The Control of the Exercise Hyperpnoea

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The experimental work required to produce the results included in this thesis were the product of my own work in the laboratory, aided where necessary by Mr John Wilson, Mrs Heather Collin, Mr Mattew Parker and Mr Anthony Turner. The composition of the written work is my own.

Andrew James Cathcart.

Abstract

Despite in excess of a century's searching, the key which will unlock the secrets of the exercise ventilatory control system remains an elusive aspiration. During moderate intensity (below the lactate threshold, $<\hat{\theta}_L$) sustained exercise the increase in ventilation ($\dot{V}_E$) is so closely matched to the pulmonary requirement to clear CO_2 ($\dot{V}CO_2$) that no classically defined error signal is manifest in the blood gas or acid-base composition of arterial blood (e.g. Casaburi *et al.*, 1977; Miyamoto, 1989; Wasserman et al, 1967; Whipp, 1981; Whipp *et al.*, 1982). The question therefore, is how ventilation is matched so accurately, not to the metabolic rate *per se*, but specifically to the rate of pulmonary CO_2 clearance (which is dissociated from metabolic rate by the transient storage of CO_2)?

During rapid incremental exercise tests this matching of $\dot{V}_E$ to $\dot{V}CO_2$ continues even beyond the lactate threshold; i.e. at a time when hyperventilation might be expected to provide respiratory compensation. This has been termed isocapnic buffering (Wasserman *et al.*, 1977). This cause of the remains unidentified, consequently, the time course of this response was investigated through a series of intermittent exercise protocols (Åstrand *et al.*, 1960a). The varying exercise-recovery duty cycle durations employed provided differing metabolic stress across varying durations of exercise, which allowed investigation of both the temporal and pH dependence of isocapnic buffering.

A similar (to the descriptions in the literature) sluggish or delay-like manifestation of respiratory compensation was observed, the magnitude of which increased with increasing $[La^-]_a$ (therefore presumably with decreasing pH_a) (Wasserman *et al.*, 1967). However, despite the relationship between $[La^-]_a$ and the magnitude of the hyperventilatory response no $[La^-]_a$ dependent threshold was evident (Whipp & Ward, 1991).

A relatively recent proposal suggests that a plastic element to the ventilatory control system might be held accountable for the experimentally observed matching of $\dot{V}_E$ to $\dot{V}CO_2$. That is, Martin and Mitchell (1993) reported that goats consistently over-breathed during treadmill exercise, following conditioning to treadmill exercise while breathing through an external dead space. This has been taken as evidence that the steady-state ventilatory response can be modulated.

Therefore, the existence of a similar mechanism in the human exercise hyperpnoea was investigated; replicating as closely as possible the study of Martin & Mitchell (1993). Furthermore, the requirement for such a mechanism was investigated by studying the control of the exercise hyperpnoea in the absence of such a 'learned-response'.

The results show no evidence of plasticity during phase II or phase III of the exercise ventilatory response under normal conditions. Furthermore, subjects devoid of any exercise experience were able to generate an appropriate, in terms of P_aCO_2 regulation, ventilatory response during sub-lactate threshold cycle

ergometer exercise. Therefore, under the conditions of the study, no requirement for an 'exercise-memory' in the genesis of a 'normal' exercise hyperpnoea could be found. In conclusion, there appears to be no primary role for respiratory plasticity in the control of the exercise hyperpnoea.

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otherwise dark and mystifying topic. But especially to Professor Ward, for her faith in me through some trying times, for her help, advice and for the cutting insight into exercise physiology and practising science.

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Definition of abbreviations

Variable	Description	Units
$\dot{V}_E$	Minute expired ventilation	l/min
$\dot{V}_I$	Minute inspired ventilation	l/min
$\dot{V}_A$	Minute alveolar ventilation	l/min
V_T	Tidal volume	l
V_D	Dead space volume	l
$\dot{V}_D$	Dead space ventilation	l/min
V_D/V_T	Dead space fraction of the breath	
B_f	Breathing frequency	breaths/min
$\dot{V}CO_2$	Pulmonary carbon dioxide output	l/min or ml/min
$\dot{V}O_2$	Pulmonary oxygen uptake	l/min or ml/min
$\dot{Q}CO_2$	Muscle carbon dioxide production	l/min or ml/min
$\dot{Q}O_2$	Muscle oxygen consumption	l/min or ml/min
RER	Respiratory exchange ratio	
RQ	Respiratory quotient	
$P_{ET}CO_2$	Partial pressure of end-tidal carbon dioxide	mmHg
$P_{ET}O_2$	Partial pressure of end-tidal oxygen	mmHg
P_ACO_2	Mean alveolar partial pressure of carbon dioxide	mmHg
P_aCO_2	Mean arterial partial pressure of carbon dioxide	mmHg
$CpaCO_2$	Content of CO_2 in pulmonary arterial blood	ml/100ml
$CpaO_2$	Content of O_2 in pulmonary arterial blood	ml/100ml
pH_a	Mean arterial pH	
S_aO_2	Mean arterial O_2 saturation	%
$[La^-]_a$	Arterial lactate concentration	mM

Parameter	Description	Units
τ	Fundamental time-constant	s
τ'	Mean-response time	s
δ	Delay time	s
$\phi 1, \phi 2, \phi 3$	Phase I, II and III of the pulmonary gas exchange and ventilatory response	
$\hat{\theta}_L$	Lactate threshold	l/min of $\dot{V}O_2$
θ_f	Fatigue threshold	l/min of $\dot{V}O_2$
$\dot{V}O_{2\,MAX}$	Maximum pulmonary oxygen uptake	l/min or ml/kg/min
$\mu\dot{V}O_2$	Peak pulmonary oxygen uptake	l/min or ml/kg/min
WR_{MAX}	Maximum work-rate	W

Acronym	Description
PRBS	Pseudo-random binary sequence

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Introduction

1.1 Background

Almost a century ago pioneering physiologists, Douglas, Geppert, Haldane, Krogh, Lindhard and Zeppert, seemingly stood on the brink of unraveling the secrets of the exercise hyperpnoea. Much was known about the response pattern of breathing during exercise, several convincing hypotheses had been proposed and awaited formal evaluation. However, those hypotheses are not dissimilar to the basic categories of hypotheses still under consideration today. For example Zuntz and Geppert (1886) proposed a series of hypotheses that included parallel activation of locomotion and respiration (the central command hypothesis), that excitation of nerve endings in the muscle may stimulate respiration (peripheral neurogenesis), excitation of nerve endings in the lung by the exercise altered blood composition may drive respiration (venous chemoreceptor hypothesis) and finally that an unidentified substance released as a by-product of exercise may simulate the respiratory centres (the substance X hypothesis, e.g. K^+).

In the intervening years work has refined these hypotheses uncovering more and more information regarding the ventilatory pattern of response during exercise and supporting the proposed control mechanisms. Many control structures have been proposed which attempt to combine these hypotheses in a functional framework, such as Dejours (1964) neurohumoral hypothesis whereby a combination of neural and humoral elements are suggested to account for the fast early rise in $\dot{V}_E$ (neural) and the secondary slow rise in $\dot{V}_E$ (humoral) and a combination of these maintain the increased $\dot{V}_E$ in the steady-state (figure 1.1).

However, as more and more evidence is uncovered, the layers enshrouding the secrets of the ventilatory control system have not been stripped away rather layers of complexity have been added. A century's worth of research has presented the field with detailed knowledge regarding the temporal response, and the requirements of ventilation during exercise. However, this known pattern of response is as yet seemingly incompatible with any known mechanism by which ventilation may be controlled.

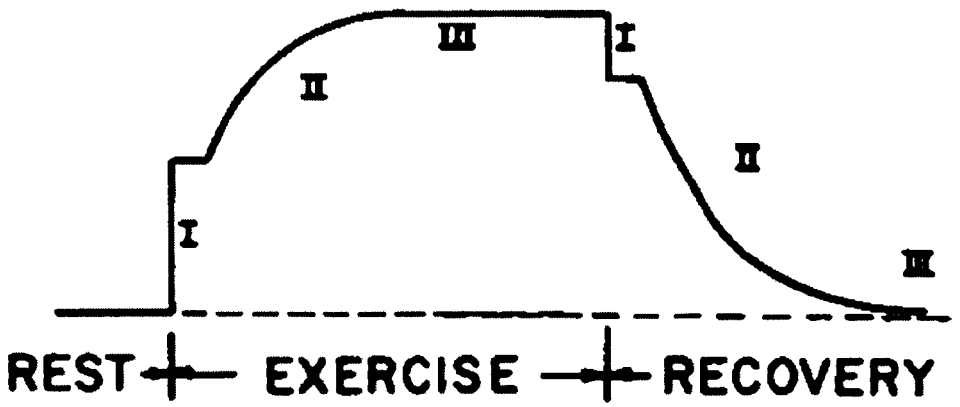


Figure 1.1: Dejours 'neurohumoral' scheme of the increase in ventilation, whereby phase I represents a fast neural response, phase II a slow humoral response and phase III a combination of phases I and II. Reproduced from Wasserman *et al.*, 1986.

The following sections of this introduction will provide a framework for interpreting the appropriateness of the ventilatory response in any given exercise situation, summarise the evidence supporting the existence of the potential mechanisms capable of driving $\dot{V}_E$ and finally review the experimental evidence to date supporting the involvement (or not) of these mechanisms in the control of the exercise hyperpnoea, with reference to that framework.

The traditional approach to investigating biological control systems involves challenging the controller while modulating the input of known, or putative, stimulants to the controller and then tracking the output response of the controller. For this approach to have any functional significance, it must be possible to accurately predict the correct response to any given challenge and therefore to know whether the system's response to augmentation or removal of a stimulus input mechanism is appropriate or not.

For the ventilatory control system there are four considerations which must be taken account of in order to predict the appropriate response to any given input. These are the intensity domain in which the exercise is being performed, control systems theory, the temporal response profiles of pulmonary gas exchange and ventilation and the ventilatory determinants (e.g. Whipp & Ward, 1980). These will be discussed in turn during the next four sections, and will provide the framework within which evidence will be interpreted.

1.2 Intensity Domains

The range of work-rates any given individual is capable of performing can be subdivided into a series of sequential intensity domains. These domains are characterised by differing metabolic requirements and their resultant effect on blood-gas and acid-base status (figure 1.2), pulmonary gas exchange and ventilation (figure 1.3) (Wasserman *et al.*, 1967; Wells *et al.*, 1957; Whipp, 1981).

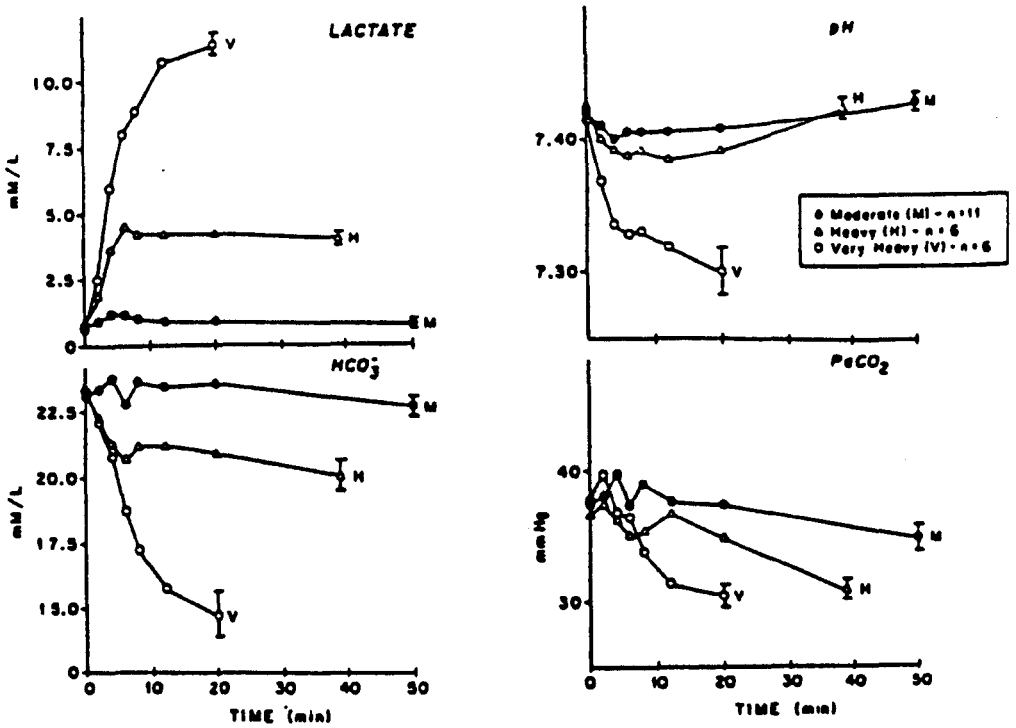


Figure 1.2: The changes in arterial blood gas and acid-base status during exercise in the moderate, heavy and very heavy intensity domains. Reproduced from Wasserman & Casaburi, 1991.

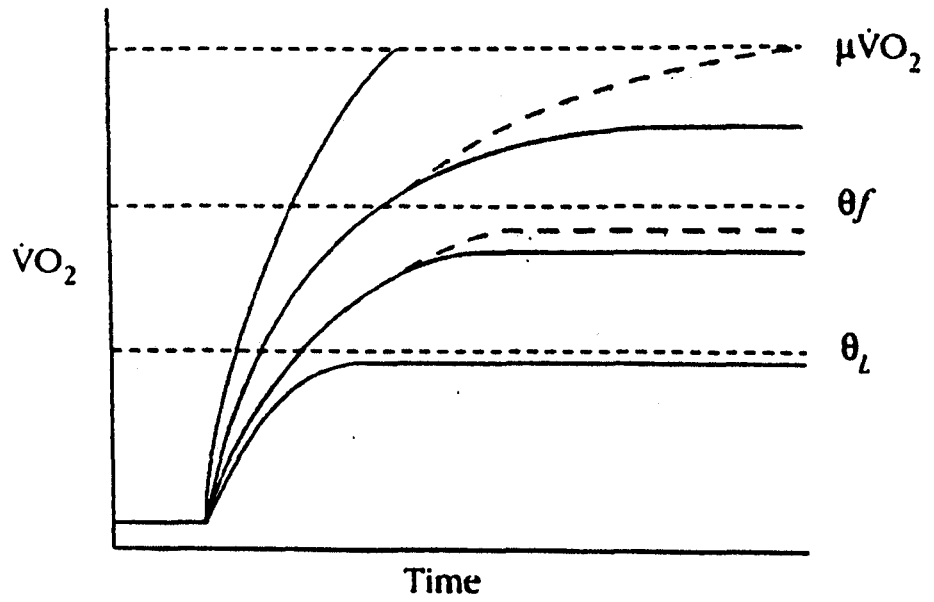


Figure 1.3: A schematic representation of the temporal response profiles of VO2 during the four different intensity domains demarcated by $\hat{\theta}_L$, $\hat{\theta}_F$ and $\mu\dot{V}O_2$. Reproduced from Whipp & Ozyener, 1998.

Work-rates lying below the lactate threshold ($\hat{\theta}_L$) are not associated with any sustained increase in arterial blood lactate concentration $[La^-]_a$ and lead to the attainment of a steady-state in all variables of interest (e.g. $\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}_E$) with resultant stability of P_aO_2, P_aCO_2 and pH_a at or close to resting levels (Assmusen & Nielsen, 1958; Dempsey *et al.*, 1984; Lugliani *et al.*, 1971; Wasserman *et al.*, 1975a) (figure 1.2, figure 1.3). Therefore, work-rates in this domain can be performed for prolonged periods of time, with fuel supply or psychological reasons being the primary cause of exercise cessation, this work-rate range has been termed "moderate" intensity (Wasserman *et al.*, 1967; Wells *et al.*, 1957; Whipp, 1981).

Above $\hat{\theta}_L$, there is a work-rate range that engenders a sustained rise in $[La^-]_a$ and $[H^+]_a$ that can eventually stabilise; this being associated with a delayed acquisition of a $\dot{V}O_2$ steady-state (Barstow & Molé, 1991; Casaburi *et al.*, 1987b; Hansen *et al.*, 1987; Paterson & Whipp, 1991; Poole *et al.*, 1988; Roston *et a.l*, 1987; Whipp & Ozyener, 1998). The highest work-rate in this "heavy" intensity domain (Wasserman *et al.*, 1967; Wells *et al.*, 1957; Whipp, 1981) has been termed the critical power (Moritani, 1981) or fatigue threshold (θ_F) (Poole *et al.*, 1988).

Above θ_F, $[La^-]_a$ and $\dot{V}O_2$ rise continuously throughout the exercise until $\dot{V}O_{2\,MAX}$ and the limit of tolerance are reached; this range being classified as very heavy or severe (see page 20 for discussion) (figure 1.3) (Poole *et al.*, 1988;

Roston *et al.*, 1987; Wasserman *et al.*, 1967; Whipp & Ozyener, 1998; Wells *et al.*, 1957; Whipp, 1981).

The detailed temporal response profile for each variable of interest will be discussed in depth in section 1.5 (page 13).

1.3 Temporal domains

During muscular exercise there is an initial short period, or phase I (ϕ1), within which mixed venous PO_2 and PCO_2 do not change and therefore pulmonary gas exchange during this period is influenced only by increasing pulmonary blood flow (i.e. cardiac output) (e.g. Whipp *et al.*, 1982). The following more-dominant phase II (ϕ2) represents the period in which the mixed venous gas tensions are changing and therefore provide an additional influence on pulmonary gas exchange (e.g. Whipp *et al.*, 1982). Finally, phase III (ϕ3) represents the period in which both cardiac output and mixed venous gas tensions stabilise at their new exercise steady-states, with resulting stabilisation also of ventilation and pulmonary gas exchange (e.g. Whipp *et al.*, 1982).

1.4 Control systems theory

1.4.1 Forcing Function

Five basic dynamic work-rate forcings have been utilised in the investigation of the exercise hyperpnoea, the step, ramp, sinusoid, impulse (all 'deterministic') and pseudo-random binary sequence (PBRS) ('stochastic') (Ward, 2000) (figure 1.4). Each function has an appropriate use depending on the characteristics of the response under scrutiny.

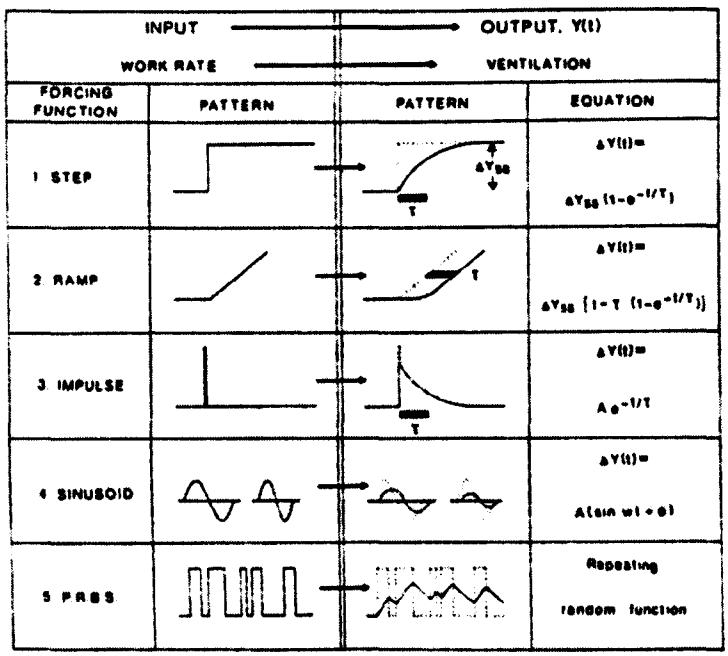


Figure 1.4: A Schematic representation of the 5 forcing functions, the input, and the expected response, the output, from a first order system. Reproduced from Whipp & Ward, 1980.

For a simple first order system with no delay (δ) a step forcing results in a simple exponential rise or fall of the form:

$$\Delta Y(t) = \Delta Y(ss)\,(1-e^{-t/\tau Y}) \qquad eq.\ 1.1$$

Where Y is the response variable (e.g. $\dot{V}_E$), Y(t) is the increase in Y above baseline, Y(ss) is the steady-state increase in Y and τY is the time constant for the response variable (Whipp & Ward, 1980). The direction of the response will depend on the direction of the step and the response τ will exhibit on-off symmetry (figure 1.4). Problems may arise as the subjects experience abrupt changes in work-rate, which increasingly becomes a problem as work-rate increases. Furthermore, if the system under scrutiny has rapid response kinetics, i.e. best stimulated by a high frequency forcing, only the actual step may be considered to be of high frequency (Ward, 2000).

The ramp function is a series of additive or subtractive steps, i.e. the ramp may be incremental (more commonly used) or decremental, and as such is the integral of the step (Whipp & Ward, 1980). For a linear system the response must therefore be the integral of the step response, i.e. a linear increase after an initial dynamic phase, thus the linear increase lags the steady-state increase by τ' (τ plus δ) (figure 1.4) (Whipp & Ward, 1980). Unlike the step, subjects are unaware of the individual work-rate changes but its repetitive nature makes it predictable (Ward, 2000). The input frequencies are, by the nature of a ramp, constrained to being low.

By contrast to the ramp the impulse function is the differential of the step, i.e. an infinite change in work-rate (for practical purposes this is usually positive and simply a large increase in work-rate ~400W) for an infinitely short time period (in practice ~10s) (e.g. Lamarra *et al.*, 1987b). Again for a linear system, as the input is the differential of a step input, the output must also be the differential of the step output, such that, after increasing during and immediately after the pulse the response is an exponential fall with a τ identical to a step (figure 1.4) (Whipp & Ward, 1980). As with a step function the abrupt work-rate change, necessarily of a large magnitude, may influence the response. The major factor in choosing the impulse is that its response (the control system output) occurs after the input ceases; in all other functions the input is operational while measuring the output response (Ward, 2000).

The sinusoidal function is exactly that, a sinusoidal variation of work-rate, the sine waves can be repeated as often as required. The output of a first order system will be sinusoidal with an identical frequency (ω) to the input function and a predictable peak to mean amplitude (A') = $(A/\sqrt{(1 + \tau^2\omega^2)})$ (where A is the system gain) and delay (ϕ) = $\tan^{-1} \tau/\omega$ (figure 1.4) (Whipp & Ward, 1980). The sinusoid allows the input frequency to be varied as appropriate, but its repeating nature makes it predictable (Ward, 2000). Furthermore, care must be taken in analysis as there is no separation of on- and off-transient responses thus resulting in lumped parameters. Therefore knowledge of system linearity is crucial before choosing this forcing function (Ward, 2000).

The PRBS function is achieved by switching randomly (in time) between two work-rates (pre-determined). The sequence is repeated after a number of switches (Whipp & Ward, 1980), hence the sequence is only pseudo-random. The output response is essentially a partial step output, therefore with an identical τ (figure 1.4). While it is only pseudo-random it is unlikely a subject would be able to predict work-rate changes. However, similar to a step and impulse, the abrupt work-rate changes may contaminate the output response (Ward, 2000). Furthermore, like the siniusoidal function, this work-rate forcing results in lumped parameters (Whipp & Ward, 1980).

For all forcing functions the 'noise' from random breath-to-breath fluctuations can be reduced, and the underlying response enhanced, by repetition and averaging of responses (Lamarra *et al.*, 1987a). The sinusoid and PBRS inherently provide repetition to allow this, for other functions repeat trials must be performed.

1.4.2 System Linearity

In terms of the ventilatory control system it is often considered that the work-rate forcing should be considered as the input and the ventilatory response as the output. The appropriateness of this consideration will be discussed later. The basic form of the input function that will be dealt with during these studies is the step, i.e. a square-wave imposition of increased work (see page 5) (figure 1.4).

For a first-order linear system, a doubling of the amplitude of the step-input function will result in a doubling of the steady-state output ventilation. The imposition of two separate inputs will result in a response that is the sum of the two inputs, and so forth. This is known as the Boltzmann principle of superposition (Milsum, 1966). Furthermore, once the output of a linear system to one input function is known, it is possible to predict the response to any other form of forcing function (see page 8).

The relationship between the work-rate input and the steady-state output ventilation has been classified as essentially linear, so long as the work-rate is below the lactate threshold (Casaburi *et al.*, 1979; Linnarsson, 1974; Miyamoto, 1992; Wasserman *et al.*, 1973; Whipp & Ward, 1991) and ignoring phase I (see section 1.5.1 for explanation) (Fujihara *et al.*, 1973). Therefore, while it is not a truly linear response, it approximates sufficiently to one that a variety of work-forcing functions may be applied, with or without additional manipulation of the controller, and the appropriate response predicted. Throughout this thesis where assumptions or deviations from linearity apply they are discussed in full. The particular function used for a given study is outlined in the individual experimental chapters, and the output response to the basic function under consideration, the step, is detailed in section 1.5 of this chapter.

1.5 Temporal response profile

Although the focus of this thesis is the control of ventilation it is still imperative to view $\dot{V}O_2$ as the underlying, readily measurable, metabolic factor dictating the requirement for increased ventilation during exercise. Therefore, considerations of the temporal response profile of $\dot{V}_E$ to dynamic exercise should take account of the corresponding $\dot{V}O_2$ response. The simplest response to consider is that to a square-wave forcing of dynamic exercise below $\bar{\theta}_L$ (i.e. moderate intensity) (Wasserman *et al.*, 1967; Wells *et al.*, 1957) (figure 1.5).

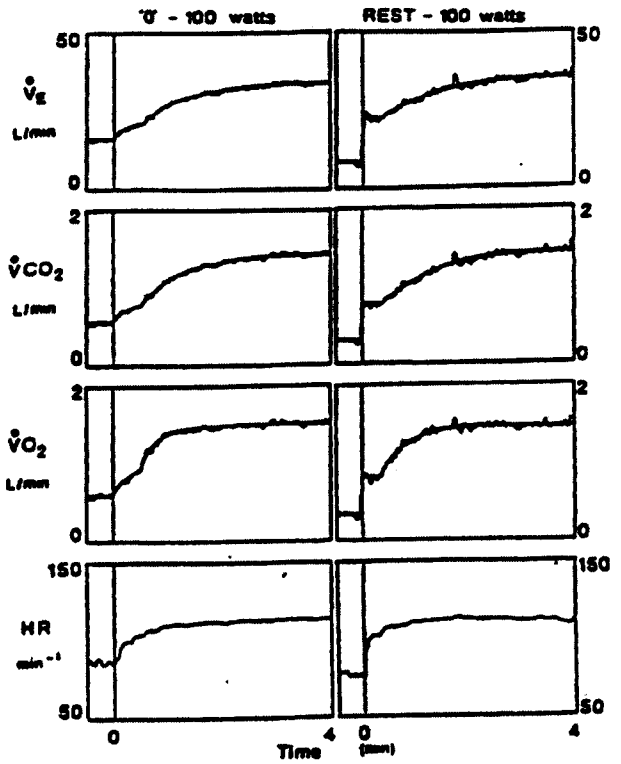


Figure 1.5: Ventilatory and cardiopulmonary responses to a square-wave forcing of work-rate, initiated either from a baseline of unloaded pedaling (left hand column) or prior rest (right hand column). Reproduced from Whipp *et al.*, 1982.

1.5.1 Below $\hat{\theta}_L$: Phase I (ϕ1)

The onset of exercise is typically accompanied by an abrupt increase in $\dot{V}_E$ within the first breathing cycle (D'Angelo & Torelli, 1971; Dejours, 1964; Jensen *et al.*, 1971; Krogh & Lindhard, 1913; Paulev, 1971; Ward, 1979b; Whipp *et al.*, 1971), followed by a plateau in $\dot{V}_E$ for the following 15-20s, until the onset of ϕ2 (Casaburi *et al.*, 1978; Dejours, 1964; Jensen, 1972; Miyamoto, 1989; Whipp *et al.*, 1982). This immediate increase in $\dot{V}_E$ is matched by equally abrupt increases in $\dot{V}O_2$ and $\dot{V}CO_2$ (Casaburi *et al.*, 1989; Krogh & Lindhard, 1913; Miyamoto, 1989; Whipp *et al.*, 1982), such that the respiratory exchange ratio (RER), and the partial pressures of end-tidal O_2 and CO_2 ($P_{ET}O_2$ and $P_{ET}CO_2$) do not change from rest across the course of ϕ1 (Casaburi *et al.*, 1978; Jensen *et al.*, 1971; Linnarsson, 1974; Ward, 1979b; Wasserman *et al.*, 1977; Whipp, 1977).

The increases in $\dot{V}O_2$ and $\dot{V}CO_2$ are unlikely to be due to exercise related changes in mixed venous PO_2 and/or PCO_2 ($P\bar{v}O_2$, $P\bar{v}CO_2$). This is because the venous effluent draining the working musculature at exercise onset is still some 10-15 seconds from reaching the lung (Barstow *et al.*, 1990; Casaburi *et al.*, 1987a; Whipp *et al.*, 1982). Rather, they are likely to represent an increase in pulmonary blood flow due to the abrupt increase in venous return and cardiac output ($\dot{Q}$) at exercise onset (Barstow *et al.*, 1990; De Cort *et al.*, 1991; Jones *et al.*, 1981; Krogh & Lindhard, 1913; Loeppky *et al.*, 1981; Weisman *et al.*, 1982). The magnitude of the ϕ1 ventilatory response is relatively unaffected by the

work-rate imposed (Dejours, 1963; Jensen, 1972; Pearce & Milhorn, 1977). Thus, the proportional contribution of $\phi 1$ to the overall ventilatory response is greatest at the lowest work-rate and declines as work-rate is increased. Indeed, the increase in $\dot{V}_E$ from rest to unloaded exercise is commonly achieved, almost totally, by the $\phi 1$ response (Assmusen, 1973).

When exercise is initiated in the supine posture, from a background of unloaded pedaling, or moderate intensity, rather than from rest the kinetics of the $\phi 1$ $\dot{V}O_2$, $\dot{V}CO_2$ and $\dot{V}_E$ responses are markedly slowed (Broman & Wigertz, 1971; Casaburi *et al.*, 1978; Weiler-Ravell *et al.*, 1983; Whipp *et al.*, 1982) (figure 1.5). In place of the abrupt first breath increase there is instead a gradual rise reaching a lower level than that achieved during transitions from rest (Asmusen, 1973; Whipp *et al.*, 1982).

This $\phi 1$ $\dot{V}_E$ response has been characterized as a first-order function, with a delay (δ) of less than one breath and a time constant (τ) of less than ten seconds (Bakker *et al.*, 1980; Bennett *et al.*, 1981; Fujihara *et al.*, 1973; Greco *et al.*, 1986; Hughson, 1990; Miyamoto, 1992; Swanson, 1978). However, such a model does not readily account for the abruptness of the $\phi 1$ $\dot{V}_E$ response from prior rest (Ward, 2000). Neither does it account for the $\phi 1$ response amplitude not being proportional to work-rate. No attempt, thus far, has been made to investigate potential competing model structures. Furthermore, the description of $\phi 1$ as first-order requires that the input to this exponential response be a square-wave, i.e. work-rate. The relative homogeneity of the $\phi 1$ $\dot{V}_E$, regardless of the

magnitude of the work-rate change, does not appear compatible with this. Therefore, at this stage it appears impossible to ascribe the status of first order system to the $\phi 1$ responses of $\dot{V}O_2$, $\dot{V}CO_2$ and $\dot{V}_E$, at least in the context of work-rate being the appropriate input.

1.5.2 Below $\hat{\theta}_L$: Phase II ($\phi 2$)

After a delay of some 15-20 seconds, commensurate with the transit delay between 'exercise' blood exiting the working muscle and reaching the alveolar capillary interface, a second slower phase of the $\dot{V}O_2$, $\dot{V}CO_2$ and $\dot{V}_E$ response is manifest at the mouth (Casaburi *et al.*, 1977; Hughson & Morrissey, 1982; Hughson, 1990; Linnarsson, 1974; Miyamoto, 1989; Whipp *et al.*, 1982). This transition from $\phi 1$ to $\phi 2$ is greeted not only by the arrival, at the lungs, of the metabolic out-pouring of the active musculature, but also by an uncoupling of the rates of change for $\dot{V}O_2$ and $\dot{V}CO_2$ (Whipp, 1977; Whipp & Ward, 1991; Whipp *et al.*, 1982).

$\dot{V}O_2$ increases as a first order, mono-exponential response (see equation 1.1) with a τ averaging ~30s, although highly variable, and a δ of 15-20s (Casaburi *et al.*, 1977; Hughson, 1990; Linnarsson *et al.*, 1974; Miyamoto, 1992; Whipp & Ward, 1991; Whipp *et al.*, 1982; Whipp *et al.*, 2002) (figure 1.5), thus a steady-state is achieved after roughly 2-3 minutes (estimate $4 \cdot \tau$ = 98% of response). The time course of this response has been shown to be quantitatively similar to that for

muscle O_2 consumption ($\dot{Q}O_2$), essentially lagging the response of the muscle by the transit delay (Barstow *et al.*, 1990). This is because there is relatively little intervening tissue capacitance for O_2 storage (Whipp, 1977).

While also responding as a simple mono-exponential, the $\phi 2$ $\dot{V}CO_2$ response is temporally dissociated, not only from that of $\dot{V}O_2$, but also from $\dot{Q}O_2$ and the muscle CO_2 production ($\dot{Q}CO_2$) (Whipp, 1977). This is due to the substantial capacity of the muscle and blood to store CO_2 (largely as bicarbonate). Therefore, following exercise onset, much of the metabolically produced CO_2 is added to the stores of CO_2 and is not evolved to the atmosphere during the on-transient of the exercise (Farhi & Rahn, 1955; Jones & Jurowski, 1979; Wasserman & Casaburi, 1991; Whipp, 1977; Whipp & Ward, 1991). Thus the $\phi 2$ kinetics for $\dot{V}CO_2$ are markedly slower than for $\dot{V}O_2$ with a τ averaging ~50-60s, although with a similar δ of 15-20s (Casaburi *et al.*, 1977; Hughson, 1990; Hughson & Morrissey, 1982; Linnarsson *et al.*, 1974; Miyamoto, 1992; Whipp & Ward, 1991; Whipp *et al.*, 1982) (figure 1.5).

For situations, such as this, when $\dot{V}_E$ cannot be appropriately matched to both the demand for O_2 uptake and CO_2 output, regulation of P_aCO_2 and hence pH_a is normally the primary goal (see page 23 for discussion). That is, the $\phi 2$ $\dot{V}_E$ response is closely linked to the slower $\phi 2$ $\dot{V}CO_2$ response, although with $\dot{V}CO_2$ slightly leading $\dot{V}_E$ (figure 1.5) (Casaburi *et al.*, 1977; Miyamoto, 1992; Whipp *et al.*, 1982; Whipp & Ward, 1991). This is likely to result in a small but

observable error in the regulation of P_aCO_2 and hence pH_a (figure 1.2) (Lamarra *et al.*, 1989; Whipp *et al.*, 1978; Whipp *et al.*, 1989). The $\phi 2$ $\dot{V}_E$ kinetics are also well described by a first order mono-exponential with a τ averaging ~55-65s and a δ of 15-20s (Broman & Wigertz, 1971; Hughson, 1990; Linnarsson, 1974; Miyamoto, 1989; Whipp *et al.*, 1982). The longer τ for $\dot{V}CO_2$ and $\dot{V}_E$ (relative to that of $\dot{V}O_2$) means that they will not attain a steady-state until at least four minutes after exercise onset.

1.5.3 Below $\hat{\theta}_L$: Phase III ($\phi 3$)

Once the storage of CO_2 has attained stability, meaning the exercise-induced increase in $\dot{Q}CO_2$ matches that of $\dot{V}CO_2$, $\dot{V}CO_2$ will reach a steady-state (figure 1.5). Shortly thereafter $\dot{V}_E$ will reach a steady-state essentially appropriate to both $\dot{V}O_2$ and $\dot{V}CO_2$, thereby effecting regulation of the arterial blood-gas tensions and acid base status (Asmussen & Nielsen, 1958; Jones, 1975; Lamb *et al.*, 1965; Lugliani *et al.*, 1971; Masson & Lahiri, 1974; Sutton *et al.*, 1976; Wasserman *et al.*, 1975; Whipp & Wasserman, 1969). This stability in $\dot{V}_E$ is maintained until another input, such as a work-rate change or a shift in substrate utilisation in prolonged exercise, perturbs the equilibrium.

During the corresponding off-transient from exercise the changes seen in $\dot{V}O_2$, $\dot{V}CO_2$ and $\dot{V}_E$ are both quantitatively and qualitatively similar although directionally opposite to the on-transient, i.e. the system is dynamically linear

(Miyamoto, 1989; Whipp & Ward, 1990; Whipp *et al.*, 1982) (see page 12) (figure 1.5).

1.5.4 Above $\hat{\theta}_L$

As the $\phi 1$ response is physically dissociated from the metabolic changes underpinning the onset of exercise, the metabolic acidosis associated with supra-$\hat{\theta}_L$ exercise would be expected to have little effect on the changes seen in $\dot{V}O_2$, $\dot{V}CO_2$ and $\dot{V}_E$ during $\phi 1$.

Marked deviations from the sub-$\hat{\theta}_L$ responses are evident in the phase II responses of $\dot{V}O_2$, $\dot{V}CO_2$ and $\dot{V}_E$, although this has only been formally characterised for $\dot{V}O_2$ (e.g. Ozyener *et al.*, 2001). The time course of $\dot{V}O_2$ begins following a similar kinetic pattern to sub-$\hat{\theta}_L$ exercise that appears to be heading to a steady-state commensurate with the absolute work-rate (Barstow & Molé, 1991; Ozyener *et al.*, 2001; Paterson & Whipp, 1991; Whipp, 1987). However, prior to reaching the $\phi 3$ plateau $\dot{V}O_2$ begins a secondary increase - the slow component (figure 1.3) (Casaburi *et al.*, 1987b; Hansen *et al.*, 1987; Ozyener *et al.*, 2001; Poole *et al.*, 1988; Roston *et al.*, 1987; Barstow & Molé, 1991; Paterson & Whipp, 1991). If the exercise is below the fatigue threshold (θ_F) this will reach a quasi-steady-state, greater than the predicted O_2 cost for the work-rate (Poole *et al.*, 1988; Roston *et al.*, 1987; Whipp & Ozyener, 1998). Otherwise the slow component will asymptote at the maximum rate of O_2 uptake ($\mu\dot{V}O_2$)

with the limit of tolerance being reached shortly thereafter (Poole *et al.*, 1988; Roston *et al.*, 1987; Whipp & Ozyener, 1998; Ozyener, *et al.*, 2001).

As mentioned earlier (see page 6) the domain lying above θ_F can be further subdivided. Work-rates where the predicted $\dot{V}O_2$ steady-state is below $\mu\dot{V}O_2$ but the slow component drives $\dot{V}O_2$ to $\mu\dot{V}O_2$ are known as very-heavy intensity (Poole *et al.*, 1988; Roston *et al.*, 1987; Whipp & Ozyener, 1998). Work-rates where the predicted O_2 steady-state lies above $\mu\dot{V}O_2$ are described as severe intensity (Whipp & Ozyener, 1998) (see figure 1.3).

Interpretation of the $\phi 2$ $\dot{V}CO_2$ response becomes even more complex as the metabolically generated CO_2, in proportion to the $\dot{V}O_2$ response outlined above, is supplemented by extra non-metabolic CO_2 generated by bicarbonate buffering of a proportion of the protons associated with lactate production (see page 27 for discussion and equation 1.4) (Beaver *et al.*, 1986; Hickham *et al.*, 1961; Naimark *et al.*, 1964; Owles, 1930; Wasserman & Casaburi, 1991; Wasserman *et al.*, 1967; Wasserman *et al.*, 1973). Furthermore, the falling pH_a is constrained by hyperventilation, potentially H^+ mediated (see page 66 for discussion), evolving CO_2 from arterial stores and therefore driving down P_aCO_2 (see equation 1.1) (Rausch *et al.*, 1991; Sutton & Jones, 1979; Wasserman & Casaburi, 1991; Wasserman & Whipp, 1975; Wasserman *et al.*, 1967).

As the CO_2 output is a combination of three discrete, although interacting, components predicting and modelling its response is an order of magnitude more

complicated than for sub-$\hat{\theta}_L$ exercise. However, while assigning specific, predictable parameters to the response is not possible without further investigation, the general patterns of response are definable. If the exercise is sub-θ_F $\dot{V}CO_2$ may attain a steady-state greater than predicted (on a sub-$\hat{\theta}_L$ basis) (Casaburi *et al.*, 1989) (figure 1.6). This can occur despite an elevated arterial lactate concentration, as it is the *rate* of change of $[La^-]_a$ that affects $\dot{V}CO_2$ rather than absolute $[La^-]_a$ (see page 27 for discussion) (e.g. Douglas, 1927, Whipp, 1981).

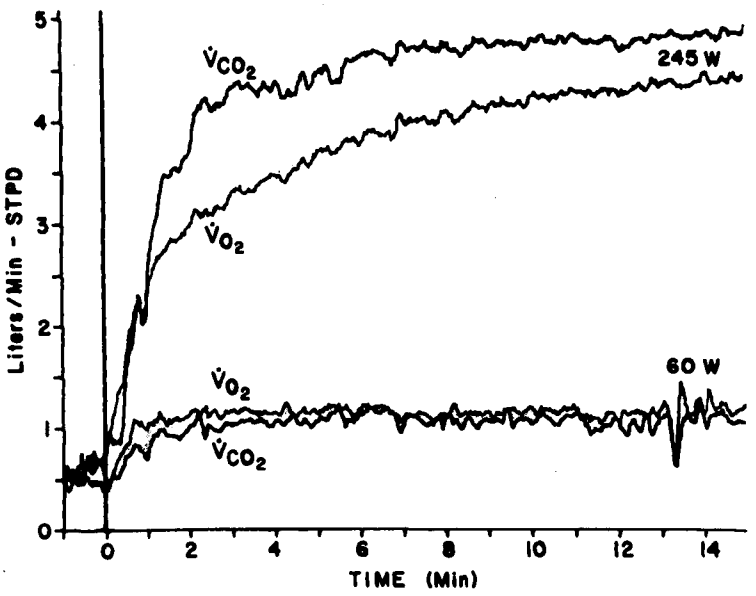


Figure 1.6: The disparate responses of $\dot{V}O_2$ and $\dot{V}CO_2$ during sub-$\hat{\theta}_L$ (60W) and supra-$\hat{\theta}_L$ (245W) exercise. Reproduced from Casaburi et al., 1989.

Above $\hat{\theta}_L$ the $\phi 2$ $\dot{V}_E$ does not reach a clear $\phi 3$ steady-state. This is due to the demand to clear CO_2, which itself does not terminate in a classical sub-$\hat{\theta}_L$ steady-state, being super-imposed by a hyper-ventilatory drive to constrain the falling pH_a (see page 23 for discussion). Therefore, if the exercise is sub-θ_F the

temporal response of $\dot{V}_E$ is a continual slow upward drift, presumably in response to the acidosis. If the work-rate lies in the domain above θ_F, it will lead to a continual steep increase in $\dot{V}_E$, again terminating when $\mu\dot{V}O_2$ is reached (Linnarsson, 1974; Wasserman *et al.*, 1981; Whipp & Mahler, 1980). Incidentally, while the limit of tolerance is not normally associated with a pulmonary limitation, if the individual in question is an elite endurance athlete, the limit of tolerance may coincide both with $\dot{V}O_{2\,MAX}$ and the maximum voluntary ventilation (MVV) (Grimby *et al.*, 1971;Klas & Dempsey, 1989; Sue & Hansen, 1984).

An interesting phenomenon is that the hyperventilation, which mediates respiratory compensation, is delayed relative to the onset of acidosis during rapidly incrementing ramp trials - termed isocapnic buffering (Wasserman *et al.*, 1977). This is in contrast to slow incrementaton rates, i.e. allowing the subject to attain a steady-state at each increment, where respiratory compensation is seen immediately at the lactate threshold ($\hat{\theta}_L$) (Wasserman *et al.*, 1977; Wasserman & Whipp, 1975).

1.6 Ventilatory determinants

The second consideration in determining the appropriateness of the ventilatory response, post a given perturbation of the system, is to know the outcome goals of the system and be able to measure them. In any situation where $\dot{V}O_2$ and

$\dot{V}CO_2$ are dissociated from each other, e.g. during exercise transients (Hughson & Morrissey, 1982; Linnarsson, 1974; Miyamoto, 1989; Whipp *et al.*, 1982), prior hyperventilation (Ward *et al.*, 1983) or substrate alterations (Brown *et al.*, 1985; Putman *et al.*, 1993; Taylor & Jones, 1979), it is clearly impossible for ventilation to be correctly matched to both. That is, ventilation may be set to accurately regulate either P_aO_2 or P_aCO_2 but not both.

The consequences of small errors in the regulation of P_aO_2 are minimal in healthy subjects at sea level. The oxy-haemoglobin dissociation curve remains relatively flat over the physiological range of arterial partial pressures, therefore the O_2 content of arterial blood (CaO_2) will be little affected by small changes in PO_2. However, the same is not true of CO_2, the effect of a regulatory error will be manifest in the regulation of pH_a, as can be seen from the Henderson-Hasselbalch equation:

$$pH_a = pK + \log \frac{[HCO_3^-]_a}{\alpha \,.\, P_aCO_2} \qquad eq.\ 1.2$$

where α is the solubility coefficient for CO_2 in blood and pK is the dissociation constant. Therefore, the variable of choice for accurate control would seem to be $\dot{V}CO_2$ (or a proxy of $\dot{V}CO_2$) hence ensuring regulation of P_aCO_2 and pH_a, this train of thought is supported by a long line of evidence (e.g. Casaburi *et al.*, 1977; Jones, 1975; Ward *et al.*, 1983; Wasserman *et al.*, 1967; Whipp & Ward, 1991) (figure 1.7).

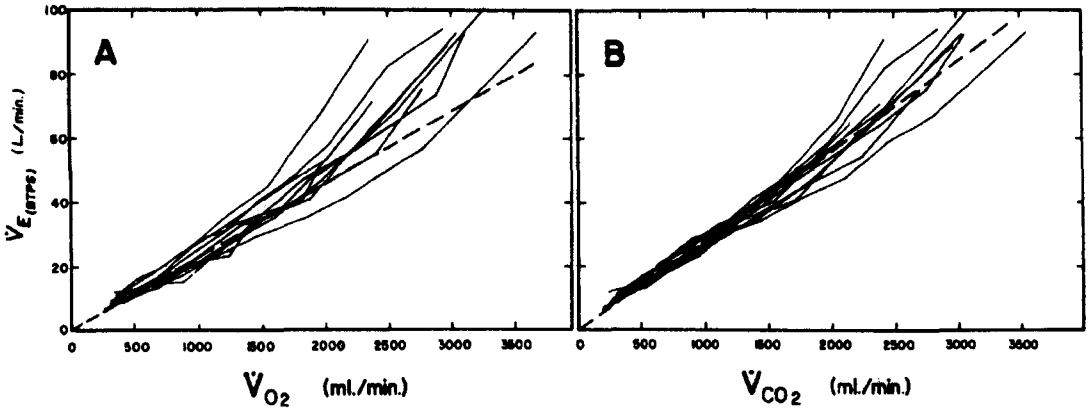


Figure 1.7: The relationship between $\dot{V}_E$ and $\dot{V}O_2$ and between $\dot{V}_E$ and $\dot{V}CO_2$ during exercise, revealing the closer association between $\dot{V}_E$ and $\dot{V}CO_2$. Reproduced from Wasserman *et al.*, 1967.

As the regulation of P_aCO_2 is the goal of the ventilatory control system, the ventilatory demands of a given exercise work-rate should be viewed with respect to: (1) P_aCO_2 set point, (2) pulmonary CO_2 clearance and (3) the dead space fraction of the breath (V_D/V_T) (e.g. Whipp, 1981).

1.6.1 P_aCO_2 set point

While P_aCO_2 is regulated at or very close to resting levels (i.e. ~40mmHg) during the steady state of moderate intensity exercise ($<\hat{\theta}_L$) (Asmussen & Nielsen, 1958; Jones, 1975; Lamb *et al.*, 1965; Lugliani *et al.*, 1971; Masson & Lahiri, 1974; Sutton *et al.*, 1976; Wasserman *et al.*, 1975), the falling pH_a associated with supra-$\hat{\theta}_L$ exercise requires a different response. In this circumstance hyperventilation, termed respiratory compensation, drives down P_aCO_2 in an attempt to constrain the falling pH_a (Rausch *et al.*, 1991; Sutton & Jones, 1979; Wasserman & Casaburi, 1991; Wasserman & Whipp, 1975;

Wasserman *et al.*, 1967) (see equation. 1.2) and thus prolong the tolerable duration of the exercise.

1.6.2 Pulmonary CO_2 clearance

The response of $\dot{V}_E$ during exercise must be proportional to the rate of pulmonary CO_2 clearance, in order to maintain P_aCO_2 at its required level (see above), rather than the actual level of work-rate or the metabolic rate (taken as $\dot{V}O_2$) (Douglas, 1927; Hansen *et al.*, 1972; Jones & Haddon, 1973; Jones *et al.*, 1965; Jones, 1975; Jones, 1976; Saltzman & Salzano, 1971). This can be described, in terms of alveolar ventilation, as:

$$\dot{V}_A = \frac{863 \cdot VCO_2}{P_ACO_2} \qquad eq.\ 1.3$$

where 863 is the constant used to correct for the convention to express ventilatory variables at body temperature and pressure saturated (BTPS), gas volumes at standard temperature (0°C) and pressure (one atmosphere) dry (STPD) and the conversion of fractional gas concentrations into partial pressures. The proportionality of $\dot{V}_E$ to $\dot{V}CO_2$ is important, because, under several circumstances $\dot{V}CO_2$ can become dissociated from both the work-rate and $\dot{V}O_2$. As discussed earlier (see page 17), during on- and off-exercise transients, the capacity of the muscle and blood to store CO_2 (but not O_2 to any great extent) means that the rate of pulmonary CO_2 clearance becomes dissociated from the rate of metabolic CO_2 production (Linnarsson, 1974; Pearce & Milhorn, 1977;

Rahn & Fenn, 1955; Wasserman *et al.*, 1977; Whipp *et al.*, 1980; Whipp *et al.*, 1982). The rates of metabolic O_2 utilisation and pulmonary O_2 uptake however are similar, only separated by the temporal tissue-to-lung transit delay. Therefore, $\dot{V}CO_2$ will be dissociated from $\dot{V}O_2$, with $\dot{V}CO_2$ slower during both transients, until both reach a steady-state and the (pulmonary) respiratory exchange ratio (RER) equals the (metabolic) respiratory quotient (RQ).

Furthermore, shifts in substrate utilisation, usually from carbohydrate to fat, across a prolonged exercise period at a given work-rate will see a slow reduction in the rate of CO_2 production. The oxidative metabolism of lipids will result in a reduction in CO_2 production of around forty percent, relative to carbohydrate metabolism, thereby reducing the ventilatory requirement to clear CO_2 and maintain P_aCO_2 at its resting level (Whipp, 1998; Whipp & Ward, 1998). However, while this would seem to make lipids a far more efficient substrate to utilise, the negative aspect is an increased O_2 cost of around six percent, relative to carbohydrate utilisation (Whipp *et al.*, 1998; Whipp & Ward, 1998). Remembering that $\dot{V}_E$ is set to the now reduced CO_2 clearance requirement, the consequence of this is that $\dot{V}_E$ must now be low for the O_2 requirement and will engender a reduced alveolar and hence arterial PO_2 (Hansen, *et al.*, 1972; Whipp, 1998). However, the effect is likely to be trivial, unless the subject is already hypoxaemic (e.g. at high altitude, or with lung disease), with little detrimental consequence as P_aO_2 will remain on the relatively flat portion of the oxy-haemoglobin dissociation curve. Thus, the unloading of O_2 to the tissue will be more or less unaffected.

An additional complexity in interpretation comes for work-rates burdened by a lactate driven metabolic acidosis. The proportion of protons produced in association with the lactate anion which are buffered, predominantly (~90%) by bicarbonate anions (HCO_3^-) both in the muscle ($KHCO_3$) and in the blood ($NaHCO_3$), lead to an increased non-metabolic production of CO_2 (Hickman *et al.*, 1961; Naimark *et al.*, 1964; Owles, 1930; Wasserman & Casaburi, 1991; Wasserman *et al.*, 1967; Wasserman *et al.*, 1973) (equation. 1.4).

$$CH_3CHOHCOO^- + H^+ + xHCO_3 \leftharpoons CH_3CHOHCOOx + H_2CO_3 \leftharpoons CO_2 + H_2O \quad eq.\ 1.4$$

A note of caution, as $\dot{V}CO_2$ is the *rate* of pulmonary CO_2 clearance it is the *rate* of lactate production, and hence the *rate* of increase in $[H^+]$ and the *rate* of fall in $[HCO_3^-]$, rather than the absolute concentration of lactate which determines the increase in the *rate* of CO_2 clearance (e.g. Douglas, 1927, Whipp, 1981).

To further cloud the issue when interpreting the response of $\dot{V}_E$ during situations when the above scenario is applicable the falling pH_a, which is partially constrained by HCO_3^- buffering, requires a further stimulation of $\dot{V}_E$ above and beyond the requirement to clear CO_2 (Sutton & Jones, 1979; Wasserman & Casaburi, 1991; Wasserman & Whipp, 1975; Wasserman *et al.*, 1967). The effect of this is to drive down P_aCO_2 and thus limit the fall in pH_a (see P_aCO_2 set-point for discussion). Therefore, except for a brief phase (isocapnic buffering) during rapidly incrementing ramp tests (see page 22 for discussion), $\dot{V}_E$ above the

lactate threshold has to respond to the metabolic CO_2 production commensurate with the metabolic cost of the work-rate, the extra non-metabolic CO_2 from HCO_3^- buffering of the metabolically-derived proton and hyperventilation to effect respiratory compensation for the metabolic acidosis. Thus, quantitative interpretation of what the appropriate ventilatory response to a given supra-$\hat{\theta}_L$ intensity exercise bout should be is at the very least complicated and at worst impossible.

1.6.3 The dead space fraction of the breath (V_D/V_T)

Finally, it must be remembered that the tidal volume (V_T) must be sufficient not only to clear its CO_2 requirement. That is, it is necessary to ventilate not only the exchanging alveoli, but also the dead space and hence clear the alveolar expirate to the atmosphere. Therefore, the requirement for total ventilation is the alveolar requirement to clear CO_2, as discussed above, plus the dead space ventilation:

$$\dot{V}_E = \dot{V}_A/(1 - V_D/V_T) \qquad eq.\ 1.5$$

During exercise, the increased pulmonary artery pressure will normally increase perfusion throughout the lung, especially at the apex the site of the greatest underperfusion at rest and hence the site of the greatest alveolar dead space. The effect of this is to reduce the alveolar contribution to the total dead space (Jones *et al.*, 1966). Conversely, increased diameter of the conducting airways may mean the anatomical dead space component is increased. This is usually more than offset, however, by greater increases in tidal volume, thus the overall effect

is to reduce V_D/V_T with increasing work-rates (Higgs *et al.*, 1967; Jones *et al.*, 1966; Wasserman *et al.*, 1967; Whipp and Wasserman, 1970).

Substituting equation 1.5 into equation 1.3 allows the level of ventilation to be viewed in terms of the constituent requirements outlined above:

$$\dot{V}_E = \frac{863 \,.\, VCO_2}{P_ACO_2 \,.\, (1 - V_D/V_T)} \qquad \textit{eq.}\ 1.6$$

The potential for differences in the requirement for $\dot{V}_E$ despite similar metabolic rate (i.e. $\dot{V}O_2$) in two different subjects can be seen in figure 1.8 (Whipp, 1996).

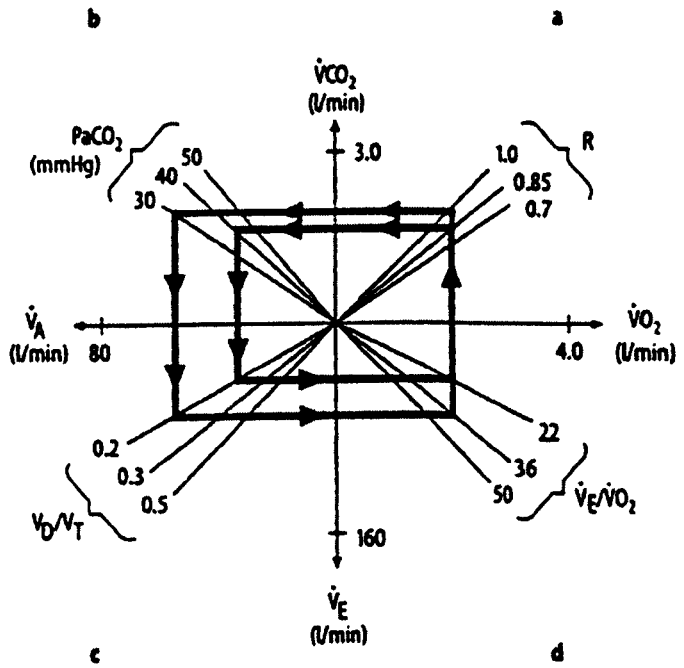


Figure 1.8: Demonstrates the effect different ventilatory determinants can have on the ventilatory response of two individuals exercising at the same VO_2. Reproduced from Ward & Whipp, 1996.

Therefore, when viewing the results of a ventilatory control experiment the simple demonstration of an altered ventilatory response is not adequate, the analysis must take account of the ventilatory response with regard to its requirements (equation 1.4) and the linearity typically displayed by the system.

1.7 Putative Ventilatory Stimulants

There is a range of mechanisms which have been demonstrated to have the potential to drive $\dot{V}_E$ during exercise. These are, on the one hand neurogenic, e.g. central command muscle reflexes and cardiovascular linkages, and on the other hand humoral, e.g. central and peripheral chemoreception. The evidence supporting the existence of each of these mechanisms will be described (section 1.6) and then the potential role each of these mechanisms may play in the control of the exercise hyperpnoea will be discussed in turn for each temporal phase (section 1.7).

1.7.1 Central Neurogenic

From the earliest studies on respiration during exercise there has been a proposal that higher neural centres capable of initiating locomotion could activate, in parallel, the respiratory centres and hence drive ventilation in proportion to metabolic rate (Zuntz & Geppert, 1886; Krogh & Lindhard, 1913). This hypothesis has subsequently been supported by numerous experimental models,

based largely around the decorticate animal, in which 'proportional' (e.g. to treadmill speed though not to $\dot{V}CO_2$) rises in $\dot{V}_E$ from stimulation of the paraventricular hypothalamic locomotor region (e.g. Orlovskii, 1969) and H2 fields of Forel (e.g. Smith *et al.*, 1960) have been reported. More recently, in humans, Fink *et al.* (1995) have demonstrated parallel activation of the motor cortex and respiratory related regions. Therefore, it appears that the genesis of locomotion is capable of providing an increased drive to $\dot{V}_E$.

1.7.2 Peripheral Neurogenic

A series of experiments by Harrison *et al.* (1932) led them to the conclusion that afferent reflexes from the exercising muscle and joints drove the exercise hyperpnoea. Many groups have since demonstrated that 'exercise' induced by peripheral neuromuscular stimulation results in an increased ventilation (e.g. Comroe & Schmidt 1943). The work of McCloskey and Mitchell (1972) and Kao (1963) demonstrated that stimulation of the group III and IV afferents, but not those of groups I and II, mediated this increased $\dot{V}_E$. The group III and IV fibers transmit information from cutaneous receptors, joint receptors and from 'free' nerve endings. While the contention that only group III and IV afferents are involved is widely accepted, there is a degree of controversy.

Direct stimulation of group I and II afferents has been shown to elicit reflex ventilatory responses (Bessou *et al.*, 1959; Carcassi *et al.*, 1983; Carcassi *et al.*, 1984; Koizumi *et al.*, 1961; Orani & Decandia, 1990; Senapati, 1966), however,

equally many experimenters find that such stimulation elicits no response (Johannson, 1962; Kumazawa & Tadaki, 1983; Mitchell *et al.*, 1968; Pitetti *et al.*, 1989; Rybicki & Kaufman, 1985; Sato *et al.*, 1981; Skoglund, 1960; Tallarida *et al.*, 1981; Tallarida *et al.*, 1983; Tallarida *et al.*, 1985). Therefore, either the stimulation paradigms used to activate group I and II afferents which elicit ventilatory responses may also recruit group III afferents, or group I and II afferents may play some minor role in reflexly driving $\dot{V}_E$.

There have been numerous proposals that a muscle chemoreflex may contribute to the peripheral neurogenic drive. However, while the free nerve endings of group IV afferents are undoubtedly chemosensitive there is much experimental data which does not support the contention of a specific muscle chemoreceptor (Asmussen & Chiodi, 1941; Brown *et al.*, 1976; Comroe & Schmidt, 1943; Dejours *et al.*, 1955; Gautier, 1969; Haouzi *et al.*, 1993; Haouzi *et al.*, 1997; Huszczuk *et al.*, 1993; Innes *et al.*, 1989; Moore *et al.*, 1934; Rowell *et al.*, 1976; Waldrop & Stremel, 1989).

1.7.3 Central and peripheral chemoreception

That the peripheral and central chemoreceptors respond to hypercapnia (Bellville *et al.*, 1979; Biscoe *et al.*, 1970; Heymans, 1929; Leusen, 1954; Von Euler & Soderberg, 1952) and that the peripheral chemoreceptors relay information regarding hypoxia (Lugliani *et al.*, 1971b; Wade *et al.*, 1970) and metabolic acidosis (Wasserman *et al.*, 1975; Winterstein & Gokhan, 1953) is essentially

unchallenged. The aortic bodies, in man, however, appear to be relatively unimportant in the control of breathing (Lugliani *et al.*, 1971a; Swanson *et al.*, 1978; Wade *et al.*, 1970).

1.7.3.2 Oscillation Hypothesis

Perhaps most intriguing of all however, is the demonstration that while the mean P_aCO_2 remains unchanged, the carotid bodies are capable of transducing information from the respiratory cycle based intra-breath oscillations of H^+-P_aCO_2 (Band *et al.*, 1980; Black & Torrance, 1971; Yamamoto, 1960). More specifically, it appears that the response is to the rate of change of pH_a during the falling phase (e.g. Cross *et al.*, 1982a).

Furthermore, it has been shown that the respiratory phase in which the carotid body stimulus is received affects the magnitude of the stimulatory outcome. Therefore, stimulation reaching the carotid bodies during expiration produced little or no ventilatory response; in comparison the same stimulus received during inspiration eliciting a marked response (Black & Torrance, 1967; Dutton *et al.*, 1967; Eldridge, 1972; Eldridge, 1976a; Cross *et al.*, 1979). Therefore, it has been proposed that a shift to a more sensitive phase receiving the H^+-P_aCO_2 oscillation signal might induce the hyperpnoeic response during exercise (Black & Torrance, 1971; Black *et al.*, 1973), this has been termed 'phase-coupling' (Whipp, 1981).

1.7.3.2 Other Stimuli

Also of interest are the other metabolites that are affected by exercise and have the ability to stimulate the known chemoreceptors. The peripheral chemoreceptors are known to be sensitive to changes in potassium concentration (Band *et al.*, 1985; Linton & Band, 1985; Burger *et al.*, 1988; Paterson & Nye, 1988), temperature (Cotes, 1955; Cunningham & O'Riordan, 1957), adenosine (McQueen & Ribiero, 1981; Griffiths *et al.*, 1990), blood pressure (Mills & Sampson, 1969), catecholamines (Cunningham *et al.*, 1958; Joels & White, 1968) and osmolality (Gallego *et al.*, 1979).

1.7.4 Cardio-dynamic

A component of the control of the exercise hyperpnoea has been argued to reside in the changes in cardiac output with exercise onset (Wasserman *et al.*, 1974). Experimental increases in cardiac output resulted in an increased $\dot{V}_E$ (Wasserman *et al.*, 1974; Juratsch *et al.*, 1982). This mechanism may operate either via a feedforward or a feedback mechanism. A feedforward mechanism has been shown by Jones *et al.* (1982), who demonstrated a reflex from receptors in the right ventricular wall, sensing right ventricular load, capable of stimulating $\dot{V}_E$. Similar reflexes have also been demonstrated by Kostreva *et al.* (1979) and by Uchida (1976), as have reflexes from the pulmonary artery (Ledsome, 1977). A feedback mechanism has been proposed involving vagal pulmonary stretch receptors, which respond to low CO_2 to decrease $\dot{V}_E$ (Banzett *et al.*, 1978;

Bartoli *et al.*, 1974). The onset of exercise will increase perfusion in some previously under-perfused areas of the lung thus reducing the stimulation of the CO_2-sensitive receptors and increasing the drive to $\dot{V}_E$.

1.8 The potential Role for each mechanism in a control scheme

1.8.1 Phase I

1.8.1.1 Peripheral and Central Chemoreception

There is no evidence available to lend any support for a humorally mediated phase I: The changes in $\dot{V}O_2$ and $\dot{V}CO_2$ during phase I are functionally isolated from the changing chemical environment of the muscle and its venous drainage, instead they are influenced by the increasing pulmonary perfusion. Not only are any classical sites of chemoreception, the carotid bodies and the ventro-lateral medullary surface, physically separated from the chemistry of exercise both at the lung and at the muscle but the ventilatory response at the onset of exercise appears proportional to $\dot{V}O_2$ and $\dot{V}CO_2$, rather than $\dot{Q}O_2$ and $\dot{Q}CO_2$. This can be seen from stable $P_{ET}O_2$, $P_{ET}CO_2$ and RER during phase I (figure 1.9) (Casaburi *et al.*, 1978b; Whipp *et al.*, 1982). A control scheme whereby $\dot{V}_E$ was controlled by an as yet unconfirmed chemoreceptor according to the O_2 consumption and CO_2 production in the muscle would not produce a $\dot{V}_E$ proportional to $\dot{V}O_2$ and $\dot{V}CO_2$.

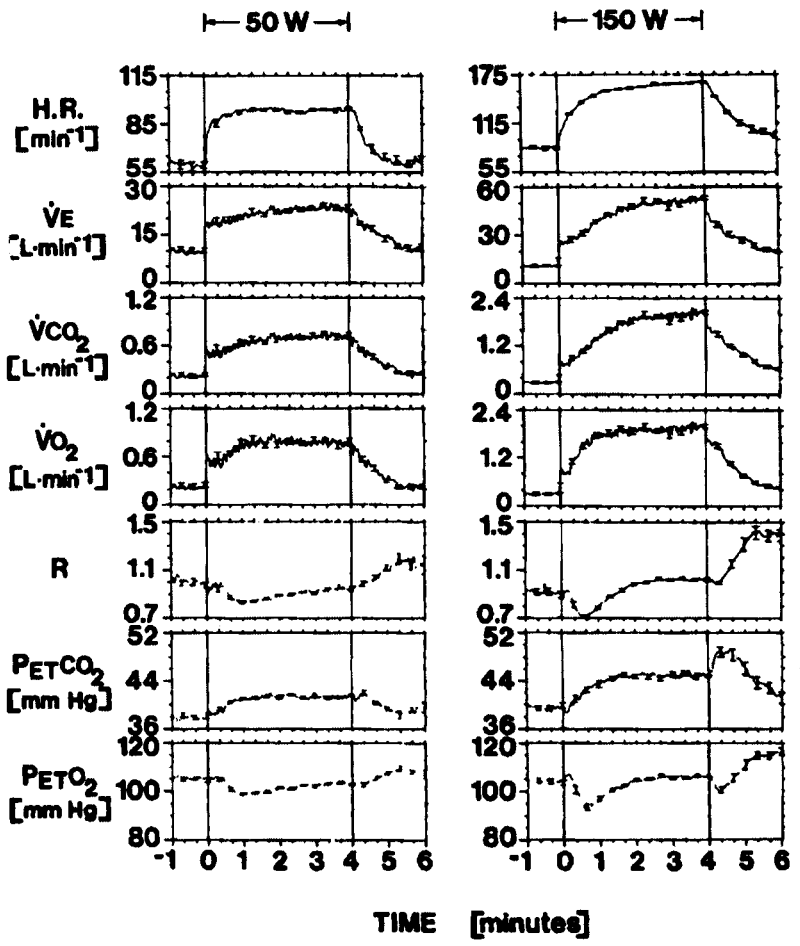


Figure 1.9: Ventilatory and pulmonary gas exchange responses to a 50W and a 150W square-wave exercise forcing, the stability of $P_{ET}O_2$, $P_{ET}CO_2$ and R during phase 1 can be seen. Reproduced from Whipp et al., 1982.

