{V}O_2$ kinetics by another process altogether (Convertino et al. 1984b; Hughson and Morrissey1983; Hughson et al. 1988).

Whilst a number of biochemical factors other than the creatine shuttle hypothesis may be responsible for the control $\dot{V}O_2$ kinetics (Balaban 1990; Mahler 1980), regulation by O_2 delivery rather than O_2 utilisation has been promoted as a more likely alternative.

2.6.2 OXYGEN TRANSPORT AND THE CONTROL OF OXYGEN UPTAKE KINETICS

During dynamic exercise, O_2 supply to the site of oxidative metabolism is dependent on a number of factors. These include arterial PO_2, $\dot{Q}T$, haemoglobin concentration, the affinity for O_2 of the haemoglobin, $\dot{Q}$ in both working and non-working tissues and the perfusion of blood through the lung and tissues that demand O_2 (Wasserman et al. 1991). Several investigators have monitored the effect that acute changes in such factors have on the rate of adjustment in $\dot{V}O_2$. In many instances, the resultant data support the premise that O_2 transport plays a major role in regulating the dynamic response in $\dot{V}O_2$. There exists, however, a great deal of evidence that contradicts this assumption.

2.6.2.1 BETA-ADRENERGIC RECEPTOR BLOCKADE

In healthy subjects, beta-adrenergic receptor blockade (β-blockade) reduces HR both at rest and during exercise (Hughson 1984a and 1984b; Hughson and Smyth 1983). Although the reduction in HR is partly offset by a rise in SV (MacFarlane et al. 1983), $\dot{Q}T$ remains below normal (Hughson and Kowalchuk 1991). To compensate for the effect of β-blockade on $\dot{Q}T$, evidence suggests that blood flow to non-working tissues ($\dot{Q}NW$) is redistributed to exercising muscles (Dumont et al. 1984; Hughson and Kowalchuk 1991).

In studies by Hughson and his colleagues (Hughson 1984a; Hughson and Kowalchuk 1991; and Hughson and Smyth 1983), a significant slowing in $\dot{V}O_2$ kinetics was observed to follow the administration of β-blockade medication. Hughson and Kowalchuk (1991) have suggested that the redistribution in $\dot{Q}NW$ that accompanies β-blockade might not be sufficient to allow uninhibited oxidative metabolism. Slower $\dot{V}O_2$ kinetics would therefore result from the effect of β-blockade on O_2 supply mechanisms.

Whilst often promoted as strong evidence of an O_2 transport limitation, studies involving β-blockade have been criticised by Hoffman et al. (1994a). These authors noted that the exercise capacity of those receiving the treatment might be reduced to such an extent that the normal LAT would be exceeded.

2.6.2.2 CIRCULATORY OCCLUSION

At the onset of exercise, $\dot{Q}$ must be redistributed to meet the demands for O_2 of the working muscle. To increase $\dot{Q}$, and therefore O_2 supply to the site of demand, the circulation in non-working areas of the body can be occluded.

In work by Hughson and Inman (1986a), circulatory occlusion was performed on the legs prior to the onset of supine arm exercise. Redistribution of $\dot{Q}$ by this method was observed to accelerate the dynamic response in $\dot{V}O_2$. Hughson and Inman (1986a) concluded that their data supported the regulation of $\dot{V}O_2$ kinetics by O_2 transport mechanisms.

2.6.2.3 SUPINE VERSUS UPRIGHT EXERCISE

During upright exercise, gravitational forces are known to slow venous return from the legs. Although greater dependence on the Frank-Starling mechanism helps to maintain SV (Bevegard et al. 1960; Holmgren and Ovenfors 1960; Poliner et al. 1980), Bevegard et al. (1960) have reported that $\dot{Q}T$ during upright exercise is still less than $\dot{Q}T$ during supine exercise. This finding is supported by evidence of a significantly lower pulse pressure and O_2 pulse in upright exercise (Convertino et al. 1984a).

If $\dot{V}O_2$ kinetics are dependent on O_2 transport, then it might be expected that reduced $\dot{Q}$ in the upright position would lead to slower $\dot{V}O_2$ kinetics. However, several studies have shown that the dynamic response in $\dot{V}O_2$ is in fact faster during upright exercise (Cerretelli et al. 1977; Convertino et al. 1984a; Hughson et al. 1991b). As the observed acceleration can not be explained by changes in mechanical efficiency (Convertino et al. 1984a) or variations in tissue metabolism (Hughson et al. 1991b), some O_2 transport mechanism external to cardiac functioning must account for the different rates of change in $\dot{V}O_2$. In explanation, it has been proposed that slower $\dot{V}O_2$ kinetics in the supine position might result from: (i) pooling of blood in the central veins that would increase the transit time from the working muscle to the lungs (Hoffmann et al. 1991); (ii) insufficient gravitational forces to augment perfusion pressure across the muscle capillary beds (Hughson et al. 1991b); and/or (iii) a decrease in sympathetic tone that

would lead to a reduction in the normal vasoconstriction seen during exercise, thus increasing blood flow through non-working tissue, particularly the viscera (Hughson et al. 1993).

In related work by Hughson et al. (1993), it was noted that the slowing in $\dot{V}O_2$ kinetics observed during supine leg exercise could be reversed by applying lower body negative pressure (LBNP). These authors have suggested that the effect of LBNP would be to raise the pressure gradient for $\dot{Q}$ from the heart to the working muscles of the leg. The resulting change in perfusion pressure (Eiken 1987 and 1988) would increase O_2 supply to the working muscles thus facilitating faster $\dot{V}O_2$ kinetics.

More recently, Williamson et al. (1996) have examined the outcome of applying lower body positive pressure (LBPP) to reduce $\dot{Q}M$ during leg exercise in the recumbent position. Following the work of Hughson et al. (1993), it might have been expected that LBPP would result in a slowing of $\dot{V}O_2$ kinetics. However, Williamson and his colleagues (1996) could find no discernible difference in the rate of change in $\dot{V}O_2$. It was therefore concluded that $\dot{Q}$, and hence O_2 supply, to the working muscles must normally be in excess of requirements.

2.6.2.4 ALTERED OXYGEN INTAKE

In investigations by Springer et al. (1991) and Xing et al. (1991), hypoxia, which reduces the PO_2 in arterial blood, was shown to result in a significant slowing of the HR response to dynamic exercise. Several authors (Engelen et al. 1996; Hughson and Kowalchuk 1995; Linnarsson et al. 1974; Murphy et al. 1989) have reported data recorded during hypoxic exercise that reveal slower $\dot{V}O_2$ kinetics compared with conditions of normoxia. Such findings suggest that the availability of O_2 acts as a rate-limiting step in $\dot{V}O_2$ kinetics. Evidence to support this claim is provided by

Linnarsson et al. (1974). These authors were able to show that exercise under conditions of hyperoxia led to a reduction in the incurred O_2 deficit. It should be noted, however, that Linnarsson and his colleagues employed WR intensities above the LAT. Furthermore, Hughson and Kowalchuk (1995) were not able to reproduce the findings of Linnarsson et al. (1974) when using moderate WR intensities. Hughson and Kowalchuk therefore acknowledged that $\dot{V}O_2$ might well be set by metabolic demand rather than the availability of O_2 under conditions of normal arterial oxygen content.

2.6.2.5 TRAINING

A number of investigators have applied chronic intervention methods to determine the process that regulates $\dot{V}O_2$ kinetics. Following the intervention, changes in the rate of the $\dot{V}O_2$ response can be compared with equivalent changes in related processes, and inferences made of possible couplings. The most common intervention involves participation in a programme of endurance training. Several authors (Cerretelli et al. 1979; Hagberg et al. 1980; Hickson et al. 1978; Phillips et al. 1995) have reported that this type of training results in a significant acceleration in $\dot{V}O_2$ kinetics.

Following a period of endurance training, Cerretelli et al. (1979) noted that even the fastest $\dot{V}O_2$ response was still slower than the rate of adjustment in central (Cerretelli et al. 1966) and peripheral (Clausen and Lassen 1971) circulation. This implies that the primary influence of training on the dynamic response in $\dot{V}O_2$ occurs distal to the capillary.

One possible source of the acceleration in $\dot{V}O_2$ kinetics would be a reduction in the diffusion distance for O_2 (Walsh 1992). This might result from a training induced increase in mitochondrial content (Davies et al. 1981; Ingjer 1979; Saltin and Gollnick 1983), capillary density and/or muscle fibre area (Andersen 1975; Andersen and

Henriksson 1977; Brodal et al. 1977). A reduction in the diffusion distance would also allow O_2 delivery to be maintained for a lower capillary P_{O_2} (Saltin et al. 1977). Alternatively, an increase in myoglobin levels following training may facilitate storage of O_2 near to the site of metabolism (Harms and Hickson 1983; Pattengale and Holloszy 1967). However, it is also possible that training induced changes in O_2 utilisation mechanisms are the cause of the faster $\dot{V}O_2$ kinetics. Such changes might include:

(i) an increase in the ATP concentration of resting muscle (Karlsson et al. 1972);
(ii) an acceleration in the rate of mitochondrial respiration as evidenced by a reduced accumulation of early lactate in trained muscle (Cerretelli et al. 1980);
(iii) a more rapid adjustment in the velocity of the reactions of the Kreb cycle and/or the transfer of electrons along the respiratory chain (Cerretelli et al. 1988);
(iv) an increase in the concentration and/or activity levels of respiratory enzymes in the cytosol and mitochondria that would facilitate oxidative phosphorylation and increase the capacity for oxidative metabolism (Andersen and Henriksson 1977; Apple and Rogers 1986; Coggan et al. 1992b; Gollnick et al. 1973; Holloszy 1967 and 1975; Holloszy and Booth 1976; Örlander and Aniansson 1980; Proctor et al. 1995; Saltin and Gollnick 1983).

Of these changes, the majority of evidence would appear to link the control of $\dot{V}O_2$ kinetics with enzymatic activity. However, a recent study by Phillips et al. (1995) suggests that this may not be the case. These authors reported acceleration in whole body $\dot{V}O_2$ kinetics as early as 4 days after the onset of a program of endurance training. This was accomplished without changes in the maximal activity of either citrate synthase (Phillips et al. 1995) or succinate dehydrogenase (Phillips et al. 1996). Phillips and his colleagues (1995) speculated that an increase in femoral artery blood velocity during the same 4-day period, leading to a more rapid acceleration in O_2 transport, might have been the source of the faster $\dot{V}O_2$ kinetics. The work of Shoemaker et al. (1996) is

in agreement with this proposal. These authors observed an increase in $\dot{Q}T$, femoral artery mean blood velocity and vascular conductance after a short period of endurance training. Nevertheless, Phillips et al. (1995) have recognised that other undetermined factors might also generate the changes observed in their study. Of these, particular consideration was given to possible adaptations in the intramuscular concentration of allosteric effectors of a variety of enzymes. Such adaptations would also explain the changes in high-energy phosphate metabolism noted by these authors.

2.6.2.6 DECONDITIONING

Following a 6 to 7 day period of bed rest, several investigators (Convertino et al. 1984b; Eßfeld et al. 1984; Stegemann et al. 1985) have detected a significant slowing in the kinetics of $\dot{V}O_2$. In the study of Convertino et al. (1984b), this slowing was only observed to occur during upright exercise. Although Chi et al. (1983) have reported that detraining induces a fall in the levels of oxidative enzymes, if changes in O_2 utilisation mechanisms had been the cause of the slowing in $\dot{V}O_2$ kinetics, then differences between pre- and post-bed rest data might have been expected during exercise in both supine and upright postures. In work by Bevegard et al. (1960) and Convertino et al. (1984a), it was suggested that $\dot{Q}T$ in the upright position might be less than in the supine position. As erythrocyte volume, plasma volume and total blood volume are known to fall during deconditioning, the resulting drop in blood volume would likely exacerbate any reduction in $\dot{Q}T$ during upright exercise (Convertino et al. 1984b). In this instance, O_2 transport would be reduced to a greater extent than in supine exercise, thus providing an explanation for the slower $\dot{V}O_2$ kinetics.

2.6.2.7 AGE

In a number of studies (Babcock et al. 1994b; Chilibeck et al. 1996; Cunningham et al. 1993) $\dot{V}O_2$ kinetics have been observed to slow with age. Several investigations have shown that, concurrent with ageing, there is a significant reduction in capillarisation (Coggan et al. 1992a; Praizkova et al. 1971), vascular conductance (Makrides et al. 1990) and arterial P_{O_2} (Sorbini et al. 1968). It has also been reported that a decrease in the elasticity, strength and capacity of the thoracic apparatus of elderly individuals may result in moderate arterial hypoxaemia (Horvath and Borgia 1984; Levitzky 1984). In other related studies, the skeletal muscle of older individuals has been shown to comprise a greater amount of 'non-muscle' tissue (Overend et al. 1992; Rice et al. 1989). This accumulated evidence has led Babcock et al. (1994b) to suggest that $\dot{Q}$ redistribution and muscle perfusion may be influential in slowing $\dot{V}O_2$ kinetics.

Prior to those studies that have revealed a slowing in $\dot{V}O_2$ kinetics with age, de Vries and his colleagues (1982) were unable to identify any significant differences between the $\dot{V}O_2$ kinetics of old and young subjects. However, the two groups tested by de Vries et al. comprised well-trained endurance athletes with approximately equal levels of age corrected $\dot{V}O_{2max}$. Following investigations by Babcock et al. (1994a), it has been shown that aerobic endurance training leads to significantly faster $\dot{V}O_2$ kinetics in previously sedentary older subjects. Data indicate that the training performed by such subjects can result in enhanced vascular conductance (Makrides et al. 1990), capillary density (Coggan et al. 1992b) and peripheral vasodilatory capacity (Martin et al. 1990). The influence of these factors on $\dot{Q}$ could therefore account for the findings reported by de Vries and his colleagues. Other data, however, document substantial increases in the oxidative capacity of skeletal muscle and mitochondrial respiratory enzymes following training in older individuals (Coggan et al. 1992b; Marsh et al. 1993b; Meredith et al.

1989; Örlander and Aniansson 1980). Such changes indicate that the control of $\dot{V}O_2$ kinetics may be attributed to mechanisms of O_2 utilisation. Additional information that demonstrates how, without training, there is a reduction with age in the activity levels of oxidative enzymes (Coggan et al. 1993; McCully et al. 1993), and a fall in the number, size and metabolic activity of animal muscle mitochondria (Beyer et al. 1984) would appear consistent with this proposal.

Further evidence that would support the impairment of $\dot{V}O_2$ kinetics by nonhemodynamic factors concerns the muscle fibre type involved in exercise. In experiments by Chilibeck et al. (1996) it was reported that estimates of $\dot{V}O_2$ kinetics obtained during plantar flexion did not slow with age. As plantar flexion is routinely used in daily activity, it is likely that the percentage of recruited type II (low oxidative) fibres (Baldwin et al. 1977) and type IIa (intermediate) fibres (Green et al. 1990) would be less than in occasionally performed exercise. The absence of any slowing in $\dot{V}O_2$ kinetics observed by Chilibeck et al. (1996) might therefore have resulted from the more efficient mechanisms of O_2 utilisation in the employed type I (high oxidative) fibres. In support of this theory, di Prampero et al. (1989) have reported that $\dot{V}O_2$ kinetics are extremely fast in animals whose glycolytic energy contribution during submaximal exercise is negligible due to their almost exclusive employment of type I muscle fibres (Hoppeler cited by Cerretelli et al. 1980). Nevertheless, it should still be noted that arm exercise, which involves a higher percentage of type IIa muscle fibres, results in similar $\dot{V}O_2$ kinetics to leg exercise at the same percentage of arm and leg $\dot{V}O_{2max}$ (Cerretelli et al. 1977).

2.6.2.8 HEART TRANSPLANTATION

Several investigators, including Cerretelli et al. (1988), Meyer et al. (1989) and Paterson et al. (1994), have noted that $\dot{V}O_2$ kinetics are prolonged in heart transplant recipients.

After heart transplantation, the HR response to exercise is delayed due to cardiac denervation (Paterson et al. 1994). It has also been reported that $\dot{Q}$ might be compromised by any period of bed rest and/or reduced physical activity experienced by transplant patients prior to, or following, their operation (Sinoway et al. 1988). Whilst these findings would appear to reflect limitation of $\dot{V}O_2$ kinetics by O_2 transport mechanisms, it is equally likely that such acute deconditioning might have led to deterioration in the oxidative machinery of skeletal muscle.

Although additional investigation involving heart transplant recipients might reveal further relevant information, it would be unwise to draw any firm conclusions from data collected in individuals who have undergone such an extreme form of surgery.

2.6.2.9 PRIOR EXERCISE

In early experiments (Casaburi et al. 1977; Diamond et al. 1977; Whipp et al. 1982), no evidence could be found to suggest that $\dot{V}O_2$ kinetics were appreciably altered by prior exercise. More recently, studies by Hughson and Morrissey (1982 and 1983) have shown the rise in $\dot{V}O_2$ to be slower in the transition from light to moderate exercise than in the transition from rest to moderate exercise. Although these findings contradict those of early experiments, Hughson and Morrissey (1983) have ascribed the discrepancy to their use of more appropriate models for characterisation of the $\dot{V}O_2$ response.

If, as has been reported by Hughson and Morrissey (1982 and 1983), $\dot{V}O_2$ kinetics are influenced by prior exercise, then this suggests that the regulatory process responsible

for the control of $\dot{V}O_2$ kinetics behaves in a non-linear manner. In their work, Hughson and Morrissey noted that the kinetics of HR were also dependent on the prior exercise state. Such evidence prompted these authors to conclude that $\dot{V}O_2$ kinetics must be controlled by haemodynamic factors.

In more recent experiments, Yoshida et al. (1995) have reported data that directly oppose those of Hughson and Morrissey (1982 and 1983). In the transition from prior exercise, Yoshida and his colleagues observed the rate of adaptation in $\dot{V}O_2$ to be significantly increased. It has been speculated that faster $\dot{V}O_2$ kinetics in the transition from one level of exercise to another would be necessary to compensate for the prior depletion of muscle O_2 stores (di Prampero 1981). This implies that $\dot{V}O_2$ kinetics are limited by the utilisation of O_2. Yoshida et al. have, however, accepted that their findings might have been influenced by the use of exercise transitions from zero load cycling rather than rest. Nevertheless, it should be noted that these authors did not identify any significant influence of prior exercise on either HR kinetics or $\dot{Q}M$ kinetics.

Whilst many investigators have argued that prior exercise has an explicit influence on $\dot{V}O_2$ kinetics, the opinions of di Prampero et al. (1989) express less certainty. These authors have proposed that prior exercise may either speed or slow $\dot{V}O_2$ kinetics. This proposal is based on work by Cerretelli et al. (1979) that shows evidence of transient anaerobic glycolysis, even in the transition to light exercise. Thus, $\dot{V}O_2$ kinetics may be accelerated or decelerated depending on whether the depletion of blood, tissue and lung O_2 stores on transition to the higher workload is greater or smaller than the corresponding anaerobic energy contribution (di Prampero et al. 1989).

2.6.2.10 BLOOD FLOW

The assumption that the availability of O_2 does not limit $\dot{V}O_2$ kinetics is well supported by data on the dynamics of $\dot{Q}$. Following the onset of exercise, the readjustment in both HR (Davies et al. 1972; Linnarsson 1974) and $\dot{Q}$ (as estimated from $\dot{Q}T$) (De Cort et al. 1991; Grucza et al. 1990; Inman et al. 1987; Miyamoto et al. 1982; Pendergast et al. 1980; Yoshida and Whipp 1994; Yoshida et al. 1993) has been shown to be quicker than the readjustment in $\dot{V}O_2$. Furthermore, in isolated animal muscle (Piiper et al. 1968) and human muscle *in vivo* (Cerretelli et al. 1978; Raynaud et al. 1973), measurements of muscle blood flow suggest that $\tau\dot{Q}M$ after the start of exercise is significantly faster than that reported for $\tau\dot{Q}O_2$ in animal muscle (Casaburi et al. 1979; Piiper et al. 1968).

More recently, advances in measurement techniques have allowed the detailed examination of $\dot{Q}$ in intact human skeletal muscle during dynamic exercise. The Doppler ultrasound technique employed by Shoemaker et al. (1994) revealed that $\dot{V}O_2$ lagged considerably behind the kinetics of femoral artery mean blood velocity. Although this finding supports the regulation of $\dot{V}O_2$ kinetics by O_2 utilisation mechanisms, Shoemaker et al. did note that the kinetics of $\dot{V}O_2$ recorded in their study were similar to those obtained during work performed above the ventilatory threshold.

In a similar investigation to that of Shoemaker et al. (1994), Grassi et al. (1996) were able to show that $\dot{Q}$Leg increased from the start of exercise. Unlike Shoemaker et al., the $\tau\dot{V}O_2$ reported by Grassi and his colleagues was not different to that expected for exercise below the ventilatory threshold. It was therefore proposed that $\dot{Q}O_2$Leg was not constrained by O_2 delivery to the leg muscles. Nevertheless, these authors have acknowledged that the factor regulating $\dot{V}O_2$ kinetics could still reside extracellularly.

That is, whilst O_2 delivery to the leg may be adequate, $\dot{Q}O_2$Leg could be limited by the poor intramuscular distribution of O_2.

Although the majority of investigations have shown that the adaptation in $\dot{Q}M$ is unrelated to $\dot{V}O_2$ kinetics, the findings of Hughson et al. (1996) are not in agreement with this notion. Using Doppler techniques, these authors determined the effects of altered $\dot{Q}M$ on estimates of muscle $\dot{V}O_2$ ($\dot{V}O_2M_{est}$) at the onset of forearm exercise. Tests were conducted in the supine position with the arm either above or below the heart to achieve different perfusion pressures. During the adaptive phase, Hughson and his colleagues observed that the rise in both $\dot{Q}M$ and $\dot{V}O_2M_{est}$ was notably faster for exercise performed below the heart. The similarity between the response times for $\dot{Q}M$ and $\dot{V}O_2M_{est}$ led Hughson et al. (1996) to conclude that muscle $\dot{V}O_2$ ($\dot{V}O_2M$) must be dependent on the dynamics of O_2 transport. To explain the conflicting findings of Grassi et al. (1996), Hughson and his colleagues claimed that the thermodilution method employed by these authors might not have been able to account for the additional contribution of pooled venous blood. Given the evidence reported by Hughson et al. (1996), the possibility that $\dot{V}O_2$ kinetics are controlled by transportation mechanisms cannot be discounted.

2.6.2.11 COMPUTER MODELLING

In an attempt to identify the process responsible for the control of $\dot{V}O_2$ kinetics, several investigators have made use of computer based physiological models. By designing models that simulate the circulatory and metabolic responses to exercise, it becomes possible to examine the likely effect on $\dot{V}O_2$ kinetics of adjusting potential limiting factors.

Using modelling techniques, Inman et al. (1987) examined the outcome of subtracting an estimate of the changes in the venous blood O_2 stores from $\dot{V}O_2A$. The resulting data suggested that mean tissue $\dot{V}O_2$ ($\dot{V}O_2T$) kinetics, representing $\dot{V}O_2M$ kinetics, were significantly faster than those of $\dot{V}O_2A$. It was further noted that the kinetics of $\dot{V}O_2T$ were similar to those of $\dot{Q}T$. These findings led Inman et al. to propose that the adaptation in $\dot{Q}T$ at the start of exercise was the rate-limiting step for $\dot{V}O_2$ kinetics.

If, as the work of Inman et al. (1987) suggests, haemodynamic factors comprise the rate-limiting step, then it might be expected that increasing $\dot{Q}M$ would result in a corresponding increase in $\dot{V}O_2M$ kinetics. However, calculations by Barstow et al. (1990), using data obtained by Corsi et al. (1975), did not result in any observable speeding in the kinetics of $\dot{V}O_2M$. Furthermore, information derived from the model of Barstow and his co-workers revealed that O_2 delivery to the muscle rises more rapidly than O_2 extraction following the onset of exercise.

Whilst the calculations of Barstow et al. (1990) might lead to the conclusion that $\dot{V}O_2$ kinetics are limited by the ability of the muscle to utilise O_2, data put forward by Cochrane and Hughson (1992) contradict this opinion. The model developed by these authors was designed to simulate the response in $\dot{V}O_2A$ to a step change in WR. Besides accounting for variations in $\dot{Q}T$ and the effect of the Bohr relationship on the oxyhaemoglobin dissociation curve, the model also detailed the distribution of $\dot{Q}$ between vascular beds of working and non-working tissue. Model data were compared with the measured response obtained from an exercising subject. The best simulation of the $\dot{V}O_2$ response generated by the model necessitated a $\tau\dot{V}O_2A$ of ~32 s and a rate of $\dot{Q}NW$ of 4.5 $l \cdot min^{-1}$. With these parameters, $\dot{V}O_2T$ was not limited by the rate of O_2 transport. By manipulating the parameters, Cochrane and Hughson were able to show

that a small adjustment in $\tau\dot{V}O_2A$ brought about an increase in $\dot{Q}NW$ to 5.0 $l \cdot min^{-1}$. This adjustment was not sufficient to influence the comparison of the model with the measured response of the subject. However, with these modifications, the model demonstrated conditions in which O_2 transport became a limiting factor in $\dot{V}O_2T$. Cochrane and Hughson therefore concluded that the balance between the availability of O_2 and its demand during exercise was quite delicate. This raises the possibility that exercising beyond particular work intensities may result in limitation of $\dot{Q}O_2$ by the processes of O_2 transport. Certainly, as was noted by these authors, such a delicate balance could account for the observed slowing in $\dot{V}O_2$ kinetics under experimental conditions such as β-blockade, circulatory occlusion and hypoxia.

2.6.3 THE CONTROL OF OXYGEN UPTAKE KINETICS BY MULTIPLE PROCESSES

Although it is generally assumed that a single process limits the rate of change in *phase II* $\dot{V}O_2$, there is evidence to suggest that this may not be the case. If $\dot{V}O_2$ kinetics are regulated by more than one process, then the $\dot{V}O_2$ response to dynamic exercise might take the form of a sigmoidal-shaped function (Jones 1973). This pattern of response was observed by Cerretelli et al. (1977) when studying $\dot{V}O_2$ kinetics during supine arm exercise. Further evidence is, however, limited. Walsh (1992) has suggested that characterisation of the $\dot{V}O_2$ response by a sigmoidal curve might not be possible due to the masking effect produced by the *phase I* component. Interestingly, data obtained from the study of Cerretelli et al. (1988) lend support to this theory. In studying gas exchange transients in heart transplant recipients (HTR), Cerretelli and his colleagues reported that the $\dot{V}O_2$ response following the onset of exercise could be characterised by a sigmoidal curve. In HTR, the rise in $\dot{Q}T$ at the start of exercise is almost entirely dependent on an increase in SV resulting from the functioning of the

muscle pump (Pope et al. 1980). The rate of change in $\dot{Q}T$ is therefore quite sluggish (Cerretelli et al. 1988). As the response in *phase I* $\dot{V}O_2$ can be largely attributed to changes in $\dot{Q}T$, it is likely that HTR will not experience this period of the response. The sigmoidal-shaped curve observed by Cerretelli and his co-workers (1988) might therefore be a more appropriate description of the *phase II* response in $\dot{V}O_2$. However, it is also feasible that the shape of the response might reflect the influence on cellular events of the slow adjustment in $\dot{Q}T$ (Cerretelli et al. 1988). Nevertheless, the possibility that $\dot{V}O_2$ kinetics is limited by more than one process should not be dismissed.

3.0 THE ANALYSIS OF OXYGEN UPTAKE KINETICS

To acquire an understanding of the response characteristics of $\dot{V}O_2$ and the underlying mechanisms of control, it is necessary to analyse the dynamic response in $\dot{V}O_2$ following a change in muscular work. An effective approach to analysis is afforded by techniques borrowed from the field of engineering. These techniques can be grouped under the general heading of *systems analysis*.

Systems analysis seeks to provide a mathematical description of cause-effect relationships occurring within a system of connected and interacting mechanisms (Marmarelis and Marmarelis 1978; Wigertz 1971). In the evaluation of $\dot{V}O_2$ kinetics, systems analysis is concerned with the relationship between a change in the intensity of muscular work and the rate of adaptation in $\dot{V}O_2$ (Barstow and Molé 1991). This relationship is influenced by the mechanisms of the cardiorespiratory system (Swanson et al. 1983).

Quantification of the dynamic relationship between work and $\dot{V}O_2$ is obtained by fitting linear mathematical models to $\dot{V}O_2$ response data. Information concerning the condition of the underlying physiological structure can be derived from analysis of the model order and model parameters (Barstow and Molé 1991). The use of linear models necessitates that a dynamically linear relationship is maintained between work and $\dot{V}O_2$ (Fujihara et al. 1973a and 1973b)

Two different types of mathematical models can be applied to characterise $\dot{V}O_2$ response data: empirical and functional. The emphasis of the empirical model is on fitting the observed response with parameter values that summarise the data regardless of whether the values might be physiologically credible (Casaburi et al. 1992; Swanson et al. 1983). In contrast to the empirical model, functional model parameters are constrained to

physiological values that can be related to aspects of the cardiorespiratory system's structure (Swanson et al. 1983).

Various input forms, or forcing functions, have been advocated to perturb the intensity of muscular work and thus stimulate a dynamic response in $\dot{V}O_2$. In addition to the onset of constant-rate exercise (Margaria et al. 1965; Sietsema et al. 1989; Whipp and Wasserman 1972), the transient response in $\dot{V}O_2$ has been estimated following step (Casaburi et al. 1989a; Hughson et al. 1988; Linnarsson 1974; Whipp et al. 1982), ramp (Davis et al. 1982; Hughson 1989; Niizeki and Miyamoto 1991; Swanson and Hughson 1988), sinusoidal (Bakker et al. 1980; Casaburi et al. 1977; Cunningham et al. 1993; Miyamoto et al. 1983) and impulse (Bakker et al. 1980; Hughson et al. 1988; Miyamoto et al. 1983) changes in WR.

Tests of $\dot{V}O_2$ kinetics are normally performed in the upright position on a cycle ergometer. This type of ergometer is suited to producing rapid changes in work rate and allows cadence to be monitored easily.