This hypothesis is further supported by the lack of effect on the phase I response found by a number of investigators studying the exercise response under conditions when the inputs from the carotid bodies and central chemoreceptors were either absent or modulated. Increasing the sensitivity of the carotid bodies or the central chemoreceptors (without input from the carotid bodies), by hypoxic and hyperoxic hypercapnic (Cunningham *et al.*, 1968; Cunningham, 1974b; Dejours, 1964; Miller *et al.*, 1974) inspirates respectively, had no effect on the magnitude or time course of the phase I response. Neither did removal of the

input from either chemoreceptor have any effect: Bilateral carotid body resection patients with near normal pulmonary function (Wasserman *et al.*, 1975b; Wasserman *et al.*, 1986) or children with congenital central hypoventilation syndromes (CCHS), i.e. who have either a total absence of or a dulled central chemosensory function, both exhibit a typical phase I $\dot{V}_E$ response (Shea *et al.*, 1993).

1.8.1.2 Peripheral and Central Neurogenesis

The rapid onset and kinetics of the phase I ventilatory response are indicative of it being mediated by a controller with a similarly fast response profile. Due to their location rather than their response kinetics, as discussed earlier, both these proposed control mechanisms lend themselves as likely candidates to influence the phase I $\dot{V}_E$ response. This subjective analysis is supported by a wealth of experimental data.

1.8.1.2.1 Feedback From Exercising Musculature

Numerous studies inducing 'exercise' (typically hindlimb contraction) by direct stimulation of α-motoneurons in the ventral spinal roots (e.g. McCloskey & Mitchell, 1972; Weissman *et al.*, 1980) or the sciatic nerve (e.g. Tibes, 1977; Bennett, 1984) in anesthetized cats and dogs have demonstrated an abrupt increase in $\dot{V}_E$ with the onset of exercise (Comroe & Schmidt, 1943).

However, this abrupt increase alone is insufficient to ascribe these responses to a muscle reflex. To isolate the muscle reflex, without influence from other potential controllers, these forms of exercise have been combined firstly with techniques to remove any humoral stimulus outwith the exercising muscle itself. This was achieved either through occlusion of the venous outflow (Comroe & Schmidt, 1943; Harrison *et al.*, 1932) or by cross perfusion with a resting animal (Kao, 1963). Neither technique produced any effect on the induced hyperpnoea. Secondly, induced exercise has been combined with experimental procedures designed to prevent the afferent traffic from the muscle reaching the central nervous system (CNS). These included dorsal root section (McCloskey & Mitchell, 1972), spinal transection (Comroe & Schmidt, 1943; Kao *et al.*, 1963) or selective blockade of the afferent groups involved (Tibes, 1977). The combination of any of these techniques with electrically stimulated exercise abolished the induced hyperpnoea.

Furthermore, while passive exercise in awake, intact humans abruptly increases ventilation (Comroe & Schmidt, 1943; Harrison *et al.*, 1932; Morikawa *et al.*, 1989), this response is absent in T5-T12 paraplegics (Morikawa *et al.*, 1989). However, a note of caution should be applied when interpreting the results of passive exercise experiments. Simply moving an individuals legs is likely to produce some form of 'startle' response regardless of how well familiarised the subject is. Therefore, the absence of a rising ventilation would be an odd finding. Experiments by Asmussen *et al.* (1965) and Galbo *et al.* (1987) investigated the ventilatory responses to exercise during partial neuromuscular blockade. These were designed to separate the muscle reflex from humoral mechanisms. The

effect of the blockade was that a given work-rate required the recruitment of more motor units than normal. This was accompanied by an increased $\dot{V}_E$, suggesting that either a muscle reflex or a central drive was mediating this increase.

However, a similar wealth of confounding evidence also abounds. While Comroe and Schmidt (1943) found that spinal cord transection abolished the hyperpnoea induced by electrically stimulated exercise in dogs, they found no effect in cats. Similarly, Lamb, (1968), Cross *et al.* (1982b), Levine (1979) and Weissman *et al.* (1980) all found no effect on the hyperpnoea of electrically evoked exercise after spinal cord transection. These results are also supported by the studies investigating electrically induced exercise in paraplegic humans. During which a similar abrupt increase in $\dot{V}_E$ at exercise onset was seen in the paraplegic subjects and the controls (Adams *et al.*, 1984; Asmussen *et al.*, 1943; Brice *et al.*, 1988; Brown *et al.*, 1990; Whipp & Ward, 1991) (figure 1.10).

1.8.1.2.2 Central Command

The evidence garnered and presented to support a role for central command in the phase I hyperpnoea has been derived from two main experimental areas. Firstly, studies whereby the genesis of exercise was achieved by direct central nervous system stimulation of either subcortical regions, such as the paraventricular locomotor region of the hypothalamus, (Orlovskii, 1969;

Eldridge *et al.*, 1985a; Waldrop *et al.*, 1986) or the H2 fields of Forel (Smith *et al.*, 1960).

Early work by Schaltenbrand and Girndt (1925) demonstrated an increased

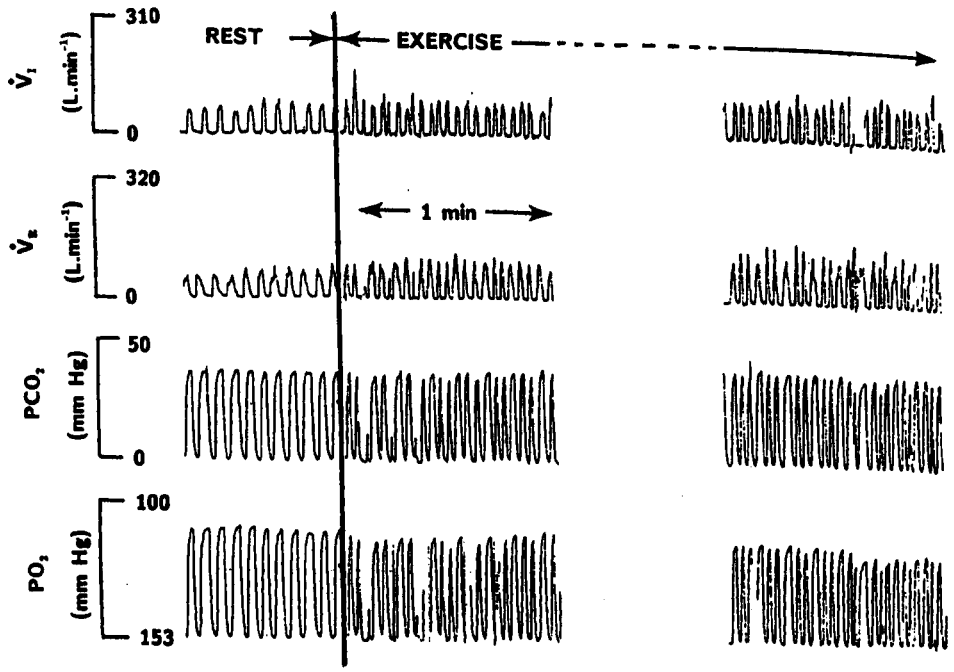


Figure 1.10: The ventilatory and respired gas tension responses during a transition from rest to exercise in a complete low level thoracic spinal cord lesion. The responses shown are at rest, the first minute of exercise and the fourth minute. Reproduced from Whipp & Ward, 1991.

respiratory response even prior to the onset of spontaneous locomotion in the decorticate cat. This pioneering work has been replicated by several other groups across the intervening years (Hinsey *et al.*, 1930; Ransom & Magoun, 1933; Eldridge *et al.*, 1981; DiMarco *et al.*, 1983; Eldridge *et al.*, 1985a). The studies of DiMarco *et al.* (1983) and Eldridge *et al.* (1985a) do not only demonstrate a respiratory response at least as fast as the onset of exercise, but also one proportional to treadmill speed, and hence theoretically proportional to the metabolic cost of the exercise (figure 1.11).

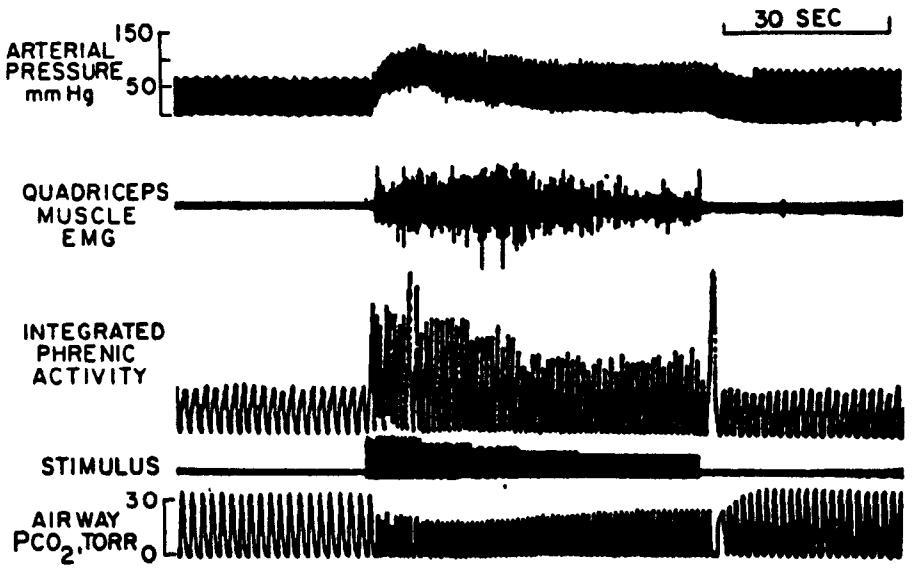


Figure 1.11: Respiratory and cardiovascular responses of a decorticate cat to electrically induced treadmill exercise. The increases in respiration and blood pressure actually precede the onset of muscular activity. Reproduced from Eldridge *et al.*, 1985.

However, while the results of these studies, as during volitional exercise, cannot be explained by the traditional chemoreceptor hypothesis (see page 32) they do not separate the influences of central command from a reflex control originating

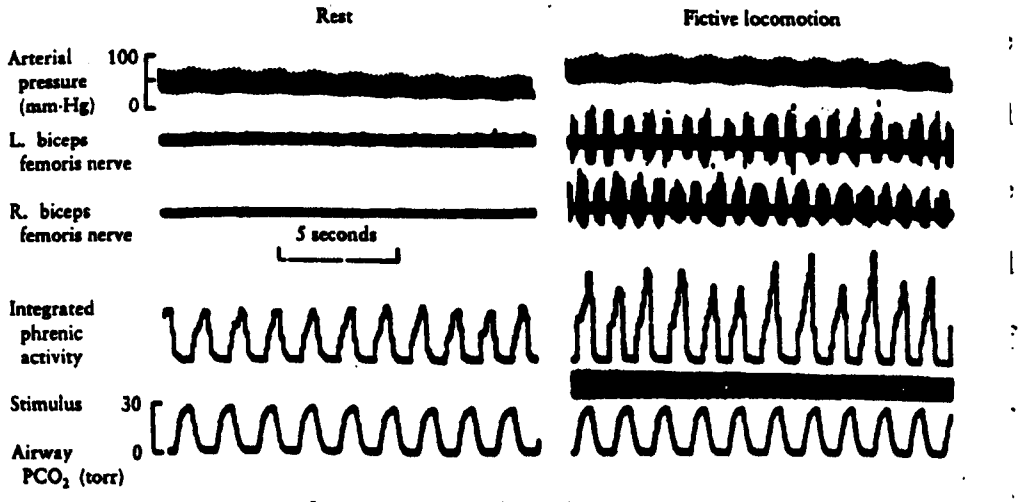


Figure 1.12: Cardiovascular and respiratory responses in a paralyzed cat when fictive locomotion was induced by stimulation of the hypothalamic locomotor region. Reproduced from Eldridge et al., 1985.

Thus the normal exercise hyperpnoea is increased ventilatory response with the onset of exercise.

Although there is little question that direct central induction of exercise can lead to a ventilatory response parallel to, or even prior to, the onset of exercise, as discussed earlier the requirements and characteristics of the ventilatory response to exercise are very precise. The simple demonstration of a stimulus to $\dot{V}_E$ does not allow quantification that it is in fact an integral part of the controller during exercise. Furthermore, the phase I ventilatory response in humans is typically isocapnic (see page 14) with a stable RER, $P_{ET}CO_2$ and $P_{ET}O_2$, i.e. it is proportional to the increases in $\dot{Q}$ (Casaburi *et al.*, 1978b; Whipp *et al.*, 1982; Whipp & Ward, 1982). However, the response generated by central electrical or pharmacological stimulation is hypocapnic (e.g. DiMarco *et al.*, 1983). This leads to a difficult interpretation, although the response is different to that in humans, it is characteristic of the volitional response of those experimental animals studied (e.g. Martin & Mitchell, 1993).

The second line of evidence generated in support of the involvement of central command in the phase I response comes from human studies where the magnitude of the central command has been dissociated from the actual work-rate performed by the muscles. Goodwin *et al.* (1972) used high frequency stimulation of the biceps tendon, known to develop a reflex tension in the muscle (De Gail *et al.*, 1966), to do this. They observed ventilation to decrease when isometric contractions were performed in the biceps, i.e. central command was reduced, and to increase when the same exercise was performed by the triceps, thus increasing central command (figure 1.13). These responses appear to occur closely with the onset of exercise.

The work of Assmusen *et al.* (1965) and Galbo *et al.* (1987), already discussed in relation to a muscle reflex, may also be viewed as demonstrating an increased central drive essentially immediately eliciting an increased ventilation. In individuals with unilateral leg weakness the ventilatory response to a given level of work was higher with the weak leg, and therefore, theoretically, with increased central command (Innes *et al.*, 1992).

The work on paraplegic subjects (Adams *et al.*, 1984b; Asmussen *et al.*, 1943; Brice *et al.*, 1988; Brown *et al.*, 1990; Whipp & Ward, 1991) (see muscle reflex section) seems to provide the most crushing evidence against the central command and muscle reflex hypotheses. Yet it has sparked considerable debate. Despite the fact that these individuals cannot volitionally generate lower limb activity it may be argued that a volitional attempt to generate exercise, and therefore a central drive, may still exist.

Furthermore, evidence against an obligatory role for either a muscle reflex or central control of the phase I ventilatory response comes from the characteristics

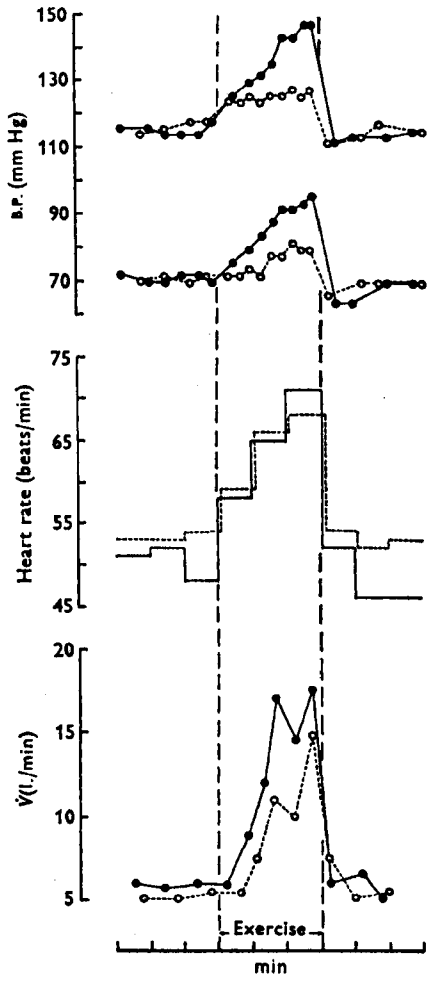


Figure 1.13: Respiratory and cardiovascular responses to static exercise under 'normal' conditions (filled circles) and the reduction in these responses when central command is reduced (open circles). Reproduced from Goodwin *et al.*, 1972.

of the response itself. When the work-rate forcing changes with a high frequency, e.g. in a sinusoidal function, there is no evidence of a fast phase I ventilatory response (Casaburi *et al.*, 1978; Wigertz, 1971). There must, presumably, still be an intrinsic drive generating the muscular contraction and therefore also reflex afferent transmission of information from the exercising muscle. Therefore, if

these mechanisms are the primary drive to phase I $\dot{V}_E$, why are they not operating under these conditions?

As mentioned earlier, the magnitude of the phase I ventilation is not proportional to the absolute work-rate, whereas the muscle reflex and the feedforward stimulus must, presumably, exhibit an innate proportionality with the intensity of the contraction and hence with work-rate (Jensen *et al.*, 1971). Transitions from rest to exercise performed in the supine position and upright work-to-work transitions evidence a slowed and reduced ventilatory response (Haouzi *et al.*, 2001; Weiler-Ravell *et al.*, 1983; Whipp *et al.*, 1982). This does not appear compatible with a control scheme whereby either a muscle reflex or central command was an obligatory control mechanism both of which should simply be proportional to work-rate.

1.8.1.3 Cardio-dynamic

The stability of $P_{ET}O_2$, $P_{ET}CO_2$ and RER across the duration of phase I is a consequence of a close temporal association of $\dot{V}_E$ to $\dot{V}CO_2$ and $\dot{V}O_2$ (Casaburi *et al.*, 1978; Whipp *et al.*, 1982). As the increases in $\dot{V}O_2$ and $\dot{V}CO_2$ during phase I are driven primarily by the increasing pulmonary blood flow, and hence by the increasing cardiac output, it has been suggested that a drive proportional to cardiac output could account for this response. This hypothesis has been termed cardio-dynamic (Wasserman *et al.*, 1974).

Despite some initial skepticism (Winn *et al.*, 1979; Eldridge & Gill-Kumar, 1980), the demonstration that experimental stimulation of cardiac output, either by a β-stimulant or by electrical pace-making, produced an immediate, appropriate rise in $\dot{V}_E$, i.e. $P_{ET}CO_2$ did not change (Wasserman *et al.*, 1974), seems consistent with a 'cardio-dynamic' control of the phase I hyperpnoea. Furthermore, the reduced phase I ventilatory response seen during supine exercise, relative to upright, can also be explained by a cardio-dynamic mechanism. In the supine position stroke volume and therefore cardiac output are increased at rest, thus reducing the magnitude of the phase I increase in cardiac output with a proportional reduction in the ventilatory response (Weiler-Ravel *et al.*, 1983).

There are, however, several lines of evidence against a cardio-dynamic involvement in the control of the phase I hyperpnoea. Firstly, Ward and co-workers (1987) have demonstrated that because of the increased slope of the intra-breath alveolar phase an unchanged end-tidal gas concentration is actually evidence of a small hyperventilation. Furthermore, this contention has been experimentally supported by Forster *et al.* (1986) who demonstrated a transient fall in P_aCO_2 during phase I. Utilising a similar exercise paradigm to Weiler-Ravel *et al.* (1983), Ishida *et al.* (1993) found a marked ventilatory response to passive exercise during sleep when cardiac output did not systematically increase and often decreased. Manual increases of cardiac output, achieved by increasing heart rate in patients fitted with permanent demand-type pacemakers, did not elicit an increase in ventilation (Jones *et al.*, 1981). This was despite increases in

$\dot{V}O_2$ and $\dot{V}CO_2$ consistent with those mediated by the increased pulmonary blood flow during phase I of exercise. Furthermore, humans (Banner *et al.*, 1988; Grassi *et al.*, 1993; Theodore *et al.*, 1987) and animals (Huszczuk *et al.*, 1990) that had undergone heart transplantation elicited an essentially normal phase I $\dot{V}_E$.

To summarise, while there are many potent stimuli to $\dot{V}_E$, many of which appear capable of driving the phase I hyperpnoea, no single control structure has as yet been proposed that can account for all the known experimental evidence.

1.8.2 Phase II

There is little debate that the onset of the phase II rise in ventilation, both during the on- and off- transient, does not occur prior to the metabolic consequences of the exercise, or recovery, arriving at the lung. The close kinetic association between $\dot{V}_E$ and $\dot{V}CO_2$, described in detail earlier, is equally well established in the literature (e.g. Casaburi *et al.*, 1977; Casaburi *et al.*, 1978b; Miyamoto, 1989; Whipp *et al.*, 1982). Demonstrations of this relationship must be interpreted cautiously, due to the inherent ability of $\dot{V}_E$ to influence $\dot{V}CO_2$. However, all experimental evidence reported here demonstrates that $\tau\dot{V}_E$ lags rather than leads $\tau\dot{V}CO_2$. Therefore, it is unlikely the observed $\dot{V}CO_2$ response is a consequence rather than a mediator of the $\dot{V}_E$ response. These observations have been taken as evidence of a humorally mediated control of the phase II

hyperpnoea; the following sections will review the potential sites of this mediation.

1.8.2.1 Peripheral chemoreflex

The carotid bodies appear prime candidates to mediate the transition from phase I to II, as experimental suppression of the carotid bodies, by hyperoxic inspirate, delayed the onset of phase II (Ward *et al.*, 1987). However, to rigorously demonstrate that the carotid bodies actively drive the change from phase I to II for $\dot{V}_E$ requires that the delay between the onset of phase II for pulmonary gas exchange, which occurs at a time consistent with the limb-lung transit delay (Whipp *et al.*, 1982), and the corresponding change in $\dot{V}_E$ is consistent with the lung-carotid body delay. Such a demonstration has not yet been made. Therefore, while they are the likely mediator of the phase change, it is as yet not possible to satisfactorily conclude that the carotid bodies serve in this role.

The postulate that the $\dot{V}_E$ kinetics during phase II are governed by those of $\dot{V}CO_2$ requires that changes in tissue CO_2 capacitance, i.e. the cause of the $\dot{V}CO_2$ response being dissociated from the rest of the metabolic processes involved in the exercise, have consequences apparent in the $\dot{V}_E$ response (Whipp & Ward, 1991). This has been demonstrated both for increased, prior hyperventilation (Ward *et al.*, 1983), and decreased, prior moderate exercise (Hughson & Morrisey, 1982), CO_2 capacitance during an exercise bout.

Furthermore, procedures designed to increase the carotid chemosensitivity, hypoxic inspirates (Griffiths *et al.*, 1986; Ward *et al.*, 1987) and ammonium chloride ingestion (Oren *et al.*, 1982), reduce $\tau\dot{V}_E$ absolutely and relative to $\tau\dot{V}O_2$ and $\tau\dot{V}CO_2$. The opposite outcome is true when the carotid bodies are removed, by carotid body resection (CBR) (Wasserman *et al.*, 1975; Whipp *et al.*, 1993; Whipp *et al.*, 1994), or 'silenced' by hyperoxic inspirates (Griffiths *et al.*, 1986; Ward *et al.*, 1987) (figure 1.14), sodium bicarbonate ingestion (Oren *et al.*, 1982) and sub-clinical intravenous dopamine infusion (Boetger & Ward, 1986).

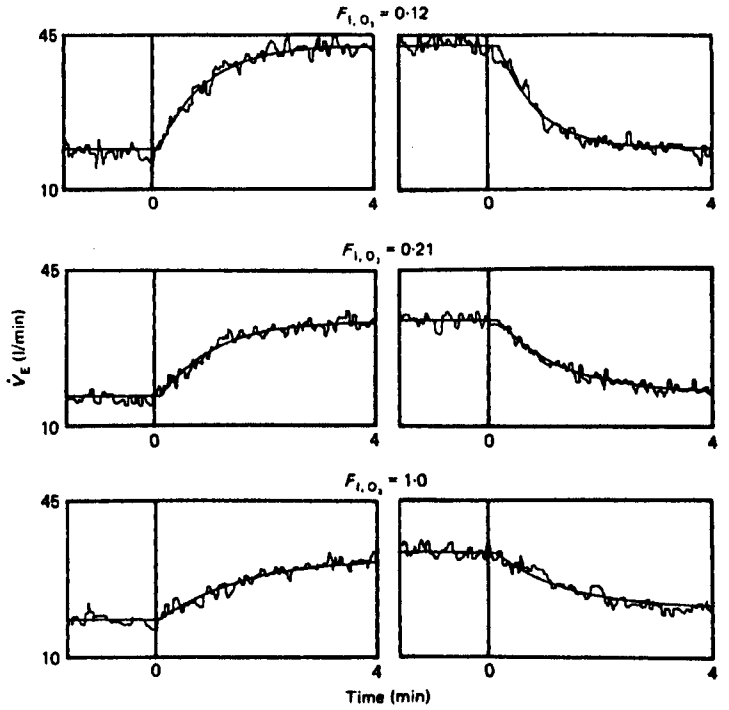


Figure 1.14: The ventilatory responses during a square-wave exercise trial breathing either a hypoxic (top), a normoxic (middle) or a hyperoxic (bottom) inspirate. Note the speeding of the ventilatory response during hypoxia and the slowing during hyperoxia. Reproduced from Griffiths *et al.*, 1986.

However, despite the repeated demonstrations that the carotid bodies are important in the phase II hyperpnoea, a satisfactory stimulus has yet to be

ascertained. The lag of $\dot{V}_E$ with respect to $\dot{V}O_2$ and $\dot{V}CO_2$ is likely to result in a transient fall in P_aO_2 and rise in P_aCO_2 (e.g. Lamarra *et al.*, 1989; Whipp *et al.*, 1978; Whipp *et al.*, 1989). These errors, however, are unlikely to be of sufficient magnitude, or time course, to mediate the phase II kinetics of $\dot{V}_E$. Based upon consideration of conventional ventilatory hypercapnic and hypoxic responsiveness, several novel alternative hypotheses have been put forward. Potassium, a known carotid body stimulant, see page 34, has been shown to increase with a time course resembling, though not formally modeled, the $\dot{V}_E$ response (Band *et al.*, 1982; Patterson *et al.*, 1989). However, formal analysis of the kinetic response of $[K^+]$ has now shown $\tau[K^+]$ to be substantially faster ($\Delta\tau$~20s) than $\tau\,\dot{V}_E$ (Casaburi *et al.*, 1995).

Another proposal (see page 33) revolves around changes in the amplitude and/or rates of change in the respiratory cycle linked oscillation in H^+ and P_aCO_2, despite a stable mean (Yamamoto, 1960). However, while the appearance of such signals in the pulmonary capillary blood is intuitively sensible, the transmission of such signals to the systemic circulation is not so straightforward. The variation in transit time and region dependent mismatching of $\dot{V}_A/\dot{Q}$ will lead to a reduction in the resolution of the oscillation in the pulmonary venous blood. The clarity of the signal is likely to be further reduced by mixing in the chambers of the left heart and in the aorta.

Despite this inevitable reduction in the signal to noise ratio, there would seem to be sufficient response characteristic of the signal remaining intact in the systemic

arterial blood to detect such oscillations in P_aO_2 (Kreuzer, 1975; Kreuzer, 1976; Purves, 1966; Yokota and Kreuzer, 1973) and pH_a (Band *et al.*, 1969a; Band *et al.*, 1969b; Band *et al.*, 1970; Band *et al.*, 1976; Grant & Semple, 1976; Honda & Ueda, 1961; Nims & Marshall, 1938; Torrance, 1968; Wolff, 1977). However, it appears that the remaining oscillatory signal in the arterial blood becomes further obscured as work-rate increases (Murphy *et al.*, 1987). Therefore, while there is little doubt that these changes do occur in the systemic arterial blood to some extent during exercise, albeit not as markedly as in the pulmonary capillaries, (Band *et al.*, 1980) and have the potential to stimulate the carotid bodies; a signal proportional to the increasing CO_2 clearance requirement with increasing work-rate does not appear to exist.

Furthermore, it has not been possible to demonstrate a consistent change in the 'phase-coupling' relationship (see page 33 for discussion) from rest to exercise, or with increasing exercise which would provide a proportional drive for $\dot{V}_E$ (Ward *et al.*, 1984; Ward *et al.*, 1995). Other known carotid body stimulants, e.g. plasma osmolality (Gallego *et al.*, 1979), adenosine (McQueen & Ribiero, 1981; Griffiths *et al.*, 1990) and temperature (Gallego *et al.*, 1979), await formal experimental analysis to determine any potential role in the phase II hyperpnoea.

1.8.2.2 Central chemoreflex

Even allowing for the technical difficulties involved in studying the central chemoreceptors, due to their inaccessibility in the intact model, there is no

convincing evidence available to support their involvement in the phase II control of $\dot{V}_E$. The delaying of the phase I-II transition on breathing hyperoxic inspirates, toward one compatible with the limb-medulla transport time, serves only to suggest the central chemoreceptors are capable of mediating the change in the absence of the primary mechanism (Ward *et al.*, 1987). Although the transient error in the regulation of P_aCO_2 during phase II may be sufficient to provide a stimulus in the CSF, the slow kinetics evidenced by the central chemoreceptors obviate their involvement as a primary drive to $\dot{V}_E$ during phase II (Bellville *et al.*, 1979). Furthermore, neither sensitisation nor attenuation of the central chemosensitivity has any affect on the phase II $\dot{V}_E$ kinetics per se, or the relationship with $\dot{V}CO_2$ (Ward *et al.*, 1987; Shea *et al.*, 1993 respectively).

1.8.2.3 Central command

The evidence presented earlier in support of a role for central command in the control of the phase I ventilatory response has also been taken to indicate the potential for a central neurogenic drive to ventilation during phase II. While it is undoubtedly the case that the experiments outlined earlier detail a feedforward mechanism capable of stimulating an increase in $\dot{V}_E$ during phase II, several experimental demonstrations make it difficult to support a central neurogenic mediation of phase II. Firstly, it is difficult to envisage a respiratory controller that is able to integrate the, presumably, constant central input into the bi-phasic increase consistently seen as the ventilatory response to a square-wave work–rate input. Similarly, the kinetic proportionality across phase II between $\dot{V}_E$ and

$\dot{V}CO_2$ which is maintained in the face of substantial experimental manipulations of $\dot{V}CO_2$ kinetics, as discussed earlier, while the central command drive remains unaltered is seemingly incompatible with such a model. Furthermore, this kinetic association is maintained during the off-transient, at a similar rate to the on-transient, during recovery to rest, i.e. when there is no volitional exercise and therefore no central command.

When the work forcing is implemented sinusoidally, the response kinetics for $\dot{V}_E$, $\dot{V}CO_2$ and $\dot{V}O_2$ appear similar to square-wave exercise, as predicted (see section 1.4.2 system linearity). Furthermore, the amplitude of the $\dot{V}_E$ response varies as a linear function of the $\dot{V}CO_2$ amplitude, a relationship which crosses the origin (Casaburi *et al.*, 1977). Therefore, in the absence of any CO_2 amplitude change there would be no ventilatory response, this would not be the outcome of a fast neurogenic system, either one driven centrally or by a muscle reflex.

Additionally, the work already discussed in relation to phase I separating the ventilatory requirement from the central drive, while measurements were not actually made during phase II, is still indicative of a ventilatory response that is not integrally coupled to the central drive (e.g. Krishnan *et al.*, 1996). More critically, studies on SCI individuals not only showed a 'normal' early ventilatory response but also a continued slow rise in $\dot{V}_E$ appropriate to the demands of the experiment (Adams *et al.*, 1984; Asmussen *et al.*, 1943; Brice *et al.*, 1988; Brown *et al.*, 1990; Whipp & Ward, 1991).

1.8.2.3.1 Short-Term Potentiation (STP)

An alternative centrally acting neurogenic drive proposed by Eldridge (1976a), although first demonstrated by Gesell *et al.* (1942), that exhibits slow kinetics may be capable of accounting for these observations. They demonstrated that the respiratory centres have a slowly decaying drive to $\dot{V}_E$ existing after the original stimulus has been removed. Furthermore, a potentiation in response to a continuous stimulus has also been observed (Eldridge & Gill-Kumar, 1978; Eldridge & Gill-Kumar, 1980) (figure 1.15).

This short term potentiation (STP) has been demonstrated in animals with stimulatory input from most of the currently proposed mediators of the exercise hyperpnoea, the carotid bodies (Eldridge, 1976b; Gesell *et al.;* 1942; Dutton *et al.*, 1967), the central chemoreceptors (Loeschke *et al*, 1970, Eldridge *et al*, 1987, Millhorn *et al*, 1982; Vis *et al*, 1981), central command (Eldridge *et al*, 1985b), muscle reflex drive (Eldridge, 1974; Eldridge, 1976b; Waldrop *et al.*, 1982) and in humans on ceasing volitional hyperventilation, where isocapnia was maintained by altering inspired PCO_2 (Swanson *et al.*, 1976; Tawadrous & Eldridge, 1974).

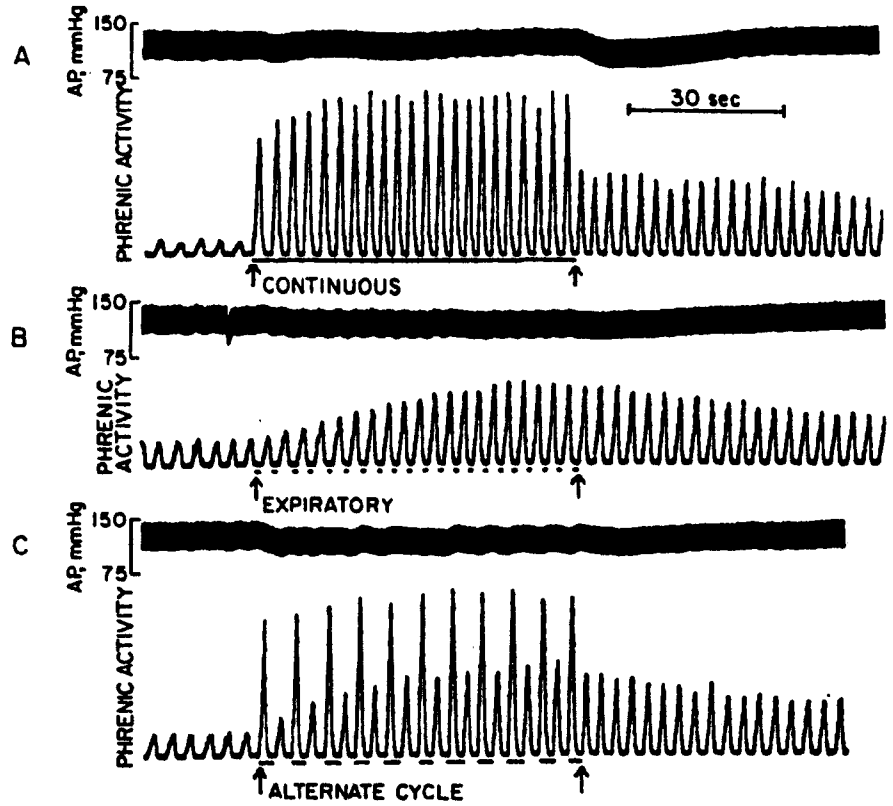


Figure 1.15: Demonstration of the presence of STP in response to a continuous input (a), one received only during expiration (reduced magnitude) (b) and to an intermittent input (demonstrating the underlying τ of the potentiation) (c). Reproduced from Eldridge & Gil-Kumar, 1980.

When interpreting the influence of this mechanism over the phase II hyperpnoea on first consideration it appears that this cannot be a primary drive, at least during the on-transient. Several confounding observations regarding the kinetics, both of the exercise hyperpnoea and of the STP, prevent simple interpretation of the role STP plays in governing the magnitude and time course of the phase II hyperpnoea. Firstly, the phase-2 τ for $\dot{V}_E$ has been shown repeatedly to be similar during the on- and off- transients, regardless of whether the off-transient is performed at rest, i.e. without any muscle reflex or central command stimulatory input (e.g. Whipp *et al.*, 1982; Griffiths *et al.*, 1986). However, the kinetic response of STP exhibits dynamic on- off- asymmetry, quite uncharacteristic of the phase II hyperpnoea, with a τ on- of ~9s (Wagner &

Eldridge, 1991) and a τ off- of 40-60s (Eldridge, 1974; Eldridge & Gill-Kumar, 1980b; Lawson & Long, 1984).

Further adding to the complexity is the demonstration by Eldridge and Gill-Kumar (1980) that the rapid onset τ is actually a product of the stimulation plus the STP, rather than τ for the development of STP (figure 1.15). As yet no quantitative characterisation has been undertaken for the time course of the potentiation alone, although it has been suggested that symmetry exists in the on-off-responses of the STP (Eldridge & Gill-Kumar, 1980). Although the development and decline of the STP appears to have similar intrinsic kinetics, the experimental demonstrations that the time course of $\dot{V}_E$ during the off-transient is unaltered between active recovery and rest (Whipp *et al.*, 1982) and during impulse forcing (Lamarra *et al.*, 1987), in addition to the reports that $\tau \dot{V}_E$ alters appropriately with experimental manipulation of $\tau \dot{V}CO_2$ (Hughson & Morrisey, 1982; Ward *et al.*, 1983) (see peripheral chemoreceptor section) mean that this issue awaits resolving.

1.8.2.4 Muscle reflex

Similar arguments apply in this context as for central command, the basic tenet being that the muscle reflex, as with the central drive, must remain roughly proportional to the work-rate across phase I, II and III. Therefore, despite the wealth of evidence already presented which clearly demonstrates the ability of the group III and IV limb afferents to reflexly drive $\dot{V}_E$, a primary reflex drive

from the exercising musculature seems unlikely to account for the dynamic bi-phasic increase in $\dot{V}_E$.

While the demonstration that SCI subjects display normal phase II $\dot{V}_E$ kinetics with respect to $\dot{V}CO_2$ can be argued only to assume there is no central drive, assuming correct clinical diagnosis of the level and completeness of lesion, there can have been no afferent feedback in these experiments. Therefore, it appears that a muscle reflex is not required for a normal control of the phase II hyperpnoea.

An interesting recent development stemming from the work of Haouzi *et al.* (1999, 2001) is that changes in intra-muscular perfusion pressure, probably detected by group IV afferents at the venular end of the circulation of the exercising muscles (Haouzi *et al.*, 1999), can reflexly drive $\dot{V}_E$ (Haouzi *et al.*, 2001). They propose that a drive proportional to the level of muscle hyperaemia could account for the humoral linkage apparent in the phase II ventilatory response. However, two lines of experimental evidence argue against such a mechanism being of primary importance. Firstly, the matching of $\dot{V}_E$ is to $\dot{V}CO_2$ rather than metabolic rate (see page 23 for discussion), whereas it appears more likely the changes in blood flow detected in such a manner would bear closer proportionality to the actual metabolic rate. Secondly, the demonstration that SCI individuals have essentially normal phase II responses must argue against a reflex afferent drive however it arises (Adams *et al.*, 1984b; Asmussen

et al., 1943; Brice *et al.*, 1988; Brown *et al.*, 1990; Whipp & Ward, 1991), unless of course the level of entry to the spinal cord is above the level of the lesion.

1.8.2.5 Cardio-dynamic

The close linkage of $\dot{V}_E$ to $\dot{Q}$ that lead investigators to propose a cardio-dynamic mediation of the phase I hyperpnoea is only evident during phase I. The transition to phase II, as already discussed, sees a close kinetic relationship between $\dot{V}_E$ and $\dot{V}CO_2$ independent of other cardio-pulmonary responses. The relative normalcy of the exercise ventilatory response in calves with artificial pneumatically driven hearts is inconsistent with a cardiovascular linkage driving phase II $\dot{V}_E$ (Huszczuk *et al.*, 1990). Furthermore, patients who have undergone heart-lung transplantation exhibit essentially normal phase II ventilatory kinetics (Grassi *et al.*, 1993). There seems little likelihood therefore that the slow phase II rise in $\dot{V}_E$ is primarily mediated by cardiac output or associated responses.

1.8.3 Phase III

Similarly to phase II, there is extensive evidence of a CO_2-linked control mechanism. Firstly, accurate regulation of P_aO_2, P_aCO_2 and hence pH_a at, or closely around, resting levels is achieved across the range of $<\hat{\theta}_L$ work-rates any given individual is capable of performing (Asmussen & Nielsen, 1958; McIlroy, 1964; Jones, 1975; Lamb *et al.*, 1965; Lugliani *et al.*, 1971; Masson & Lahiri,

1974; Sutton *et al.*, 1976; Wasserman *et al.*, 1975; Whipp & Wasserman, 1969). Secondly, while there is a reasonable proportionality between $\dot{V}O_2$ and $\dot{V}_E$ the dissociation between $\dot{V}CO_2$ and $\dot{V}O_2$ due to varying substrate utilisation has shown that $\dot{V}_E$ and $\dot{V}CO_2$ are more closely related (Douglas, 1927; Jones, 1975; Hansen *et al.*, 1972; Taylor & Jones, 1979; Wasserman *et al.*, 1986). However, as during phase II the actual mechanism of this proposed linkage is not so clear. The stability the linkage creates in the arterial blood gas tensions and pH removes any classical error signal from which this relationship might 'traditionally' stem.

1.8.3.1 Peripheral chemoreception

Despite the stability in the peripheral chemoreceptors traditional stimuli, as peripheral chemosensitivity has been repeatedly demonstrated to be increased during the steady-state of moderate intensity exercise, in comparison to rest, the unchanged blood gas and acid-base status may still provide some increased drive to $\dot{V}_E$ (Dejours, 1962; Griffiths *et al.*, 1986; MacDonald *et al.*, 1990; Weil & Swanson, 1991).

Additionally, the other carotid body stimulants not traditionally associated with respiration, $[K^+]$, osmolality, adenosine and the H^+-P_aCO_2 oscillation (specifically its rate of change but, as during phase II, not its phase coupling characteristic) have all been shown to be elevated, when compared to rest, during

the steady-state of exercise (Paterson, 1992; Gallego *et al.*, 1979; McQueen & Ribiero, 1981; Cross *et al.*, 1982a).

However, using the Dejours test, the carotid body contribution to the phase III hyperpnoea has been estimated to be ~20% (Dejours, 1962; Griffiths *et al.*, 1986; Macdonald *et al.*, 1990) (figure 1.16). Although, due to the sluggish response kinetics of the carotid bodies (relative to the onset of a corrective central chemoreceptor mediated response) it has been argued the ~20% value may be an underestimate (MacDonald *et al.*, 1990; Ward, 1994). This is further confirmed by the experimental demonstration of an essentially normal phase III response elicited by CBR subjects (Wasserman *et al.*, 1975). Therefore, it appears the carotid bodies are normally active during the steady-state and provide a proportional input capable of fine tuning or correcting any errors generated, but do not account for the entire response.

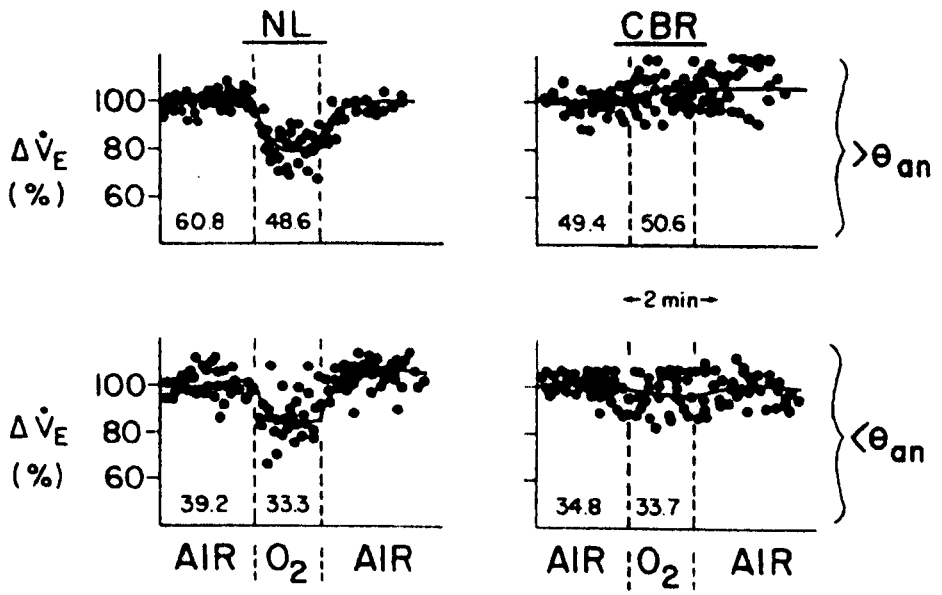


Figure 1.16: The effect of breathing a 100% O_2 inspirate for 1 minute during steady-state exercise in normal subjects (NL) and subjects who had undergone carotid body resection (CBR). Reproduced from Whipp & Davis, 1979.

1.8.3.2 Central chemoreception

The stability of the blood gas and acid base status throughout the steady-state of moderate intensity exercise may be indicative of a humoral control mechanism. However, the stability in the arterial blood also seems to be transcribed to the cerebral spinal fluid (CSF), Bisgard *et al.* (1978) have shown this to be the case in ponies. As described in phase II, the oscillation of H^+-P_aCO_2 only appears to maintain fidelity in the arterial blood perfusing the central chemoreceptors when the breathing frequency is low (Millhorn *et al.*, 1984).

While there is undoubtedly an elevated arterial $[K^+]$, it is unlikely to drive a centrally mediated response. Firstly, as Davson (1970) contended, there is likely to be no elevation of CSF $[K^+]$ and secondly, because Band *et al.* (1985) have demonstrated that the stimulatory effect of $[K^+]$ on $\dot{V}_E$ was abolished in the absence of the peripheral chemoreceptors. Finally, the relative normality of the phase III ventilatory response displayed by CCHS individuals argues against a primary phase III $\dot{V}_E$ drive being under central chemoreception (Gaudio *et al.*, 1969; Lugliani *et al.*, 1979; Paton *et al.*, 1993; Shea *et al.*, 1993) (figure 1.17).

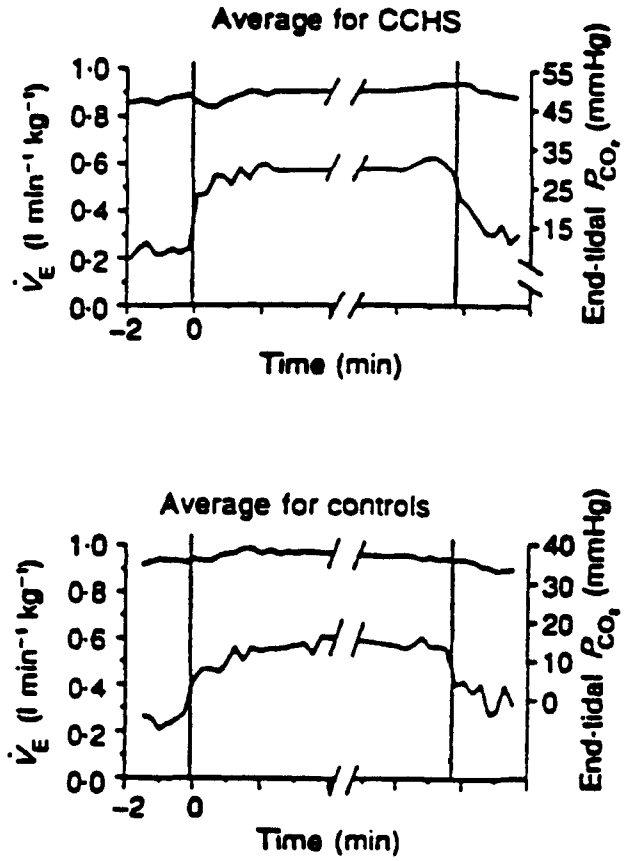


Figure 1.17: Ventilatory and $P_{ET}CO_2$ responses to a square-wave forcing of exercise in subjects with congenital central hypoventilation syndromes (CCHS) and controls. Reproduced from Shea *et al.*, 1993.

1.8.3.3 Central and Peripheral Neurogenesis

Clearly if the carotid bodies provide ~20% and under normal conditions the central chemoreceptors serve essentially no role in the phase III hyperpnoea, then the remainder of the stimulus must lie outwith the traditional humoral stimuli. As the ventilatory response is closely associated with the absolute work-rate, though more specifically with $\dot{V}CO_2$ (as already discussed), a stimulus mechanism proportional to work-rate is intuitively attractive. As such, the central command

and muscle reflex mechanisms already discussed to be present during exercise and capable of providing a continual drive to $\dot{V}_E$, in proportion to the work-rate, might drive a proportion of the remaining ~80% of the response.

However, this appears not to be essential for a normal phase III hyperpnoea as Adams *et al.* (1984) have demonstrated that SCI individuals have an essentially normal phase III ventilatory response. In addition, the report by Poon *et al.* (1987) that reducing the volitional requirement for $\dot{V}_E$, using an assisted ventilator, led firstly to an increase in $\dot{V}_E$ then a return to the level appropriate for the CO_2 output argues against a purely central or peripheral neural drive during phase III.

1.8.3.4 Cardio-dynamic

The demonstration that humans (Banner *et al.*, 1988; Theodore *et al.*, 1987) and animals (Huszczuk *et al.*, 1990) who have undergone heart transplantation have appropriate phase III ventilatory responses, leading to regulation of arterial blood gas tensions and pH, suggests that a cardiac afferent mediated drive is not required during phase III. However, in 'normal' individuals it is impossible to conclude that the stimulus to $\dot{V}_E$ which is known to exist from cardiovascular linkages plays no role in the ~80% of the response not mediated humorally (Banzett *et al.*, 1978; Bartoli *et al.*, 1974; Juratsch *et al.*, 1982; Kostreva *et al.* 1979; Ledsome, 1977; Uchida 1976; Wasserman *et al.*, 1974).

1.8.4 Supra-$\hat{\theta}_L$

Up to this point the discussion has focused around work-rates which do not engender a sustained metabolic acidosis. When a subject is exercising at a work-rate in the heavy, very heavy or severe domain (see earlier for descriptions), i.e. above their lactate threshold, regardless of the metabolic reasons for the onset of lactate accumulation, the consequence is an added challenge for the respiratory system (see page 27).

1.8.4.1 Phase I

There is little to distinguish the phase I response during supra-$\hat{\theta}_L$ exercise in comparison to sub-$\hat{\theta}_L$ exercise. This is a predictable consequence of phase I being unaffected by the metabolic cost of performing the work. Therefore, the control of $\dot{V}_E$ during phase I is likely to be little different during supra-$\hat{\theta}_L$ intensity exercise than below $\hat{\theta}_L$, although this has not yet been well studied.

1.8.4.2 Phase II & III

Due to the lack of formal analysis of the temporal response patterns of $\dot{V}_E$ and $\dot{V}CO_2$ above $\hat{\theta}_L$, the indistinct phase II to III transition and the absence of a steady state at work-rates in the very heavy and severe domains the control processes for these phases will be considered together.

1.8.4.2.1 Central and Peripheral Neurogenic Drives

As the ventilatory response to exercise performed above $\hat{\theta}_L$ is so markedly affected by the metabolic acidaemia the conventional focus of discussions on the control processes often revolve around humoral mechanisms. While the weight of experimental evidence points to a chemoreflex control of the respiratory compensation, it should be noted that this is the control of the extra ventilatory response above the normal metabolic (work-rate related) component. Therefore the putative neurogenic drives, already outlined, that are believed to provide both a feedforward, central command, and feedback, muscle reflex and cardio-dynamic, drive to breathing could still be operational providing a stimulus proportional to the underlying work-rate.

1.8.4.2.2 Central chemoreflex

There is no demonstrable sustained CO_2 stimulus to $\dot{V}_E$ in the arterial blood during supra-$\hat{\theta}_L$ exercise, P_aCO_2 either remains unchanged during isocapnic buffering (Wasserman *et al.*, 1977) or falls during respiratory compensation (Wasserman *et al.*, 1967). Combining this with the limited permeability of the blood-brain barrier to protons (Cestan *et al.*, 1925; Robin *et al.*, 1958; Winterstein & Gökhan, 1952) and there is little evidence to support a major role for the central chemoreceptors in the supra-$\hat{\theta}_L$ ventilatory response.

However, the falling P_aCO_2, resulting in an alkalotic CSF (Bisgard *et al.*, 1978), would be expected to reduce central chemoreceptor activity. This has been argued to influence the respiratory compensation for the metabolic acidosis in two ways, firstly, by reducing the total chemoreflex drive. Secondly, stimulation of the efferent fibers of Hering's nerve, by superperfusion of the CSF with alkaline solutions, has been shown to inhibit the peripheral chemoreceptors (Majcherczyk & Willshaw, 1973).

Finally, similarly to the notion of redundancy in the ventilatory controller proposed for sub-$\hat{\theta}_L$ exercise, in the absence of the peripheral chemoreceptors, achieved by hyperoxic inspirate, there was gradual slow increase in $\dot{V}_E$ leading towards, although not achieving, restoration of pH_a (Rausch *et al.*, 1991). Rausch et al. (1991) proposed this 'secondary' compensation in the absence of the primary drive might be mediated by the central chemoreceptors responding to the slow leakage of H^+ into the CSF (Teppema *et al.*, 1984; Eldridge *et al.*, 1985a).

1.8.4.2.3 Peripheral chemoreflex

During supra-$\hat{\theta}_L$ exercise pH_a falls (Assmusen & Nielsen, 1947) and circulating catecholamine levels rise (Banister & Griffiths, 1972; Flandrois *et al.*, 1977; Haggendal *et al.*; 1970; Euler & Hellner, 1952). These are both known to be potent carotid body stimulants, although β-adrenergic blockade did not attenuate the hyperventilation during supra-$\hat{\theta}_L$ exercise (Dodd *et al.*, 1989). Regardless,

the contention that at least the falling pH_a drives the hyperventilation is supported by the close correlation between the fall in pH_a and the level of hyperventilation (Dempsey *et al.*, 1972; Dempsey & Rankin, 1967).

Furthermore, sensitisation of the carotid bodies, by hypoxic inspirate with matched degrees of lactic acidaemia, speeds the return of pH_a towards normal (Rausch *et al.*, 1991) and absence of carotid bodies, in CBR asthmatics, abolishes the compensation (figure 1.18) (Wasserman *et al.*, 1975b). Additionally, desensitisation of the carotid bodies by hyperoxic inspirates reduces the hyperventilation (McLoughlin *et al.*, 1993; Rausch *et al.*, 1991; Wilson & Welch, 1975; Asmussen & Nielsen, 1958). While it has been argued that the effect of high O_2 on the degree of acidosis may account for this, the work of Rausch and colleagues (1991) showed the greatest fall in pH_a while breathing hyperoxic inspirates (when compared to euoxic and hypoxic mixtures). Therefore, the reduced hyperventilation in the presence of high O_2 cannot be ascribed simply to a reduced acidotic requirement to hyperventilate.

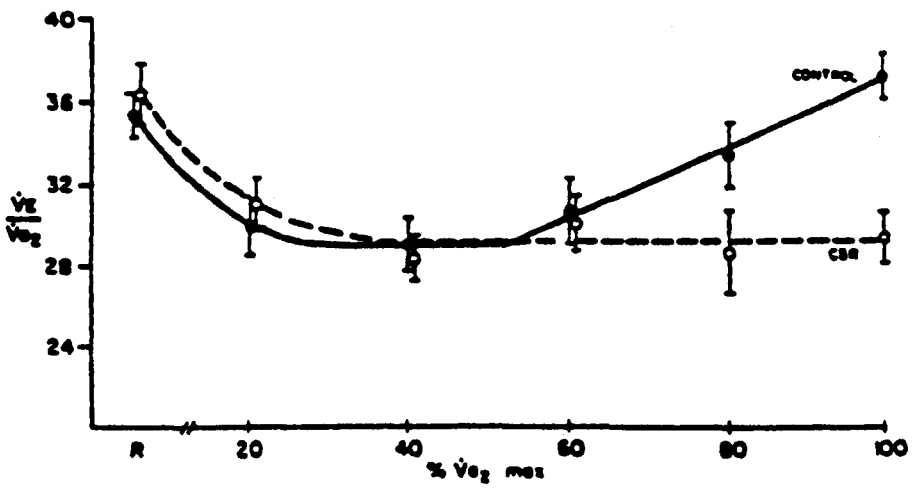


Figure 1.18: The response of the ventilatory equivalent for O_2 during a rapidly incrementing exercise test for a group of subjects who have under gone carotid body resection (CBR) and their controls. The stability of $\dot{V}_E / \dot{V}O_2$ in the CBR subjects illustrates the lack of respiratory compensation for the metabolic acidosis. Reproduced from Wasserman *et al.*, 1975.

A portion of the hyperventilatory drive may arise from carotid body stimulation by increased arterial [K^+] seen during exercise of heavy intensity and above (Newstead *et al.*, 1990; Paterson *et al.*, 1990). Additionally it is possible the increased circulating [K^+] may also provide a drive through stimulation of the group III and IV limb afferents (Busse *et al.*, 1989; Tibes *et al.*, 1976).

There are however, several lines of evidence which, while not totally incompatible with the carotid bodies as sole mediators of the hyperventilation, appear to argue against such a mechanism. Firstly, brief hyperoxic inspirates, the Dejours test (1962), only resulted in a decrease in $\dot{V}_E$ of ~20% (Jeyaranjan *et al.*, 1987; MacDonald *et al.*, 1990). However, Macdonald *et al.* (1990) have argued that this is due to the secondary consequences of high inspired O_2 masking the later stages of the transient fall in $\dot{V}_E$ and thus underestimating the fall in $\dot{V}_E$. Also, McLoughlin *et al.* (1993) contend that hyperoxia is not fully effective during acidemia in silencing the carotid bodies. Furthermore, if the carotid body contribution to the ventilatory response is the actual hyperventilatory portion of the response (i.e. that above the CO_2 clearance requirement) plus ~20% of the CO_2 clearance requirement (taken from sub-$\hat{\theta}_L$ estimates, page 60), then this magnitude of decrease may well account for the majority of this portion of the response.

Secondly, patients with McArdle's syndrome, deficient in myophosphorylase b and therefore unable to produce lactic acid, hyperventilate at work-rates near

their syndrome-limited maximum (Hagberg *et al.*, 1982; Hagberg *et al.*, 1990). However, as these individuals tend to hyperventilate across all work-rates they are capable of performing this phenomenon is likely to be pain or anxiety related and have no bearing on the supra-$\hat{\theta}_L$ hyperventilation in normal subjects (Riley *et al.*, 1993; Whipp, 1983a).

Another potential challenge to the carotid body control of the supra-$\hat{\theta}_L$ respiratory compensation is the demonstration that for rapidly incrementing tests the respiratory compensation is delayed, with regard to the onset of metabolic acidosis, in comparison to steady-state incremental protocols (figure 1.19). This domain of exercise has been termed isocapnic buffering (Wasserman *et al.*, 1977). The finding is inconsistent with the rapid kinetics of the carotid bodies demonstrated in response to experimental reductions in pH_a (Schmidt *et al.*, 1939). However, this finding is also inconsistent with any other classical drive to $\dot{V}_E$. A tentative hypothesis revolves around a theoretical time or amplitude related threshold to H^+ stimulation (Whipp & Ward, 1991).

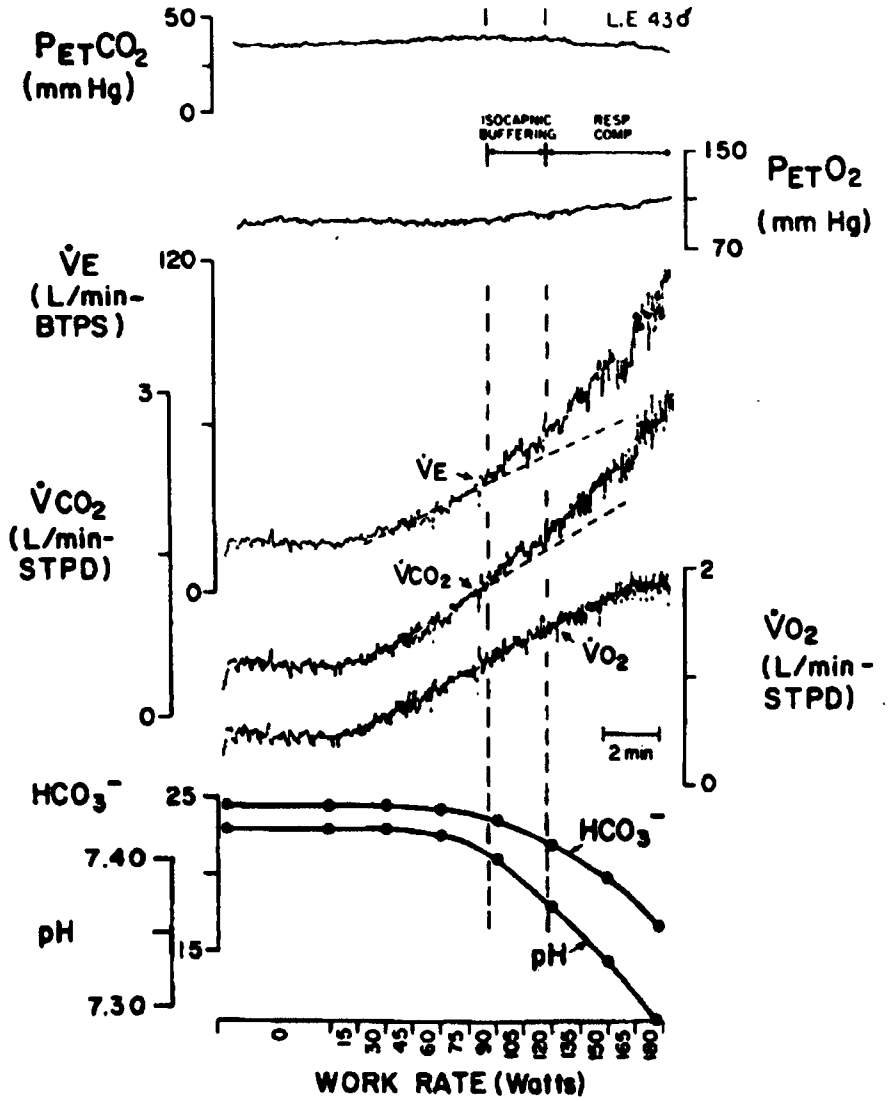


Figure 1.19: The ventilatory, pulmonary gas exchange and arterial blood acid-base responses during a rapid incremental exercise test illustrating the delayed onset of respiratory compensation relative to the lactate threshold. Reproduced from Wasserman *et al.*, 1986.

1.9 Alternative control theories

The inability of classical ventilatory control theories to explain the matching of $\dot{V}_E$ to $\dot{V}CO_2$ has led several investigators to search for novel control structures capable of explaining the seemingly errorless $\dot{V}_E$ - $\dot{V}CO_2$ coupling.

1.9.1 Optimisation

The first of these proposals is that ventilatory control may occur through optimisation. That is to say the ventilatory controller, presumably within the ponto-medullary respiratory integrating regions, drives ventilation to an optimal level; i.e. one which balances the 'cost' of humoral regulation of P_aCO_2 with the mechanical 'cost' of generating of the appropriate level of ventilation (Poon *et al.*, 1983; Poon, 1987; Swanson & Robbins, 1986; Young & Poon, 1998). This would require the controller to be capable of continually cycling between perturbation and evaluation in order to find the optimal response.

However, there are several experimental observations which are not compatible with such an optimising controller. Firstly, the model requires 'knowledge' of the actual ventilation to calculate the mechanical 'cost', the absence of afferent vagal feedback prevents such 'knowledge' but does not affect the exercise ventilatory response (Grassi *et al.*, 1993). Furthermore, the controller also requires 'knowledge' of the metabolic 'cost' of P_aCO_2 regulation. This is traditionally accepted as stemming from the $\dot{V}_E$ -P_aCO_2 slope. Patients with a zero slope of the $\dot{V}_E$ -P_aCO_2 relationship in response to CO_2 inhalation have an essentially normal exercise response (Shea *et al.*, 1993). Finally, with an additional experimental CO_2 challenge (e.g. additional external dead space) the optimization hypothesis predicts an isocapnic response, the experimental evidence shows a hypercapnic response (Ward & Whipp, 1980).

1.9.2 Redundancy

The consistent demonstration (see earlier) that in the absence of one known drive to $\dot{V}_E$ the response is essentially normal. This repeated finding lends itself to the notion of redundancy in the ventilatory controller first raised by Barcroft (1934). Albeit his view was only of two possible drives, one chemical and one neural, but the suggestion was that in the absence of either mechanism the other would subserve the combined role. Several more formal models have been proposed in recent years (Yamamoto, 1980; Swanson, 1992). However, both these models suffer from issues surrounding the humoral controllers as all simple models still do, due to the lack of classical stimulus from which they may operate (see earlier for discussion). Regardless, what is important to note is not the specific models that have been proposed. Rather, it is the general concept that the experimental observations detailed throughout this discussion cannot be explained by a single mechanism, only by an as yet unresolved cluster of interacting mechanisms which must in the absence of one primary controller have the potential to compensate for the missing input.

1.9.3 Long Term Modulation

The paradoxical stability of P_aCO_2 during the steady-state of moderate exercise brought about by a close linkage between $\dot{V}_E$ and $\dot{V}CO_2$, which seemingly

precludes a classical CO_2 driven mediation, has led several investigators to the postulate of Somjen (1992) and the work of Martin and Mitchell (1993). The demonstration that volitionally exercising goats could be taught, using a associative conditioning paradigm, a new ventilatory response for a standard treadmill task was interpreted in terms of a respiratory memory (Martin & Mitchell, 1993). The coincidental relationship between this finding and the proposition that functionally error-free control systems may operate by anticipating present and future needs based on past experience (Somjen, 1992) intriguingly parallels that of the $\dot{V}_E$ - $\dot{V}CO_2$ relationship. Therefore, it appears possible that a plastic element in the ventilatory control system, possibly operating through the same pathways demonstrated by Eldridge and co workers (see page 54) (e.g. Eldridge *et al.*, 1985) in relation to STP within an exercise period, would need only to provide a close approximation of the response with humoral sensors able to correct for any errors generated.

To date much conflicting evidence abounds regarding the existence of LTM in the control of the exercise hyperpnoea. Several studies have reported the ability to modulate both the early (phase I) and the steady-state (phase III) level of ventilation in humans, using both increased dead space (Turner, 1997; Adams *et al.*, 1992; Turner *et al.*, 1996; Sumners & Turner, 1999) and increased resistance (Stewart & Turner, 2000). However, these are only preliminary reports and they do not actually demonstrate even an increased absolute level of ventilation, let alone an increased $\dot{V}_E$ in relation to $\dot{V}CO_2$. Their conclusions are drawn mainly from a fall in P_aCO_2, estimated from $P_{ET}CO_2$, from rest to exercise during post-

conditioning. Both Helbling *et al.* (1997) and Turner and Sumners (2002) demonstrated an augmented $\dot{V}_E$ during the early stages (phase I) of exercise following associative conditioning of exercise paired with increased dead space. However they were unable to demonstrate any effect on the steady-state (phase III) response. In contrast, Moosavi *et al.* (2001) were unable to demonstrate any effect of associative conditioning on the ventilatory response to treadmill running during any phase of the exercise.

Although the goats employed by Martin and Mitchell (1993) and Johnson and Mitchell (2001) did consistently over-breathe during treadmill exercise, subsequent to exercise paired with increased external dead space (hypercapnic challenge), the typical exercise ventilatory response in goats is such that P_aCO_2 falls from rest to steady-state moderate exercise, i.e. they become hypocapnic. This is in contrast to the isocapnia that typifies the response in humans. Therefore the possibility exists that a simple species difference could account for the inability of authors to demonstrate the existence of LTM in the phase III response to moderate exercise in humans.

Clearly this field requires further research to begin to clarify many of the equivocal issues raised thus far. To this end, two separate studies were carried out to investigate the role of respiratory memory in the control of the exercise hyperpnoea. The specific characteristics and results of those studies are detailed in the following two chapters

1.10 Summary

To the casual observer it may appear that where we stand now is not altogether different to where Krogh and Lindhard stood in 1913 or even Zuntz and Geppert as far back as the 1880's. However, this is far from a fair reflection, considerably more evidence has been amassed both supporting and refuting all the mechanisms proposed during the infancy of this topic. Therein however lies the fundamental problem; the complexities of the ventilatory control system and its seemingly layered structure render it fascinatingly complicated to study. Therefore, while there is much knowledge regarding the mechanisms potentially involved in the control of the exercise hyperpnoea, we are as yet unable to propose a coherent structure by which these known ventilatory drives may combine to account for the complex pattern of ventilatory response to exercise. As such the challenge for the next hundred years of investigation is likely not to be one of discovering a new mechanism by which $\dot{V}_E$ can be driven. Rather, it is likely to be one to explain the many seemingly incompatible observations and to decipher the specific combination of mechanisms which control $\dot{V}_E$ and allow appropriate regulation of P_aCO_2.

1.11 Aims

Therefore, the aim of these studies is not to search for another system which may drive $\dot{V}_E$, but to attempt to resolve potential control structures. Firstly, further investigating the varying importance of central command versus humoral control through another exercise paradigm, intermittent exercise, which dissociates work-rate from the exercise intensity (as classically defined). Furthermore, and possibly more importantly, this work-rate forcing will allow investigation of the temporal characteristics of the respiratory compensation for metabolic acidemia. Thus the experimental protocol has the possibility of revealing a time related threshold for H^+ stimulation of the carotid bodies (Whipp & Ward, 1991); with the aim of shining some light into the cloud of mystery veiling the phenomenon of isocapnic buffering during rapid incremental tests.

Secondly, one recent observation which does open up a genuinely new, to Krogh and Lindhard's generation anyway, avenue of research is that of Martin and Mitchell's LTM phenomenon. Therefore, the aim is to investigate whether a modulation of the ventilatory response, similar to that seen in goats (Martin & Mitchell, 1993), can be elicited in humans through associative conditioning.

Finally, to determine whether a learned ventilatory response to a particular exercise mode is obligatory for the genesis of a 'normal' ventilatory response to that mode of exercise. Studies two and three therefore aim to clarify the potential role of LTM in the control of the exercise ventilatory response.

Chapter 2

General Methods

2.1 Subjects

The differing requirements of the studies included meant a separate cohort of subjects participated in each of the studies, although one subject participated in both study one and study two. The specific characteristics of each subject group are detailed in the individual experimental chapters. All subjects volunteered to participate, with no incentive (financial or otherwise) offered, and provided written informed consent as approved by the University of Glasgow Ethics Committee. Furthermore, subjects were required to complete an approved medical questionnaire (appendix 2.1) in order to identify any pre-existing conditions which may increase the risk factor associated with performing exercise tests. Any subjects who met one of the exclusion criteria (e.g. an asthmatic, or a history of sudden death in the family) were thanked for their time but advised that they could not be included in the study. Additionally, for studies involving maximal exercise tests all subjects were medically screened for resting ECG and blood pressure prior to commencing the study.

To minimise the influence of extraneous factors during different tests in any given subject, the subjects were instructed to adhere to several criteria prior to each laboratory session:

1. No strenuous exercise for 24 hours prior to each visit
2. No alcohol to be consumed in the 24 hour period prior to testing
3. No caffeine ingestion during the 4 hours preceding a test
4. No food consumption for the 2 hours leading up to a test

This was confirmed by means of a questionnaire completed by subjects prior to each test (appendix 2.2). If any subject did not meet these criteria on a particular visit, or was showing signs of illness or injury, the test was postponed until the subject was fully recovered. Further precautions to prevent extraneous contamination of data were employed in individual studies and are outlined in those chapters.

2.2 Protocols

2.2.1 Familiarisation

Any new environment provokes a reaction from the conscious human, regardless of whether this is pleasure and excitement, arrival at a holiday destination for example, or anxiety and concern in an environment perceived as being hostile. Such reactions could therefore prevent accurate characterisation of the underlying physiological responses to the activity being performed, whether seated on a plane flying to a holiday destination (rest plus elation) or walking through a dark unfamiliar alley (moderate exercise plus "fight and flight" response).

The exercise laboratory environment can be viewed by many prospective subjects as intimidating, and, as such, runs the risk of eliciting the latter response. Therefore, in an attempt to prevent contamination of the underlying

physiological responses to any given protocol, all subjects underwent a thorough familiarisation visit. This involved familiarising the subject with the laboratory environment, equipment, personnel and protocols. The measures taken to counter each of these areas of concern are detailed as follows:

1. To make the laboratory a less intimidating background, music was played at a low level; where possible, research staff did not wear laboratory coats; all monitoring equipment was kept out of the subjects view while exercising and all audible alarms were turned off.

2. The purpose and operation of each item of equipment used during a given study was explained to the subjects. All equipment that the subjects were interfaced with (the cycle ergometer, for example) was adjusted to be as comfortable and unobtrusive as possible. These settings were recorded and replicated for subsequent visits.

3. All members of staff involved in each study were personally introduced to the subjects.

4. The exact details of each protocol were thoroughly explained to the subjects, along with what was required of them. The subjects then performed trial runs of each experimental protocol comprising the study in question.

Each study required slight variations in the familiarisation sessions, depending on its purpose; these alterations are explained in the relevant experimental chapters. Furthermore, the potential not only of the laboratory environment *per se*, but also of any non-specific event during the exercise test (e.g. a phone ringing, a temperature change in the laboratory or a conversation between experimenters) to provoke a response from the subject was taken account of. In an attempt to contend with this the laboratory was organised so as to provide the subject with, as close as possible, a consistent level of visual, auditory and thermal stimulation. For all studies, the subject's height and weight were measured during the familiarisation session (Leicester Height Measure, Invicta Plastics Ltd., Leicester, UK and Salter Weigh-Tronix, Avery, Birmingham, UK)

2.2.2 Incremental Exercise Test

To determine subject characteristics and to assign work-rates for subsequent tests, all subjects performed a rapid incremental exercise test to the limit of tolerance (t_{lim}) on a cycle ergometer. This facilitated non-invasive estimation of the lactate threshold ($\hat{\theta}_L$) from a cluster of pulmonary gas exchange and ventilatory responses and measurement of peak $\dot{V}O_2$ ($\mu\dot{V}O_2$). The computer controlling the cycle ergometer was pre-programmed with a series of incremental protocols which increased the work-rate by the smallest possible time division; thus preventing the subject from perceiving the individual increments in work-rate. The appropriate incrementation rate for each subject was chosen on the basis of subjective analysis of fitness level, e.g. 5W/min imposed as 1W/12s,

10W/min implemented as 1W/6s and 15W/min applied as 3W/12s. Thus the protocol (figure 2.1b) more closely approximated a ramp (figure 2.1a) than a staircase (figure 2.1c).

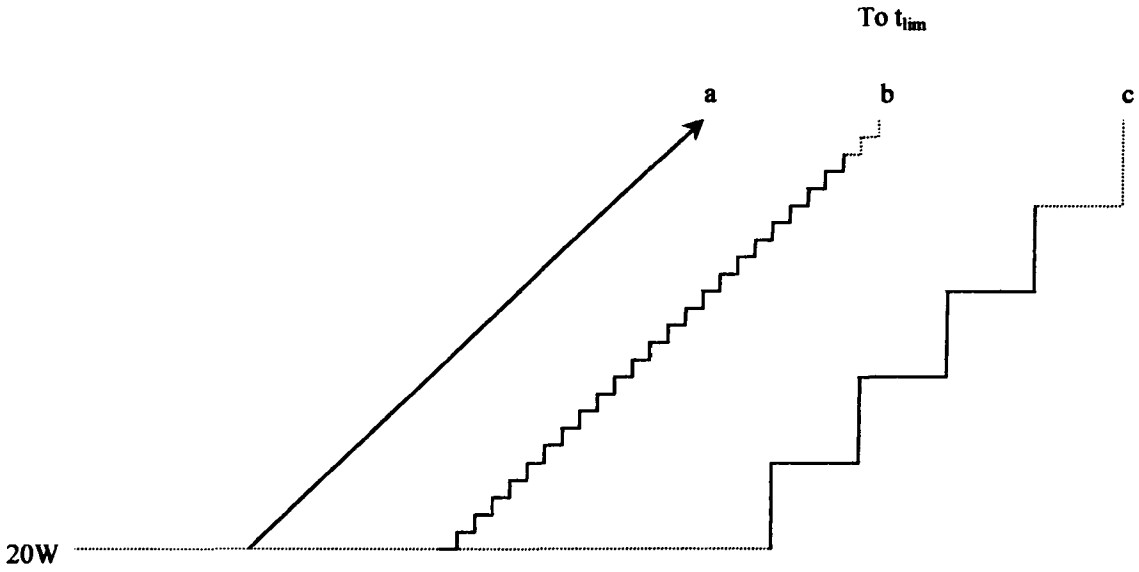


Figure 2.1: a, a true ramp profile; b, a rapidly incrementing test which closely approximates to a ramp (e.g. 3W/12s); c, a traditional stepwise incremental test (e.g. 15W/min).

To begin each test, subjects sat quietly at rest on the ergometer for a period of ~3 minutes. This allowed them to become accustomed to breathing through the mouthpiece, as, despite thorough familiarisation, there can be a tendency to hyperventilate on insertion of the mouthpiece. This period of rest was essential to monitor the subjects' breathing to ensure that they were not hyperventilating, or had not been in the period immediately prior to monitoring. For non-invasive estimation of the lactate threshold, it is critical that the subject does not hyperventilate prior to the test commencing as this can predispose to a false positive or "pseudo" threshold (ψ_L) (Ozcelik *et al.*, 1999; Ward & Whipp, 1992;

Whipp *et al.*, 1987). That is, hyperventilation, reducing the body's stored CO_2 content, will result in an initially lower slope of the $\dot{V}CO_2$-$\dot{V}O_2$ relationship as the CO_2 stores are refilled. When the deficit in stored CO_2 is overcome there will be an acceleration in $\dot{V}CO_2$. This acceleration in $\dot{V}CO_2$ will appear similar to $\hat{\theta}_L$ although at a lower $\dot{V}O_2$ than normally expected.

To minimise the likelihood of this happening, the following criteria were set for the resting baseline: a stable resting RER of 0.7-0.9, a $P_{ET}CO_2$ in the range of 37-43mmHg and a $\dot{V}_E$ of 5-10 l/min. Furthermore, not only these specific criteria were monitored. As mentioned earlier, a subject may hyperventilate while breathing through the breathing apparatus and/or during the period they are in the laboratory prior to monitoring. The former is relatively easy to diagnose, as a progressively rising RER will be evident as CO_2 additional to its metabolic rate of production is washed out of the body stores (Farhi & Rahn, 1955; Jones & Jurkowski, 1979; Khambatta & Sullivan, 1974). However, the latter condition proves more complicated, as the increasing RER will not be seen due to this occurring prior to monitoring. In this scenario, as the subject's CO_2 stores will already be depleted, the symptom is a low RER, typically ~0.7 or less, followed by a slow return to normal resting levels. Only once stability around an RER of 0.8 had been restored, the other criteria outlined above met and with stable baselines established for all of them would the subject be instructed to commence cycling.

Following the resting phase all exercise tests were initiated with as functionally close to 'unloaded' pedalling as the ergometer could provide, i.e. ideally where no external resistance was applied, with the only energy being expended to rotate the mass of the legs. However, despite applying no external resistance to the ergometer's flywheel, power must still be expended to overcome the inertia of the flywheel plus the internal friction of the flywheel itself. Therefore, in practice (unless otherwise specified) for 'unloaded' pedalling a work-rate of 20W was employed; as this was the lowest power output for which the ergometer input-output relationship was reliably linear (figure 2.2).

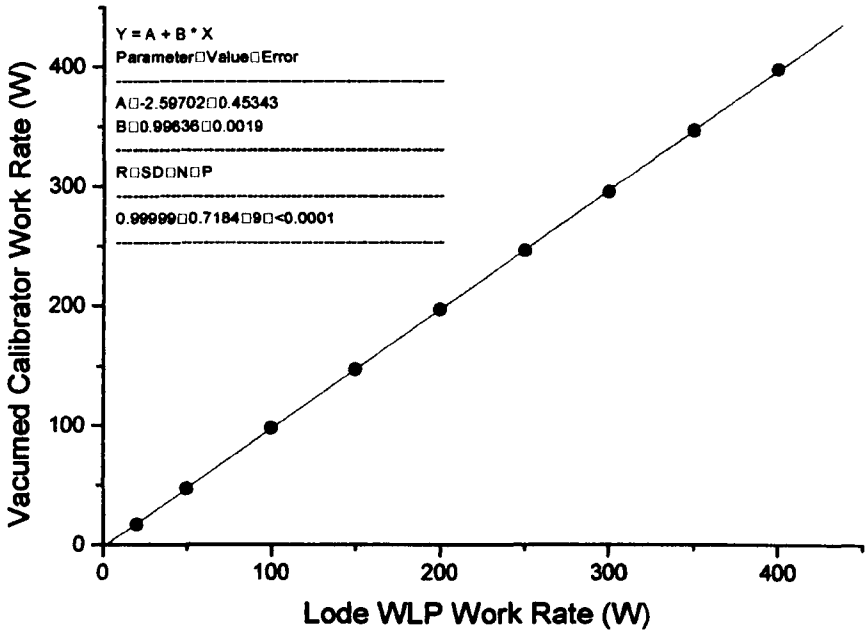


Figure 2.2: The relationship between the measured power output from the lode cycle ergometer (from a Vacumed motor driven torque calibrator) and the stated work-rate (from the Lode work load programmer, WLP) during a sample calibration.

Subjects were instructed initially to pedal around 40-50 rpm, although for all power outputs above 'unloaded' the ergometer's computer controller manipulated resistance inversely to cadence, thus maintaining power output constant (Lode,

Excalibur Sport Operator Manual V2.0, 1994). For the essentially zero external resistance applied during 'unloaded' pedalling, increasing cadence increases the total O_2 cost of subjects moving their legs against the fixed internal resistance of the flywheel:

Total work/min = (Internal resistance + work of rotating legs) . cadence *eq.* 2.1

Therefore, the subjects were instructed to keep a comfortable rhythm around 40-50 rpm firstly to prevent engendering an increased O_2 cost and secondly, as, through pilot work, this range of cadences was found to provide the smoothest pedalling motion. Subjects continued cycling at 'unloaded' pedalling until a stable response had been attained for all variables (typically three to four minutes) and the criteria outlined earlier had been met, allowing for a small elevation in $\dot{V}_E$ and possibly in $P_{ET}CO_2$ (see page x for discussion) compared to resting.

Typically, the incremental test protocol was initiated on the ergometer's computer controller, without the subjects' knowledge, 2-3 minutes after these criteria had been attained. As the individual work-rate increments were imperceptible it typically took several minutes before the subject became aware that the "ramp" phase had begun; thus denying them any time reference for the duration of the "ramp" period.

The subjects were instructed to increase the cadence at will after the lactate threshold had been passed, particularly if it was felt their cadence was

inappropriate (i.e. too low for the work-rate). As the computer varies resistance inversely with cadence, it can appear easier to produce higher power outputs via a fast cadence and relatively low resistance than *vice versa*. Although increasing cadence at a given work-rate would engender an additional O_2 cost, from the extra cost of rotating the legs (regardless of the inverse relationship between cadence and resistance), this was acceptable as the final outcome of the test was the maximal uptake of O_2. Therefore any such additional (but modest) O_2 cost more than offset the potential for a subject fatiguing early through mechanical limitations associated with producing high power via low-cadence, high-resistance cycling.

The end point of the incremental test was deemed to be when the subject could no longer maintain a cadence above 50 rpm. The subjects were also at liberty to request the cessation of the test at any point, although this did not happen during any study. At the end-point the external resistance was removed by exiting from the ramp protocol to allow the subject active recovery at 'unloaded' pedalling; aiming to maintain venous return by keeping the muscle pump operational and thus prevent venous pooling and its detrimental consequences on cardiac output and arterial blood pressure. This was continued for at least six minutes, or until the experimenters deemed it was appropriate for the subject to stop cycling.

2.2.3 Square-Wave Exercise Tests

As with the incremental tests, all square-wave exercise tests were initiated from a background of quiet rest followed by 'unloaded' pedalling (figure 2.3), after the criteria outlined previously had been met. From 'unloaded' pedalling the work-rate was increased abruptly, without prior warning, to the pre-programmed required level (specific to the individual test) in less than 1 second (power slope 1000W/s) (Lode, Excalibur Sport Operator Manual V2.0, 1994). Depending on the size of the work-rate increment, the subject was instructed to maintain the cadence if the change was relatively small, or to transiently increase the pedalling speed, to overcome the inertia, if the increase in power output was relatively large.

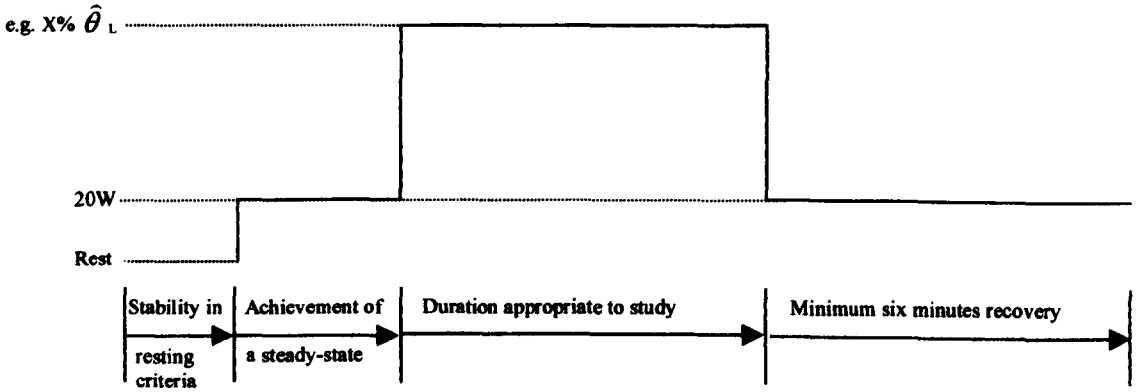


Figure 2.3: Schematic representation of a square-wave exercise trial. Commencing with a period of monitored quiet rest, until stability at the desired level in RER $P_{ET}CO_2$ and $\dot{V}_E$ had been achieved. Followed by a period of unloaded pedalling for sufficient time to attain a steady-state in all variables of interest. A step increase in power output to the desired level for the study, maintained as required by the study design. Finally a step decrease in power output, returning to 20W to facilitate recovery with a minimum duration of 6 minutes.

Once working at the new work-rate the subject continued at a constant cadence until the next phase of the protocol (see each experimental chapter for specific details). That is, the work-rate could be increased, decreased or returned to 'unloaded' as required by the specific study; again these changes were made by the ergometer's work load programmer essentially instantaneously. If the work-rate was exhaustive the same end-point criteria as used for incremental tests were applied for termination of the test. The final phase of any constant load test was always 'unloaded' pedalling for a minimum of six minutes. This allowed both measurement of the off-transient responses from the particular work-rate employed and active recovery from the higher level of exercise (see earlier for discussion).

The selection of work-rates for each study is outlined in the individual experimental chapters but all followed the same basic criteria to ensure the subjects were all working at the same exercise intensity: i.e. moderate, heavy, very heavy or severe, demarcated by the lactate threshold ($\hat{\theta}_L$) and fatigue threshold (θ_F) (see section 1.2 , figure 1.2 & figure 1.3). The appropriate intensity domain was selected for the purposes of each study and subjects assigned work-rates within that domain.

2.3 Equipment

2.3.1 Cycle Ergometer

The cycle ergometer (Excalibur Sport, Lode BV, Gronigen, The Netherlands) used in these studies is controlled by a work load programmer computer (Lode BV, Gronigen, The Netherlands), allowing cadence-independent imposition of work-rates; i.e. the computer controls the resistance applied to the flywheel (in the form of an electromagnetic brake) inversely proportionally to changes in cadence. The power output is linear over the range of 10-1000W, independent of cadence between 30 and 120 rpm, and is accurate to $\leq$ 2% from 20W (Lode, Excalibur Sport Operator Manual V2.0, 1994). Changes in power output of up to a rate of 1000W/s can be achieved; this setting was fixed for all studies. The ergometer was calibrated using a motor-driven torque calibrator (VacuMed, model 17800, Ventura, California, USA); a sample calibration is shown in figure 2.2.

2.3.2 Heart Rate and Arterial O_2 Saturation

Heart rate was measured beat-by-beat continuously throughout all tests as the R-R interval from a six-lead ECG (Q710, Quinton, Kent, UK). Paper recordings of the ECG profile were made during the tests for detection of any cardiac abnormalities that would predispose the subject to risk from maximal exercise

testing. These printouts were annotated with the work-rate being performed by the subject and the saturation of oxygen in arterial blood (S_aO_2) measured by a near-infra-red pulse oximeter (Satlite trans, Datex Engstron, Helsinki, Finland) from the ring finger of the subject's left hand.

2.3.3 Turbine Flow Sensor

During all tests the subjects breathed through a low resistance, low dead space mouthpiece assembly which had an integrated turbine flow sensor and gas sample line. Housed inside the turbine flow sensor is a rapidly responding ($\tau_{Final\ Speed}$ 30 ms) lightweight (5 mg), low resistance (< 1.5 $cmH_2O/l/s$ at 3 l/s) impeller (Interface Associates Inc., Laguna Niguel, California, USA) which is driven in opposite directions by inspired and expired gas flow. Four light emitting diodes send infrared light beams across the internal bore of the flowmeter which are sequentially broken by the spinning impeller. The interruptions in the light beam are detected by four phototransistors, the voltage output generated by these transistors being a measure of respired gas flow. This output is processed to determine both the rate and direction of flow, and the volume for each respiratory half-cycle (Interface Associates Inc.).

The output signal from the transducer is linear from 0.1 to 12 l/s with an accuracy of ±2%, and is insensitive to changes in gas viscosity (Interface Associates Inc.). Each transducer is individually calibrated by Interface Associates to produce a calibration factor (K-factor) of 500 impulses/l. As the K-

factor is strictly dependent on the geometrical shape of the impeller it will change if the impellars internal features change. Therefore, to prevent saliva contamination of the impeller changing its mass a screen and saliva trap were placed between the impeller and the subject. Calibration of the system must be performed before each experiment to allow for the influence of changes in ambient pressure, temperature and humidity on the transformation of the output voltage to volume.

2.3.4 Mass Spectrometer

Measurement of respired gas concentrations was performed every 20 ms by a quadrupole mass spectrometer (QP9000, Morgan Medical, Kent, England). An inlet rotary pump drew a continuous sample of gas along the capillary sampling-line to the mass spectrometer. In addition to transporting gas from the subject toward the mass spectrometer, the pressure drop across the capillary provides a suitable driving force across the molecular leak to introduce a very small flow of the gas sample into the high vacuum of the analyser. Once inside the analyser, the sample gas molecules are ionised by electron bombardment and then injected down the axis of the quadrupole analyser. The electrostatic fields of the quadrupole lens separate the ions by their mass-to-charge ratio; at a given instant in time only one mass to charge ratio can pass through the analyser. The effect of this, therefore, is to 'electrically filter' the component species of the inflowing gas, so that only one species at a time can pass through the analyser to the electron multiplier and finally reach the ion detector. Therefore, each species of

gas emerges, one at a time, from the ion detector as a small voltage proportional to the fractional concentration of that gas species in the original sample. This output is amplified and passed onto the analogue-to-digital converter.

The outputs from the mass spectrometer and turbine transducer were digitized and relayed to the computer, where they were phase-aligned (Beaver *et al.*, 1973) (see page 95) allowing on-line computation of breath-by-breath pulmonary gas exchange and ventilation by the algorithms detailed below.

2.3.5 Gas Exchange Algorithm

The computation of ventilation and pulmonary gas exchange was performed online, breath-by-breath, using the algorithms of Beaver *et al.* (1973). These algorithms calculate pulmonary gas exchange over the duration of a single breath. The basic concept is the same as for gas collection methods (e.g. Douglas bags), whereby the continuously-measured expired flow is divided into consecutive temporal samples at the same frequency the gas concentrations are being analysed. Therefore, in the limit, the volume of gas expired over a given period (T) is given by:

$$V_E = \int_{t=0}^{T} \dot{V}\,exp(t)dt \qquad eq.\ 2.2$$

where $\dot{V}exp$ is the expired flow during an infinitesimally short time interval dt, and the corresponding increase in expired volume is given by the product $\dot{V}exp$

(t) x dt. However, in practice dt is replaced by a constant Δt and the mean flow across the time interval $(t + \Delta t)$ replaces the instantaneous flow at (t):

$$V_E = \sum_{t=0}^{T} \dot{V} \exp (t + \Delta t) \, . \, \Delta t \qquad \textit{eq.}\ 2.3$$

where $\dot{V} \exp$ is the mean flow rate during the time interval $t + \Delta t$. Minute ventilation ($\dot{V}_E$) is obtained by summing V_E across the duration of an expiration (T_E) and that sum divided by T_E. To calculate $\dot{V}O_2$ and $\dot{V}CO_2$ the same process is applied to the product of the gas concentration and the expired flow for each small sampling period, such that in the limit:

$$VO_2 = \int_{t=0}^{T} \dot{V} \exp (t)dt \, . \, [(\Delta FO_2)true] \qquad \textit{eq.}\ 2.4$$

and, assuming F_ICO_2 to be quantitatively negligible,

$$VCO_2 = \int_{t=0}^{T} \dot{V} \exp (t)dt \, . \, F_ECO_2 \qquad \textit{eq.}\ 2.5$$

However, in practice dt is substituted for the sampling time interval Δt:

$$VO_2 = \sum_{t=0}^{T} \dot{V} \exp (t + \Delta t) \, . \, \Delta t \, . \, [(\Delta FO_2)true] \qquad \textit{eq.}\ 2.6$$

and:

$$VCO_2 = \sum_{t=0}^{T} \dot{V} \exp (t + \Delta t) \,.\, \Delta t \,.\, F_E CO_2 \qquad eq.\ 2.7$$

where the true O_2 difference is $[(\Delta FO_2)\text{true}] = \dfrac{(F_IO_2 - F_EO_2 - F_IO_2 \,.\, F_ECO_2)}{(1-F_IO_2)}$

Thus $\dot{V}O_2$ and $\dot{V}CO_2$ are the summed VO_2 and VCO_2's across the duration of an expiration (T_E) and subsequently divided by T_E.

Through the principles of these equations and a correction to account for the small dead space of the turbine assembly, it is possible to measure accurately on-line the pulmonary exchange of oxygen and carbon dioxide on a breath-by-breath basis. A note of caution which must be remembered when dealing with respiratory variables is the convention to express $\dot{V}_E$ as body temperature, pressure and saturated with water vapour (BTPS), i.e. its natural conditions, but to standardise $\dot{V}O_2$ and $\dot{V}CO_2$ to standard temperature and pressure dry (STPD). Therefore, the computer applied the appropriate correction factors to take account of the atmospheric conditions during each experiment.

2.3.6 Calibration

Prior to each test, the turbine transducer and the mass spectrometer were calibrated using known standards. A known volume (3l) was 'inspired' and 'expired' repeatedly (10 times per direction) from a precision syringe (Hans Rudolph, Kansas City, USA) through the turbine transducer across a range of flow rates. The 'true' values were then compared with the measured volumes and accepted if the mean was within 0.2% (i.e. between 2.995 and 3.005l) of the 'true' volume.

To calibrate the mass spectrometer, two gases of known, precision-analysed concentrations were sampled. The gases were chosen to cover the range of fractions of CO_2, O_2 and N_2 typically seen during exercise (i.e. mixtures that cover high and low CO_2, O_2 and N_2). A calibration check was repeated at the end of each experiment. In the rare event of more than 1% change in the measurement of the gases from pre- to post-experiment the results were discarded.

As a subject expires, the gas passes from the mouth into the mouthpiece assembly where a small portion is continuously drawn off by the capillary sampling line, the remainder drives the impeller housed in the turbine transducer. The information regarding flow and volume is relayed, essentially instantaneously, to the computer. Meanwhile the gas sample is only beginning the physical transit along the capillary sample line to reach the mass spectrometer

before being analysed and the gas compositions being passed to the computer. Therefore, to facilitate correct computation of breath-by-breath pulmonary gas exchange these signals must be time-aligned. A measurement of the delay associated with the sample line and the response time of the spectrometer was undertaken prior to every test. The delay was measured by passing a high velocity, high CO_2 concentration bolus of gas down the sample line, this was achieved using a small dead-space solenoid valve (Beaver *et al.*, 1973). The voltage signal from the switch was recorded on the chart (running at 100 $mm.s^{-1}$) and used as time zero. The delay was measured as the latency from time zero to 63% of the spectrometer response (i.e. one time constant) (figure 2.4). This was chosen as the spectrometer exhibited first order dynamics, i.e. an exponential output in response to the square wave input. Three repeats were performed before every test, the mean recorded and imputed into the breath-by-breath software.

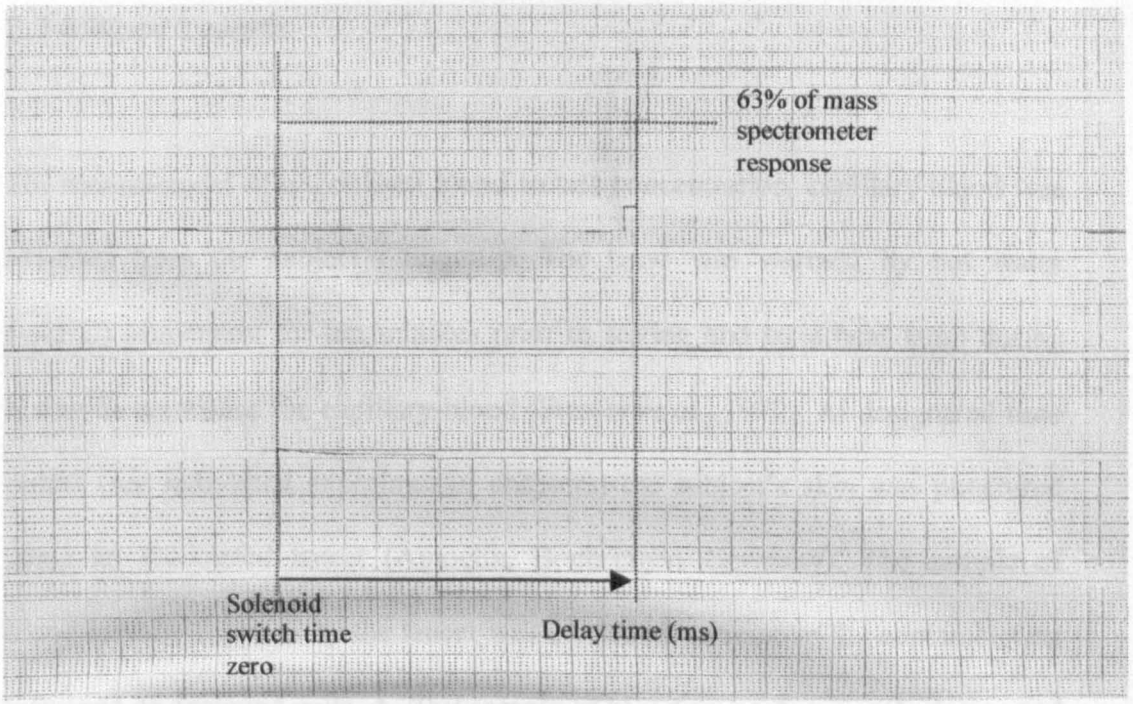


Figure 2.4: A representative delay estimation for the QP9000. The signal from the solenoid switch is taken as time zero and the delay recorded as the latency until 63% off the mass spectrometers steady-state response is achieved (Beaver *et al.*, 1973).

2.3.7 Data capture

Respired O_2, CO_2 and volume profiles were also continuously recorded using a digital chart recorder (Dash 10, Astro-Med Inc., Rhode Island, USA), together with beat-by-beat heart rate (Quinton 710), work-rate (Lode Excalibur sport) and perceptual ratings of leg fatigue and dyspnoea from a visual analogue scale (VAS) (Glasgow University, Electronic Engineering Department) (Whipp & Ward, 1992). Throughout all tests the chart was run at 1 $mm.s^{-1}$ (a resolution sufficient to observe individual breath characteristics) for editing purposes (see section 2.4). Additionally, for short periods during the steady-state, typically 5-6 breaths, the chart was run at 25 $mm.s^{-1}$. This increased resolution allowed for graphical estimation of mean alveolar PCO_2 (P_ACO_2) (see page 107).

2.3.8 Blood Lactate

For measurement of arterialised blood lactate concentration, capillary blood was sampled from the subject's fingertip. The hand was warmed by hot water (~42°C) immersion for ten minutes prior to testing and by a heat lamp during testing to arterialise the capillary blood (Froster *et al.*, 1972). At designated time points (see individual experimental chapters) the subject's skin was punctured using an automated lancet (Autoclix, Boehringer, Germany). The sample of blood initially released by the lancet was discarded, to remove the contaminating influence of damaged cells. A 30µl sample of blood was subsequently drawn and

collected in a 50µl capillary tube prepared with heparin (an anti-coagulant), fluoride (a glycolysis inhibitor) and nitrite (an anti-oxidant). Following thorough mixing the sample was either immediately analysed or capped and stored on ice for subsequent analysis (delay < 1 hour post sampling).

All analysis was performed using an automated lactate analyser (Analox GM-7, Analox Instruments, London, UK). An oxygen electrode was used to detect changes in PO_2 brought about when lactate is oxidised to pyruvate in the presence of lactate oxidoreductase:

$$\text{Lactate} + O_2 \leftharpoons \text{Pyruvate} + H_2O_2 \qquad eq.\ 2.8$$

Analysis of a sample was accepted when agreement of less than 0.1 mM between readings was reached. Calibration was performed with an 8 mM standard and repeated post-analysis as a calibration check. Only if measurement of the standard was within +/- 0.2 mM were the results accepted.

2.4 Analysis

2.4.1 Data editing

Breaths that are not part of the physiological response data set occur primarily for two reasons. Either the subject voluntarily takes a breath uncharacteristic of the current breathing pattern (e.g. a particularly large breath, or a long inspiration

and very short expiration) or the on-line software 'mis-triggers' a breath. The latter can occur if the subject coughs or swallows during a breath, as the determination of a breath is made by the software 'looking' for a change in the direction of the volume signal above a certain threshold: this value can be adjusted by the experimenter prior to a study. However, a careful balance must be met: higher volumes chosen for accurate breath detection will mean less 'mis-triggering' of breaths, but will lead to underestimation of exhaled O_2 and CO_2 volumes and to more breaths with small tidal volumes (e.g. at rest) being missed.

To deal with 'mis-triggered' breaths, the raw signals of respired O_2, CO_2 and volume recorded on a chart were analysed in conjunction with numerical print-outs of the constituent parts of the breath, i.e. inspired time, expired time, tidal volume and end tidal PCO_2 and PO_2. From this, breaths that were clearly atypical of the surrounding breathing pattern were removed from the data set (figure 2.5). Breaths where there was any degree of uncertainty over the classification of a 'mis-trigger' were left in the data set.

The 'noise' typically associated with breath-by-breath gas exchange has been extensively classified as an uncorrelated Gaussian distribution (Lamarra *et al.*, 1987, Rossiter *et al.*, 2000; Puente-Maestu *et al.*, 2001). Therefore, it is reasonable to conclude that any breaths lying outwith prediction bands enveloping 4 standard deviations from the mean are unlikely to be part of the underlying physiological response to the imposed work-rate forcing (Lamarra *et al.*, 1987, Rossiter *et al.*, 2000; Puente-Maestu *et al.*, 2001). Based on these criterion breaths that were not part of the underlying response, either through

'mis-triggering' or the subject voluntarily taking an uncharacteristic breath, were removed from the data set.

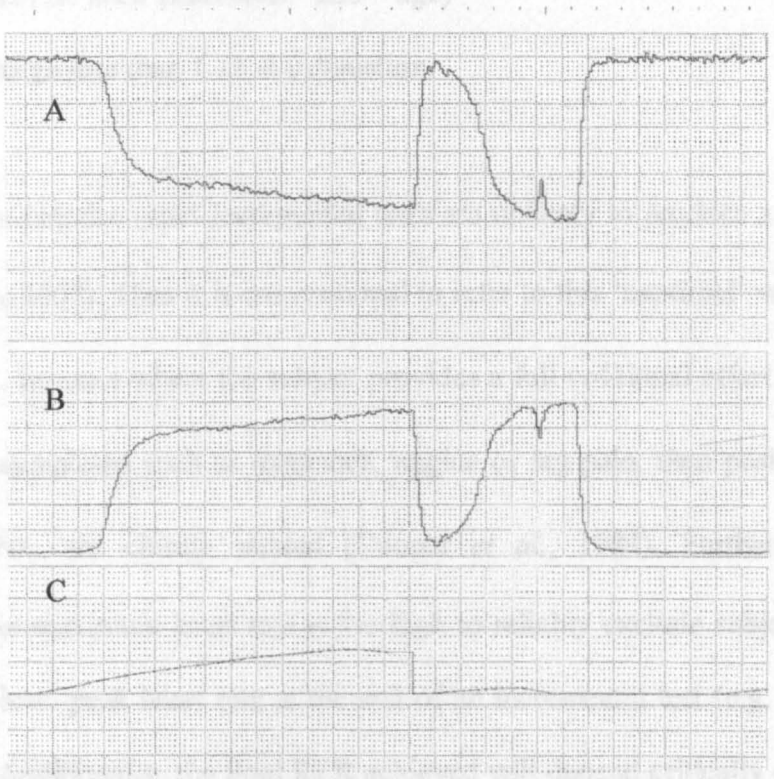


Figure 2.5: A recording of respired O_2 (A), CO_2 (B) and volume (C) demonstrating how an event such as a cough can create non-physiological breaths. The breath detection software has split this 'breath' into two separate breaths (C). The values for both these breaths were not representative of the surrounding response.

2.4.2 Calculation of $\dot{V}O_2$ Peak

Peak $\dot{V}O_2$ ($\mu\dot{V}O_2$) was calculated as the mean $\dot{V}O_2$ during the final 30s of an incremental test. It is often reported in the literature that two of the three following criteria should be met before the test can be classified as having being maximal (e.g. Hale *et al.*, 1998; ACSM, 2000):

1. a plateau in $\dot{V}O_2$ was achieved.
2. the peak heart-rate was within +/-10 bpm of the age-predicted maximum heart rate (i.e. max. heart rate = 220 – age)
3. RER was greater than 1.15 at exhaustion.

However, in practice rapid-incremental tests are unlikely to produce a plateau in $\dot{V}O_2$ (Coats, 2003). Thus it is conventional to refer to this 'maximal' value as the 'peak' $\dot{V}O_2$. In cases where the subject provides a full volitional effort and is not limited by symptoms such as dyspnoea, angina or leg pain, then peak $\dot{V}O_2$ and maximal $\dot{V}O_2$ are closely related (Cooper *et al.*, 1984). Furthermore, the variability in maximum heart rate is too high to reliably exclude subjects on the basis of a 'low' peak heart rate at the end of an incremental test (e.g. Cooper *et al.*, 1984). Additionally, the final RER achieved will depend critically on the rate at which stored CO_2 is 'unloaded' as a result of HCO_3^- buffering of metabolic acid and also because of respiratory compensation, both of which are highly dependent on the work-rate incrementation rate (e.g. Whipp, 1987). Therefore, all values reported are peak $\dot{V}O_2$ rather than $\dot{V}O_{2\,MAX}$. When this parameter was of specific importance to the study design, rather than simply part of the subject demographics, the incremental tests were repeated in an attempt to ensure a maximal effort had been given.

2.4.3 Lactate Threshold Estimation

The lactate threshold ($\hat{\theta}_L$) was estimated non-invasively from the pulmonary gas exchange and ventilatory consequences of the proton produced in association with the lactate anion. For rapid incremental exercise tests, these changes can be detected using a cluster of indices that include the V-slope (Beaver *et al.*, 1986) and a series of ventilatory-based variables (Davis *et al.*, 1982; Whipp *et al.*, 1981). The underpinning physiology is the production of extra non-metabolic CO_2 during the bicarbonate buffering reaction:

$$La^- + H^+ + xHCO_3 \leftharpoons H_2CO_3 \leftharpoons CO_2 + H_2O \qquad eq.\ 2.9$$

where La^- is the lactate anion and x is Na in the muscle or K in the blood. The result of this is an acceleration from $\hat{\theta}_L$ onwards of $\dot{V}CO_2$ relative to $\dot{V}O_2$ as the work-rate continues to increase (figure 2.6).

The increased rate of CO_2 clearance above $\hat{\theta}_L$ is associated with a proportional increase in $\dot{V}_E$, such that $\dot{V}_E$ immediately increases out of proportion to $\dot{V}O_2$. This change can be observed as the $\dot{V}O_2$ at which $\dot{V}_E/\dot{V}O_2$ and $P_{ET}O_2$ begin to increase while there is no concomitant rise in $\dot{V}_E/\dot{V}CO_2$ or $P_{ET}CO_2$, i.e. hyperventilation relative to O_2 but not to CO_2, which has been termed isocapnic buffering (Wasserman *et al.*, 1977; Whipp *et al.*, 1978; Whipp *et al.*, 1989). The increase in $\dot{V}_E/\dot{V}O_2$ without change in $\dot{V}_E/\dot{V}CO_2$ is important as it is atypical of

hyperventilation caused by non-specific factors unassociated with the increasing work-rate (e.g. anxiety or hypoxia).

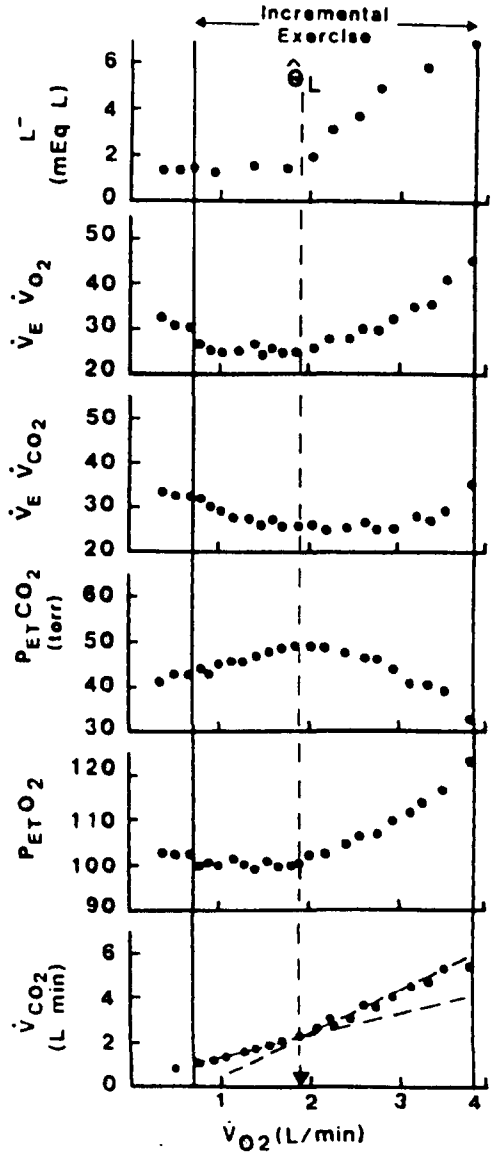


Figure 2.6: Ventilatory and pulmonary gas exchange responses during an incremental exercise test (15W/min). The solid vertical line is the onset of the incremental phase and the dashed vertical line is $\hat{\theta}_L$. Reproduced from Whipp, 1994.

Typically, 2-3 minutes after $\hat{\theta}_L$ a further increase in $\dot{V}_E$, out of proportion to $\dot{V}CO_2$, is observed. This is known as the 'respiratory compensation point' (RCP) (Wasserman *et al.*, 1977) and has been argued to reflect H^+ stimulation of

the carotid bodies although the reason for the delay is as yet not fully understood (Rausch *et al.*, 1991; Whipp, 1981; Whip & Ward, 1991). While the RCP does not directly impinge on the determination of $\hat{\theta}_L$, it is used to set the range of data over which the determination of $\hat{\theta}_L$ will be made (Beaver *et al.*, 1986). That is, the data from resting, 'unloaded' pedalling, the early kinetic lag phase and above the RCP is excluded, leaving only the two 'regions of interest' (Beaver *et al.*, 1986).

The V-slope method estimates the lactate threshold as the intersection of best-fit linear regression to the upper and lower regions of interest of the $\dot{V}O_2$-$\dot{V}CO_2$ plot (figure 2.6). The effects of $\hat{\theta}_L$ on ventilatory-based variables can be seen by viewing the changes in $\dot{V}_E/\dot{V}O_2$ and $P_{ET}O_2$ with an absence of change in $\dot{V}_E/\dot{V}CO_2$ and $P_{ET}CO_2$ (detailed above) from plots of $\dot{V}_E/\dot{V}O_2$ and $P_{ET}O_2$ vs $\dot{V}O_2$ with $\dot{V}_E/\dot{V}CO_2$ and $P_{ET}CO_2$ vs $\dot{V}O_2$ (figure 2.6).

The $\dot{V}O_2$ obtained from this cluster of indices can then be converted to a work-rate from the incremental test. This, however, is not equal to the steady-state work-rate associated with this $\dot{V}O_2$ value, based on the steady-state $\dot{V}O_2$-WR relationship. It has been shown that after the initial lag phase, the slope of the $\dot{V}O_2$ response during a ramp-incremental test is the same as the steady-state $\dot{V}O_2$ work-rate relationship (figure 2.7) (Whipp *et al.*, 1981). Therefore, at any point during the linear phase of the incremental response $\dot{V}O_2$ lags behind the steady-state response by the mean response time (τ`) (i.e. the sum of the phase I delay

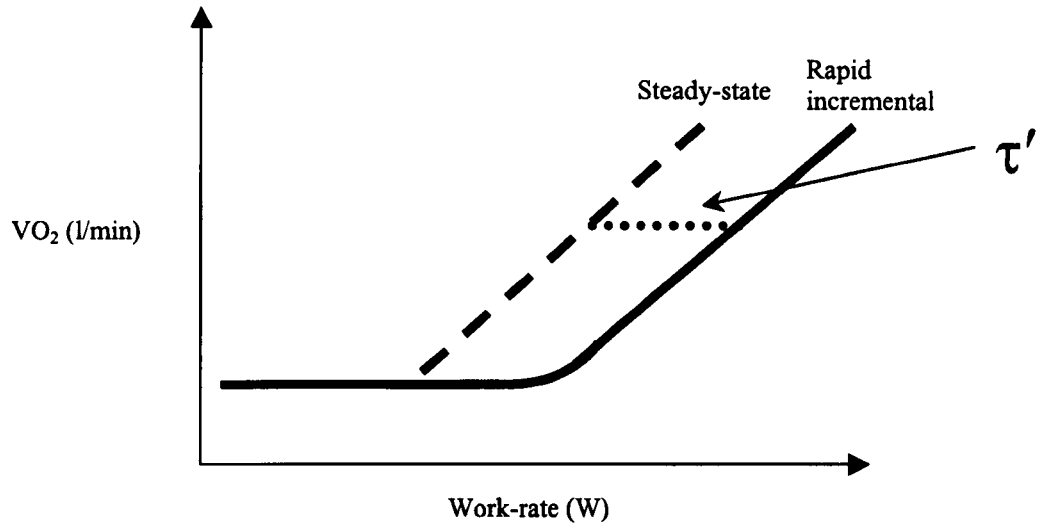


Figure 2.7: Schematic illustration depicting how $\dot{V}O_2$ during a rapid incremental exercise test lags behind the steady-state $\dot{V}O_2$ response by τ' (Whipp *et al.*, 1981).

plus phase II τ) (Whipp & Ward, 1980; Whipp *et al.*, 1981). Furthermore, it should be noted that $\hat{\theta}_L$ occurs at the same $\dot{V}O_2$ regardless of work-rate, i.e. the larger ΔWR/Δt (the incrementation rate) the higher the work-rate at $\hat{\theta}_L$ (e.g. Whipp, 1987). Thus the steady-state work-rate equivalent to $\dot{V}O_2$ at $\hat{\theta}_L$ can be calculated as:

$$WR_{\hat{\theta}L} = \text{actual work-rate} - \Delta WR/\Delta t \,.\, \tau' \qquad eq.\ 2.10$$

where $WR_{\hat{\theta}L}$ is the steady-state work-rate equivalent to the $\dot{V}O_2$ corresponding to the lactate threshold and 'actual work-rate' is the work-rate during an incremental exercise test at which $\hat{\theta}_L$ occurs. As a safe practical approximation a value of 60s is taken for τ' for normal healthy young subjects. Therefore, as Δt can also be expressed as 60s equation 2.10 simplifies to:

$WR_{\hat{\theta}L}$ = actual work-rate – ΔWR *eq.* 2.11

2.4.4 Kinetic analysis

In order to minimise the influence of the breath-to-breath 'noise' on the estimation of phase II τ multiple repeats of the transition in question were averaged (Lamarra *et al.*, 1987; Whipp *et al.*, 1982). To achieve this, each individual edited data set was linearly interpolated, i.e. the value for each breath was continuously assigned to sequential one-second time bins, until the following breath occurred (Lamarra *et al.*, 1987; Whipp *et al.*, 1982). Thus each breath was correctly 'time weighted' and there were no phases with a higher density of data which would bias the fitting procedure. The interpolated data sets were then time-aligned and averaged; thus creating an 'average' data set for a given work-rate step. These mean response data sets were then averaged (e.g. across each 10s period) further smoothing the 'noise'. To avoid contamination of the phase II response with phase I data, no fitting procedure was initiated prior to 20s after the change in work-rate (i.e. in excess of the phase I duration) (Whipp *et al.*, 1982). A single exponential (equation 2.12) was used to fit the phase II response (Whipp *et al.*, 1982):

$$X(t) = X_{(BL)} + \Delta X_{(ss)} \cdot [1-e^{-(t-\delta)/\tau}] \quad \textit{eq.}\ 2.12$$

where X is the variable of interest (e.g. $\dot{V}_E$), $X_{(BL)}$ is the mean steady-state response of the given variable from the previous work-rate, $\Delta X_{(ss)}$ is the amplitude of the phase II response, δ is a non-physiological time delay and τ is the phase II time constant. The best-fit exponential was modelled by iterative least-squares non-linear regression (Lamarra, 1987; Whipp *et al.*, 1982) using commercial software (Origin, Microcal Software, USA). The best-fit response was determined when further alteration of the model parameters did not further reduce the residual sum square of errors (χ^2).

2.4.5 Mean Alveolar PCO_2

From a continuous chart recording of respired PCO_2, run at fast speed (Ward & Whipp, 1980), it is possible to 'reconstruct' graphically the alveolar PCO_2 profile (DuBois *et al.*, 1952). This is done by extrapolating the 'alveolar' phase back to the onset of expiration, corrected for the sample line transit delay (see page x). This phase is typically linear, but occasionally curvilinear; in all situations the best-fit contour of the response was used to extrapolate to expiratory onset.

As the final portion of the expirate remains as the dead-space gas and the initial inspirate being dead space gas, the alveolar PCO_2 will continue to rise after the peak value has been obtained at the mouth and even into inspiration (Saunders & Cumin, 1992). To account for the peak alveolar values never reaching the mouth, it has been argued that the alveolar profile should be offset leftward, by a small period appropriate to the alveoli to mouth transit and the alveolar profile also

extrapolated to the actual end of expiration (Saunders & Cumin, 1992; Whipp *et al.*, 1990). However, due to the minor impact of this correction on the actual values estimated and the difficulty in actually measuring the delay (given its dependence on instantaneous flow), this was not taken account of during these studies. Therefore. all measures of P_ACO_2 are associated with a small but consistent underestimation, whose magnitude has been estimated to be of the order of 1-2 mmHg at most (Saunders & Cumin, 1992).

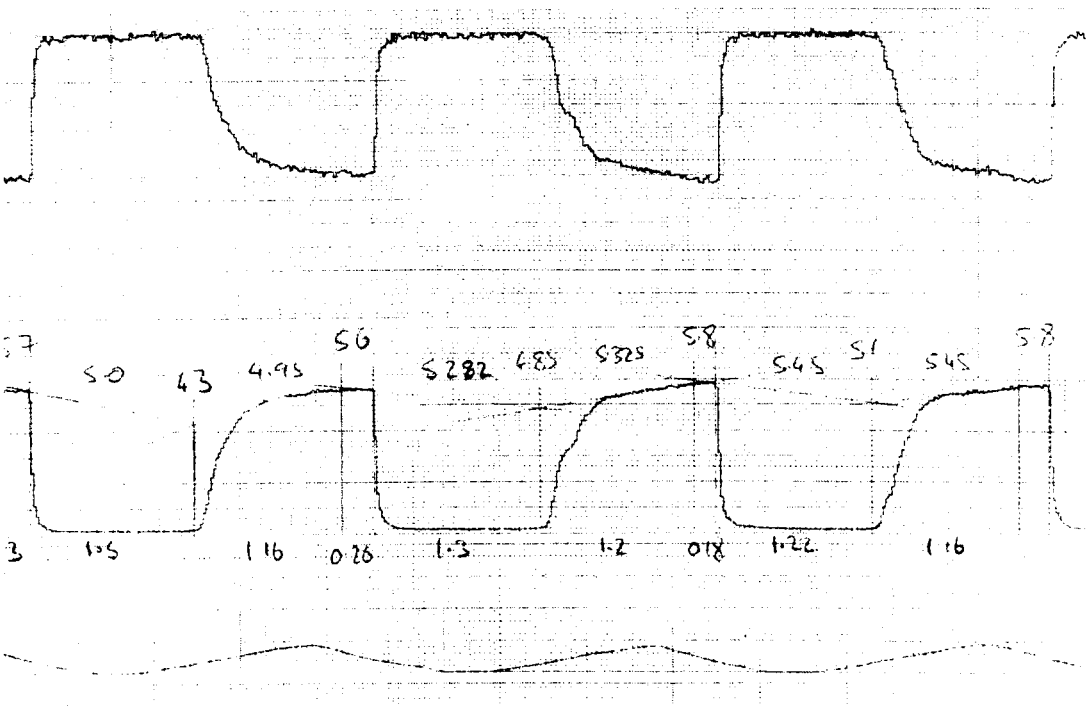


Figure 2.8: An actual example of an estimation of P_ACO_2. Shown are three consecutive breaths with the mean $[CO_2]$ values during inspiration, expiration and a transiently stable phase prior to the onset of inspiration annotated and the durations of each phase. This data was then used to calculate a time weighted value for P_ACO_2.

The inspiratory phase is assumed to be linear as the falling phase of P_aCO_2 oscillation is thought to be linear (DuBois *et al.*, 1952; Yamamoto, 1960). This is supported by the demonstration that the rising phase of the intra-breath pH_a oscillation is linear (e.g. Murphy *et al.*, 1987). Therefore, P_ACO_2 was estimated

as the time-weighted means of the rising (expiratory) and falling (inspiratory) plus the transiently stable phase (after inspiratory onset, if present) (figure 2.8) (e.g. Whipp *et al.*, 1990). Although included, the stable phase had a quantitatively negligible effect on the estimated value of P_ACO_2.

2.4.6 Statistical analysis

The particular statistical tests employed to analyse the results in each study are outlined in the individual experimental chapters.

Chapter 3

Intermittent exercise

3.1 Introduction

The seemingly complex interaction of mechanisms which underlie the exercise hyperpnoea, although as yet not fully understood, confound the interpretation of responses generated from stimulation of single mechanisms, such as direct central nervous system stimulation (e.g. Orlovskii, 1969). Therefore, it is difficult to ascribe mechanistic significance to much of the experimental data supporting the role of a central feedforward neurally-driven hyperpnoea which comes from animal preparations quite unlike, and eliciting responses quite unlike, the intact exercising human (e.g. Orlovskii, 1969). Furthermore, the ability of the ventilatory control system to operate effectively without any one of its putative primary drives, taken by some to indicate the existence of redundancy within the system (e.g. Swanson, 1992; Yamamoto, 1980), is an additional challenge to resolving the underlying control processes.

Secondly of interest is the paradoxical observation that the onset of compensatory hyperventilation is delayed, relative to the onset of metabolic acidosis, during rapidly incrementing exercise tests (Scheuermann & Kowalchuck, 1998; Wasserman *et al.*, 1977; Whipp & Ward, 1991). This is despite the rapid response kinetics typically exhibited by the carotid body chemoreceptors, the putative mediators of the hyperventilation (see page 66) (e.g. Wasserman et al., 1975), in animals to acute primary changes in arterial pH (e.g. Biscoe et al., 1970; Ponte and Purves, 1974).

Both of these issues remain unresolved. Therefore, an exercise protocol for intact humans that dissociated metabolic cost from power output and allowed detailed inspection of the temporal response profile exhibited by the compensatory hyperventilation was sought.

This search led to intermittent exercise; that is to say exercise that is characterised by short periods of exercise interspersed with periods of recovery. This format of exercise is prevalent in many major sporting disciplines and so has received much attention in regard to exercise performance although little with regard to ventilatory control. Saltin and colleagues (1976) have shown that the relative metabolic cost of performing exercise intermittently is affected not only by the amplitude of the exercise-recovery oscillations, but also by the durations of the exercise and recovery phases and the ratio of the exercise to recovery duty cycle.

Furthermore, the work of Åstrand, Christensen and colleagues (1960) investigated the responses to high intensity cycling and running when performed intermittently (Åstrand *et al.*, 1960a; Åstrand *et al.*, 1960b; Christensen *et al.*, 1960a; Christensen *et al.*, 1960b). Their results showed that manipulating the exercise-recovery duty cycle durations, but maintaining their ratio with the same work-rate amplitude, markedly affected physiological cost of performing the exercise; evidenced by the blood lactate responses (figure 3.1) (Åstrand *et al.*, 1960b). The shortest exercise period, 10s exercise 20s recovery, incurred essentially no sustained elevation in blood lactate concentration compared to rest (Åstrand *et al.*, 1960b). The intermediate duration, 30s exercise 60s recovery, led

to a sustained elevation in lactate concentration that reached a steady-state (Åstrand *et al.*, 1960b). Finally, the longest exercise bout, 60s exercise 120s recovery, resulted in a continually rising lactate concentration until fatigue ensued and the test could not be completed (Åstrand *et al.*, 1960b).

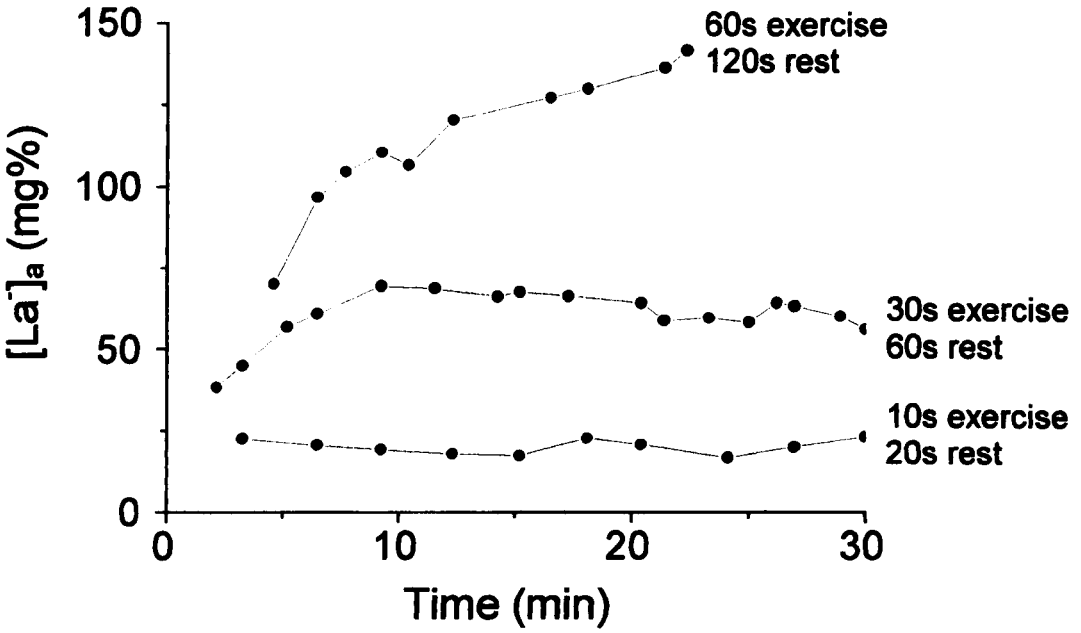


Figure 3.1: The capillary blood lactate response to three different exercise-rest duty cycle duration intermittent cycling tests. Adapted from Åstrand *et al.* (1960b).

These lactate response profiles are qualitatively similar to those obtained during sustained constant-load exercise in the moderate (below the lactate threshold), heavy (between the lactate and fatigue thresholds) and very heavy (above the fatigue threshold) intensity domains respectively (see page 4 for discussion) (Wasserman *et al.*, 1967; Wells *et al.*, 1957; Whipp *et al.*, 1981). Therefore, if the pulmonary gas exchange responses also appeared to lie in different intensity domains, as defined by the lactate response, then the different exercise-recovery duty cycle durations could perhaps reasonably be classified as being in different intensity domains. Thus, the requirement for ventilation (see earlier for

discussion of appropriate ventilation), based on the metabolically-defined intensity domain, might be expected to vary across the duty cycle durations; hence dissociating the presumably constant neurogenic drives from the metabolic requirements of performing the exercise.

Furthermore, across the different exercise-recovery duty cycle durations the neural stimulus is presumed to be essentially constant, and, the 10s exercise 20s recovery excepted, there is a substantial metabolic acidosis presumably present (Åstrand *et al.*, 1960a). Therefore, it should be possible to determine the onset point of compensatory hyperventilation and relate it either to a time-dependent or $[H^+]_a$, using $[La^-]_a$ as a qualitative "proxy" (e.g. Casaburi &Wasserman, 1991; Wasserman *et al.*, 1967), dependent mechanism (Whipp & Ward, 1991).

3.2 Aims

Therefore, the aims of the study were to investigate whether or not the pulmonary gas exchange consequences of the exercise also lay in the moderate, heavy, and very heavy domains. Then, further to that, to investigate the appropriateness of the ventilatory responses to the metabolic demand of these differing intensities of exercise; despite the same absolute work-rate being performed.

A second aim was to determine the presence of compensatory hyperventilation in each protocol and to establish the time-course of its onset and relate that to the degree of acidosis present, using $[La^-]_a$ as a proxy (e.g. Casaburi &Wasserman, 1991; Wasserman *et al.*, 1967). Thus establishing whether an amplitude or time related threshold could be implicated in the delayed respiratory compensation typically associated with rapidly incrementing exercise tests (Whipp & Ward, 1991).

3.3 Hypothesis

The ventilatory response during each protocol is hypothesised to be appropriate to the metabolic demands provided by the 'intensity domain' the exercise-recovery duty-cycle duration lies in, despite the presumably constant neurogenic drive. As such during the 10s:20s trials the ventilatory response will be appropriate for the requirement to clear CO_2. Whereas during the 30s:60s,

60s:120s and 90s:180s trials a progressively increasing level of hyperventilation to effect respiratory compensation will be seen. During all trials where respiratory compensation is present there will be a delay following the first increase in work-rate before the onset of hyperventilation as already seen during incremental exercise (Wasserman *et al.*, 1977).

3.4 Methods

Outlined here are the specific details of the subjects, apparatus, protocols and analysis techniques employed during this study, the basic principles underlying these protocols and measurements have been explained in detail already (see chapter 2).

3.4.1 Subjects

Six recreationally active males mean age 25.2 (s.d. 4.5) (table 3.1) who regularly participated in intermittent type sports (football and hockey) volunteered and provided written informed consent (appendix 3.1), approved by the Glasgow University Ethics Committee, before participating in the study. The subjects were required to adhere to the criteria outlined on page 78 prior to each visit.

Subject	Age (years)	Height (cm)	Weight (kg)	$\mu\dot{V}O_2$ ($l\,min^{-1}$)	$\mu\dot{V}O_2$ ($ml\,kg^{-1}\,min^{-1}$)	$\hat{\theta}_L$ ($l\,min^{-1}$)	μWR (Watts)
1	33	186	95.5	4.26	44.6	2.84	374
2	22	172	66.3	3.30	49.8	1.61	258
3	22	186	83.2	4.30	51.7	2.60	401
4	24	191	73.5	3.23	43.9	1.95	254
5	28	166	71.5	3.69	51.6	1.75	314
6	22	180	74.1	3.50	47.2	1.53	290
Mean	25.2	180	77.4	3.71	48.1	2.05	315
± S.D.	4.5	10	10.4	0.47	3.4	0.55	61

Table 3.1: Subject characteristics detailing both individual and group mean values for age, height, weight, absolute peak oxygen uptake ($\mu\dot{V}O_2$ l/min), peak oxygen uptake relative to body weight ($\mu\dot{V}O_2$ ml/kg/min), oxygen uptake at the estimated lactate threshold ($\hat{\theta}_L$ l/min) and peak work-rate (μWR) all functional indices measured during an during an incremental exercise test.

3.4.2 Apparatus

All exercise tests took place in the Laboratory of Human Physiology on an electromagnetically-braked, computer-controlled cycle ergometer (Excalibur sport, Lode, Netherlands). The R-R interval from a six-lead electrocardiogram (Quinton 670, USA) was used to determine heart rate, on a beat-by-beat basis. Arterial oxygen saturation was measured in the fingertip using pulse oxymetry (Datex Ohmeda). Inspired and expired gas flow and volume were measured via turbinometry (Interface Associates, Laguna Niguel, CA, USA). Respired gas concentrations (O_2, CO_2, and N_2) were measured at a frequency of 50Hz by a quadropole mass spectrometer (QP9000, Morgan Medical, Kent, England). Breath-by-breath pulmonary gas exchange was calculated on-line from this data using the algorithms of Beaver *et al.* (1973). Arterialised capillary blood was sampled at pre-determined time points throughout all tests and analysed for lactate concentration (Analox GM-7, Analox instruments, London, UK) (see general methods for detailed descriptions).

3.4.3 Protocols

Following a familiarisation session, which included both incremental and square-wave exercise tests, the subjects completed five exercise sessions all performed on the cycle ergometer (described on page 89) and separated by at least 48 hours. The first session was an incremental exercise test (15W/min) to the limit of

tolerance (t_{lim}) allowing estimation of the lactate threshold ($\hat{\theta}_L$), measurement of peak $\dot{V}O_2$ ($\mu\dot{V}O_2$) and peak work-rate (μWR) (see page 100 for discussion).

Then, in a randomly assigned order and on separate days, the subjects all performed four intermittent tests. Following 4 minutes at 20W, the work-rate for all tests was switched intermittently between 120%μWR and 20W, with a fixed exercise-recovery duty cycle ratio of 1:2 and a test duration of 30 minutes or t_{lim}, whichever came first (figure 3.2). The exercise-recovery durations, in seconds, were 10:20, 30:60, 60:120 and 90:180. Thus, all tests that reached completion required the same total amount of work to have been performed.