With the obvious exception of rest-to-work transitions, the onset of a forcing is usually preceded by 4 to 5 minutes of loadless pedalling or light intensity exercise. This period helps to reduce any initial transients in $\dot{V}O_2$ (Bakker et al. 1980). To minimise the contribution of non-linear influences to the observed response, changes in work intensity must be rigorously constrained to the sub-LAT domain.

In estimating the kinetic parameters of $\dot{V}O_2$, the limitations of traditional measurement methods (see Hamar 1991) can be overcome by employing the breath-by-breath measurement technique. However, a major concern of breath-by-breath measurement is inherent breath-to-breath noise. Insofar as noise is a physiological component of the cardiorespiratory system there is no need for correction (Eßfeld et al. 1987). If, on the

other hand, noise is excessive, then the true response in $\dot{V}O_2$ can be obscured. To minimise the influence of breath-to-breath noise and thus enhance parameter accuracy, the $\dot{V}O_2$ responses from several identical tests can be averaged (Lamarra et al. 1987). The number of test repetitions required to achieve a specified parameter confidence varies considerably between subjects (Lamarra et al. 1987). In studies by Casaburi et al. (1989a), Hughson et al. (1988) and Whipp et al. (1982), subjects completed between 6 and 9 test repetitions. Casaburi et al. (1989a) have advocated a minimum of 45 minutes rest between tests.

3.1 STEP WORK RATE FORCINGS AND THE ONSET OF CONSTANT-RATE EXERCISE

Most of the information available on $\dot{V}O_2$ kinetics is based on the study of changes in $\dot{V}O_2$ following either a step increase in WR or the onset of constant-rate exercise. It is generally acknowledged that the transient $\dot{V}O_2$ response resulting from these forcing types can be characterised by a low-order exponential function (Linnarsson 1974; Miyamoto et al. 1982; Whipp 1971; Whipp et al. 1982).

For an exponential process that manifests first-order kinetics, the instantaneous rate of change is proportional to the distance to the new steady state. The speed of the response may therefore be described by a single parameter (Whipp and Casaburi 1982). Perhaps the most commonly employed parameter in the study of $\dot{V}O_2$ kinetics is τ, the time constant (Babcock et al. 1994b; Casaburi et al. 1989a; Di Prampero et al. 1970; Hughson et al. 1988; Linnarsson 1974; Whipp et al. 1982; Whipp and Wasserman 1970). Previously, other investigators have favoured the use of $t_{½}$ (Diamond et al. 1977) or the rate constant (k) (Whipp and Casaburi 1982). It should be noted, however, that all of these parameters are related: $\tau = 1/k = t_{½}/0.693$ (Hughson and Morrissey 1982).

In fitting a single exponential model to $\dot{V}O_2$ response data, the oldest view (Hill and Lupton 1923) proposes that the model should extend from the point at which WR was increased. This implies that changes in muscle metabolism are instantaneously expressed at the lung (Barstow et al 1990). Given the evidence presented in Chapter 2, such a proposal is clearly incorrect. The second approach to fitting $\dot{V}O_2$ response data uses a model that incorporates both an exponential component and a time delay. By including a time delay, the exponential component is not constrained to begin coincident with the increase in WR. The model takes the form:

$$\dot{V}O_2(t) = A_0 + A_1 \cdot (1 - e^{(-(t-TD)/\tau)}) \quad (3.1)$$

where A_0 represents baseline $\dot{V}O_{2ss}$, A_1 is the difference between baseline $\dot{V}O_{2ss}$ and the new $\dot{V}O_{2ss}$, τ is the time constant and *TD* represents the time delay in the appearance of the response at the lung. Fitting of the model to data is achieved using least-squares regression techniques. The model was noted by Whipp et al. (1982) to provide an acceptable description of $\dot{V}O_2$ in the transition from light to moderate intensity work. The potential of the model to fit the adaptation in $\dot{V}O_2$ following a step increase in WR is shown in Figure 3.1 (overleaf).

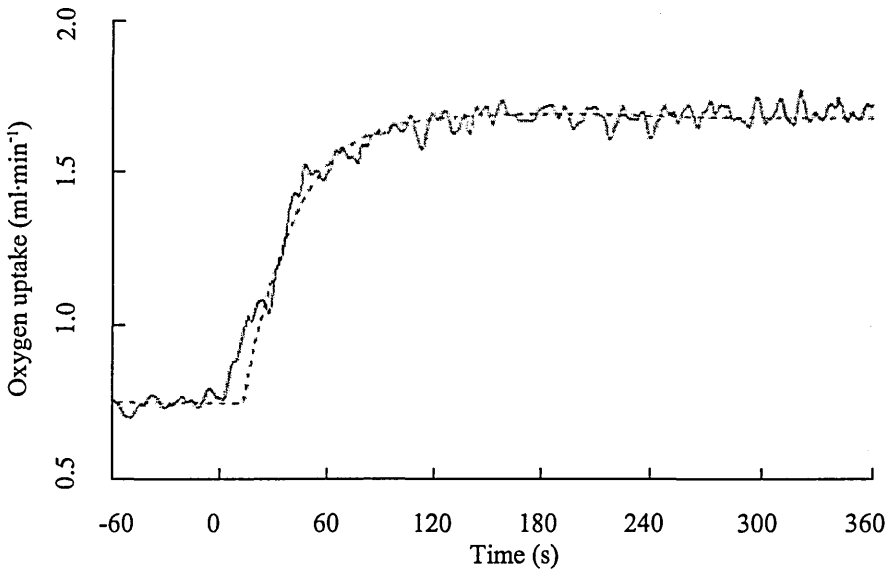


Figure 3.1. The change in oxygen uptake resulting from the transition between unloaded cycling and an exercise intensity equivalent to 80% of the lactic acid threshold. The data are the mean responses of 5 subjects. Data are fitted with a first-order exponential model incorporating a time delay constrained to start after the *phase I* component of the response (broken line). The illustration is taken from Barstow et al. (1994).

In most instances, a single-component exponential model adequately fits the data obtained from step WR forcings. However, evaluation of the dynamic characteristics of $\dot{V}O_2$ have revealed that the predominant exponential rise in $\dot{V}O_2$ is preceded by a small early increase, know as *phase I* (see Chapter 2). This phase is most evident when data is recorded in the transition from rest-to-exercise (Whipp et al. 1982). To accommodate the *phase I* rise in $\dot{V}O_2$, data can be fitted with a two-component exponential model (Hughson et al. 1988; Hughson and Swanson 1988):

$$\dot{V}O_2(t) = A_0 + \{A_1 \cdot (1 - e^{(-(t-TD_1)/\tau_1)}) \cdot u(t - TD_1)\} + \{A_2 \cdot (1 - e^{(-(t-TD_2)/\tau_2)}) \cdot u(t - TD_2)\} \quad (3.2)$$

This model has two terms in parallel, each with its own amplitude (A_1, A_2), time constant (τ_1, τ_2) and time delay (TD_1, TD_2). The function $u(t - TD_n)$ acts to turn on each term when $t - TD_n \geq 0$. The first term in the model fits the rapid initial increase in

phase I $\dot{V}O_2$. The second term fits the slower increase in $\dot{V}O_2$ (*phase II*) as it extends towards a new $\dot{V}O_{2ss}$ (*phase III*).

Figure 3.2, overleaf, shows $\dot{V}O_2$ data obtained from several step forcings fitted by the model described in Equation 3.2.

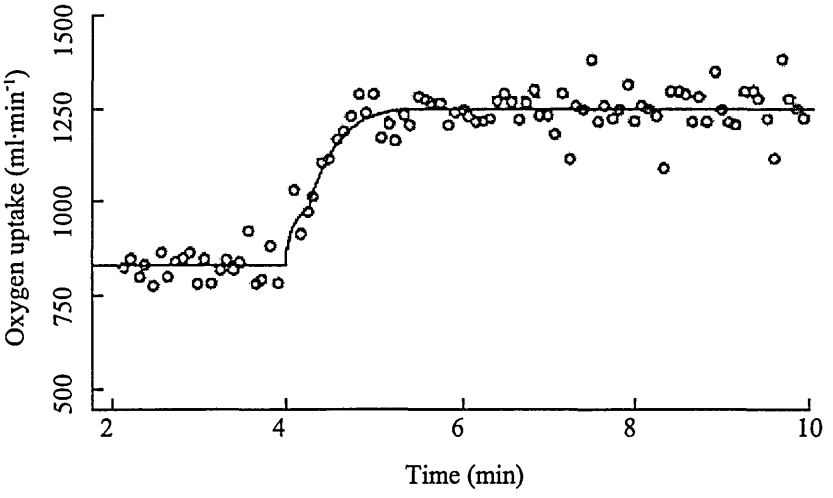


Figure 3.2. The response in oxygen uptake resulting from a step work rate forcing. Data comprise six repetitions of 4 minutes of cycling at 25 watt followed by 6 minutes at an intensity of 65 watt. Superimposed on the oxygen uptake data is a second-order exponential model (solid line). Data for the figure were collected during investigations by Hughson et al. (1988).

The parameters that characterise the dynamic response in $\dot{V}O_2$ provide information that permits comparison between conditions and individuals. To summarise this information, the mean response time (MRT) has been put forward as a simple measure of the overall rate of change in $\dot{V}O_2$ (Linnarsson 1974). The MRT is calculated as:

$$\text{MRT} = \tau + TD \tag{3.3}$$

where τ and TD are derived from the fitting of a first-order function to the $\dot{V}O_2$ response data (Linnarsson 1974).

For a second-order function, Swanson and Hughson (1988) have proposed the total lag time (TLT) as an equivalent to the MRT. Using the assumption of linearity, TLT is

derived from the model used to fit response data obtained from a ramp forcing. The method of calculation is as follows:

$$\text{TLT} = (A_1/(A_1+A_2)) \cdot (TD_1 + \tau_1) + (A_2/(A_1+A_2)) \cdot (TD_2 + \tau_2) \quad (3.4)$$

A detailed explanation of the derivation of the TLT is given in the paper by Swanson and Hughson (1988).

3.2 RAMP WORK RATE FORCINGS

Increasing WR as a ramp function of time affords a simple approach to determining $\dot{V}O_2$ kinetics (Whipp et al. 1981). In subjects with a reduced exercise tolerance, the ramp protocol is an attractive option as the stress imposed by the test can be increased gradually (Murphy et al. 1989). The advantage of the ramp protocol is that it may be extended to include determinations of both $\dot{V}O_2$ at the LAT and $\dot{V}O_{2max}$ (Davis et al. 1982).

To determine the kinetic parameters of $\dot{V}O_2$ from ramp WR forcings, response data may be fitted with linear mathematical models. These models are obtained by integration of the corresponding step models (Fujihara et al. 1973a). Equation 3.5 gives the two-component form of a ramp model:

$$\dot{V}O_2(t) = A_0 + [A_1 \cdot \{t - TD_1 - \tau_1 \cdot (1 - e^{(-(t-TD_1)/\tau_1)})\} \cdot u(t - TD_1)] +$$
$$[A_2 \cdot \{t - TD_2 - \tau_2 \cdot (1 - e^{(-(t-TD_2)/\tau_2)})\} \cdot u(t - TD_2)] \quad (3.5)$$

The model comprises two separate terms, each with its own amplitude (A_1 and A_2), time constant (τ_1 and τ_2) and time delay (TD_1 and TD_2). The function $u(t - TD_n)$ acts to turn on each term when $t - TD_n \geq 0$.

Although the model described in Equation 3.5 can be employed to characterise the $\dot{V}O_2$ response to ramp WR forcings, analysis is more frequently performed using a different

approach. This involves fitting the asymptotically linear portion of the $\dot{V}O_2$ response using linear regression techniques. To avoid the influence of initial transients, data recorded during the first ~2 min following the start of the ramp are normally excluded from the fit. Non-linear influences can be minimised by terminating the regression below the point equivalent to a subject's LAT (Swanson and Hughson 1988). Back extrapolation of the linear regression line yields an intersect on the time axis. The difference in time between the onset of the ramp and the intersection point equals the TLT (Murphy et al. 1989; Swanson and Hughson 1988).

As with other forms of forcing, reliable estimates of $\dot{V}O_2$ kinetics can only be achieved by averaging the data derived from several repetitions of a ramp input (Hughson and Inman 1987). Figure 3.3 (overleaf) illustrates the averaged response in $\dot{V}O_2$ obtained from a number of identical ramp WR forcings. Also included in the figure are the linear regression line and derived TLT.

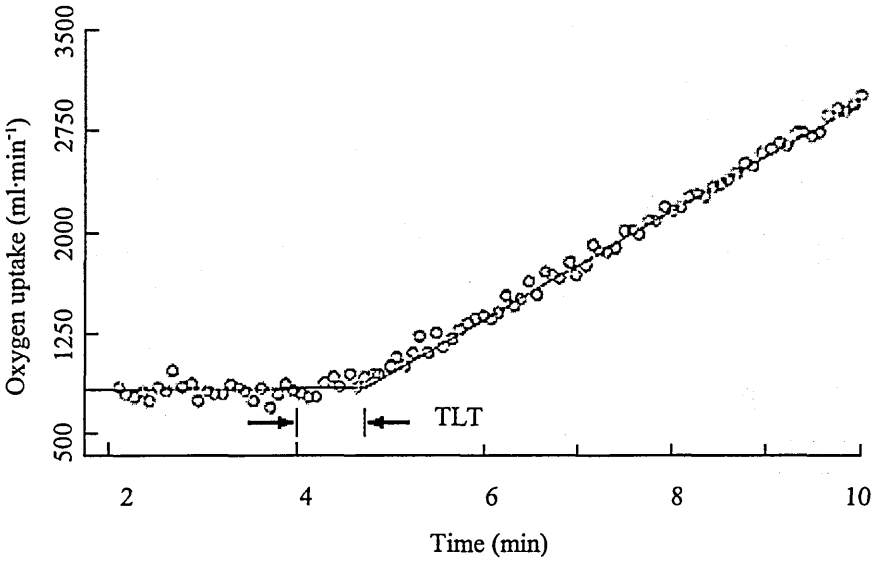


Figure 3.3. The change in oxygen uptake resulting from a ramp work rate forcing. Data comprise six repetitions of 4 minutes of cycling at a constant intensity of 25 watt followed by a 40 watt·minute^{-1} ramp. The onset of the ramp is at 4 minutes (only the last 2 minutes of cycling at 25 watt are shown in the figure). The linear regression line is fitted to the data from 2 minutes following the start of the ramp, and terminated at 10 minutes. Derivation of total lag time (TLT) is included in the figure. The figure is taken from work published by Swanson and Hughson (1988).

Davis et al. (1982) have proposed that valid assessments of $\dot{V}O_2$ kinetics are possible using ramp slopes between 20 W·min^{-1} and 50 W·min^{-1}. However, in tests by Swanson and Hughson (1988) the kinetic parameters of $\dot{V}O_2$ derived from 20 W·min^{-1} and 40 W·min^{-1} ramp forcings were shown to be significantly different and highly dependent on the slope of the ramp. Furthermore, values of TLT derived from ramp WR forcings were noted to be poorly correlated with estimates of TLT obtained from step WR forcings. In a related study, Murphy et al. (1989) examined the dynamic response in $\dot{V}O_2$ during step and ramp exercise tests under conditions of both normoxia and hypoxia. Murphy and her colleagues reported that the expected slowing of $\dot{V}O_2$ kinetics under hypoxia did not occur during tests using ramp forcings. Inman and Hughson (1984)

have also found that changes in $\dot{V}O_2$ kinetics due to β-blockade cannot be detected when using ramp as opposed to step WR forcings.

The findings of Inman and Hughson (1984), Murphy et al. (1989) and Swanson and Hughson (1988) suggest that the cardiorespiratory system does not behave in a dynamically linear manner following a ramp change in WR. Therefore, the kinetic parameters obtained from ramp tests should not be used to elicit a meaningful interpretation of the integrity of the cardiorespiratory system (Murphy et al. 1989).

3.3 SINUSOIDAL WORK RATE FORCINGS

Although less prevalent than other input forms, sinusoidal WR forcings are well suited to the evaluation of $\dot{V}O_2$ kinetics. A sinusoidal forcing has the advantage of evoking a cyclical response. After an initial period of stabilisation, data recorded during each cycle can be averaged. Using this approach, a series of separate tests is not required to accentuate the underlying response (Casaburi et al. 1977).

If the relationship between a sinusoidal forcing and responding variable is approximately linear, then the response will be a sinusoid of the same period, but of generally different amplitude and phase angle (Casaburi et al. 1977). To evaluate the dynamic response in $\dot{V}O_2$, the amplitude and phase angle must be determined. This can be achieved by fitting averaged data with the model described in Equation 3.6.

$$\dot{V}O_2(t) = A_0 + A \cdot \sin(2p/T \cdot (t - \phi)) \qquad (3.6)$$

where A_0 is the mid-point of the sinusoidal $\dot{V}O_2$ response, A is the amplitude (mid-point to peak) and ϕ is the relative phase angle. The value T equals the period of the sinusoidal forcing.

The dynamic relationship between a sinusoidal forcing and resultant output can be quantified by calculating the amplitude ratio (α), that is, output to input, and phase shift (φ), that is, output minus input (Eßfeld et al. 1987). This process is illustrated in Figure 3.4.

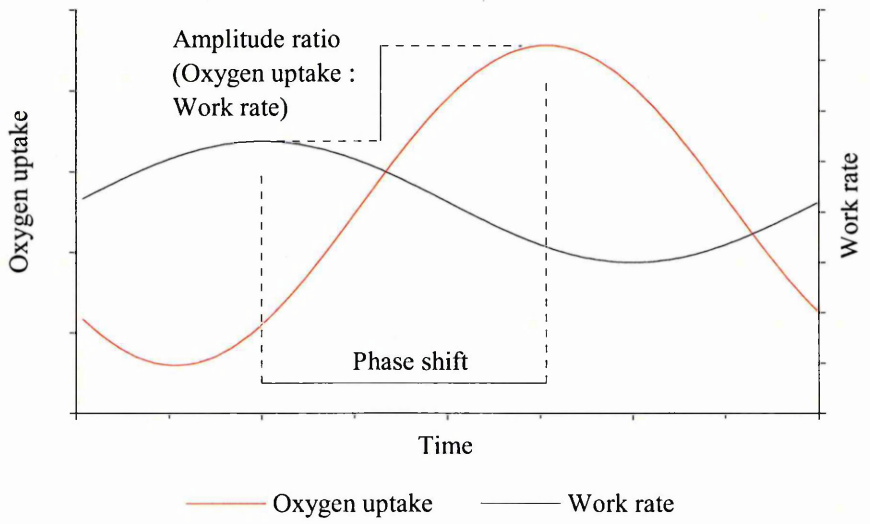


Figure 3.4. Formulation of the kinetic parameters of amplitude ratio and phase shift resulting from the dynamic relationship between a sinusoidal forcing (work rate) and responding variable (oxygen uptake). The figure is adapted from work published by Kerlin (1974).

It is also possible to use frequency analysis techniques to derive the best estimates of α and φ. This method of analysis is described in more detail in Chapter 7. In brief, frequency analysis determines the harmonic components of the forcing and corresponding response. For each component, the analysis yields values for amplitude and phase angle. These can then be used to compute α and φ parameters.

In studies using sinusoidal WR forcings, frequency analysis is the preferred method of estimating the kinetic parameters of $\dot{V}O_2$ (Bakker et al. 1980; Casaburi et al. 1977; Fukuoka et al. 1995; Haouzi et al. 1992; Whipp et al. 1980). Figure 3.5 (overleaf) shows the $\dot{V}O_2$ response to a sinusoidal WR forcing. The fundamental component, constructed after frequency analysis, is superimposed on the data.

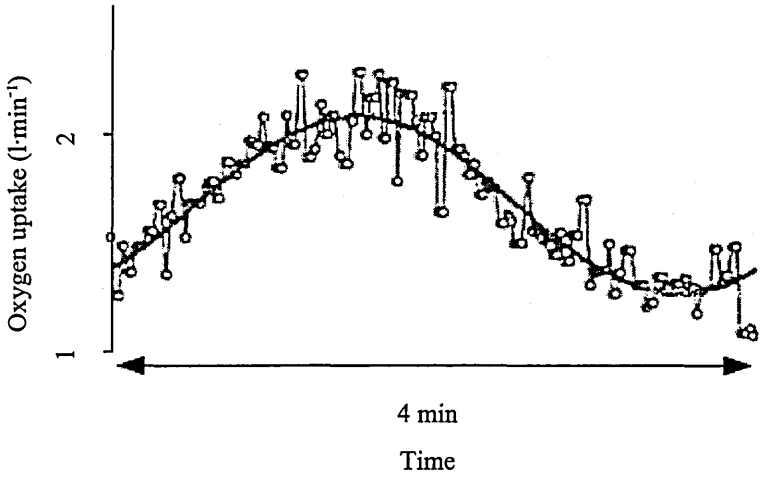


Figure 3.5. Fundamental component superimposed on the response in oxygen uptake resulting from a sinusoidal work rate forcing with a cyclical period of 4 minutes. The forcing had a total duration of 16 minutes (4 cycles) and oscillated between two work rates equal to those observed at 90% and 30% of the subject's lactic acid threshold. The forcing was preceded by 4 minutes of constant work at a rate equal to the midpoint of the sinusoid. The information for constructing the fundamental component was acquired using techniques of frequency analysis (see Chapter 7). The figure is taken from work by Haouzi et al. (1993).

Where frequency analysis has been employed to determine the kinetic parameters of $\dot{V}O_2$, there have been reports of additional components in the response. In a linear system, frequency analysis should elicit only a fundamental component (Bennett et al. 1981; Marmarelis and Marmarelis 1974; Wiener 1958). Evidence of additional components would therefore indicate non-linear influences in the regulation of $\dot{V}O_2$ kinetics. Although non-linear control is feasible, it is more likely that the appearance of additional components results from breath-to-breath noise (Hoffman et al. 1994b). Thus, α and φ parameters derived from the fundamental component should be considered to provide an acceptable representation of the dynamic response in $\dot{V}O_2$.

Although the $\dot{V}O_2$ response to an individual sinusoidal WR forcing provides a description of $\dot{V}O_2$ kinetics for a single input frequency, the evaluation of overall system

$\dot{V}O_2$ kinetics requires that α and φ are known for a range of frequencies (Eßfeld et al. 1987; Marmarelis and Marmarelis 1978). Tests must therefore be conducted of the dynamic response in $\dot{V}O_2$ to a number of sinusoidal forcings. Once completed, the information derived from these tests can be displayed in a Nyquist plot (Bakker et al. 1980; Casaburi et al. 1977; Casaburi et al. 1980; Whipp et al. 1980), though both polar diagrams (Wigertz 1970) and bode plots (Cunningham et al. 1993) may be used as alternatives. In the Nyquist plot, α parameters are used to determine the distance of the data point from the origin, whilst corresponding φ parameters are used to determine the angle of the data point from the abscissa (Casaburi et al. 1977). In this way, those points nearest the abscissa express the data obtained from low frequency inputs, and those nearest the ordinate reflect the data derived from high frequency inputs (Miyamoto et al. 1983).

An example of a Nyquist plot is depicted in Figure 3.6 (overleaf). The figure illustrates how, as the frequency of the input rises, α decreases whilst φ increases. The steady state amplitude (half the difference between $\dot{V}O_{2ss}$ at the upper and lower WR limits) is included in the figure and plotted on the abscissa (no phase shift).

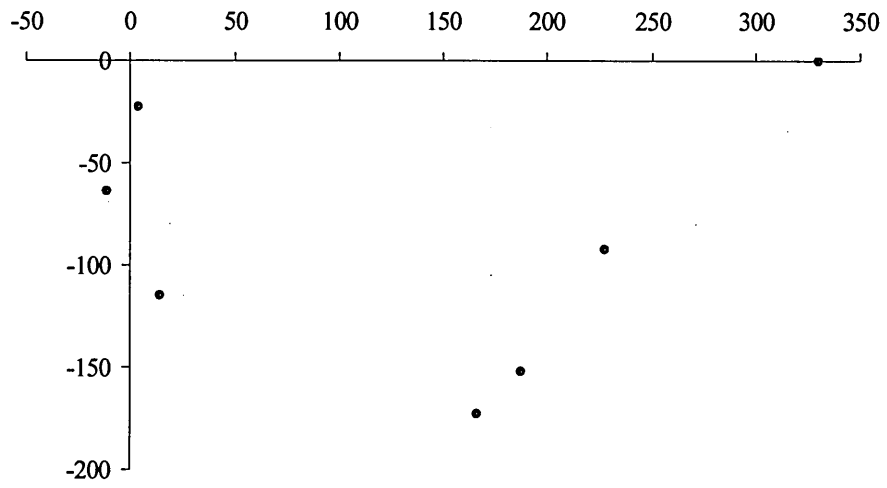


Figure 3.6. Nyquist plot of amplitude ratio and phase shift parameters computed from the oxygen uptake ($\dot{V}O_2$) response to sinusoidal work rate forcings with periods of 42, 60, 120, 240, 360 and 600 seconds. The steady state amplitude, that is, half the difference in steady state $\dot{V}O_2$ between the upper and lower work rate limits is included in the figure and plotted on the abscissa (no phase shift). Data for the figure were collected during tests performed by subject BW in the study of Casaburi et al. (1977).

To quantify system $\dot{V}O_2$ kinetics, the data presented in the Nyquist plot may be fitted with mathematical models that express the dynamic interrelation involved (Casaburi et al. 1977; Casaburi et al. 1980). If experiments are performed with a range of relatively low frequency sinusoidal forcings, then the derived data will tend to reflect the dynamic response in *phase II* $\dot{V}O_2$ (see also Chapter 4). Thus, a low-order model should provide an adequate fit to the data.

In the study of Casaburi et al. (1977), $\dot{V}O_2$ response data were obtained from a series of sinusoidal WR forcings with periods ranging from 10.0 to 0.7 min. The data were shown to be well described by a first-order model without time delay. To conform to physiological understanding, it might be expected that the relationship between $\dot{V}O_2$ and WR would be better expressed using more complex models, that is, ones incorporating a time delay. Yet, although this approach was employed by Bakker et al. (1980), the

estimated time delay parameters were observed to be negative for each subject (mean -7.1 s). This implies that the response in $\dot{V}O_2$ occurs prior to the onset of the forcing, an outcome that is not physiologically possible.

To characterise the $\dot{V}O_2$ response to sinusoidal WR forcings with a positive time delay, Sherrill and Swanson (1981) have advocated the use of a non-rational (four-parameter transfer function) model. Unlike the model employed by Bakker et al. (1980), the non-rational model is not limited by the property that, given the amplitude response to a sinusoidal input, a unique minimum phase shift must exist (Guilleman 1963; Sherrill and Swanson 1981). Therefore, if the response data yield a phase shift below the theoretical minimum, the non-rational model will not assume an inappropriately high alternative that can only be compensated for by a negative time delay (Sherrill and Swanson 1981).

In its simplest arrangement, the model described by Sherrill and Swanson (1981) takes the form:

$$G_4(s) = (C_1 + C_2 \cdot e^{sTD})/(s\tau + 1) \qquad (3.7)$$

where $s = i\omega$, τ is the time constant, TD is the time delay, C_1 indicates the relative response of a mode without time delay and C_2 indicates the relative response of a mode with a time delay. For $s = i\omega$, i is the symbol for the imaginary domain of numbers and ω is the angular frequency, $2\pi/T$. The angular frequency acts as a parameter linking the real and imaginary parts of G, thereby defining the curve which is fitted to the Nyquist plot. The real (Re) and imaginary (Im) parts of G can be computed from:

$$\mathrm{Re}(G(\omega)) = \{C_1 + C_2 \cdot \cos(\omega TD) - \omega\tau C_2 \cdot \sin(\omega TD)\}/(\omega^2\tau^2 + 1) \qquad (3.8)$$

and

$$\mathrm{Im}(G(\omega)) = [-\omega\tau \cdot \{C_1 + C_2 \cdot \cos(\omega TD)\} - C_2 \cdot \sin(\omega TD)]/(\omega^2\tau^2 + 1) \qquad (3.9)$$

Sherrill and Swanson (1981) have noted that the fit of the non-rational model is generally better than that of the model used by Bakker and his colleagues (1980). Figure 3.7 illustrates the fit of the model to the $\dot{V}O_2$ response data shown in Figure 3.6. The model was applied using a least squares algorithm.

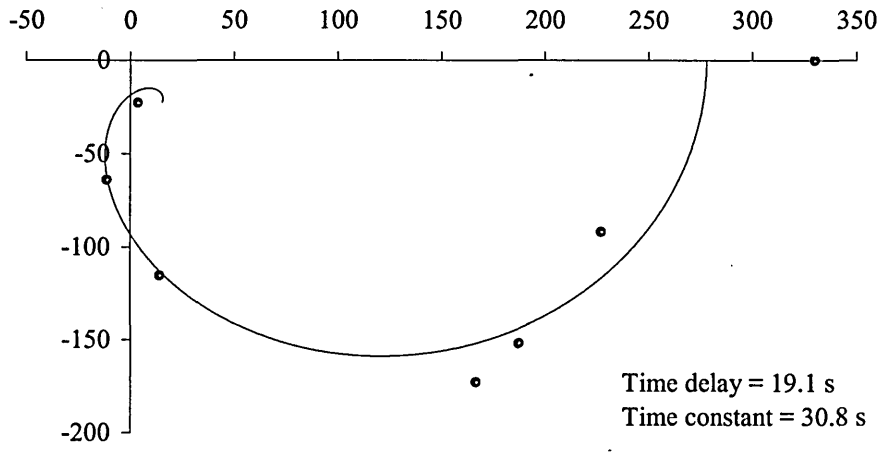


Figure 3.7. Nyquist plot of amplitude ratio and phase shift parameters computed from the oxygen uptake ($\dot{V}O_2$) response to sinusoidal work rate forcings with periods of 42, 60, 120, 180, 240, 360 and 600 seconds. Steady state amplitude of $\dot{V}O_2$ is included in the figure. The data are fitted with the model described in Equation 3.7. Best fit time delay and time constant parameters resulting from the model fit are included in the figure. Data for the figure were collected during tests performed by subject BW in the study of Casaburi et al. (1977).

If the nature of an investigation demands information regarding the *phase I* component of the dynamic response in $\dot{V}O_2$, then the responses to a range of high frequency sinusoidal WR forcings must be evaluated (see also Chapter 4). In the assessment of $\dot{V}O_2$ kinetics, careful consideration must be given to the amplitude of high frequency WR inputs. If the amplitude is too low, then the response may become obscured by breath-to-breath noise (Haouzi et al. 1992). If, however, the amplitude is too great, then non-linear influences may become apparent (Haouzi et al. 1993). In either case, the desired information will not be returned.