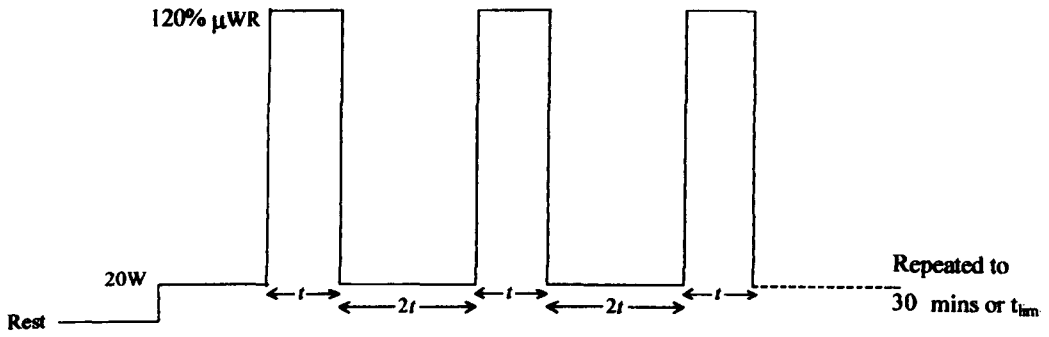


Figure 3.2: Schematic representation of the intermittent exercise protocol. Following a period of monitored rest and 20W cycling the work-rate was elevated to 120%μWR for t seconds (where t was 10s, 30s, 60s or 90s) then reduced to 20W for 2t (i.e. 20s, 60s, 120s or 180s). This pattern was repeated until 30 minutes or volitional exhaustion.

Fingertip arterialised capillary blood samples were taken at rest, during unloaded pedaling and at set intervals throughout the intermittent tests. The samples were taken on average every three minutes, at specific time points so that each test of different exercise-recovery duty cycle durations would be on the same phase and to provide both exercising and recovery values.

3.4.4 Analysis

3.4.4.1 Editing

The typical procedure for dealing with breath-by-breath 'noise', caused by random fluctuations in breathing pattern and mis-triggering of breaths (outlined earlier), requires estimation of the mean response in order to exclude the data which lies beyond 4 s.d. from that mean. However, due to the potential complexities introduced by the metabolic acidaemia the mean response could not be modelled. Therefore, to deal with erroneous values the composition of a breath was compared to those immediately surrounding it. Those breaths clearly not typical of the current response were excluded (see page 98); any breaths where there was a degree of uncertainty were left in the dataset.

3.4.4.2 Ventilatory and pulmonary gas exchange responses

The first transition from 20W to 120%μWR was viewed individually; the subsequent transitions, after stable end-exercise and end-recovery values had been achieved in a given variable, were graphically overlaid to improve signal-to-noise characteristics.

The onset of compensatory hyperventilation was investigated by estimating the onset of an increase in slope of the $\dot{V}_E$ - $\dot{V}CO_2$ relationship, for each exercise and recovery cycle separately, as a linear regression calculated by a commercial analysis package (Microcal Origin). The exception to this was the 10s exercise

20s recovery where specific oscillations in $\dot{V}_E$ and $\dot{V}CO_2$ were not seen in tandem with the work-rate changes. Therefore, where appropriate, the $\dot{V}_E$-$\dot{V}CO_2$ relationship was estimated across the entire intermittent segment of the test using the same analysis technique as for the longer cycle duration tests.

3.4.4.3 Statistical analysis

To establish whether varying the work-recovery duty cycle duration had any effect on $[La^-]_a$, the values obtained for the four intermittent tests were compared using a repeated measures One-Way Analysis of Variance (ANOVA). Post-hoc analysis (paired t-tests) was conducted when significance, accepted when $P < 0.05$, was revealed by ANOVA. All further comparisons detailed in the results section were made using paired t-tests.

3.5 Results

3.5.1 Incremental exercise test

Typical ventilatory and pulmonary gas exchange responses to a ramp incremental exercise test are displayed in figure 3.3 (subject 1), including the cluster of changes used to non-invasively estimate $\hat{\theta}_L$ (Beaver *et al.*, 1986; Davis *et al.*, 1982; Whipp *et al.*, 1981). The work-rates utilised for the remaining trials were set relative to $\hat{\theta}_L$ and μWR as determined in figure 3.3 and displayed in table 3.1.

3.5.2 Intermittent exercise 90s exercise 180s recovery

No subject was able to complete the full 30-minute duration of a 90s:180s trial; in fact one subject (number 6) was not even able to complete a single exercise-recovery duty cycle, despite completing all other intermittent trials. Two subjects fatigued on the second exercise period (subject numbers 3 & 5), one during the third (subject number 1) and one during the fourth (subject number 2). A progressive rise in $[La^-]_a$ was seen throughout the duration of the 90s:180s trials which did not peak until shortly into recovery (figure 3.4). For all variables of interest ($\dot{V}_E$, $\dot{V}CO_2$, $\dot{V}O_2$, R, $P_{ET}CO_2$ and HR) distinct oscillations in synchrony with the work-rate changes were observed and peak $\dot{V}_E$ rose progressively with each duty cycle (figure 3.5, appendix 3.2).

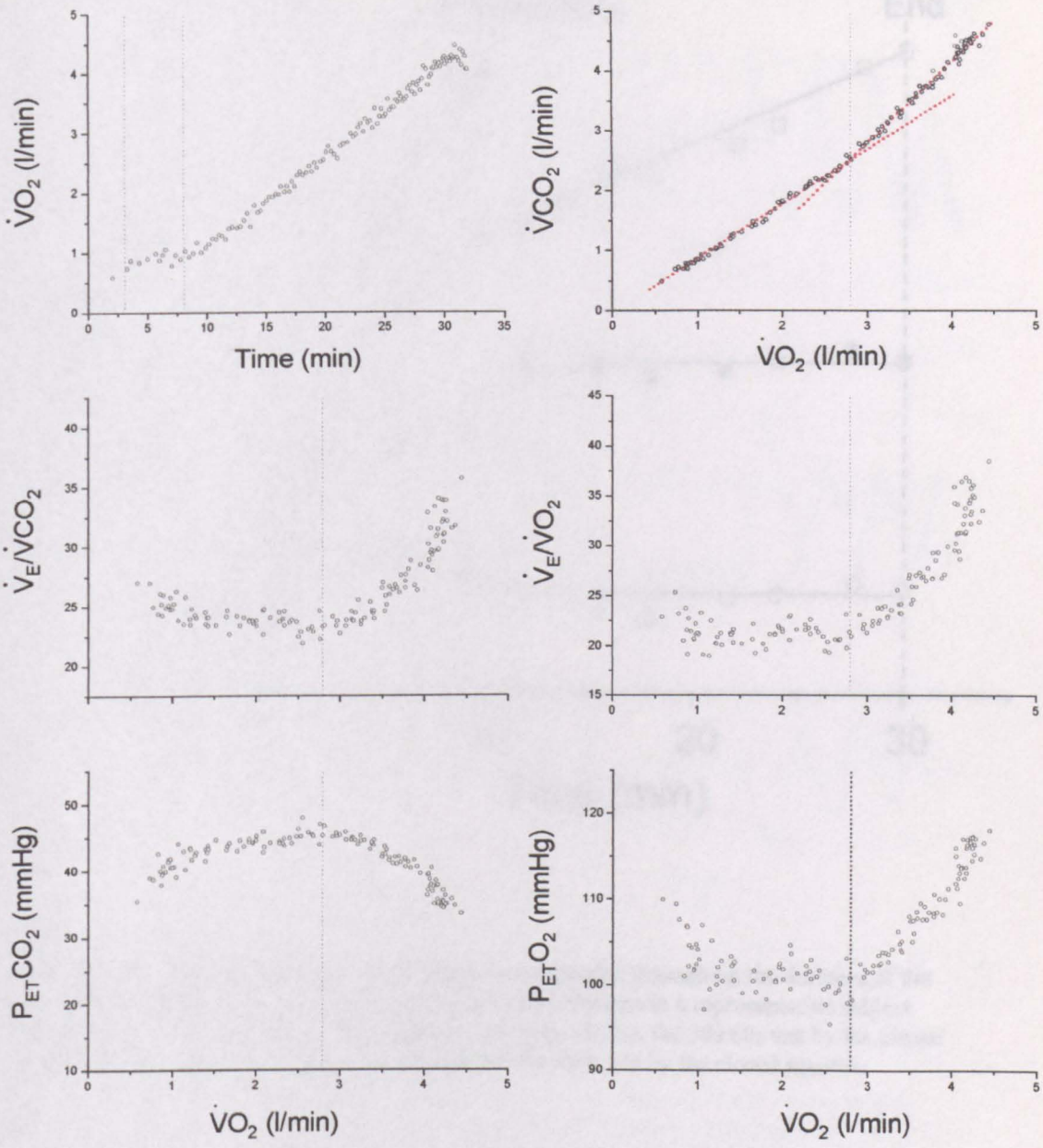


Figure 3.3: The cluster of responses used to non-invasively estimate the lactate threshold from an incremental exercise test (subject number 1). The first vertical line on the top left hand panel is the start of unloaded pedalling and the second line is the onset of the incremental phase. In all other panels the vertical lines indicate the lactate threshold.

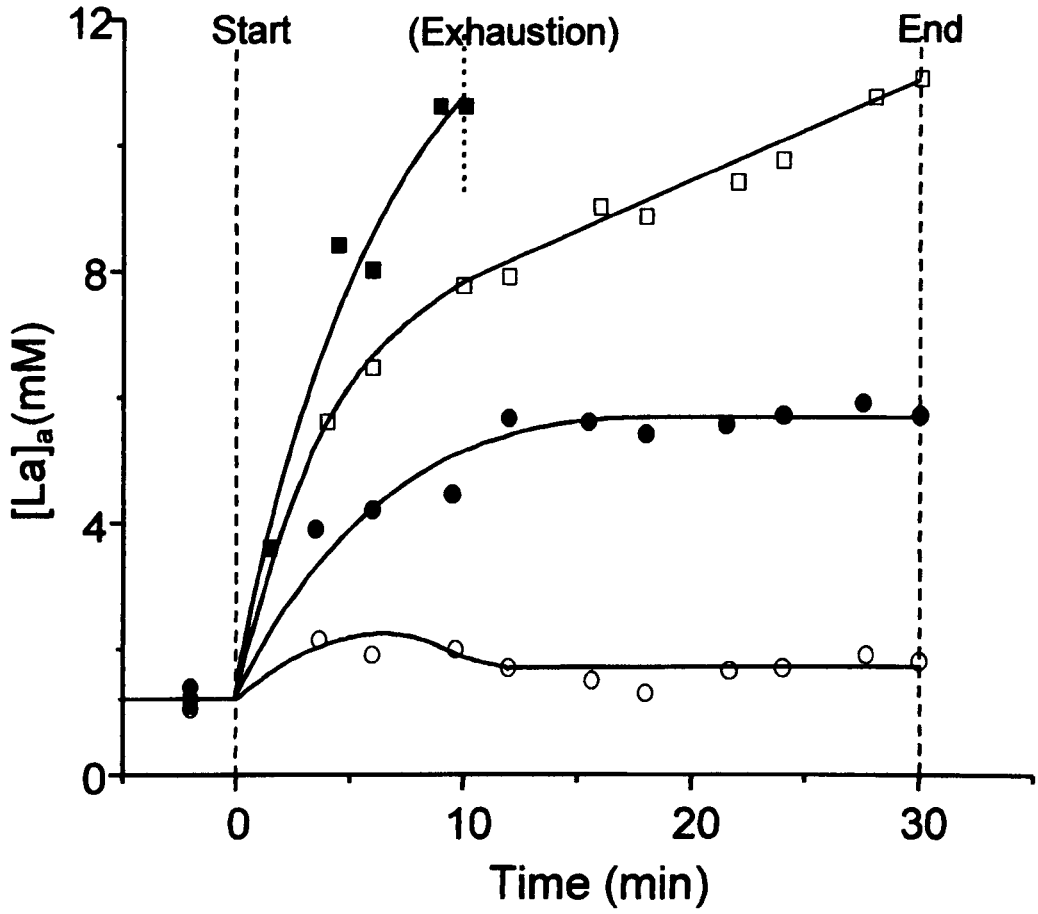


Figure 3.4: Arterialised capillary blood lactate concentration throughout the duration of the four different intermittent work: recovery duty cycle durations in a representative subject (subject 1). The 10s:20s test is represented by the open circles, the 30s:60s test by the closed circles, the 60s:120s by the open squares and the 90s:180s test by the closed squares

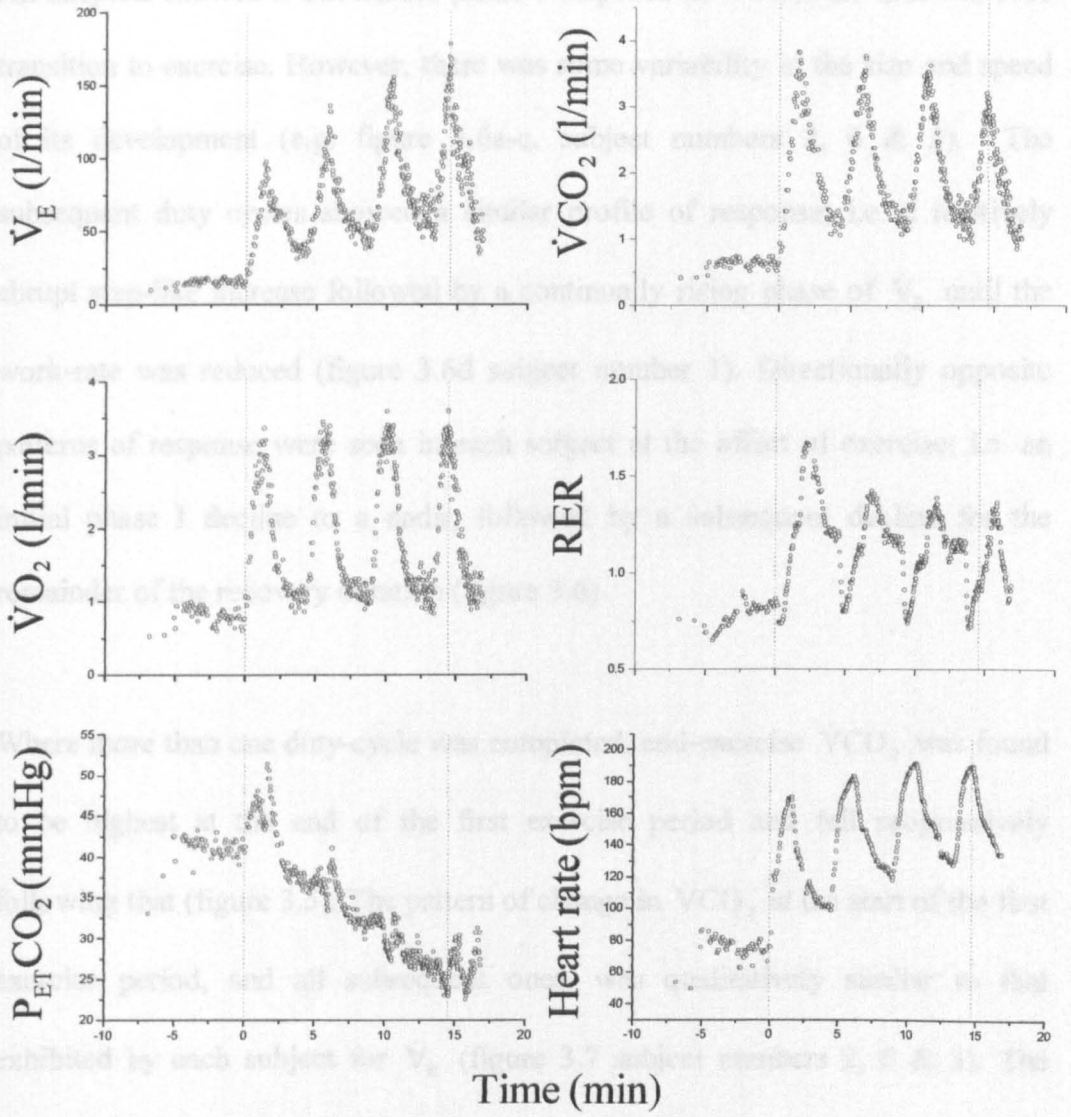


Figure 3.5: Breath-by-breath ventilatory, pulmonary gas exchange and heart rate responses from subject 2 during a 90s:180s intermittent exercise test. The first vertical dashed line indicates the onset of the first intermittent work phase and the second dashed line indicated exhaustion. Distinct oscillations with the changes in work-rate are again visible in all variables from the first work cycle.

All subjects showed a discernible phase I response in $\dot{V}_E$ on the first 90s:180s transition to exercise. However, there was some variability in the size and speed of its development (e.g. figure 3.6a-c, subject numbers 2, 6 & 3). The subsequent duty cycles showed a similar profile of response; i.e. a relatively abrupt step-like increase followed by a continually rising phase of $\dot{V}_E$ until the work-rate was reduced (figure 3.6d subject number 1). Directionally opposite patterns of response were seen in each subject at the offset of exercise; i.e. an initial phase I decline to a nadir, followed by a subsequent decline for the remainder of the recovery duration (figure 3.6).

Where more than one duty-cycle was completed, end-exercise $\dot{V}CO_2$ was found to be highest at the end of the first exercise period and fell progressively following that (figure 3.5). The pattern of change in $\dot{V}CO_2$ at the start of the first exercise period, and all subsequent ones, was qualitatively similar to that exhibited by each subject for $\dot{V}_E$ (figure 3.7 subject numbers 2, 6 & 3). The proportionality between the phase I rises in $\dot{V}CO_2$ and $\dot{V}_E$ was fairly constant between subjects, but there was no specific relationship between work-rate and magnitude of the initial change in $\dot{V}_E$ (table 3.2).

Subject	ΔWR (watts)	$\Delta \dot{V}_E$ (ϕ1 trans. 1) (l/min)	$\Delta \dot{V}CO_2$ (ϕ1 trans.1) (l/min)	$\Delta \dot{V}_E$ (ϕ1 trans. 2) (l/min)	$\Delta \dot{V}CO_2$ (ϕ1 trans.2) (l/min)	$\Delta \dot{V}_E / \Delta \dot{V}CO_2$ (ϕ1 trans. 1)	$\Delta WR / \Delta \dot{V}_E$ (ϕ1 trans. 1)	$\Delta \dot{V}_E / \Delta \dot{V}CO_2$ (ϕ1 trans. 2)	$\Delta WR / \Delta \dot{V}_E$ (ϕ1 trans. 2)
1	429	37.2	1.2	32	0.8	31	11.5	40	13.4
2	290	8.3	0.3	11.4	0.3	27.7	34.9	38	25.4
3	461	36.2	1.1	28.5	0.6	32.9	12.7	47.5	16.2
4									
5	357	13.3	0.5	16.1	0.4	26.6	26.8	40.3	22.2
6	328	5.9	0.2			29.5	55.6		
Mean	373	20.2	0.7	22	0.5	29.5	28.3	41.4	19.3
± S.D	70.8	15.31	0.46	9.82	0.22	2.52	18.13	4.17	5.49

Table 3.2: The relationship between V_E and both WR and VCO_2 during phase 1 of a 90s:180s intermittent trial

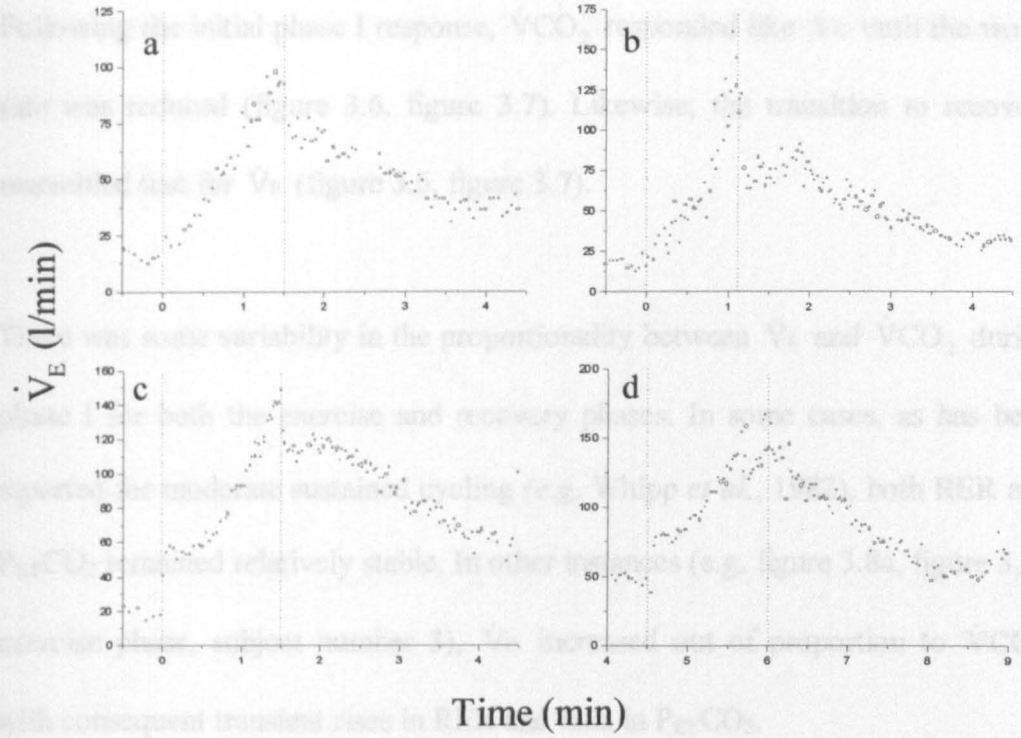


Figure 3.6: The different responses of $\dot{V}_E$ exhibited by different subjects during the first work: recovery duty cycle from a 90s:180s intermittent trial panels a, b and c. Panel d is a representative second transition from a 90s:180s trial. The first vertical dashed line is the onset of exercise and the second is the onset of recovery.

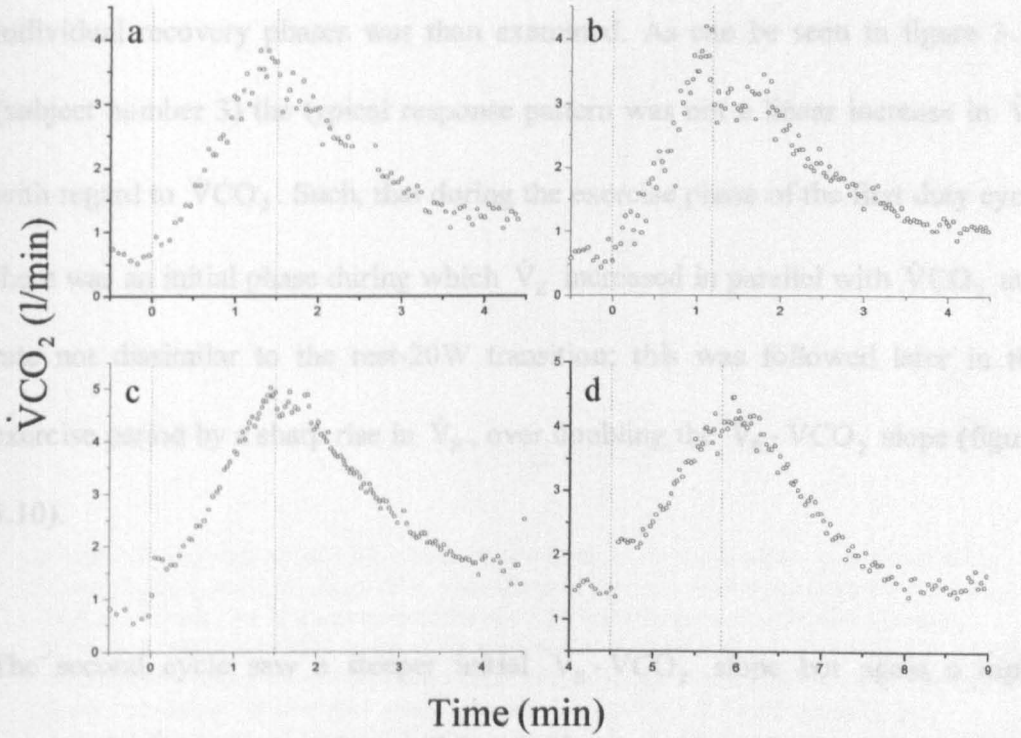


Figure 3.7: The different responses of $\dot{V}CO_2$ exhibited by different subjects during the first work: recovery duty cycle from a 90s:180s intermittent trial panels a, b and c. Panel d is a representative second transition from a 90s:180s trial. The first vertical dashed line is the onset of exercise and the second is the onset of recovery.

Following the initial phase I response, $\dot{V}CO_2$ responded like $\dot{V}_E$ until the work-rate was reduced (figure 3.6, figure 3.7). Likewise, the transition to recovery resembled that for $\dot{V}_E$ (figure 3.6, figure 3.7).

There was some variability in the proportionality between $\dot{V}_E$ and $\dot{V}CO_2$ during phase I for both the exercise and recovery phases. In some cases, as has been reported for moderate sustained cycling (e.g. Whipp *et al.*, 1982), both RER and $P_{ET}CO_2$ remained relatively stable. In other instances (e.g. figure 3.8a, figure 3.9a exercise phase, subject number 3), $\dot{V}_E$ increased out of proportion to $\dot{V}CO_2$ with consequent transient rises in RER and falls in $P_{ET}CO_2$.

To ascertain the extent to which respiratory compensation for the metabolic acidosis was present, the $\dot{V}_E$ - $\dot{V}CO_2$ relationship across individual exercise and individual recovery phases was than examined. As can be seen in figure 3.10 (subject number 3) the typical response pattern was not a linear increase in $\dot{V}_E$ with regard to $\dot{V}CO_2$. Such, that during the exercise phase of the first duty cycle there was an initial phase during which $\dot{V}_E$ increased in parallel with $\dot{V}CO_2$ at a rate not dissimilar to the rest-20W transition; this was followed later in the exercise period by a sharp rise in $\dot{V}_E$, over doubling the $\dot{V}_E$ - $\dot{V}CO_2$ slope (figure 3.10).

The second cycle saw a steeper initial $\dot{V}_E$ - $\dot{V}CO_2$ slope but again a rapid increase in $\dot{V}_E$ not occurring until late in the cycle was observed (figure 3.11, figure 3.31). Although only two subjects managed to start a third duty cycle and

only one a fourth no second phase $\dot{V}_E$-$\dot{V}CO_2$ response was observed (figure 3.11, figure 3.31).

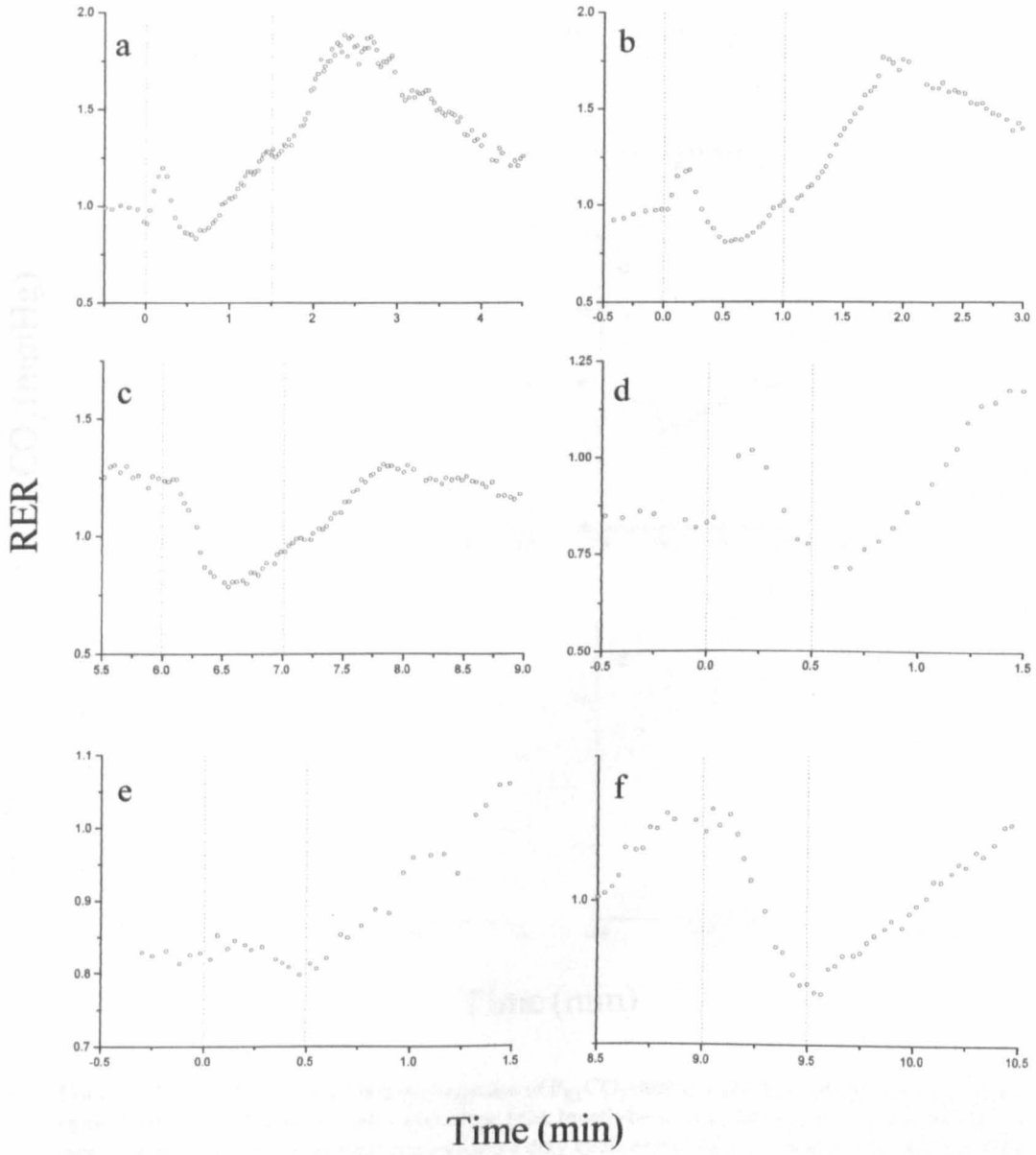


Figure 3.8: The response of RER during the first transition to the 90s:180s intermittent work-rate is shown in panel (a). Panel (b) is representative of a subject who showed an increase in RER shortly after the first transition to the 60s:120s intermittent work-rate, while panel (c) shows brief stability in RER during the first transition of a 60s:120s trial. Panel (d) shows RER from a 30s:60s intermittent trial during which RER increased. Panel (e) however, is the first transitions during a 30s:60s trial when there was stability in RER. Finally panel (f) is a representative subsequent transition during a 30s:60s trial. The first vertical dashed line is the onset of exercise and the second is the onset of recovery.

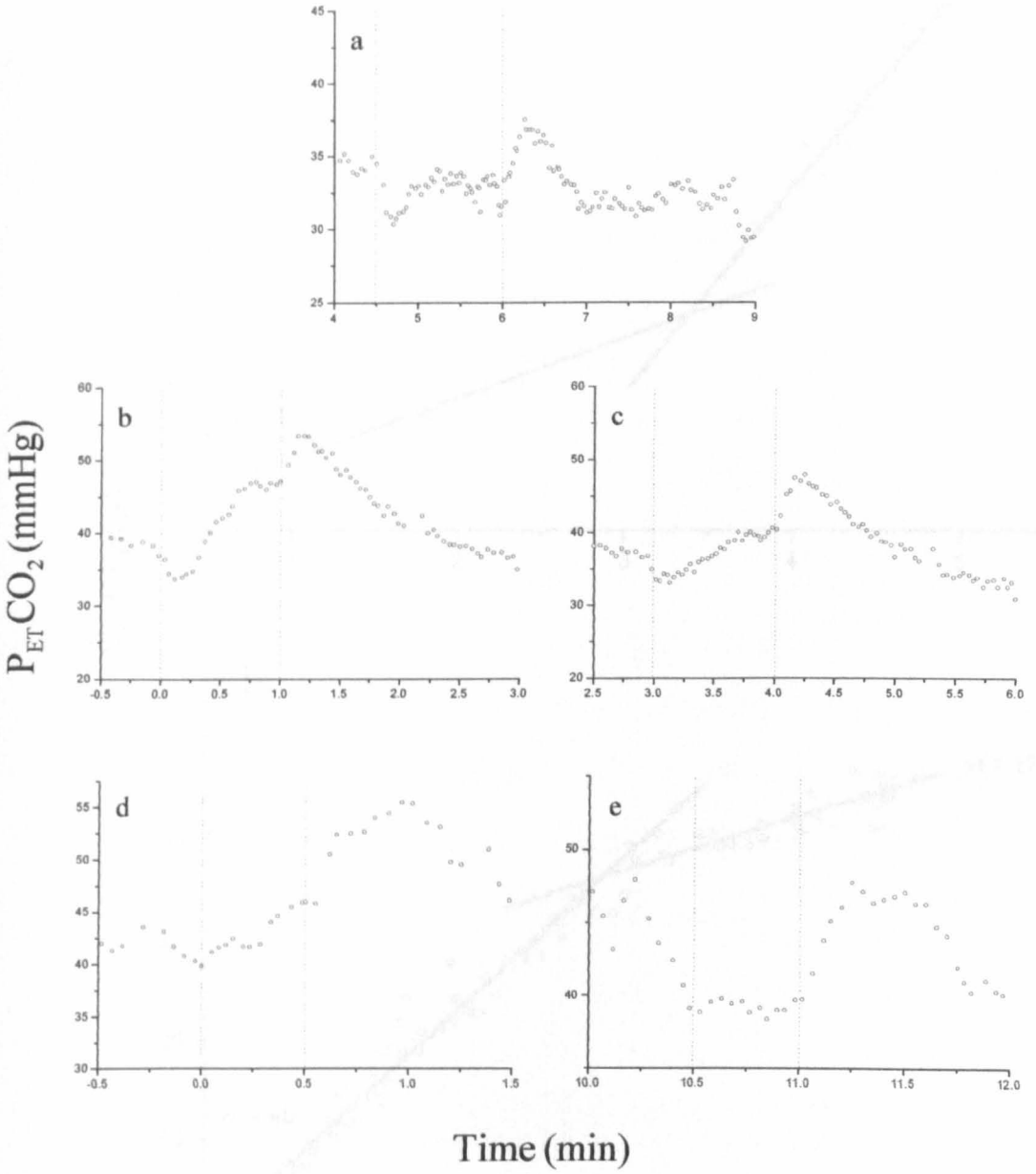


Figure 3.9: Panel (a), a representative response of $P_{ET}CO_2$ during a single exercise: recovery duty cycle from a 90s:180s intermittent trial. Pane l (b), breath-by-breath changes in $P_{ET}CO_2$ during a representative first 60s:120s exercise: recovery duty cycle and during a representative cycle during a 60s:120s intermittent trial, panel (c). The response of $P_{ET}CO_2$ during the first 30s:60s intermittent transition panel (d) and during a representative transition later on during a 30s:60s intermittent trial panel (e). The first vertical dashed line is the onset of exercise and the second is the onset of recovery.

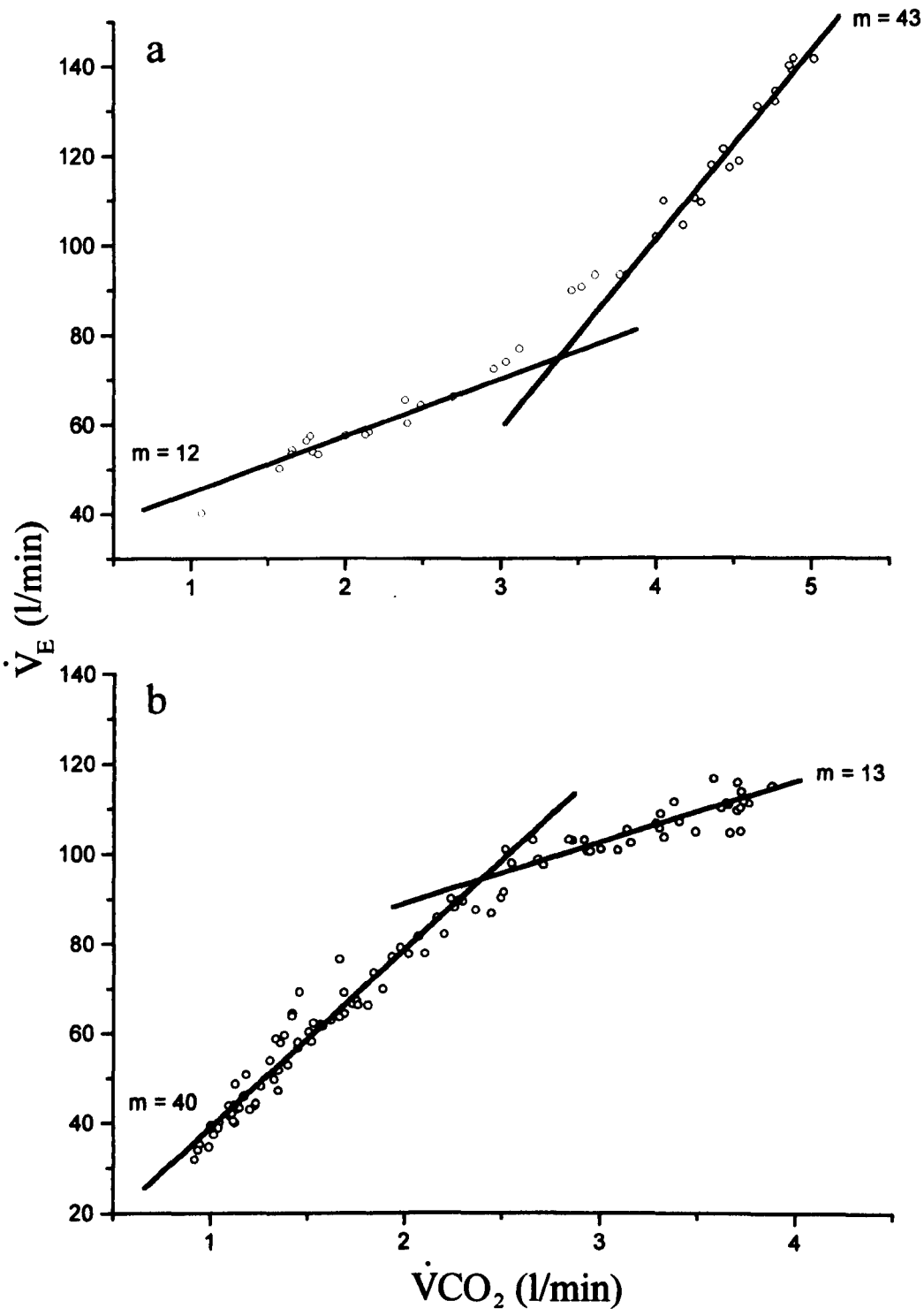


Figure 3.10: Two compartment response of the $\dot{V}_E$ - $\dot{V}CO_2$ relationship during a representative 'on' transient from a 90s:180s intermittent trial (panel a). Two compartment response of the $\dot{V}_E$ - $\dot{V}CO_2$ relationship during a representative 'off' transient from a 90s:180s intermittent trial (panel b). The slope (m) of each relationship is indicated on the plot.

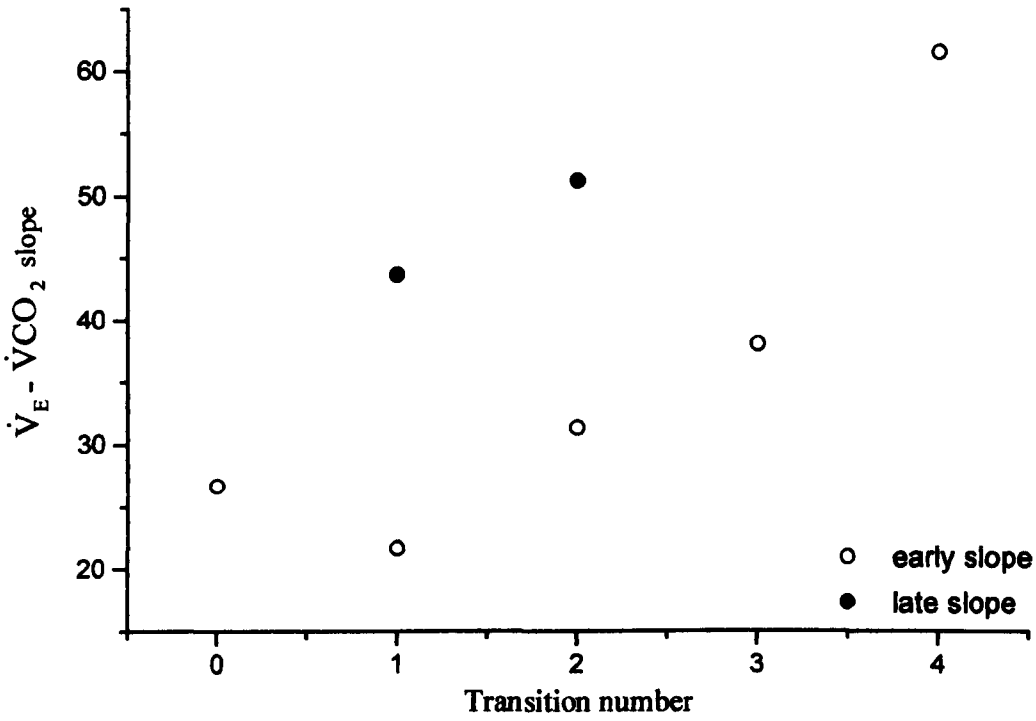


Figure 3.11: The slope of the $\dot{V}_E$ - $\dot{V}CO_2$ relationship during each 'on' transition during a 90s:180s trial, the plot displays both the early (open circle) and late phase (closed circle).

A similar pattern occurred during the off-transition, whereby there was a very slow fall in $\dot{V}_E$ after the work-rate decreased, despite $\dot{V}CO_2$ recovering rapidly (figure 3.10b). This was followed by a much steeper slope to the $\dot{V}_E$ - $\dot{V}CO_2$ relationship as $\dot{V}_E$ began to decline rapidly (figure 3.10b). The continued elevation of $\dot{V}_E$ early in recovery with very little decrease meant the $\dot{V}_E$ - $\dot{V}CO_2$ slope was close to being flat.

3.5.3 Intermittent exercise 60s exercise 120s recovery

The reduction of the exercise duration to 60s again lead to a marked and continuous rise in $[La^-]_a$ throughout the tests; which typically peaked early in recovery and was not significantly lower than during the 90s:180s trials ($p=0.169$) (figure 3.4). This sustained elevation of lactate resulted in only four of the six subjects completing all 10 of the exercise-recovery duty cycles. Distinct oscillations in tandem with the work-rate changes were again evident in all variables (figure 3.12, appendix 3.3). The amplitude of the $\dot{V}_E$ oscillation increased progressively with every exercise period and there was also a tendency for the nadir to rise slightly across the duration of a trial (figure 3.12). A pattern similar to that observed during the 90s:180s trials was seen in $\dot{V}_E$ at the onset and offset of exercise both during the first cycle and during all subsequent cycles (figure 3.6 & figure 3.13, subject number 3).

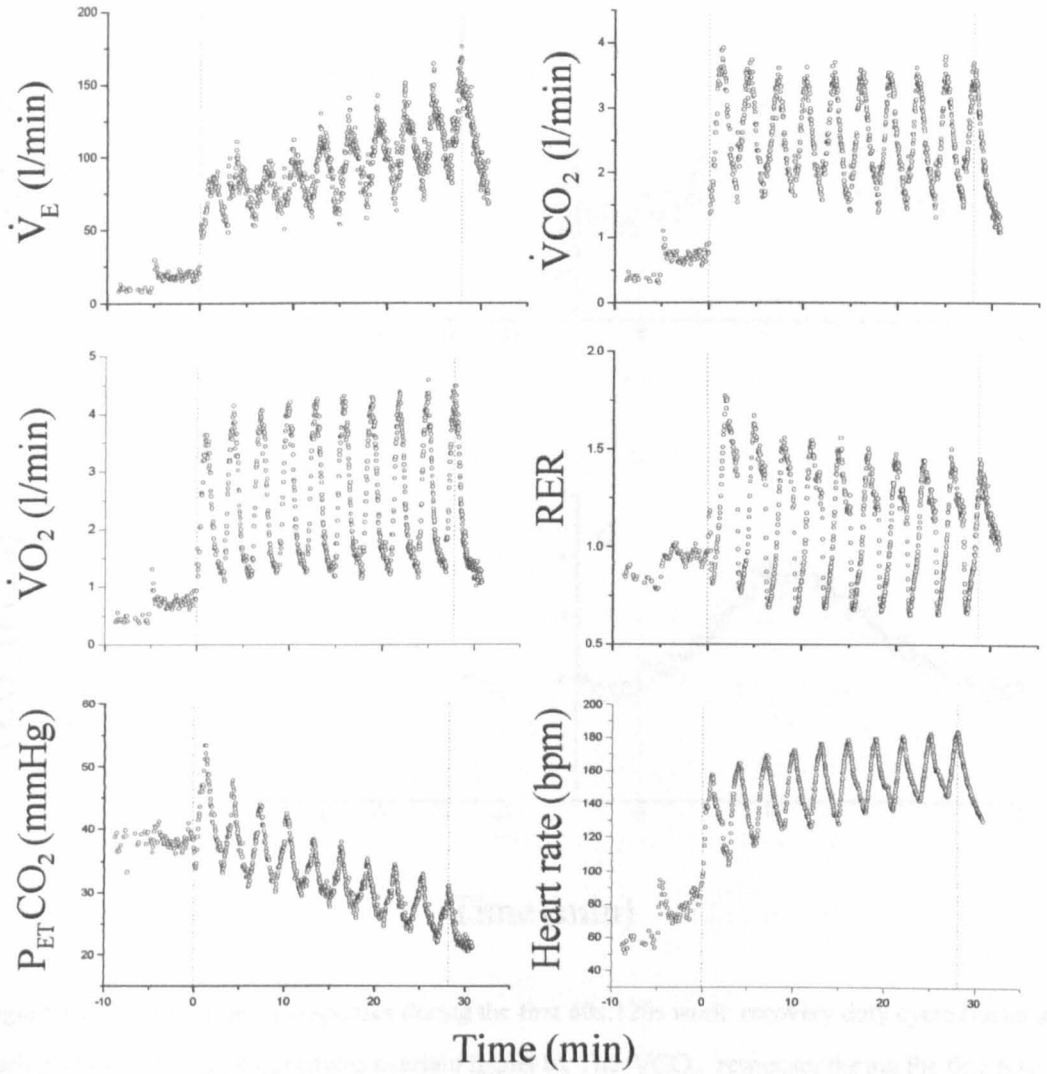


Figure 3.12: Breath-by-breath ventilatory, pulmonary gas exchange and heart rate responses from a representative subject (subject 1) during a 60s:120s intermittent exercise test. Distinct oscillations with the changes in work-rate are again visible in all variables from the first work cycle. The first vertical dashed line is the onset of exercise and the second is the onset of recovery.

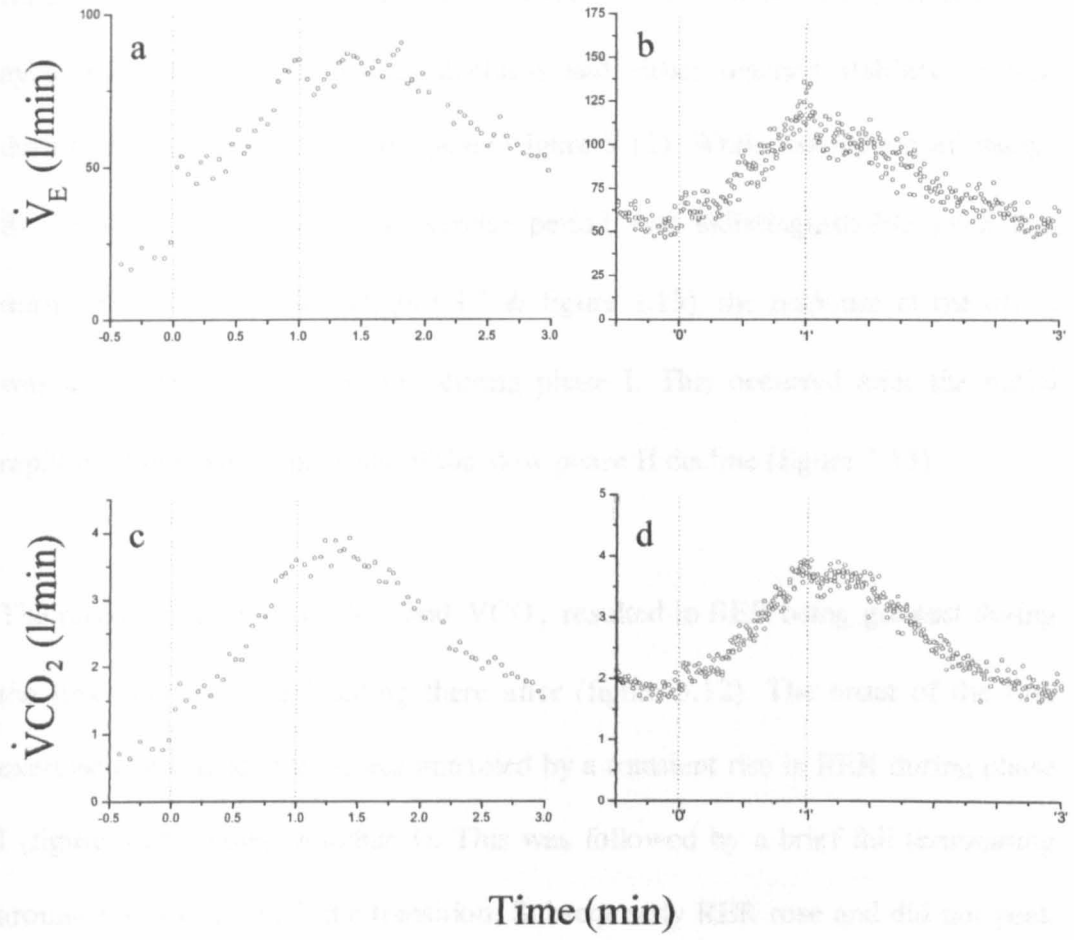


Figure 3.13: The ventilatory responses during the first 60s:120s work: recovery duty cycle (panel a) and during a 60s:120s trial, 4 transitions overlaid (panel b). The $\dot{V}CO_2$ responses during the first 60s:120s work: recovery duty cycle (panel c) and during a 60s:120s trial, 4 transitions overlaid (panel d). The first vertical dashed line is the onset of exercise and the second is the onset of recovery.

Similarly to the 90s:180s trials $\dot{V}CO_2$ tended to be greatest during the first duty cycle then end-exercise values declined and either reached stability or rose during the final couple of duty cycles (figure 3.12). While the pattern of change for $\dot{V}CO_2$ at the onset of a exercise period was indistinguishable from that during the 90s:180s trials (figure 3.7 & figure 3.13), the response at the offset was a secondary rise in $\dot{V}CO_2$ during phase I. This occurred after the initial rapid fall but prior to the onset of the slow phase II decline (figure 3.13).

The response patterns of $\dot{V}O_2$ and $\dot{V}CO_2$ resulted in RER being greatest during the first duty cycle and falling there after (figure 3.12). The onset of the first exercise phase tended to be accompanied by a transient rise in RER during phase I (figure 3.8b, subject number 3). This was followed by a brief fall terminating around midway through the transition. Subsequently RER rose and did not peak until well into recovery, before finally falling until the next duty cycle began (figure 3.8b). The transition from exercise to recovery tended to be accompanied by a slowing of the increase in RER, although generally not actually achieving stability. All duty cycles after the first were typically characterised by a stable RER during the 'on' phase I, i.e. a normal phase I response, and a similar pattern of response thereafter (figure 3.8c, subject number 2).

Increases were seen in $P_{ET}CO_2$ with each exercise phase followed by larger decreases during recovery. This lead to $P_{ET}CO_2$ peaking at the end of the first exercise period and both the amplitude of oscillation and the mean falling throughout the remainder of the trial (figure 3.12). During the first exercise cycle $P_{ET}CO_2$ fell slightly with the onset of exercise and remained at that level for the

duration of phase I before rising throughout the remainder of the exercise period (figure 3.9b subject number 3). It was also observed that $P_{ET}CO_2$ characteristically rose early during phase I of recovery; such that a distinct change in the rate of increase was evident in comparison to the rise during exercise. After eventually attaining stability $P_{ET}CO_2$ declined throughout the remainder of phase II (figure 3.9b). The response pattern during subsequent duty cycles was not substantially different (figure 3.9c subject number 3).

During all 60s:120s trials end-exercise and end-recovery heart rates rose continually throughout the test (figure 3.12). At the onset of an exercise period heart rate tended to remain stable over the first 10s and then rose steadily for the reminder of the exercise period (figure 3.14, subject number 1). This increase was maintained early into recovery before decreasing until the next duty cycle was initiated (figure 3.14).

The regression slope of the $\dot{V}_E$ - $\dot{V}CO_2$ relationship decreased with respect to baseline during the first duty cycle, both during exercise and recovery (figure 3.15 & figure 3.31). Thereafter both exercise and recovery $\dot{V}_E$ - $\dot{V}CO_2$ slopes increased with each duty cycle (figure 3.15, figure 3.16 & figure 3.31), although the recovery phase always exhibited a shallower slope than during exercise (figure 3.15 & figure 3.31). In some subjects a non-linear increase in $\dot{V}_E$ was observed late during either an exercise phase or recovery phase, although this was most common during recovery. This typically occurred as $\dot{V}_E$ fell little early in recovery while $\dot{V}CO_2$ recovered resulting in a relatively flat $\dot{V}_E$ - $\dot{V}CO_2$ relationship, this was followed by a sharp decline in $\dot{V}_E$ with a continual steady

falling $\dot{V}CO_2$ resulting in a sharply falling $\dot{V}_E$ - $\dot{V}CO_2$ slope (figure 3.17, subject number 3).

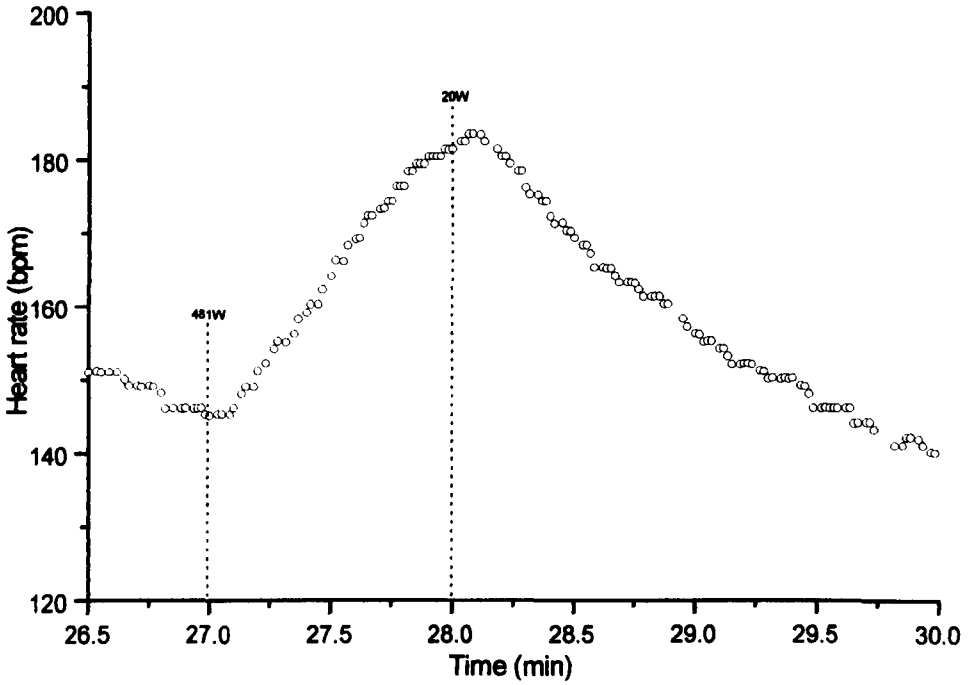


Figure 3.14: A representative (subject 1) response of heart rate from a single work: recovery duty cycle during a 60s:120s intermittent trial. The first vertical dashed line is the onset of exercise and the second is the onset of recovery.

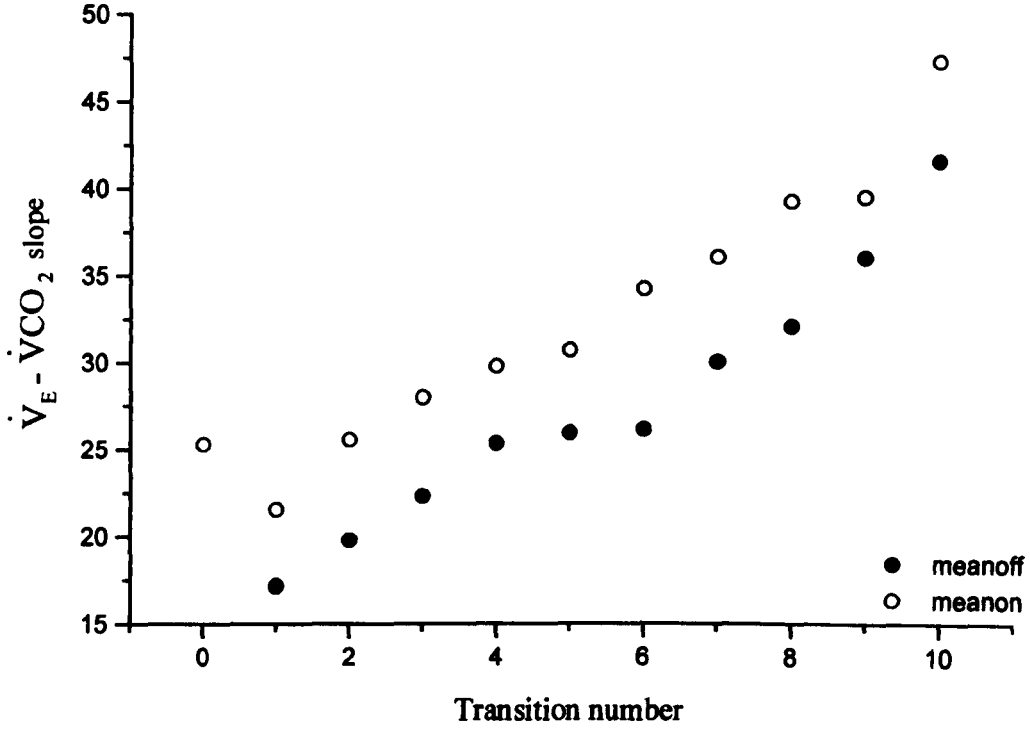


Figure 3.15: The group mean $\dot{V}_E - \dot{V}CO_2$ regression slopes from both 'on' and 'off' transitions from the 60s:120s intermittent trials.

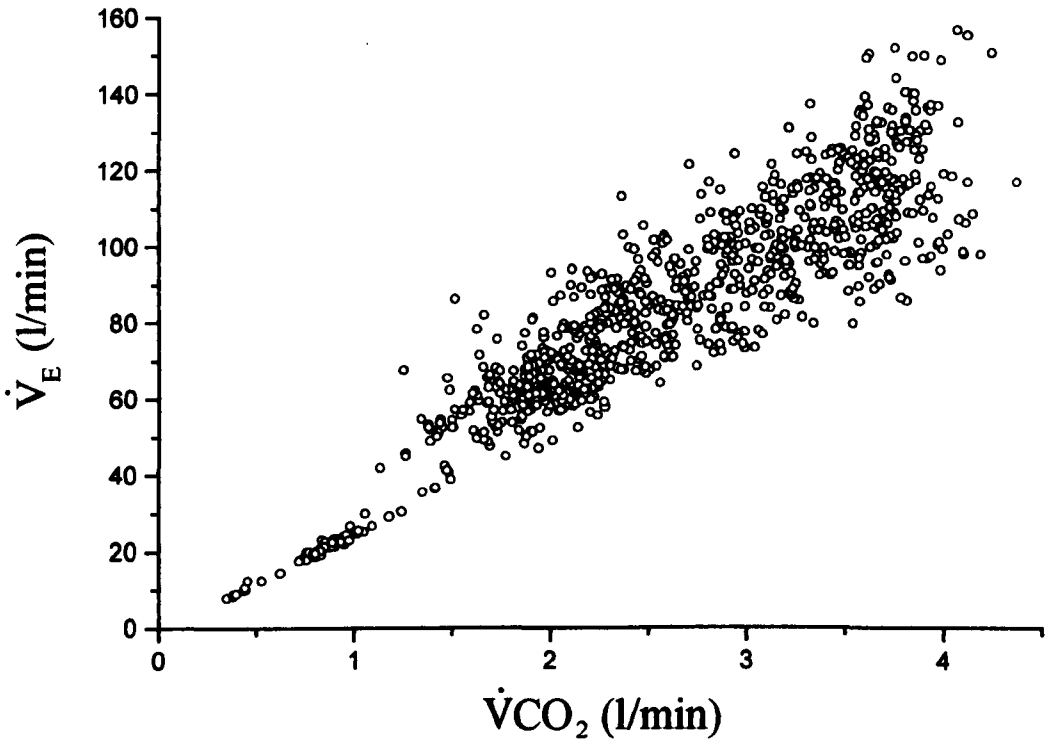


Figure 3.16: The $\dot{V}_E$ - $\dot{V}CO_2$ relationship from a representative subject (subject 3) during a 60s:120s intermittent trial, baseline and all work: recovery duty cycles included.

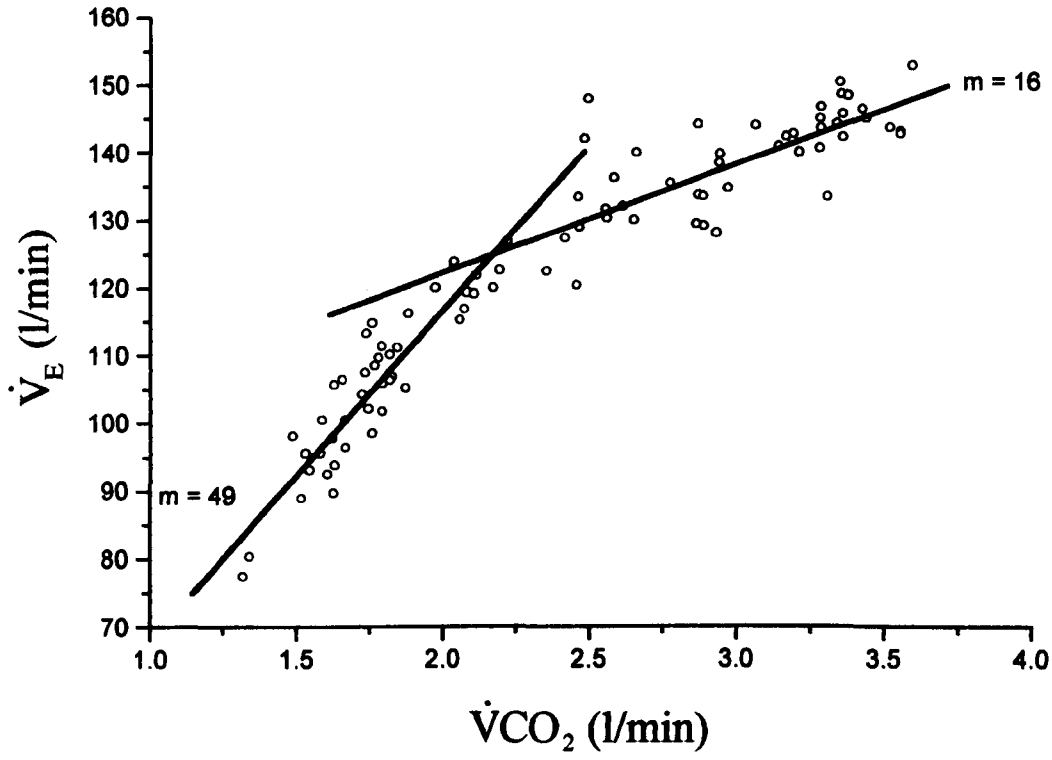


Figure 3.17: Two compartment response of $\dot{V}_E$ - $\dot{V}CO_2$ relationship during a representative off-transient from a 60s:120s intermittent trial (subject 3). The slope (m) of each relationship is indicated on the plot.

3.5.4 Intermittent exercise 30s exercise 60s recovery

Unlike the 60s:120s and 90s:180s trials all subjects were able to complete the entire 30 minute duration (20 duty cycles) of the 30s:60s test. While $[La^-]_a$ initially rose sharply during the trials and then continued to rise slowly it did eventually reached stability between 4mM and 6mM (figure 3.4) (table 3.3), i.e. elevated above baseline levels, and significantly lower than during either the 60s:120s or 90s:180s tests ($p=0.001$, $p=0.013$).

Subject	10s:20s	30s:60s	60s:120s	90s:180s
1	1.1	4.5	9.95	10.55
2	0.3	6.15	9.9	8.6
3	0.75	5.25	10.55	9.2
4	0.2	2.9	4.45	-
5	1.0	5.55	10.85	9.1
6	2.0	5.25	9.05	-
Mean	0.9	4.9*	9.1*§	9.4*§
± S.D.	0.7	1.1	2.4	0.8

* significantly different from 10s:20s test. § significantly different from 30s:60s test.

Table 3.3: The increases in $[La^-]_a$ during each of the four intermittent exercise protocols, calculated as the difference from baseline to peak exercise value.

The shortening of the exercise-recovery duty cycle still resulted in distinct oscillations in $\dot{V}_E$, $\dot{V}CO_2$, $\dot{V}O_2$, RER and $P_{ET}CO_2$ synchronous with the changes in work-rate (figure 3.18 & appendix 3.4). While these oscillations do not appear as distinct as during the 60s:120s or 90s:180s trials they can be more easily viewed when fewer transitions are presented, thus stretching out the changes (figure 3.19). The mean of the $\dot{V}_E$ oscillation rose steadily over the first few duty cycles attaining a steady mean around the 5th duty cycle (figure 3.18 & appendix

3.4). The amplitude of the oscillation then remained relatively constant across the duration of the test.