3.4 IMPULSE WORK RATE FORCINGS

Impulse WR forcings are deterministic input functions that differ from sinusoidal forcings in that a full scale of frequencies is tested simultaneously (Bakker et al. 1980). For systems that are linear and time invariant, the response to an impulse completely characterises a system (Bennett et al. 1981).

Impulse WR forcings employed in the examination of $\dot{V}O_2$ kinetics are not true impulses in the sense of infinite amplitude and zero width (Hughson et al. 1988). Rather, they take the form of a pulse in which WR rises abruptly from a baseline for a brief interval of perhaps 5 to 10 s, before returning to baseline for a period of several minutes.

Although it is possible to use a first-order model to describe the $\dot{V}O_2$ response resulting from an impulse forcing, Hughson et al. (1988) have shown that a second-order model provides a marked improvement in fit. This model takes the following form:

$$\dot{V}O_2(t) = A_0 + \{A_1 \cdot (1 - (e^{(-(t-TD_1)/\tau_1)})/\tau_1) \cdot u(t - TD_1)\} + \{A_2 \cdot (1 - (e^{(-(t-TD_2)/\tau_2)})/\tau_2^2) \cdot u(t - TD_2)\} \quad (3.10)$$

where A_0 represents baseline $\dot{V}O_2$, and A_n, τ_n and TD_n are the amplitude, time constant, and time delay parameters for each component, respectively. The function $u(t - TD_n)$ acts to turn on each term when $t - TD_n \geq 0$.

The fit of the model described in Equation 3.10 to $\dot{V}O_2$ response data is shown in Figure 3.8 (overleaf). The model was fitted using least-squares regression techniques.

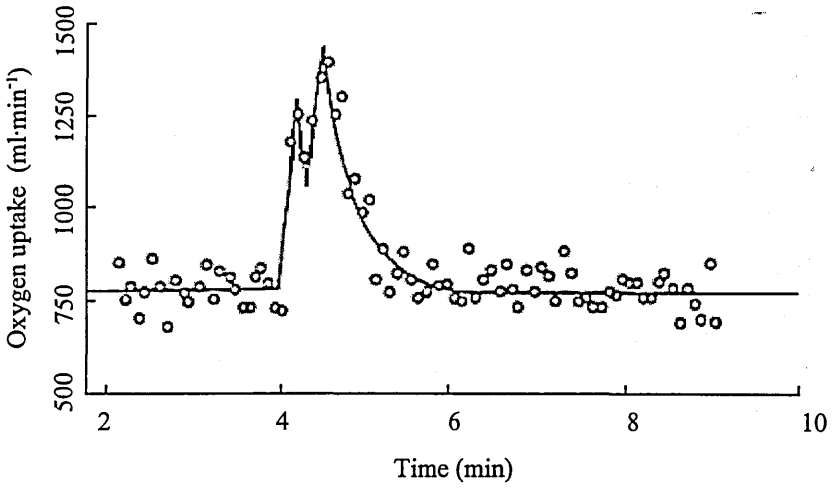


Figure 3.8. The oxygen uptake ($\dot{V}O_2$) response resulting from an impulse work rate forcing. The forcing comprised two periods (4 minutes and 6 minutes duration, respectively) of cycling at an intensity of 25 watt, separated by a 10 second pulse of 260 watt. $\dot{V}O_2$ response data are the average of eight repetitions (data points are calculated as 5 second averages). Data are fitted with a second-order model (solid line). The figure is taken from work published by Hughson et al. (1988).

Although the range of frequencies tested by impulse forcings is greater than that which can be achieved using step, ramp or sinusoids, there is concern that the system response may be influenced by the use of large work loads (Bennett et al. 1981).

3.5 PRACTICAL LIMITATIONS IN THE EVALUATION OF OXYGEN UPTAKE KINETICS

Of those forms of WR forcing that provide an effective means of evaluating $\dot{V}O_2$ kinetics, it is generally accepted that tests must be repeated several times in each subject. Data derived from the tests can then be averaged to emphasise the true underlying $\dot{V}O_2$ response. Although the cyclical nature of the data obtained from a sinusoidal WR forcing obviates the need for repeated tests, quantification of the system response in $\dot{V}O_2$ requires that the kinetic parameters are known for a range of different frequency sinusoidal forcings. Thus, at least two sets of tests must still be undertaken.

A practical disadvantage of conducting a series of tests is that it is time consuming and may necessitate testing on different days (Cunningham et al. 1993; Hoffmann et al. 1994a). For many subjects, obtaining a description of $\dot{V}O_2$ kinetics by this method can prove inconvenient or impractical.

4.0 PSEUDORANDOM BINARY SEQUENCE WORK RATE FORCINGS AND THE STUDY OF OXYGEN UPTAKE KINETICS

Of the various input disturbances used to study the dynamic response characteristics of a physiological system, perhaps the most useful is the sinusoid. Although this type of input may be applied in a number of forms (Hahn et al. 1993; Robbins 1984; Stoll 1969; Williams et al. 1996), in the exploration of cardiorespiratory responses the sinusoidal WR forcing is the preferred choice of many investigators (Bakker et al. 1980; Casaburi et al. 1977; Fukuoka et al. 1995; Haouzi et al. 1993; Miyamoto et al. 1983; Wigertz 1971).

Quantification of the overall system response to sinusoidal forcings requires that α and φ parameters are known for all relevant input frequencies (Eßfeld et al. 1987). System response kinetics are determined by fitting the two sets of parameters with a mathematical model that expresses the dynamic interrelation involved (Casaburi et al. 1977; Casaburi et al. 1980). To avoid the time consuming process of collecting the necessary information from a series of individual tests, the responses to several input frequencies may be studied simultaneously using multifrequent forcings (Carter et al. 1967; Kerlin 1974; Godfrey 1969b).

Multifrequent inputs should be considered as the sum of a number of sinusoidal inputs of different amplitude, frequency and relative phase angle (Lynn 1973). The response data obtained from multifrequent inputs can be considered in terms of both time and frequency. Frequency response results are often seen as more convenient because their interpretation is simple and a system's response kinetics may be computed in an algebraic way without the need for explicit mathematical modeling (Eßfeld et al. 1987; Hoffmann et al. 1994a).

As the sinusoidal content of different multifrequent inputs can vary, care must be taken to choose only those inputs that give results over frequency ranges of interest. Since it is not always possible to find a multifrequent input comprising the exact desired sinusoidal content, the processes of signal break down may be reversed. Using this method, the sinusoidal components can be tailored to obtain input signals optimised with regard to the number of frequencies tested, the power distribution over these frequencies and the test duration (Eßfeld et al. 1987). This chapter, however, will only consider the use of pre-constructed inputs, specifically the PRBS.

4.1 PSEUDORANDOM BINARY SEQUENCE INPUT SIGNALS

To date, the most commonly used signal in the measurement of physiological responses to a range of sinusoidal inputs is the PRBS. The first application of a PRBS in the biological field was by Ponte and Purves in 1974. These investigators successfully determined the dynamic response of the carotid body in cat to pseudorandom changes in arterial blood CO_2 tension, O_2 tension and pH. In 1980, the PRBS was extended to the examination of responses in man by Sohrab and Yamashiro. By varying the levels of inspired CO_2 on a pseudorandom breath-by-breath basis, Sohrab and his colleague were better able to study the dynamic responses mediated by peripheral chemoreceptors. It was not until the work of Bennett et al. in 1981 that the dynamic responses of the cardiorespiratory system were examined using PRBS WR forcings.

In the study of cardiorespiratory system kinetics, the use of a PRBS to perturb WR has a

number of advantages:

i) A PRBS can be realised easily as it only requires switching between two levels of work.

ii) A PRBS contains many sinusoidal inputs, permitting determination of the responses to a wide range of different frequencies in a single test (Kerlin 1974).

iii) Unlike truly random binary signals, the binary level transitions of a PRBS can occur only at specific times. These transitions are separated by intervals during which the binary state is fixed (Hampton 1965). The importance of this will be discussed in Part 4.4.1 of this chapter.

Pseudorandom binary sequences may be easily generated with a digital shift register using modulo-2 addition. Modulo-2 addition requires that each stage of the shift register be set with the digits 0 or 1. The selection and order of these digits are unimportant, except that the digit 1 must be chosen at least once.

The digits at particular stages are added together according to the rules defined in Table 4.1.

Table 4.1. Modulo 2 addition (Kerlin 1974).

First digit (m)	Second digit (n)	$(m + n)_{Mod\text{-}2}$
1	0	1
0	1	1
1	1	0
0	0	0

Digits resulting from the addition are used to generate a sequence that repeats itself periodically. The number of digits, or units, required to construct a complete sequence varies according to the number of stages on the shift register and the order in which the stages are added together. Although it is possible to construct sequences of any length,

only those composed from the maximum number of units (Z) have the desired pseudorandom character.

The maximum number of units required to construct a PRBS is given by:

$$Z = 2^n - 1 \tag{4.1}$$

where n is the number of stages in the shift register. The choice of which stages to add to generate Z has already been established for several PRBSs. A selection of the required arrangements appears in Table 4.2.

Table 4.2. Arrangements required to construct maximum length pseudorandom binary sequences (Kerlin 1974).

Stages (n)	Z, ($2^n - 1$)	Stages to be added, modulo-2
2	3	1, 2
3	7	1, 3
4	15	1, 4
5	31	2, 5
6	63	1, 6
7	127	1, 7
8	255	1, 2, 8
9	511	4, 9
10	1023	3, 10
11	2047	2, 11
12	3095	2, 7, 12

As an example, to generate the maximum number of units from a 3-stage digital shift register, the operations are as follows:

a) Add the digits from stages 1 and 3 under the definitions of Table 4.1.

b) Shift all digits one stage to the right removing the last digit.

c) Insert the resultant digit from step (a) into stage 1.

d) Repeat steps (a) to (c) until the original pattern is obtained.

With the initial conditions 1 0 0, these operations result in the following:

Stages on register	1	2	3
Initial pattern	1	0	0
	1	1	0
	1	1	1
	0	1	1
	1	0	1
	0	1	0
	0	0	1
Cycle repeats	1	0	0

Thus a 7-unit PRBS is *1 1 1 0 1 0 0*. The 0 and 1 indicate the change of levels within the signal. For example, should the sequence be *1 0 1 0*, then this will denote a signal of either *high low high low* or *low high low high*, depending upon the association of each digit. If 1 is associated with high and 0 with low, then, for the 7-unit PRBS described above, the resultant input signal will be as illustrated in Figure 4.1.

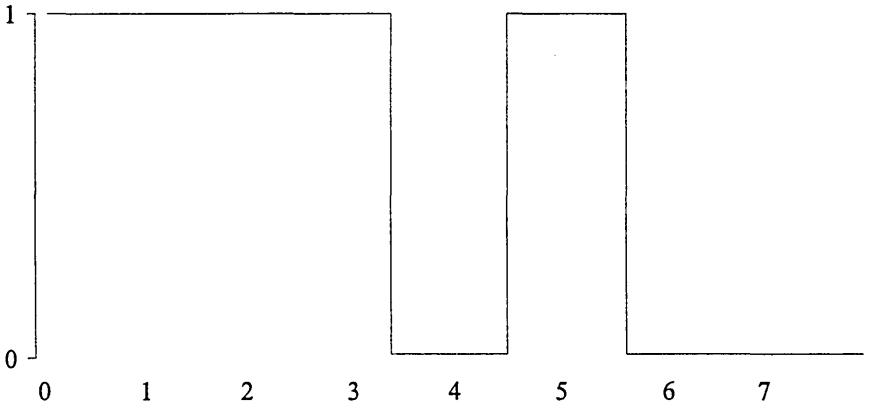


Figure 4.1. A 7-unit pseudorandom binary sequence input signal generated using a 3-stage digital shift register.

By allocating a specific variable, for example work load, to each of the two levels, the PRBS may be applied in the form of a forcing function.

4.2 PROPERTIES OF THE PSEUDORANDOM BINARY SEQUENCE

To understand how the PRBS may be applied in response testing, it is necessary to consider the basic properties of this type of signal.

4.2.1 THE AUTO-CORRELATION FUNCTION

One of the main features of a PRBS is its characteristic auto-correlation function (ACF). The ACF is an average measure of a signal's time domain properties and is formally defined as:

$$\mathrm{ACF}_{aa}(TD) = (1/T) \int_0^T x(t) \cdot x(t + TD)\, dt \qquad (4.2)$$

where T is the period of the PRBS, TD is the time delay and $x(t)$ is a signal multiplied by a time shifted version of itself, $x(t + TD)$, and then integrated.

The ACF of a PRBS can be practically estimated from:

$$\mathrm{ACF}_{aa}(TD) = 1 - [(Z + 1)/T] \cdot TD \quad \text{for} \quad 0 \le TD \le T/Z \qquad (4.3)$$

$$= -1/Z \quad \text{for} \quad T/Z \le TD \le (Z - 1) \cdot T/Z \qquad (4.4)$$

$$= -Z + [(Z + 1)/T] \cdot TD \quad \text{for} \quad (Z - 1) \cdot T/Z \le TD \le T \qquad (4.5)$$

The general form of the ACF appears in Figure 4.2 (overleaf). The ACF has a spike at 0, T, $2T$, ... and a negative bias between spikes of $1/Z$. The width of the spike is equal to 2ω, where ω is the unit duration. As ω decreases and the spike becomes sharper, the ACF tends towards a delta function.

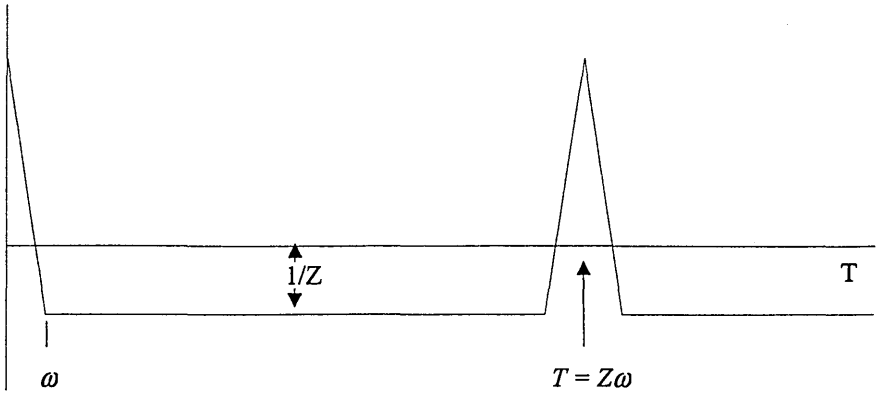


Figure 4.2. Auto-correlation function of a Z-unit pseudorandom binary sequence input signal (ω= unit duration).

4.2.2 THE FOURIER SERIES

Although its form suggests otherwise, it is important to consider a PRBS as the sum of a number of sinusoids of varying amplitude, frequency and relative phase angle (Lynn 1973). This concept is central to the theory of frequency domain analysis.

The summation of the sinusoids may be represented by the following:

$$f(\mathrm{t}) = A_0 + \sum_{k=1}^{\infty} A_k \sin(k\theta \mathrm{t} + \phi_k) \qquad (4.6)$$

where A_o is a steady level to which the sinusoidal terms are added, $\theta = 2\pi/T$, k is the number of the sinusoid, A is its amplitude (mid-point to peak) and ϕ is its relative phase angle. The process of summation is depicted in Figure 4.3 (overleaf).

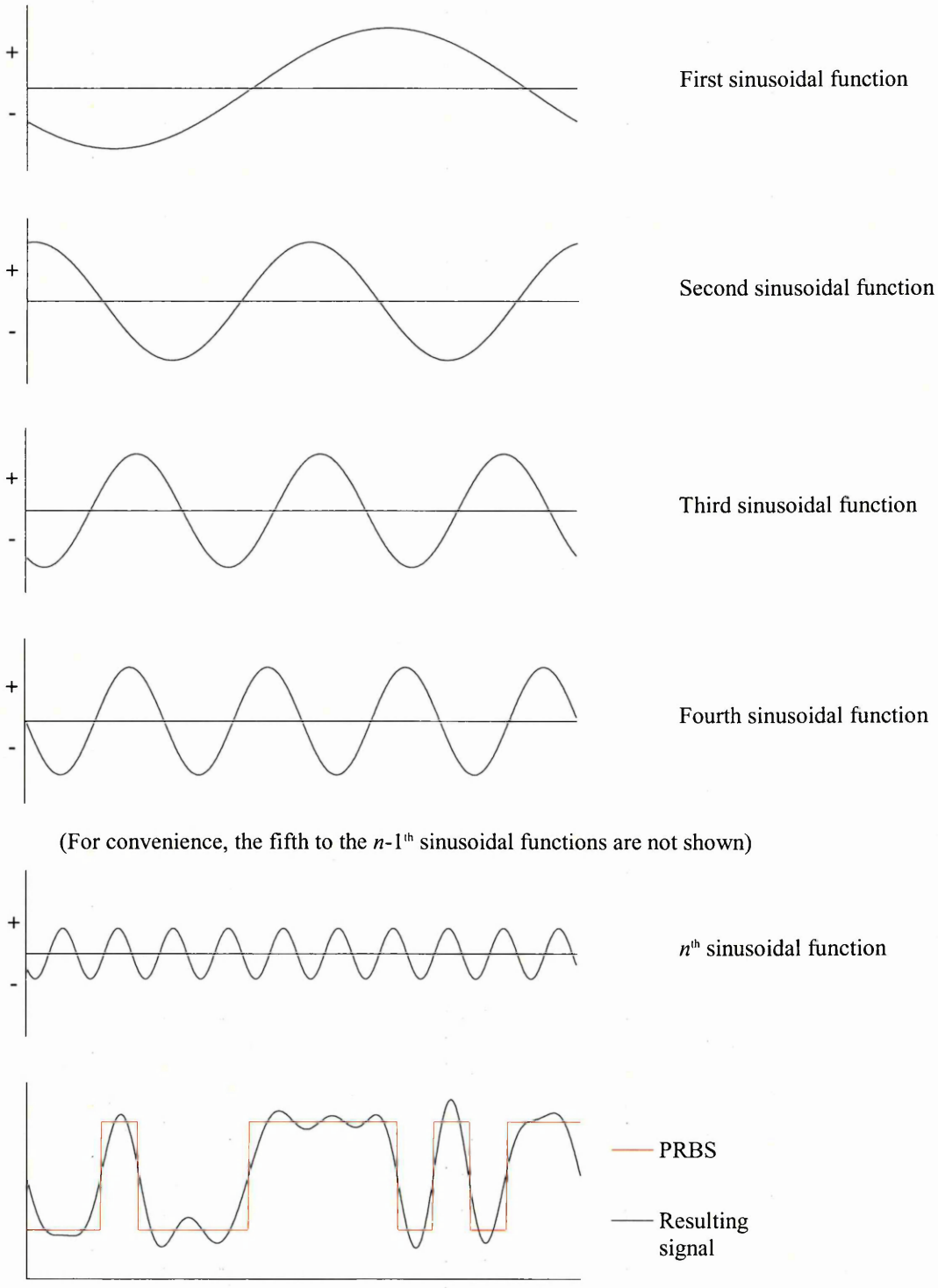


Figure 4.3. Synthesis of a pseudorandom binary sequence input signal by the summation of n sinusoidal functions. From Equation 4.6, each data point on the first sinusoidal function is summed with its equivalent data point on the second, third, fourth, ... and n^{th} functions. Each end value is then added to a steady level to generate a complete signal. The figure shows the resulting signal with a 15-unit pseudorandom binary sequence (PRBS).

As the number of sinusoids included in the summation increases, the approximation to the PRBS can be seen to become more accurate (Figure 4.4).

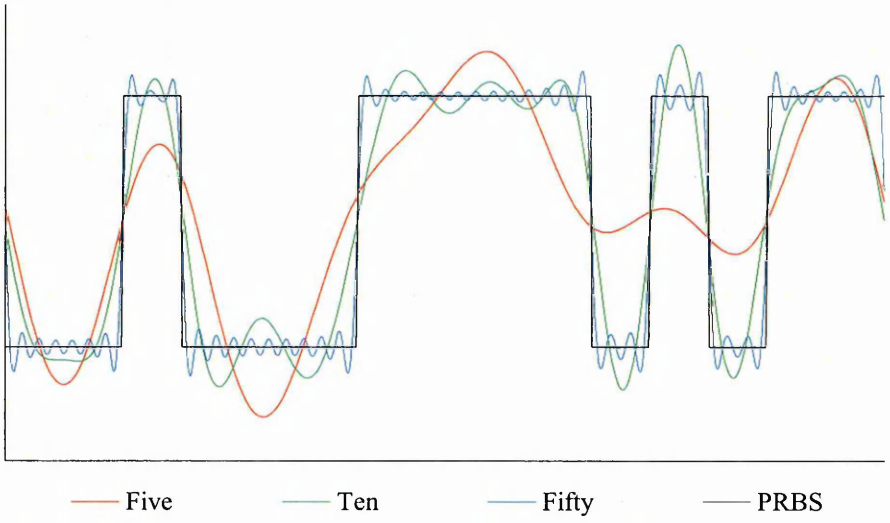


Figure 4.4. Construction of a pseudorandom binary sequence (PRBS) input signal from the summation of five, ten and fifty sinusoidal functions.

If an infinite number of sinusoids were included in the summation, then the result would be a perfect reconstruction of the PRBS.

The sinusoids that form a PRBS constitute its harmonic content. The first sinusoid in the summation is known as the first or fundamental harmonic and has a period equal to that of the signal. Its frequency in Hz is:

$$f_1 = 1/T \qquad \text{Hz} \tag{4.7}$$

Further sinusoids have periods and frequencies that are integral multiples (a half, a third, a quarter, ...) of the fundamental harmonic. These sinusoids are referred to as the second harmonic, third harmonic, fourth harmonic, and so on.

A PRBS may be separated into its individual harmonics using the method of Fourier analysis. The derived series of harmonics are known as a Fourier series and can be used to describe the frequency domain properties of a PRBS. The trigonometric form of the

Fourier series is:

$$f(t) = A_0/2 + \sum_{k=1}^{\infty} A_k \cos k\theta t + \sum_{k=1}^{\infty} B_k \sin k\theta t \qquad (4.8)$$

where $\theta = 2\pi/T$, k is the harmonic number and A_0 is a steady level to which the sine and cosine terms are added.

The coefficients A_k and B_k can be obtained from:

$$A_k = 2/T \int_{-T/2}^{T/2} f(t) \cdot \cos(2k\pi t/T)\, dt \qquad \text{for} \qquad 0 \le k \le \infty \qquad (4.9)$$

$$B_k = 2/T \int_{-T/2}^{T/2} f(t) \cdot \sin(2k\pi t/T)\, dt \qquad \text{for} \qquad 1 \le k \le \infty \qquad (4.10)$$

Amplitude (Amp) and phase angle (ϕ) terms for each harmonic are determined from the above coefficients as follows:

$$\text{Amp}_k = \sqrt{(A_k^2 + B_k^2)} \qquad (4.11)$$

$$\phi_k = \tan^{-1}(B_k/A_k) \qquad (4.12)$$

4.2.3 THE POWER SPECTRUM

The power spectrum defines the distribution of power over the harmonic components of a PRBS. The power spectrum of a PRBS as a function of harmonic number may be calculated from:

$$P_k = (M/2)^2/Z^2 \qquad \text{for} \qquad k = 0 \qquad (4.13)$$

$$P_k = [2(Z+1)(M/2)^2]/Z^2 \cdot \{[\sin(k\pi/Z)]/(k\pi/Z)\}^2 \qquad \text{for} \qquad k \ne 0 \qquad (4.14)$$

where P_k is the power in the kth harmonic, k is the harmonic number and M is the magnitude of the PRBS (difference between the upper and lower binary settings).

The power spectra of several PRBSs are shown as amplitude spectra in Figure 4.5. To make comparisons, the spectra are all based on the same magnitude and fundamental period.

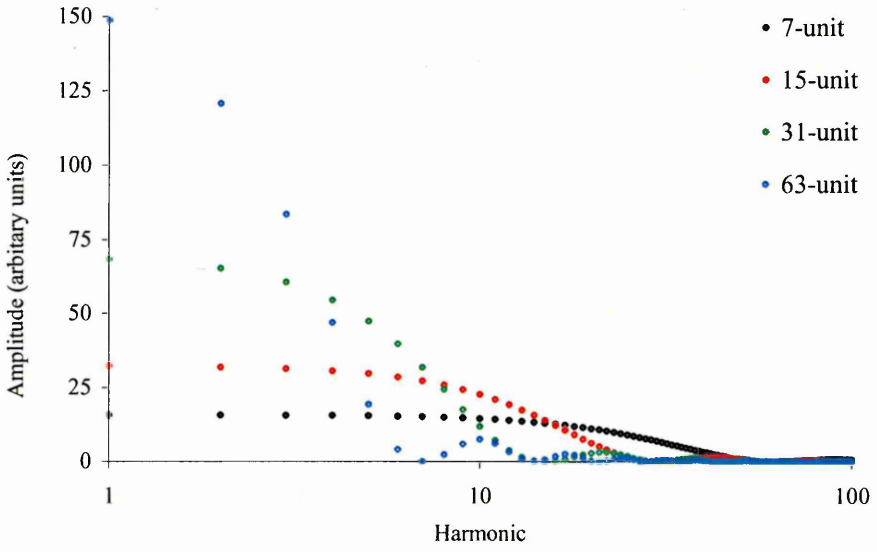


Figure 4.5. Normalised amplitude spectra for 63-unit, 31-unit, 15-unit and 7-unit pseudorandom binary sequence input signals. A logarithmic scale is used for the harmonic axis.

Figure 4.5 shows how the power is unevenly distributed, with the main concentration apportioned to a specific range of harmonics. This range is defined by the bandwidth of a signal (Kerlin 1974) and comprises the fundamental to the $k_{½}$th harmonic, where $k_{½}$ denotes the harmonic number at which the power of the PRBS is down to a half of its maximum value. Beyond this harmonic, the power falls rapidly.

As $k_{½}$ is given by:

$$k_{½} = 0.44Z \tag{4.15}$$

it follows that those PRBS with the greatest number of units will also have the largest bandwidth. In such cases, the power must be distributed across more harmonics. This is achieved by reducing the power at each harmonic (see Figure 4.5).

4.3 THE EXAMINATION OF OXYGEN UPTAKE KINETICS USING PSEUDORANDOM BINARY SEQUENCE WORK RATE FORCINGS

Since the early work of Eßfeld et al. (1982), PRBS WR forcings have been widely employed in the examination of $\dot{V}O_2$ kinetics. Besides comparisons between subjects differing in aerobic capacity (Eßfeld et al. 1987), investigations have included assessments of the effects of deconditioning (Eßfeld et al. 1984; Stegemann et al. 1985), β-blockade (Kowalchuk and Hughson 1990), different body positions (Hughson et al. 1991b; Hoffmann et al. 1991), different arterial O_2 content (Hughson and Kowalchuk 1995; Xing et al. 1991) and different training strategies (Jarvis et al. 1997a - see Appendix 1)

4.3.1 SYSTEM CONSTRAINTS

Although PRBSs have been used to provide information in fields as diverse as response testing in nuclear reactors (Carter et al. 1967) and temperature variation in distillate splitters (Godfrey 1969a), in most of these cases the choice of sequence is wholly dependent on the response component under investigation. In the assessment of $\dot{V}O_2$ kinetics, the complex nature of the cardiorespiratory system places restrictions on the sequences that might be used in testing. The major sources of constraint that must be considered include system linearity, breath-to-breath noise, exercise duration and breathing frequency.

4.3.1.1 SYSTEM LINEARITY

In the study of $\dot{V}O_2$ kinetics, an important concept is that of system linearity (see also Chapter 2). Only if the response to an input shows evidence of linearity, can linear mathematical models be used to quantify the cardiorespiratory system's response kinetics (Fujihara et al. 1973a and 1973b).

In work by Haouzi et al. (1993), sinusoidal WR forcings were used to examine the $\dot{V}O_2$ responses above versus below the LAT. The study identified significantly reduced α and increased φ parameters above compared to below the LAT. These findings indicate the influence of non-linear behaviour and agree with previous investigations that have examined the $\dot{V}O_2$ responses to high intensity step changes in WR (Barstow and Molé 1991; Casaburi et al. 1989a; Linnarsson 1974; Whipp and Wasserman 1986). To date, no evidence can be found of studies that have examined the $\dot{V}O_2$ responses to PRBS WR forcings above versus below the LAT. Nevertheless, if system linearity is to be maintained, then care should be taken to avoid the use of WR intensities that might exceed a subject's LAT.

4.3.1.2 BREATH-TO-BREATH NOISE

In estimating the kinetic parameters of $\dot{V}O_2$, a chief source of concern is inherent breath-to-breath noise. If the magnitude of this noise is excessive, then the underlying responses that result from changes in an input signal may be obscured. In turn, this can lower confidence in the results of an investigation (Goodwin and Payne 1977).

To achieve a desired parameter accuracy, the effect of excessive breath-to-breath noise can be reduced by averaging the responses to several identical test sequences (Lamarra et al. 1987). It can also prove beneficial to maintain a high response-to-noise ratio. To accomplish this, it is necessary to employ a signal that has an adequate distribution of power. Since this distribution is primarily dependent upon the signal's overall magnitude, the limits for the upper and lower work loads must be set as wide apart as possible. However, in most studies of $\dot{V}O_2$ kinetics the workload settings are restricted by the capabilities of the ergometer and the need to avoid exceeding a subject's LAT.

Consequently, when choosing a PRBS input signal, careful consideration must be given to the amount of power available at each harmonic.

4.3.1.3 EXERCISE DURATION

To obtain comparable assessments of $\dot{V}O_2$ kinetics during PRBS testing, the characteristics of the system controlling $\dot{V}O_2$ responses must remain relatively unchanged over time (Bennett et al. 1981). For exercise intensities at or below the LAT, extending the duration of an experiment has been shown to cause slow increases in body temperature (Saltin and Hermansen 1966) and lead to raised blood concentrations of cortisol (Kindermann et al. 1982). As these changes may influence the kinetics of $\dot{V}O_2$ (Solomon and Taylor 1994), care must be taken to choose a sequence that is not unnecessarily long.

4.3.1.4 BREATHING FREQUENCY

An additional restriction in the choice of a suitable PRBS is the need to consider the rate of breathing. Unless the time spent at either of the binary levels of a PRBS WR forcing exceeds the breathing frequency of a subject, important response data can be omitted (Bennett et al. 1981). The need to set ω at or above the rate of breathing is the main reason why truly random binary sequences are not suitable for use in $\dot{V}O_2$ response testing. With this form of input signal, the binary transitions can occur at any time. Consequently, the interval between transitions may not exceed that of the breathing frequency.

4.3.2 SELECTION OF AN APPROPRIATE PSEUDORANDOM BINARY SEQUENCE

To date, a number of different PRBSs have been used in the examination of cardiorespiratory system kinetics. These range from the 15-unit (30 s·unit^{-1}) input employed by Eßfeld et al. (1987) to the 127-unit (5 s·unit^{-1}) input of Greco et al. (1986).

In the determination of $\dot{V}O_2$ kinetics, the choice of which PRBS to employ is largely dependent on whether the investigator wishes to examine *phase I* and/or *phase II* of the $\dot{V}O_2$ response to dynamic exercise. To examine a system's response characteristics during a particular phase, the physiological mechanisms that initiate and control that phase must be 'excited' or 'challenged' (Bennett et al. 1981; Hughson et al. 1990a). Previous investigations have shown that certain input frequencies are able to excite particular mechanisms (Bennett et al. 1981; Hughson et al. 1990a). More specifically, those PRBS inputs comprising a greater proportion of high frequency harmonics excite the mechanisms operating during *phase I*, whilst those inputs comprising a greater proportion of low frequency harmonics challenge the mechanisms that function during *phase II* (Hughson et al. 1990b; Xing et al. 1991). By extending the bandwidth of the input to include a relatively equal distribution of high and low frequency harmonics, it is also possible to excite the mechanisms that operate during both phases of the $\dot{V}O_2$ response, simultaneously.

The first study of cardiorespiratory system kinetics to use a PRBS as the basis for varying a WR forcing was conducted by Bennett et al. (1981). These investigators determined the kinetics of $\dot{V}I$ using a 63-unit (5 s·unit^{-1}) PRBS WR input. The bandwidth of this PRBS comprised harmonics in the frequency range 0.0032 to 0.0857 Hz. The data obtained by Bennett and his co-workers were analysed in the time domain (see Chapter 7), and the cross-correlation function of $\dot{V}I$ with WR fitted with either single- or two-component exponential models typical of those used to characterise

impulse responses in linear systems. The results showed the two-component model to provide a statistically better fit than the single-component model. It was therefore concluded that the frequency content of the PRBS had been sufficient to excite the mechanisms operating during both *phase I* and *phase II* of the $\dot{V}_I$ response to exercise. In support of this conclusion, Bennett and his colleagues noted that the $\dot{V}_I$ response during *phase I* was attenuated when the upper frequency range of the PRBS was reduced.

Although the study of Bennett et al. (1981) was confined to the examination of $\dot{V}_I$ kinetics, identical PRBS inputs have also been used to investigate the $\dot{V}_{O_2}$ response to dynamic exercise (Hughson et al. 1991a; Xing et al. 1991). Whilst these investigations have been successful, estimates of $\dot{V}_{O_2}$ kinetics are more normally obtained using a 15-unit (30 s·unit^{-1}) PRBS (Eßfeld et al. 1987; Hughson and Kowalchuk 1995; Stegemann et al. 1992). This PRBS has a frequency range of 0.0022 to 0.0133 Hz. Hughson et al. (1990b) have noted that such a relatively low range will tend to excite the physiological mechanisms that function during *phase II* of the $\dot{V}_{O_2}$ response to dynamic exercise.

In investigations by Hughson and his colleagues (1991a), it was reported that estimates of TLT obtained from tests employing a 15-unit (30 s·unit^{-1}) PRBS WR forcing were not significantly different to estimates derived from a 63-unit (5 s·unit^{-1}) PRBS. Furthermore, in almost fifty comparisons, including tests on subjects in supine and upright positions and under conditions of hypoxia and normoxia, no significant differences could be identified between mean estimates of TLT obtained from PRBS tests and mean estimates calculated from the $\dot{V}_{O_2}$ response to step changes in WR.

Currently, the most common method of assessing $\dot{V}O_2$ kinetics involves the use of step WR forcings (Babcock et al. 1994b; Barstow et al. 1994; Casaburi et al. 1992; Hughson et al. 1993; Phillips et al. 1995; Whipp et al. 1982). Although step forcings are relatively straightforward to apply, subjects must be tested on several occasions so that the resulting data can be averaged (Lamarra et al. 1987). Whilst this approach helps elucidate the 'true' response in $\dot{V}O_2$, the time required to complete investigations can prove inconvenient or impractical (Cunningham et al. 1993; Hoffmann et al. 1994a).

Using the PRBS method, an estimate of $\dot{V}O_2$ kinetics in the sub-LAT domain can be obtained from a single test session of ~30 min duration (Hughson et al. 1990d). Hughson et al. (1991a) have reported that there is no significant difference between estimates of the TLT derived from PRBS WR forcings and step WR forcings. This finding suggests that PRBS tests provide the same determination of $\dot{V}O_2$ kinetics as the established step method, but without the need for repeated testing.

5.1 THE EVALUATION OF OXYGEN UPTAKE KINETICS IN CLINICAL, UNTRAINED OR YOUNG SUBJECTS

Although PRBS tests may prove advantageous in physiological assessment, when examining patients, young subjects or individuals with low physical fitness, it can be necessary to reduce the upper work limit of a forcing. This undertaking helps to lessen the discomfort of testing and to minimise the possibility of the subject exceeding the LAT. Due to the pre-determined nature of a PRBS, however, any reduction in the upper work limit will cause a fall in the distribution of power across the bandwidth of the sequence. If the power across the bandwidth falls below a critical level, then the effect on the response-to-noise ratio can make it difficult to elicit discernible responses from

the forcing (Eßfeld et al. 1987). In this situation, valid estimates of $\dot{V}O_2$ kinetics become impossible to obtain (Cooper et al. 1992).

An alternative approach to evaluating $\dot{V}O_2$ kinetics might be to employ a multifrequent signal optimised with regard to the power available at each harmonic, the number of harmonics tested and the range of frequencies over which testing is conducted (Bakker et al. 1980; Eßfeld et al. 1987). Perturbing WR according to this form of signal may provide a suitable means of estimating $\dot{V}O_2$ kinetics in subjects whose level of exercise tolerance demands a critical reduction in the upper work limit of a PRBS forcing (Bakker et al. 1980).

To construct a multifrequent WR forcing that enhances identification of the underlying $\dot{V}O_2$ response, the most effective method would be to raise the power in each harmonic comprising the frequency range of interest. Although this could be accomplished by increasing the magnitude of the overall signal, given subjects with a limited tolerance to exercise, this approach is clearly not feasible. Therefore, to achieve the required increase in power, it would be necessary to minimise the percentage of signal energy in all harmonics other than those comprising the frequency range of interest and/or reduce the total number of harmonics over which the power is distributed (Kerlin 1974). By constructing a signal solely from those harmonics in the desired frequency range, the maximum increase in power would thus be achieved. However, simply adding together a select number of harmonics would not necessarily generate a suitable forcing (see Figure 5.1 overleaf).

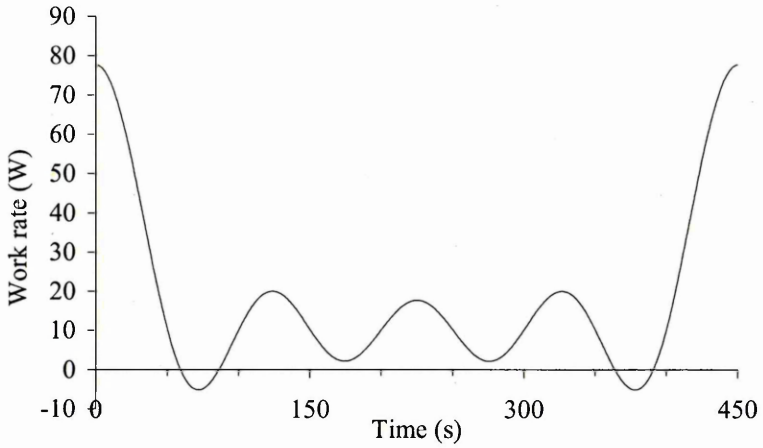


Figure 5.1. The outcome of adding four sinusoidal forcings with frequencies of 0.0033, 0.0067, 0.0100 and 0.0133 hertz, respectively. All sinusoids have a 15 watt amplitude and zero degree phase angle. The summation was accomplished using the formula presented in Equation 4.6.

Aside of exceeding the operational parameters of the ergometer, the signal shown in Figure 5.1 may well generate a non-linear response in $\dot{V}O_2$.

To construct a practical multifrequent signal with the potential to elicit discernible and valid cardiorespiratory system responses, it is necessary to consider the form of a PRBS. If a signal is constructed from those harmonics comprising the bandwidth of a PRBS, then the sequence will take a form similar to that depicted in Figure 5.2.

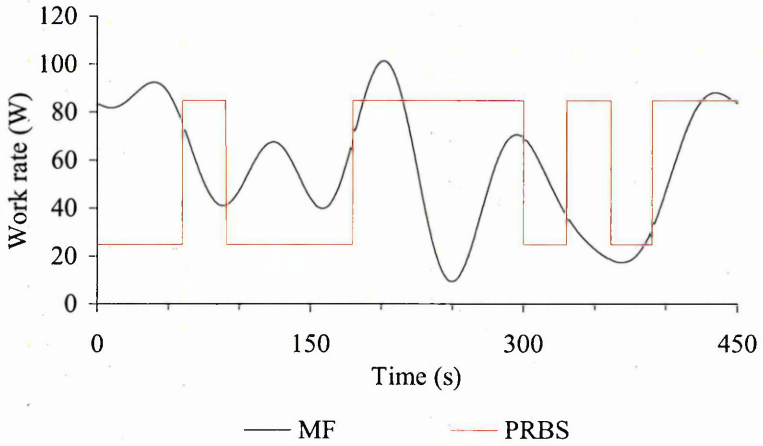


Figure 5.2. The multifrequent form (MF) taken by a 15-unit (30 s·unit^{-1}) pseudorandom binary sequence (PRBS) work rate forcing after removal of the harmonic components outside of the bandwidth. The lower and upper work limit settings for the PRBS were 25 and 85 watt, respectively.

From Figure 5.2, it can be deduced that the magnitude of a PRBS forcing is maintained within pre-determined limits by the negating effect of the infinite number of extra-bandwidth harmonics that form the PRBS. Thus, to create a multifrequent signal that does not exceed practical limits, harmonics outside the frequency range of interest must be included in the signal's construction.

If the power necessary to construct the desired form of multifrequent signal cannot be derived from those harmonics outside the frequency range of interest, then an alternative source must be considered. One option would be to re-allocate the power distributed across the range of interest to a select number of harmonics (Buckner 1970). Whilst this approach has been employed in the analysis of engineering based systems (Kerlin 1974), it is not known whether it can be applied to the assessment of $\dot{V}O_2$ kinetics.

5.1.1 VALIDATION OF NEW MEASUREMENT METHOD

If a specially adapted multifrequent WR forcing could be developed to evaluate $\dot{V}O_2$ kinetics in clinical, untrained or young subjects, then the first step would be to assess the validity of the resulting data. As measures of $\dot{V}O_2$ kinetics obtained *in vivo* require the use of invasive methods (for example, Grassi et al. 1996), it might be more appropriate to compare the data with that derived from another form of forcing. Since multifrequent inputs fall under the same signal classification (discrete-level, periodic) as PRBS inputs, the same analytical techniques can be used to evaluate response data. Therefore, rather than make initial comparisons with the output of a more established WR forcing, it would be more practical to compare the data with that obtained from a PRBS test. Furthermore, in work by Hughson and his colleagues (1991a), it was reported that, in almost fifty comparisons, no significant differences could be identified between mean estimates of TLT obtained from PRBS tests and mean estimates

calculated from the $\dot{V}O_2$ response to step changes in WR. This suggests that PRBS WR forcings provide an acceptable description of a system's $\dot{V}O_2$ kinetics and are therefore suitable for evaluating the validity of data derived from a specially adapted multifrequent WR forcing.

When comparing two methods for measuring the same quantity, the aim is to assess whether the methods show adequate agreement (Altman 1991). If the agreement between the methods is sufficiently high, then one method can be confidently used as a surrogate of the other (Lee 1992). Examining the agreement between two methods in this manner necessitates that the between trials variability in measurements must also be considered.

5.1.2 VARIABILITY IN ESTIMATES OF OXYGEN UPTAKE KINETICS

In clinical or physiological examination, assessment methods often have large measurement errors associated with them (Bland and Altman 1995). These errors tend to arise from the variability that occurs due to a combination of biological, technological and environmental factors (Bland and Altman 1995; Katch et al. 1982; Sale 1991). In order to compare the agreement between two methods, it is important to consider the amount of variability between trials. If an established method has poor repeatability, then any new method with which it is compared will show little agreement, regardless of how good the new method is (Bland and Altman 1995).

The number of reproducibility studies that have considered the effect of variability on estimates of $\dot{V}O_2$ kinetics is relatively small (Claxton et al. 1996; Hughson and Inman 1986b; Whipp et al. 1981). Of these, only Claxton et al. (1996) have investigated variability in estimates of $\dot{V}O_2$ kinetics derived from PRBS WR forcings. Claxton and his colleagues observed wide limits of agreement (Altman 1991) in parameter estimates, and expressed concern over the possible influence of inherent biological variability.

Prior to investigating the development of a new multifrequent signal, it would therefore seem wise to gain a more comprehensive knowledge of the between trials variability in estimates of $\dot{V}O_2$ kinetics obtained from PRBS WR forcings.

5.2 SUMMARY OF EXPERIMENTAL AIMS AND OBJECTIVES

To evaluate $\dot{V}O_2$ kinetics in clinical, untrained or young subjects, it can be necessary to lower the upper limit of a PRBS WR forcing. This undertaking effects a fall in the distribution of power across the harmonic content of the sequence. The consequent reduction in the response-to-noise ratio means that valid estimates of $\dot{V}O_2$ kinetics may be difficult to obtain. Accordingly, the main aim of this thesis will be to investigate the potential for developing a multifrequent WR forcing for use with subjects whose level of exercise tolerance demands a critical reduction in the magnitude of a PRBS forcing.

For validation purposes, the new method will be used to evaluate $\dot{V}O_2$ kinetics initially in normal healthy individuals. The resulting data will then be compared with estimates of $\dot{V}O_2$ kinetics derived from the same individuals using an established PRBS WR forcing. Statistical analysis will be employed to assess how closely estimates derived from the two methods agree.

When evaluating the agreement between two methods, it is important to consider repeatability. If a measurement method shows poor repeatability, then it will not agree well with another method, however accurate the other method might be. Prior to investigating the development of a specially adapted multifrequent forcing, this thesis will therefore seek to quantify the degree of variability in estimates of $\dot{V}O_2$ kinetics derived from an established PRBS WR forcing.

In summary, the experimental section of this thesis will seek to address the following

questions:

i) What is the degree of variability in estimates of $\dot{V}O_2$ kinetics derived from PRBS WR forcings?

ii) Could a specially adapted multifrequent WR forcing be used to evaluate $\dot{V}O_2$ kinetics in clinical, untrained or young subjects?

6.0 MATERIALS AND METHODS

6.1 SUBJECTS

All subjects were drawn from healthy, non-smoking populations and volunteered to participate in testing. Anthropometric data are presented in the methods section of each study. Additional information concerning each subject appears in Appendix 2.

Before undertaking a test, each subject was screened for the presence of medical contraindications and informed of the possible risks associated with testing. All subjects were briefed on the nature and general purpose of the study, and asked to provide written consent to their participation. An example of the medical questionnaire and consent form is included in Appendix 3.

Subjects were requested to avoid any form of heavy exercise and to refrain from consuming alcohol and caffeinated products in the 12 hours prior to each test. If the study required completion of more than one test, then the same requests were also applied to the intervening period.

The experimental protocol and consent forms were reviewed and approved by a senior member of the research team. Testing was performed under the supervision of at least two researchers, one of whom was trained to provide first aid in the event of an emergency. To ensure the safety of all subjects, equipment checks were undertaken on a regular basis by trained personnel.

6.2 EXERCISE PROTOCOL

All tests were performed in the upright body position on an electrically braked cycle ergometer (Bosch, 550 ERG). The ergometer was linked to a computer based control system (First Breath Associates, Software Version 2.0, 1992) that varied the work rate according to a programmable signal. Programming of the signal was accomplished

using a sub-routine of the respiratory measurement program employed in the studies (First Breath Associates).

To simplify the comparison of both intra- and inter-experiment response data, the range of harmonic frequencies over which testing was performed did not alter. The frequency range of interest was from 0.0022 to 0.0133 Hz. In previous investigations, a 15-unit (30 s·unit^{-1}) PRBS with a bandwidth equivalent to this range was shown to elicit a good description of the *phase II* response in $\dot{V}O_2$ (Hughson 1991a; Hughson et al. 1990b).

The lower and upper work limits employed in tests were either 25 and 85 W or 25 and 105 W, respectively. The ability of the ergometer and associated equipment to administer these WRs was assessed using a rotary torque transducer (Industrial Measurements limited, TRSC-100). In the dynamic transition from 25 to 85 to 25 W, the assessment revealed that the actual measured power was 21.3 (5.9) to 83.6 (10.2) to 25.6 (8.5) W, respectively (where values are the mean (standard deviation) of twenty tests). In the dynamic transition from 25 to 105 to 25 W, the actual measured power was shown to be 20.6 (6.8) to 103.1 (10.4) to 23.8 (8.2) W, respectively (mean (standard deviation) of twenty tests). Calibration of the ergometer was performed locally by a trained technician. Further details regarding the accuracy and stability of the ergometer can be found in work by Claxton (1999, unpublished).

The step increases and decreases in WR for the transitions between 25 to 85 to 25 W and 25 to 105 to 25 W were met by the ergometer at a rate of ~100 W·s^{-1}. The rate was determined by monitoring the direct current (DC) input signal and the resultant DC output of the ergometer using a flat bed chart recorder (Scientific and Medical products Ltd., Linseis LS/L 600/L 650).

All subjects were given several minutes to become familiar with the exercise protocol before the commencement of testing. Additionally, each protocol incorporated a

warm-up period. Detailed descriptions of test protocols are given in the methodological section of each experiment. Post-test examination of the responses obtained from each subject did not identify any un-expected deviations in the data.

A pedal rate of 60 revolutions per minute (rpm) was requested during each test. To assist subjects in maintaining this rate, cadence was displayed visually on a control panel situated on the handlebars of the ergometer. In addition, an electronic control circuit ensured that the work required of the subject remained constant regardless of cadence. This compensated for the small variations in pedal rate (typically ±5 rpm) that occurred both within and between subjects.

Throughout testing, no information was given of impending changes in WR.

6.3 DATA COLLECTION

For the measurement of inspiratory and expiratory volumes and flows, subjects breathed room air through a low dead-space, low resistance turbine volume transducer (Alpha Technologies YMM110). This device was calibrated prior to each test using a 3000 ml syringe (Hans Rudolph Incorporated) manually pumped at flow rates approximating those encountered during the test. A small sample of respiratory gas was continuously drawn from the turbine and analysed for fractional concentrations of O_2, CO_2 and N_2 by mass spectrometry (Marquette MGA-1100). Pre-test calibrations were performed on the mass spectrometer using high accuracy gases (tolerance ±0.3%) of known composition. Selected performance specifications of the mass spectrometer are presented in Appendix 4.

The electrical signals from the turbine and mass spectrometer were sampled every 5 ms. To achieve on-line time alignment of the volume and gas concentration signals, the time delay from the turbine to the mass spectrometer was determined before each test. After analogue to digital conversion, signals were stored on computer for the breath-by-breath

computation of $\dot{V}O_2$ (STPD) using algorithms described by Beaver et al. (1981). These algorithms solve $\dot{V}O_2$ as the difference between the inspired and expired volume over one breath, with adjustments made for fluctuations in alveolar gas composition and in nominal lung volume (NLV). NLV was assumed to be equivalent to functional residual capacity (FRC) as predicted from height and weight and therefore end-expiratory lung volume. Data recorded by Wessel et al. (1979) show that end-expiratory lung volume varies from one breath to the next and that this variation can have a significant effect on measurements of $\dot{V}O_2$. Therefore, it should be noted that variations in error sensitivity might contribute to breath-to-breath noise.

During each test, attempts were made to minimise increases in body temperature by improving the conditions for evaporative heat loss. This was achieved by use of an electric fan. The fan was positioned behind the subject so as not to affect airflow through the turbine.

6.4 DATA PREPARATION

To reduce the influence of initial transients, breath-by-breath data recorded during the warm-up period were not included in the analysis. The remaining data were automatically inspected for any abnormal breaths collected during the experiment. Such breaths typically result from swallowing or other disturbances in the sensed airflow and are usually associated with an opposing companion breath. To avoid the unnecessary elimination of data, where possible, abnormal breaths were edited by combining with the previous or following companion breath (Pórszász and Riley 1995). Any remaining abnormal breaths were deleted from the data. The number of combined and deleted breaths was small, amounting to an average of 0.3% of the total recorded breaths.

Before proceeding, recorded data were checked visually to assess for plausibility. Although it was not expected that this process would identify small errors, it was hoped to find major errors that might significantly influence any subsequent analysis.

Following pre-processing, breath-by-breath data were linearly interpolated to obtain $\dot{V}O_2$ values at equidistant time intervals (1 s). The corresponding WR at each 1 s was the actual WR according to the input signal that was used for the test. Interpolated data were split into separate files each of a length equivalent to the duration of one test cycle. The files were then time aligned and ensemble averaged to produce one representative set of data for each test. The process of averaging helped to de-emphasise random fluctuations, that is, breath-to-breath noise, in the $\dot{V}O_2$ response. It was assumed that breath-to-breath noise could be characterised as uncorrelated and Normally distributed. Noise was also accepted as largely independent of the work rates employed in each test (Lamarra et al. 1987).

The averaged data were analysed in the frequency domain using Fourier analysis. The methodology for this approach is described in detail in Chapter 7. Additional noise reduction was achieved by using the power spectral density (PSD) of the input and the cross-power spectral density (CPSD) of the input and output to calculate the frequency response parameters (Eßfeld et al. 1987; Hoffmann et al. 1992). To determine the PSD and CPSD, the Fourier analysis was performed on the ACF and CCF of each data set (see Chapter 7). The resulting Fourier series was then used to yield α and φ parameters for the dynamic relationship between WR and $\dot{V}O_2$. By incorporating the CCF into calculations of the kinetic parameters, the response-to-noise ratio was improved by attenuation of those components not correlated with the WR signal, specifically breath-to-breath noise (Eßfeld et al. 1987).

6.5 DATA PRESENTATION

The α and φ parameters derived from testing were presented in a Nyquist plot (see Chapter 3). In the plot, α parameters were used to determine the distance of the data point from the origin, whilst corresponding φ parameters were used to determine the angle of the data point from the abscissa.

6.6 ESTIMATION OF THE KINETIC PARAMETERS OF OXYGEN UPTAKE

The discrete frequency range of the WR forcings used in each experiment was chosen to excite primarily the physiological mechanisms that function during *phase II* of the $\dot{V}O_2$ response to dynamic exercise (Hughson et al. 1990b). In previous studies using individual sinusoidal forcings, the system response to a similar range of frequencies has been described by several different order models, both with and without time delay (Bakker 1980; Casaburi et al. 1977; Cunningham et al. 1993). The objective of this thesis was not to test any particular model, but rather to estimate the kinetic parameters of the dynamic response in $\dot{V}O_2$. Accordingly, it was decided that the non-rational (four-parameter transfer function) model advocated by Sherrill and Swanson (1981) would be used to characterise the $\dot{V}O_2$ responses to all protocols. This model has been shown to yield both a time constant and a positive time delay when used to express the relationship between WR and $\dot{V}O_2$. A more detailed description of the model is provided in Chapter 3.

The non-rational model was fitted to data using an iterative least squares method that combined visual and analytical techniques. The data and model were displayed graphically on a computer screen. Initial estimates of parameter values were entered until the shape of the model approximated that of the data. Using the estimated parameters as starting values, a computer program sought to fit the model to the data by randomised variation of the parameters. A best fit was formed on the basis of

minimising the sum of the distances of each fitted point from the corresponding measured data point. The program continued through at least 100 iterations before generating a model that described the data.

As the non-rational model employed in this thesis yielded only single τ and *TD* parameters, it was not possible to use estimates of the TLT (Equation 3.4) as a means of summarising the $\dot{V}O_2$ response to testing. Instead, parameters derived from the model fitting were used to calculate estimates of the MRT of $\dot{V}O_2$ (Equation 3.3).

6.7 STATISTICAL ANALYSIS

The statistical analyses performed in the following studies were based on the assumption that the within-subject differences had an approximately Normal distribution.

An analysis of variance (ANOVA) with repeated measures on one factor was used to identify significant differences between the data obtained from the subjects. ANOVA was performed using the SPSS statistics package (SPSS, Software Version 8.0, 1998).

Where appropriate, quantification of the agreement between tests was performed by calculating the 95% limits of agreement (mean difference between test 1 and test 2 ± the standard deviation of the differences between test 1 and test 2 multiplied by 1.96). The limits of agreement provide information about the maximum difference likely to occur between two test methods (Bland and Altman 1986). The technique is also suited to the examination and quantification of between trials variability (Atkinson 1995). Unlike more traditional methods, limits of agreement allow variability to be expressed in the same units of measurement as the data that are being investigated (Altman 1991).

Statistical significance for all analyses was set at $P<0.05$. Where practicable, actual values of P are given in the results. Results are expressed as mean (standard deviation).

7.0 THE ANALYSIS OF OXYGEN UPTAKE RESPONSES TO PSEUDORANDOM BINARY SEQUENCE WORK RATE INPUTS

To incorporate the information necessary to complete the experimental chapters of this thesis, the following sections will consider the techniques used to analyse the $\dot{V}O_2$ response data derived from PRBS WR forcings. The techniques described can be separated into two different analytical domains: frequency and time. Due to the similarity between multifrequent signals and PRBS signals, the analysis of data from multifrequent forcings can be performed in the same domains using identical techniques. However, analysis in the time domain demands that the signal employed in data collection possesses several specific characteristics. In general, these characteristics are only present in PRBS signals. It should therefore be noted that the analysis of data from a multifrequent forcing is only likely to be possible in the frequency domain. The materials and methods used to collect the data presented in the figures accompanying the description of each technique are the same as those described in the previous chapter.

7.1 ANALYSIS OF RESPONSES IN THE FREQUENCY DOMAIN I

In Chapter 4, it was noted how a PRBS should be considered as the sum of an infinite number of sinusoids. The same principle also applies to any input to a system that takes the form of a PRBS, and to any output generated by such an input.

To separate a PRBS into its harmonic components, a Fourier analysis can be performed on the signal (see Chapter 4). The same method of analysis may also be used to separate a PRBS input and any ensuing output. The derived Fourier series will yield coefficients of the sine and cosine functions at each harmonic of the input and output (Kerlin 1974). These coefficients can be used to compute amplitude and phase angle terms over the harmonic range of interest (Equations 4.11 and 4.12). When all the

terms are known, a relationship can be established between the input and output at each harmonic. The relationship is obtained by dividing the amplitude of the output by that of the input to yield an α parameter, and by subtracting the phase angle of the input from that of the output to yield a φ parameter (Jenkins and Watts 1968). The process of establishing a relationship between a PRBS input and a selected output is illustrated in Figure 7.1.

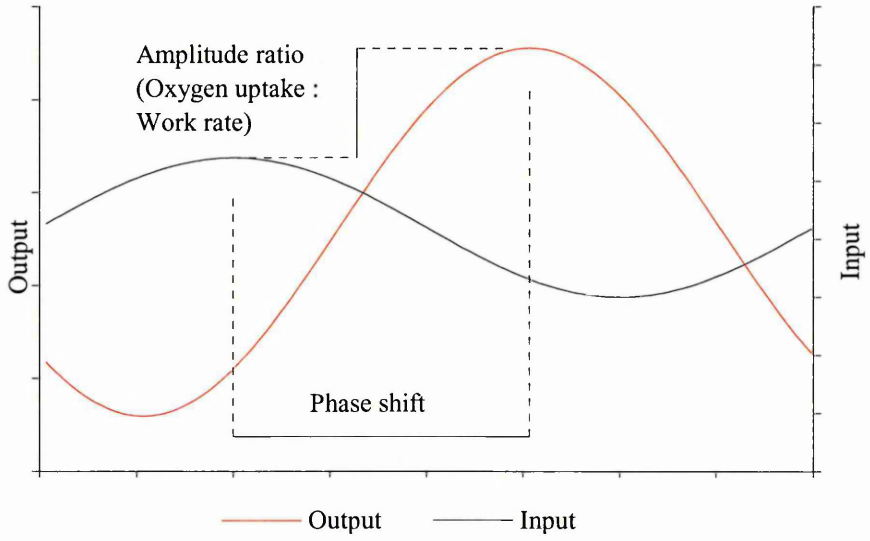


Figure 7.1. Diagrammatic representation of how the relationship between a pseudorandom binary sequence input harmonic (fundamental) and selected output is established in the frequency domain.