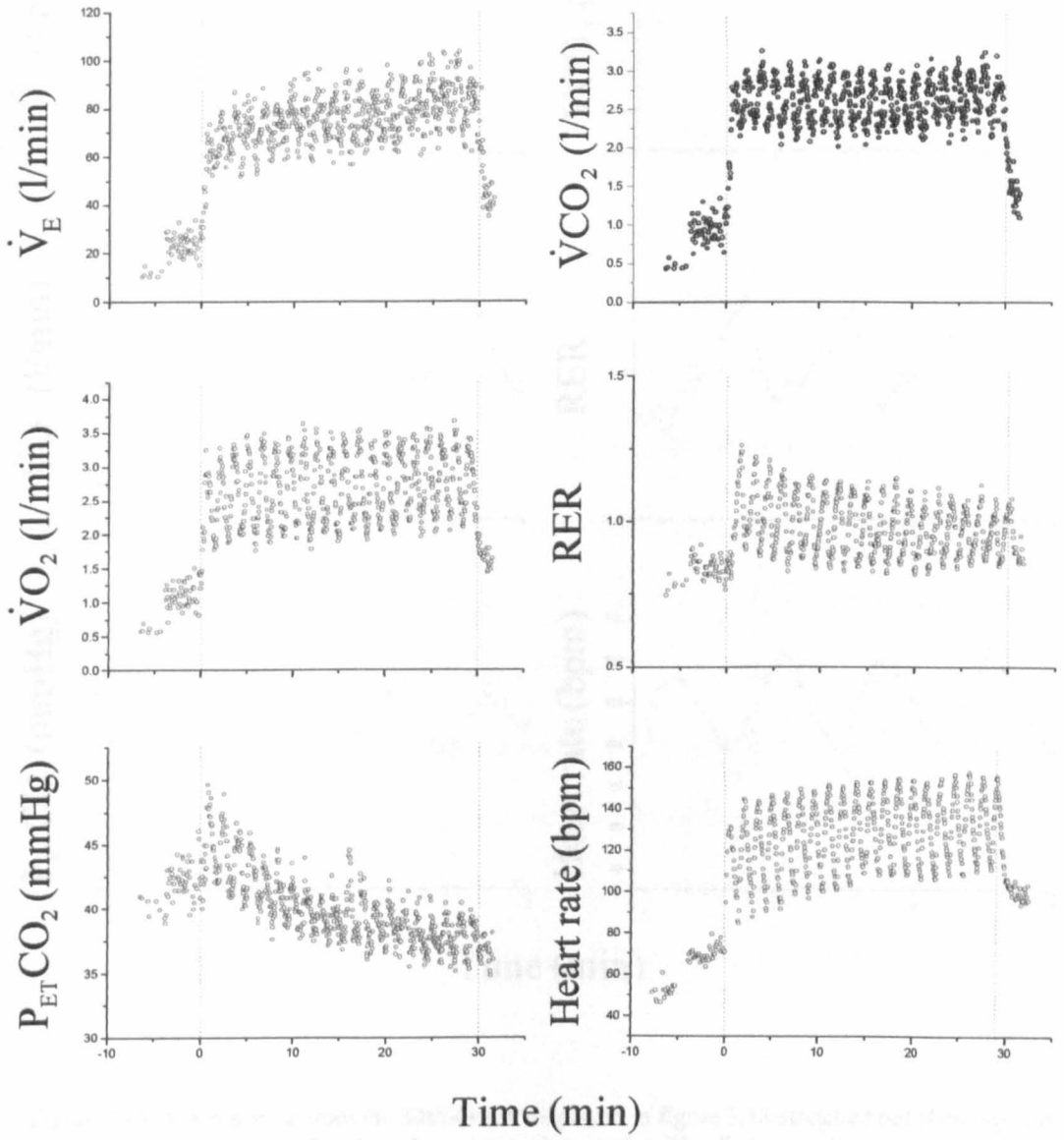


Figure 3.18: Breath-by-breath ventilatory, pulmonary gas exchange and heart rate responses from a representative subject (subject 1) during a 30s:60s intermittent exercise test. Distinct oscillations with the changes in work-rate can now be seen in all variables from the first work cycle. The first vertical dashed line is the onset of exercise and the second is the onset of recovery.

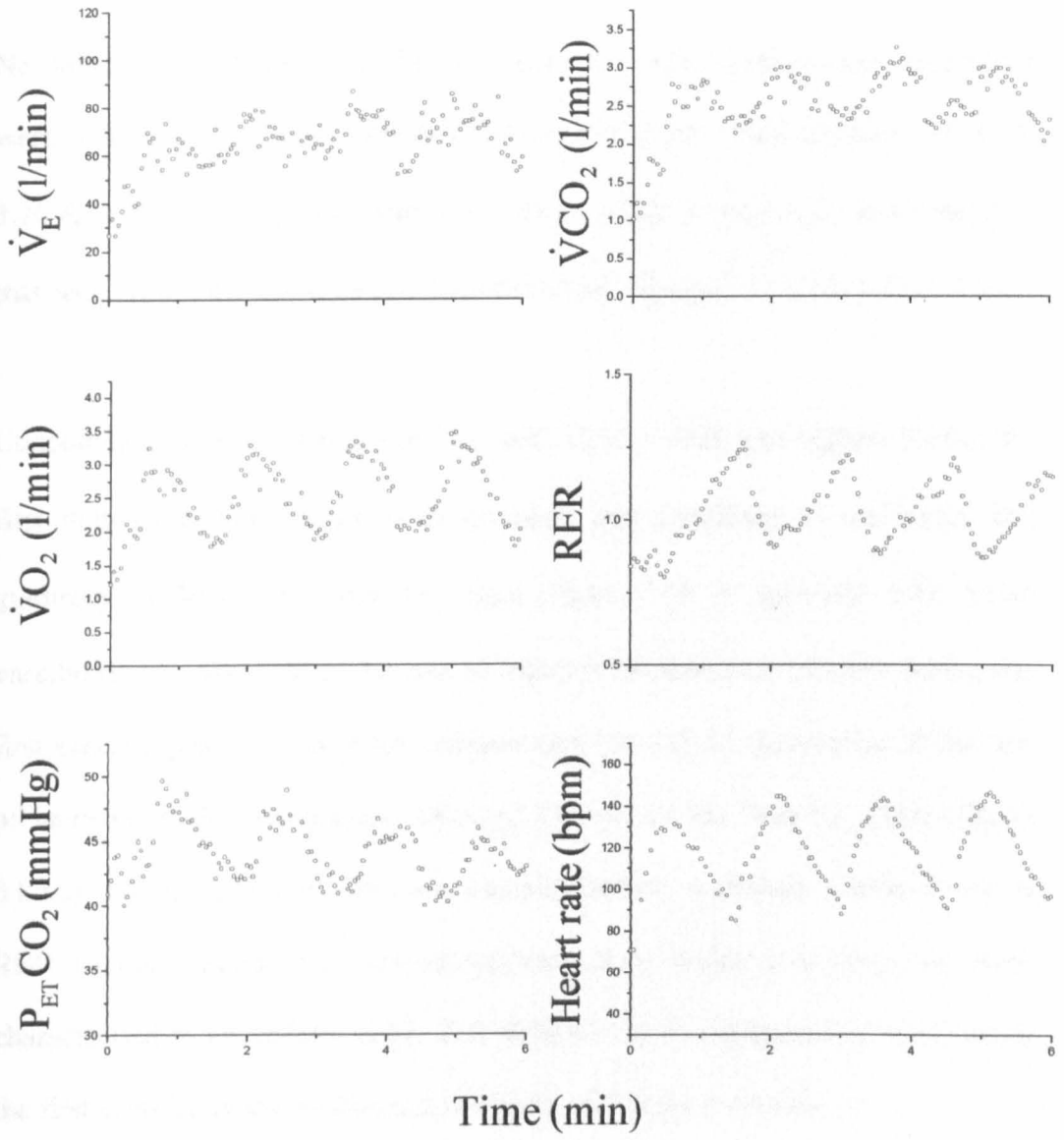


Figure 3.19: The response from the 30s:60s test displayed in figure 3.18 stretched out showing only the first 9 minutes, i.e. the first 6 work: recovery duty cycles. The distinct oscillations with the work-rate changes now are clearly evident in all variables.

No substantial differences in the $\dot{V}_E$ response pattern during each duty cycle were seen in comparison to the 60s:120s and 90s:180s trials (figures 3.6, 3.13, 3.20 & 3.21). The response pattern of $\dot{V}CO_2$ within a duty cycle was similar to that seen during the 60s:120s and 90s:180s trials (figures 3.7, 3.13, 3.22 & 3.23).

Concomitant with the changes in $\dot{V}O_2$ and $\dot{V}CO_2$, RER was highest during the first duty cycle after which both its mean and amplitude of oscillation fell gradually as $\dot{V}CO_2$ fell and $\dot{V}O_2$ rose (figure 3.18 & appendix 3.4). Some variability was observed in the rate at which RER increased initially during the first exercise phase (figure 3.8d,e subject numbers 3 & 1). Regardless of the rate of increase, RER subsequently fell until the end of the exercise phase (figure 3.8d,e). The transition to 20W was accompanied by a gradual continual rise in RER (figure 3.8d,e). All exercise-recovery duty cycles following this were characterised by an initially stable RER followed by the same pattern seen during the first exercise-recovery duty cycle (figure 3.8f, subject number 1).

All subjects exhibited an increase from baseline in $P_{ET}CO_2$ by the end of the first exercise cycle; following this the mean $P_{ET}CO_2$ across each duty cycle fell (figure 3.18 & appendix 3.4). In some subjects the amplitude of change across a duty cycle also fell, in others it remained relatively constant. The first step change from 20W to the intermittent work-rate was accompanied by a slow rise $P_{ET}CO_2$ pausing midway through the transition before continuing to rise until the work-rate was reduced (figure 3.9d, subject number 3). The return to 20W saw a further rise in $P_{ET}CO_2$ again reaching a plateau before beginning to decline back

toward baseline for the remainder of the 60s (figure 3.9d). A similar pattern was observed during recovery in all subsequent duty cycles, however no change was typically seen during exercise (figure 3.9e, subject number 3).

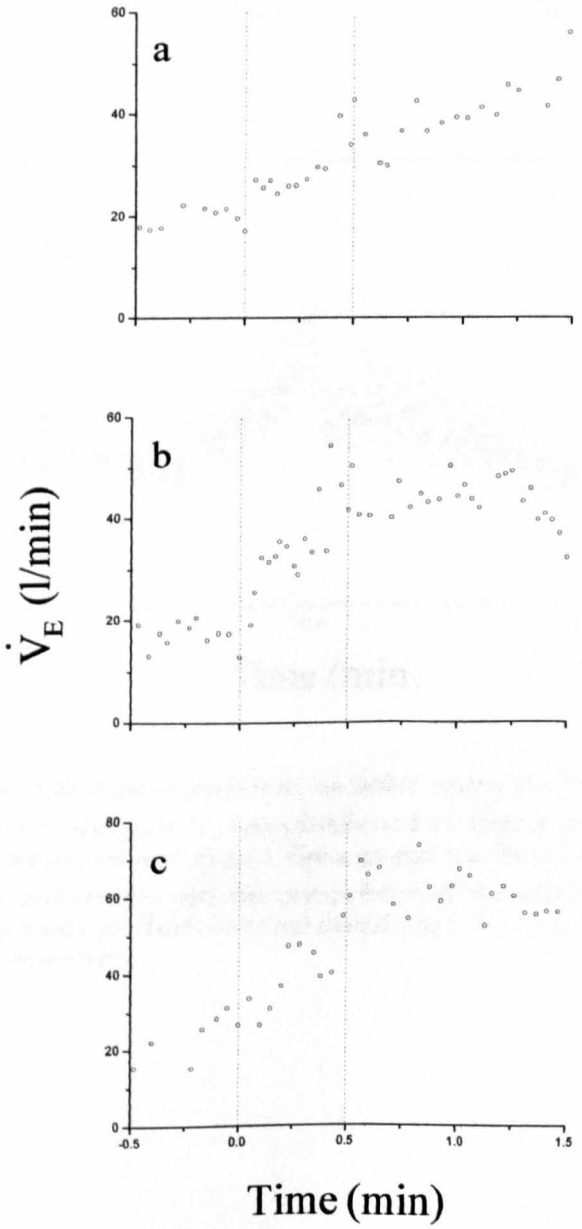


Figure 3.20: Three different patterns of $\dot{V}_E$ response during the first 30s:60s work: recovery duty cycle. Panel A illustrates a in immediate increase parallel with the work rate change, followed by a brief plateau and then a continual rise. Panel B shows an immediate increase in $\dot{V}_E$ followed by a rising $\dot{V}_E$ over the next few breaths before plateauing then rising throughout the remainder of the exercise period. Panel C depicts a subject who showed no $\dot{V}_E$ increase at the onset of exercise, rather an increase was seen some 10s later, this increase lead to a plateau throughout the remainder of the trial. The first vertical dashed line is the onset of exercise and the second is the onset of recovery.

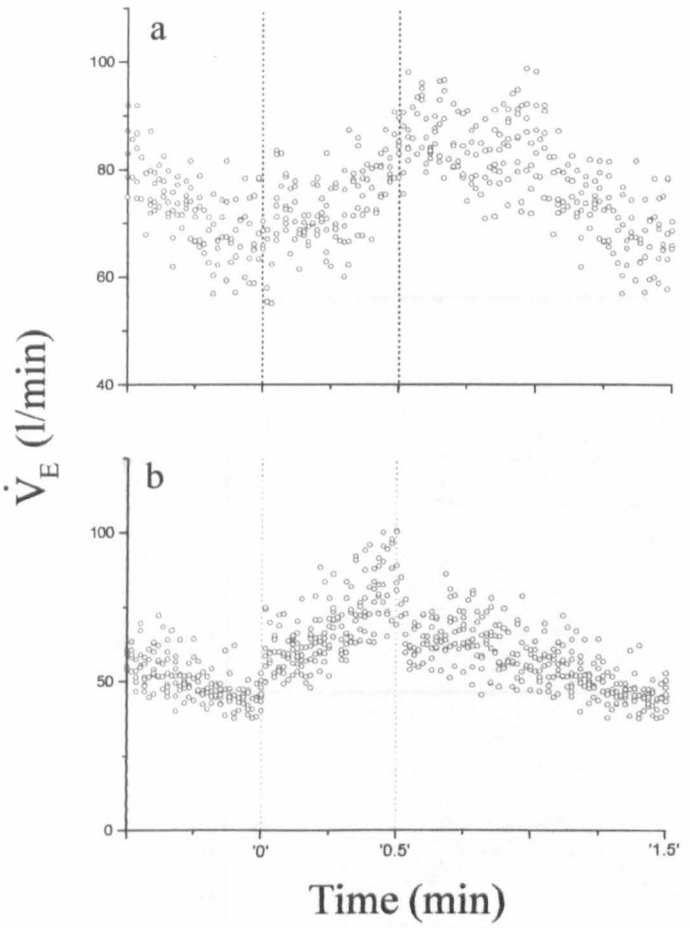


Figure 3.21: Ten $\dot{V}_E$ transitions overlaid from the 'stable' region of a 30s:60s test, panel (a) depicts a subject with no change in $\dot{V}_E$ immediately on the transition to exercise, followed by a very slight rise for the remainder of phase 1, then a gradual rise. Panel (b) shows a subject who increased $\dot{V}_E$ in tandem with the work-rate change followed by a plateau for the remainder of phase 1, then a continued rise. The first vertical dashed line is the onset of exercise and the second is the onset of recovery.

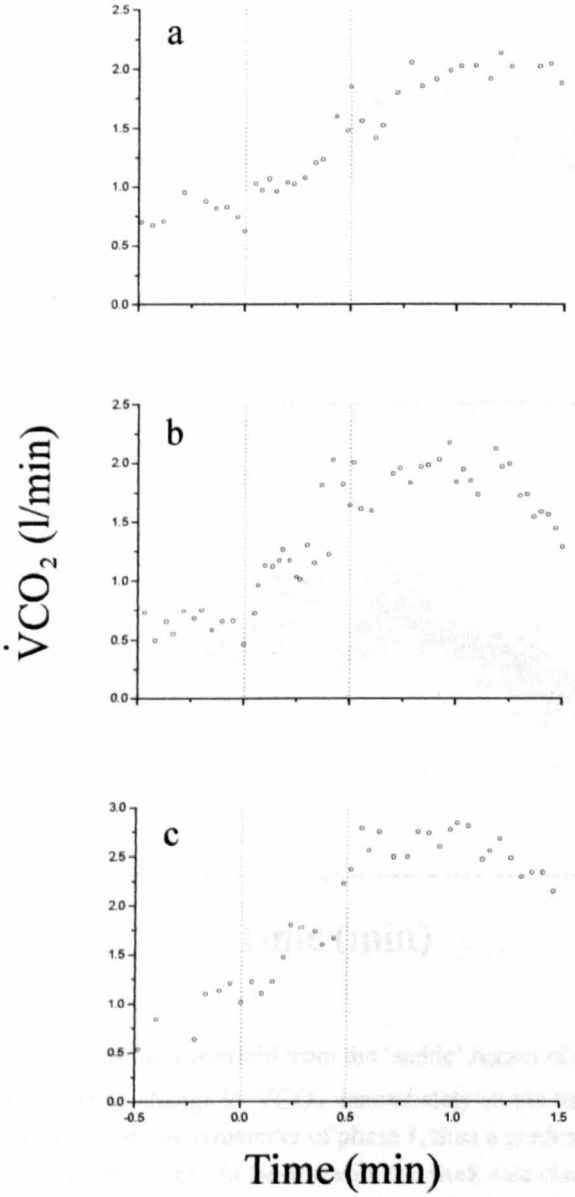


Figure 3.22: Three different patterns of $\dot{V}CO_2$ response during the first 30s:60s work: recovery duty cycle. Panel (a) illustrates an immediate increase parallel with the work rate change, followed by a brief plateau and then a continual rise. Panel (b) shows an immediate increase in $\dot{V}CO_2$ followed by a rising $\dot{V}CO_2$ over the next few breaths before plateauing then rising throughout the remainder of the exercise period. Panel (c) depicts a subject who showed no $\dot{V}CO_2$ increase at the onset of exercise, rather an increase was seen some 10s later, this increase lead to a plateau throughout the remainder of the trial. The first vertical dashed line is the onset of exercise and the second is the onset of recovery.

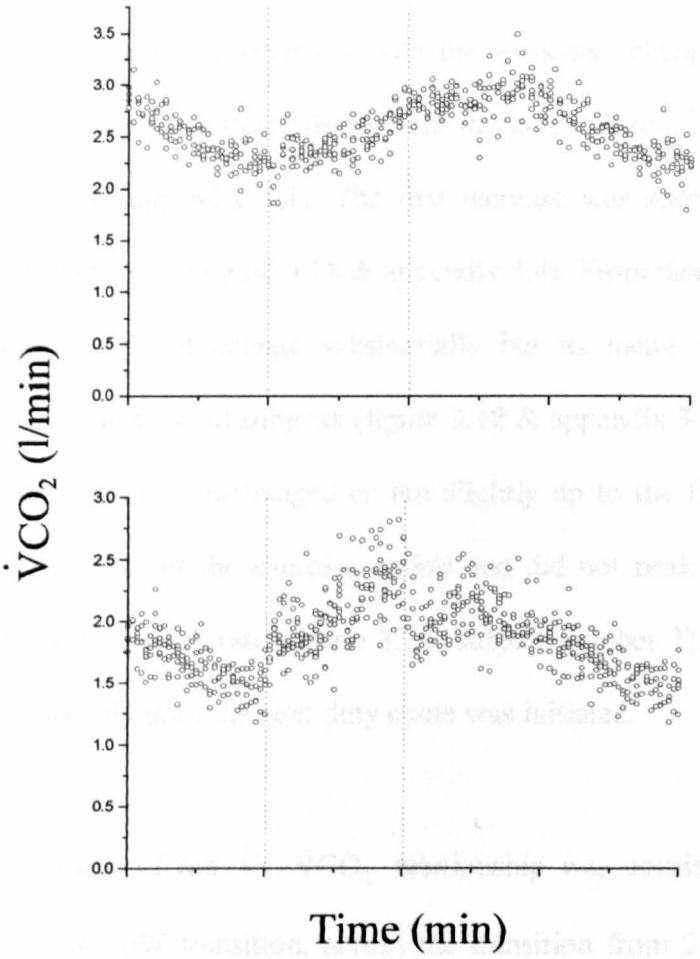


Figure 3.23: Ten $\dot{V}CO_2$ transitions overlaid from the 'stable' region of a 30s:60s test, the top panel depicts a subject with no change in $\dot{V}CO_2$ immediately on the transition to exercise, followed by a very slight rise for the remainder of phase 1, then a gradual rise. The lower panel shows a subject who increased $\dot{V}CO_2$ in tandem with the work-rate change followed by a plateau for the remainder of phase 1, then a continued rise. The first vertical dashed line is the onset of exercise and the second is the onset of recovery.

Again, a clear oscillation, in harmony with the work-rate changes, in the heart rate response was always present throughout the entire duration of the 30s:60s tests (figure 3.18 & appendix 3.4). The first increase was seen shortly, 5-10s, after the onset of exercise (figure 3.18 & appendix 3.4). From there the amplitude of the oscillation did not change substantially but its mean value tended to increase across the tests in all subjects (figure 3.18 & appendix 3.4). In each duty cycle heart rate remained unchanged or fell slightly up to the first 5s and then rose for the remainder of the exercise period and did not peak until some 10s after the reduction in work-rate (figure 3.24, subject number 1). The heart rate then fell progressively until the next duty cycle was initiated.

The regression slope of the $\dot{V}_E$ - $\dot{V}CO_2$ relationship was consistently reduced, relative to the rest-20W transition, across the transition from 20W to the first intermittent exercise cycle (figure 3.25 & figure 3.31). The following duty cycles exhibited a steady increase in the slope, despite stability being reached in $[La^-]_a$ (figures 3.25, 3.31, 3.26 & 3.4). Furthermore, the $\dot{V}_E$ - $\dot{V}CO_2$ regression slope was consistently and significantly lower during the recovery segment of each duty cycle ($p<0.001$) (figure 3.25 & figure 3.31).

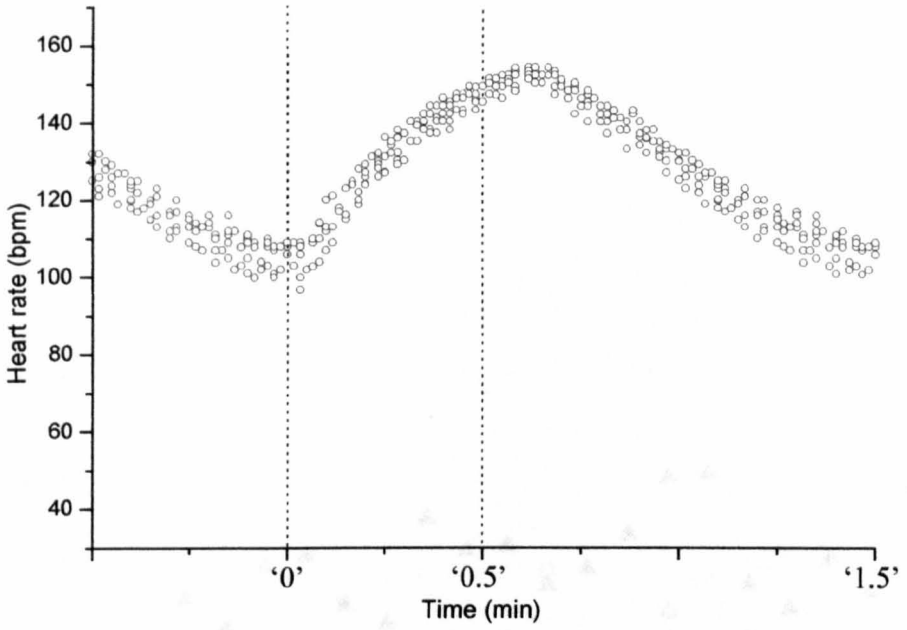


Figure 3.24: The heart rate response during 10 consecutive cycles overlaid of a 30s:60s intermittent test from a representative subject (subject 1).

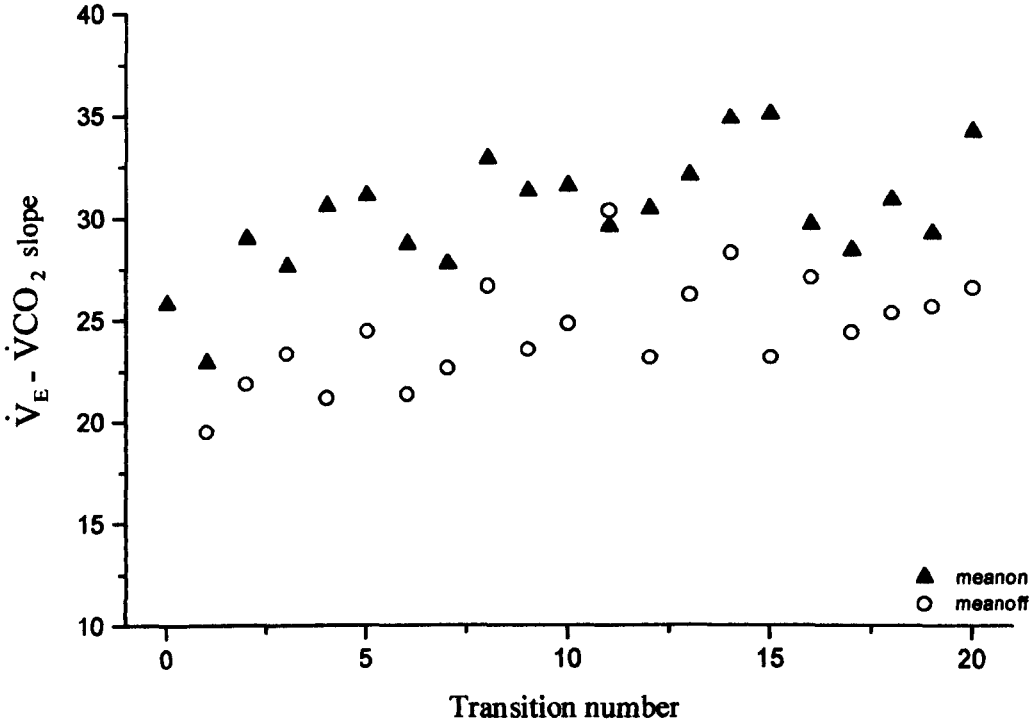


Figure 3.25:The group mean $\dot{V}_E$ - $\dot{V}CO_2$ regression slopes during on and off transitions from the 30s:60s intermittent trials.

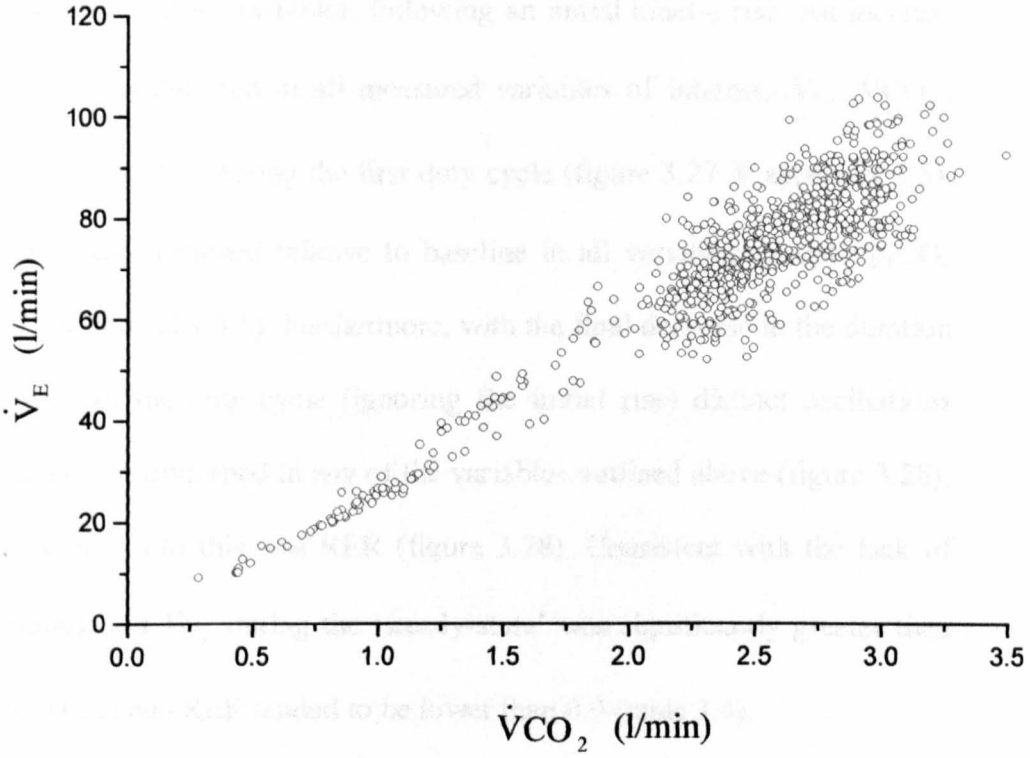


Figure 3.26: The $\dot{V}_E$ - $\dot{V}CO_2$ relationship during the whole 30s:60s trial in a representative subject (subject 3).

3.5.5 Intermittent exercise 10s exercise 20s recovery

Similarly to the 30s:60s trials all subjects were able to complete the full 30-minute duration of the 10s:20s test, however, there was little accumulation of lactate above baseline values (figure 3.4). The lack of an increased $[La^-]_a$ despite the relatively high work-rate lead to attainment of stability in all pulmonary gas exchange and ventilatory variables, following an initial kinetic rise. An increase from baseline was detected in all measured variables of interest, $\dot{V}_E$, $\dot{V}CO_2$, $\dot{V}O_2$, RER and $P_{ET}CO_2$, during the first duty cycle (figure 3.27 & appendix 3.5). This increase was sustained relative to baseline in all variables except $P_{ET}CO_2$ (figure 3.27 & appendix 3.5). Furthermore, with the final decrease in the duration of each phase of the duty cycle (ignoring the initial rise) distinct oscillations could no longer be discerned in any of the variables outlined above (figure 3.28). The only exception to this was RER (figure 3.28). Consistent with the lack of $[La^-]_a$ accumulation $\dot{V}O_2$ during the 'steady-state' was significantly greater than $\dot{V}CO_2$ ($p<0.001$) and RER tended to be lower than 0.9 (table 3.4).

In the case of heart rate clear oscillations were again evident in tandem with the work-rate changes during the 10s:20s trials (figures 3.27, 3.28 & appendix 3.5).

A significant rise in breathing frequency from 16 breaths per minute during the 20W baseline to 26 breaths per minute during the 10s:20s intermittent phase was seen in all subjects ($p=0.001$).

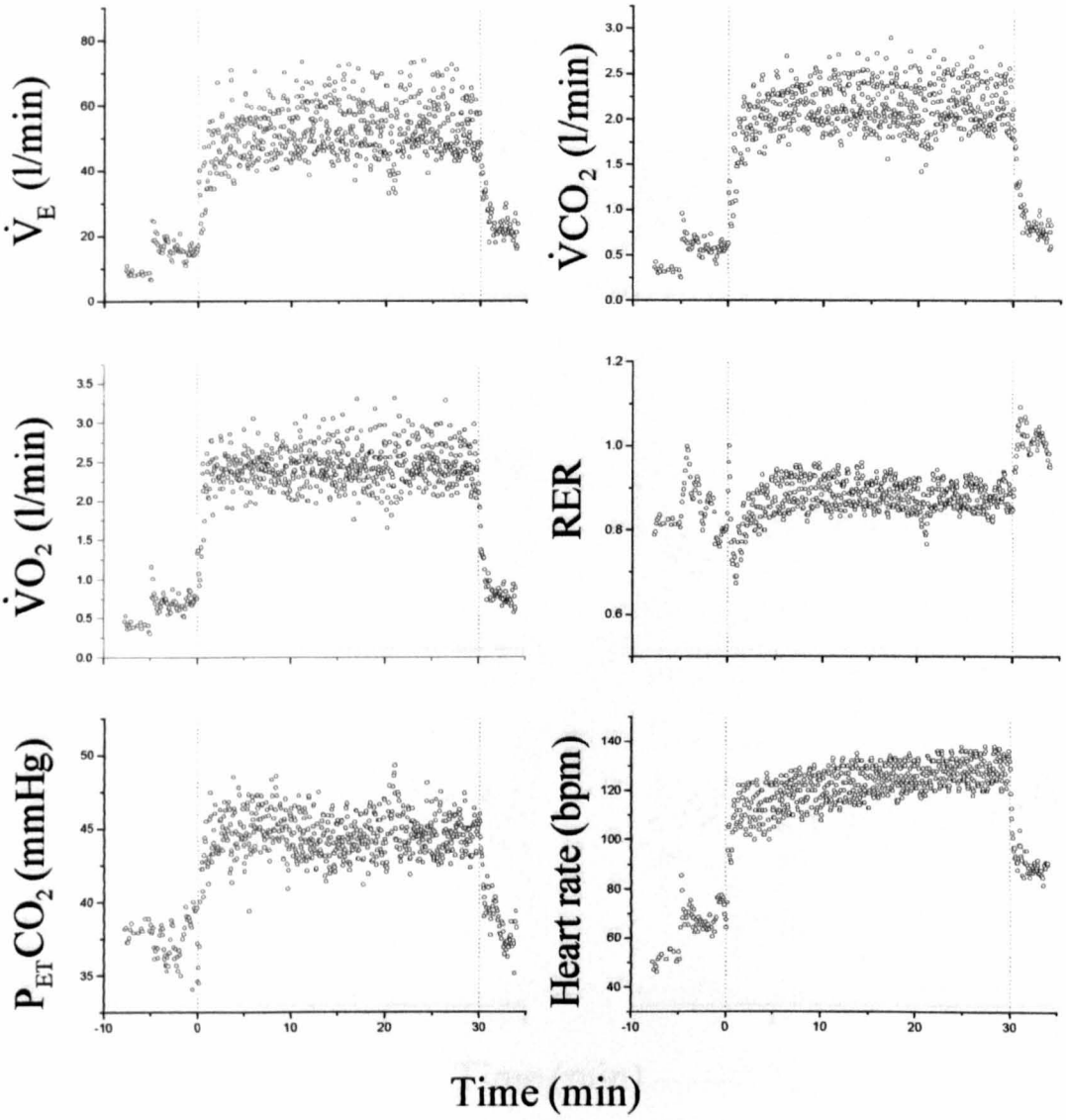


Figure 3.27: Breath-by-breath ventilatory, pulmonary gas exchange and heart rate responses from a representative subject (subject 1) during a 10s:20s intermittent exercise test. While distinct oscillations with the changes in work-rate are not obvious, except in heart rate, a clear elevation above baseline (20W) beginning in tandem with the first work cycle can be seen in all variables. The first vertical dashed line is the onset of exercise and the second is the onset of recovery.

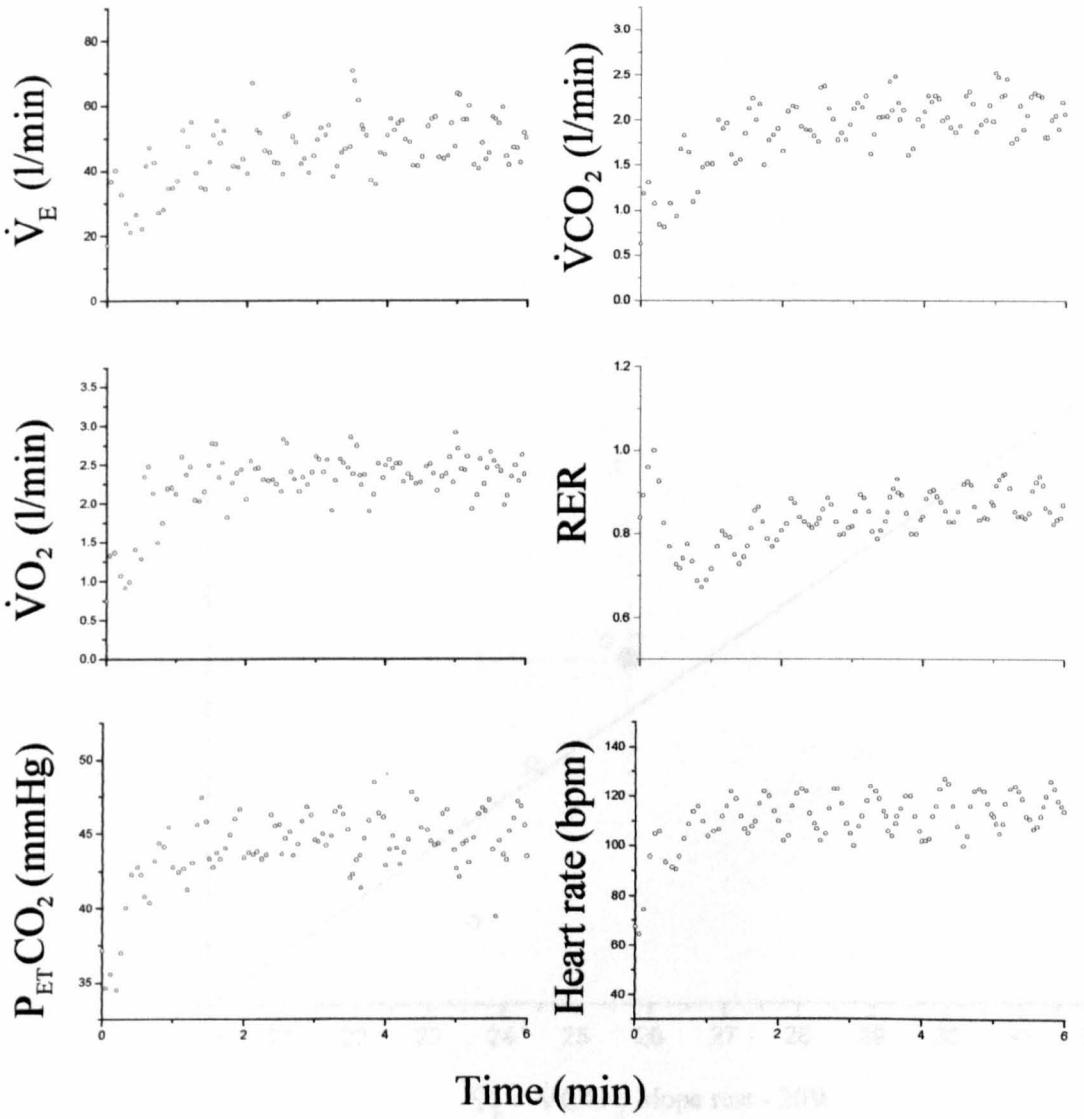


Figure 3.28: The response from the 10s:20s test displayed in figure 3.27 stretched out showing only the first 6 minutes, i.e. the first 12 exercsie: recovery duty cycles. The distinct oscillations with the work-rate changes are evident in RER and heart rate while only seemingly random fluctuations can be discerned in all other variables.

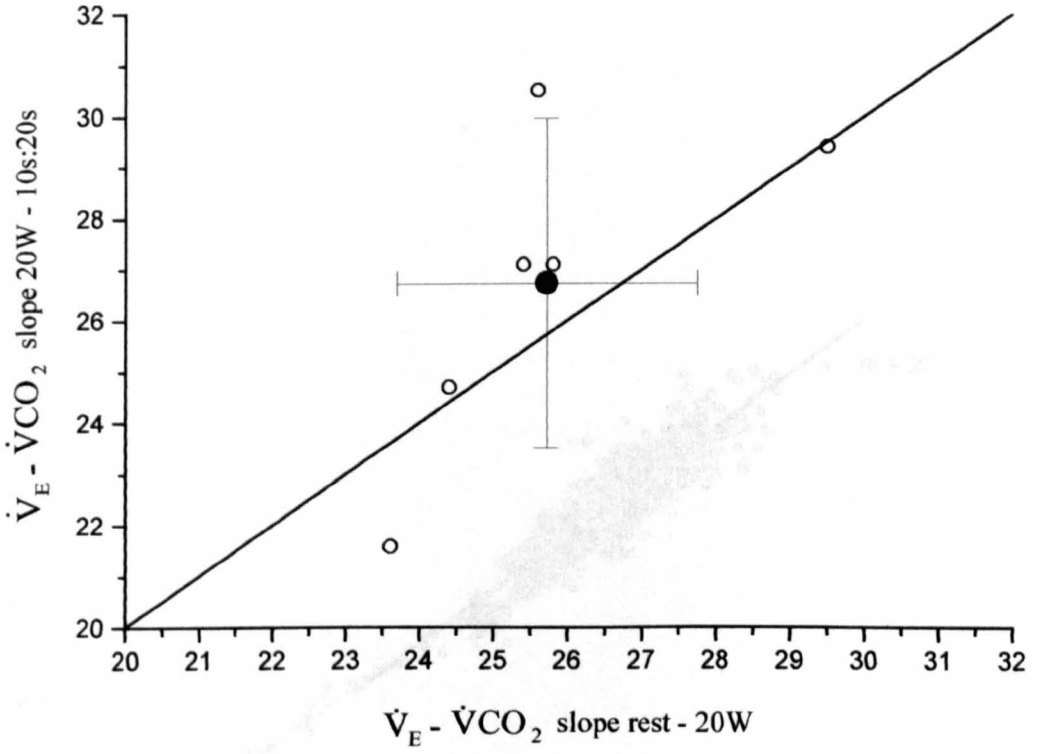


Figure 3.29: The plot displays the slope of the $\dot{V}_E$ - $\dot{V}CO_2$ relationship during the rest to 20W transition plotted against the corresponding response from the 10s:20s intermittent test. Open circles represent individual subjects while the closed circle is the group mean.

There was a trend, although significance was not reached, for an increase in the regression slope of $\dot{V}_E$ - $\dot{V}CO_2$ comparing the baseline response, taken as the transition from rest to 20W, and that across all duty cycles of the 10s:20s test (figure 3.29 & figure 3.31) (p=0.329). However, the mean increase in slope, relative to baseline, during the 10s:20s tests was only 1.02 (figures 3.29, 3.30 & 3.31).

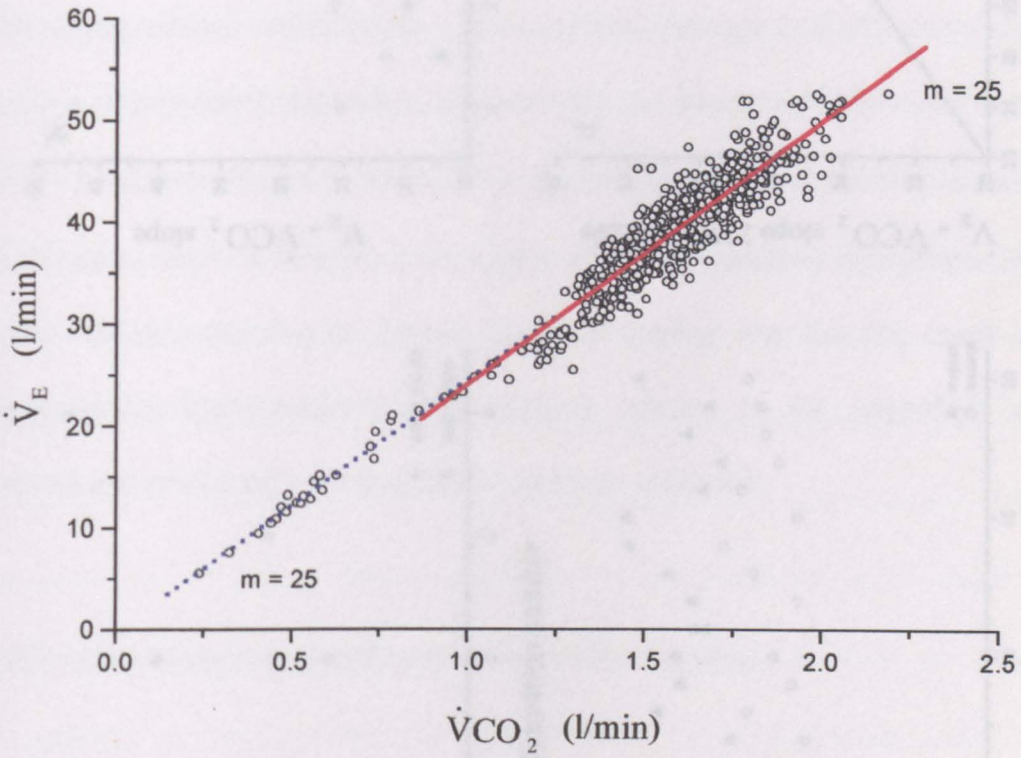


Figure 3.30: The $\dot{V}_E$ - $\dot{V}CO_2$ relationship during the transition from rest to 20W, dashed line, and the $\dot{V}_E$ - $\dot{V}CO_2$ relationship during the 10s:20s intermittent test in a representative subject (subject 3). The slopes (m) of each relationship are noted at the relevant ends of the plot

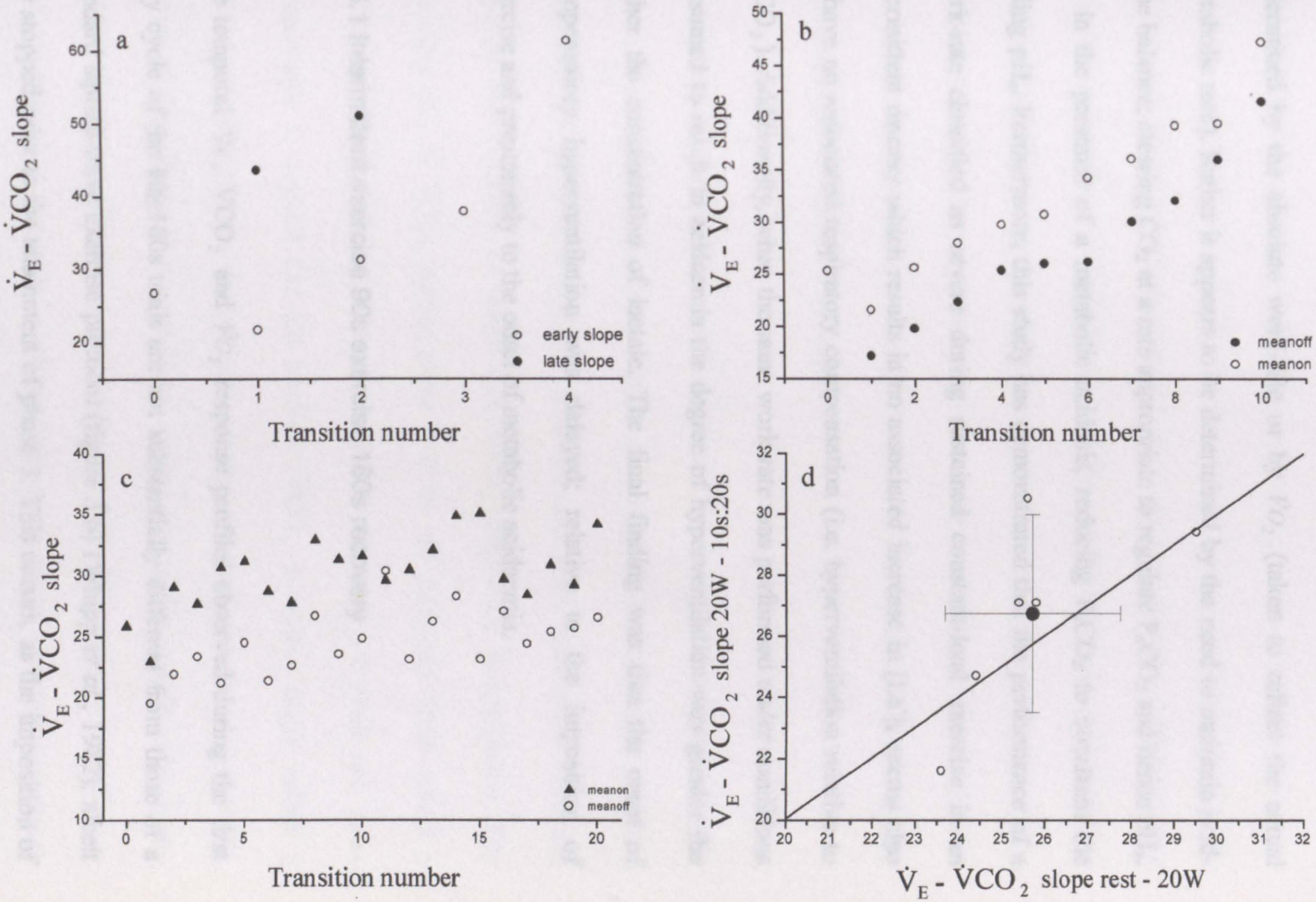


Figure 3.31: A comparative figure illustrating the change in slope across the duration of the 90:180 (a), 60:120 (b) and 30:60 (c) trials and showing the change in slope from the rest to 20W transition compared to the 20W intermittent exercise transition during the 10:20 (d) trials. On panel d the open circles are the individual responses while the closed circle is the group mean

3.6 Discussion

This study has demonstrated that the ventilatory response during exercise is not determined by the absolute work-rate or by $\dot{V}O_2$ (taken to reflect the actual metabolic rate). Rather it appears to be determined by the need to maintain acid-base balance; clearing CO_2 at a rate appropriate to regulate P_aCO_2 and hence pH_a, or, in the presence of a metabolic acidosis, reducing P_aCO_2 to constrain the falling pH_a. Furthermore, this study has demonstrated that the performance of a work-rate classified as severe during sustained constant-load exercise in an intermittent manner which results in no associated increase in $[La^-]_a$ seems also to have no associated respiratory compensation (i.e. hyperventilation relative to $\dot{V}CO_2$). Additionally, when the same work-rate was performed under conditions presumed to result in acidaemia the degree of hyperventilation was greater the higher the concentration of lactate. The final finding was that the onset of compensatory hyperventilation was delayed; relative to the imposition of exercise and presumably to the onset of metabolic acidaemia.

3.6.1 Intermittent exercise 90s exercise 180s recovery

The temporal $\dot{V}_E$, $\dot{V}CO_2$ and $\dot{V}O_2$ response profiles observed during the first duty cycle of the 90s:180s trials are not substantially different from those of a standard square-wave exercise protocol (figure 3.6) (Whipp *et al.*, 1982), albeit one stopped prior to the attainment of phase 3. This occurs, as the imposition of

the first intermittent exercise period is, of course, identical to a supra-$\widehat{\theta}_L$ square-wave trial.

3.6.1.1 Phase I

An important feature of the temporal pattern of response was the observation that the transition to recovery resulted in phase I $\dot{V}_E$ and $\dot{V}CO_2$ responses essentially indistinguishable, except possibly in magnitude, from those seen during a sustained moderate intensity square-wave exercise test (Whipp *et al.*, 1982). This is in contrast to the secondary transient rise seen in $\dot{V}CO_2$ but not $\dot{V}_E$ during the 30s:60s and during the 60s:120s trials.

The explanation for this, however, is not clear. This is in part due to the complex nature of the $\dot{V}CO_2$ response, which comprises CO_2 from three sources: metabolic CO_2 production ($\dot{Q}CO_2$), CO_2 released from bicarbonate buffering reactions and evolution of stored CO_2 during respiratory compensation. The secondary rise of $\dot{V}CO_2$ during phase I of the off transients could reflect a continuing rise in any of these components. However, it does not seem unreasonable to suggest that the transient $\dot{V}CO_2$ overshoot might reflect a residual contribution from the buffering reaction continuing to increase the CO_2 content of the pulmonary arterial blood during the 30s:60s and 60s:120s trials. This overshoot is simply absent during the longer 90s:180s trials as the buffering reactions may have proceeded close to completion during the exercise period. Thus, when the work-rate is reduced with a concomitant fall in $\dot{Q}$ and hence

pulmonary blood flow, as the CO_2 content of the pulmonary arterial blood should remain relatively unchanged throughout phase I (see above), $\dot{V}CO_2$ will fall with the reduction in $\dot{Q}$ (figure 3.32) (Barstow *et al.*, 1990; De Cort *et al.*, 1991; Jones *et al.*, 1981; Krogh and Lindhard, 1913; Loeppky *et al.*, 1981; Weisman *et al.*, 1982; Yoshida & Whipp, 1995). However, the pattern of ventilatory response is similar in the three longest exercise-recovery duty cycle trials. Therefore, the $\dot{V}_E$ response during phase I under these conditions of intermittent exercise does not seem to be related solely to the requirement to clear CO_2 at the lungs. The reasons for this are presently unclear.

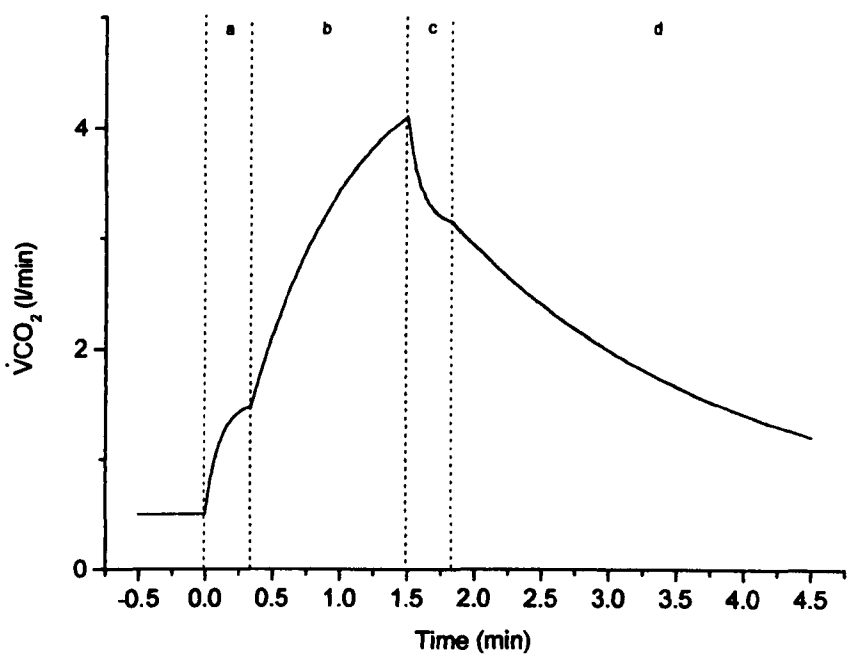


Figure 3.32: 90:180 schematic showing the on-transient rise in $\dot{V}CO_2$ during phase I (a), followed by the secondary phase II rise (b). This is followed by a falling $\dot{V}CO_2$ during phase I off the off-transient (c) and slowly declining phase II (d).

Furthermore, a lack of consistency was seen in the magnitude of the phase I response. The same subject did not always exhibit the same magnitude of

increase during phase I in the first duty cycle for all four intermittent trials, for example. This lack of proportionality between the work-rate and the increase in $\dot{V}_E$ appears incompatible with classical forms of neurogenesis (e.g. central command and muscle reflex) (e.g. Zuntz and Geppert, 1886; Krogh and Lindhard, 1913). The subjects were unaware of the duration of exercise periods in the intermittent trial they were undertaking and additionally as there was no specific order effect it seems unlikely this was a volitional or conditioned response.

3.6.1.2 $\dot{V}_E$ - $\dot{V}CO_2$ Relationship

A particularly interesting feature of the longest duty-cycle utilised (i.e. 90s:180s) is evident in the $\dot{V}_E$ - $\dot{V}CO_2$ relationship. For the first exercise period the $\dot{V}_E$ - $\dot{V}CO_2$ relationship had a slope lower than that for baseline (taken as the rest-20W transition), the same pattern as observed during the 30s:60s and 60s:120s trials; then after about a minute the slope increases dramatically. Following this the next exercise period again starts with only a slightly increased slope with regard to baseline just as the 30s:60s and 60s:120s trials did, again about a minute into the trial the slope increases sharply. Only one subject completed a third duty cycle and while no secondary increase was seen there was a marked increase in the slope from the onset of exercise. No subject was able to complete a fourth transition; therefore, as the secondary increase appeared time delayed it was not expected that one would be seen and as during the third duty cycle the slope was increased throughout.

While $[La^-]_a$ was first measured just prior to the end of the first exercise period, by this point it was already elevated in the blood to nearly 4mM. It seems unlikely that the point of inflection in the $\dot{V}_E$-$\dot{V}CO_2$ relationship represents a sudden outpouring of lactate and H^+ from the muscle as it would appear sensible to assume the increase in the lactate concentration during the 90s of exercise followed a similar pattern to that observed during square-wave exercise tests, i.e. curvlinearily (Wasserman *et al.*, 1967). So, during the period when there was no elevated $\dot{V}_E$-$\dot{V}CO_2$ slope the $[La^-]_a$ was already close to the maximum seen during the 30s:60s trials during which there was a marked hyperventilation as the trial progressed. Therefore, it seems unlikely that the onset of hyperventilation was concentration related, but rather delayed in time for some unknown reason.

The same principle holds true for the second exercise period and is even less open to debate as there can be no question that the blood $[La^-]_a$ was substantially elevated at the beginning of that exercise period. It is clear from figure 3.4 that immediately before the end of the preceding recovery period that $[La^-]_a$ was substantially elevated, to around 8mM. Therefore, while the initial slope during the second exercise period was slightly elevated it was not even as steep as observed toward the end of the 30s:60s trials when $[La^-]_a$ was not as high (see page 138 and page 151). Furthermore if the delay in the onset of compensatory hyperventilation was concentration-dependent it would be expected that the $[La^-]_a$ might have increased across the second exercise period to cause the delayed rise in the $\dot{V}_E$-$\dot{V}CO_2$ slope. However, the $[La^-]_a$ actually fell from the end of the first recovery period to the end of the second exercise period. The outcome of this is that the onset of compensatory hyperventilation appears to be

delayed in time both relative to the onset of exercise and to the onset of acidaemia, and is not dependent strictly on the $[La^-]_a$ and therefore presumably the $[H^+]_a$.

The off-transient responses observed showed that $\dot{V}_E$ remained high initially while $\dot{V}CO_2$ declined, in other words the $\dot{V}_E$ - $\dot{V}CO_2$ slope was close to flat, and then declined steeply later into the exercise period (figure 3.10). First appraisal suggests that this may have nothing to do with compensatory hyperventilation as an increased $\dot{V}_E$ - $\dot{V}CO_2$ slope is the conventional symptom of hyperventilation (e.g. Wasserman *et al.*, 1967). However, a reduced slope as $\dot{V}_E$ is recovering means that $\dot{V}_E$ is remaining elevated with respect to CO_2 clearance. Although a reduced slope could also, under 'normal' circumstances, simply indicate appropriate $\dot{V}_E$ if $\dot{V}_D$ had decreased; a likely consequence of a recovery transition as the decrease in $\dot{V}_E$ will be partly achieved through a decrease in breathing frequency (Beaver and Wasserman, 1970; Dejours, 1964; Whipp and Wasserman, 1973). However, under these conditions the $\dot{V}_E$-intercept was substantially elevated, not reduced, i.e. this was hyperventilation. Later into recovery $\dot{V}_E$ decreased more rapidly and consequently the $\dot{V}_E$ - $\dot{V}CO_2$ slope increased.

3.6.2 Intermittent exercise 60s exercise 120s recovery

A similar set of considerations discussed with regard to the 90s:180s trials can be applied here, as the 60s exercise period, during the first duty cycle any way, is

simply a reduction of the 90s exercise period. Therefore, as the subjects were unaware of which protocol they were beginning the lack of difference in the early responses to the onset of exercise is a predictable outcome (see introduction page 11). After the first exercise period passes beyond 30s the metabolic consequences of the exercise become separated from those of the 30s:60s test but remain the same as during the 90s:180s trial. This can be seen again in the steeply rising profile of $[La^-]_a$, and the continued rise in end-exercise $\dot{V}_E$ throughout all 60s:120s trials similar to the 90s:180s trials.

3.6.2.1 Phase I

That the transition from recovery to exercise or exercise to recovery always resulted in a ventilatory response similar in pattern to $\dot{V}CO_2$, regardless of whether that was a step change, a gradual change or even no change in $\dot{V}CO_2$, is worthy of consideration (e.g. figures 3.6 & 3.7). As the initial step change in $\dot{V}_E$ and $\dot{V}CO_2$ occurred on the same breath, it is possible that a neurogenic drive (central or peripheral) proportional to the absolute work-rate drove the step change in $\dot{V}_E$ irrespective of error driven feed-back control, thus causing hyperventilation (e.g. Zuntz & Geppert, 1886; Krogh & Lindhard, 1913). Therefore, as CO_2 is highly soluble in the blood the increased $\dot{V}CO_2$ would have been a result of the increased $\dot{V}_E$ rather than a cause of the increased $\dot{V}_E$. However, careful consideration of the pattern of response also shows that RER was initially stable and then fell progressively throughout all duty cycles, after the first (figure 3.5). An increase in $\dot{V}CO_2$ driven by $\dot{V}_E$, i.e. hyperventilation,

would have caused an initial rise in RER. The initial stability in RER during the on-transient is, however, consistent with a cardiodynamic rise in $\dot{V}CO_2$ that is matched by $\dot{V}_E$ (Casaburi *et al.*, 1978; Jensen *et al.*, 1971; Linnarsson, 1974; Ward, 1979b; Wasserman *et al.*, 1977; Whipp, 1977). The fact that heart rate does not show immediate changes at the transitions (e.g. figure 3.14) does not rule out this proposition of a changing cardiac output mediating the increased $\dot{V}CO_2$. It is quite possible that a rapid change in stroke volume, possibly mediated through changes in venous return brought about by alterations in the muscle pump with the changes in work-rate (Frank, 1895; Patterson, 1914), could effect the appropriate change in cardiac output to account for the observed $\dot{V}CO_2$ responses.

After the initial decrease in $\dot{V}CO_2$ following the transition to recovery (figure 3.33 'c') there was a small rise in $\dot{V}CO_2$ (figure 3.33 'd') before $\dot{V}CO_2$ began the 'phase II' decrease (figure 3.33 'e'); this small rise was not matched by $\dot{V}_E$ (figure 3.6). The origin of this rise in $\dot{V}CO_2$ is uncertain. However, it is possible that it represents the still rising PCO_2 in the pulmonary arterial blood (the product of the exercising muscle some 15s-20s ago) in combination with a residual contribution from bicarbonate buffering reactions being sufficient to offset the slowing decline in cardiac output and hence pulmonary blood flow (Miyamoto *et al.*, 1982).

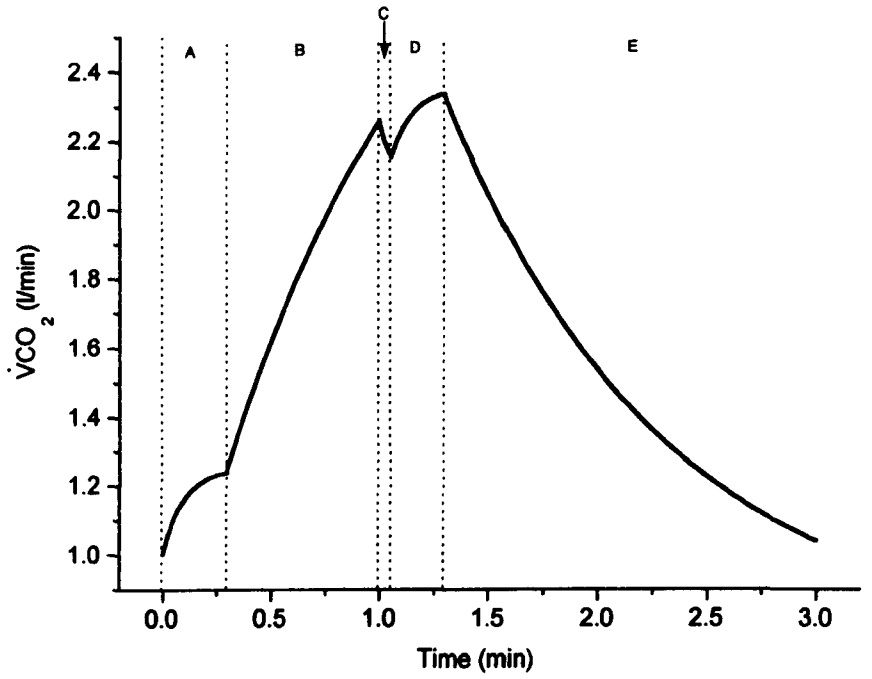


Figure 3.33: 60s:120s single transition schematic illustrating the phase I increase at exercise onset (A), the slower phase II rise (B) during the on-transition. At exercise off-set there is an immediate phase I fall in $\dot{V}CO_2$ (C) followed by a secondary rise in $\dot{V}CO_2$ (D) and finally a phase II decline in $\dot{V}CO_2$ (E).

However, what is not clear is why $\dot{V}_E$ becomes dissociated from the secondary rise in $\dot{V}CO_2$? There seems little likelihood that it involves an input to the ventilatory controller from the carotid body chemoreceptors responding to the acidaemia (a hyperventilatory stimulus) as the level of $\dot{V}_E$ is low, not high, for the CO_2 output (Wasserman *et al.*, 1975; Winterstein and Gokhan, 1953). This fact both argues against a $[H^+]_a$ linkage driving this phase I response and suggests that this result may be transferable to considerations of control of the moderate intensity exercise hyperpnoea.

The remaining classical possibilities are that $\dot{V}_E$ is being controlled by a central neural drive, muscle reflex (both presumed to be essentially proportional to work-rate) or a cardiovascular linkage possibly proportional to cardiac output (e.g. Zuntz & Geppert, 1886; Krogh & Lindhard, 1913; Wasserman *et al.*, 1974). No specific relationship was seen between work-rate and the magnitude of the phase I $\dot{V}_E$ and in fact there was a considerable variation between the 'on' phase I increase in $\dot{V}_E$ and the corresponding 'off' decrease in $\dot{V}_E$. Neither of these findings appears compatible with either a reduction in central command or a muscle reflex stimulus reducing and/or maintaining the drive to $\dot{V}_E$ during phase I at a time when $\dot{V}CO_2$ is rising again.

It is important to note that this discussion is primarily focusing around a secondary event during phase I. That is $\dot{V}CO_2$ does decrease rapidly with the offset of exercise, over the first few breaths, but then begins to rise again. Therefore, conceptually it is possible that the initial decline in $\dot{V}_E$ could have been mediated by the control system on the basis of information from a receptor "aware" of the falling $\dot{V}CO_2$. However, the wealth of available evidence argues against such a mixed venous chemoreceptor (Asmussen and Chiodi, 1941; Brown *et al.*, 1976; Comroe and Schmidt, 1943; Dejours *et al.*, 1955; Gautier, 1969; Haouzi *et al.*, 1993; Haouzi *et al.*, 1997; Huszczuk *et al.*, 1993; Innes *et al.*, 1989; Moore *et al.*, 1934; Rowell *et al.*, 1976). The declining $\dot{V}CO_2$ response is likely to have been caused by a fall in cardiac output and it is likely, though not confirmed through measurement, that cardiac output would have continued to decline throughout recovery (e.g. De Cort *et al.*, 1991). Therefore,

despite no evidence of a falling cardiac output during phase I in terms of the heart rate response (see page 168 for discussion) the most plausible, known, drive to $\dot{V}_E$ during phase I of the transition from exercise to recovery may be one linked to cardiac output.

How this is compatible with several lines of evidence arguing against an obligatory role for a cardio-dynamic drive during phase I, e.g. the normality of the phase I response after heart and heart-lung transplantation (Banner *et al.*, 1988; Grassi *et al.*, 1993; Huszczuk *et al.*, 1990 Theodore *et al.*, 1987), is unclear. Regardless it would appear that the ventilatory controller is unaware of the pulmonary requirement to clear CO_2 during this unusual phase I response, thus further arguing against a humoral stimulus driving phase I $\dot{V}_E$.

3.6.2.2 $\dot{V}_E$ - $\dot{V}CO_2$ Relationship

The small progressive increase in the slope of the $\dot{V}_E$ - $\dot{V}CO_2$ relationship that was seen during each exercise-recovery duty cycle, both during the exercise and the recovery phases (although steeper during exercise) and following an initial decrease in slope relative to baseline, was qualitatively similar to that observed during the 30s:60s trials. The only difference being the magnitude of the increase in the slope which was consistent with the increased $[La^-]_a$ (taken as a surrogate for an increased $[H^+]_a$) during the 60s:120s trials. However, there was no evidence of a hyperventilatory response (i.e. an increased slope of the $\dot{V}_E$ - $\dot{V}CO_2$ relationship) until the third duty cycle and even then only a very mild hyperventilation. By the third duty cycle the $[La^-]_a$ had already risen above the

peak value obtained during the 30s:60s trials yet the compensatory hyperventilation was markedly less. Therefore it appears that a strictly $[H^+]_a$ (based on $[La^-]_a$) dependent mechanism is unlikely to account for the delay in the onset of compensatory hyperventilation, i.e. isocapnic buffering (Wasserman *et al.*, 1977).