Analysis in the frequency domain allows the system response to be determined without the fitting of mathematical models (Eßfeld et al. 1987; Hoffmann et al. 1994a). Since the choice of an appropriate model is not always straightforward, this approach can prove advantageous. To determine the system response, α and φ parameters are used to predict directly the response to another form of input. Consequently, the system under study must behave in a linear manner (see Chapter 2). The method of computation for predicting the response to a step input is as follows:

$$Y(t) = \sum_{k=0}^{n} A_k \cdot \alpha_k \cdot \sin(k\theta t + \phi_k + \varphi_k) \qquad (7.1)$$

where $\theta = 2\pi/T$, k is the number of a square wave (representing a step) input harmonic, A_k and ϕ_k are the amplitude and phase angle of that harmonic and α_k and φ_k are the amplitude ratio and phase shift obtained from a PRBS input versus output relationship at a harmonic of equivalent frequency. The value n is the number of the square wave input harmonic with a frequency that corresponds to that of the $k_{½}$th harmonic of the PRBS input. However, as the harmonic frequencies of the PRBS are unlikely to match those of the square wave input, n is taken as the number of the harmonic with the next lowest frequency compared to that of the $k_{½}$th harmonic. Where there are no corresponding frequencies from which to extract values of α_k and φ_k, estimates may be obtained by linear interpolation from the known α and φ parameters (Hoffmann et al. 1994a). Figure 7.2 demonstrates how the $\dot{V}O_2$ response following the onset of exercise compares with the predicted response computed using the α and φ parameters resulting from a 15-unit (30 s·unit^{-1}) PRBS WR forcing. The predicted response has been adjusted to the correct scale by taking into account the static gain.

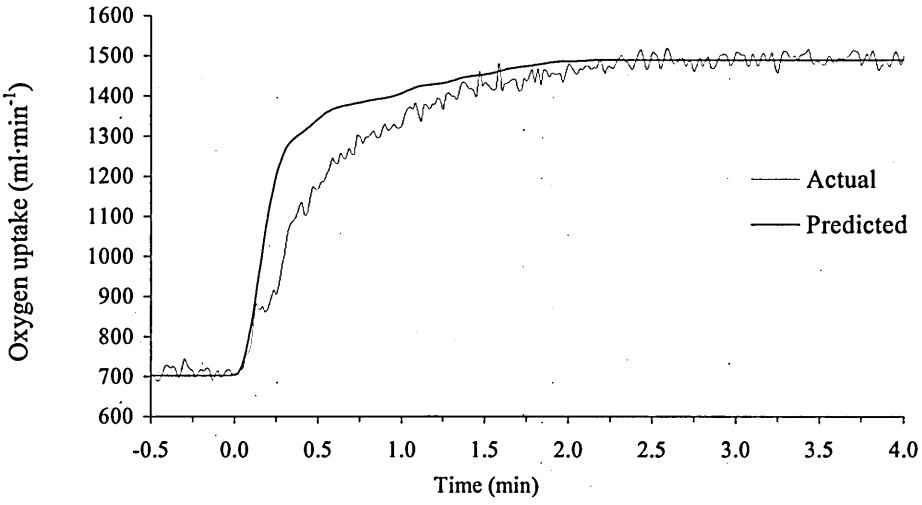


Figure 7.2. Comparison of the predicted and actual oxygen uptake responses following the onset of exercise. The predicted response has been computed using information obtained from a 15-unit (30 second·unit^{-1}) pseudorandom binary sequence work rate forcing. Exercise onset is at 0 minutes. Data for the figure were collected during tests undertaken as part of this thesis. The data are unpublished.

It can be seen from Figure 7.2 that the predicted response resembles a single-exponential curve constrained to start at the onset of the input. Consequently, the method is perhaps better suited to the analysis of less complex system responses.

7.2 ANALYSIS OF RESPONSES IN THE FREQUENCY DOMAIN II

An alternative method of establishing a system's response kinetics in the frequency domain involves charting α and φ parameters on a Nyquist plot. This approach is generally used to communicate the results obtained from sinusoidal testing (Bakker et al. 1980; Casaburi et al. 1977; Casaburi et al. 1980), but can also be employed to express the data obtained from PRBS tests (Bennett et al. 1981).

The use of Nyquist plots has already been described in Chapter 3. In brief, α parameters are used to determine the distance of the data point from the origin, whilst corresponding φ parameters are used to determine the angle of the data point from the abscissa (Casaburi et al. 1977). An example of a Nyquist plot using data obtained from PRBS testing is depicted in Figure 7.3 (overleaf). The figure illustrates how, as the frequency at each harmonic of the input rises, α decreases whilst φ increases.

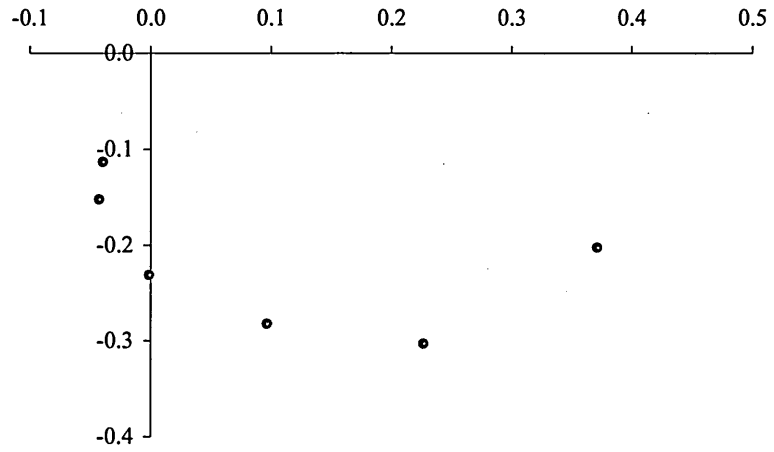


Figure 7.3. Nyquist plot of amplitude ratio and phase shift parameters computed from the oxygen uptake response to a 15-unit (30 second·unit^{-1}) pseudorandom binary sequence (PRBS) work rate forcing. The plot incorporates data from the bandwidth of the PRBS, that is, from the fundamental to the 6th harmonic. Data for the figure were collected during tests undertaken as part of this thesis. The data are unpublished.

To quantify a system's response kinetics, data may be fitted with mathematical models (see Chapter 3). Figure 7.4 (overleaf) illustrates the outcome of using a non-rational (four-parameter transfer function) model (Equation 3.7) to describe $\dot{V}O_2$ response data derived from a 15-unit (30 second·unit^{-1}) PRBS WR forcing. The τ and *TD* parameters resulting from the best fit of the model are included in the figure.

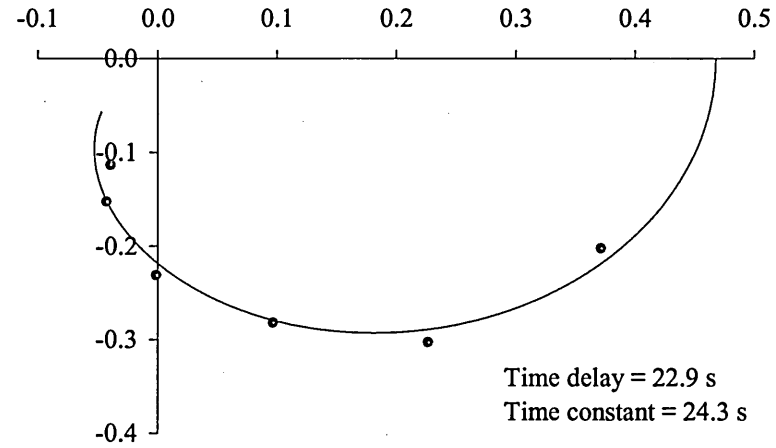


Figure 7.4. Nyquist plot of amplitude ratio and phase shift parameters computed from the oxygen uptake response to a 15-unit (30 second·unit^{-1}) pseudorandom binary sequence work rate forcing shown with best fit to a non-rational (four-parameter transfer function) model. Best fit kinetic parameters are included in the figure. Data for the figure were collected during tests undertaken as part of this thesis. The data are unpublished.

7.3 ANALYSIS OF RESPONSES IN THE TIME DOMAIN

The time domain analysis of the output to a PRBS input may be accomplished using auto- and cross-correlation techniques (Kerlin 1974; Marmarelis and Marmarelis 1978). In Chapter 4, the ACF of a PRBS was shown to have a spike that tended towards a delta function as ω decreased. For relatively small values of ω, the spike can be regarded as an approximation of an impulse forcing. It is well known that when the ACF takes this form, the cross-correlation function (CCF) of the output with the input may be treated as an estimate of the impulse response (Godfrey 1969b; Hill and McMurtry 1964).

The CCF is formally defined as:

$$CCF_{ab}(TD) = (1/T) \int_0^T x(t) \cdot y(t + TD)\, dt \qquad (7.2)$$

where $x(t)$ and $y(t)$ are two signals separated by a time delay, TD. Figure 7.5 (overleaf) shows a typical CCF as might be derived from the $\dot{V}O_2$ response to a 63-unit (5 s·unit^{-1}) PRBS WR forcing.

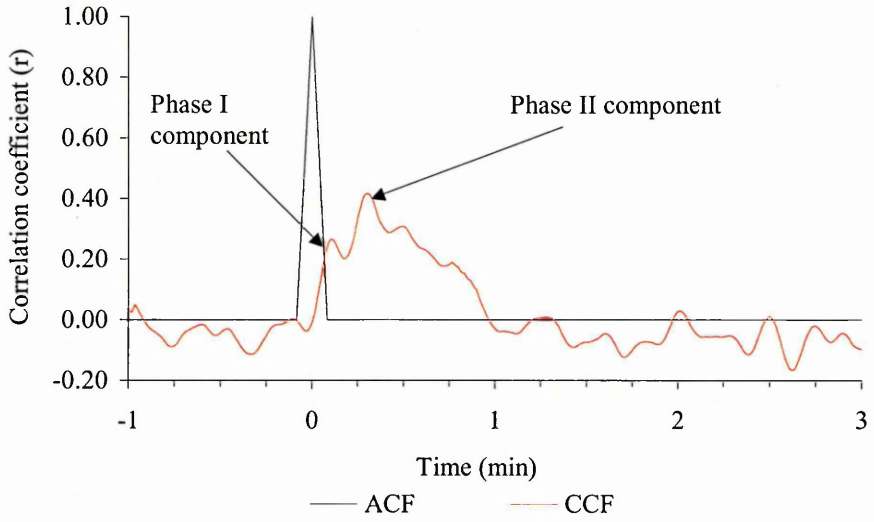


Figure 7.5. Auto-correlation function (ACF) of a 63-unit (5 second·unit^{-1}) pseudorandom binary sequence work rate forcing with corresponding cross-correlation function (CCF) of oxygen uptake with work rate. Data for the figure were collected during tests undertaken as part of this thesis. The data are unpublished.

The shape of the CCF shown in Figure 7.5 is characteristic of the response pattern obtained from a true impulse forcing (Bakker et al. 1980; Hughson et al. 1988; Sherrill et al. 1983), that is, an initial abrupt component followed, after a short delay, by a second component. If the system under study shows evidence of linear behaviour, then the response can be quantified by fitting mathematical models, typical of those used to describe impulse responses (see Chapter 3), to the CCF data.

Although for relatively small values of ω the spike of an ACF can be observed to approximate an impulse, for larger values of ω the spike tends towards a much broader triangular function (Figure 7.6 overleaf).

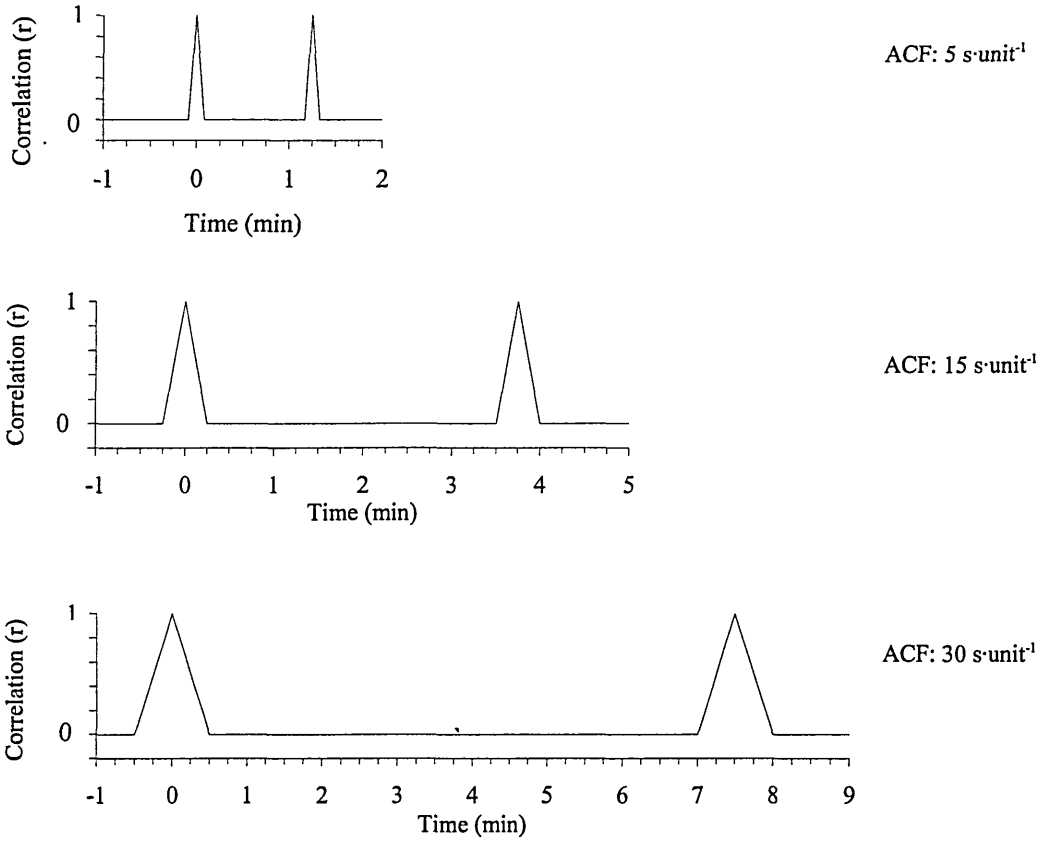


Figure 7.6. Auto-correlation functions of three, 15-unit pseudorandom binary sequence signals with unit durations of 5, 15 and 30 s.

Clearly, the ACFs relating to the 15 and 30 s·unit^{-1} PRBSs cannot be considered to approximate impulses. Instead, this form of the ACF may be viewed as the equivalent of a ramp increase followed by a ramp decrease (Hughson et al. 1991a). Therefore, the CCF data can be fitted with a model comprising the linear summation of three, two-component exponential equations of the form used to characterise the response to ramp inputs (Hughson et al. 1991a; Swanson and Hughson 1988). Since a ramp is the integral of a step, the two-component form of a single ramp model, as derived from Equation 3.2, is as follows:

$$Y_r(t) = A_0 + \{A_1 \cdot [t - TD_1 - \tau_1 \cdot (1 - e^{-(t - TD_1)/\tau_1})] \cdot u(t - TD_1)\} + \{A_2 \cdot [t - TD_2 - \tau_2 \cdot (1 - e^{-(t - TD_2)/\tau_2})] \cdot u(t - TD_2)\} \quad (7.3)$$

where A_0 is the baseline, A_1 and A_2 are the amplitude terms and TD_n and τ_n are the time delay and time constant for each component, respectively. The function u(t - TD_n) acts to turn on each component when t - $TD_n \geq 0$ (Hughson et al. 1991a). The linear summation is conducted as follows:

$$Y_{CCF}(t) = Y_0 + Y_{r1}(t) \cdot u(t - \omega) - 2\, Y_{r2}(t) \cdot u(t) + Y_{r3}(t) \cdot u(t + \omega) \qquad (7.4)$$

where $Y_m(t)$ is obtained from Equation 4.18 such that A_1, A_2, TD_1, TD_2, τ_1 and τ_2 are common to each of the equations. The functions u(t + ω), u(t) and u(t - ω) act to turn on each term when u(t + ω), u(t) and u(t - ω) ≥ 0 (Hughson et al. 1991a). The summation is depicted schematically in Figure 7.7.

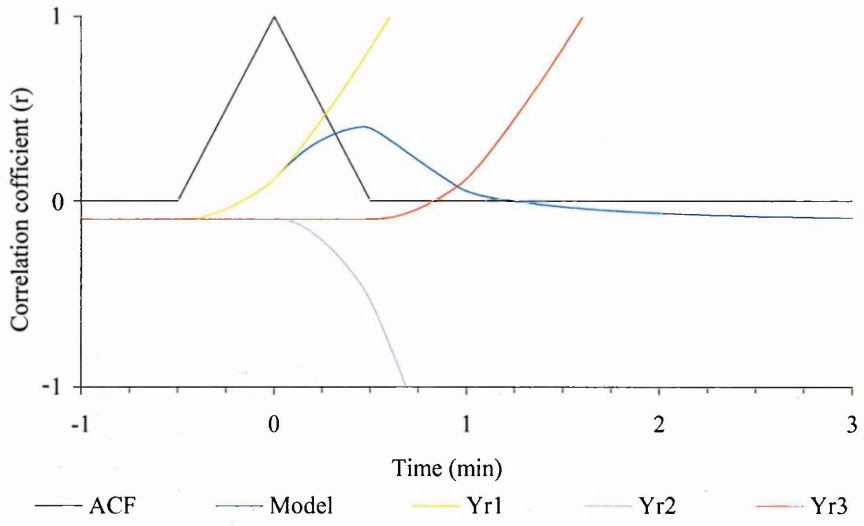


Figure 7.7. Model composed from the linear summation of three ramp components Yr1, Yr2, and Yr3, shown with corresponding auto-correlation function (ACF).

The potential of the model to fit the CCF data resulting from the $\dot{V}O_2$ responses to a 15-unit (30 s·unit^{-1}) PRBS WR forcing is shown in Figure 7.8 (overleaf).

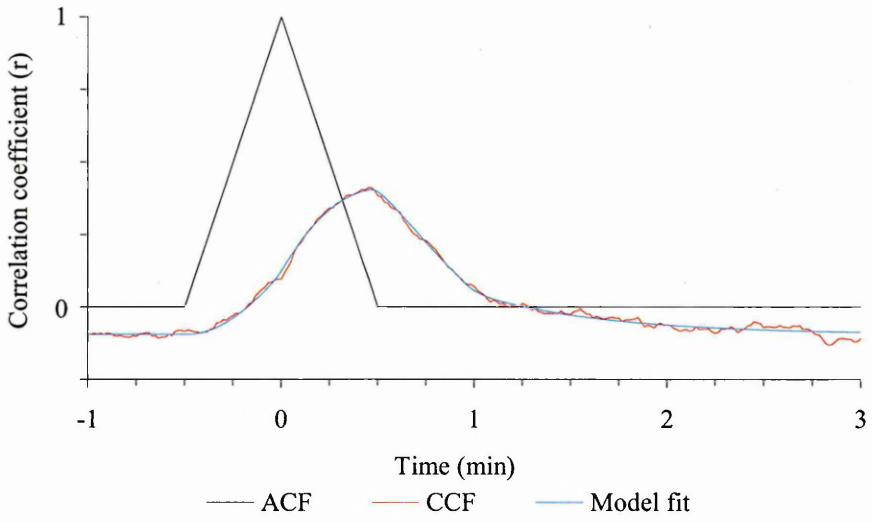


Figure 7.8. Cross-correlation function for data obtained from a 15-unit (30 second·unit^{-1}) pseudorandom binary sequence work rate forcing fitted by the ramp model of Hughson et al. (1991a). The figure includes the corresponding autocorrelation function and best fit parameter values. Data for the figure were collected during tests undertaken as part of this thesis. The data are unpublished.

Hughson et al. (1991) have shown that the three-part ramp model can also provide a satisfactory fit to response data that would have been normally described by impulse models.

7.4 SUMMARISING THE DYNAMIC RESPONSE IN OXYGEN UPTAKE TO PSEUDORANDOM BINARY SEQUENCE WORK RATE FORCINGS

The kinetic parameters that describe the $\dot{V}O_2$ response to PRBS WR forcings provide information that allows comparison between conditions and individuals. Hughson et al. (1991a) have shown that it is possible to summarise this information by determining the TLT (Equation 3.4). Assuming the principle of linearity, estimates of the TLT can be calculated using the parameters obtained from the time domain analysis of $\dot{V}O_2$ responses to PRBS forcings.

8.0 AN INVESTIGATION OF BETWEEN TRIALS VARIABILITY IN ESTIMATES OF OXYGEN UPTAKE KINETICS

8.1 INTRODUCTION

Previously, step (Whipp et al. 1982), impulse (Bakker et al. 1980), ramp (Whipp et al. 1981) and sinusoidal (Casaburi et al. 1977) forcing functions have been used to study $\dot{V}O_2$ kinetics. More recently, the PRBS WR forcing has provided an alternative method of analysing the dynamic response in $\dot{V}O_2$ (Hoffmann et al. 1991; Hughson et al. 1990d; Stegemann 1992; Xing et al. 1991).

When examining patients, young subjects or individuals with low physical fitness, it can prove necessary to reduce the upper work limit of a PRBS forcing. This action causes a reduction in the response-to-noise ratio. Should the ratio fall below a critical level, then it may not be possible to elicit discernible responses from the forcing (Eßfeld et al. 1987). One solution would be to perturb WR according to a multifrequent signal tailored to enhance identification of the underlying response (Bakker et al. 1980; Eßfeld et al. 1987). If it is possible to develop such a signal, then the kinetic data derived from the resulting WR forcing would need to be validated. To achieve this, the data could be compared with the output obtained from a PRBS WR forcing.

In clinical or physiological examination, assessment methods often have large measurement errors associated with them (Bland and Altman 1995). Consequently, when comparing the data from two methods it is necessary to consider the amount of variability between trials. If the established method against which comparisons are made has poor repeatability, then even a new method that is perfect will not agree well with it (Bland and Altman 1995).

Although variability in both maximal and submaximal $\dot{V}O_2$ has been extensively investigated (Cunningham et al. 1977; Daniels et al. 1984; Katch et al. 1982;

Nordrehaug et al. 1988; Williams et al. 1991), only a small number of repeatability studies can be found that have considered variability in estimates of $\dot{V}O_2$ kinetics (Claxton et al. 1996; Hughson and Inman 1986b; Whipp et al. 1981). Of these, only Claxton et al. (1996) have considered variability in the kinetic parameters of $\dot{V}O_2$ obtained from PRBS WR forcings. The parameters recorded by Claxton and his colleagues were noted to be prone to considerable variability between trials.

In the study of Claxton et al. (1996), the degree of variability in kinetic parameter estimates was shown to be mildly dependent on the frequency of the input harmonic. The bandwidth of the PRBS signal employed by Claxton and his colleagues comprised harmonics in the frequency range 0.0032 to 0.0857 Hz. As the main experiment in this thesis is concerned with the dynamic $\dot{V}O_2$ response to a different range of frequencies, the repeatability of kinetic parameters may differ from that observed by Claxton et al. (1996). Accordingly, the objective of this experiment was to examine and quantify the between trials variability in estimates of $\dot{V}O_2$ kinetics obtained from two PRBS WR forcings. The chosen forcings comprised harmonics in the required frequency range, that is, from 0.0022 to 0.0133 Hz.

8.2 METHODS

Subjects. Seventeen healthy subjects (9 male and 8 female) gave their informed consent to participate in this experiment. The subjects were drawn from a population of physical education students, regularly involved in physical activity. Anthropometric data are presented in Table 8.1 (overleaf). Further information concerning each subject appears in Appendix 2

TABLE 8.1. Subject data: mean value (SD).

Group	*n*	Age (yr)	Height (cm)	Body mass (kg)
All	17	22.1 (1.0)	172.4 (7.1)	71.5 (9.2)
Female	8	22.0 (0.9)	166.6 (4.2)	65.1 (4.6)
Male	9	22.1 (1.1)	178.2 (3.7)	80.0 (6.6)

Exercise protocol. The experiment was carried out using two identical exercise tests (P1 and P2). The exercise protocol consisted of three consecutive cycles of a 15-unit (30 s·unit^{-1}) PRBS. The bandwidth of this PRBS comprises harmonics in the frequency range 0.0022 to 0.0133 Hz. Tests were preceded by a 4 min warm-up period composed from the last 4 min of a single PRBS cycle. As the length of each PRBS equalled 7.5 min, this gave a total test duration of 26.5 min. Throughout the test, the WR was automatically switched between 25 and 85 W. Figure 8.1 provides a graphic representation of the WR protocol. The timing of each switch was the same in both P1 and P2.

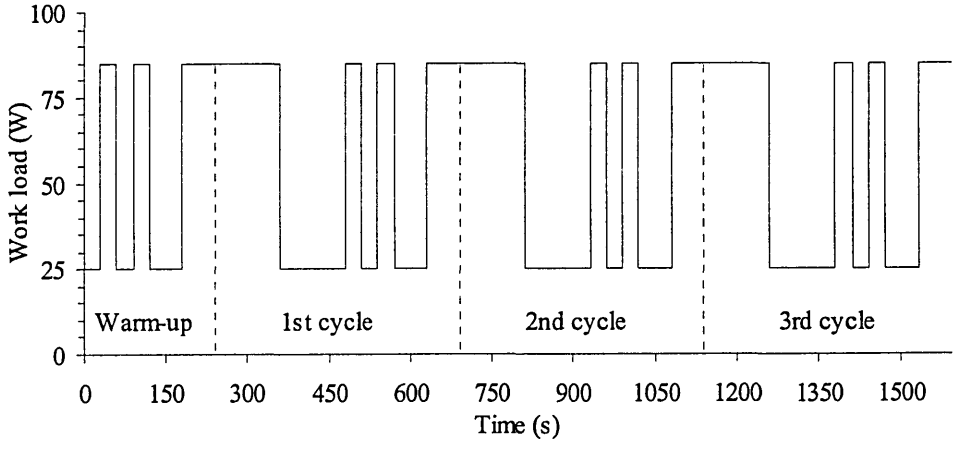


Figure 8.1. Graphical representation of the pseudorandom binary sequence work rate protocol used in the experiment.

To allow for sufficient recovery between tests and to minimise the influence of circadian fluctuations, testing was performed at the same time of day on two consecutive days. Every effort was made to maintain the same experimental conditions

throughout testing. Over the duration of the experiment, the maximum difference in ambient temperature experienced by any of the subjects was ±2°C.

Data collection. $\dot{V}O_2$ was measured on a breath-by-breath basis using a respiratory mass spectrometer. Pre-test calibrations were performed on the mass spectrometer using high accuracy gases (tolerance ±0.3%) of known composition.

Data analysis. Breath-by-breath data from the three complete PRBS cycles were averaged and analysed in the frequency domain using Fourier methods. To further reduce the magnitude of breath-to-breath noise, the PSD of the input and the CPSD of the input and output were used to calculate frequency response parameters (Eßfeld et al. 1987; Hoffmann et al. 1992). The analysis yielded α and φ parameters for the relationship between WR and $\dot{V}O_2$ at each harmonic of the PRBS input. Individual α and φ parameters were used to determine data points on a Nyquist plot. The data points were fitted with a non-rational (four-parameter transfer function) model. Fitting of the model returned τ and *TD* parameters. Parameter values were used to calculate an estimate of the MRT of $\dot{V}O_2$.

Statistical analysis. Quantification of the between trials variability in estimates of the MRT was performed by calculating the 95% limits of agreement (mean difference in MRT between P1 and P2 ± the standard deviation of the differences in MRT between P1 and P2 multiplied by 1.96).

In addition to quantifying between trials variability, ANOVA was used to identify any significant differences between the τ, *TD* and MRT obtained from the two tests.

8.3 RESULTS

The mean WR of the PRBS test was 57.0 W and the mean values for $\dot{V}O_2$ prior to computation of the PSD and CPSD were 1052.7 (98.4) and 1065.0 (118.4) ml·min^{-1} for P1 and P2, respectively.

The mean $\dot{V}O_2$ response to the 3 PRBS cycles comprising P1 is shown in Figure 8.2 for *subject A*.

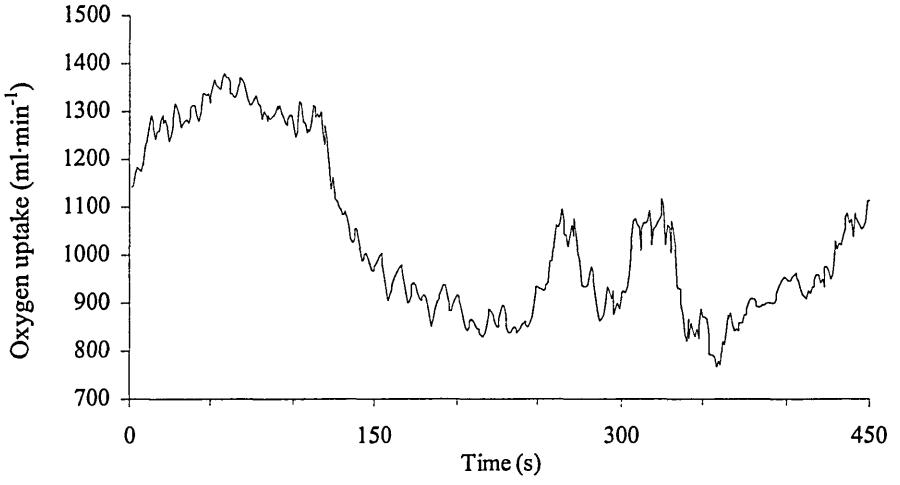


Figure 8.2. The dynamic response in oxygen uptake for *subject A* resulting from the first of two identical pseudorandom binary sequence work rate forcings. Response data are the average of three consecutive cycles.

The comparative mean values of α and φ parameters for P1 and P2 following determination of the PSD and CPSD are shown in Table 8.2 (overleaf). The table comprises data from the bandwidth of the PRBS, that is, from the fundamental to the sixth harmonic.

For each subject, α and φ parameters derived from the two PRBS tests were used to determine data points in a Nyquist plot (Figure 8.3 overleaf). Included in Figure 8.3 are the corresponding best fits to the data of the non-rational model used in the experiment.

Table 8.2. Comparison of amplitude ratio and phase shift parameters from the fundamental to the sixth harmonic for the relationship between work rate input and oxygen uptake output. Values are presented as mean (standard deviation) of the 17 subjects and follow computation of the power spectral density and the cross-power spectral density.

Harmonic no.	Frequency (Hz)	Amplitude Ratio (arbitrary units)		Phase shift (degrees)	
		P1	P2	P1	P2
1	0.0022	0.43 (0.04)	0.43 (0.06)	-28.6 (4.5)	-28.9 (4.3)
2	0.0044	0.36 (0.04)	0.34 (0.05)	-54.4 (5.4)	-54.1 (7.5)
3	0.0067	0.27 (0.04)	0.25 (0.04)	-70.7 (14.6)	-78.1 (9.7)
4	0.0089	0.24 (0.05)	0.23 (0.06)	-90.6 (13.7)	-90.8 (19.3)
5	0.0111	0.14 (0.05)	0.14 (0.04)	-113.0 (20.2)	-107.4 (24.8)
6	0.0133	0.12 (0.03)	0.10 (0.04)	-111.5 (13.6)	-122.0 (20.0)

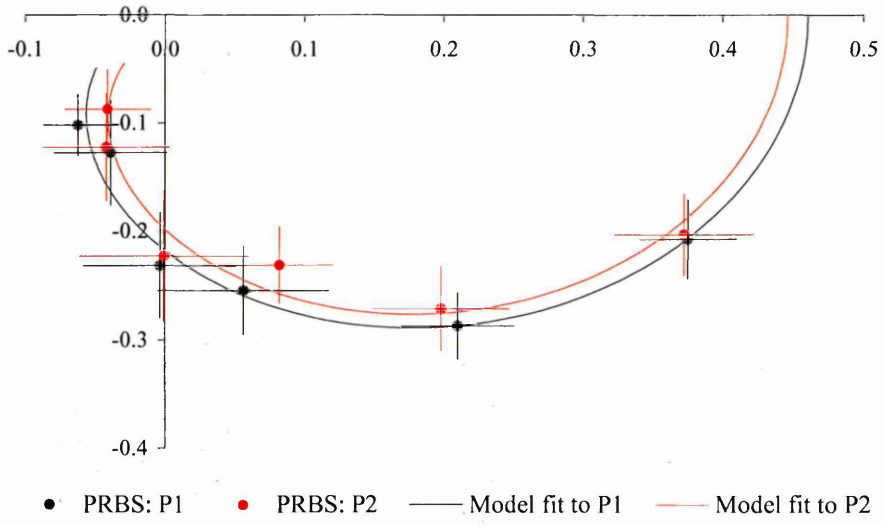


Figure 8.3. Nyquist plot illustrating the data points determined from the mean (standard deviation) amplitude ratio and phase shift parameters computed from the first (P1) and second (P2) pseudorandom binary sequence work rate forcings (number of subjects = 17). Included in the figure are the corresponding best fits to the data of the non-rational model employed in the experiment. Error bars depict standard deviation.

Mean parameter values resulting from the fitting of mathematical models to individual data are shown in Table 8.3. Included in the table are the calculated MRTs of $\dot{V}O_2$ for P1 and P2.

Table 8.3. Comparison of mean (standard deviation) parameter values derived from the first (P1) and second (P2) pseudorandom binary sequence tests (number of subjects = 17). The data are the result of the best fits to the response data of a non-rational (four-parameter transfer function) model. Included in the table is the calculated mean response time (MRT) of oxygen uptake.

Test no.	C_1	C_2	Tau, τ (s)	Time Delay, TD (s)	MRT, $\tau + TD$ (s)
P1	0.21 (0.07)	0.25 (0.06)	25.8 (5.8)	23.1 (5.0)	48.9 (6.5)
P2	0.24 (0.06)	0.21 (0.05)	25.3 (7.5)	25.3 (4.4)	50.7 (6.5)

To examine and quantify the between trials variability in estimates of the MRT, the 95% limits of agreement were calculated. Although the mean difference between the trials was small (-1.8 s), the range of values comprising the limits remained wide (-11.6 to 8.0 s). Of the ~5% of individual differences that fell outside of the limits, no difference exceeded the range by more than 2.2 s.

The ANOVA did not identify any statistically significant differences between estimates of τ, TD and the MRT of $\dot{V}O_2$ obtained from the two tests (P = 0.19, 0.86 and 0.44, respectively).

8.4 Discussion

The primary aim of this thesis is to investigate the development of a new signal for perturbing WR during tests of $\dot{V}O_2$ kinetics in subjects with a limited tolerance of exercise. If investigations reveal that a new signal can be developed, then its potential to provide valid assessments of $\dot{V}O_2$ kinetics would need to be evaluated. As it is not

possible to make comparisons with exact physiological measures, it is proposed that data are compared with the output from an established PRBS WR forcing.

When comparing data from different methods, problems arise if the degree of repeatability is not known. Should one method exhibit poor repeatability, then it may never agree well with the other method (Bland and Altman 1995). Consequently, prior to commencing investigations, the present experiment sought to determine the between trials variability in estimates of $\dot{V}O_2$ kinetics obtained from the PRBS method.

To assess variability in estimates of $\dot{V}O_2$ kinetics, repeated measurements were taken from a series of subjects. For each test, a description of the dynamic response in $\dot{V}O_2$ was provided by fitting response data with a non-rational mathematical model. In work by Sherrill and Swanson (1981), an identical model was used to evaluate data obtained from the study of Bakker et al. (1980). The values reported by Sherrill and Swanson for τ and *TD* are very similar to those recorded in the present experiment.

A summary of individual response data was provided by calculating an estimate of the MRT of $\dot{V}O_2$. Variability in the MRT was quantified using the 95% limits of agreement. The limits comprise a range of values that include approximately 95% of the individual differences between measurements obtained in the two tests. The results show that, for any new subject drawn from a similar population, the MRT can be expected to vary by up to ~10 s between tests, with variation being equally likely in either direction. Given this knowledge, it becomes possible to establish whether a new method will provide the same determination of $\dot{V}O_2$ kinetics as the PRBS method. That is, if the difference between the MRT obtained using the new method and the PRBS method is less than or equal to 10 s, then the two methods are likely to be interchangeable. Such supposition would necessitate that the between trials variability

in the MRT obtained from a new method is no greater than that observed in the present experiment.

To establish the validity of estimates of $\dot{V}O_2$ kinetics obtained using a new method, it would be necessary to make direct comparisons with estimates derived from the PRBS method. Given the wide limits of agreement observed in the present experiment, this would not appear feasible. A possible solution would be to seek a reduction in the magnitude of the limits. This would necessitate that the main sources of variability between trials are identified. From previous investigations (Bland 1995; Katch et al. 1982; Sale 1991) it is known that repeatability in physiological measures is influenced by both biological variation and technological variation. Biological variation relates to the relative consistency with which an individual can perform, whilst technological variation relates to the way in which a test is conducted (Sale 1991). Although every care was taken to minimise the contribution to variability of technological variation, very little could be done to account for changing environmental influences and fluctuations in the accuracy and stability of the equipment. However, evidence derived from work by Katch et al. (1982) would suggest that the contribution to overall variability of technological variation is liable to be small.

If technological variation has only a limited influence on variability, then minimising biological variation may help to reduce the limits of agreement. In studies by Morgan et al. (1991) and Williams et al. (1991), it was suggested that biological variation was primarily dependent on circadian fluctuations, fatigue and habituation. Whilst attempts were made to minimise the influence of circadian fluctuations and fatigue during the present experiment, it was not known whether repeatability of the MRT might vary according to any learning or training effect of the repeat testing (Henry 1959). To investigate this possibility, the mean difference between estimates of the MRT obtained in the two trials was examined. The mean difference reflects the average bias of the

values obtained in the first trial relative to those obtained in the second. Large mean differences would be indicative of undesirable trends that might result from, for example, procedural accommodation. Should such trends be identified, their effect on variability could be subtracted out (Katch et al. 1982). Following inspection of the results, no substantive evidence of any trends could be detected in the MRT. Nevertheless, as only two tests were employed in the experiment, any progressive trend may have been obscured. Therefore, using data from a previous study by this laboratory (Cooke et al. 1995, unpublished), a more detailed examination was performed of the repeatability of estimates of the MRT in four subjects. The subjects were from a similar population to that of the current experiment and had not been involved in any previous testing. Each subject completed four identical PRBS tests on four separate days. The protocol used in the study was the same as the one used in the present experiment. An ANOVA revealed no statistically significant difference in the MRT derived from the four tests. Further examination of both individual and mean differences between the results of each test did not identify any particular upward or downward trends. These results were taken to infer that variability in the present experiment was not subject to the influence of habituation.

Given that habituation was unlikely to have had an effect on estimates of the MRT, it is possible that substantial levels of individual variability may have been cause of the poor repeatability (Daniels et al. 1984; Morgan et al. 1987; Nordrehaug et al. 1991). As the limits of agreement used in the present experiment represent overall variability between trials, the influence of large individual variations may go undetected. Since the greatest individual variability would necessarily be included in the ~5% of differences that lie outside the range of the limits, these values were identified and submitted to a separate examination. The examination revealed that the range of values specified by the limits was not exceeded by more than 2.2 s. Thus, the limits of agreement would appear to be

a fair reflection of both individual and overall variability between trials during PRBS tests.

One other possible source of variation was identified by Claxton et al. (1996). The data reported by these authors indicated a mild trend towards wider limits of agreement at those harmonics with the highest frequencies. In investigations by Hoffmann et al. (1994b), the effect of breath-to-breath noise on $\dot{V}O_2$ kinetics was shown to rise with increasing frequency of input. Thus, breath-to-breath noise may have an adverse effect on the magnitude of variability between trials. In the present experiment, noise reduction was achieved by averaging the responses from three consecutive PRBS WR forcings and by employing the PSD and CPSD in the calculation of frequency response parameters (Eßfeld et al. 1987; Hoffmann et al. 1992). In accordance with the work of Lamarra et al. (1987), an increase in the number of sequences from which data are averaged may help to further de-emphasise random fluctuations. Unfortunately, this would add greatly to the duration of a test. Therefore, unless another approach to reducing noise and hopefully variability can be found, it may have to be accepted that information from the PRBS method will only allow a general determination of the validity of data derived from a new method. Consequently, should this thesis reveal that a new method can be developed to assess $\dot{V}O_2$ kinetics, it may prove necessary to determine the validity of response data by comparing it with those data obtained from another established assessment method. Until such investigations have been completed, care would need to be taken when using response data derived from the new method to make inferences of a physiological nature.

8.5 Conclusion

The primary objective of this experiment was to assess the between trials variability in estimates of $\dot{V}O_2$ kinetics obtained from two identical PRBS tests. The wide limits of

agreement were indicative of the poor repeatability of the kinetic parameters derived from testing. Evidence from other studies suggests that that the primary cause of poor repeatability may be breath-to-breath noise. Although the wide limits do not restrict comparisons with data derived from any new method, unless variability between trials can be reduced, accurate assessments of validity using PRBS data will not be possible.

8.6 EXPERIMENTAL SHORTCOMINGS

During the composition of this thesis, every effort was made to augment understanding of the techniques employed in multifrequent testing and analysis. This course of action was undertaken to ensure that data presented in the thesis were both accurate and valid. As part of the process, the computer software supplied for data analysis and protocol generation was subject to detailed investigation. The investigation revealed a flaw in the algorithm used to generate PRBS protocols. As a result, protocols produced using the software did not possess the properties of a true PRBS. Crucially, the power distribution over the bandwidth of the resulting sequences was relatively uneven, with the main concentration apportioned to the first two harmonics. The flaw was also evident in the ACF of the PRBS. Rather than the definite form described in Figure 4.2, that is, a spike at 0, T, $2T$, ... and a negative bias between spikes of $1/Z$, each spike in the ACF was separated by a series of small fluctuations. As similar characteristics can be seen in work published by Hughson et al. (1991a) (Figures 4 to 6) and Kowalchuk and Hughson (1990) (Figures 4 and 5), it may be that the PRBS generated by the algorithm would not have had a detrimental influence on resulting data. Certainly, given that the values reported by Sherrill and Swanson (1981) for τ and TD are very similar to those recorded in the present experiment, it would appear that the power in the remaining harmonics comprising the bandwidth was sufficient to elicit valid responses in $\dot{V}O_2$.

Nevertheless, this assumption cannot be confirmed without comparative data from an experiment using true PRBS WR forcings.

Due to the time-consuming nature of the investigation into the software, the flaw in the algorithm was only discovered after the completion of the experiment presented in this chapter. The possibility therefore exists that the resulting experimental data are invalid. Accordingly, the findings outlined above can only be considered to represent the probable degree of variability in estimates of $\dot{V}O_2$ kinetics derived from PRBS exercise tests.

9.0 THE ANALYSIS OF OXYGEN UPTAKE KINETICS USING A MODIFIED MULTIFREQUENT WORK RATE FORCING

9.1 INTRODUCTION

In recent years, assessments of $\dot{V}O_2$ kinetics have been shown to provide a suitable means of evaluating the effects of various experimental treatments and training strategies (Hagberg et al. 1980; Hughson 1984a and 1989; Hughson and Inman 1986a; Palange et al. 1995; Phillips et al. 1995; Stegemann et al. 1985). Although the changes that occur as a result of these interventions are most commonly quantified using step WR forcings, the same information can be extracted using a suitably chosen PRBS WR forcing (Hughson et al. 1991a). PRBS tests have the advantage of being able to yield the necessary information in a single session of ~30 min or less duration (Hughson 1990c). The minimal imposition experienced by subjects during PRBS tests means that the approach is ideal for clinical assessment.

When examining patients, young subjects or individuals with low physical fitness, it can be necessary to reduce the upper work limit of a PRBS forcing. This undertaking not only lessens the discomfort of testing, but also helps to minimise the possibility of the subject exceeding the LAT. Due to the pre-determined nature of a PRBS, however, any reduction in the upper work limit will cause a fall in the distribution of power across the bandwidth of the sequence. If the distribution of power falls below a critical level, then the effect on the response-to-noise ratio may make it difficult to elicit discernible responses from the forcing (Eßfeld et al. 1987). In this situation, valid estimates of $\dot{V}O_2$ kinetics will become impossible to obtain (Cooper et al. 1992).

An alternative approach to determining $\dot{V}O_2$ response kinetics would be to perturb WR according to a multifrequent input signal optimised with regard to the number of frequencies tested, the power distribution over these frequencies and the test duration

(Eßfeld et al. 1987). This form of signal could help to enhance the response-to-noise ratio, raising confidence in kinetic parameter estimates (Bakker et al. 1980).

Although modified multifrequent signals are prevalent in the field of engineering (see Kerlin 1974), they have not been employed in the assessment of $\dot{V}O_2$ kinetics. Accordingly, the objective of this experiment will be to investigate the potential for developing a multifrequent WR forcing specially adapted for evaluating $\dot{V}O_2$ kinetics in subjects with a reduced level of exercise tolerance. To assess the validity of kinetic parameters derived from the adapted forcing, comparisons will be made with the response data obtained from a comparable PRBS WR forcing.

9.2 METHODS

Subjects. Sixteen healthy subjects (10 male and 6 female) gave their informed consent to participate in this experiment. The subjects were drawn from a population of physical education students, regularly involved in physical activity. As a result of a lactate analysis (see *lactate sampling* below), the data obtained from two subjects were excluded from analysis. Anthropometric data for the remaining 14 subjects are presented in Table 9.1. Further information concerning each subject appears in Appendix 2

TABLE 9.1. Subject data: mean value (SD).

Group	*n*	Age (yr)	Height (cm)	Body mass (kg)
All	14	22.9 (1.9)	172.6 (6.7)	74.3 (10.3)
Female	4	22.3 (1.0)	169.3 (5.6)	65.3 (6.9)
Male	10	23.3 (2.3)	175.8 (1.4)	79.8 (8.4)

Exercise protocol. For the purposes of method comparison, each subject completed a single PRBS exercise protocol. The protocol consisted of three consecutive cycles of a 15-unit (30 s·unit^{-1}) PRBS. The bandwidth of this PRBS comprises harmonics in the frequency range 0.0022 to 0.0133 Hz. To ensure compliance with the properties of a

true PRBS, the sequence was generated using manual techniques. The exercise protocol included a warm-up period composed from the last 4 min of a single PRBS cycle. This gave a total protocol length of 26.5 min. Throughout each test, the WR was automatically switched between 25 and 85 W. Figure 9.1 shows a graphical representation of the protocol.

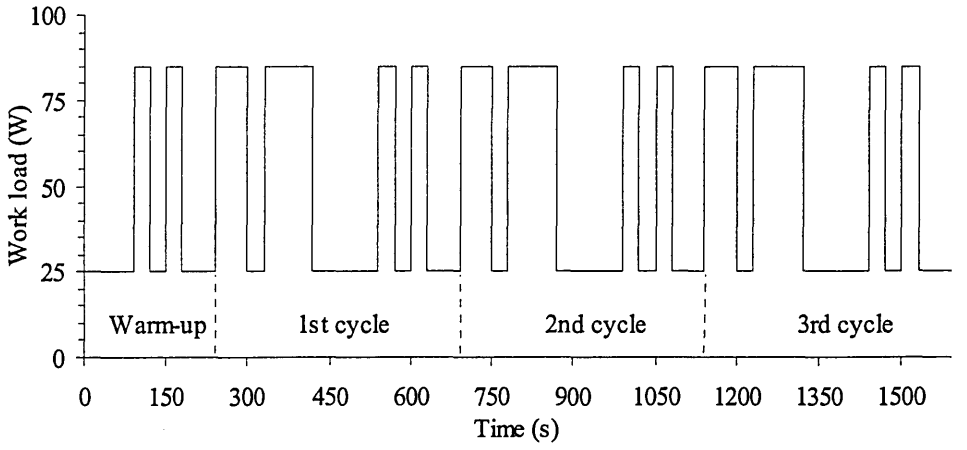


Figure 9.1. Graphical representation of the pseudorandom binary sequence work rate protocol used in the experiment.

To evaluate the potential for developing a multifrequent WR forcing for use with subjects in whom exercise intolerance is a feature, an optimisation routine was used to construct a multifrequent signal adapted to enhance identification of the underlying $\dot{V}O_2$ response. The method of construction requires that several factors be taken into consideration. These factors include frequency range of interest, power distribution, lower and upper work limit settings, harmonic phase angle and switching frequency.

To facilitate method comparison, the frequency characteristics of the harmonics used in the construction of the multifrequent signal were chosen so that they matched those of the 15-unit (30 s·unit^{-1}) PRBS. Thus, the frequency range of interest was from 0.0022 to 0.0133 Hz. It has been reported that this range will elicit a good description of the *phase II* response in $\dot{V}O_2$ (Hughson 1991a; Hughson et al. 1990b).

The extent to which the available signal power can be distributed across the frequency range of interest is dependent on the magnitude of the signal, the percentage of signal energy in those harmonics outside the range of interest and the number of harmonics over which the power must be distributed. For a 15-unit PRBS with lower and upper work limits of 25 and 85 W, respectively, the power distribution is such that the harmonics comprising the bandwidth have amplitudes ranging from 15.9 W at the fundamental harmonic to 12.1 W at the sixth harmonic (mean 14.3 W). The aim of the present experiment is to formulate a multifrequent signal that assists identification of the underlying $\dot{V}O_2$ response. In investigations by Hoffmann et al. (1994b), it was noted that the influence of breath-to-breath noise on $\dot{V}O_2$ responses increased with the frequency of input. To generate a signal that enhances the response-to-noise ratio at higher frequencies, it would be necessary to employ a power distribution that is the inverse of that observed across the bandwidth of a PRBS. The additional power required to achieve this form of distribution was acquired from the fifth harmonic in the frequency range of interest. Since the frequency of the fifth harmonic (0.0111 Hz) is similar to that of the fourth (0.0089 Hz) and sixth (0.0133 Hz) harmonics, it was speculated that the dynamic response characteristics of $\dot{V}O_2$ at these harmonics would also be similar. Therefore, exclusion of information from the fifth harmonic should have little influence on estimates of the overall system response in $\dot{V}O_2$.

For the purposes of comparison, the limits chosen for the lower and upper work settings of the multifrequent forcing were 25 and 85 W, respectively. As a starting point, the amplitudes of the fundamental to the sixth harmonics were set at 16.0, 16.5, 17.0, 17.5, 0.0 and 18.5 W, respectively (mean 14.3 W). This distribution of power across the frequency range of interest is displayed in the form of an amplitude spectrum in Figure 9.2 (overleaf). Included in the figure is the amplitude spectrum of the 15-unit (30 s·unit^{-1}) PRBS for the equivalent range of frequencies.

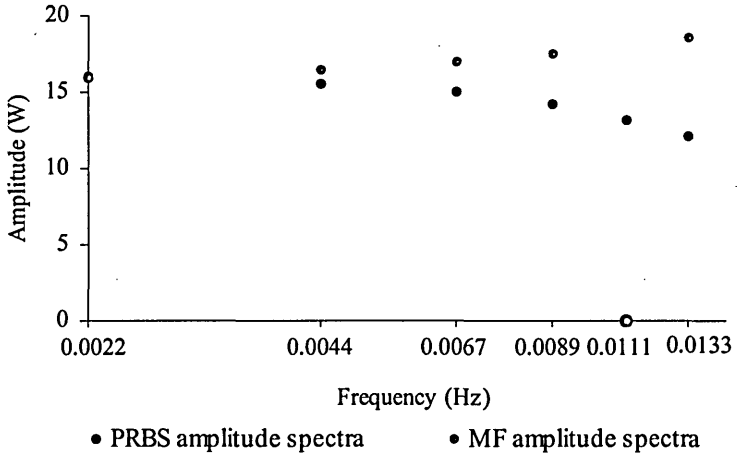


Figure 9.2. Amplitude spectra of a proposed multifrequent (MF) work rate forcing and a standard 15-unit (30 second·unit^{-1}) pseudorandom binary sequence (PRBS) work rate forcing. The frequencies shown encompass the range of interest of the MF forcing and the bandwidth of the PRBS forcing. The lower and upper work limits chosen for each forcing were 25 and 85 watts, respectively

Signal construction was accomplished by adding together the chosen harmonic components according to the following formula:

$$f(\mathrm{t}) = A_0 + \sum_{k=1}^{\infty} A_k \sin(k\theta \mathrm{t} + \phi_k) \qquad (9.1)$$

where A_o is a steady level to which the harmonic terms are added, $\theta = 2\pi/T$, k is the number of the harmonic, A its amplitude (mid-point to peak), ϕ its relative phase angle and T its period in seconds. This formula was originally presented and described in Chapter 4 of this thesis (see Equation 4.6). The outcome of the summation with $A_o = 55$ W and $\phi_k = 0°$ is shown in Figure 9.3 (overleaf).

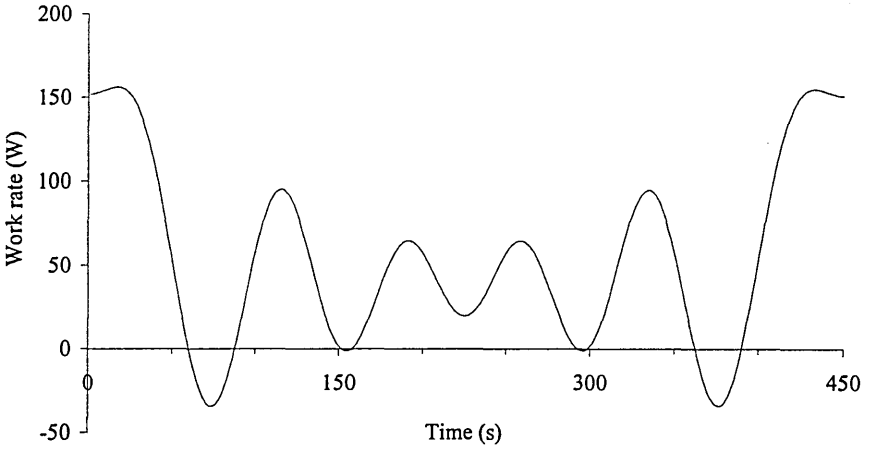


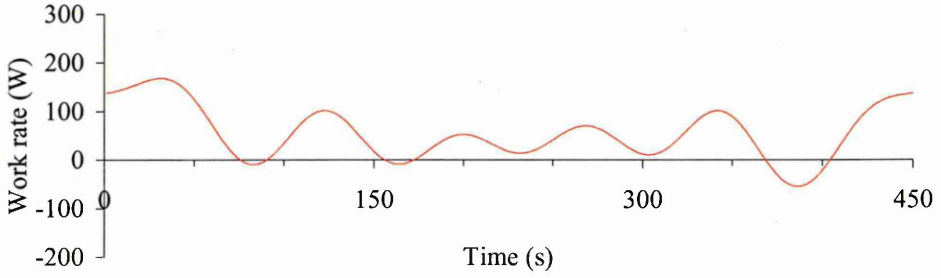
Figure 9.3. Signal generated by summing five harmonics with amplitudes of 16.0, 16.5, 17.0, 17.5 and 18.5 watt and 0 degree relative phase angle. The harmonics are added to a steady level of 55 watt. The summation was accomplished using the formula presented in Equation 9.1.

As a first step to curtailing the lower and upper limits of the multifrequent signal shown in Figure 8.3, the data and resultant signal were displayed graphically on a computer screen. Estimates of A_o and ϕ_k were then entered until a reasonable reduction in signal magnitude had been achieved. Using the chosen power distribution and estimated A_o and ϕ_k as starting values, an optimisation routine was employed to generate a signal that comprised the desired power distribution without exceeding acceptable limits. The routine involved examining each of the 450 data points (time interval = 1 s) comprising the signal to identify values that exceeded the pre-determined lower and upper limits. Any values less than 25 W or greater than 85 W were replaced with values of 25 W or 85 W, respectively. The amended signal data were then subjected to a Fourier analysis. From the resultant harmonic information, the distribution of power from the fundamental to the sixth harmonic was recorded and compared with the desired distribution of 16.0, 16.5, 17.0, 17.5, 0.0 and 18.5 W, respectively. If the power at any of the harmonics was above or below that desired, then the harmonic data were altered in a compensatory manner. For example, if the power at the second harmonic was less than 16.5 W, then A_2 would be replaced by a value greater than 16.5 W. The method

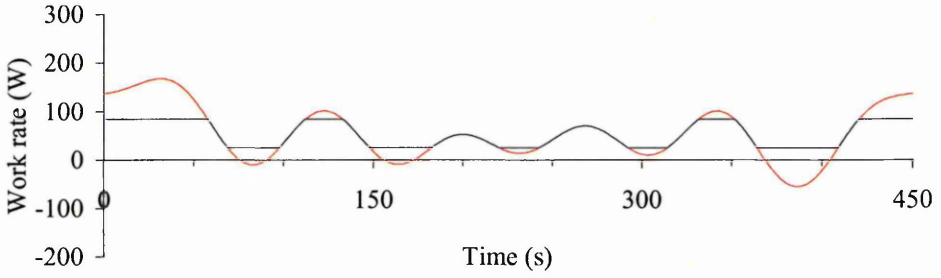
demanded the use of replacement values considerably greater than or less than the original value. No changes were made in ϕ_k. Using the amended harmonic data, the process of signal break down was then reversed and a second signal constructed from the formula described in Equation 9.1. Only amplitude and phase angle data from the fundamental to the fiftieth harmonic were used in the construction of the second signal. The data comprising the signal were then inspected, and values exceeding the pre-determined lower and upper limits replaced as described previously. The new signal was then subjected to a Fourier analysis, and the resulting power distribution compared with the desired distribution. Unless a close approximation to the desired distribution was achieved, the process of signal construction and breakdown was repeated. Generation of a suitable signal by this approach required that the process was repeated ~40 times. The optimisation routine described above is depicted in Figure 9.4 (overleaf), steps (a) to (e).

To ensure that the interval between WR transitions did not fall below that of an expected breathing frequency, signals generated by the optimisation routine with intervals less than 10 s were rejected. New starting signals were constructed using the same desired power distribution (16.0, 16.5, 17.0, 17.5, 0.0 and 18.5 W, for the fundamental to the sixth harmonic), but with differing ϕ_k.

(a)



(b)



(c) Fourier analysis of the data at step (b).

(d) Compensatory adjustments to the power distribution generated at step (c).

(e)

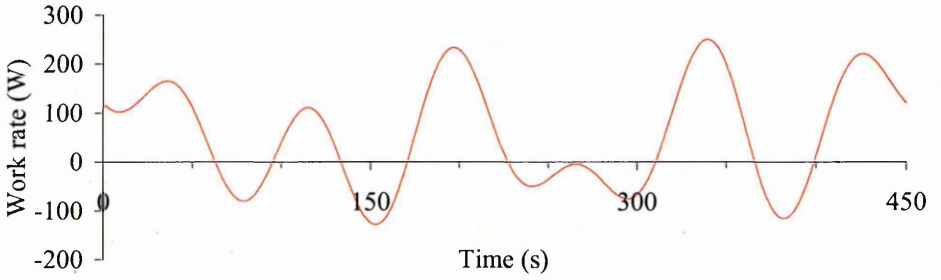


Figure 9.4. Signal generation routine: The data comprising the initial signal (a) were inspected to identify values that exceeded the pre-determined lower and upper limits. Offending values were replaced by values equal to the nearest limit (b). The new data were subjected to a Fourier analysis (c) and the power distribution over the first 6 harmonics recorded. If the power at any of the 6 harmonics was above or below that desired, compensatory adjustments were made to the data (d). Using the amended data, the signal was then reconstructed (e). (b) through (e) were then repeated until the Fourier analysis yielded a close approximation of the desired power distribution at step (c).

The amplitudes of the first six harmonics comprising the final multifrequent signal generated by the optimisation routine were 14.6, 15.3, 16.3, 18.3, 4.0 and 19.5 W, respectively. This gives a mean amplitude of 14.7 W compared with 14.3 W over the same number of harmonics in the PRBS. The corresponding phase angles for each harmonic were -13.5, -29.0, +76.1, -24.7, -56.5 and +21.5 degrees, respectively. A_o was 53 W. The signal is depicted graphically in Figure 9.5.

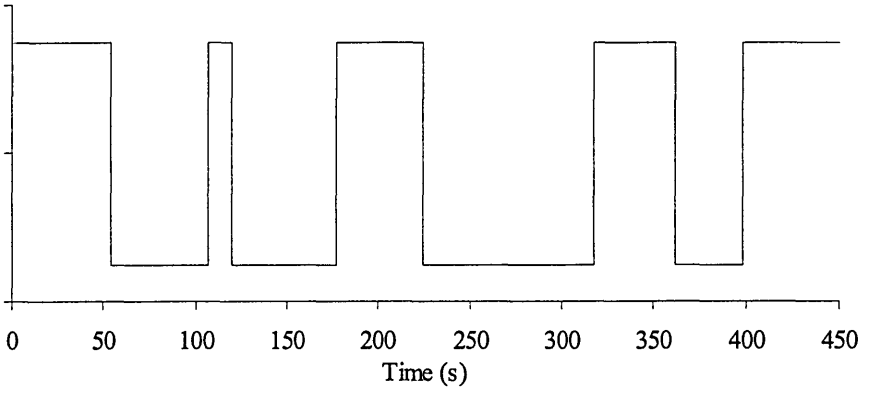


Figure 9.5. Multifrequent signal generated from the optimisation routine described above and in Figure 9.4, (a) to (e).