3.6.3 Intermittent exercise 30s exercise 60s recovery

The maintenance of distinct oscillations in all variables despite the reduction of the exercise period to 30s is a consequence of both the duration and intensity of the exercise period in addition to the response kinetics of the variables of interest (e.g. Lamarra *et al.*, 1987). The reduction of the exercise periods to 30s meant that each exercise period is still analogous to the first 30s of a square-wave trial, rather than an impulse which the 10s exercise period more closely equates to (e.g. Whipp and Ward, 1981). It should be noted that while the oscillating nature of the intermittent trials may draw parallels with a sinusoidal exercise forcing, it is actually quite different as the intermittent trial is composed of a series of discrete step forcings, with the possible exception of the 10s:20s trial (see later for discussion). Therefore, phase I responses typically absent in a ramp or sinusoidal forcing are present in these trials (Casaburi *et al.*, 1977; Fujihara *et al.*, 1973; Karlson and Wigertz, 1971; Wigertz, 1970; Wigertz, 1971).

Therefore, unlike the shorter exercise duration in the 10s:20s trials and similar to the two longer intermittent trials (already discussed) the metabolic consequences of the increased work-rate will begin reaching the lung, and be exchanged, at a time when the work-rate is still elevated (figure 3.34) (Casaburi *et al.*, 1987; Whipp *et al.*, 1982). Thus there will be the development of both phase I and the beginning of the slow exponential phase II rise during the 30s exercise period (figure 3.34 'a1' 'b1') (Casaburi *et al.*, 1977; Hughson & Morrissey, 1982; Hughson, 1990; Linnarsson, 1974; Miyamoto, 1989; Whipp *et al.*, 1982).

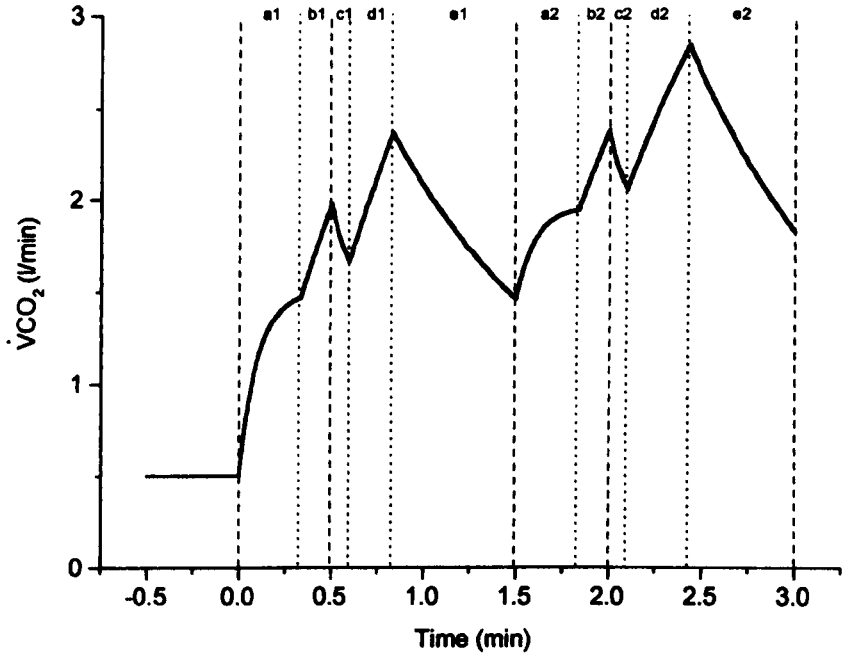


Figure 3.34: 30s:60s schematic showing the first two transitions, a1 represents the phase I increase at exercise onset, b1 is the onset of phase II which terminates as the work-rate is reduced and $\dot{V}CO_2$ falls with the reduction in $\dot{Q}$ (c1). A secondary rise was observed during phase I of the off-transient (d1). This was followed by the phase II decline (e1). The responses are repeated in a2, b2, c2, d2 and e2.

3.6.3.1 Phase I

However, while the decreased arterial lactate concentration (in comparison to the 90s:180s & 60s:120s trials) was accompanied by a substantially reduced ventilatory response throughout the 30s:60s trials; the general pattern of response was not substantially different to the 60s:120s trials (figures 3.6, 3.13 3.20 & 3.21). As such, during the 30s:60s trials the same dissociation of $\dot{V}_E$ from $\dot{V}CO_2$ during phase I of recovery as seen during the 60s:120s trial was observed (figure 3.34).

Again this is likely to have occurred due to the conflicting changes in cardiac output and increased mixed venous CO_2 content and decreased O_2 content from the exercising muscle and bicarbonate buffering. The decrease from 60s exercise to 30s exercise presumably resulted in $\dot{Q}O_2$ and $\dot{Q}CO_2$ being on the steeper portion of their exponential increase and the bicarbonate buffering reactions being further from equilibrium; therefore, it is likely the proposition made with regard to the 60s:120s trials is also applicable here (see page 168). The ventilatory controller again appears unaware of the CO_2 demand during phase I and is responding to another input, possibly related to cardiac output.

3.6.3.2 $\dot{V}_E$ - $\dot{V}CO_2$ Relationship

The decrease in the slope of the $\dot{V}_E$ - $\dot{V}CO_2$ relationship typically seen during the first exercise period, in comparison to the rest-20W transition, may simply reflect the rapid kinetics of $\dot{V}CO_2$; brought about by the bicarbonate buffering reactions

(e.g. figure 1.6) (Rausch et al., 1991). The progressive rise in the $\dot{V}_E$ - $\dot{V}CO_2$ slope throughout the remainder of the 30s:60s trials may well be a result of the slowly rising $[La^-]_a$ and therefore presumably the slowly falling pH_a, i.e. respiratory compensation (Wasserman *et al.*, 1975; Winterstein and Gokhan, 1953).

What is of interest is the consistently lower $\dot{V}_E$ - $\dot{V}CO_2$ slope during recovery (see page 166); the reduced slope in recovery may be indicative of compensatory hyperventilation. Furthermore, if the degree of hyperventilation was consistent across exercise and recovery the slope should not change. Therefore, the decreased slope in recovery actually indicates a greater hyperventilatory response during recovery than during exercise. It is highly unlikely that the transition to recovery was the actual cause of this increased hyperventilation. Rather, it would appear that much of the hyperventilatory response is considerably delayed with respect to both the increase in work-rate and the onset of metabolic acidaemia (presumed to be present due to an increased $[La^-]_a$).

Similarly to the 60s:120s and 90s:180s trials the ventilatory response as viewed through the $\dot{V}_E$ - $\dot{V}CO_2$ relationship does not appear to be driven neurally through either central command or muscle reflexes. Rather the ventilatory response again appears appropriate to the 'metabolic' demand, again the need to reduce P_aCO_2.

3.6.4 Intermittent exercise 10s exercise: 20s recovery

For the first 10s:20s cycle in any typical 10s:20s trial, $\dot{V}O_2$, $\dot{V}CO_2$ and $\dot{V}_E$ all increased with the increase in work-rate. This response was perhaps predictable, as the imposition of the first 10s exercise period is equivalent to the initial 10s of a square-wave and is identical to a "standard" 10s impulse (or more properly "pulse") exercise test (e.g. Lammara *et al.*, 1987). Therefore, the expected response would be the appearance of a normal work-to-work phase I response (see page 14) with an accompanying stable RER response (Casaburi *et al.*, 1978; Jensen *et al.*, 1971; Linnarsson, 1974; Ward, 1979b; Wasserman *et al.*, 1977; Whipp, 1977). In these trials however, RER tended to increase during the first 10s exercise period; this is likely to have been an artefact of the relatively high work-rates eliciting a 'startle' response (e.g. Whipp and Wasserman, 1970).

3.6.4.1 Oscillations

Thus, it is appropriate to consider the first duty cycle of the trial as a standard impulse protocol. Whereby, 15-20s after the onset of exercise, following the initial phase I increase (figure 3.35 'a'), a secondary rise in $\dot{V}_E$, $\dot{V}CO_2$ and $\dot{V}O_2$ would be expected. This would be caused as the metabolic effluent of the exercising musculature reaches the pulmonary circulation and begins contributing to the expirate (figure 3.35 'b') (Casaburi *et al.*, 1977; Hughson & Morrissey, 1982; Hughson, 1990; Linnarsson, 1974; Miyamoto, 1989; Whipp *et al.*, 1982). During an impulse trial, the period during which $\dot{V}CO_2$ and $\dot{V}O_2$ rise as a consequence of the exercise-altered composition of mixed venous blood is

likely to be similar to the duration of the exercise period. Therefore, this secondary rise will shortly be replaced by a falling phase in $\dot{V}_E$, $\dot{V}CO_2$ and $\dot{V}O_2$ as the exercising musculature recovers (figure 3.36) (Lamarra *et al.*, 1987b).

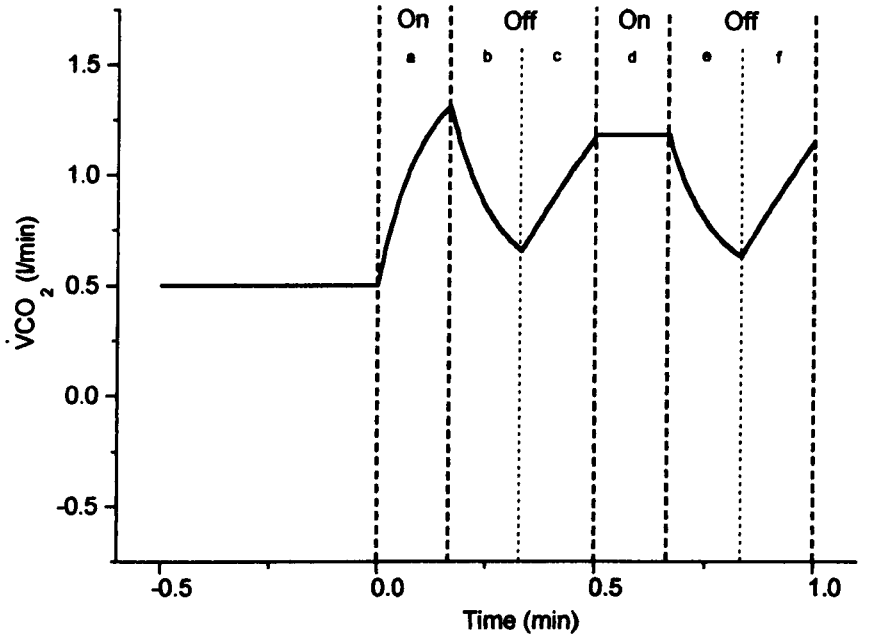


Figure 3.35: Schematic representation of the changes occurring in $\dot{V}CO_2$ during the first two exercise: recovery duty cycles of a 10s:20s intermittent trial. During phase (a) $\dot{V}CO_2$ rises due to an increased $\dot{Q}$ while $CpaCO_2$ remains unaltered. During phase b the subject is recovering, $\dot{Q}$ is falling and $CpaCO_2$ is still unchanged from baseline. However, in phase (c), later into recovery, while $\dot{Q}$ continues to fall $CpaCO_2$ begins to rise as a consequence of the first exercise period. At the onset of the next exercise period, phase d, $CpaCO_2$ begins to decline, reflecting the period of recovery begun 20s previously, while $\dot{Q}$ begins to rise once more. The changes seen during phase e and phase f are similar to those during phases (b) and (c).

However, in the 10s:20s intermittent test this is not likely to occur. Assuming that phase I lasts 20s (Casaburi *et al.*, 1978b; Dejours, 1964; Jensen *et al.*, 1972; Miyamoto, 1989; Whipp *et al.*, 1982) and the period during which $\dot{V}CO_2$ and $\dot{V}O_2$ are being increased by the venous drainage of the exercising musculature is similar to the duration of the exercise period, i.e. 10s, then, as the falling phase should begin to occur (30s after exercise onset) the work-rate will be increased

again (figures 3.35 'd' & 3.36). Therefore, the falling PCO_2 and rising PO_2 in the pulmonary arterial circulation, as the metabolic consequences of recovery reach the lung, will be offset by a rise in pulmonary blood flow at the onset of the second-exercise-recovery duty cycle (figure 3.35 'd'). Clearly in the intact exercising human variances in transit time for example will mean that such perfect timing of responses will not occur, thus further blurring the responses measured at the mouth.

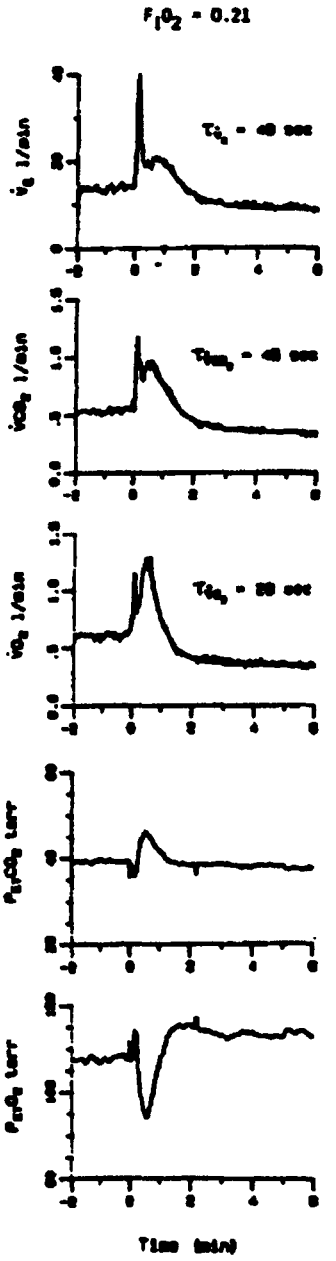


Figure 3.36: Ventilatory and pulmonary gas exchange responses during and impulse exercise test (modified from Lamarra *et al.*, 1987b)

3.6.4.2 Quasi-Steady-State

The lack of sustained rise in $[La^-]_a$ and therefore presumably of $[H^+]_a$ was evident in the stability of $\dot{V}O_2$, $\dot{V}CO_2$ and RER throughout all 10s:20s intermittent trials. Following the initial 'kinetic' rise in $\dot{V}O_2$ and $\dot{V}CO_2$, both attained a 'steady-state'. A similar pattern of response was also evident in RER with a small increase above the level seen at 20W. This potentially suggests either a shift towards a greater proportion of energy being supplied through the catabolism of carbohydrate rather than fat or a change in CO_2 capacitance allowing a reduced storage of the metabolically produced CO_2 (Brown *et al.*, 1985; Farhi, 1964; Farhi and Rahn, 1965; Farhi and Rahn, 1960; Putman *et al.*, 1993; Taylor and Jones, 1979). In two subjects there was a decrease in RER around 20 minutes into the trial though always remaining elevated with respect to baseline exercise. As there was no indication of hypoventilation in these subjects it is possible that the reduction in RER indicated either a switch toward a greater utilization of fat as the substrate for metabolism or an increased tissue CO_2 capacitance, thus reducing $\dot{V}CO_2$ relative to $\dot{Q}CO_2$ (Brown *et al.*, 1985; Farhi, 1964; Farhi and Rahn, 1965; Farhi and Rahn, 1960; Putman *et al.*, 1993; Taylor and Jones, 1979).

Following an initial rise in $P_{ET}CO_2$ all subjects exhibited a small decrease of the order of 2mmHg before attaining stability. While this may appear indicative of hyperventilation and a concomitant fall in P_aCO_2, as it was accompanied by an increase in breathing frequency this is unlikely to be substantial, if at all. That is, the reduction in inspired and expired time, with increased breathing frequency, will serve to reduce the duration of the "alveolar phase" of the expired PCO_2

profile, thus reducing the peak value obtained, i.e. the end tidal value (see page 227 for discussion) (Ward, 1987). However, it is not possible to confirm this hypothesis as P_aCO_2 was not measured during these trials. This was the case as the validity of non-invasive methods for estimating P_ACO_2 and, thence, P_aCO_2 has not been examined under non-steady-state conditions of the kind encountered in the present study. Furthermore, we elected not to perform sequential arterial or arterialised blood sampling at the high density that this study would demand, because of the substantial technical challenges.

3.6.4.3 $\dot{V}_E$ - $\dot{V}CO_2$ Relationship

While there was no significant difference in the group mean $\dot{V}_E$ - $\dot{V}CO_2$ relationship between 'baseline' and during the intermittent phase of the 10s:20s trials, three subjects did exhibit small increases in the slope of the $\dot{V}_E$ - $\dot{V}CO_2$ relationship. This may be interpreted as hyperventilation. However, the high breathing frequencies observed during this trial will be likely to have produced a correspondingly large increase in $\dot{V}_D$; thus increasing the requirement for $\dot{V}_E$ to clear a given load of CO_2 (Jones *et al.*, 1966; Whipp, 1977). Furthermore, the increased $\dot{V}_E$ - $\dot{V}CO_2$ slope was accompanied by an increased $P_{ET}CO_2$, while hyperventilation would have been accompanied by a decreased $P_{ET}CO_2$. Therefore the increased slope is not necessarily inconsistent with $\dot{V}_E$ being appropriate for regulation of P_aCO_2.

The other subjects in whom the $\dot{V}_E$ - $\dot{V}CO_2$ slope was unchanged from the rest-20W transition to the 10s:20s intermittent phase exhibited a more prominent tidal

volume contribution (and therefore a smaller increase in breathing frequency) to the $\dot{V}_E$ increase. Thus, despite $\dot{V}_D$ increasing with the increase in breathing frequency its overall contribution to each breath (i.e. V_D/V_T) may have remained relatively unchanged (Higgs *et al.*, 1967; Jones *et al.*, 1966; Wasserman *et al.*, 1967; Whipp, 1977; Whipp and Wasserman, 1970). Therefore, in these cases, that the unchanged $\dot{V}_E$-$\dot{V}CO_2$ slope could also represent an appropriate regulation of P_aCO_2.

Consequently, it appears that despite a high level of central command and muscle reflex drive (at least in response to mechanical changes) proportional to the absolute work-rate, the ventilatory response is likely to be proportional to, and appropriate for, the total requirement to clear CO_2 and thus to maintain P_aCO_2.

3.6.5 Respiratory compensation and isocapnic buffering

While this study has again demonstrated the existence of a delay-like or slow kinetic manifestation of respiratory compensation following the onset of acidotic exercise and that this phenomenon is not $[La^-]_a$, and therefore presumably not $[H^+]_a$, dependent it is unable to shed light on its cause. As this sluggish hyperventilatory response is not instantly compatible with the known properties of the carotid bodies (see introduction page 69) (the proposed mediators of respiratory compensation e.g. Wasserman *et al*, 1975b) this may be taken as evidence refuting the role of the carotid bodies in mediating the hyperventilatory response. However, other proposals such as an increased central command, out of proportion to the exercise work-rate, due to fatigue of muscle fibres (e.g.

Assmusen, 1947) seem equally incompatible with such a slow response. To resolve this issue it is imperative to map the response profile of carotid body activity under the conditions experienced during heavy exercise. With this information it may be possible to understand why appropriate respiratory compensation does not occur at the onset of metabolic acidosis.

3.6.6 Future directions

It could be argued that this study requires a suitable control, potentially in the form of moderate work-rates implemented in these exercise-recovery duty cycles. This would rule out any effect from the manner the exercise was imposed as a cause of the responses observed, rather than the responses being a consequence of the exercise itself. Furthermore, this would establish whether the dissociation of $\dot{V}_E$ from $\dot{V}CO_2$ during phase I of a recovery period was a normal response and a consequence of the ventilatory controller being unaware of the CO_2 clearance requirement, rather than being an artefact of the acidosis.

With regard to the dissociation of $\dot{V}_E$ from $\dot{V}CO_2$ during phase I observed during this study two possible paths are immediately obvious. Firstly, it is postulated here that central command and peripheral neurogenic drives were not likely to be mediating this response as the work-rate, and therefore the neurogenic drive, was fixed yet there was substantial variability in $\dot{V}_E$. However, this could be experimentally confirmed by repeating these studies with paraplegic subjects and externally generated exercise, thus potentially removing these drives. A second approach would be to investigate the potential of a cardio-

dynamic mechanism to provide a drive capable of accounting for these observations. This could be achieved firstly by attempting to measure cardiac output with sufficient temporal density to track the changes during phase I in these protocols. An alternative would be to manipulate cardiac output, e.g. by altering venous return either by changing body position or through changes in lower body pressure, during such a protocol and examine the responses of $\dot{V}_E$.

3.7 Conclusions

This study has demonstrated that the ventilatory response remains appropriate to the metabolic demands, be that maintenance of P_aCO_2 or lowering of P_aCO_2 to effect respiratory compensation for a metabolic acidosis, in the face of a substantial, presumably constant, neurogenic drive. Secondly, that the ventilatory response during phase I is not proportional to the classical neurogenic drives, nor is it aware of the specific CO_2 clearance requirements; thus potentially implicating a cardio-dynamic mechanism. Though this is only by default rather than through experimental demonstration of such a mechanism. Finally, that the delayed onset of compensatory hyperventilation, referred to as isocapnic buffering in a rapid incremental test, is delayed both relative to the onset of exercise, i.e. the onset of neurogenic drives, the onset of the metabolic acidaemia and it is seemingly not mediated by a concentration dependent threshold. Although to qualify the final remark, in general, the hyperventilation is more marked the higher the $[La^-]_a$. Furthermore, it should be noted that the delayed onset of compensatory hyperventilation can only be seen within a duty cycle during the longer duty-cycle trials (i.e. 60:120 and 90:180).

Chapter 4

Long Term Modulation (LTM) of the exercise hyperpnoea

4.1 Introduction

In light of the inconsistent outcomes of the studies performed thus far regarding the role of respiratory memory in the control of the exercise hyperpnoea (see page 73) and the lack of information on the effect of LTM on the phase II kinetics of $\dot{V}_E$, this study revisited the problem. To do so the experimental design focused around associative conditioning of the 20W-80W cycle ergometry transition with an added external dead space while utilising components from the various experimental protocols that have been successful in demonstrating the existence of LTM in the exercise hyperpnoea. Therefore, the dead space volume, number of conditioning trials, rest between conditioning trials and delay between conditioning and post-conditioning measurements are drawn from studies describing the existence of LTM.

In order to limit the potential of confounding factors to influence the outcome, several changes were made to previous studies. Firstly, a period of thorough familiarisation and sufficient baseline repeats to ensure the measured responses were truly representative of the requirements of the exercise were undertaken. Secondly, the work-rates chosen were all determined to be of moderate intensity. Thus ruling out the complicating effects of the metabolic acidosis associated with work-rates above $\hat{\theta}_L$. Thirdly, P_aCO_2 was estimated experimentally, whereas previous studies had only utilised estimations of P_aCO_2 based on $P_{ET}CO_2$; which typically only provide accuracy at the population level. Furthermore, the nature of the conditioning paradigm requires that it lasts for several hours. Therefore it

is important to take into account the effect of shifts in substrate utilisation, manifested in RQ. This has not been accounted for in previous work. Finally, the utilisation of rigorous kinetic analysis will allow investigation of the existence of LTM during the phase II response.

4.2 Aims

The aim, therefore, is to return to the protocols utilised in the existing demonstrations of LTM in the exercise hyperpnoea but to account for the potentially confounding influences of substrate utilisation, subject familiarisation, classification of intensity domain and its effect on attainment of a steady-state. This aims to add clarity to the inconsistency that currently ensnares the literature regarding the phase III response. Furthermore, through rigorous kinetic analysis it will be investigated whether LTM is present in the phase II response and therefore whether it may be involved in the close kinetic matching of $\dot{V}_E$ to $\dot{V}CO_2$ typically observed during phase II.

4.3 Hypothesis

The consistent demonstration that $\dot{V}_E$ follows the lead of $\dot{V}CO_2$ during phase II, almost irrespective of the situation, is incompatible with the imposition of a pre-determined learned response at the onset of exercise. Therefore, it is hypothesised that the phase II pattern of ventilatory response, or more

importantly its appropriateness with regard to P_aCO_2 regulation, will be unaffected in any way by the conditioning. That is to say the ratio of $\tau \dot{V}_E / \tau \dot{V}CO_2$ will be unaffected by the conditioning. The phase III response however, in the absence of a sustained demonstrable error signal is the more likely candidate to derive information and thus drive from memory. Consequently it is plausible that the phase III exercise ventilatory response may exhibit plasticity in response to classical conditioning. Thus an increased phase III ventilatory response may be observed during the post-conditioning trials.

4.4 Methods

4.4.1 Subjects

Nine healthy physically active males (table 4.1) aged between 20 and 42 years provided written informed consent appropriate to the study (as approved by the Institutional Ethics Committee: Appendix 4.1) and volunteered to participate in the investigation. The subjects were informed that some tests would involve an increased breathing challenge. However, they were not informed how this would be implemented, or during which tests it would be implemented. Furthermore, they were otherwise unaware as to the purpose of the study.

Subject	$\mu\dot{V}O_2$ *(L/min)*	$\mu\dot{V}O_2$ *(ml/kg/min)*	$\dot{V}O_{2\,\theta L}$ *(L/min)*	*Height (m)*	*Weight (kg)*	*Age (years)*
1	3.61	47.0	1.99	1.81	76.8	21
2	3.68	54.2	1.95	1.77	67.9	21
3	3.70	44.2	2.15	1.82	83.7	22
4	2.79	42.7	1.63	1.73	65.3	21
5	3.92	47.9	1.89	1.81	81.9	22
6	4.30	51.7	2.6	1.86	83.1	22
7	2.64	30.6	1.42	1.79	86.4	42
8	3.15	40.3	2.03	1.77	78.2	25
9	3.37	35.5	1.76	1.68	94.8	26
Mean	3.46	43.79	1.94	1.78	79.79	24.67
(s.d.)	0.53	7.54	0.33	0.05	9.10	6.75

Table 4.1: Subject characteristics detailing both individual and group mean values for age, height, weight, absolute peak oxygen uptake ($\mu\dot{V}O_2$ l/min), peak oxygen uptake relative to body weight ($\mu\dot{V}O_2$ ml/kg/min) and oxygen uptake at the estimated lactate threshold ($\dot{V}O_2\,\hat{\theta}L$ l/min) all functional indices measured during an during an incremental exercise test.

4.4.2 Apparatus

All experiments took place in the Laboratory of Human Physiology on an electronically-braked, computer-controlled cycle ergometer (Excalibur Sport, Lode, Netherlands). The following variables were monitored continuously throughout the experiments (see chapter 2): Heart rate and ECG; arterial oxygen saturation; inspired and expired gas volumes and respired O_2, CO_2 and N_2 concentrations.

4.4.2.1 External Dead Space

The external dead space (V_D = 1.4l) and sham dead spaces (equal inspired and expired resistance (ΔP = 0.1 cmH_2O) were imposed by fixing a rigid cylindrical tube (ID 3.5cm) onto the inspired and expired ports of a two-way non-rebreathing valve (Hans Rudolph Inc., Missouri, USA). In the case of the sham dead space the valve functioned as intended (figure 4.1a); therefore the only additional dead space was that of the valve assembly itself. For the actual external dead space, the valves segregating inspiration and expiration were removed and airflow through one port occluded; thus forcing inspiration and expiration to occur through the same port. In effect, this created a single passage for airflow equivalent to creating the dead space with a single fixed piece of tubing, while retaining the external appearance of the sham apparatus (figure 4.1b). The purpose of this was that the subject had no visual cue on entering the laboratory as to any difference in set-up between pre-conditioning, conditioning and post-conditioning. Additionally, as the resistances of both set-ups were

matched, there was no change in the respiratory impedance at a given ventilation (Whipp & Ward, 1980).

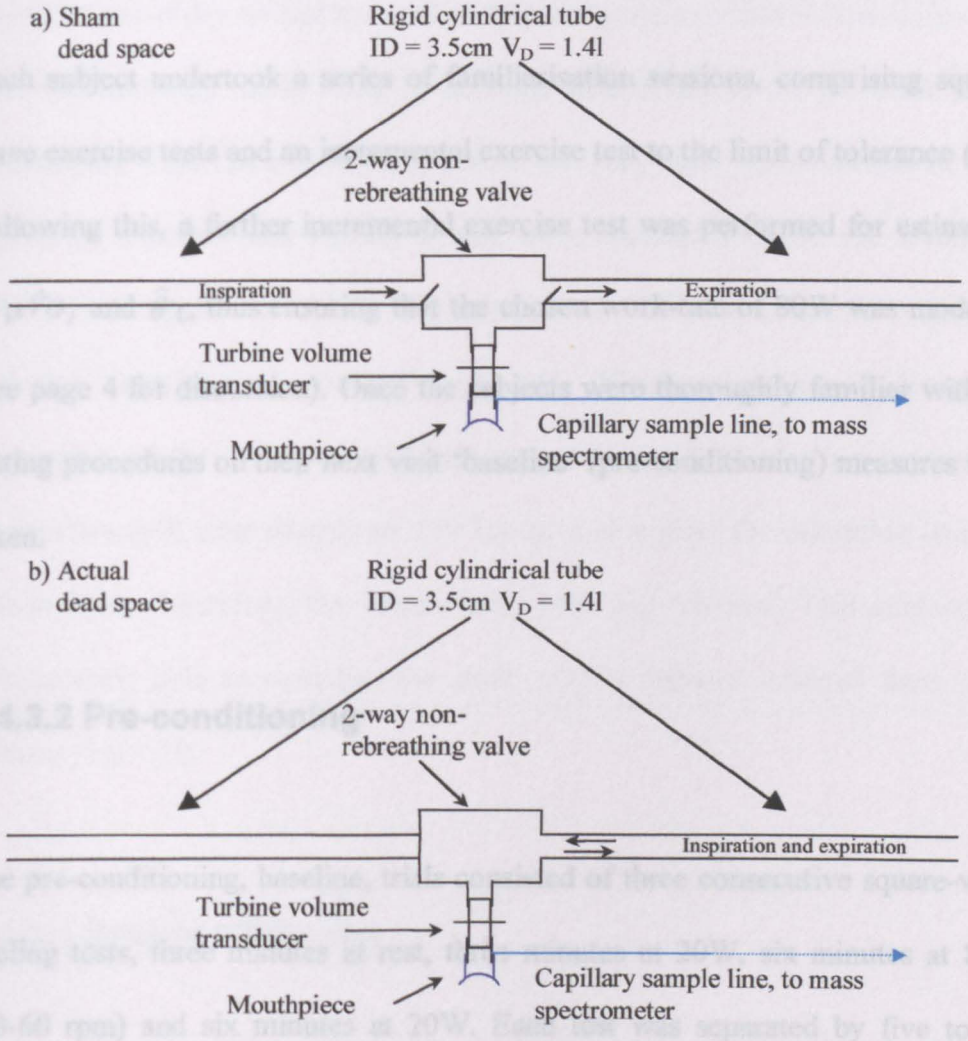


Figure 4.1: a) A schematic representation of the sham dead space set-up with inspiration and expiration occurring through different ports. Therefore the only effect of the tubing was increased resistance. b) The actual dead space set-up, externally identical to the sham apparatus. However airflow could only occur through one port, thus increasing both the resistance and the external dead space.

4.4.3 Protocols

4.4.3.1 Familiarisation

Each subject undertook a series of familiarisation sessions, comprising square-wave exercise tests and an incremental exercise test to the limit of tolerance (t_{lim}). Following this, a further incremental exercise test was performed for estimation of $\mu\dot{V}O_2$ and $\hat{\theta}_L$, thus ensuring that the chosen work-rate of 80W was moderate (see page 4 for discussion). Once the subjects were thoroughly familiar with the testing procedures on their next visit 'baseline' (pre-conditioning) measures were taken.

4.4.3.2 Pre-conditioning

The pre-conditioning, baseline, trials consisted of three consecutive square-wave cycling tests, three minutes at rest, three minutes at 20W, six minutes at 80W (50-60 rpm) and six minutes at 20W. Each test was separated by five to ten minutes of rest. During these tests the subjects breathed through the 'sham' dead space which was matched to the resistance of the dead space, as described earlier.

4.4.3.3 Conditioning

The conditioning trials were conducted on the day following the pre-conditioning and at a time of day so that the post-conditioning trials (which followed one hour after conditioning) would occur at the same time of day as the pre-conditioning tests (figure 4.2). The conditioning period involved eight repeated trials of cycle ergometry exercise (one minute at rest, three minutes at 20W, six minutes at 80W (50-60 rpm) and finally six minutes at 20W) in association with the imposed external dead space (V_D = 1.4l). However, subject 3 found the imposition of the 1.4l external dead space during 80W cycling beyond his capabilities (HR rose sharply to 177 b.p.m. and arterial O_2 saturation dropped below 90%). Therefore, the conditioning trial was aborted. This subject was subsequently able to complete the study with a reduced external dead space volume (V_D = 1l).

4.4.3.4 Post-conditioning

One hour after the final conditioning trial the subjects performed three post-conditioning 20W-80W-20W square-wave tests, identical to the three pre-conditioning tests.

In an attempt to standardise the substrate utilisation profile over the pre and post-conditioning trials, the subjects were instructed to eat no sooner than six hours prior to entering the laboratory for pre-conditioning and no sooner than one hour

prior to the conditioning/post-conditioning session. No food was allowed during the four hours of conditioning, or during the one hour resting between conditioning and post-conditioning. Additionally, the subjects were instructed as far as possible to maintain light activity during the five hours prior to their pre-conditioning trials, to attempt to match the energy expenditure of the conditioning trials (figure 4.2).

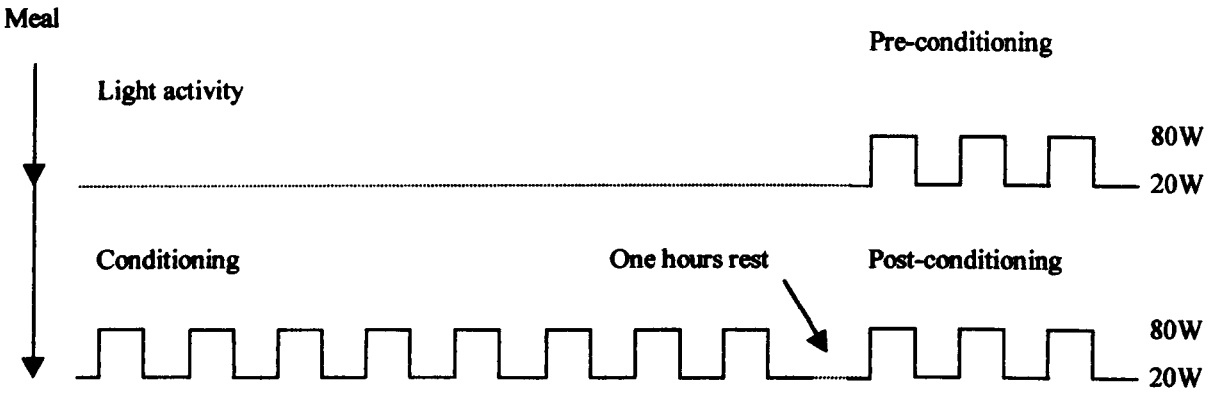


Figure 4.2: Schematic representation of the pre-conditioning day and of the conditioning/post-conditioning day.

4.4.4 Analysis

Prior to calculating the mean of these responses, the raw breath by breath data was edited to remove all breaths mis-triggered by the on-line software and which fell beyond four standard deviations from the mean (see page 98 for discussion). Once edited, the breath-by-breath data was interpolated into 1s time bins allowing the three pre-conditioning tests to be averaged. The baseline response for each work period was taken from this average of three tests.

In order to determine whether or not the conditioning to exercise with an added external dead space had augmented the ventilatory response during subsequent bouts of exercise alone, steady-state measurements were made for all respiratory variables. From the responses collected during the three pre-conditioning trials data from the final minute of each work period, i.e. 20W and 80W, was used to determine the mean steady-state responses of all pulmonary gas exchange and ventilatory variables. However, in situations when this data set was not representative of the preceding temporal response pattern, the preceding minute's data was substituted.

Additionally, the relationship between $\dot{V}_E$ and $\dot{V}CO_2$ was interrogated both in terms of its slope, estimated as a linear regression using commercial analysis software, (Microcal Origin) and its $\dot{V}_E$-intercept.

As stability of P_aCO_2 is normally associated with increasing $P_{ET}CO_2$ with increasing work-rate, up to $\hat{\theta}_L$, and because $P_{ET}CO_2$ shows day to day variation, the variable of interest is $\Delta P_{ET}CO_2$ rather than the absolute $P_{ET}CO_2$. Therefore, comparisons were made not of phase III $P_{ET}CO_2$ between pre- and post-conditioning, but more importantly of $\Delta P_{ET}CO_2$. Mean alveolar PCO_2 was also estimated using the method of DuBois *et al.* (1952) (see page 107 for discussion).

Kinetic analysis was performed on the phase II transient responses of $\dot{V}_E$, $\dot{V}CO_2$ and $\dot{V}O_2$ by non-linear least squares regression in order to estimate τ (Microcal Origin) (see page 106 for detailed description).

The data from the three post-conditioning tests was analysed in the same manner for kinetic analysis. This was done, firstly, to provide similar 'weighting' to pre- and post-conditioning and secondly as the 'noise' associated with a single repeat precluded fitting most of the subjects' data with sufficient confidence in τ. However, for steady-state measurements where averaging over 60s reduced the influence of the 'noise' the three post-conditioning tests were treated as separate entities. This was as exercise without an external dead space should produce a 'de-conditioning' effect, this would, of course, only occur if the conditioning trials had produced a long-term modulatory effect.

One-way analysis of variance, with time as the repeated measure, was used for statistical comparisons of all variables between pre-conditioning and conditioning, across the conditioning trials and from pre- to the three sequential post-conditioning trials. Where a significant difference was found ($p < 0.05$) post-hoc analysis (paired t-tests) was used to locate the difference.

4.5 Results

4.5.1 Incremental exercise test

Figure 4.3 shows a representative series of plots for subject 2 from an incremental exercise test used to estimate $\hat{\theta}_L$ and hence the steady-state work-rate equivalent to that oxygen cost. The $\dot{V}O_2$ and work-rate values calculated to correspond to each subject's $\hat{\theta}_L$ can be seen in table 4.1. This confirms that the chosen work-rates of 20W and 80W for the square-wave cycle-ergometry were moderate, i.e. $<\hat{\theta}_L$, for all subjects.

4.5.2 Square-wave exercise test

4.5.2.1 Conditioning

Prior to such considerations as, did the conditioning modulate the steady-state $\dot{V}_E$ response, the most important question is: Did the conditioning provide a sufficient hypercapnic challenge to augment the steady-state $\dot{V}_E$ requirement for cycle ergometry at 20W and 80W?

A comparison of the average pre-conditioning response and the first conditioning trial, for subject 2, can be seen in figure 4.4. This subject exhibited a three-fold increase in $\dot{V}_E$ at 20W and a two-fold increase at 80W during steady-state

exercise in the first conditioning trial. Increases on a similar scale were found for all subjects, with $\dot{V}_E$ increasing on average at 20W from 17l/min to 38l/min and at 80W from 29l/min to 56l/min (table 4.2 & table 4.3). However, these increases in steady-state $\dot{V}_E$ from baseline to conditioning trial 1 were still not sufficient for effective CO_2 clearance.

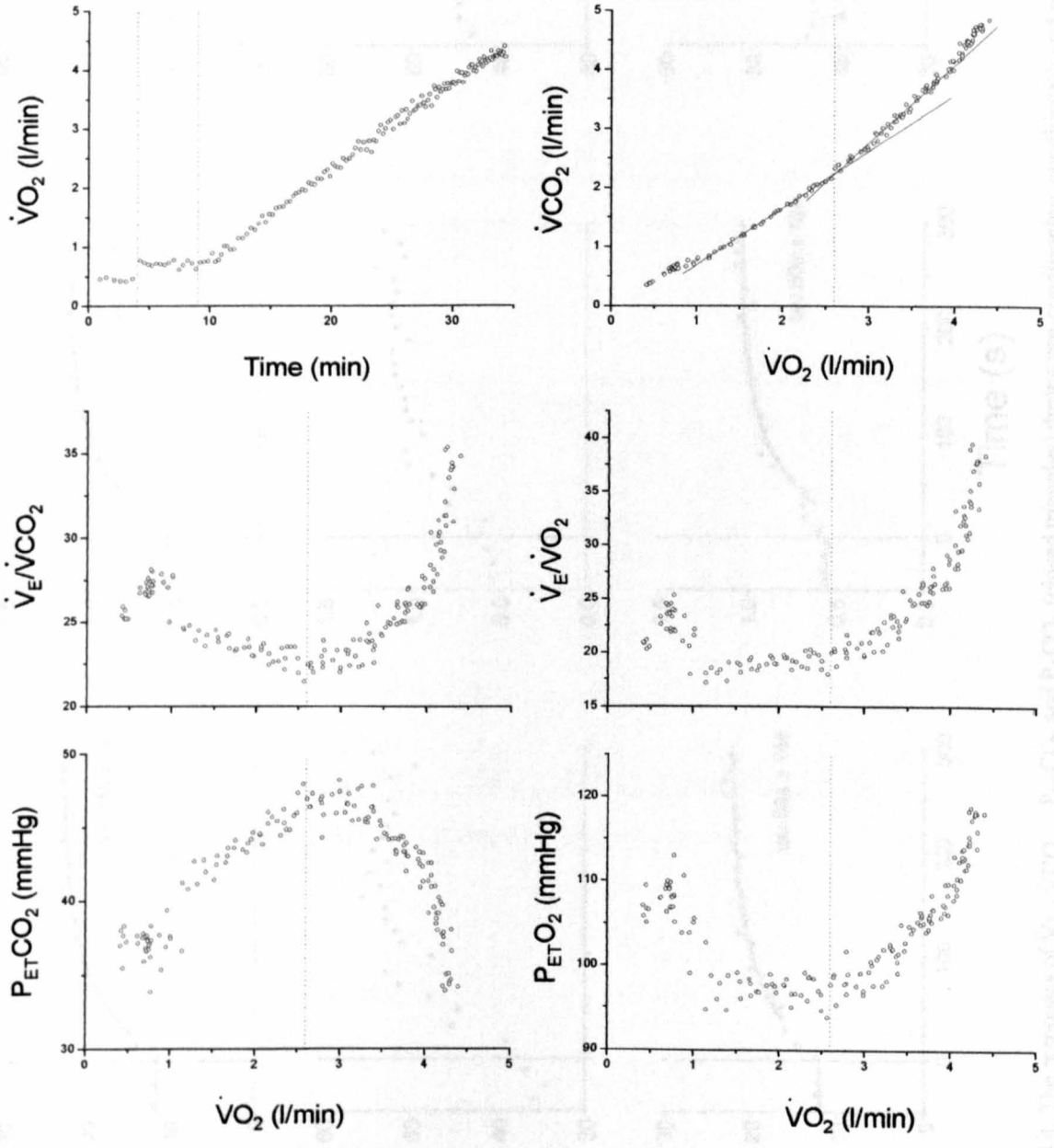


Figure 4.3: The cluster of responses used to non-invasively estimate the lactate threshold from an incremental exercise test (subject number 6). The first vertical line on the top left hand panel is the start of unloaded pedalling and the second line is the onset of the incremental phase. In all other panels the vertical lines indicate the lactate threshold.

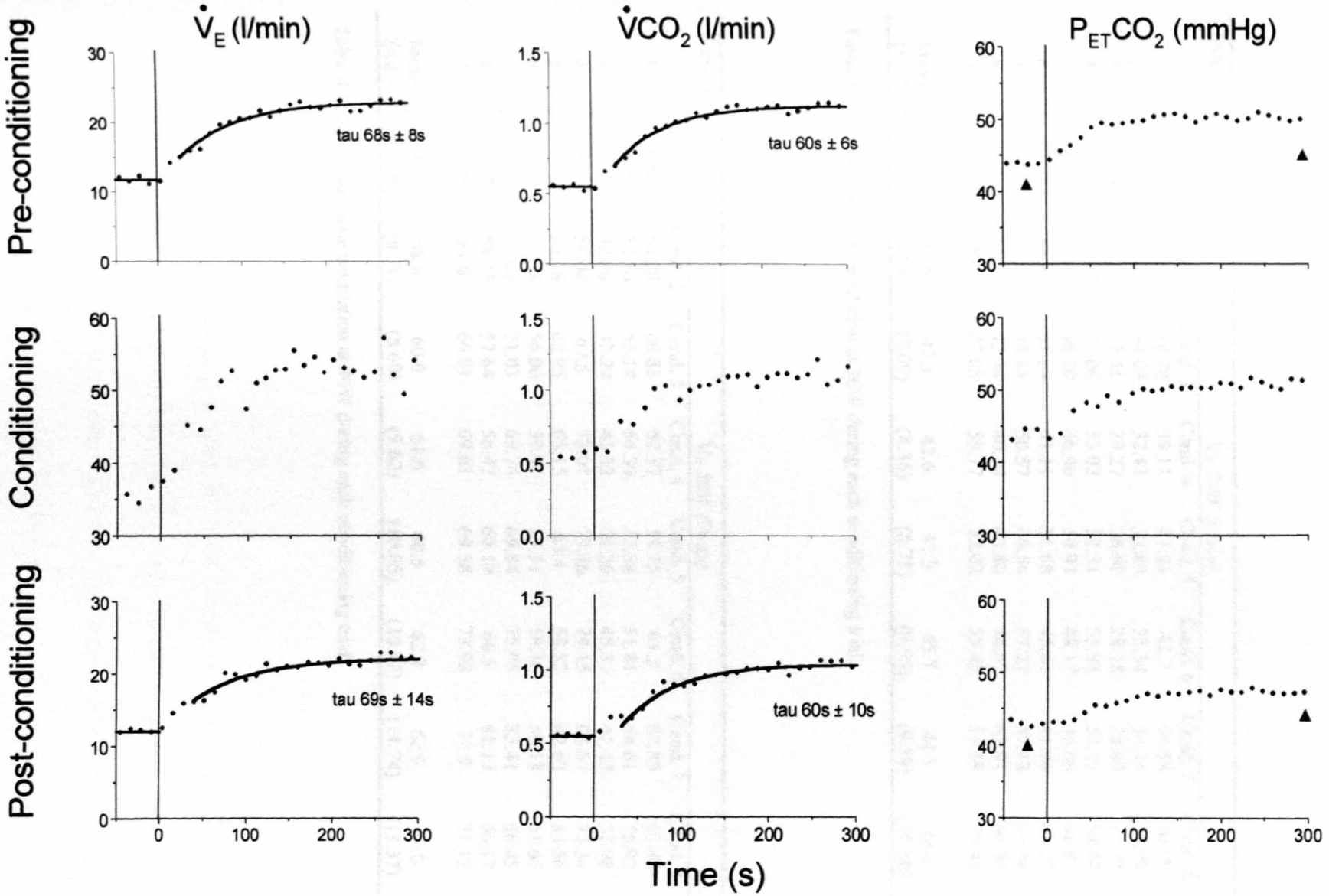


Figure 4.4: The responses of $\dot{V}_E$, $\dot{V}CO_2$, $P_{ET}CO_2$ and $P_{\bar{A}}CO_2$ (closed triangles) during pre-conditioning, conditioning and post-conditioning from subject 2. The vertical lines indicate the onset of 80W exercise

Subject	$\dot{V}_E$ *20W (l/min)*							
	Cond. 1	Cond. 2	Cond. 3	Cond. 4	Cond. 5	Cond. 6	Cond. 7	Cond. 8
1	34.48	41.68	38.78	39.11	41.01	42.2	40.54	40.69
2	31.83	31.87	34.09	32.43	32.05	33.34	34.14	36.09
3	23.16	25.27	27.41	29.27	28.69	28.78	28.69	27.72
4	30.2	47.42	52.06	53.02	52.31	55.94	53.15	58.42
5	48.11	46.83	48.29	46.96	50.93	48.17	46.06	46.87
6	30.77	35.28	37.29	41.11	36.68	45.61	41.24	47.77
7	48.87	45.4	53.44	50.57	46.54	57.27	57.43	61.04
8	49.6	43.37	42.44	40.14	40.88	44.67	43.91	44.94
9	42.12	48.41	47.05	50.77	52.02	55.43	54.89	55.45
Mean	37.7	40.6	42.3	42.6	42.3	45.7	44.5	46.6
(s.d.)	(9.71)	(8.04)	(8.69)	(8.36)	(8.75)	(9.95)	(9.59)	(10.80)

Table 4.2: The steady-state ventilation at 20W during each conditioning trial.

Subject	$\dot{V}_E$ *80W (l/min)*							
	Cond. 1	Cond. 2	Cond. 3	Cond. 4	Cond. 5	Cond. 6	Cond. 7	Cond. 8
1	58.62	64.28	60.83	62.37	59.75	61.3	62.85	65.45
2	50.49	52.56	55.18	56.39	52.36	51.81	54.61	55.92
3	42.35	42.06	42.29	41.32	41.26	45.37	41.43	37.98
4	54.52	58.04	67.5	73.01	73.36	74.15	68.67	75.34
5	65.31	63.18	62.95	65.15	63.4	58.82	60.63	64.66
6	51.1	55.25	56.06	59.92	55.31	58.16	59.13	61.65
7	67.25	71.62	71.03	69.74	69.84	75.95	83.41	86.45
8	59.23	58.72	57.64	56.47	60.19	66.5	62.17	66.17
9	57.09	61.6	66.91	69.61	69.58	73.92	72.9	71.12
Mean	56.2	58.6	60.0	61.6	60.6	62.9	62.9	65.0
(s.d.)	(7.69)	(8.31)	(8.60)	(9.62)	(10.05)	(10.62)	(11.74)	(13.37)

Table 4.3: The steady-state ventilation at 80W during each conditioning trial.

Evidence of CO_2 retention was seen from an accompanying increase in $P_{ET}CO_2$ (figure 4.4) of the order of 4-5mmHg (table 4.4 & table 4.5). Furthermore, as the conditioning trials progressed, there was a progressive adaptation to the dead space, with steady-state $\dot{V}_E$ increasing by a further 10l/min from conditioning trial 1 to trial 8 (20W p=0.029, 80W p=0.014) (table 4.2 & table 4.3). This was accompanied by a reduction in CO_2 retention, evidenced by a fall in $P_{ET}CO_2$ at both 20W and 80W from conditioning trial 1 to 8 of 3-4mmHg (20W p=0.016, 80W p=0.000), i.e. a return towards baseline values (table 4.4 & table 4.5).

4.5.2.2 Pre- vs. post-conditioning

There was no significant difference in the absolute level of ventilation across the three pre-conditioning tests, at either 20W or 80W, for any subject (20W p=0.521, 80W p=0.374). Therefore baseline measures were obtained by averaging the mean steady-state response to each work-rate for $\dot{V}_E$, $\dot{V}CO_2$, $\dot{V}O_2$, $P_{ET}CO_2$ and P_ACO_2.

4.5.2.2.1 Steady-state $\dot{V}O_2$

The conditioning session was found to have no effect on the O_2 cost of steady-state cycling at any work-rate or between any trial (20W p=0.969, 80W p=0.954). The mean $\dot{V}O_2$s were 0.87l/min, 0.88l/min, 1.51l/min and 1.53l/min at 20W during pre- and post- and at 80W during pre- and post-conditioning respectively (table 4.6).

Subject	$P_{ET}CO_2$ *20W (mmHg)*							
	Cond. 1	Cond. 2	Cond. 3	Cond. 4	Cond. 5	Cond. 6	Cond. 7	Cond. 8
1	41.2	39.0	41.2	37.3	39.7	40.1	36.8	40.8
2	43.5	45.5	46.2	45.6	42.2	42.5	43.6	44.6
3	50.8	47.1	44.1	46.9	47.6	49.1	48.1	46.9
4	49.5	47.4	45.0	43.3	42.4	43.5	43.1	43.1
5	42.9	42.0	40.6	40.6	40.1	40.3	40.2	39.1
6	39.7	40.5	39.7	40.0	40.2	39.4	40.3	38.7
7	39.4	39.6	39.9	38.8	39.9	36.0	38.2	38.5
8	44.8	47.4	45.8	46.5	43.6	42.6	43.8	42.6
9	42.1	43.8	43.2	41.0	39.8	39.8	38.8	39.2
Mean	43.8	43.6	42.9	42.2	41.7	41.5	41.4	41.5
(s.d.)	(4.02)	(3.43)	(2.57)	(3.50)	(2.62)	(3.62)	(3.52)	(2.98)

Table 4.4: The end tidal partial pressure of CO_2 during the 20W steady-state of each conditioning trial.

Subject	$P_{ET}CO_2$ *80W (mmHg)*							
	Cond. 1	Cond. 2	Cond. 3	Cond. 4	Cond. 5	Cond. 6	Cond. 7	Cond. 8
1	48.6	46.4	46.6	45.1	46.1	45.8	44.5	45.3
2	55.8	53.8	52.6	51.8	51.8	52.2	51.5	51.0
3	59.3	55.0	52.7	51.7	56.5	56.5	57.0	56.0
4	54.8	51.5	48.8	48.0	47.0	46.3	47.5	46.5
5	45.3	44.4	43.7	43.0	43.8	44.2	42.8	41.6
6	45.1	44.2	43.8	43.2	43.9	44.5	44.3	43.4
7	42.8	43.4	42.7	42.1	42.2	40.6	40.9	40.4
8	51.3	51.5	51.6	51.1	50.1	48.6	49.6	48.4
9	49.9	48.4	48.0	46.8	45.5	44.9	45.3	45.1
Mean	50.3	48.7	47.8	47.0	47.4	47.1	47.0	46.4
(s.d.)	(5.52)	(4.38)	(3.92)	(3.89)	(4.57)	(4.75)	(4.98)	(4.85)

Table 4.5: The end tidal partial pressure of CO_2 during the 80W steady-state of each conditioning trial.

Subject	$\dot{V}O_2$ 20W (l/min)		$\dot{V}O_2$ 80W (l/min)		RER 20W		RER 80W		Heart-rate 20W (bpm)		Heart-rate 80W (bpm)	
	Pre-	Post-1	Pre-	Post-1	Pre-	Post-1	Pre-	Post-1	Pre-	Post-1	Pre-	Post1
1	0.93	0.87	1.65	1.55	0.73	0.7	0.82	0.78		84.9		112.4
2	0.73	0.72	1.35	1.44	0.75	0.67	0.85	0.75	72.6	80.4	108.8	122
3	0.92	1.12	1.58	1.67	0.88	0.7	0.86	0.8	75.8	82.8	92	103.8
4	0.76	0.75	1.42	1.47	0.81	0.77	0.84	0.76	102	103.2	127.9	130.2
5	0.96	0.84	1.45	1.45	0.77	0.69	0.8	0.78	83.1	67.1	100.6	90.4
6	0.71	0.74	1.26	1.36	0.8	0.75	0.82	0.78	67.6	70.7	89.3	89.2
7	1.08	1.3	1.74	1.89	0.68	0.64	0.76	0.71	71.1	81	88.2	95.2
8	0.91	1.01	1.62	1.59	0.78	0.71	0.79	0.79		89.8		113.8
9	0.82	0.77	1.52	1.41	0.87	0.69	0.89	0.75	74.9	79.3	98.4	102.8
Mean	0.87	0.9	1.51	1.54	0.79	0.7*	0.83	0.77*	78.16	82.1	100.74	106.64
(s.d.)	(0.12)	(0.2)	(0.15)	(0.16)	(0.06)	(0.04)	(0.04)	(0.03)	(11.56)	(10.49)	(13.98)	(14.13)

Table 4.6: Steady-state comparisons of $\dot{V}O_2$, RER and heart rate between pre-conditioning and post-conditioning 1 (* significantly different from pre-conditioning).

4.5.2.2.2 Steady-state $\dot{V}CO_2$

There was a tendency for $\dot{V}CO_2$ to be lower at both 20W and 80W during the post-conditioning trials, 20W pre- 0.68l/min, 20W post- 0.64l/min, 80W pre- 1.24l/min and 80W post-conditioning 1.14l/min. However, this difference was not statistically significant between any trial (20W p=0.796, 80W p=0.177) (table 4.7).

4.5.2.2.3 Steady-state RER

Despite the lack of significant difference in each of its components, $\dot{V}CO_2$ and $\dot{V}O_2$, it is possible that RER may still be affected. This was in fact the case with a significant difference being found both at 20W (p=0.028) and at 80W (p=0.000). At 20W RER fell on average from 0.79 during pre-conditioning to 0.7 during the first post conditioning trial, but interestingly then rose progressively to 0.72 and 0.75 during the second and third post-conditioning trials (table 4.6). However, at 80W the fall was maintained across the duration of post-conditioning from a group mean of 0.83 during pre-conditioning to 0.77, 0.76 and 0.75 throughout the post-conditioning session (table 4.6).

Subject	$\dot{V}_E$ *20W (l/min)*		$\dot{V}_E$ *80W (l/min)*		$\dot{V}CO_2$ *20W (l/min)*		$\dot{V}CO_2$ *80W (l/min)*		$\Delta\dot{V}_E/\Delta\dot{V}CO_2$		$\tau\dot{V}_E$		$\tau\dot{V}_E/\tau\dot{V}CO_2$	
	Pre-	Post-1	Pre-	Post-1	Pre-	Post-1	Pre-	Post-1	Pre-	Post-1	Pre-	Post1	Pre-	Post-1
1	15.66	13.5	30.61	26.15	0.68	0.61	1.35	1.21	22.31	21.08	45	47	1.05	1.12
2	11.75	10	22.98	22.59	0.55	0.48	1.14	1.08	19.03	20.98	64	66	1.07	1.03
3	20	18.81	29.7	31.35	0.81	0.78	1.37	1.34	17.32	22.39	47	53	1	0.98
4	14.85	13.89	24.97	22.55	0.61	0.58	1.19	1.12	17.44	16.04	123	121	1.34	1.34
5	21.49	16.71	29.12	27.55	0.75	0.58	1.15	1.13	19.08	19.71	43	42	1.02	1.02
6	15.53	14.78	27.2	27.36	0.57	0.55	1.04	1.07	24.83	24.19	49	48	1.02	1.02
7	19.5	23.93	34.22	37.41	0.74	0.84	1.33	1.33	24.95	27.51	80	77	1.21	1.18
8	16.97	18.22	28.63	31.33	0.71	0.71	1.28	1.25	20.46	24.28	68	69	1.08	1.28
9	18.45	17.04	34.46	26.6	0.71	0.54	1.35	1.05	25.02	18.75	58	57	1.07	1.08
Mean	17.13	16.32	29.1	28.1	0.68	0.63	1.24	1.18	21.16	21.66	64.1	64.4	1.1	1.12
(s.d.)	(3.03)	(3.95)	(3.81)	(4.69)	(0.09)	(0.12)	(0.12)	(0.11)	(3.2)	(3.4)	(25.3)	(24.1)	(0.04)	(0.04)

Table 4.7: Comparisons of ventilation and CO_2 output both between pre-conditioning and post-conditioning 1 in the steady-state and between pre-conditioning and post-conditioning during the transient.

4.5.2.2.4 Steady-state $\dot{V}_E$

From figure 4.4 there is no difference in the steady-state $\dot{V}_E$, either at 20W or 80W, from pre-conditioning to post-conditioning for subject 2. Furthermore, when the ventilatory responses for 20W and 80W during pre-conditioning are plotted against the corresponding first post-conditioning responses, it is clear that the trend is actually for $\dot{V}_E$ to be lower during the first post conditioning trial (figure 4.5). However, this did not reach significance (20W p=0.361, 80W p=0.424) (table 4.7). Additionally, there was no significant difference in $\dot{V}_E$, at either work-rate, between pre-conditioning and any of the three post-conditioning trials (20W p=0.963, 80W p=0.814) (table 4.7).

4.5.2.2.5 $\dot{V}_E$ - $\dot{V}CO_2$ relationship

While the conditioning had no effect on the absolute $\dot{V}_E$, it is possible that a reduction in $\dot{V}CO_2$ and therefore a reduction in the requirement for $\dot{V}_E$ could mask the effect of the conditioning on absolute $\dot{V}_E$. This can be viewed in terms of the $\dot{V}_E$ - $\dot{V}CO_2$ relationship, specifically its slope and $\dot{V}_E$-intercept (figure 4.6). Therefore, the average baseline slope of the $\dot{V}_E$ - $\dot{V}CO_2$ relationship ($\Delta\dot{V}_E/\Delta\dot{V}CO_2$) was plotted against $\Delta\dot{V}_E/\Delta\dot{V}CO_2$ from the first post-conditioning trial (figure 4.7). This revealed, as with absolute $\dot{V}_E$, that the conditioning trials had no significant effect on the $\dot{V}_E$ - $\dot{V}CO_2$ relationship during

the first post-conditioning trial (p=0.848), or any of the subsequent trials (p=0.372) (table 4.7).

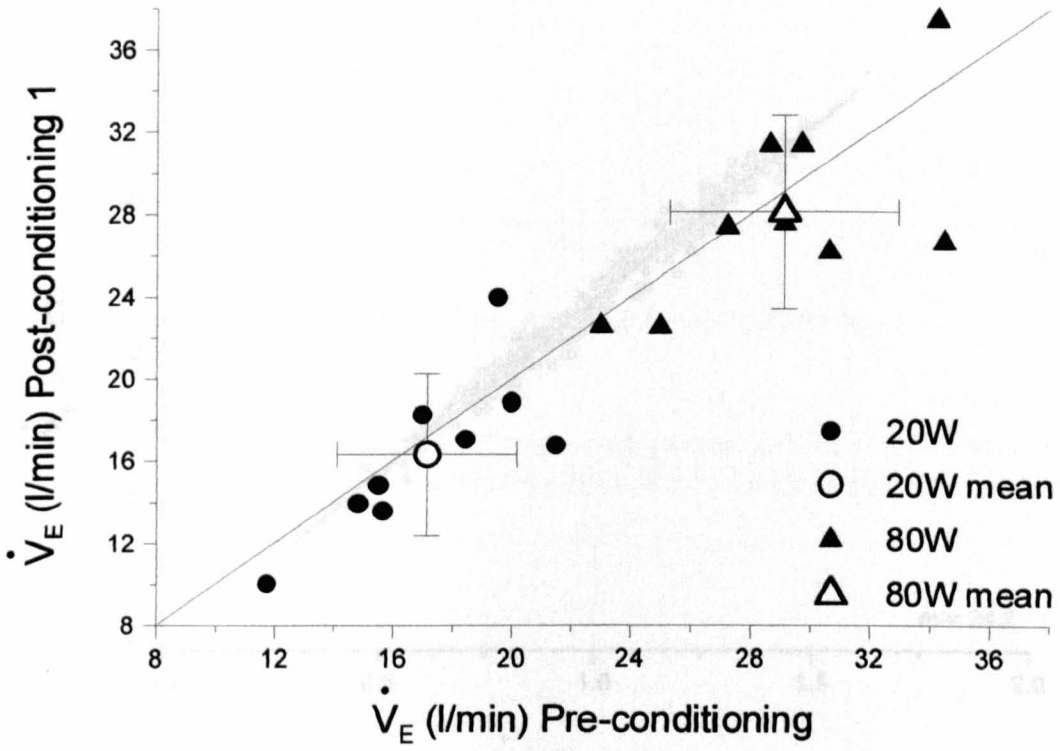


Figure 4.5: The mean steady-state $\dot{V}_E$ at 20W and 80W during pre-conditioning, plotted against the corresponding responses during post-conditioning-1. The closed symbols are individual responses and the open symbols are group means.

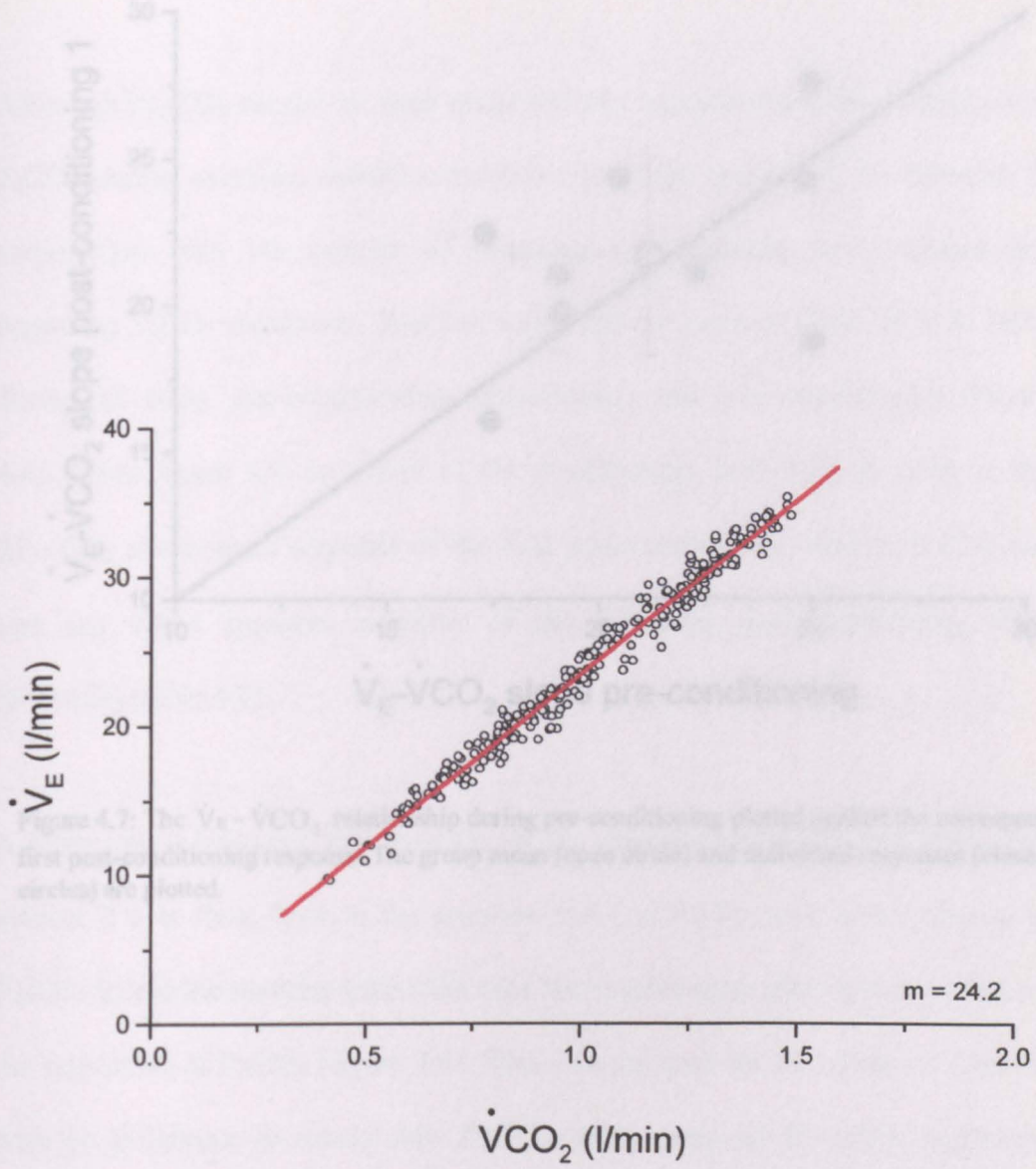


Figure 4.6: A representative example of the linear relationship typically found between $\dot{V}_E$ and $\dot{V}CO_2$ (subject 6 post-conditioning 1)

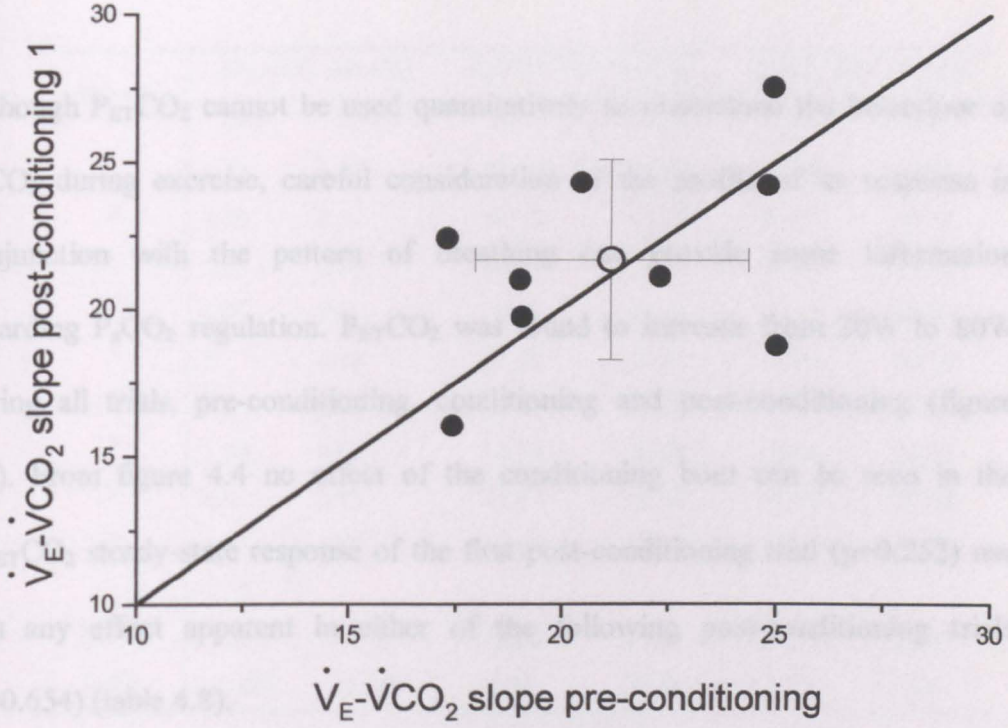


Figure 4.7: The $\dot{V}_E$ - $\dot{V}CO_2$ relationship during pre-conditioning plotted against the corresponding first post-conditioning response. The group mean (open circle) and individual responses (closed circles) are plotted.

4.5.2.2.6 Steady-state $P_{ET}CO_2$ and P_ACO_2

Although $P_{ET}CO_2$ cannot be used quantitatively to understand the behaviour of P_aCO_2 during exercise, careful consideration of the profile of its response in conjunction with the pattern of breathing can provide some information regarding P_aCO_2 regulation. $P_{ET}CO_2$ was found to increase from 20W to 80W during all trials, pre-conditioning, conditioning and post-conditioning (figure 4.4). From figure 4.4 no effect of the conditioning bout can be seen in the $\Delta P_{ET}CO_2$ steady-state response of the first post-conditioning trial ($p=0.252$) nor was any effect apparent in either of the following post-conditioning trials ($p=0.654$) (table 4.8).

A more robust indicator, than $P_{ET}CO_2$, of the regulation of P_aCO_2 is P_ACO_2. For subject 2 it is clear, both in the absolute level of P_ACO_2 and in the change in P_ACO_2 across the exercise transition, that the conditioning trials had no effect on the regulation of P_ACO_2 (figure 4.4). This was the case for the group as a whole with no difference in steady-state P_ACO_2 being observed for either work-rate (20W $p=0.955$, 80W $p=0.979$) or in ΔP_ACO_2 ($p=0.815$) (table 4.8).

Subject	$\Delta P_{ET}CO_2$ (mmHg)		P_ACO_2 20W (mmHg)		P_ACO_2 80W (mmHg)	
	Pre-	Post-1	Pre-	Post-1	Pre-	Post-1
1	1.83	3.59	42.28	42.85	42.19	43.08
2	6.29	4.24	40.78	41.36	45.33	44.15
3	5.33	2.28	39.79	43	48.44	44.98
4	7.67	8.4	40.7	41.16	45.46	46.94
5	4	4.93	37.46	37.54	38.42	40.59
6	2.6	2.62	37.79	34.84	37.95	40.19
7	2.59	1.02	38.79	34.08	39.54	35.67
8	4.6	2.13	41.47	38.13	41.17	37.98
9	1.75	4.78	37.67	38.33	38.86	39.69
Mean	4.07	3.78	39.64	39.03	41.93	41.47
(s.d.)	(2.08)	(2.18)	1.79	3.28	3.71	3.6

Table 4.8: The changes in end tidal and mean alveolar PCO_2 between pre-conditioning and post-conditioning 1.

4.5.2.2.7 Steady-state breathing pattern

Despite no change in the actual level of ventilation during the steady-state, it is possible that the conditioning trials had modified the pattern of breathing. However, from figure 4.8 it is clear subject 2 is achieving the necessary increase in ventilation through the same pattern of breathing. This is confirmed by the lack of change in either tidal volume or breathing frequency at either work-rate between pre-conditioning and all three post-conditioning trials (B_f 20W p=0.938, 80W p=0.958; V_T 20W p=0.756 80W p=0.917) (table 4.9).

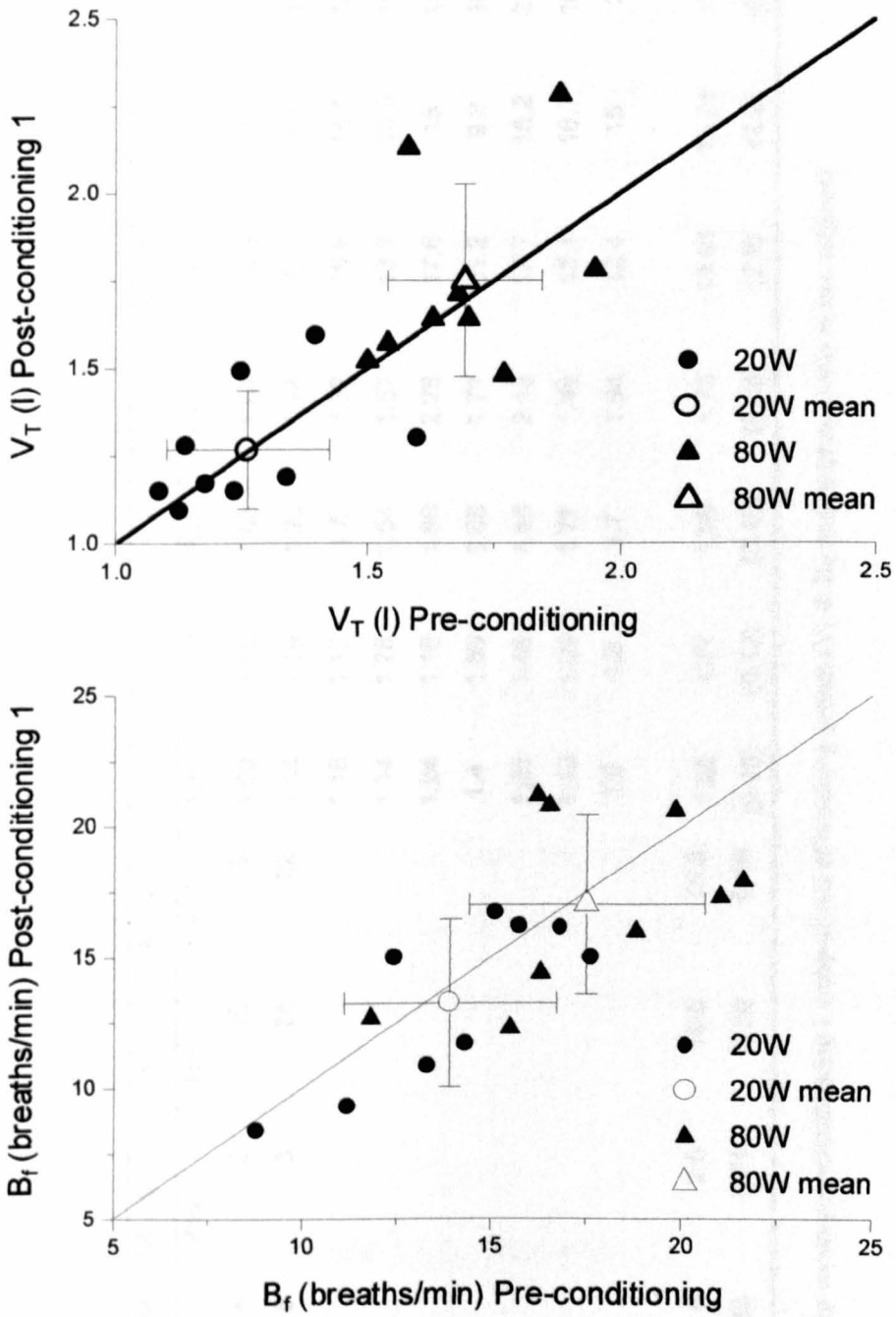


Figure 4.8: Plots of breathing frequency and tidal volume during pre-conditioning against post-conditioning. This illustrates that the same pattern of breathing was used to achieve the required ventilation during pre- and post-conditioning.

Subject	*Dyspnoea 20W (%)*		*Dyspnoea 80W (%)*		*V_T 20W (l)*		*V_T 80W (l)*		*B_f 20W (breaths/min)*		*B_f 80W (breaths/min)*	
	Pre-	Post-1	Pre-	Post-1	Pre-	Post-1	Pre-	Post-1	Pre-	Post-1	Pre-	Post-1
1	4	2	15	9	1.09	1.15	1.63	1.64	14.3	11.7	18.8	15.98
2	8	3	24	22	1.34	1.19	1.95	1.78	8.8	8.4	11.8	12.7
3					1.18	1.17	1.5	1.52	16.8	16.1	19.8	20.6
4					1.14	1.28	1.54	1.57	13.3	10.9	16.3	14.4
5					1.24	1.15	1.88	2.28	17.6	15	15.5	12.3
6					1.4	1.59	1.68	1.71	11.2	9.3	16.5	20.8
7					1.25	1.49	1.58	2.13	15.7	16.2	21.6	17.9
8					1.13	1.09	1.77	1.48	15.1	16.7	16.2	21.2
9					1.6	1.3	1.7	1.64	12.4	15	21	17.3
Mean	6	2.5	19.5	15.5	1.26	1.27	1.69	1.75	13.91	13.26	17.5	17.02
(s.d.)	2.83	0.71	6.36	9.19	(0.16)	(0.17)	(0.15)	(0.28)	(2.8)	(3.2)	(3.1)	(3.43)

Table 4.9: Pre-conditioning versus post-conditioning 1 comparisons of breathing pattern (V_T & B_f) and dyspnoea (only in two subjects)

4.5.2.2.8 Steady-state heart rate

There was no significant difference in heart rate between pre-conditioning and any of the post conditioning trials, either at 20W ($p=0.788$) or 80W ($p=0.748$). Despite the lack of statistical significance, there was a tendency for heart rate to be higher at each work-rate as post conditioning progressed; thus suggesting that cardiac output may also have been elevated. The group means for heart rate during pre-conditioning were 78 bpm at 20W and 101 bpm at 80W while during post conditioning this rose to 82, 80 and 84 bpm at 20W and 107, 108 and 110 bpm at 80W across the three post-conditioning trials (table 4.6).

4.5.2.2.9 Steady-state perceptual ratings

Analogue ratings of dyspnoea were only taken for two subjects; therefore statistical comparisons were not made. However, the results do reveal a greater perception of effort during post-conditioning compared to pre-conditioning, at each work-rate (table 4.9).

4.5.2.2.10 Phase II $\dot{V}_E$ and $\dot{V}CO_2$ kinetics

Figure 4.4 shows a typical interpolated three trial average pre-conditioning response for $\dot{V}_E$ and $\dot{V}CO_2$ with the phase II transition fitted and the corresponding τ displayed. The slightly faster response of $\dot{V}CO_2$, in comparison to $\dot{V}_E$, can be seen for both pre- and post-conditioning in subject 2 (figure 4.4).

Additionally, in this subject it is apparent there was little effect on the phase II kinetics of any variable as a result of the conditioning paradigm. Comparisons of $\tau \dot{V}_E$ and $\tau \dot{V}CO_2$ pre- to post- conditioning revealed no significant difference in the phase II time course ($\tau \dot{V}_E$ p=0.724, $\tau \dot{V}CO_2$ p=0.712) (table 4.7). Further to this, there was no significant difference in the relationship of $\tau \dot{V}_E$ to $\tau \dot{V}CO_2$ ($\tau \dot{V}_E / \tau \dot{V}CO_2$) from pre- to post-conditioning (p=0.417) (figure 4.9) (table 4.7).

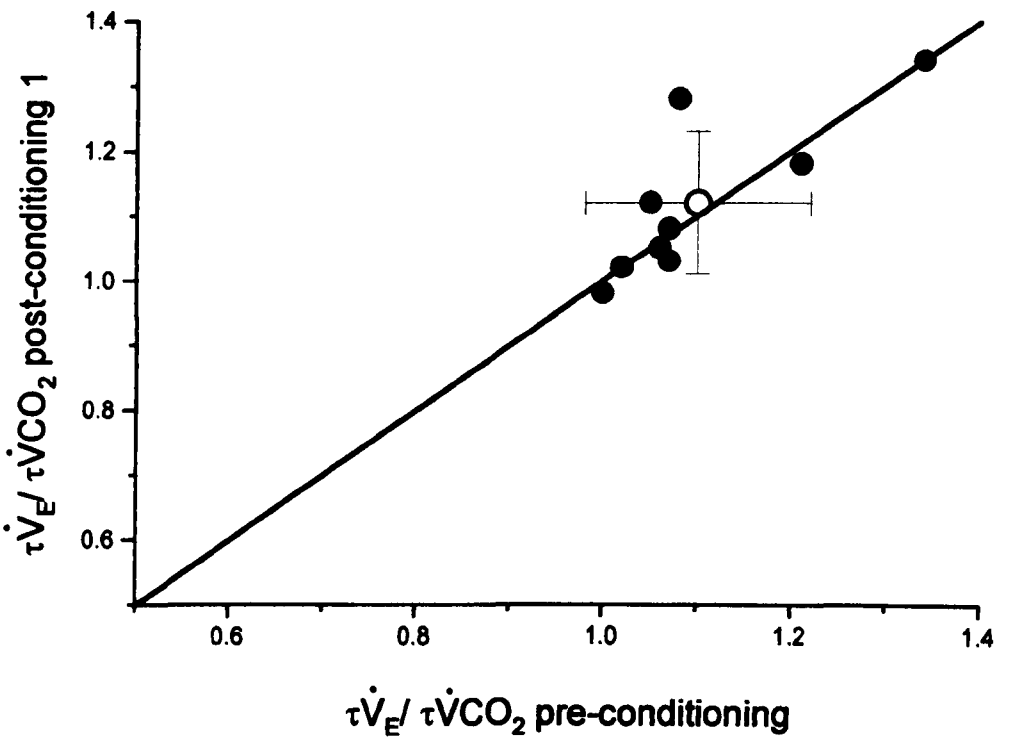


Figure 4.9: The ratio of $\tau \dot{V}_E$ to $\tau \dot{V}CO_2$ during pre-conditioning versus post-conditioning. The group mean response (closed circle) and individual responses (open circles) are shown.

4.6 Discussion

The results of this study show that no modulation of the phase II or III ventilatory response occurred as a result of associative conditioning of the 20W-80W cycle ergometry transition with a hypercapnic challenge in the form of additional external dead space. There was, therefore, no demonstration of the existence of LTM in the control of phases II and III of the exercise hyperpnoea. So, does this lead to the conclusion that there is no role for LTM in the control of the exercise hyperpnoea? How can the ambiguity between current studies on LTM be resolved? To answer these questions there are three main issues that must be resolved before conclusions can be drawn.

4.6.1 Current evidence regarding LTM

The work of Martin & Mitchell (1993), showing that the magnitude of the phase III exercise hyperpnoea could be modulated in goats, by means of an associative learning paradigm, was taken as the first experimental demonstration of LTM in the exercise hyperpnoea. However, due to the inherent species difference, goats typically exhibit a hypocapnic phase III response, rather than the isocapnia that characterises the human phase III response, it is possible this result is specific to this species. Furthermore, it is possible, given the trend for exercise P_aCO_2 to be falling in Martin and Mitchell's goats during the baseline trials, as well as between the baseline and post conditioning trials (figure 4.10,B), that this was a

demonstration of progressively increasing anxiety in the experimental animals; rather than of LTM.

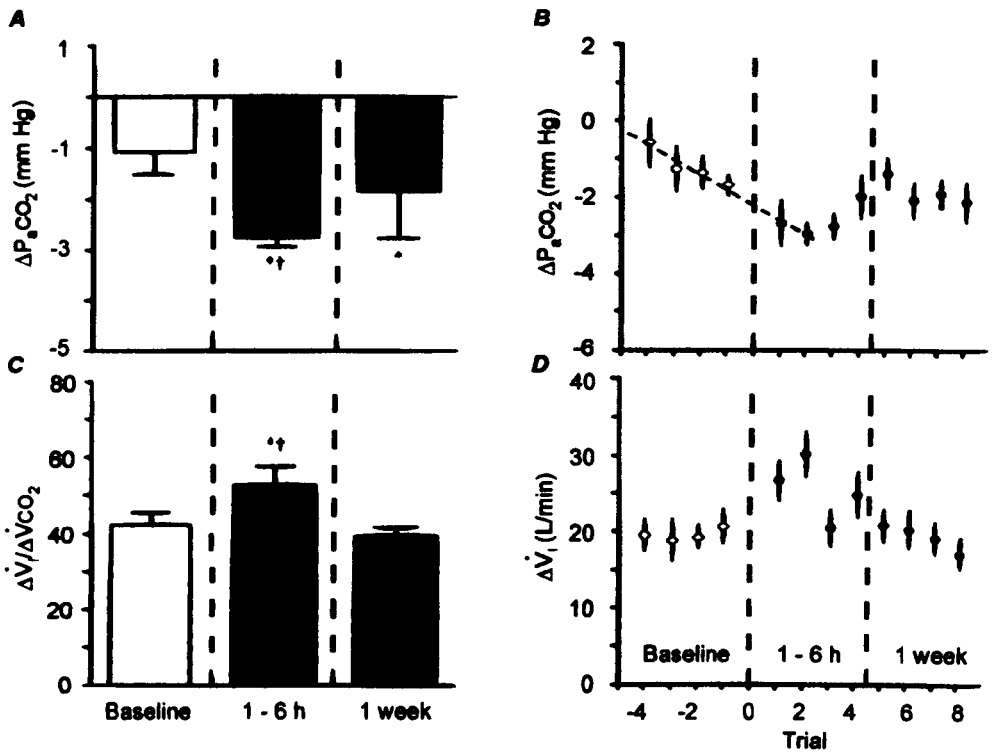


Figure 4.10: The baseline, 1-6 hours post-conditioning and 1 week post-conditioning ventilatory and pulmonary gas exchange responses of treadmill exercising goats. Panel B indicates the progressive hypocapnia seen with advancing trial number. Modified from Martin & Mitchell, 1993.

On the back of such speculation this area has seen a renewed effort to demonstrate the existence of a long-term memory phenomenon during exercise in humans. Several studies in humans report the existence of LTM, however, all these preliminary reports only detail decreases in rest to exercise P_aCO_2, estimated from $P_{ET}CO_2$, with no concomitant report of increased $\dot{V}_E$ (Adams *et al.*, 1992; Hughes *et al.*, 1997; Turner *et al.*, 1996). Furthermore, Moosavi *et al.* (2002) reinterpreted their preliminary report (Adams *et al.*, 1992) suggesting that rather than demonstrating associative learning, all they had shown were subjects becoming familiar with the laboratory environment, possibly rather like Martin

& Mitchell's (1993) goats? When Moosavi *et al.* (2002) repeated their experiments, controlling for this phenomenon by employing a non-conditioning control session, they found that the test-control differences they had initially reported as LTM were equally pronounced during the first experimental session regardless of whether this was the conditioning or sham-conditioning session.

4.6.2 The conditioning paradigm

Assuming that there is the capacity for plasticity in the exercise ventilatory controller, for which there is ample precedent in other areas of ventilatory control (see page 286 for discussion), for the successful genesis of a 'LTM' response it is imperative that the correct tools are employed. Namely, it must be ensured that the conditioning paradigm is appropriate for the task in hand. For associative conditioning to be effective there are three areas of potential concern, firstly, was the stimulus presented during conditioning sufficient? Secondly, inexorably linked with the first concern and more difficult to interpret, was the conditioning stimulus presented often enough? Finally, was sufficient time allowed for the response to develop?

The first issue is relatively straightforward to deal with. The requirement must be that the conditioning paradigm necessitates an increased ventilatory response to a given work-rate; which is then 'learned' as the appropriate response to that work-rate. This study employed a dead space of 1.4l, which required an increase of ~20l/min at 20W and 30l/min at 80W. This was accompanied by CO_2 retention, evidenced by an increase in $P_{ET}CO_2$ of 4-5mmHg. Therefore, the dead space

employed for this study was successful in providing an increased ventilatory requirement to both 20W and 80W cycle ergometry. Most other studies that have been successful in demonstrating an elevated ventilatory response post-conditioning have employed similar volume dead spaces (e.g. Turner & Sumners, 2002). However, Moosavi *et al.* (2001) who found no alteration in post-conditioning $\dot{V}_E$ used a dead space of only 0.6l. This was done so as to remain imperceptible to the subjects and while this aspect was seemingly successful, they may have failed to elicit LTM due to an insufficient conditioning stimulus. An alternative approach to minimise the volitional contribution, as utilised in this study, is to match the increased resistance of the dead space in a sham dead space that is visually identical to the actual dead space; thus removing some of the perceptual cues to the imposition of the dead space.

Was the conditioning stimulus presented often enough? The CO_2 retention present for all subjects during the first conditioning trial suggested that short-term potentiation of the exercise hyperpnoea was insufficient to meet the demands imposed by the added dead space. However, by trial 8 there is an increased ventilatory response, which is now sufficient to have (all but) abolished CO_2 retention. This may be evidence of a long term sharpening of STP. Therefore, as STP has been implicated as a possible pathway mediating LTM (Turner & Sumners, 2002) it is evidence that the mechanisms putatively believed to be responsible for LTM were active during this study.

Other studies in this area have presented many more paired trials, e.g. 14-20 trials (Martin & Mitchell, 1993) and 16 trials (Helbling *et al.*, 1997), or a few

more trials, e.g. 10 trials (Stewart & Turner, 2000; Turner *et al.*, 1996), and found evidence of an elevated steady-state ventilatory response post-conditioning. While others employing a similar number, or slightly more, to this study also found no effect in the steady-state response, e.g. 10 trials (Turner & Sumners, 2002), 8-11 trials (Moosavi *et al.*, 2001).

Finally, was sufficient time allowed for the response to develop? Again this can only be answered by comparing the effectiveness of other studies. Successful demonstrations of LTM have employed rest periods between conditioning trials ranging from 20 minutes (Martin & Mitchell, 1993; Stewart & Turner, 2000) to 24 hours (Helbling *et al.*, 1997). While other studies employing similar breaks between trials, 5-10 minutes (Moosavi *et al.*, 2001) and 20 minutes (Turner & Sumners, 2002), have found no resultant effect on the steady-state exercise hyperpnoea. Similarly, delays between the end of the final conditioning trial and the first post-conditioning trial of 1 hour (Martin & Mitchell, 1993, Stewart & Turner, 2000) to 24 hours (Helbling *et al.*, 1997) have been successful in modulating the post-conditioning phase III response. Yet, Turner & Sumners (2002) were unable to demonstrate any effect despite employing similar breaks between trials (20 minutes) and one hours delay between the end of conditioning and the beginning of post-conditioning. The only study to date not to employ at least one hours delay before undertaking the post-conditioning measurements was unable to find any evidence of LTM in the exercise hyperpnoea (Moosavi *et al.*, 2001).

The current study employed similar rests between conditioning trials (5-10 minutes) and a similar delay before commencing the post-conditioning trials (1 hour) as employed by successful studies. Therefore, it seems unlikely that insufficient time for the LTM response to develop can account for these results. However, until a conditioning paradigm is found that can robustly and repeatably be demonstrated to modulate the post-conditioning exercise hyperpnoea, we cannot be certain of the components required for such a paradigm.

Therefore, there is no evidence to suggest that the conditioning protocol utilised in this study was insufficient, in any facet, to have elicited LTM of the exercise hyperpnoea.

4.6.3 Potential mis-interpretation of results

The complexity of the ventilatory control system, and its outcomes, mean there are many potential pitfalls for the unprepared investigator. The most vital nugget of information when attempting to interpret data on LTM, or any other potential control system, is the repeated demonstration that $\dot{V}_E$ is intrinsically linked, not to absolute work-rate or metabolic rate, but specifically to the requirement to clear CO_2. Thus providing accurate regulation of P_aCO_2, which in turn leads to regulation of pH_a; linking of $\dot{V}_E$ to work-rate or metabolic rate, expressed as $\dot{V}O_2$, cannot provide this regulation of pH_a. Therefore, increases in $\dot{V}_E$ (e.g. Turner & Sumners, 2002) without some frame of reference cannot simply be

interpreted as modifications in the ventilatory control structure. To this end the current study goes to great lengths to avoid these pitfalls.

4.6.3.1 Familiarisation

To remove much of the inter-trial variability associated with human exercise testing it is important to make measurements, such as steady-state $\dot{V}_E$, as the mean response from several consecutive trials (three in this study). However, prior to doing that it is necessary to be sure that all the trials are representative of the underlying physiological response to the exercise. If, for example, no familiarisation was undertaken it is possible that the subject may have become either progressively more relaxed, or anxious, in the laboratory environment as the baseline trials progressed. This would lead either to a diminishing or an increasing level of hyperventilation as the trials progressed. One of the key differences between this study and others in the literature was the care taken in familiarising the subjects prior to commencing with actual measurements. Furthermore, it is vital to be aware of the time course of the ventilatory response and ensure that the subject has actually attained a stable phase III response before making steady-state measurements. Therefore, it is appropriate that so little variability was observed in the steady-state level of $\dot{V}_E$ between the three pre-conditioning trials.

4.6.3.2 Influence of protocol duration on substrate utilisation

The nature of the conditioning experiment meant that the subjects were unable to eat from one hour before entering the lab until finishing the post-conditioning trials, some 6 hours later. The likelihood therefore, is that the subjects will be moving toward a greater proportion of ATP being supplied through fat metabolism as the trials progressed. In an attempt to counter this, the subjects were also instructed not to eat for five hours prior to coming to the laboratory for the pre-conditioning session and to maintain light activity throughout that time. This was designed to match the subjects' energy balance in the hours leading up to the pre- and post-conditioning trials. To examine how successful this study design was, or possibly to explore subject compliance, the O_2 cost, CO_2 output and respiratory exchange ratio of steady-state cycling at 20W and at 80W was calculated for the pre-conditioning bouts against the three individual post-conditioning trials.

4.6.3.2.1 Steady-state O_2 cost

As, during moderate exercise, $\dot{V}_E$ is driven in accordance to the metabolic demand to clear CO_2, created by the exercise intensity, the primary drive to consider must therefore be $\dot{V}O_2$. Consequently, it is imperative to know the relative O_2 costs of pre- and post-conditioning bouts before the appropriateness of the ventilatory response can be determined. While the underlying O_2 cost of cycle ergometry is well established in the literature at 10ml/min/W (e.g.

Wasserman & Whipp, 1975) irrespective of age, fitness or gender, the O_2 cost is dependent on the substrate mixture being metabolised. A shift from a predominantly glycolytic to a lypolytic metabolic flux will increase the O_2 cost (see page 26 for discussion). Despite there being a tendency for $\dot{V}O_2$ to be higher at both work-rates during pre-conditioning this did not reach statistical significance. Therefore, while there was no definite indication that a shift in substrate utilisation had occurred, it could not be ruled out.

4.6.3.2.2 Steady-state $\dot{V}CO_2$

On first glance it would appear that an increasing O_2 utilisation would increase waste CO_2 production, and therefore increase the CO_2 clearance demand, ultimately requiring an increased $\dot{V}_E$. However, on reflection, the increased $\dot{V}O_2$, albeit not statistically significant, is likely to indicate a shift from glycolysis toward lypolysis. The metabolism of fat is not only associated with an increased O_2 cost but also a reduced CO_2 production and therefore a reduced requirement for CO_2 clearance. Consequently, the shift in substrate mixture is likely to actually result in a lower $\dot{Q}CO_2$ and hence actually require a reduced $\dot{V}_E$. Potentially a reduction in $\dot{V}_E$ brought about by a change in the substrate mixture being metabolised could be masked by an increase brought about through LTM; resulting in an unchanged $\dot{V}_E$. Therefore, failure to account for shifts in substrate utilisation is actually likely to hide, rather than augment, the potential effects of LTM. Again, despite not reaching statistical significance, there is a definite tendency for $\dot{V}CO_2$ to be lower during post-conditioning. This suggests that a

shift to generating a greater proportion of ATP through fat metabolism had occurred, therefore probably reducing the ventilatory requirement.

4.6.3.2.3 Steady-state RER

Despite no significant difference being found in either $\dot{V}O_2$ or $\dot{V}CO_2$, it is possible that the changes expected to occur with a shift in substrate utilisation may still be evident in R. This is due to the fact that the changes in both $\dot{V}O_2$ and $\dot{V}CO_2$ associated with a shift to fat utilisation will decrease R, i.e. a decrease in the numerator and an increase in the denominator. This was in fact the case, as a statistically significant fall in RER was found at both 20W and 80W. Thus it was evident that either the experimental design, or the subjects adherence to it, had failed to standardise the substrate mixture being utilised to provide energy between pre- and post-conditioning trials. However, this simply means that this difference must be taken account of when viewing the post-conditioning ventilatory response, an issue not addressed by other studies (e.g. Turner & Sumners, 2002).

4.6.3.3 Steady-state $\dot{V}_E$

Similar to $\dot{V}CO_2$, the phase III level of ventilation during the post-conditioning trails tended to be lower than pre-conditioning. However, despite the shift toward fat metabolism, evidenced by a reduced RER during post-conditioning, providing a reduced CO_2 clearance requirement, this does not necessitate that $\dot{V}_E$ should be

reduced. The requirement for ventilation at any given time is based not only on the CO_2 clearance requirement, but also on the level at which P_aCO_2 is to be regulated and the dead space fraction of the breath, i.e. how efficient a breath is. Therefore, while the absolute level of ventilation does not appear to have been affected by the conditioning paradigm, it is not possible to conclude that LTM has not affected $\dot{V}_E$ from the data discussed thus far.

4.6.3.4 $\dot{V}_E$ - $\dot{V}CO_2$ relationship

While there was a tendency for both $\dot{V}_E$ and $\dot{V}CO_2$ to be lower during pre-conditioning this does not quantify that $\dot{V}_E$ was still responding appropriately for the CO_2 clearance requirement. This is classically observed through a linear regression of the $\dot{V}_E$ - $\dot{V}CO_2$ relationship. While the demonstration that the slope of this relationship did not change from pre- to post-conditioning is not inconsistent with appropriate ventilation and regulation of P_aCO_2, it must be remembered that the dead space fraction of the breath is also key to the appropriate ventilation. From figure 4.11 it can be seen that the dead space volume (V_D) governs the position of the $\dot{V}_E$ - $\dot{V}CO_2$ relationship. Furthermore, remembering that V_D increases from rest to exercise (e.g. Jones *et al.*, 1966) the $\dot{V}_E$ - $\dot{V}CO_2$ relationship is actually likely to be indicated by line 2. However, should V_D be reduced during the post-conditioning trials the demonstration that $\dot{V}_E$ - $\dot{V}CO_2$ was unchanged would actually be indicative of hyperventilation, i.e. the measured response similar to line 2 should actually have been line 1 and

would now be inappropriate. Therefore, it is imperative also to investigate the regulation of P_aCO_2 alongside the slope of the $\dot{V}_E$ - $\dot{V}CO_2$ relationship.

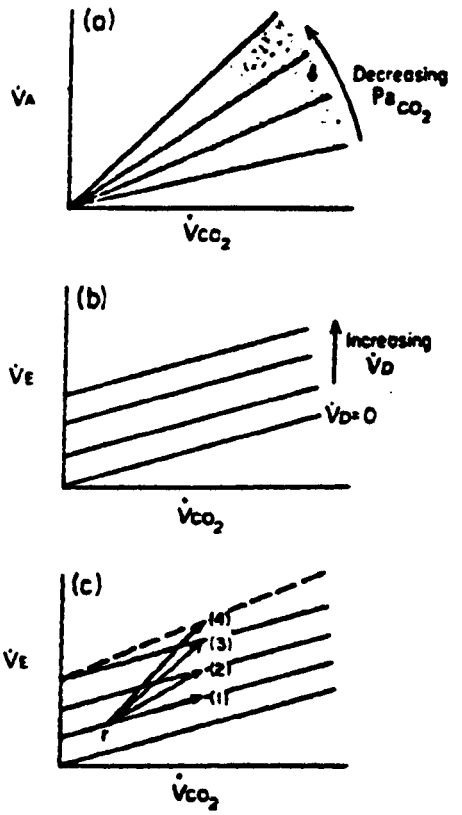


Figure 4.11: Panel (a) shows the increasing requirement for alveolar ventilation as the P_aCO_2 set-point is reduced. Panel (b) shows the appropriate $\dot{V}_E$ - $\dot{V}CO_2$ response to regulate P_aCO_2 at a given $\dot{V}_D$. Therefore, the reality during exercise is panel (c) where the actual response is unlikely to be 1 as $\dot{V}_D$ will increase, the magnitude of the increase (response 2 or 3) will vary. Furthermore, in supra-threshold situations a steeper slope is required (4) to reduce P_aCO_2. Reproduced from Whipp, 1981.

4.6.3.5 Steady-state $P_{ET}CO_2$ and P_ACO_2

Many of the early reports of LTM in the steady-state ventilatory response relied upon estimating P_aCO_2 either directly from $P_{ET}CO_2$ (e.g. Hughes *et al.*, 1997) or from the equation of Jones *et al.* (1979) (e.g. Turner *et al.*, 1996). However,

$P_{ET}CO_2$ is a complex variable both in terms of its origin and its analysis and therefore should be handled carefully. For any given breath the end tidal value is composed of three parts (figure 4.12):

1. The absolute P_aCO_2, which typically does not change from rest to steady-state moderate intensity exercise ($<\hat{\theta}_L$) (Asmussen & Nielsen, 1958; McIlroy, 1964; Jones, 1975; Lamb *et al.*, 1965; Lugliani *et al.*, 1971; Masson & Lahiri, 1974; Sutton *et al.*, 1976; Wasserman *et al.*, 1975; Whipp & Wasserman, 1969) but falls during supra-$\hat{\theta}_L$ exercise (respiratory compensation) in an attempt to constrain the fall in pH_a (Rausch *et al.*, 1991; Sutton and Jones, 1979; Wasserman and Casaburi, 1991; Wasserman and Whipp, 1975; Wasserman *et al.*, 1967).

2. The rate of metabolic CO_2 production, though actually more importantly the rate of delivery to the lung (i.e. the metabolic rate or the work-rate). This means that the intra-breath alveolar PCO_2 slope will become steeper with increasing work-rate (Whipp *et al.*, 1990).

3. The breathing frequency. While this will have little effect between rest and moderate exercise, due to the relatively shallow alveolar slope, between rest or moderate exercise and severe ($>\hat{\theta}_L$) work-rates the potential large difference in breathing frequency and the relatively steep alveolar PCO_2 slope can dramatically affect $P_{ET}CO_2$.

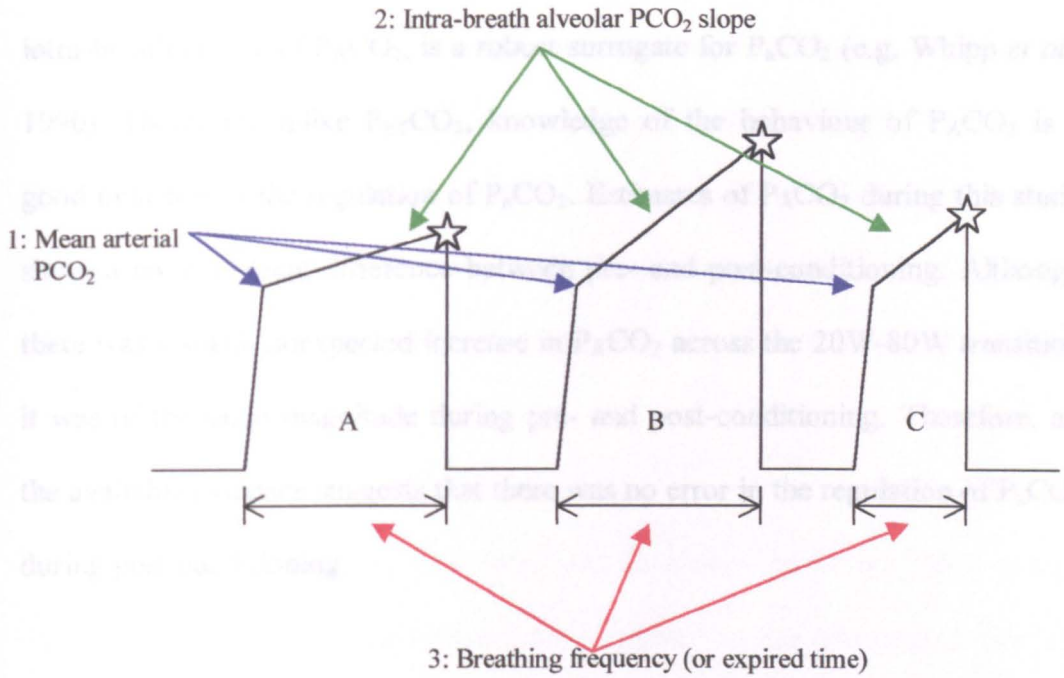


Figure 4.12: The effect of changing the rate of metabolic CO_2 production or the breathing frequency on $P_{ET}CO_2$ (indicated by the stars) despite an unchanged P_aCO_2 (1). Breaths A and B have the same breathing frequency (3), however in B the rate of CO_2 production and hence the slope of the intra-breath alveolar PCO_2 profile (2) has increased. This results in $P_{ET}CO_2$ for breath B being larger than for breath A. Breath C has the same rate of CO_2 production (2) as breath B but a much greater breathing frequency (3). This results in the $P_{ET}CO_2$ being reduced, almost to the level in breath A.

Therefore, drawing conclusions regarding the regulation of P_aCO_2 based on the behaviour of $P_{ET}CO_2$ is fraught with risk. However, careful analysis of the response pattern exhibited by $P_{ET}CO_2$ during moderate exercise transitions can shed some light, although not conclusively, on the behaviour of P_aCO_2. A step change in work-rate normally results in a small rise in $P_{ET}CO_2$ followed by stability. This comes from parts 1 and 3 above not changing markedly but part 2, CO_2 flux, increasing with increased metabolic rate. Therefore, with knowledge of the pattern of breathing across an exercise on-transition small increases in $P_{ET}CO_2$, as observed in this study, may not be inconsistent with regulation of P_aCO_2.

However, P_ACO_2, which can be estimated from a graphical reconstruction of the intra-breath profile of P_ACO_2, is a robust surrogate for P_aCO_2 (e.g. Whipp *et al.*, 1990). Therefore, unlike $P_{ET}CO_2$, knowledge of the behaviour of P_ACO_2 is a good indicator of the regulation of P_aCO_2. Estimates of P_ACO_2 during this study showed no significant difference between pre- and post-conditioning. Although there was a small, unexpected increase in P_ACO_2 across the 20W-80W transition it was of the same magnitude during pre- and post-conditioning. Therefore, all the available evidence suggests that there was no error in the regulation of P_aCO_2 during post-conditioning.

4.6.3.6 Steady-state breathing pattern

Minute ventilation ($\dot{V}_E$) is simply the product of tidal volume (V_T) and breathing frequency (B_f); therefore increases in $\dot{V}_E$ can be achieved through increases in either V_T or B_f independent of the other or a combination of both. The pattern of breathing by which the subjects made the increases in ventilation across the 20W-80W transition is deserving of investigation for two reasons. Firstly, any consideration regarding regulation of P_aCO_2 from $P_{ET}CO_2$ must take breathing pattern into account and, secondly, could the conditioning paradigm have modulated the pattern of breathing rather than the magnitude of the ventilatory response?

With regard to using $P_{ET}CO_2$ as an indication that P_aCO_2 has been accurately regulated, while small increases in $P_{ET}CO_2$ with an increase in work-rate are not inconsistent with P_aCO_2 regulation, neither are they necessarily consistent. For

example, hypoventilation causing an increase in P_aCO_2 brought about by a reduction in breathing frequency might also lead to small rises in $P_{ET}CO_2$. This would occur partly because of the increased pulmonary capillary to alveoli partial pressure gradient. However, more importantly for this discussion, because the reduction in breathing frequency might lead to a prolongation of the alveolar phase of the breath (figure 4.12) this will also give rise to a greater end-tidal value.

Therefore, the demonstration that there was no change in the breathing pattern between pre- and post-conditioning adds credence to the observation that the change in $P_{ET}CO_2$ from 20W to 80W is not inconsistent with regulation of P_aCO_2.

Furthermore, in the absence of an effect on the steady-state level of ventilation, it is possible that the conditioning had affected the pattern by which the subject's breathing was changed to increase ventilation going from 20W to 80W. This is plausible, as the subject was required to breathe with abnormally high tidal volumes during the conditioning period to provide sufficient $\dot{V}_E$ for the appropriated level of $\dot{V}_A$. For example, a 'normal' exercise tidal volume might be of the order of 1-2l with the dead space portion of that being only around 0.15l. However, during conditioning the dead space portion became around 1.55l (the subjects dead space, the dead space of the breathing apparatus and the added external dead space). There was, therefore, around a ten-fold increase in the dead space portion of the breath. This magnitude of increase meant that increasing breathing frequency to provide the extra $\dot{V}_E$ required at 80W, over 20W, would

simply have ventilated the dead space. However, as can be seen from table 4.9 and figure 4.8, just like the level of ventilation, no effect was seen on the breathing pattern for any subject.

4.6.3.7 Steady-state heart-rate and perceptual ratings

There was a tendency for heart rate to be higher during post-conditioning, although it did not reach statistical significance. However, despite the lack of statistical significance, these increases were present and it is possible they may reflect an increased level of effort performing the exercise as the trials progressed. Equally, they may reflect boredom, hunger or discomfort on the subjects' part. Either way, despite the possible cardio-dynamic link to drive $\dot{V}_E$ during phase I there is little evidence for such a mechanism being involved during the steady-state (see chapter 1). Therefore, the raised heart rate without a concomitant increase in $\dot{V}_E$ is unlikely to represent an error in the ventilatory response.

Similarly, while the perceptual ratings, only taken in two subjects, appear to suggest the post-conditioning tests required greater effort: the recorded changes are still at the mild end of the range, and do not affect the analysis regarding how appropriate the ventilatory response was during post-conditioning.

Although no formal investigation was undertaken, many of the subjects reported that they believed they were being 'fed' a different gas mixture to breathe. The reason given for this conclusion was that they experienced mild headaches and

dizziness during and immediately following the conditioning period, which they attributed to breathing a gas mixture other than air.

4.6.3.8 Control of Phase II

No other study to date has attempted to characterise the phase II temporal response of $\dot{V}_E$ following associative conditioning. Therefore the same concerns must apply over the interpretation of the data as for phase III. However, what is clear is that the close kinetic linkage between $\dot{V}_E$ and $\dot{V}CO_2$ was not disturbed by the conditioning trials. Again this is best viewed in terms of the relative response of $\dot{V}_E$ to $\dot{V}CO_2$, i.e. $\tau\dot{V}_E/\tau\dot{V}CO_2$. This is important both because no change in $\tau\dot{V}_E$ in the face of an altered $\tau\dot{V}CO_2$ would be interpreted as no evidence of LTM if $\tau\dot{V}CO_2$ was not accounted for; but also because of the inherent day to day variability expected in τ (Lamarra *et al.*, 1987). Basing conclusions on the ratio of $\tau\dot{V}_E$ to $\tau\dot{V}CO_2$ removes this uncertainty.

4.6.4 Redundancy of control mechanisms

In addition, the proposition of redundancy in the respiratory controller (see page 72 for discussion) whereby there is provision for the absence of a normal control mechanism is important to consider. The goal of the conditioning trials was to modulate a hypothesised 'learned' component of the exercise ventilatory response. Therefore, if such a 'learned' component is important in the normal

maintenance of P_aCO_2, manipulating its input would be analogous to the removal of an appropriate control mechanism, thus creating an error in the regulation of P_aCO_2. The ventilatory control system has consistently been shown to be capable of functioning in the absence of one of its purported primary controllers (see page 72). Consequently, it is not possible to rule out the possibility that another component of the ventilatory control system, e.g. CO_2 mediated peripheral chemoreception, detected an, unmeasured here, error in arterial blood gas tensions created by LTM, early after the onset of exercise, and adjusted the response accordingly. Therefore, despite the absence of evidence of LTM in either the non-steady or the steady-state response, it may actually be there simply buried by the efficiency of alternative control mechanisms.

4.7 Future directions

Several issues raise their heads on the basis of this study. With regard to redundancy, could the steady-state response be conditioned in the absence of error feed-back control (e.g. in CBR subjects or with high inspired PO_2).

With regard to the conditioning paradigm, the stimulus for increased ventilation needs to be addressed. That is, is it appropriate to manipulate the breathing pattern so markedly? Hyperventilating, during post-conditioning, by such large tidal volumes would surely be so consciously obvious as to cause the subject to volitionally constrain their breathing without need for error feedback related control. While respiratory resistive loads have also been utilised (e.g. Stewart & Turner, 2000) this will still provide an increased stimulus to breathing

consciously obvious to the subject. Therefore it is possible that CO_2 infusion in the inspirate, for example, may be sufficient to provide the hypercapnic challenge required, while remaining less perceptible to the subject.

In terms of subject compliance and time constraints, the question may be asked, is a pre-conditioning session necessary? The outcome measure could, and possibly should, be based simply on the question: Is the ventilatory response during post-conditioning appropriate, or not?

Finally, the area deserving of most attention is the phase I response, this is the most obvious candidate to be exclusively neurally driven. Furthermore, given the demonstration of Beaver & Wasserman (1970) that subjects less familiar with exercise testing tended to show blunted phase I responses there may already be some evidence of LTM in phase I in the literature.

4.8 Conclusions

Investigation of both the absolute level of $\dot{V}_E$ and the level relative to $\dot{V}CO_2$ revealed no difference between pre- and post-conditioning. The close dynamic coupling typically observed between $\dot{V}_E$ and $\dot{V}CO_2$ was still present during post-conditioning. Furthermore, while other studies relied on estimating P_aCO_2 from $P_{ET}CO_2$ (Turner *et al.*, 1996), which can be unreliable, the present study utilised the 20-80W $\Delta P_{ET}CO_2$, removing concerns over day-to-day variability in absolute $P_{ET}CO_2$, and P_ACO_2, a reliable surrogate for P_aCO_2 (DuBois *et al.*,

1952). No change in either of these variables was found from pre- to post-conditioning. Thus confirming that $\dot{V}_E$ was still operating at the 'correct' level to regulate P_aCO_2 and not an artificially increased level out of proportion to the actual demand. Furthermore, no change in the breathing pattern utilised to achieve the increase in $\dot{V}_E$ required going from 20W to 80W was observed. Therefore, the present study has been unable to demonstrate any evidence of a role for LTM in the control of phases 2 and 3 of the exercise hyperpnoea.

However, while the conditioning paradigm employed during this study should have been sufficient to elicit LTM, the issues outlined in this discussion await definitive resolution and therefore still cloud the analysis. Further studies into the participation of LTM in the phase I response will both help clarify that temporal domain and possibly shed light on the specific requirements for a successful conditioning paradigm. Until such details are known, despite the weight of evidence to the contrary, it is impossible to rule out the involvement of LTM in the control of the exercise hyperpnoea.

Chapter 5

The role of an 'exercise-memory' in the control of the exercise hyperpnoea

5.1 Introduction

The difficulty encountered by several experimenters in attempting to demonstrate the existence of LTM in the steady-state ventilatory response has been taken as indicative of the difficulty in modulating an existing memory, rather than absence of the pathway (Moosavi *et al.*, 2002). Therefore, it was reasoned that uncovering the role of memory in the exercise hyperpnoea might be more successfully achieved utilising a paradigm that does not include such a reinforced memory. To this end it was proposed that repetitive performance of a given mode of exercise, presumably from an early age, would be required to generate the hypothesized 'memory-bank' able to mediate such a control of the exercise hyperpnoea.

Given the inconsistencies in the literature (e.g. Moosavi *et al.*, 2002, Turner *et al.*, 1996) and the uncertainty regarding the appropriateness of any given conditioning paradigm, it was thought that attempting to elicit LTM in a group lacking an 'exercise-memory' might be fruitless. Therefore, it was proposed that studying the ability of such a cohort of subjects so devoid of experience of a given mode of exercise, and exercise in general, that they could not have generated such a theoretical 'memory bank' to appropriately control $\dot{V}_E$ would be less susceptible to such protocol related deficiencies (see chapter 4).

5.2 Aims

The aim of this experiment therefore, rather than attempting to manipulate an existing memory, was to investigate the hyperpnoeic response of subjects to a particular mode of exercise, with essentially no established 'exercise-memory'. The goal of this was to determine whether a 'learned' ventilatory response was a requisite component of the ventilatory control system in its quest to regulate P_aCO_2 appropriately. To this end the hyperpnoeic responses to moderate intensity cycle ergometry of 'cycling naïve' subjects were investigated.

5.3 Hypothesis

The consistent demonstration of a close linkage between $\dot{V}_E$ and $\dot{V}CO_2$, providing accurate regulation of P_aO_2, P_aCO_2 and pH_a, is indicative of a predominantly humoral control mechanism operating during phase II and III (see introduction). Therefore, it is hypothesised that these subjects will show a 'normal' ventilatory response during phase II closely linked to $\dot{V}CO_2$ and presumably under an as yet unconfirmed humoral control mechanism. However, given the lack of a sustained error signal in the steady-state, it is possible that some integration of signals proportional to the work-rate with the humoral signal, essentially unchanged from rest, may require to be 'learned' in order to provide the appropriate increased drive to $\dot{V}_E$ during moderate exercise. Therefore, it is hypothesised that a progressive improvement in the appropriateness of the

ventilatory response (in terms of accuracy of P_aCO_2 regulation) of these subjects may be seen across the exercise transitions as STP begins the 'learning' process within the exercise bout (Eldridge *et al.*, 1976a).

5.4 Methods

5.4.1 Subjects

The experimental group consisted of 9 healthy (8 female and 1 male), inactive volunteers with an average age of 41 years (range 23-57), none of whom had ever learned to ride a bicycle and all of whom were habitually inactive. All provided both informed consent (as approved by the institutional ethics committee) and written evidence from a family member or close long-term friend of their inability to ride a bicycle.

A second, control group consisted of 9 age, sex, build and activity matched volunteers, who had learned to ride a bicycle but were also sedentary. Again all provided informed consent (as approved by the institutional ethics committee) but no additional documentation was required.

5.4.2 Apparatus

All exercise tests were performed on an electronically-braked cycle ergometer (Lode, Excalibur Sport, Netherlands) (see page 89), customized to allow a comfortable riding position to those inexperienced with the sitting position typically associated with cycling. During all tests ventilatory and pulmonary gas exchange parameters were determined breath-by-breath (QP9000, Morgan

Medical, England) utilising the algorithms of Beaver *et al.* (1973) (for detailed description see earlier). Respired O_2, CO_2 and volume signals were also transcribed to a paper trace (Dash 10, Astro-Med Inc., Rhode Island, USA) to allow reconstruction of alveolar PCO_2 profiles and hence estimation of mean alveolar PCO_2 ($P_{\bar{A}}CO_2$), using the 'DuBois' method (DuBois *et al.*, 1952). For safety purposes heart rate and ECG responses were measured continuously (Q710, Quinton, England) throughout exercise, as was arterial O_2 saturation (Satlite Trans, Datex Engstrom, Finland).

5.4.3 Protocols

To allow monitoring of the responses to cycle ergometry while the subjects were naïve to that particular mode of exercise, familiarisation tests included no exercise. During this session the purpose of all monitoring equipment, the practicalities of performing the exercise tests, but not the purpose of the study were explained to the subjects. Furthermore, the subject was allowed a period of resting breathing while connected to the mouthpiece and seated on the cycle ergometer. This allowed the subjects to become familiar with both the laboratory environment and equipment without tainting the results. The control group underwent an identical familiarisation procedure containing no exercise.

The order of the exercise visits was reversed in comparison to the other studies in this thesis, i.e. the incremental exercise test was performed last. This was done so that the response to square-wave exercise could be recorded without the influence of a preceding exercise session. Therefore, the work-rates used during

the constant load exercise tests had to be estimated to be sub-$\hat{\theta}_L$. The subsequent performance of an incremental exercise test allowed classification of the intensity domain in which the work-rates chosen for each subject lay (see page 4).

The profile chosen for the square-wave exercise tests was one resembling that of a ziggurat pyramid (figure 5.1), such that the first exercise visit involved the subject pedaling at a constant rate (50-60rpm) across a range of increasing and subsequently decreasing work-rates estimated to be sub $\hat{\theta}_L$.

Figure 5.1: A ziggurat is a pyramidal structure composed of receding tiers built upon a square, oval or rectangular base, with a shrine at the summit.

Following an initial rest and unloaded exercise period, the work-rate was elevated and maintained for 6 minutes, after which the level was again increased, and held constant for 6 minutes. Subsequently the work was reduced by the same

increment, for a further 6 minutes, before returning to unloaded exercise for the final 6-minute stage. Thus creating a ziggurat profile (figure 5.2) and allowing analysis of both the on- and off-transient responses to each work-rate. The second exercise session involved the subjects undertaking a sub-maximal incremental exercise test (ramp 5W/min-15W/min to accommodate varying fitness levels), capped at 80% of predicted maximum heart rate. At least one month after the first exercise session the subjects performed a repeat of the ziggurat profile exercise test.

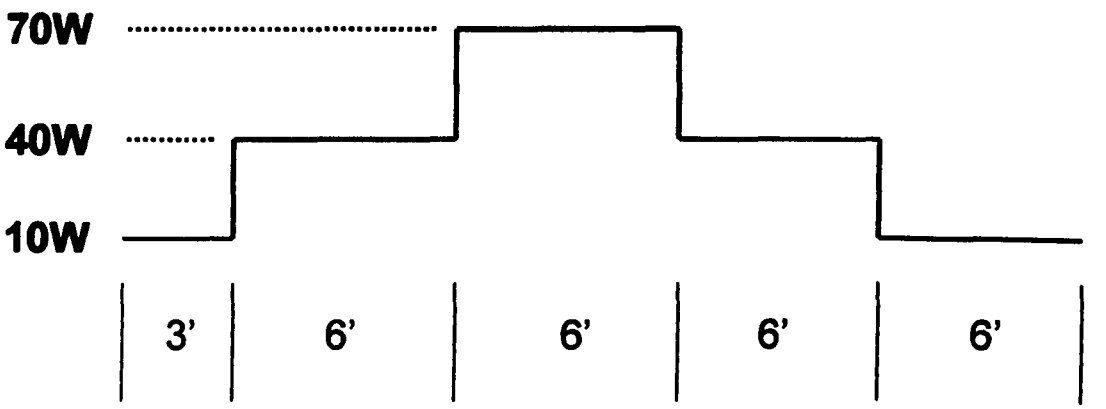


Figure 5.2: A schematic representation of the ziggurat work forcing utilised during this study.

5.4.4 Analysis

The question of interest was not whether the steady-state ventilation was the same for a naïve subject and their control at a given work-rate, but whether the naïve subjects ventilatory response was normal, both in relation to their control and to the established pattern of response (see page 13). Therefore, as in chapter 4, the analysis was based around the appropriateness of the ventilatory response during exercise; defined by the accuracy of P_aCO_2 regulation (see page 78).

All comparisons of steady-state ventilatory and pulmonary gas exchange responses between work-rates, between tests and between groups, were performed on data averaged over the final minute of each work-rate stage. Analysis of P_ACO_2 was performed on the average of three sequential breaths from the steady-state of each work-rate. For comparisons between work-rates, within a subject group, paired t-tests were performed and between tests a one-way analysis of variance with repeated measures was employed. While for comparisons between groups, two-sample t-tests were used. Statistical significance was accepted at $p < 0.05$.

5.5 Results

5.5.1 Ziggurat exercise test

5.5.1.1 Steady-state $\dot{V}O_2$

A typical example of a 'cycling naïve' subject's breath-by-breath pulmonary gas exchange and ventilatory responses to a ziggurat work forcing is illustrated in figure 5.3. This dataset has been edited to remove data pertaining to breaths mis-calculated by the on-line breath-by-breath software (Morgan Medical) and any breaths lying beyond the range enclosed by 4 standard deviations from the mean (see page 98). From figure 5.3, it appears that the steady-state oxygen cost of a given exercise intensity is constant for this subject. Furthermore, table 5.1, table 5.2 and figure 5.4 illustrate that all experimental-subjects, and their controls, exhibit an oxygen cost per watt of work which is unaltered across the duration of a test. Their oxygen cost, as expected, appears in-line with the response for a 'normal' individual (Hansen *et al.*, 1987; Wasserman & Whipp, 1975).

5.5.1.2 Steady-state $\dot{V}CO_2$

Similarly to $\dot{V}O_2$, steady-state $\dot{V}CO_2$ appears unaltered between on- and off-transitions to the same work-rate (figure 5.3). This was found to be the case for

both groups, between on- and off-transitions and between trial 1 and trial 2 (table 5.3, table 5.4).

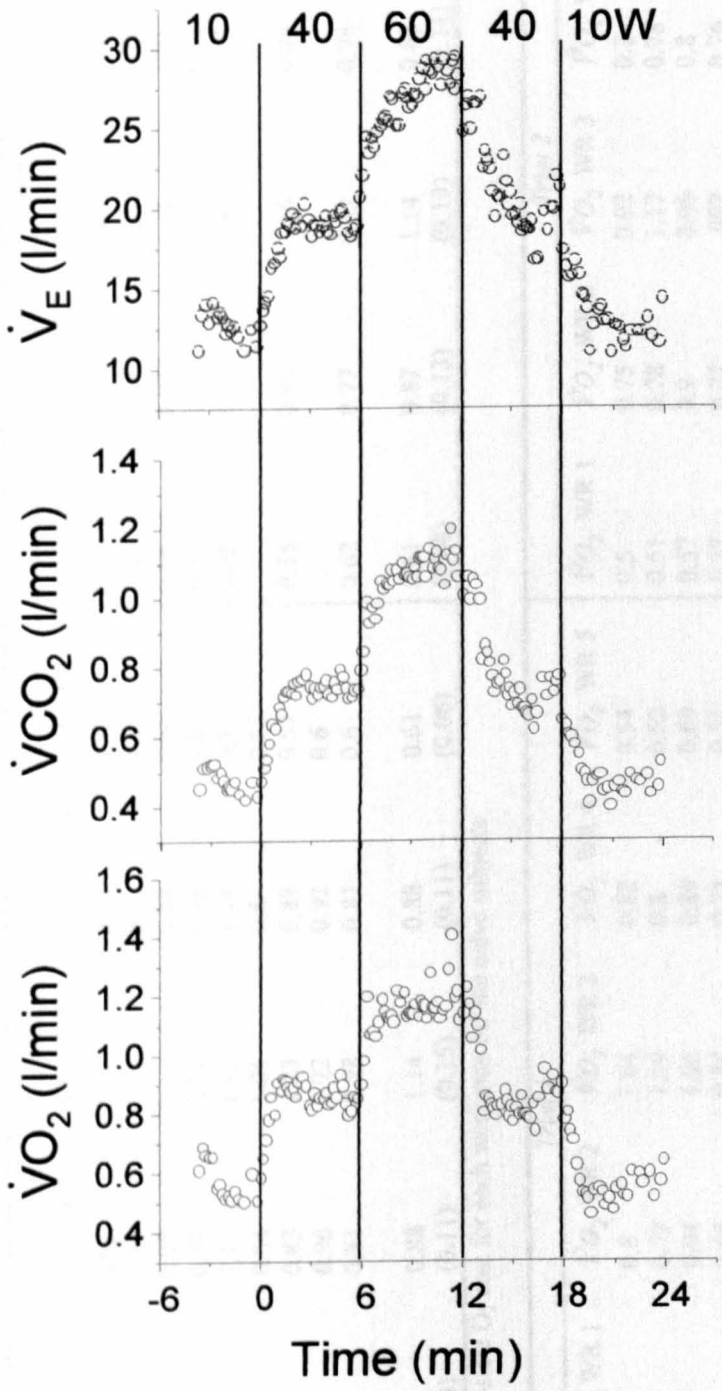


Figure 5.3: Pulmonary gas exchange and ventilatory responses of a cycling naïve subject to a ziggurat square-wave protocol.

	Trial 1					*Trial 2*				
Naïve	$\dot{V}O_2$ WR 1	$\dot{V}O_2$ WR 2	$\dot{V}O_2$ WR 3	$\dot{V}O_2$ WR 4	$\dot{V}O_2$ WR 5	$\dot{V}O_2$ WR 1	$\dot{V}O_2$ WR 2	$\dot{V}O_2$ WR 3	$\dot{V}O_2$ WR 4	$\dot{V}O_2$ WR 5
1	0.71	0.93	1.1	0.93	0.72	0.6	0.89	1.09	0.85	0.62
2	0.56	0.79	1.23	0.8	0.54	0.46	0.77	1.21	0.77	0.46
3	0.64	0.95	1.1	0.88	0.64	0.66	0.97	1.13	1.01	0.64
4	0.63	0.74	0.92	0.78	0.6	0.68	0.75	0.98	0.75	0.55
5	0.73	1.1	1.42	1.13	0.7	0.68	1.1	1.5	1.13	0.65
6	0.51	0.84	1.05	0.8	0.55					
7	0.53	0.83	1.23	0.89	0.57	0.55	0.86	1.16	0.89	0.54
8	0.57	0.96	1.22	0.92	0.6					
9	0.72	0.81	0.98	0.81	0.6	0.62	0.77	0.92	0.78	0.63
Mean	0.62	0.88	1.14	0.88	0.61	0.61	0.87	1.14	0.88	0.58
(SD)	(0.08)	(0.11)	(0.15)	(0.11)	(0.06)	(0.08)	(0.13)	(0.19)	(0.14)	(0.07)

Table 5.1: The steady-state O_2 cost for each work-rate for the naïve subjects

	Trial 1					*Trial 2*				
Control	$\dot{V}O_2$ WR 1	$\dot{V}O_2$ WR 2	$\dot{V}O_2$ WR 3	$\dot{V}O_2$ WR 4	$\dot{V}O_2$ WR 5	$\dot{V}O_2$ WR 1	$\dot{V}O_2$ WR 2	$\dot{V}O_2$ WR 3	$\dot{V}O_2$ WR 4	$\dot{V}O_2$ WR 5
1	0.54	0.8	1.04	0.82	0.54	0.5	0.75	0.92	0.8	0.49
2	0.59	0.79	1.19	0.8	0.52	0.51	0.78	1.17	0.78	0.48
3	0.67	0.94	1.06	0.89	0.69	0.57	0.9	0.96	0.8	0.56
4	0.53	0.66	0.84	0.71	0.51	0.57	0.77	.092	0.76	0.52
5	0.90	1.38	1.76	1.29	0.89	0.76	1.27	1.81	1.27	0.82
6	0.46	0.72	0.97	0.70	0.46	0.53	0.80	1.11	0.72	0.49
7	0.67	0.86	1.19	0.91	0.57	0.53	0.81	1.04	0.78	0.59
8	0.55	0.77	1.15	0.86	0.60	0.89	0.91	1.16	0.85	0.67
9	0.55	0.73	0.90	0.77	0.56	0.60	0.70	0.88	0.66	0.46
Mean	0.61	0.85	1.12	0.86	0.59	0.61	0.85	1.02	0.82	0.56
(SD)	(0.13)	(0.21)	(0.27)	(0.18)	(0.13)	(0.13)	(0.17)	(0.44)	(0.18)	(0.12)

Table 5.2: The steady-state O_2 cost for each work-rate for the control subjects

	Trial 1					*Trial 2*				
Naive	$\dot{V}CO_2$ WR 1	$\dot{V}CO_2$ WR 2	$\dot{V}CO_2$ WR 3	$\dot{V}CO_2$ WR 4	$\dot{V}CO_2$ WR 5	$\dot{V}CO_2$ WR 1	$\dot{V}CO_2$ WR 2	$\dot{V}CO_2$ WR 3	$\dot{V}CO_2$ WR 4	$\dot{V}CO_2$ V
1	0.58	0.80	0.93	0.77	0.59	0.52	0.80	1.01	0.73	0.54
2	0.44	0.66	1.14	0.64	0.46	0.35	0.64	1.14	0.61	0.37
3	0.55	0.87	1.02	0.75	0.53	0.50	0.81	0.97	0.80	0.49
4	0.51	0.62	0.78	0.66	0.48	0.54	0.62	0.82	0.59	0.42
5	0.57	0.97	1.31	0.95	0.59	0.53	0.9	1.33	0.93	0.52
6	0.41	0.65	0.93	0.64	0.42					
7	0.44	0.73	1.11	0.75	0.47	0.43	0.72	1.00	0.74	0.43
8	0.47	0.79	1.07	0.77	0.49					
9	0.55	0.69	0.84	0.64	0.46	0.52	0.67	0.80	0.65	0.53
Mean	0.50	0.75	1.01	0.73	0.50	0.48	0.74	1.01	0.72	0.47
(SD)	(0.06)	(0.12)	(0.16)	(0.10)	(0.06)	(0.07)	(0.10)	(0.18)	(0.12)	(0.07)

Table 5.3: The steady-state CO_2 output at each work-rate for the naïve subjects

	Trial 1					*Trial 2*				
Control	$\dot{V}CO_2$ WR 1	$\dot{V}CO_2$ WR 2	$\dot{V}CO_2$ WR 3	$\dot{V}CO_2$ WR 4	$\dot{V}CO_2$ WR 5	$\dot{V}CO_2$ WR 1	$\dot{V}CO_2$ WR 2	$\dot{V}CO_2$ WR 3	$\dot{V}CO_2$ WR 4	$\dot{V}CO_2$ V
1	0.37	0.64	0.90	0.64	0.42	0.39	0.64	0.8	0.63	0.40
2	0.44	0.62	1.07	0.60	0.41	0.41	0.64	1.02	0.65	0.39
3	0.51	0.76	0.92	0.74	0.55	0.45	0.77	0.85	0.68	0.46
4	0.44	0.57	0.72	0.60	0.40	0.48	0.63	0.79	0.63	0.44
5	0.80	1.06	1.41	1.07	0.71	0.61	0.94	1.48	1.01	0.68
6	0.39	0.64	0.90	0.60	0.40	0.41	0.67	1.02	0.62	0.43
7	0.52	0.68	1.00	0.73	0.44	0.48	0.69	0.98	0.66	0.47
8	0.40	0.62	1.02	0.69	0.45	0.58	0.69	0.97	0.68	0.52
9	0.43	0.58	0.75	0.62	0.45	0.51	0.58	0.76	0.56	0.39
Mean	0.48	0.69	0.97	0.70	0.47	0.48	0.69	0.96	0.68	0.46
(SD)	(0.13)	(0.15)	(0.20)	(0.14)	(0.10)	(0.08)	(0.11)	(0.22)	(0.13)	(0.09)

Table 5.4: The steady-state CO_2 output at each work-rate for the control subjects

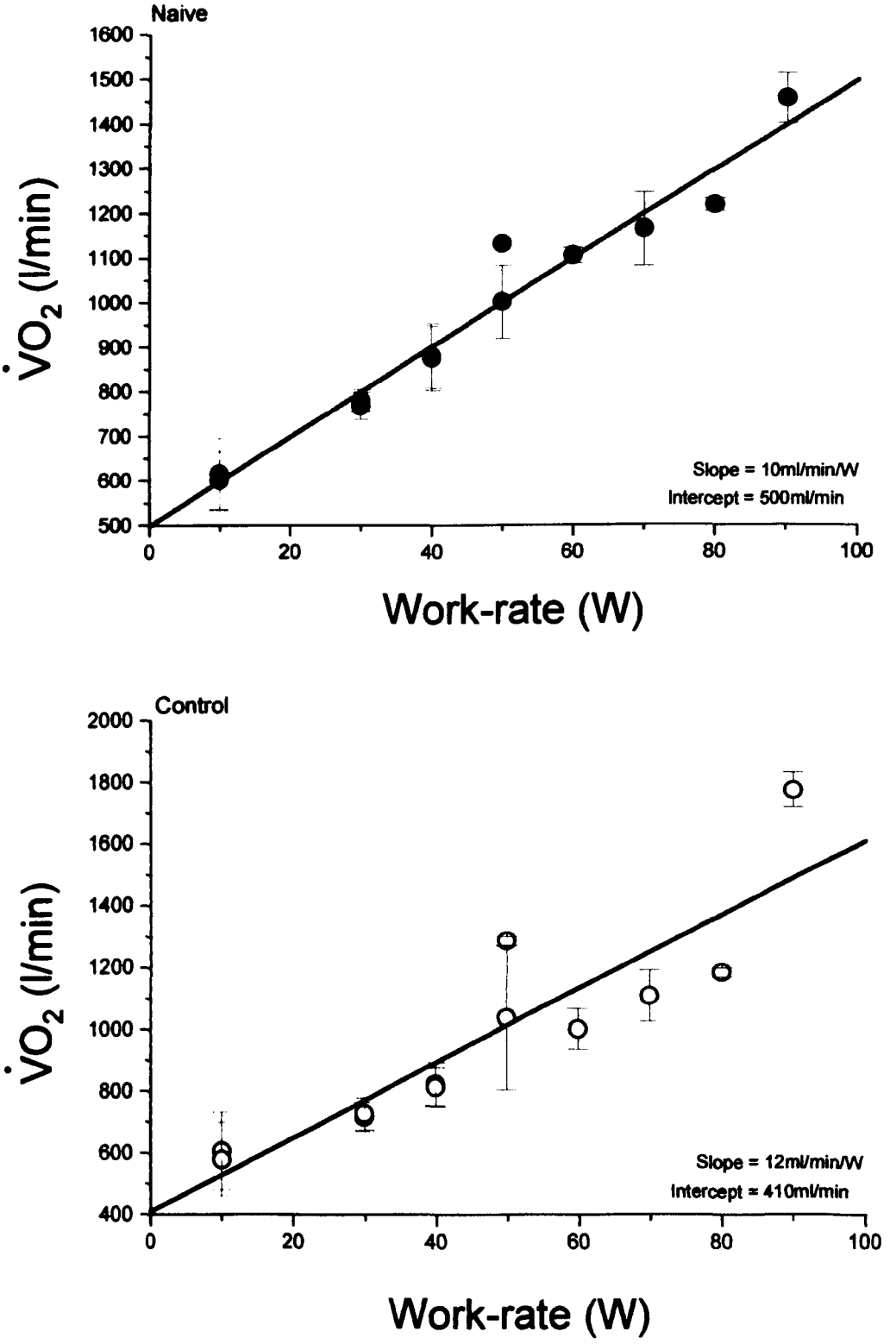


Figure 5.4: Displays the $\dot{V}O_2$-work relationship for both the naïve subject group (panel a) and their controls (panel b). The relationship is linear in both cases with a slope of ~10ml/W. However the control data is skewed by one subject, markedly heavier than the others. His O_2 cost lies on the same slope but with an increased $\dot{V}O_2$-intercept (i.e. greater resting rate).