The binary form of the signal depicted in Figure 9.5 suggests that the term multifrequent binary sequence (MFBS) may be used for descriptive purposes.

The power distribution of the MFBS signal shown in Figure 9.5 is presented as an amplitude spectrum in Figure 9.6 (overleaf). Included in the figure is the amplitude spectrum of the PRBS signal against which comparisons were made. The figure illustrates the gradual increase in power in the harmonics of interest comprising the MFBS compared with the bandwidth harmonics of the PRBS.

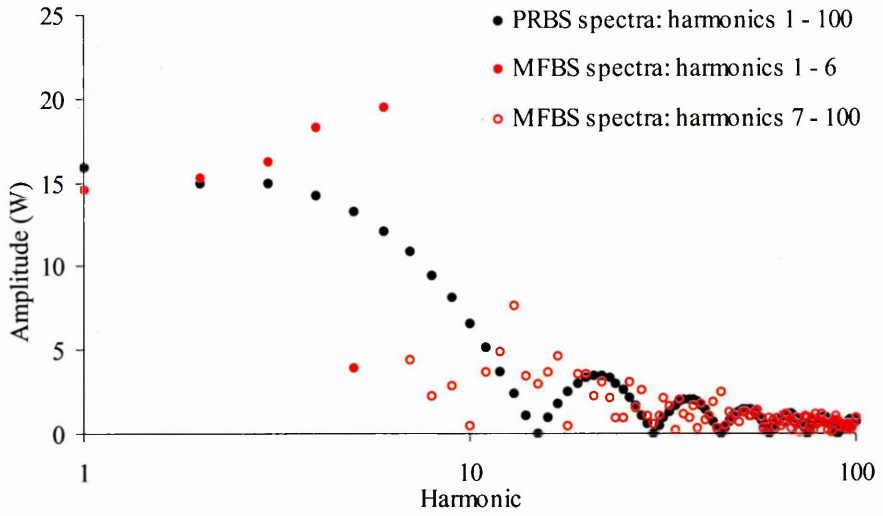


Figure 9.6. Amplitude spectra covering the first 100 harmonics of a multifrequent binary sequence (MFBS) work rate forcing and a 15-unit (30 second·unit^{-1}) pseudorandom binary sequence (PRBS) work rate forcing. The MFBS was generated from the optimisation routine described in Figures 9.4 (a) to (e). The lower and upper work limits chosen for each forcing were 25 and 85 watts, respectively. A logarithmic scale is used for the harmonic axis.

To evaluate $\dot{V}O_2$ kinetics using the MFBS, each subject completed three consecutive cycles of a WR forcing perturbed according to the MFBS signal. The influence of initial transients was minimised by using a shortened MFBS with an identical switching rate to the protocol as a pre-test warm-up. The duration of the warm-up period was 4 min. Figure 9.7 provides a description of the complete protocol.

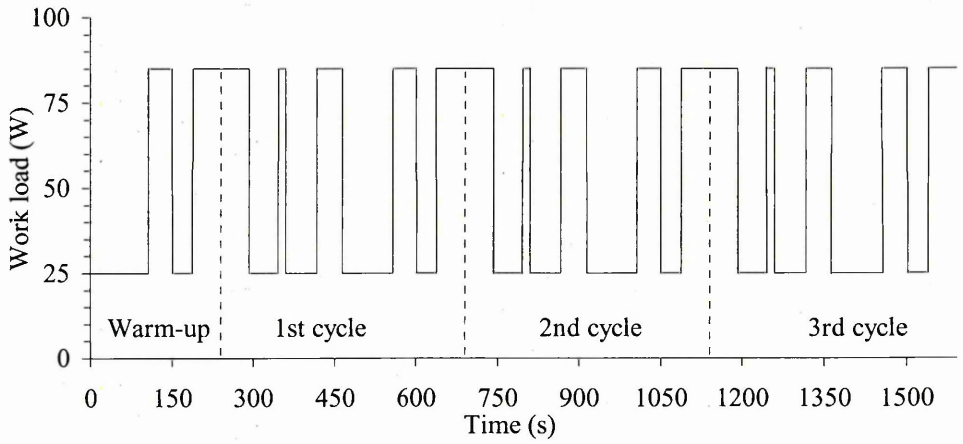


Figure 9.7. Graphical representation of the multifrequent binary sequence work rate protocol used in the experiment.

Subjects completed the two separate exercise tests in random order. To allow for sufficient recovery between tests and to minimise the influence of circadian fluctuations, testing was performed at the same time of day on two consecutive days. Every effort was made to maintain the same experimental conditions throughout testing. Over the duration of the experiment, the maximum difference in ambient temperature experienced by any of the subjects was ±3°C.

Data collection. $\dot{V}O_2$ was measured on a breath-by-breath basis using a respiratory mass spectrometer. Pre-test calibrations were performed on the mass spectrometer using high accuracy gases (tolerance ±0.3%) of known composition.

Lactate sampling. During dynamic exercise above the LAT, non-linear influences are known to be expressed in estimates of $\dot{V}O_2$ kinetics (Barstow et al. 1993; Casaburi et al. 1987a; Casaburi et al. 1989a; Whipp 1994b; Whipp and Wasserman 1986). To reduce the influence of non-linearities, Hoffman et al. (1994a) have proposed that the upper WR used in tests should not elicit blood lactate concentrations above 2 $mmol \cdot l^{-1}$. Accordingly, blood lactate concentrations were measured in a number of subjects. Sampling was performed immediately after the end of testing, with blood specimens drawn from a finger. The lactate concentration in each specimen was measured using a lactate analyser (Analox instruments, GM7). All subjects participating in the experiment were required to provide blood specimens.

Data analysis. Breath-by-breath data from the three complete PRBS and MFBS cycles were averaged to provide a representative set of data for each test. As the ACF of the MFBS signal does not take the form of an impulse or ramp-on/ramp-off function, it is not possible to analyse responses in the time domain (Bennett et al. 1981; Hughson et al. 1991a). Thus, data were analysed in the frequency domain using Fourier methods. To reduce the magnitude of breath-to-breath noise, the PSD of the input and the CPSD

of the input and output were used to calculate frequency response parameters (Eßfeld et al. 1987; Hoffmann et al. 1992). The analysis yielded α and φ parameters for the relationship between WR and $\dot{V}O_2$ at each harmonic of the PRBS and MFBS input. Individual α and φ parameters were used to determine data points on a Nyquist plot. As data at the fifth harmonic of the MFBS were excluded from the analysis, the Nyquist plot formed from the MFBS parameters comprised only five data points.

The Nyquist data were fitted with a non-rational (four-parameter transfer function) model. Fitting of the model returned τ and *TD* parameters. Parameter values were used to calculate an estimate of the MRT of $\dot{V}O_2$.

To assess whether the exclusion of data at the fifth harmonic of the MFBS would have an influence on estimates of $\dot{V}O_2$ kinetics, τ and *TD* parameters were also calculated from the PRBS response data using information from the fundamental to the fourth and the sixth harmonics only.

Statistical analysis. Quantification of the agreement between estimates of the MRT obtained from the PRBS and MFBS methods was performed by calculating the 95% limits of agreement (mean difference in MRT between PRBS and MFBS ± the standard deviation of the differences in MRT between MFBS and PRBS multiplied by 1.96).

In addition to quantifying between trials variability, ANOVA was used to identify any significant differences between the τ, *TD* and MRT obtained from the two tests. The ANOVA technique was also employed to determine whether τ and *TD* parameters derived from the PRBS forcing were influenced by exclusion of data at the fifth harmonic.

9.3 RESULTS

As a result of the lactate analysis, data obtained from two subjects were excluded from further analysis. In the remaining subjects, the mean (standard deviation) blood lactate concentration for the two tests was 1.7 (0.4) $mmol \cdot l^{-1}$.

The mean WRs of the PRBS and MFBS tests were 57.0 and 53.0 W, and the mean values for $\dot{V}O_2$ of the 14 subjects investigated were 1063.6 (112.5) and 1055.0 (109.4) $ml \cdot min^{-1}$, respectively (mean values of $\dot{V}O_2$ are prior to computation of the PSD and CPSD). The mean $\dot{V}O_2$ responses of *subject A* to the 3 cycles comprising the PRBS and MFBS WR forcings are shown in Figure 9.8 (overleaf).

The comparative mean values of α and φ parameters for the PRBS and MFBS methods following determination of the PSD and CPSD are shown in Table 9.2. The table comprises data from the bandwidth of the PRBS forcing and the frequency range of interest of the MFBS forcing, that is, from the fundamental to the sixth harmonic.

Table 9.2. Comparison of amplitude ratio and phase shift parameters derived from the pseudorandom binary sequence (PRBS) and multifrequent binary sequence (MFBS) methods. Data are shown from the fundamental to the sixth harmonic for the relationship between work rate input and oxygen uptake output. Values are presented as mean (standard deviation) of the 14 subjects and follow computation of the power spectral density and the cross-power spectral density.

Harmonic no.	Frequency (Hz)	Amplitude Ratio (arbitrary units)		Phase shift (degrees)	
		PRBS	MFBS	PRBS	MFBS
1	0.0022	0.42 (0.05)	0.42 (0.03)	-28.6 (4.4)	-27.2 (3.7)
2	0.0044	0.38 (0.05)	0.37 (0.02)	-53.2 (4.8)	-57.9 (4.9)
3	0.0067	0.30 (0.04)	0.31 (0.03)	-71.2 (5.8)	-73.6 (4.7)
4	0.0089	0.23 (0.03)	0.22 (0.03)	-90.9 (9.7)	-89.3 (7.6)
5	0.0111	0.16 (0.03)	Not applicable	-106.4 (11.7)	Not applicable
6	0.0133	0.12 (0.03)	0.13 (0.03)	-108.8 (14.4)	-111.7 (8.5)

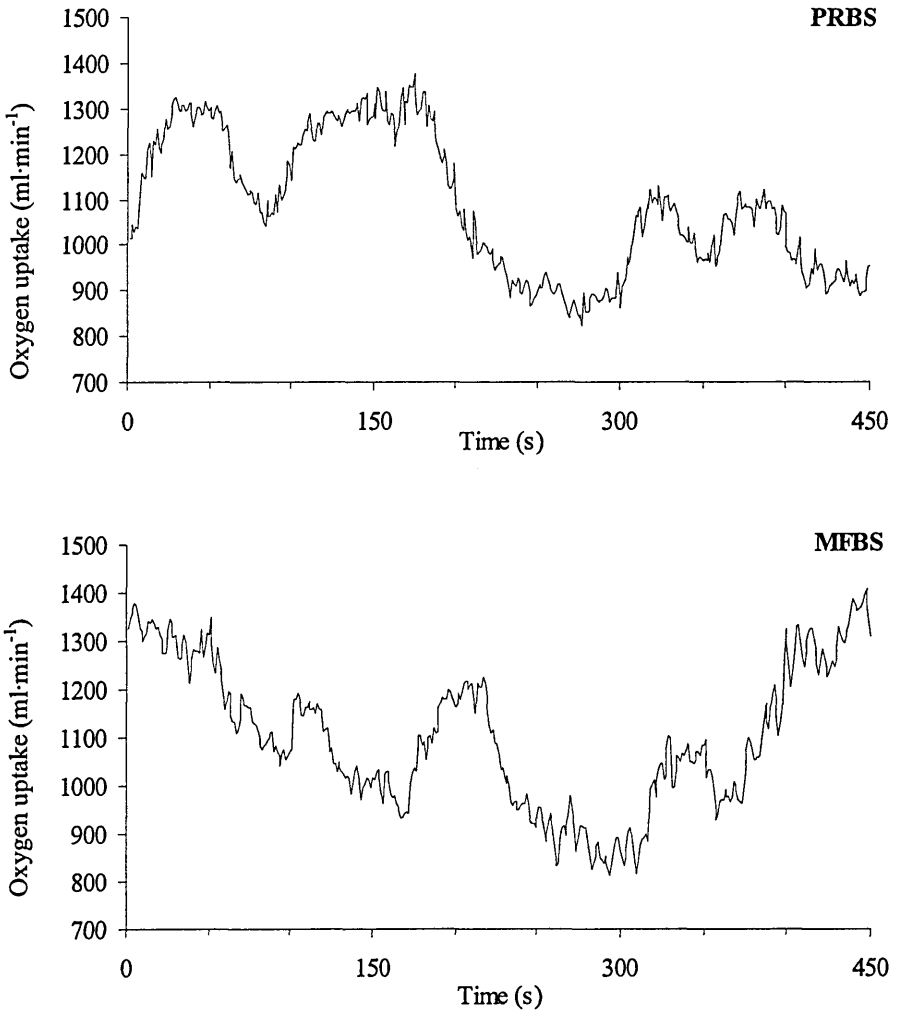


Figure 9.8. Comparison between the dynamic responses in oxygen uptake for *subject A* resulting from the pseudorandom binary sequence (PRBS) work rate forcing and the multifrequent binary sequence (MFBS) work rate forcing. In each case, response data are the average of three consecutive cycles.

For each subject, α and φ parameters derived from the two PRBS tests were used to determine data points in a Nyquist plot (Figure 9.9 overleaf). Included in Figure 9.9 are the corresponding best fits to the data of the non-rational model used in the experiment.

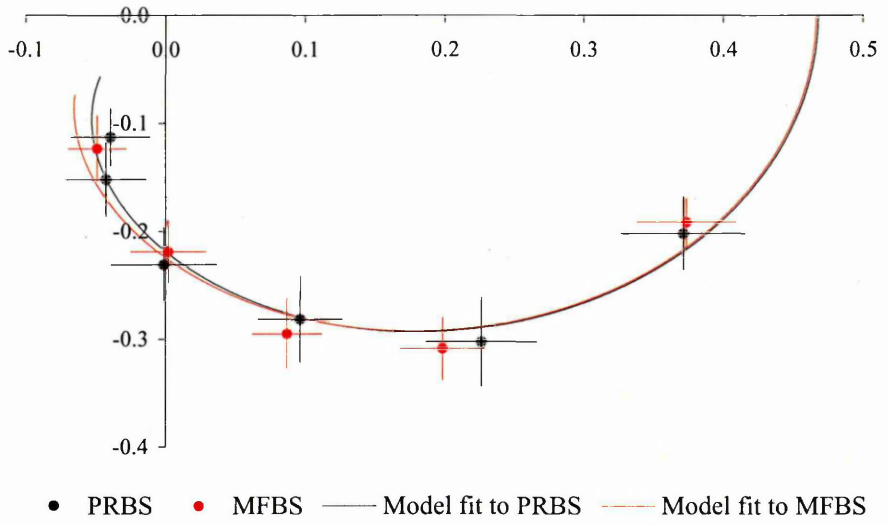


Figure 9.9. Nyquist plot illustrating the data points determined from the mean (standard deviation) amplitude ratio and phase shift parameters computed from the pseudorandom binary sequence (PRBS) and multifrequent binary sequence (MFBS) work rate forcings (number of subjects = 14). Included in the figure are the corresponding best fits to the data of the non-rational model employed in the experiment. Error bars depict standard deviation.

Mean parameter values resulting from the fitting of mathematical models to individual data are shown in Table 9.3. Included in the table are the calculated MRTs of $\dot{V}O_2$ for the PRBS and MFBS methods.

Table 9.3. Comparison of mean (standard deviation) parameter values (number of subjects = 14) derived from the pseudorandom binary sequence (PRBS) and multifrequent binary sequence (MFBS) methods. The data are the result of the best fits to the response data of a non-rational (four-parameter transfer function) model. Included in the table is the calculated mean response time (MRT) of oxygen uptake.

Test	C_1	C_2	Tau, τ (s)	Time Delay, TD (s)	MRT, $\tau + TD$ (s)
PRBS	0.23 (0.04)	0.24 (0.04)	24.4 (5.0)	22.4 (5.6)	46.8 (4.2)
MFBS	0.18 (0.08)	0.29 (0.08)	26.6 (3.3)	18.6 (5.6)	45.2 (5.0)

To examine and quantify the agreement in estimates of the MRT obtained from the PRBS and MFBS methods, the 95% limits of agreement were calculated. The limits ranged from -6.5 to 9.6 s with a mean difference between the methods of 1.5 s. Of the ~5% of individual differences that fell outside of the limits, no difference exceeded the range by more than 0.2 s.

An ANOVA did not identify any statistically significant differences between estimates of τ, *TD* and the MRT of $\dot{V}O_2$ obtained from the two methods (P = 0.08, 0.16 and 0.36, respectively).

The mean τ and *TD* parameters calculated from the PRBS response data, excluding information at the fifth harmonic, were 25.0 (5.1) and 21.2 (6.3) s, respectively. ANOVA did not reveal any significant differences between these parameter values and those obtained from the PRBS using information from all six harmonics comprising the bandwidth (P = 0.75 and 0.61 for τ and *TD*, respectively).

9.4 Discussion

The objective of this experiment was to assess the potential for developing a multifrequent WR forcing specially adapted for evaluating $\dot{V}O_2$ kinetics in subjects with a reduced level of exercise tolerance. To achieve this objective, an optimisation routine was used to generate a signal that took the form of a MFBS. The routine enabled construction of a signal optimised with regard to the power distribution in a specific number of harmonics with a chosen frequency range. The routine also produced a signal that remained within pre-determined binary limits.

In a previous investigation (Hoffmann et al. 1994b), the influence of breath-to-breath noise on the dynamic response in $\dot{V}O_2$ was noted to increase with increasing frequency of sinusoidal input. By formulating a signal that minimised the waste of available power, the aim was to enhance the response-to-noise ratio at the higher frequency

harmonics. It was expected that the resulting signal would assist identification of the underlying response in $\dot{V}O_2$.

The method of signal construction demanded that a percentage of the total power remained in the harmonics outside of the range of interest. This allowed for construction of a multifrequent signal within acceptable limits. To achieve the desired distribution form, the necessary power was acquired from the fifth harmonic in the frequency range of interest. The premise for choosing this particular harmonic was based on the similarity of frequencies at the fourth, fifth and sixth harmonics. To ensure that omission of data from the fifth harmonic would not have an adverse influence on estimates of the overall system response in $\dot{V}O_2$, data from the fundamental to the fourth and sixth harmonics of the PRBS forcing were used to determine τ and *TD* parameters. Comparisons with the kinetic parameters obtained from the PRBS forcing using information from all six harmonics comprising the bandwidth did not reveal any significant differences. Thus, the use of the fifth harmonic as a source of additional power would appear acceptable.

Analysis of variance did not identify significant differences between any of the kinetic parameters obtained from the PRBS forcing and those derived from the MFBS forcing. This suggests that the two different methods provided similar assessments of $\dot{V}O_2$ kinetics. However, inspection of the data revealed that the mean *TD* derived from the MFBS WR forcing was less than that calculated from the PRBS forcing (18.6 versus 22.4 s, respectively). It was noted that the *TD* yielded by the MFBS forcing was closer to that which might be expected from step WR forcings (Hughson et al. 1982; Whipp et al. 1982). In tests using sinusoidal inputs, the *TD* is necessarily a derivative of φ (Sherrill and Swanson 1981). As φ is known to increase with the frequency of input, information from those harmonics with the highest frequencies will have a greater influence on determinations of the *TD*. Since the power in the higher frequency

harmonics of the MFBS was increased above that in the equivalent harmonics of the PRBS, it is possible that the enhanced response-to-noise ratio in the MFBS would have helped to elicit better estimates of the true φ, and thus *TD*. To establish whether this might be the case, it would be necessary to carry out investigations using the data derived from other MFBS with different power distributions.

In addition to the ANOVA, the 95% limits of agreement were calculated to assess the agreement between estimates of the MRT derived from the MFBS and PRBS methods. The limits comprise a range of values that include approximately 95% of the individual differences between measurements obtained in the two tests. The results show that the MRT varied between the two methods by ~8 s, with differences being equally likely in either direction. Previously, the between trials variability in the MRT derived from a PRBS forcing with identical bandwidth to the one employed in the present experiment was shown to be ± ~10 s (see Chapter 8). As the difference likely to occur between the two methods is less than that expected due to variability, this suggests that the MFBS forcing provides the same determination of $\dot{V}O_2$ kinetics as the PRBS forcing. Examination of both the mean and individual differences in estimates of the MRT between the two methods did not reveal any information that would contradict this assumption. However, the improper PRBS used to perturb WR in Chapter 8 may have given rise to misleading information concerning the degree of variability between trials. Therefore, the possibility exists that the between trials variability in the MRT derived from PRBS tests could be less than the difference between measurements obtained using the MFBS and PRBS method. Certainly, until the experiment presented in Chapter 8 is repeated using a true PRBS, it would be unwise to draw any firm conclusions from the resulting information.

If further experiments were to be conducted, then it would prove advantageous to include a repeatability study using two identical MFBS protocols. Data from the study

could then be used to facilitate determination of the agreement between the PRBS and MFBS methods (Bland and Altman 1986). Evidence also suggests that it may be beneficial to compare the MFBS method with another established assessment method, for example, a step WR forcing. This approach is likely to provide a better determination of the validity of the $\dot{V}O_2$ response data derived from the MFBS method.

In working to establish the validity of $\dot{V}O_2$ response data, it should be noted that the dynamic response in $\dot{V}O_2$ below the LAT has been shown to be mildly dependent on WR (Casaburi et al. 1989a; Hughson et al. 1988). If it transpires that $\dot{V}O_2$ kinetics are also dependent on the power distribution of a signal, then this may make it difficult to investigate changes in $\dot{V}O_2$ kinetics that might occur following, for example, an experimental intervention or training strategy (Casaburi et al. 1989a). In a previous experiment by this laboratory (Jarvis et al. 1997b – see Appendix 5), findings were reported that suggest changing the power distribution of a PRBS forcing by increasing its overall magnitude will induce a slowing in $\dot{V}O_2$ kinetics. However, a formal assessment of the LAT was not included in the experiment. It is therefore possible that the data were not representative of $\dot{V}O_2$ responses in the aerobic range. That is, the reported trend towards slower $\dot{V}O_2$ kinetics may well have been the result of non-linear influences that occur above the LAT. The similarity in the MRT calculated from the MFBS and PRBS data in the present experiment would appear to confirm that alterations in the power distribution of a signal are unlikely to have an effect on estimates of $\dot{V}O_2$ kinetics. However, the tendency towards a slightly longer τ obtained from the MFBS forcing gives some cause for concern. Information from the lactate analyses indicates that non-linearities in the response data should have been avoided. Therefore, the differences observed in τ might simply be the result of variability between the two methods. Alternatively, it is possible that the varying influence of

transient glycolytic ATP production (Cerretelli et al. 1979; Cerretelli et al. 1980) is the source of the dissimilarity. Certainly, further investigation concerning the possible influence on $\dot{V}O_2$ kinetics of varying power distributions would seem important.

Given the outcome of the lactate analysis in the present experiment, it is likely that the 85W upper limit would almost certainly be associated with a loss of system linearity in clinical patients, young subjects or individuals of reduced physical fitness. Using the MFBS method, selective redistribution of the available signal power would allow the upper work setting to be reduced to greater extent than in a PRBS forcing before the $\dot{V}O_2$ response became indistinguishable from breath-to-breath noise. Nevertheless, great care would still need to be taken when choosing the work limits of the forcing. A similar level of consideration would also be required when selecting the frequency range over which assessments are performed. In the present experiment, the frequency range of interest for the MFBS was 0.0022 to 0.0133 Hz. Hughson et al. (1990b) have noted that this range will tend to excite the physiological mechanisms that function during *phase II* of the $\dot{V}O_2$ response to dynamic exercise. To examine the *phase I* response in $\dot{V}O_2$, it would be necessary to incorporate higher frequency components in the construction of the MFBS. To achieve this, the available power would need to be distributed over additional harmonics. When testing populations that demand a reduction in the overall magnitude of a signal, there may not be sufficient signal power to ensure that all relevant harmonics will elicit $\dot{V}O_2$ responses that are discernible from breath-to-breath noise. An alternative option would be to perform two separate MFBS tests comprising harmonics at frequencies that cover different portions of the range of interest. However, it should be noted that the switching rate of a MFBS comprised entirely of high frequency harmonics is likely to be faster than the breathing rate of a subject. This factor could lead to the omission of vital information during data

collection (Bennett et al. 1981). Consequently, high frequency MFBS tests may not be suited to the evaluation of $\dot{V}O_2$ kinetics.

Although the assessment of $\dot{V}O_2$ kinetics using MFBS WR forcings may be limited to a relatively low range of frequencies, evidence suggests that future investigations would still benefit by considering further developments in signal design. In work by Bakker et al. (1980), it was proposed that the choice of harmonic frequencies might influence the validity of kinetic parameter estimates. Accordingly, Engeman et al. (1981) have developed a method for optimally selecting both the frequency and amplitude of input harmonics to assist interpretation of the underlying system response. Although optimal harmonic characteristics were not considered as part of this thesis, the process is likely to prove advantageous.

To produce a signal that comprises harmonics with very specific characteristics, it would be necessary to consider a new method of signal construction. Although the optimisation routine used in the present experiment is straightforward, it only allows limited control over how the power is distributed across the harmonic content of a signal. In work by Jensen (1959), it was proposed that binary approximation of harmonic summations could provide an alternative method of signal generation (Figure 9.10 overleaf).

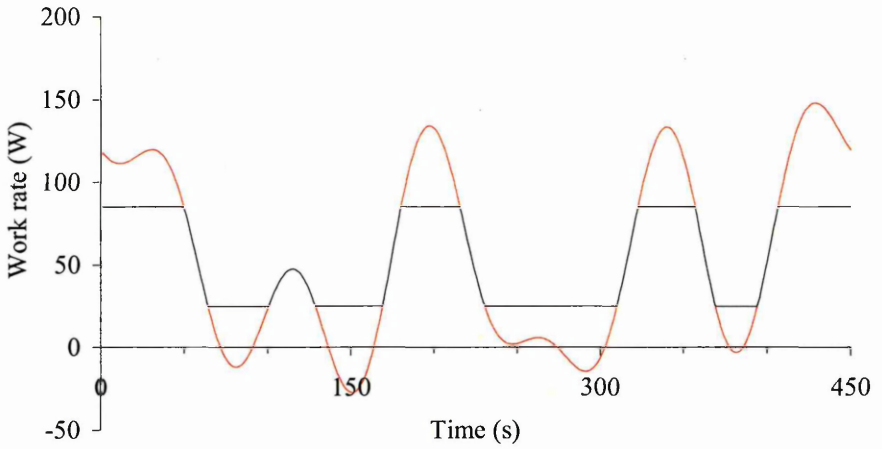


Figure 9.10. A 25 and 85 watt binary approximation of a multifrequent signal formed from the summation of five harmonics of different amplitude, frequency and phase angle.

Although binary approximation yields a signal that does not compromise practical and physiological limitations, the distribution of signal power amongst the selected harmonics is likely to be far from that which is desired (Kerlin 1974). To gain greater control over the power distribution of a MFBS forcing, it is possible that the only solution would be the use of a computerised optimisation process. In work by Buckner (1970), a computer code was developed that achieves a concentration of signal power in the required harmonics and a good agreement between the actual and desired power distribution. Therefore, the application of this code to the generation of signals for use in the examination of $\dot{V}O_2$ kinetics would appear worthy of further investigation.

9.5 Conclusion

The main objective of this experiment was to investigate the potential for developing a multifrequent WR forcing specially adapted for the assessment of $\dot{V}O_2$ kinetics in subjects with a reduced level of exercise tolerance. The results suggest that, by redistributing the available signal power amongst a select number of harmonics, and minimising the percentage of energy in all other harmonics, it is possible to generate a

signal that provides similar estimates of $\dot{V}O_2$ kinetics as an established PRBS WR forcing. Although tests were conducted on healthy individuals, the method of redistributing the available signal power should enable the upper work limit of a forcing to be reduced for clinical, untrained or young subjects with little undue influence on assessments of the underlying $\dot{V}O_2$ response. Whilst further investigations are needed to confirm the validity of response data, early evidence indicates that a multifrequent WR forcing with optimally selected harmonic characteristics may prove valuable in the assessment of $\dot{V}O_2$ kinetics for clinical or physiological purposes.

10.0 OVERALL DISCUSSION

After the onset of constant-rate exercise, investigations have shown that $\dot{V}O_2$ kinetics in the sub-LAT domain may be separated into three temporal phases (Linnarsson 1974; Whipp et al. 1982). It is widely accepted that the dominant second phase is well described by a monoexponential function of time (Linnarsson 1974). Analysis of the time constant of this function can be used to provide important diagnostic information concerning the behaviour of the respiratory system (Lamarra and Whipp 1995).

Research has shown that the rate at which $\dot{V}O_2$ accelerates to a new steady state can vary according to a number of factors. These factors include aerobic capacity (Cerretelli et al. 1979; Eßfeld et al. 1987; Powers et al. 1985; Stegemann et al. 1985; Zhang et al. 1991), different forms of physical training (Berry and Moritani 1985; Fukuoka et al. 1995; Jarvis et al. 1997a), age (Babcock et al. 1994b; Chilibeck et al. 1996; Cunningham et al. 1993) and cardiovascular and pulmonary disease states (Casaburi et al. 1997; Cooper et al. 1992; Koike et al. 1994; Nery et al. 1982; Sietsema et al. 1994; Spiro 1977).

To evaluate the kinetics of $\dot{V}O_2$, exercise intensity can be perturbed according to a step, impulse or ramp function of time. Simple linear mathematical models can then be used to characterise the dynamic response in $\dot{V}O_2$. To reduce uncertainty in the parameters resulting from the fit of the model, tests are usually repeated several times in each subject so that the responses can be averaged. The time-consuming nature of this approach makes investigations outside a research setting difficult to conduct (Hoffmann et al. 1994a).

An alternative approach to obtaining a description of $\dot{V}O_2$ kinetics employs sinusoidal WR forcings (Bakker et al. 1980; Casaburi et al. 1977). A sinusoidal input has the advantage of generating a cyclical response. The data from each cycle can then be

separated and averaged to give a measure of $\dot{V}O_2$ kinetics from a single test. However, a description of the system response to sinusoidal forcings requires that the kinetic parameters are known for all relevant input frequencies (Eßfeld et al. 1987).

More recently, the PRBS WR forcing has been used to evaluate the dynamic response in $\dot{V}O_2$ (Eßfeld et al. 1982; Hoffmann et al. 1991; Hughson et al. 1990a; Stegemann et al. 1985; Xing et al. 1991). By perturbing WR according to a PRBS, the responses at several sinusoidal frequencies can be measured simultaneously. If the system that controls $\dot{V}O_2$ kinetics behaves in a linear manner, then the information obtained from a PRBS test can be used to derive an estimate of $\dot{V}O_2$ kinetics in a single test session of ~30 min or less duration (Hughson et al. 1990d).

Whilst PRBS WR forcings can be applied with minimal imposition to a subject, to minimise the contribution of non-linear influences, changes in work intensity must be constrained to the sub-LAT domain (Henson et al. 1989). For subjects in whom exercise intolerance is a feature, the necessary reduction in the upper work limit is likely to effect a significant fall in the distribution of power across the bandwidth of the sequence. If the distribution of power falls below a critical level, then it may not be possible to elicit discernible responses from the forcing (Eßfeld et al. 1987). To resolve this problem, this thesis investigated the potential for developing a multifrequent WR forcing adapted to enhance identification of the underlying $\dot{V}O_2$ response.

The approach taken to meet the aim of the thesis involved constructing a multifrequent signal modified with regard to the power available at each harmonic, the number of harmonics tested and the range of frequencies over which testing was conducted. Construction of the signal was accomplished by redistributing the available signal power to specific harmonics in a chosen frequency range of interest. The power

distribution of the signal was selected to increase the response-to-noise ratio at the higher frequency harmonics of the input.

In accordance with the work of Hughson et al. (1990b), it was noted that the frequency range chosen for the multifrequent signal should excite the physiological mechanisms that function during *phase II* of the $\dot{V}O_2$ response to dynamic exercise. To characterise this response, data were fitted with a non-rational (four-parameter transfer function) model. Parameter values reported for the fit of this model to sinusoidal $\dot{V}O_2$ response data (Sherrill and Swanson 1981) are similar to those recorded in the present experiments. Although it is not within the scope of this thesis to consider the merits of the non-rational model, it was noted that the confidence limits associated with the parameter values might be wider than those of a lower order model (Cunningham et al. 1993). However, unlike the second-order model proposed by Bakker et al. (1980), the non-rational model returns a positive time-delay when used to characterise $\dot{V}O_2$ response data from sinusoidal WR forcings.

Results revealed that the *TD* yielded by the model fit to the MFBS response data was similar to that expected in healthy subjects during tests involving step WR forcings (Hughson et al. 1982; Whipp et al. 1982). From previous work (Sherrill and Swanson 1981), it is known that *TD* is necessarily a derivative of φ. As φ increases with the harmonic frequency of the input, information from those harmonics with the highest frequencies is likely to have a greater influence on determinations of the *TD*. Since the power distribution of the MFBS forcing was selected to increase the response-to-noise ratio at higher frequency harmonics, it was proposed that this would have aided derivation of better estimates of φ and thus *TD*. Whilst the same effect was not observed in estimates of τ, further investigation of the proposal would appear worthwhile.

Although the subjects involved in the present experiments were drawn from a population of healthy and active individuals, the main purpose of the MFBS WR forcing would be to enhance determinations of $\dot{V}O_2$ kinetics in clinical, untrained or young subjects. To accommodate individuals with a low exercise tolerance, the power distribution across the harmonic range of the MFBS forcing was chosen to increase the response-to-noise ratio, particularly at higher frequencies of the input. It was hypothesised that this would allow the upper work limit of the forcing to be reduced with little undue influence on assessments of the underlying $\dot{V}O_2$ response. To substantiate this assumption, it would be necessary to assess whether a MFBS forcing could provide valid estimates of $\dot{V}O_2$ kinetics. Due to the wide limits of agreement observed in estimates of the MRT obtained from PRBS WR forcings (see Chapter 8), only a general determination of the validity of the MFBS method could be established. Whilst the improper PRBS employed in Chapter 8 may have been the cause of the wide limits, evidence from other studies (Hughson and Inman 1986b; Nordrehaug et al. 1991) would suggest that the reported findings accurately reflect the poor repeatability of kinetic parameters derived from PRBS tests. Certainly, comparison of the MFBS method with the established step method would be advised in any further experiments to determine the validity of the $\dot{V}O_2$ response data.

Although this thesis was not able to assess the validity of the response data obtained using the MFBS method, estimates of the MRT derived from the MFBS forcing and a true PRBS forcing were shown to be well matched. To obtain a statistical assessment of the agreement, the between trials variability in the kinetic parameters computed from the MFBS response data would have to be established. If a repeatability study were to be performed using the MFBS WR forcing, then comparing the standard deviation of the differences with that observed in data derived from PRBS forcings would identify which method exhibits the least variability. As evidence suggests that variability during

multifrequent tests is influenced by breath-to-breath noise, the increased response-to-noise ratio afforded by the design of the MFBS forcing should yield parameters that are more repeatable. If MFBS tests are less susceptible to variability, then the method may prove more effective in establishing the efficacy of an intervention method or strategy.

In previous investigations (Casaburi et al. 1989a; Hughson et al. 1988; Hagberg et al. 1978b), $\dot{V}O_2$ kinetics below the LAT were shown to be dependent on the magnitude of a step change in WR. As this finding does not comply with Boltzmann's principle of superposition (Fujihara et al. 1973a & 1973b), it is possible that the behaviour of the process controlling $\dot{V}O_2$ kinetics is non-linear (see also Chapter 2). This makes it difficult to interpret changes in $\dot{V}O_2$ kinetics that might result from, for example, a specific intervention therapy or training strategy (Casaburi et al. 1989a). If the $\dot{V}O_2$ response generated at each harmonic of a MFBS WR forcing is also shown to demonstrate a dependency on WR, then changes in the distribution of power across the harmonic range of interest may lead to variations in assessments of $\dot{V}O_2$ kinetics. Not only would this make differences in response kinetics harder to interpret, but validating the response data against those obtained using another established method might also prove difficult. Although Hughson et al. (1991a) have reported data that suggest the process controlling $\dot{V}O_2$ kinetics behaves in a linear manner during multifrequent testing, Eßfeld et al. (1991) have suggested that estimates of $\dot{V}O_2$ kinetics derived from such tests might be subject to a form of quasi-linearity. Under these conditions, the outcome of a test could be dependent on the range of frequencies that are examined. This theory is supported by research conducted by Hoffmann et al. (1994b). These investigators identified additional components in the $\dot{V}O_2$ responses to sinusoidal forcings with frequencies higher than ~0.0100 Hz. It was proposed that the additional components reflected the contribution from non-linearities generated by processes

within the cardiorespiratory system. In an earlier study, Hoffmann et al. (1992) had shown good agreement between the kinetic parameters derived from individual harmonics of a PRBS and those obtained from sinusoidal forcings of equivalent frequency. It was therefore concluded that the system response to harmonic components with frequencies higher than ~0.0100 Hz must also be non-linear. As the method of analysis in the present experiment requires the inclusion of information from harmonic frequencies above the limit proposed by Hoffmann et al. (1994b), this gives cause for concern. However, the time domain analysis of PRBS response data necessarily incorporates information from the complete harmonic range of a forcing. Consequently, it might be expected that $\dot{V}O_2$ kinetics determined using this method would be similarly affected. Yet, Hughson et al. (1991a) have reported that the kinetic parameters derived from the time domain analysis of the $\dot{V}O_2$ response to PRBS forcings are comparable with those obtained from step forcings. The findings reported by Hughson and his colleagues are even more contentious as the respective bandwidths of the 15-unit PRBS and 63-unit PRBS used in investigations comprised 33% and 85% of harmonics with frequencies higher than 0.0100 Hz, respectively. Clearly, further investigation is necessary to establish the reasons why the time domain analysis of $\dot{V}O_2$ response data is not influenced by the inclusion of high frequency harmonic components. Information derived from such investigations may also prove advantageous in establishing whether estimates of $\dot{V}O_2$ kinetics are likely to be dependent on the distribution of signal power in a MFBS forcing. In any future experiments, care would need to be taken to ensure that all work intensities are rigorously constrained to the sub-LAT domain. Only after these experiments have been completed would it be possible to determine whether the general agreement observed between the kinetic parameters obtained from the MFBS and PRBS methods is indicative of compliance with the criterion accorded by dynamic linearity.

If the process controlling $\dot{V}O_2$ kinetics may be regarded as linear during MFBS tests, then opportunities exist for further development of the method. One particular area of interest concerns the frequency location and amplitude characteristics of input harmonics. If these characteristics are optimally selected, then the accuracy of estimates of $\dot{V}O_2$ kinetics may be enhanced (Bakker et al. 1980). In addition to assisting the assessment of the dynamic response in $\dot{V}O_2$, optimal signal design may also facilitate interpretation of the underlying physiology (Engeman et al. 1981). Consequently, MFBS tests could prove beneficial in the advancement and investigation of different hypotheses concerning the control mechanisms of $\dot{V}O_2$ kinetics.

To accommodate specific harmonic characteristics in the design of a MFBS signal, it would be necessary to consider other methods of signal construction. The optimisation routine used in this thesis achieved a power distribution closely resembling that desired at each harmonic. However, a computerised routine (for example, Buckner 1970) is likely to offer more control over the shape of the distribution. It may also assist in minimising the waste of signal energy in harmonics outside of the frequency range of interest.

In addition to generating MFBS signals with specific characteristics, a computerised routine would facilitate the construction of a much wider range of signal forms. Whilst these might not all be suited to the evaluation of $\dot{V}O_2$ kinetics, alternative applications include the assessment of the dynamic response in $\dot{V}I$ (Bennett et al. 1981; Greco et al. 1986) and the basis on which levels of inspired gas concentrations can be varied for the assessment of chemoreceptor responsivity (Bellville et al. 1976; Dhawale and Bruce 1995).

As well as assisting with the evaluation of different physiological responses, a MFBS WR forcing could prove advantageous in the assessment of $\dot{V}O_2$ kinetics in subjects

other than those with a reduced exercise tolerance. In previous investigations, PRBS tests have been used to detect differences in $\dot{V}O_2$ kinetics resulting from a variety of interventions (Eßfeld et al. 1984; Hoffmann et al. 1991; Hughson et al. 1991b; Kowalchuk and Hughson 1990; Stegemann et al. 1985; Xing et al. 1991). Due to the design of MFBS WR forcings, it is possible that the approach will offer improved sensitivity to changes that might occur in the kinetic parameters of $\dot{V}O_2$. Consequently, MFBS tests may be well suited to the assessment of physical performance, providing information necessary to develop individual profiles, evaluate training methods and monitor progress during rehabilitation.

11.0 FINAL CONCLUSION

The main aim of this thesis was to investigate the potential for developing a multifrequent WR forcing specially adapted for the assessment of $\dot{V}O_2$ kinetics in clinical, untrained or young subjects. The findings of the experiments show that:

i) A multifrequent WR forcing with a power distribution chosen to enhance identification of the underlying $\dot{V}O_2$ response can provide similar estimates of $\dot{V}O_2$ kinetics to an established PRBS forcing.

ii) Due to the poor repeatability of the kinetic parameters obtained from the PRBS WR forcing, it is not possible to establish the validity of estimates of $\dot{V}O_2$ kinetics derived from a MFBS forcing.

Whilst these findings suggest that perturbing WR according to a MFBS signal could be used to assess $\dot{V}O_2$ kinetics in subjects with a reduced level of exercise tolerance, it is clear that further investigations are necessary to establish the validity of the resulting $\dot{V}O_2$ response data. Although repeating the experiment presented in Chapter 8 using a true PRBS to perturb WR may provide the required information, evidence suggests that direct comparisons with the kinetic parameters obtained from step WR forcings may be better suited to the task.

In addition to validating the information from MFBS WR forcings, there is a need to determine whether estimates of $\dot{V}O_2$ kinetics are invarient with the power distribution across a sequence. Compliance with the criteria accorded by static and dynamic linearities would facilitate the interpretation of changes in $\dot{V}O_2$ kinetics that might result from, for example, a specific intervention therapy.

Prior to performing further investigations, it is likely that construction of a MFBS signal would benefit from the inclusion of harmonics with optimal characteristics. This would necessitate the use of a computerised optimisation routine. In addition to offering

greater control over the power in those harmonics comprising the frequency range of interest, a computerised routine may also help minimise the waste of signal energy in other harmonics.

In future studies that employ MFBS tests, it should be possible to reduce the upper work limit of the forcing and still elicit $\dot{V}O_2$ responses that are discernible from breath-to-breath noise. In healthy, active subjects, this technique may allow more accurate determinations of the kinetic parameters of $\dot{V}O_2$ in the sub-LAT domain. The resulting response data could then be used to provide detailed descriptions of the underlying physiology of the respiratory system. As MFBS forcings have the advantage of being able to yield the required information in a single session of less than 30 min duration, the approach is likely to prove very beneficial in the assessment of $\dot{V}O_2$ kinetics for both clinical and physiological purposes.

AN ASSESSMENT OF THE OXYGEN UPTAKE KINETICS OF SPRINT AND ENDURANCE TRAINED ATHLETES

David R. Jarvis, Neil V. Challis, Janet H. Chapman, David B. Claxton, Mary L. Fysh

INTRODUCTION

The influence of different training intensities on $\dot{V}O_2$ kinetics has been well documented (see Phillips et al. 1995). In studying these influences, previous investigators have tended to employ either step or single frequency sine wave forcings. As these forcings only allow the examination of finite responses, it was hypothesised that certain training effects may not be detected.

Multifrequent exercise tests offer an alternative approach to the assessment of $\dot{V}O_2$ kinetics. These tests apply several different sine wave forcings simultaneously.

One example of a multifrequent test is the pseudo random binary sequence (PRBS) exercise test. Investigations by Hoffmann et al. (1994) have shown a good agreement between the results of PRBS tests and those obtained from single frequency sine wave forcings.

The aim of this experiment was to use a multifrequent PRBS test to examine the effect of two different training intensities on $\dot{V}O_2$ kinetics.

METHODS

Twelve trained male subjects, mean age 24.6 (SD 3.6) years gave their informed consent to participate in this study. The subjects were divided into two groups: $Group_S$, sprint trained (n = 6) and $Group_E$, endurance trained (n = 6). Tests were performed in the upright body position on an electrically braked cycle ergometer.

The exercise protocol incorporated four identical PRBS sequences each consisting of 15 units of 30 s duration. Throughout each sequence, the work rate was automatically switched between 20 W and 80 W. The upper work rate was set so as not to exceed the work rate at the ventilatory threshold of each subject. A pedalling rate of 60 revolutions per minute was maintained throughout each test. Gas exchange was measured on a breath-by-breath basis using a respiratory mass spectrometer.

Breath-by-breath data from each test were analysed in the frequency domain using Fourier techniques. The analysis yielded amplitude and phase shift parameters for the relationship between work rate input and $\dot{V}O_2$ output. Assessment of inter-group variability was made using the analysis of variance (ANOVA) technique. Only those parameters in the frequency range 0.0022-0.0089 Hz were considered suitable for analysis (Hoffmann et al. 1994). These frequencies correspond to sine waves with periods ranging from 450 s to 112.5 s. Unless stated otherwise, statistical significance was set at $P<0.05$. Results are expressed as means (SD).

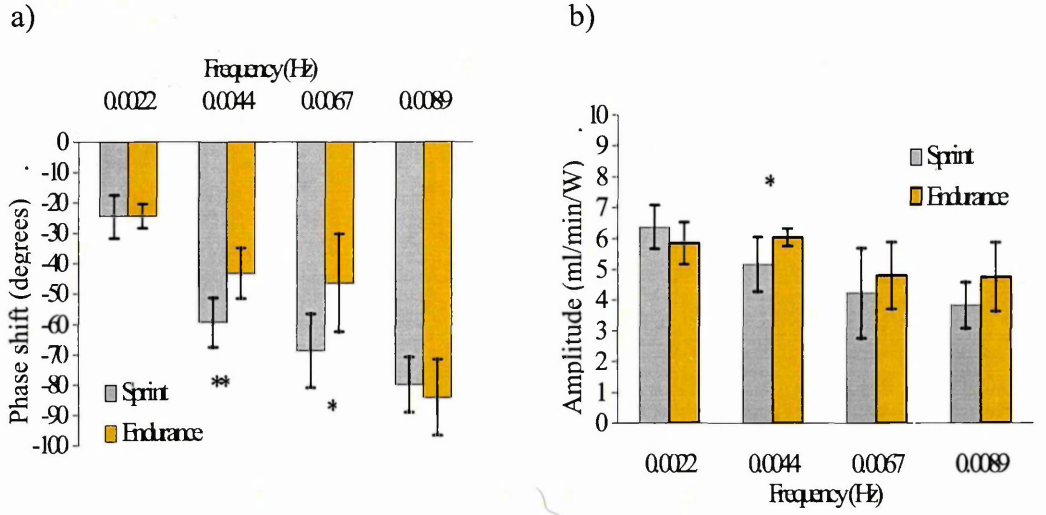
RESULTS

The mean work rate of the PRBS test was 53.0 W and the mean values for $\dot{V}O_2$ were 1020.2 (69.0) and 945.7 (74.9) $ml \cdot min^{-1}$ for $Group_S$ and $Group_E$ respectively. This resulted in a $\dot{V}O_2$ to work rate ratio of 19.2 (1.3) and 17.8 (1.4) $ml \cdot min^{-1} \cdot W^{-1}$. Mean values for phase shift and amplitude ratio parameters are shown in Figure 1 (overleaf).

At the lowest frequency (0.0022 Hz), the mean phase shift for $Group_S$ was almost identical to that of $Group_E$. For frequencies of 0.0044 and 0.0067 Hz, ANOVA revealed

significantly longer phase shifts in $Group_S$. This trend was reversed at the highest frequency, though not to a statistically significant extent.
With the exception of the lowest frequency, there was a trend towards greater amplitude ratios in $Group_E$. This difference was only significant at a frequency of 0.0044 Hz.

Figure 1. Comparison of phase shift (a) and amplitude ratio (b) values for the relationship between work rate and oxygen uptake: mean values (SD).



* significant difference at P<0.05 ** significant difference at P<0.01

DISCUSSION

In contrast to individual work rate forcings, PRBS tests enable $\dot{V}O_2$ kinetics to be examined over a wide range of frequencies.

In this experiment, no significant difference in $\dot{V}O_2$ kinetics was detected between $Group_S$ and $Group_E$ at the lowest frequency. As this frequency corresponds to a sine wave with a period of 450 s, it is possible that any similarities may be due to the passive nature of the forcing.

Faster $\dot{V}O_2$ kinetics, as indicated by significantly larger amplitude ratios and/or shorter phase shifts, were observed in $Group_E$ at frequencies of 0.0044 and 0.0067 Hz. This is in direct contrast to the study of Berry and Moritani (1985). Employing rest to work step forcings, these investigators noted that interval training, similar to that performed by $Group_S$, resulted in significantly faster $\dot{V}O_2$ kinetics than endurance training.
The findings of this study would suggest that different training intensities might have variable influences on $\dot{V}O_2$ kinetics. These differences may only become apparent if a wide range of frequencies is examined in addition to individual work rate forcings.

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SUBJECT DETAILS

Experiment 1: An investigation of between trials variability in estimates of oxygen uptake kinetics

Subject code	Sex	Age (yr)	Body Mass (kg)	Height (cm)
A	m	21	77	179
Ba	f	22	67	166
Bob	f	22	64	165
Bos	f	24	69	172
Ca	m	24	88	182
Cl	m	23	74	180
L	m	22	90	178
Ma	m	22	82	182
Me	m	21	78	178
Mu	f	21	71	172
P	f	21	62	156
R	m	22	76	179
Sh	f	22	67	159
Sp	f	22	55	167
St	m	22	73	178
We	f	22	66	165
Wh	m	22	82	179

Blood lactate samples were not recorded during the experiment. In a group of subjects (n = 16) drawn from the same population as those participating in the experiment, the mean (SD) $\dot{V}O_{2max}$ and ventilatory threshold recorded during cycle ergometry were 2943 (667) $ml \cdot min^{-1}$ and 2694 (412) $ml \cdot min^{-1}$, respectively. The mean (SD) WR at $\dot{V}O_{2max}$ was 304 (59) W.

Experiment 2: The analysis of oxygen uptake kinetics using a modified multifrequent work rate forcing

Subject code	Sex	Age (yr)	Body Mass (kg)	Height (cm)	Lactate* ($mmol \cdot l^{-1}$)
Bu	m	23	83	181	1.1
Cr	m	23	88	181	2.0
Ed	m	27	85	191	1.3
Ha	f	23	78	177	2.1
Hod	m	22	84	180	1.4
How	m	28	65	175	1.3
Hu	m	23	73	186	1.2
Pr	m	22	73	167	1.7
Ri**	f	22	65	160	4.2
Ro**	f	24	61	162	4.5
Sh	m	22	74	181	2.0
Sm	f	22	66	162	2.2
St	m	22	80	171	1.5
Tud	f	21	60	171	1.6
Tur	m	21	93	184	1.5
Wa	f	22	60	170	2.1

* Blood lactate concentration at end of exercise. Samples were taken after completion of one work rate protocol (pseudorandom binary sequence or multifrequent binary sequence) on a random basis.

** Subjects excluded from experiment due to high blood lactate concentration following work rate protocols.

THE ANALYSIS OF OXYGEN UPTAKE KINETICS

INVESTIGATOR: DAVID R. JARVIS
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Tel: 0114 2532454

CONSENT FORM AND INFORMATION SHEET

Purpose of this investigation

Measurements of oxygen uptake kinetics can be used to assess the physiological responses to a variety of experimental treatments and training strategies. As tests do not require subjects to exercise at maximum capacity, the method of assessment is ideal for individuals with a reduced exercise tolerance.

At present, information on variability between measurements of oxygen uptake kinetics is limited. Unless variability can be quantified, it may not be possible to establish the true efficacy of an intervention method.

The purpose of this investigation is to examine and quantify the between trials variability in measurements of oxygen uptake kinetics obtained from two pseudorandom binary sequence exercise tests.

Explanation of investigation

i) After a short warm-up, you will be required to perform an exercise test on a cycle ergometer. The test will last approximately 30 minutes.

ii) The exercise work load will alternate randomly between 25 and 85 watts, every 30 to 180 seconds.

ii) You are required to pedal at a constant rate of 60 rpm (the rate will be displayed on the cycle).

iv) Oxygen consumption and heart rate will be measured throughout the test.

In order to evaluate variability, you will be required to complete the test on two consecutive days. Both sessions will be held within the Health and Fitness Laboratory.

Special instructions

Participants are asked to:-

i) abstain from consuming large quantities of alcohol and caffeinated products prior to the tests.

ii) avoid heavy exercise in the 24 hours before the study.

iii) consume a light meal at about 2 hours before the start of the tests.

iv) wear suitable clothing (training shoes, shorts, tracksuit, T-shirt, etc.)

Discomforts

The protocol used in the study is sub-maximal. This means that it is unlikely to cause any discomfort or pose a risk to health. Full supervision will be given

throughout the tests and signs of well-being monitored. However, **should you experience any unusual feelings associated with the physical effort, then please stop the test.**

Medical screening

You are requested to complete a simple health and exercise questionnaire prior to testing. This information is used to assess your aptitude to undertake the tests. Only current health problems indicated on this form will exclude you from the study. All records are strictly confidential.

Inquiries

Any questions about the procedures used in this study are encouraged. If you have any doubts or queries then please feel free to ask.

Freedom of consent

Your permission to perform these tests is required. Your participation is strictly voluntary and you are free to deny consent and withdraw from this investigation at any time.

Name _____ (please print)

Signature _____ Date _____

Witness _____ Date _____

A) Personal details

Surname _____

First name(s) _____

Date of birth _____

Age _____

Contact address

_____

_____

_____

_____

Telephone No. _____

B) Medical details

i) Have you been told that you have any of the following health problems?

	Yes	No
i) asthma	☐	☐
ii) high blood pressure	☐	☐
iii) diabetes	☐	☐
iv) anaemia	☐	☐
v) cardiovascular diseases	☐	☐

ii) Have you had any of the following in the last 6 months?

	Yes	No
i) viral or bacterial infections	☐	☐
ii) musculoskeletal injuries	☐	☐
iii) operations involving a general anaesthetic	☐	☐

iii) Are you taking any medications prescribed by your G.P.?

Yes	No
☐	☐

If yes, then please give details

_____

iv) Do you have any other disabilities that might impair your exercise performance in these tests?

Yes	No
☐	☐

If yes, then please give details

_____

_____

B) Exercise participation

i) Which activities do you participate in regularly?

_____

_____

_____

_____

_____

iii) Approximately how much time do you spend each day, week or month involved in physical exercise?

_____

_____

_____

_____

_____

_____

APPENDIX 4

SELECTED PERFORMANCE SPECIFICATIONS

MGA-1100 mass spectrometer (Marquette Electronics)

Accuracy:

Gas	Range	Accuracy (% full scale)
N_2	100% or 1000 mmHg	±1%
O_2	100% or 1000 mmHg	±1%
CO_2	10% or 100 mmHg	±2%

Stability:

Breath-to-breath ±0.1%
Daily ±1%
Monthly ±2%

THE INFLUENCE OF WORK RATE ON OXYGEN UPTAKE KINETICS DETERMINED BY PSEUDORANDOM BINARY SEQUENCE EXERCISE TESTS

David R. Jarvis, Neil V. Challis, Janet H. Chapman, David B. Claxton, Mary L. Fysh

INTRODUCTION

The study of oxygen uptake ($\dot{V}O_2$) kinetics has been shown to provide valuable information about aerobic capacity.

An important concept in the study of $\dot{V}O_2$ kinetics is dynamic linearity. Dynamic linearity implies that, for exercise intensities not associated with a sustained increase in blood lactate, $\dot{V}O_2$ kinetics should remain largely independent of work rate, of prior conditions and of the type of work rate forcing function (Hughson 1990).

One method of assessing $\dot{V}O_2$ kinetics involves the use of pseudo random binary sequence (PRBS) exercise tests. For PRBS tests that employ set work rate changes, $\dot{V}O_2$ kinetics have been shown to exhibit dynamic linearity over a specific range of frequencies (Hoffmann et al. 1992 & 1994). The aim of this experiment was to investigate whether dynamic linearity is significantly compromised if the work rate range of a PRBS test is extended.

METHODS

Fourteen subjects, mean age 22.1 (SD 0.9) years gave their informed consent to participate in this study. Tests were performed in the upright body position on an electrically braked cycle ergometer.

Subjects completed two separate PRBS exercise tests. Each test incorporated a series of four PRBS sequences consisting of 15 units of 30 s duration. Throughout each test the work rate was automatically switched between 25 and 85 W for PRBS1 and 25 and 105 W for PRBS2. The timing of each switch was the same in both tests. The upper work rate was set so as not to exceed the work rate at the ventilatory threshold of each subject. A pedalling rate of 60 revolutions per minute was maintained during each test. Gas exchange was measured on a breath-by-breath basis using a respiratory mass spectrometer.

Breath-by-breath data from each test were analysed in the frequency domain using Fourier techniques. The analysis yielded phase shift and amplitude parameters for the relationship between work rate input and $\dot{V}O_2$ output. Assessment of inter-test variability was made using the analysis of variance (ANOVA) technique. Following the work of Hoffmann et al. (1994), only those parameters in the frequency range 0.0022-0.0089 Hz were considered suitable for analysis. Unless stated otherwise, statistical significance was set at $P<0.05$. Results are expressed as means (SD).

RESULTS

The mean values for work rate and for $\dot{V}O_2$ were 57.0 and 67.7 W and 1029.7 (97.8) and 1167.4 (98.5) $ml \cdot min^{-1}$ for PRBS1 and PRBS2 respectively. This resulted in a $\dot{V}O_2$ to work rate ratio of 20.3 (1.9) and 19.5 (1.6) $ml \cdot min^{-1} \cdot W^{-1}$. Mean values for phase shift and amplitude parameters are shown in Table 1 (overleaf).

Except at the highest frequency, that is 0.0089 Hz, there was a trend towards longer phase shifts and greater amplitudes in PRBS2. Although ANOVA revealed no statistically significant difference between any of the phase shift parameters, statistically

significant differences ($P<0.001$) between amplitude parameters in the frequency range 0.0022-0.0067 Hz were observed.

Table 1. Mean phase shift and amplitude values for the relationship between work rate and oxygen uptake.

Frequency (Hz)	Phase shift (degrees)		Amplitude ($ml \cdot min^{-1} \cdot W^{-1}$)	
	PRBS1	PRBS2	PRBS1	PRBS2
0.0022	-27.47 (3.94)	-29.63 (4.38)	7.83 (0.57)	8.90 (0.52)*
0.0044	-52.51 (4.08)	-55.96 (5.23)	6.42 (0.52)	7.12 (0.50)*
0.0067	-69.97 (9.78)	-75.47 (8.05)	4.45 (0.58)	5.46 (0.67)*
0.0089	-89.64 (12.67)	-89.61 (7.11)	4.48 (1.05)	4.40 (0.90)

* significant difference at $P<0.001$

DISCUSSION

The aim of this experiment was to investigate whether extending the work rate range of a PRBS test might induce appreciable non-linearities in the output response.

The results generated by PRBS1 are very similar to those obtained by Hoffmann et al. (1992 & 1994) using an identical PRBS test. This relationship suggests that the results of PRBS1 exhibit dynamic linearity.

The trend towards longer phase shifts in PRBS2 is in accordance with the study of Casaburi et al. (1989) amongst others. These investigators have identified a tendency for $\dot{V}O_2$ kinetics to be slower at higher work rates, even when the work rates are not associated with lactic acidosis.

In the lower frequency range (0.0022-0.0067 Hz) there were statistically significant differences between the amplitude parameters of PRBS1 and those of PRBS2. In a concurrent experiment (unpublished data) the intra-subject variability resulting from two identical PRBS tests was examined. The results of this experiment suggest that the statistically significant differences in amplitude may be attributable in part to inherent biological variability. However, it is also possible that dynamic linearity has been compromised by extending the range of work rates to those employed in PRBS2. Care should therefore be taken when quantitating $\dot{V}O_2$ kinetics obtained from PRBS tests.

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