5.5.1.3 Steady-state RER

The directionally opposite changes seen in $\dot{V}O_2$ and $\dot{V}CO_2$ with substrate alterations mean that RER may exhibit changes despite seemingly unchanged $\dot{V}O_2$ and $\dot{V}CO_2$ responses. However, during these trials there was no significant change in RER for a given work-rate in a given subject: Either between on- and off-transitions or between trial 1 and trial 2 (table 5.5, table 5.6).

5.5.1.4 Steady-state $\dot{V}_E$

There is, however, a degree of variation in the steady-state ventilatory response to a given work-rate, within each subject group and between the naïve subjects and their controls. For two naïve subjects performing the same ziggurat protocol with similar O_2 costs, the ventilatory responses can be markedly different (figure 5.5a) or almost identical (figure 5.5b).

Figure 5.6 shows the absolute level of ventilation for a given work-rate for the naïve subjects during the 'on' phase of the ziggurat test compared to the same work-rate during the 'off' phase. There was no significant difference in the naïve or control subjects' steady-state $\dot{V}_E$ when the 'on' transition to a given work-rate is compared to the corresponding 'off' transition (table 5.7, table 5.8) (trial 1 WR1vs5 p=0.237, WR2vs4 p=0.92, trial 2 WR1vs5 p=0.354, WR2vs4 p=0.495). From this it is apparent that most naïve and control subjects were able to accurately match $\dot{V}_E$ to the absolute work-rate during any given trial.

	Trial 1					*Trial 2*				
Naive	RER WR 1	RER WR 2	RER WR 3	RER WR 4	RER WR 5	RER WR 1	RER WR 2	RER WR 3	RER WR 4	RER WR
1	0.82	0.86	0.85	0.83	0.82	0.86	0.90	0.93	0.86	0.88
2	0.79	0.83	0.93	0.79	0.84	0.75	0.82	0.94	0.79	0.81
3	0.85	0.92	0.93	0.86	0.83	0.77	0.84	0.85	0.79	0.76
4	0.81	0.84	0.85	0.85	0.81	0.79	0.83	0.83	0.79	0.77
5	0.79	0.88	0.92	0.84	0.84	0.79	0.82	0.89	0.82	0.80
6	0.81	0.78	0.89	0.8	0.76					
7	0.87	0.88	0.91	0.84	0.82	0.78	0.85	0.86	0.83	0.8
8	0.82	0.83	0.88	0.84	0.81					
9	0.76	0.85	0.86	0.79	0.77	0.83	0.87	0.87	0.83	0.85
Mean	0.81	0.85	0.89	0.83	0.81	0.80	0.85	0.88	0.82	0.81
(SD)	(0.03)	(0.03)	(0.03)	(0.02)	(0.02)	(0.03)	(0.03)	(0.04)	(0.03)	(0.04)

Table 5.5: The steady-state respiratory exchange ratio at each work-rate for the naive subjects

	Trial 1					*Trial 2*				
Control	RER WR 1	RER WR 2	RER WR 3	RER WR 4	RER WR 5	RER WR 1	RER WR 2	RER WR 3	RER WR 4	RER WR
1	0.69	0.80	0.86	0.79	0.77	0.78	0.84	0.87	0.80	0.81
2	0.74	0.78	0.90	0.75	0.79	0.81	0.82	0.87	0.84	0.81
3	0.76	0.81	0.87	0.83	0.80	0.80	0.85	0.89	0.84	0.83
4	0.82	0.87	0.86	0.84	0.79	0.83	0.81	0.86	0.83	0.84
5	0.89	0.77	0.82	0.83	0.80	0.80	0.74	0.82	0.80	0.84
6	0.84	0.89	0.92	0.87	0.88	0.78	0.83	0.92	0.86	0.87
7	0.78	0.79	0.85	0.80	0.77	0.90	0.85	0.94	0.84	0.80
8	0.72	0.81	0.88	0.80	0.77	0.65	0.76	0.83	0.80	0.78
9	0.77	0.80	0.83	0.81	0.81	0.85	0.84	0.87	0.85	0.85
Mean	0.78	0.81	0.87	0.81	0.80	0.80	0.82	0.87	0.83	0.83
(SD)	(0.06)	(0.04)	(0.03)	(0.03)	(0.03)	(0.07)	(0.04)	(0.04)	(0.02)	(0.03)

Table 5.6: The steady-state respiratory exchange ratio at each work-rate for the control subjects

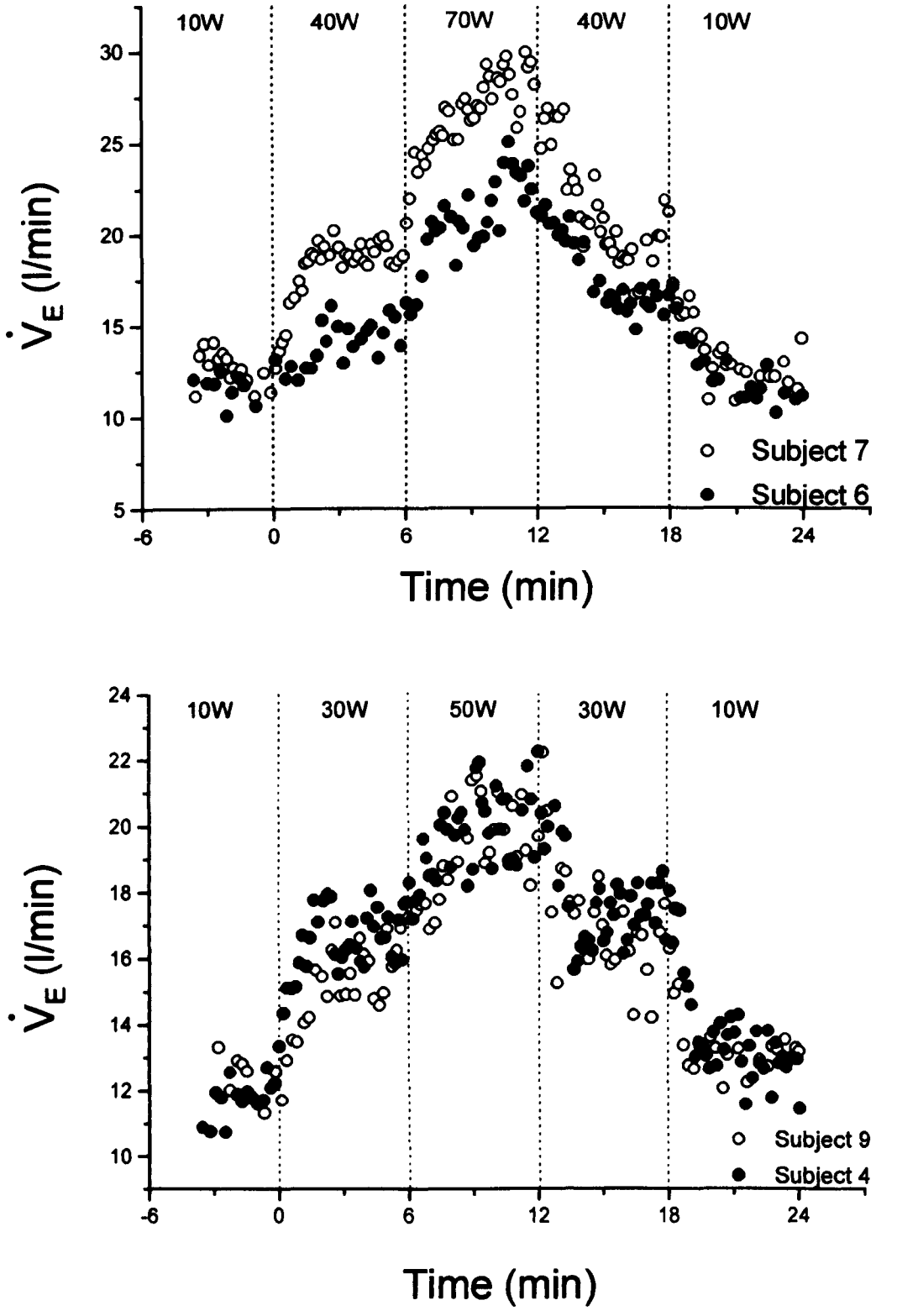


Figure 5.5: Panel a depicts two naïve subjects who have markedly different steady-state ventilatory responses for the same work-rate. Whereas panel b illustrates two naïve subjects with near identical ventilatory responses to the same work rate.

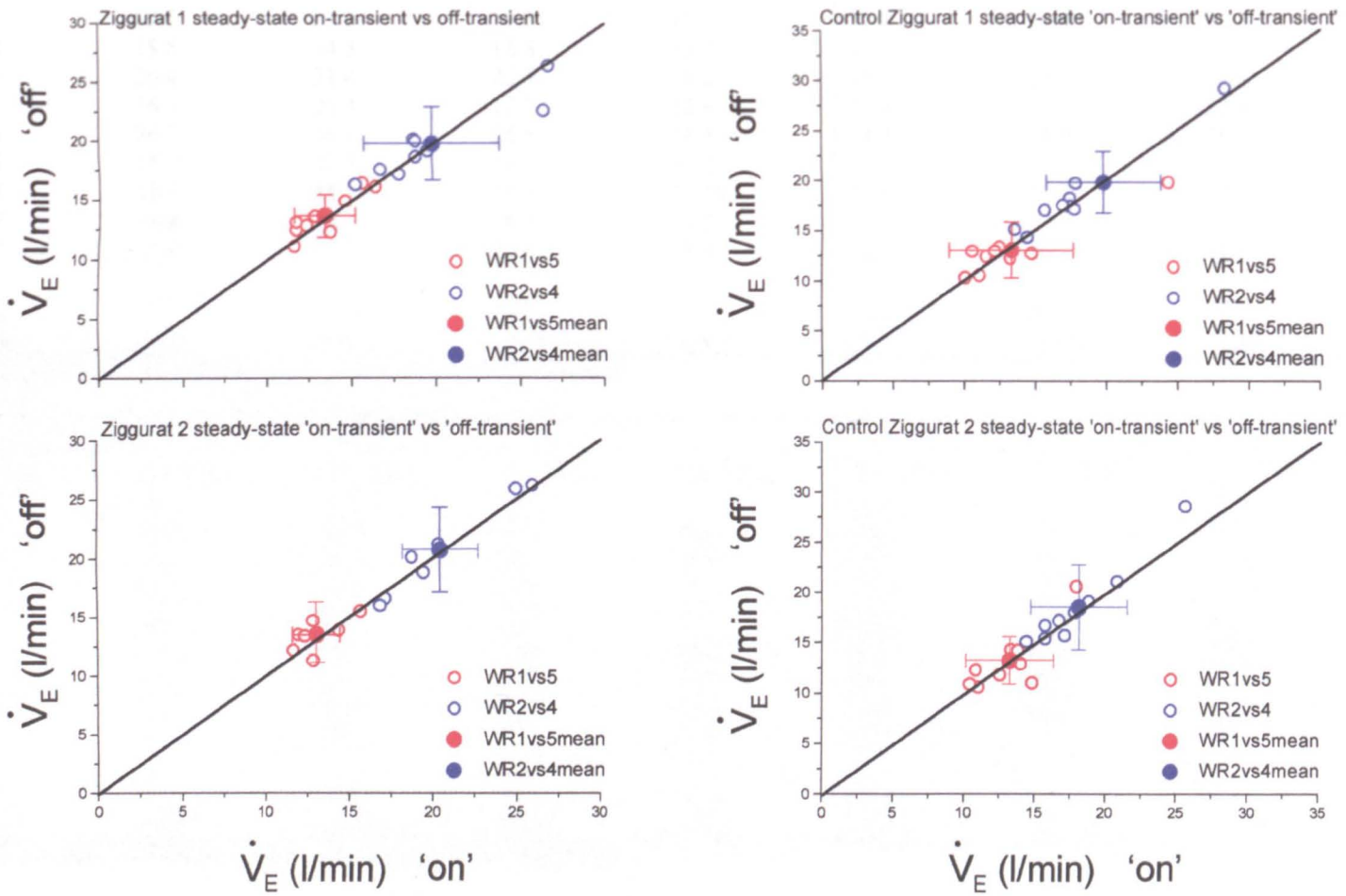


Figure 5.6: Comparisons of the steady-state ventilatory response to a given work-rate during the 'on-transition' compared to the 'off-transition'. For naïve subjects trial 1 + 2 (panels a + b) and their controls trial 1 + 2 (panels c + d). The open circles represent the first versus fifth work rate stages, the open triangles the second versus fourth, while the closed circles and triangles are the respective group means.

	Trial 1					*Trial 2*				
Naive	$\dot{V}_E$ WR 1	$\dot{V}_E$ WR 2	$\dot{V}_E$ WR 3	$\dot{V}_E$ WR 4	$\dot{V}_E$ WR 5	$\dot{V}_E$ WR 1	$\dot{V}_E$ WR 2	$\dot{V}_E$ WR 3	$\dot{V}_E$ WR 4	$\dot{V}_E$ WR
1	14.6	19.5	21.1	19.3	15	12.8	19.4	24.8	18.8	14.7
2	12.8	18.8	34.3	18.8	13.7	11.9	20.3	39.7	21.2	13.5
3	16.9	26.4	31.4	22.7	16.2	15.6	25.9	31.1	26.2	15.5
4	12.3	16.7	20.4	17.7	12.9	12.8	17.1	22.9	16.6	11.3
5	15.6	26.7	36.1	26.5	16.5	14.3	24.9	36.2	25.9	13.9
6	11.6	15.2	22.7	16.4	11.2					
7	11.7	18.7	28.2	20.3	12.58	11.6	18.7	25.5	20.1	12.2
8	11.7	18.8	26.1	20.1	13.2					
9	13.7	17.8	22.7	17.3	12.4	12.3	16.8	19.5	16.0	13.4
Mean	13.4	19.8	27.0	19.9	13.7	13.0	20.4	28.5	20.7	13.5
(SD)	(1.91)	(4.02)	(5.84)	(3.10)	(1.80)	(1.42)	(3.61)	(7.38)	(4.09)	(1.42)

Table 5.7: The steady-state ventilation at each work-rate for the naive subjects

	Trial 1					*Trial 2*				
Control	$\dot{V}_E$ WR 1	$\dot{V}_E$ WR 2	$\dot{V}_E$ WR 3	$\dot{V}_E$ WR 4	$\dot{V}_E$ WR 5	$\dot{V}_E$ WR 1	$\dot{V}_E$ WR 2	$\dot{V}_E$ WR 3	$\dot{V}_E$ WR 4	$\dot{V}_E$ WR
1	10.5	17.4	25.5	18.3	13	13.3	20.8	27.4	21.2	14.4
2	11.0	14.4	25.6	14.4	10.6	11.0	15.7	24.9	16.8	10.7
3	12.4	17.4	21.5	17.5	13.4	10.4	17.1	19.5	15.8	11.0
4	13.2	16.9	20.8	17.6	12.3	14.0	17.8	22.6	18.1	13.0
5	24.3	28.3	37.3	29.3	19.9	17.9	25.6	40.6	28.7	20.7
6	11.5	17.7	23.6	17.2	12.5	10.8	16.7	28.8	17.3	12.4
7	14.7	17.8	26.1	19.8	12.8	13.8	18.8	27.6	19.2	14.3
8	10.0	13.5	20.9	15.2	10.4	12.5	14.4	20.4	15.2	11.9
9	12.1	15.6	19.8	17.1	13.0	14.8	15.7	20.2	15.5	11.1
Mean	13.3	17.7	24.6	18.5	13.1	13.2	18.1	25.8	18.6	13.3
(SD)	(4.36)	(4.27)	(5.32)	(4.35)	(2.76)	(2.36)	(3.40)	(6.57)	(4.23)	(3.10)

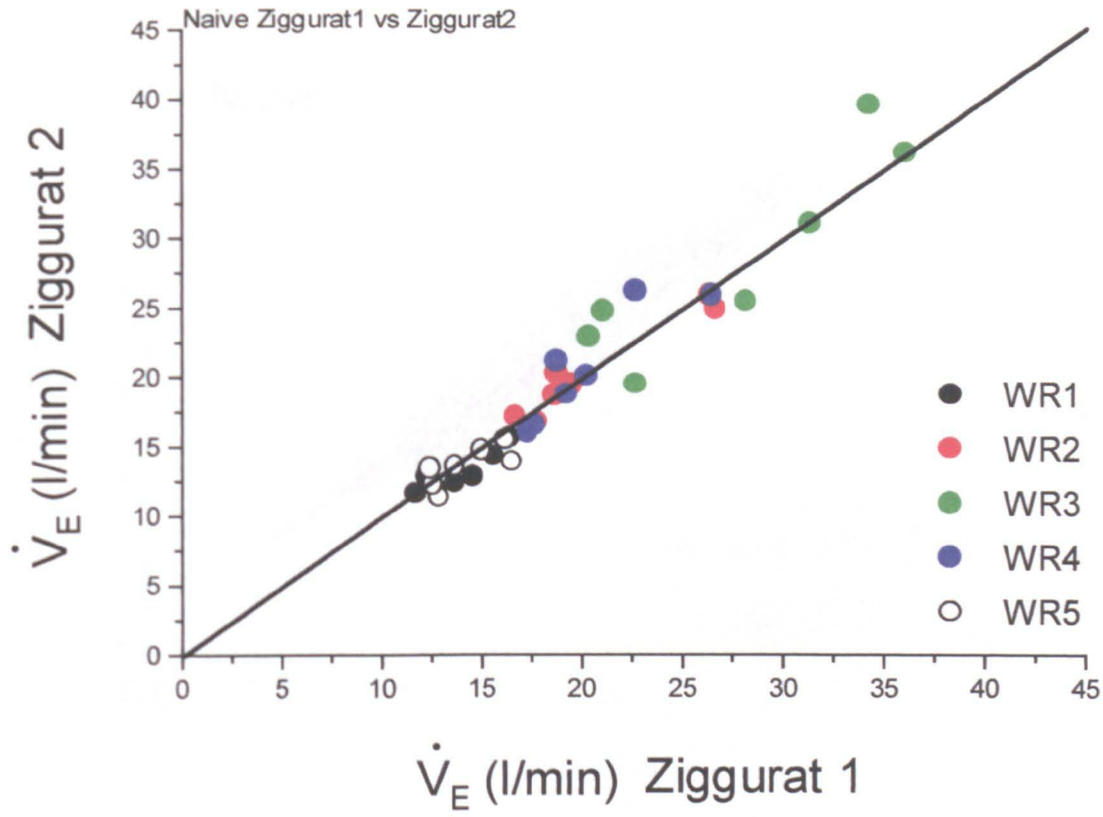
Table 5.8: The steady-state ventilation at each work-rate for the control subjects

The level of $\dot{V}_E$ for a given segment of the ziggurat however, does show variation between tests, for both naïve and control subjects (figure 5.7). Therefore, unlike $\dot{V}O_2$, there is an element of variability in the absolute level of ventilation in all comparisons, except within a given test.

5.5.1.5 $\dot{V}_E$ - $\dot{V}CO_2$

As already mentioned, it is not the absolute level of ventilation which is of most interest, rather it is the relationship between $\dot{V}_E$ and $\dot{V}CO_2$. Figure 5.8 illustrates the tight linear relationship found across the duration of the test, for all subjects, between $\dot{V}_E$ and $\dot{V}CO_2$. The slopes of this relationship were found not to be significantly different from those of their controls (p=0.19) (table 5.9, table 5.10) and lay within the accepted range (e.g. Neder *et al.*, 2001; Wasserman *et al.*, 1967).

Further to this, separating out the response for individual transitions and pairing the on- and off-transitions to a given work-rate displays the dynamic symmetry of the on- and off-responses (figure 5.9). This was found to be the case for all subjects in both naïve and control groups (figure 5.10, figure 5.11) (table 5.9, table 5.10) (Naïve trial 1 trans.1vs4 p=0.074, trans.2vs3 p=0.971, Naïve trial 2 trans1vs4 p=0.708, trans.2vs3 p=0.299).



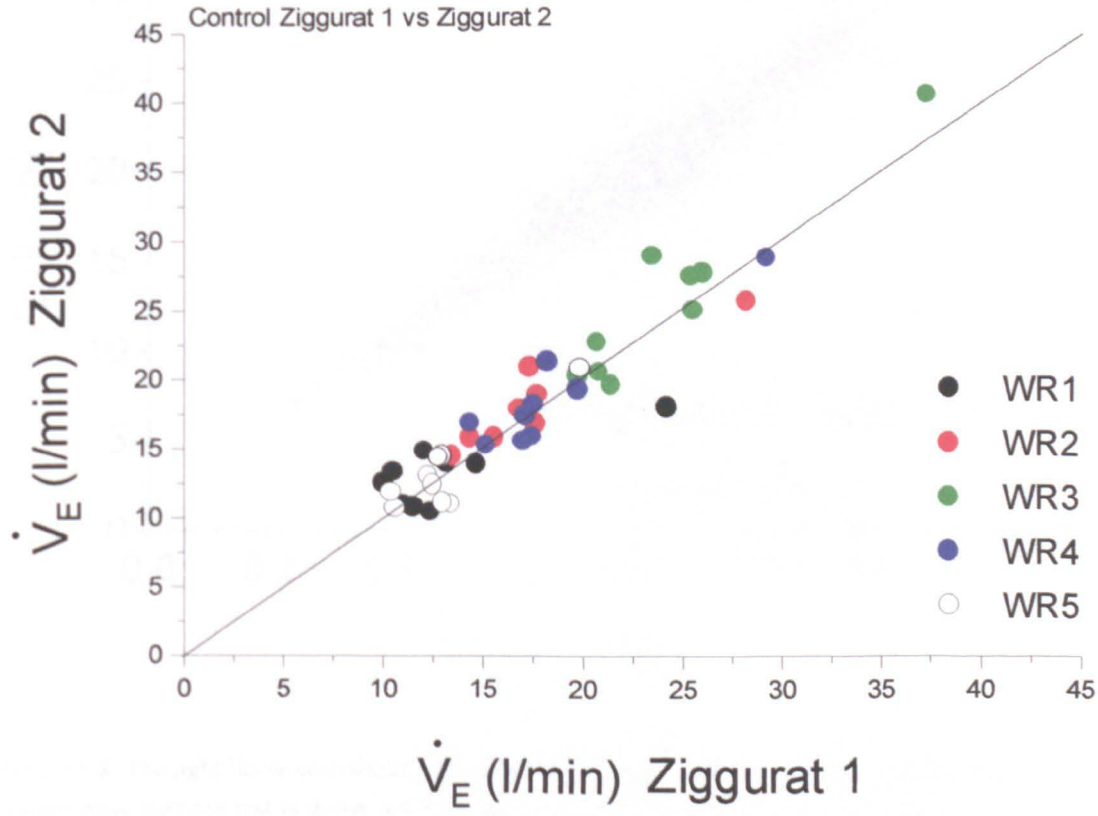


Figure 5.7: The steady-state ventilatory response to a given work-rate during the first ziggurat trial compared to the same work-rate during trial 2, for the naïve subjects and for their controls.

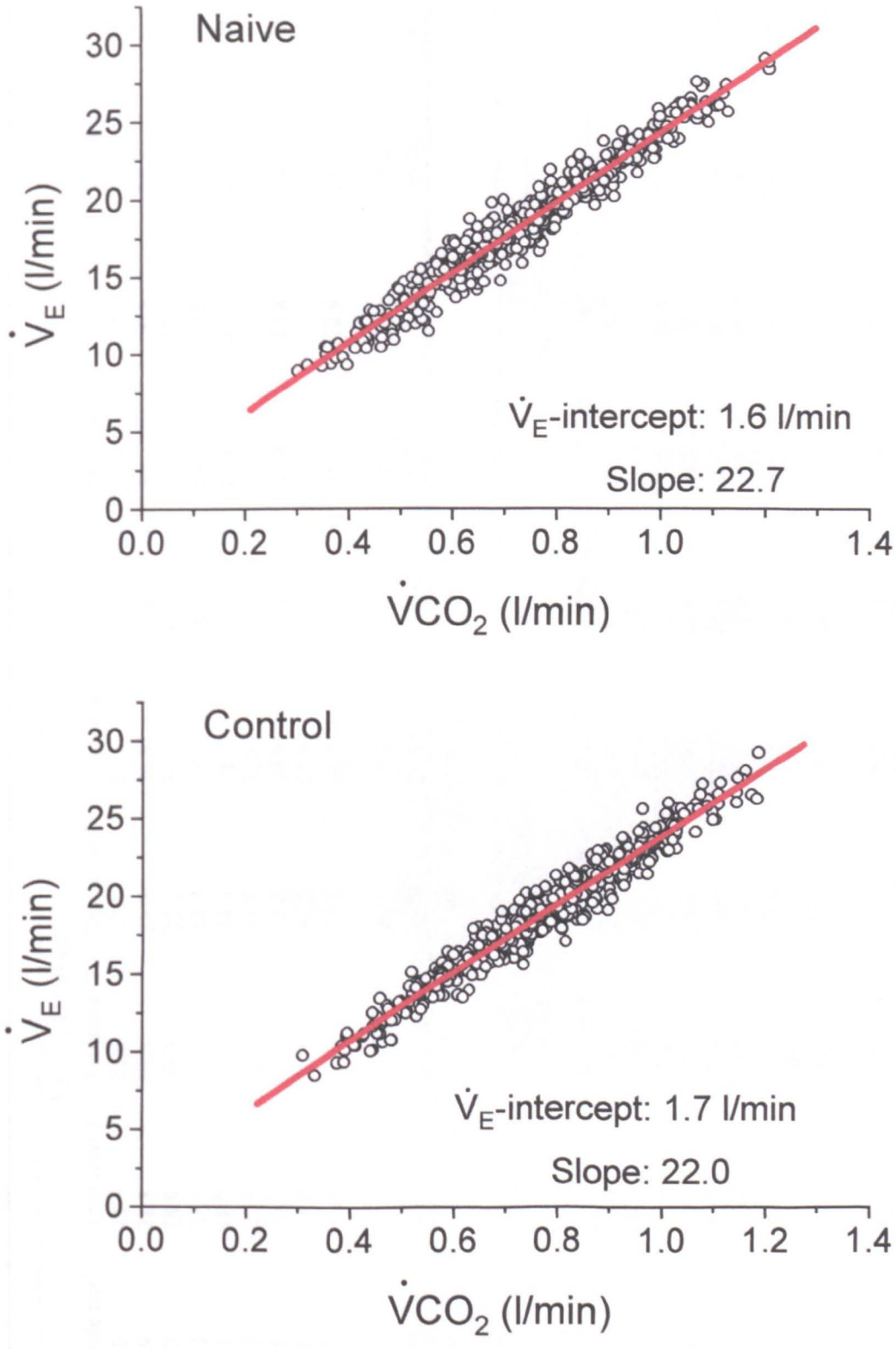


Figure 5.8: The tight linear correlation found between $\dot{V}_E$ and $\dot{V}CO_2$ across the whole ziggurat square-wave exercise test is shown for both naïve (top panel) and control (bottom panel).

Naive	*Trial 1*					*Trial 2*				
	$\dot{V}_E$ - $\dot{V}CO_2$ slope					$\dot{V}_E$ - $\dot{V}CO_2$ slope				
	Whole trial	Transition 1	Transition 2	Transition 3	Transition 4	Whole trial	Transition 1	Transition 2	Transition 3	Transition 4
1	22.0	23.7	24.5	22.9	23.5	22.7	23.9	23.5	22.1	25.3
2	29.9	28.8	30.6	27.2	30.9	33.6	30	29.3	35.2	33.9
3	30.2	28.8	30.3	32.7	30.3	32.3	34.3	33.7	31.6	32.3
4	28.0	27.1	26.2	25.7	27.3	28.7	29.7	29.2	29.4	25.7
5	27.1	28.7	27.5	28.5	28.7	26.3	27.2	27.1	25.5	29.7
6	21.2	21.8	25.5	21.7	22.9					
7	23.8	24.3	24.8	21.8	25.9	24.4	23.9	23.8	24	26
8	22.5	24.5	24.5	30.3	27.5					
9	26.2	26.9	26.2	25.8	26.3	24	25.3	22.9	24	22.6
Mean	25.7	26.1	26.7	26.3	27.0	27.4	27.8	27.1	27.4	27.9
(SD)	(3.41)	(2.57)	(2.35)	(3.81)	(2.74)	(4.24)	(3.82)	(3.96)	(4.78)	(4.12)

Table 5.9: The slope of the $\dot{V}_E$ - $\dot{V}CO_2$ relationship for each 'on' and 'off' transition for the naive subjects

Control	*Trial 1*					*Trial 2*				
	$\dot{V}_E$ - $\dot{V}CO_2$ slope					$\dot{V}_E$ - $\dot{V}CO_2$ slope				
	Whole trial	Transition 1	Transition 2	Transition 3	Transition 4	Whole trial	Transition 1	Transition 2	Transition 3	Transition 4
1	26.8	26.2	31	29.2	25.2	31.7	33.1	29.8	33.2	29.1
2	21.8	23.7	23.8	24.5	28.5	22.9	22.7	23.2	24.6	28
3	21.1	23.6	24.2	23.3	20.9	21	21.6	24.4	22.9	25.1
4	26.8	26.4	27.9	26.2	26.4	26.8	28.5	28.4	26.1	26.6
5	25	30.3	26.4	26.4	28.16	26.8	29.4	28	29.3	30.4
6	25	25.5	27.2	25.5	29.2	26.8	24.6	32.9	26.5	27.5
7	23.4	26	25.3	26.5	29.8	26.9	26.7	30.2	30	28.8
8	19	18.5	19	21.1	23.7	19.5	18.6	18.8	21.8	30.5
9	24.2	25.4	27.2	27.2	27.0	24.3	26.4	27.3	24.5	27.8
Mean	23.7	25.1	25.8	25.5	26.5	25.2	25.7	27	26.5	28.2
(SD)	(2.63)	(3.14)	(3.33)	(2.35)	(2.87)	(3.69)	(4.40)	(4.25)	(3.67)	(1.74)

Table 5.10: The slope of the $\dot{V}_E$ - $\dot{V}CO_2$ relationship for each 'on' and 'off' transition for the naive subjects

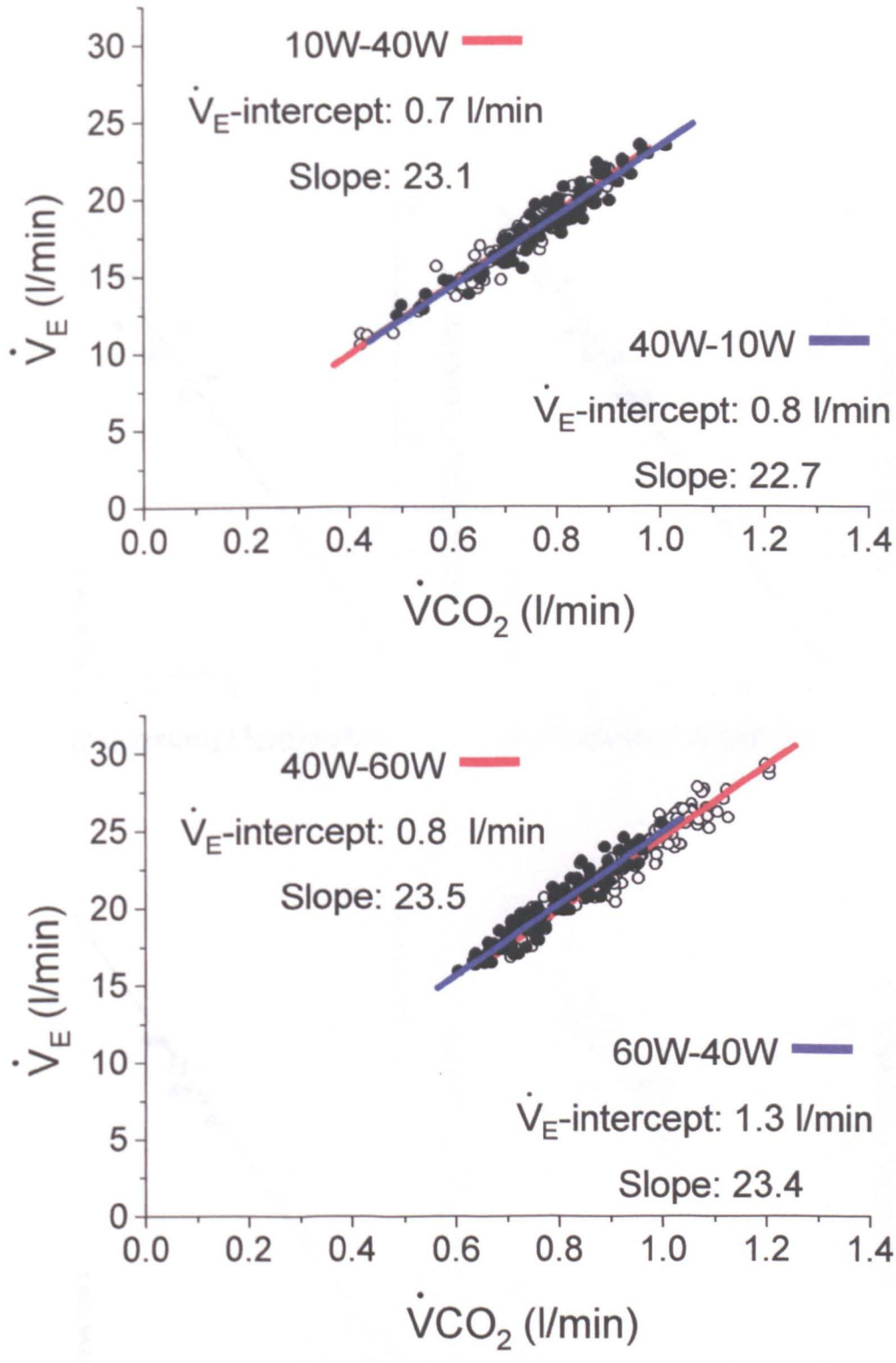


Figure 5.9: The individual on- and off-transitions for each work rate have been extracted and plotted with their pair, for the lower work-rate top panel and higher work-rate bottom panel.

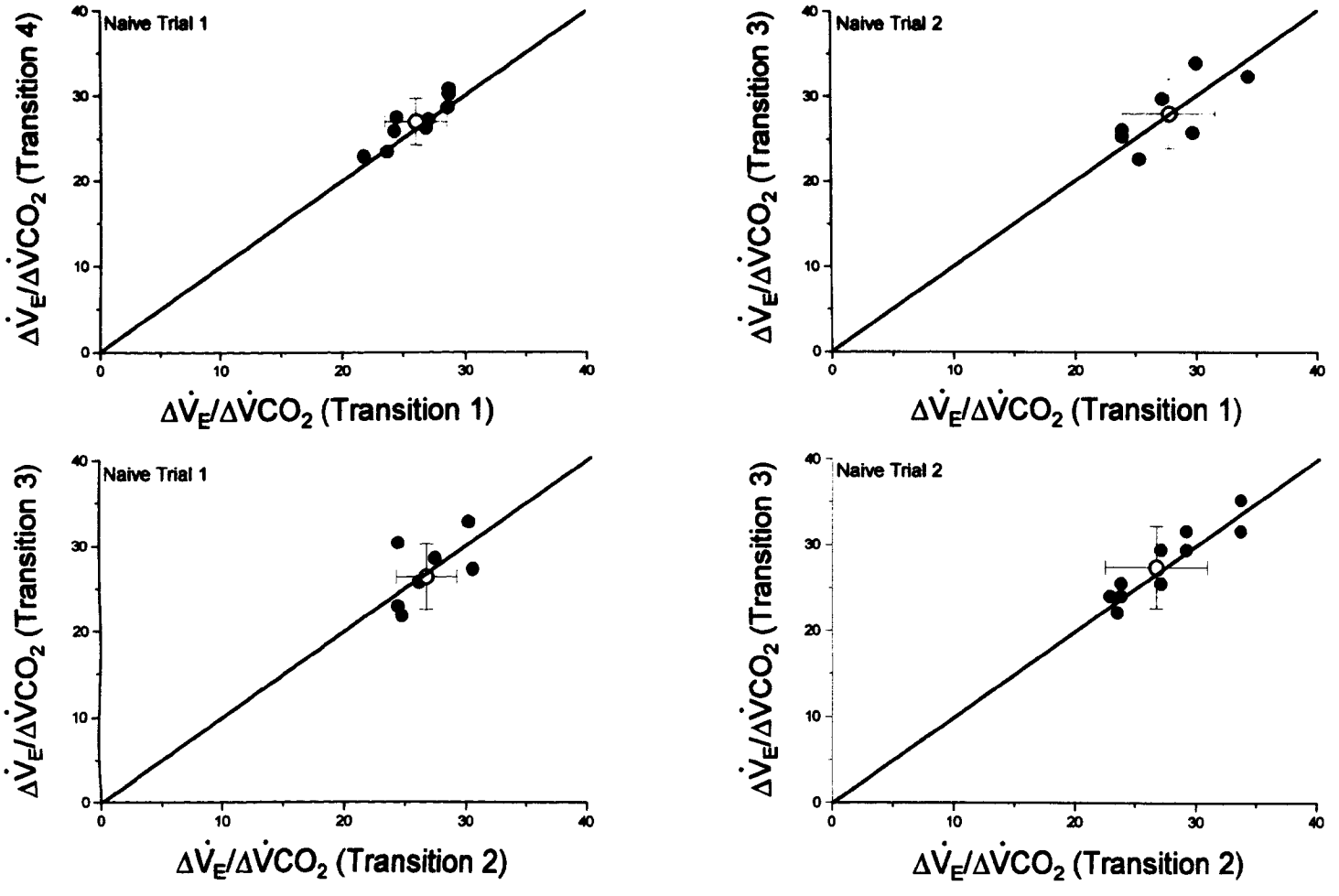


Figure 5.10: The relationship between the $\dot{V}E$ - $\dot{V}CO_2$ slope during the 'on-transient' and the 'off-transient' to and from a given work rate. All panels relate to the naive group, a + b are for the first and last transitions during trial 1 and 2 respectively. While panels c + d are the middle two transitions during trial 1 and 2 respectively

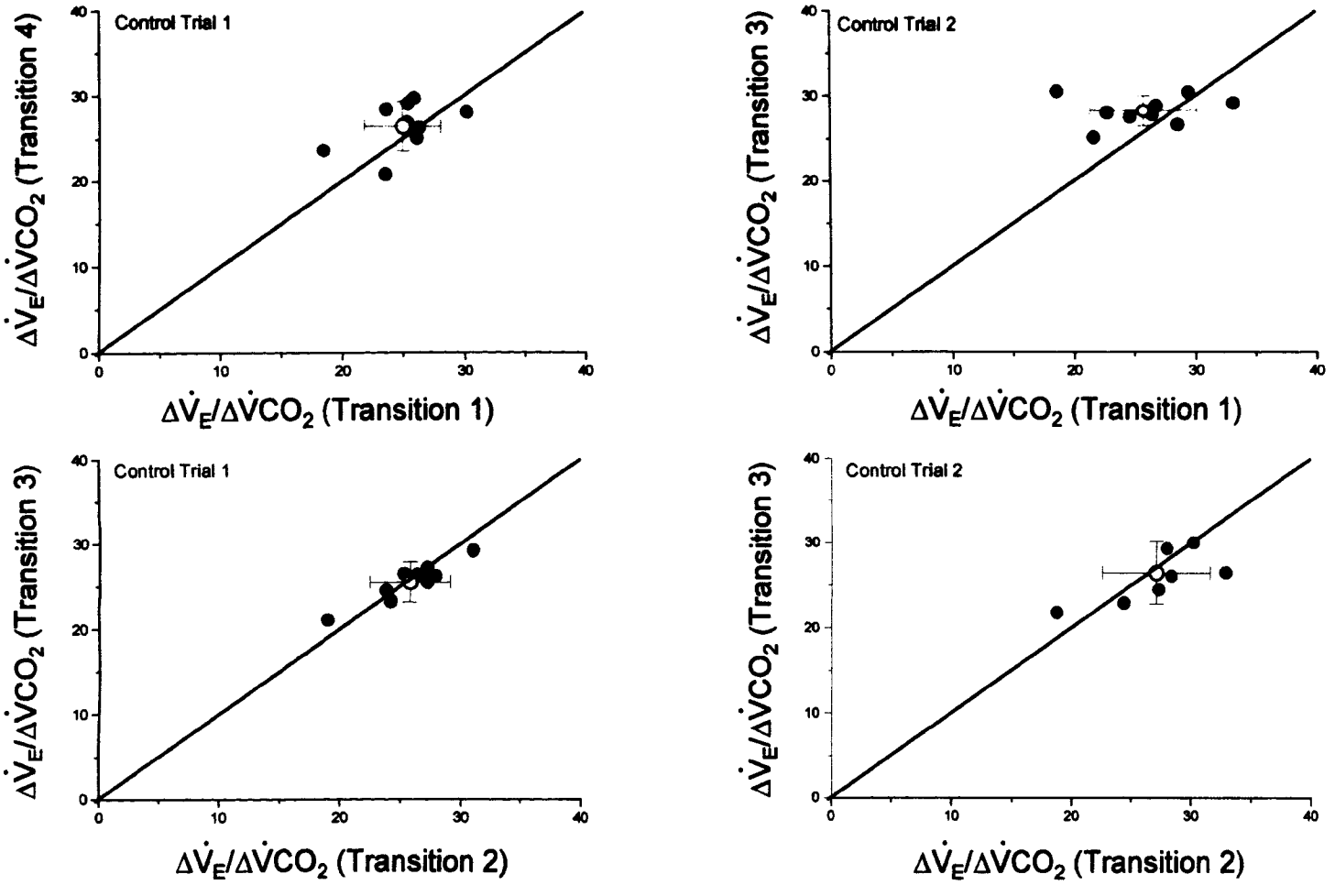


Figure 5.11: The relationship between the $\dot{V}E$ - $\dot{V}CO_2$ slope during the 'on-transient' and the 'off-transient' to and from a given work rate. All panels relate to the control group, a + b are for the first and last transitions during trial 1 and 2 respectively. While panels c + d are the middle two transitions during trial 1 and 2 respectively

As there were found to be some differences in the absolute level of $\dot{V}_E$ between trial 1 and trial 2 in many subjects, it was also of interest to see if the relationship was the same during trial 2 as trial 1. Figure 5.12a shows the group mean $\dot{V}_E$-$\dot{V}CO_2$ slopes for each transition from trial 1 for the naïve subjects, while figure 5.12b shows the controls, plotted against the corresponding values from trial 2. While there was a slight tendency, in both groups, for the second trial to have a steeper relationship, this only reached significance in the control group (table 5.9, table 5.10) (naïve p=0.376, control p=0.03).

5.5.1.6 Steady-state $P_{ET}CO_2$ and P_ACO_2

The primary goal of the ventilatory control system is the regulation of P_aCO_2, and hence pH_a. Therefore, when studying the effectiveness of the ventilatory control system the behavior of P_aCO_2 is of prime importance. While no direct measures of P_aCO_2 were made during the study, breath-by-breath recordings were made of $P_{ET}CO_2$. Typically, for both naïve and control subjects, $P_{ET}CO_2$ was found to increase on average by 1-2mmHg with each rise in work-rate and to decrease similarly with each fall in work-rate (figure 5.13) (table 5.11, table 5.12). Both naïve and control subjects were found to have significantly lower $P_{ET}CO_2$s during the 2nd ziggurat.

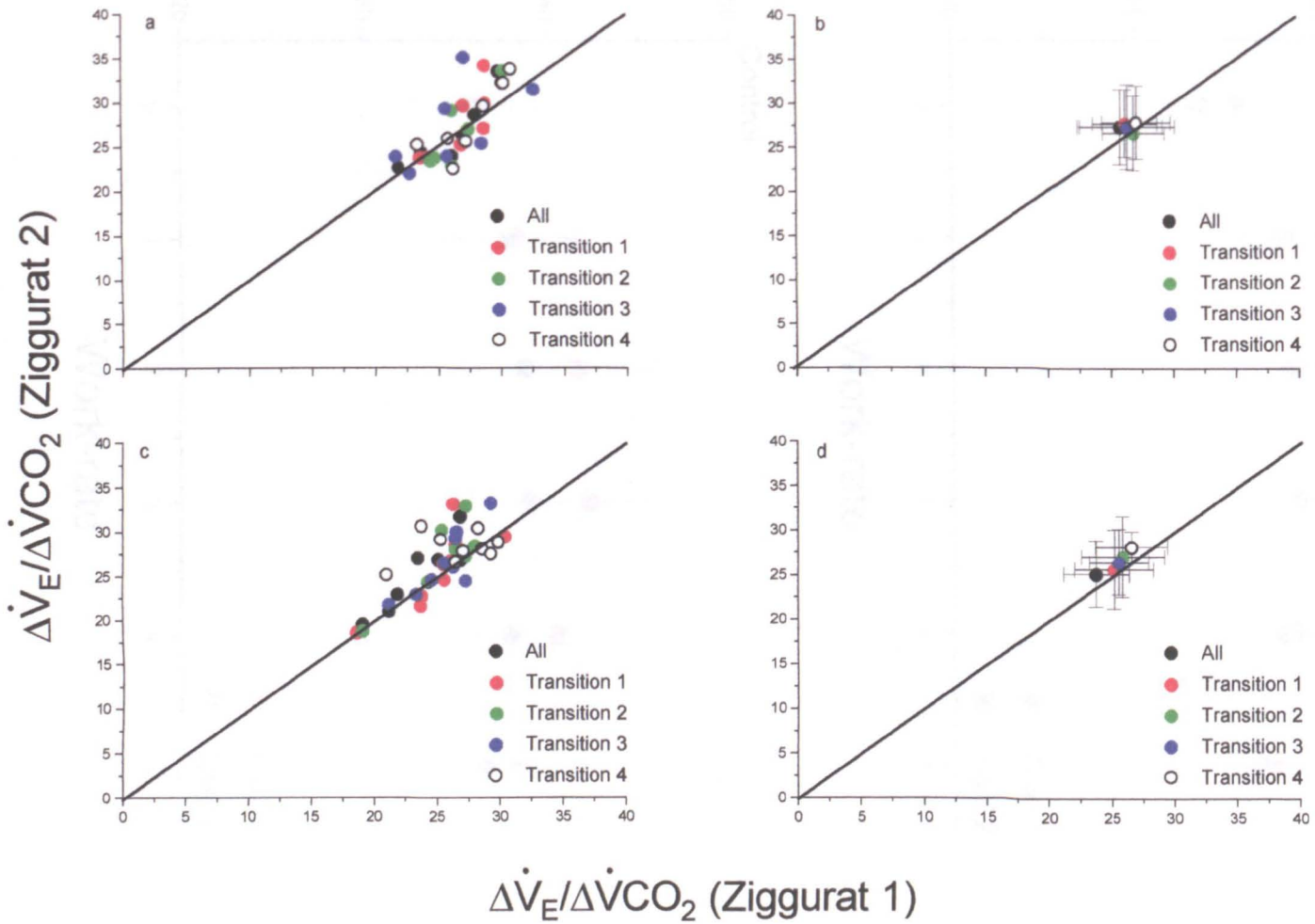


Figure 5.12: The relationship of the $\dot{V}_E$ - $\dot{V}CO_2$ slope for a given transition during the first test compared to the same transition during the second trial. Panel a + c are the naïve and controls respectively while panels b + d are the respective group means. The open symbols represent the slope across the whole trial, the red symbols transition 1, the green symbols transition 2, the blue symbols transition 3 and the open symbols transition 4.

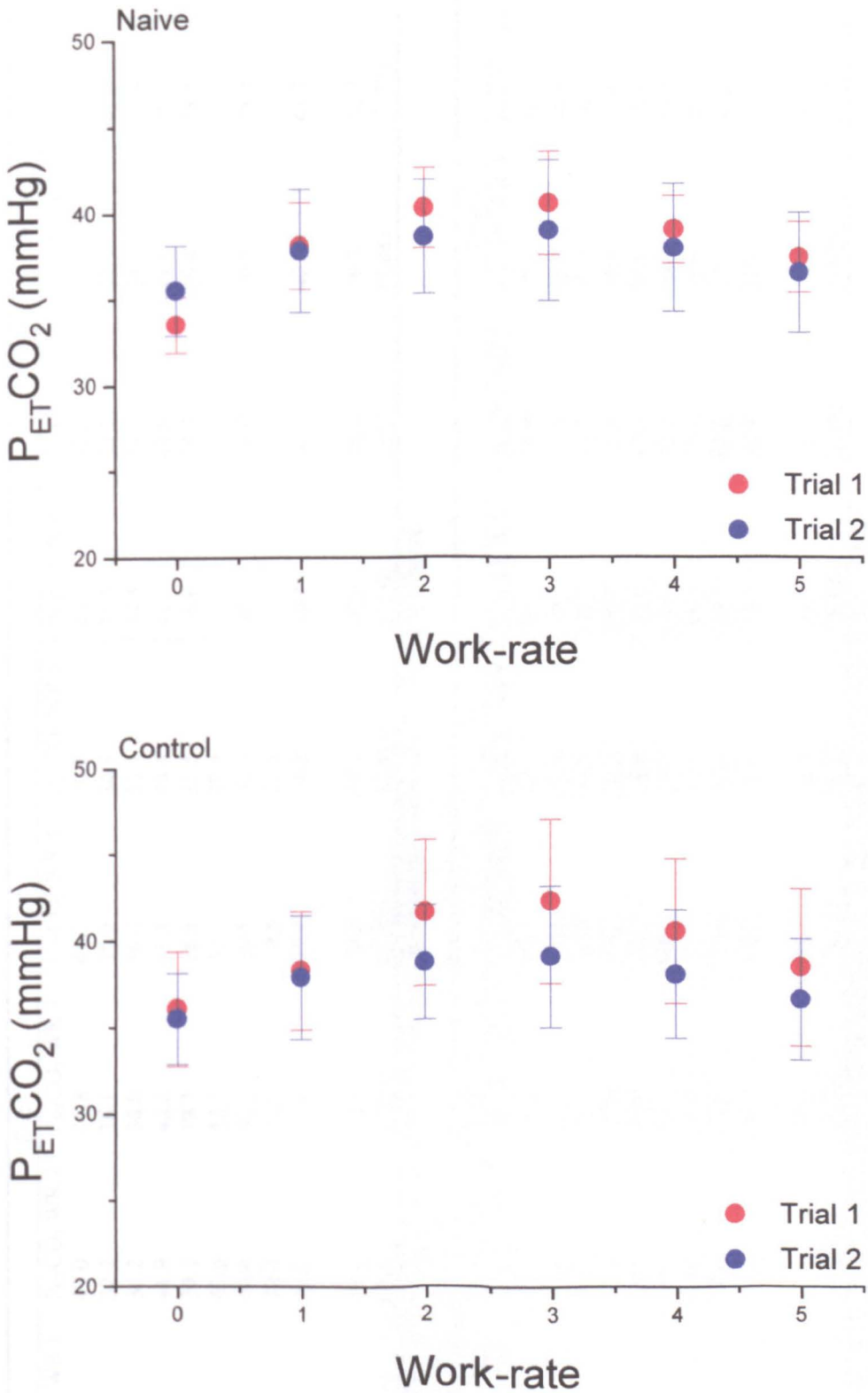


Figure 5.13: Group mean steady-state $P_{ET}CO_2$ responses across the ziggurat work forcing. The top panel displays the naïve subject group while the lower panel displays the matching responses of their controls. The first trial is represented by the red circles and the second by the blue circles.

	Trial 1					*Trial 2*				
Naive	$P_{ET}CO_2$ WR 1	$P_{ET}CO_2$ WR 2	$P_{ET}CO_2$ WR 3	$P_{ET}CO_2$ WR 4	$P_{ET}CO_2$ WR 5	$P_{ET}CO_2$ WR 1	$P_{ET}CO_2$ WR 2	$P_{ET}CO_2$ WR 3	$P_{ET}CO_2$ WR 4	$P_{ET}CO_2$ V
1	38.6	42.0	44.8	41.1	39.8	40.2	42.7	43.0	41.0	38.1
2	35.3	37.2	36.1	35.9	34.3	31.5	34.2	32.3	32.3	30.3
3	35.5	36.2	36.0	36.0	35.1	34.5	34.1	34.3	33.4	33.3
4	42.5	41.9	42.2	41.3	40.4	41.3	40.9	41.0	40.9	40.3
5	37.7	39.1	39.7	38.5	35.8	38.6	38.6	39.4	38.4	38.1
6	35.1	42.6	42.2	40	37.8					
7	38.5	40.6	41.7	39.3	38.1	38.3	39.9	40.8	38.5	37.2
8	39.4	42.4	42.9	39.9	37.5					
9	40.4	40.9	39.6	39.4	38.2	40.4	40.1	42.1	41.3	38.6
Mean	38.1	40.3	40.6	39.0	37.4	37.8	38.6	39.0	38.0	36.6
(SD)	(2.51)	(2.33)	(3.00)	(1.95)	(2.04)	(3.56)	(3.31)	(4.08)	(3.70)	(3.49)

Table 5.11: The steady-state partial pressure of CO_2 in the end tidal gas at each work-rate for the naive subjects

	Trial 1					*Trial 2*				
Control	$P_{ET}CO_2$ WR 1	$P_{ET}CO_2$ WR 2	$P_{ET}CO_2$ WR 3	$P_{ET}CO_2$ WR 4	$P_{ET}CO_2$ WR 5	$P_{ET}CO_2$ WR 1	$P_{ET}CO_2$ WR 2	$P_{ET}CO_2$ WR 3	$P_{ET}CO_2$ WR 4	$P_{ET}CO_2$ V
1	32.7	36.9	36.5	35.5	31	31.7	33.9	33.7	33.2	30.3
2	38.7	42.4	42.9	41.4	38.4	39.1	41.9	43.2	40.6	38.2
3	41.7	44.9	44.7	43.3	41.5	42.2	45.7	45.5	44.4	41.7
4	38.8	38.6	39.9	38.7	38.5	36.4	37.8	38.4	37.6	36.9
5	35	40.1	40.2	38.7	38.4	37.2	40.1	39.5	39.0	36.8
6	37.2	39.6	41.7	38.4	35.7	36.7	40.2	38.6	37.5	34.8
7	38.3	40.9	41.4	40	37.5	39.3	42.3	42.0	40.4	38.9
8	44.3	51.0	53.3	49.9	47.9	43.7	47.9	50.7	45.2	43.3
9	37.3	38.8	39.2	38.1	36.7	38.1	40.7	41.2	39.5	38.0
Mean	38.2	41.5	42.2	40.4	38.4	38.3	41.2	41.4	39.7	37.7
(SD)	(3.40)	(4.26)	(4.76)	(4.17)	(4.55)	(3.48)	(4.09)	(4.83)	(3.63)	(3.77)

Table 5.12: The steady-state partial pressure of CO_2 in the end tidal gas at each work-rate for the control subjects

While, during steady-state exercise, $P_{ET}CO_2$ becomes dissociated from mean P_aCO_2 due partly to the changes in the gradient of the intra-breath alveolar PCO_2 profile, P_ACO_2 remains inexorably linked to P_aCO_2. Although there was an element of variability in P_ACO_2 evident for some naïve, and control, subjects there was no consistent trend across a trial (table 5.13, table 5.14). This can be seen from the group means (figure 5.14) which reveal that there was no substantial difference in P_ACO_2 between any of the work-rates for either group. Statistical analysis confirmed that there were no significant differences in P_ACO_2 across either trial 1 (naïve p=0.552, control p=0.416) or trial 2 (naïve p=0.931, control p=0.503). As with $P_{ET}CO_2$, P_ACO_2 was found to be lower during the second trial for the naïve subjects (naïve p=0.000), however unlike $P_{ET}CO_2$ this was only the case for the naïve subjects (control p=0.597).

5.5.1.7 Phase II

The lack of trial repetition and the greater than normal breath-to-breath 'noise' exhibited by both subject groups prevented formal classification of the phase II time constant with any degree of confidence. Therefore, information regarding the phase II control of $\dot{V}_E$ has only been drawn from the $\dot{V}_E$ - $\dot{V}CO_2$ relationship across the transition.

	Trial 1					*Trial 2*				
Naive	P_ACO_2 WR 1	P_ACO_2 WR 2	P_ACO_2 WR 3	P_ACO_2 WR 4	P_ACO_2 WR 5	P_ACO_2 WR 1	P_ACO_2 WR 2	P_ACO_2 WR 3	P_ACO_2 WR 4	P_ACO_2 W
1	35.4	39.1	39.9	37.0	38.4	36.6	38.4	38.3	37.6	35.1
2	31.7	35.5	32.8	32.9	31.5	29.6	31.0	30.1	29.8	28.8
3	31.2	31.4	30.7	33.0	32.6	31.6	30.4	30.6	30.4	30.3
4	38.1	38.2	38.8	37.5	36.7	36.4	36.4	36	37.4	36.7
5	37.8	37.8	38.3	36.6	38.4	36.6	36.3	34.9	34.8	34.9
6		38.3	39.7	38.3	37.5					
7	35.1	37.8	39.7	36.3	36.6	35.0	36.9	36.9	34.5	35.3
8	36.4	39.7	40.4	36.2	35.6					
9	36.5	36.9	35.9	36.0	36.4	37.7	37.2	38.6	38.3	35.3
Mean	35.3	37.2	37.4	36.0	36.0	34.8	35.2	35.1	34.7	33.8
(SD)	(2.57)	(2.49)	(3.48)	(1.86)	(2.42)	(3.02)	(3.17)	(3.45)	(3.45)	(2.97)

Table 5.13: The steady-state mean partial pressure of CO_2 in the alveolar gas at each work-rate for the naive subjects.

	Trial 1					*Trial 2*				
Control	P_ACO_2 WR 1	P_ACO_2 WR 2	P_ACO_2 WR 3	P_ACO_2 WR 4	P_ACO_2 WR 5	P_ACO_2 WR 1	P_ACO_2 WR 2	P_ACO_2 WR 3	P_ACO_2 WR 4	P_ACO_2 W
1	33.8	33.9	32.7	32.3	31.0	29.7	31.0	31.2	29.7	28.4
2	35.9	39.0	38.3	38.8	36.0	37.2	29.2	40.4	36.4	36.3
3	38.6	39.9	40.0	38.5	35.4	39.2	41.8	40.5	41.2	38.6
4	34.8	35.3	35.1	34.5	35.0	33.2	34.2	35.8	34.3	32.8
5	30.4	34.4	34.5	35.1	32.9	35.2	37.6	35.5	32.8	36.0
6	35.2	37.1	37.0	35.1	33.3	34.4	37.0	35.3	33.3	34.3
7	34.9	36.1	36.7	35.5	34.6	36.3	38.7	36.5	36.8	37.0
8	39.6	45.6	46.6	47.1	42.5	40.7	43.9	48.0	41.6	39.4
9	34.9	38.1	38.2	35.0	36.7	35.0	36.1	35.4	35.8	34.1
Mean	35.3	37.7	37.7	36.9	35.3	35.7	36.6	37.6	35.8	35.2
(SD)	(2.65)	(3.59)	(4.01)	(4.32)	(3.22)	(3.25)	(4.71)	(4.80)	(3.85)	(3.32)

Table 5.14: The steady-state mean partial pressure of CO_2 in the alveolar gas at each work-rate for the control subjects.

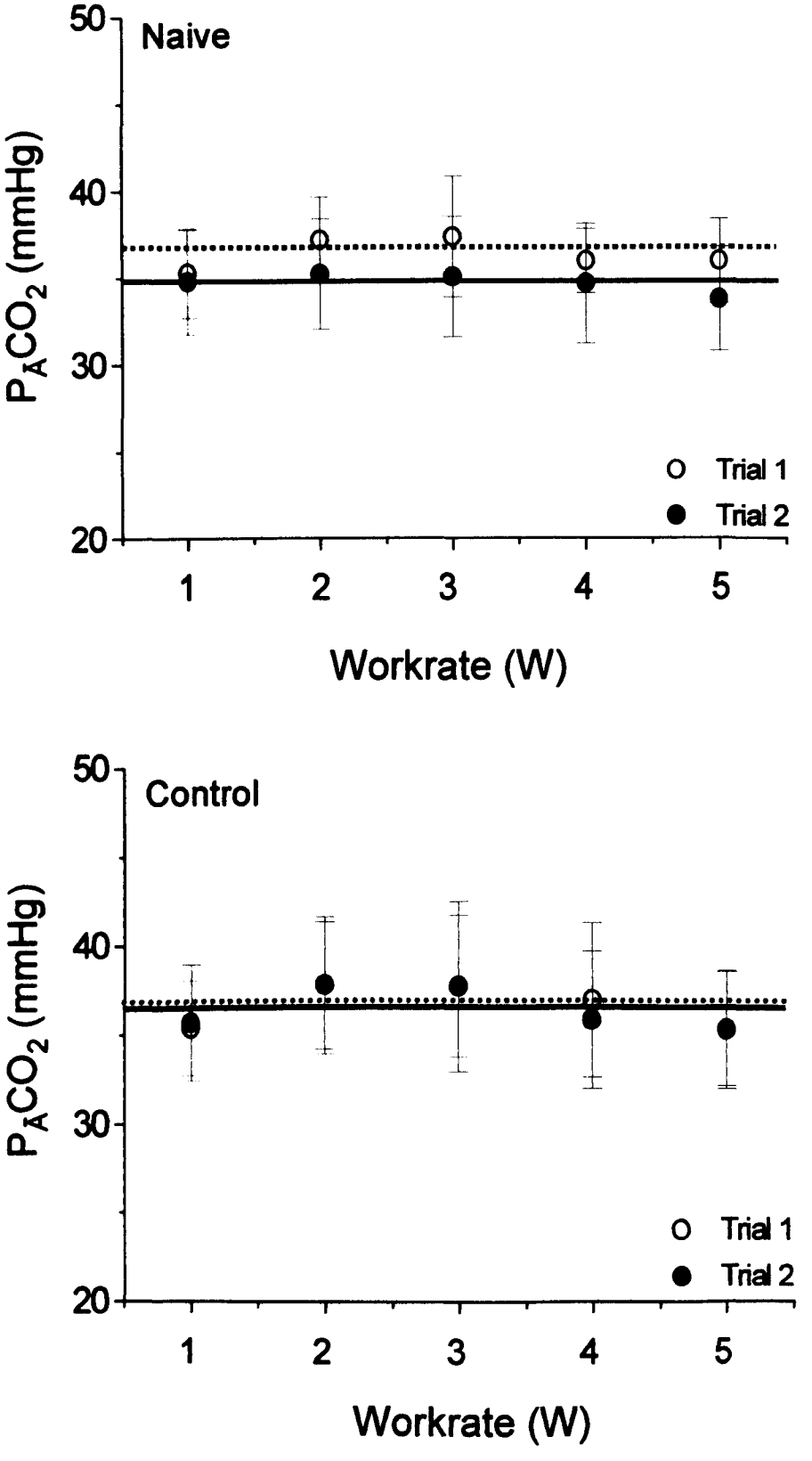


Figure 5.14: The mean alveolar PCO_2 during the steady state at each work rate for both the naïve group (top panel) and their controls (lower panel). The open symbols represent the first ziggurat trial while the closed symbols are the second. The dashed lines represent the mean during the first trial and the solid lines the mean of the second trial.

5.5.2 Incremental exercise test

The sequence of pulmonary gas exchange measurements used for parameter estimation from the incremental test ($\mu\dot{V}O_2$ and $\hat{\theta}_L$) is shown in figure 5.15. A break point in the $\dot{V}O_2$-$\dot{V}CO_2$ relationship can be seen to have occurred consistent with an increase in the ventilatory equivalent for O_2, while there was no change in the equivalent for CO_2. These changes were also matched by a sudden rise in $P_{ET}O_2$ with no equivalent fall in $P_{ET}CO_2$. These responses were all the result of the underlying metabolic consequences of the lactate threshold and therefore this is a reliable non-invasive estimate of subject 7's lactate threshold. The work-rates chosen, prior to commencing the study, for each of the naïve subjects were, in all but two cases, below the lactate threshold (table 5.15, table 5.16). The estimates of $\mu\dot{V}O_2$ and $\hat{\theta}_L$ (table 5.15, table 5.16) are a good indicator of the sedentarity (see page 240) of the naïve subject group and the appropriateness of their controls.

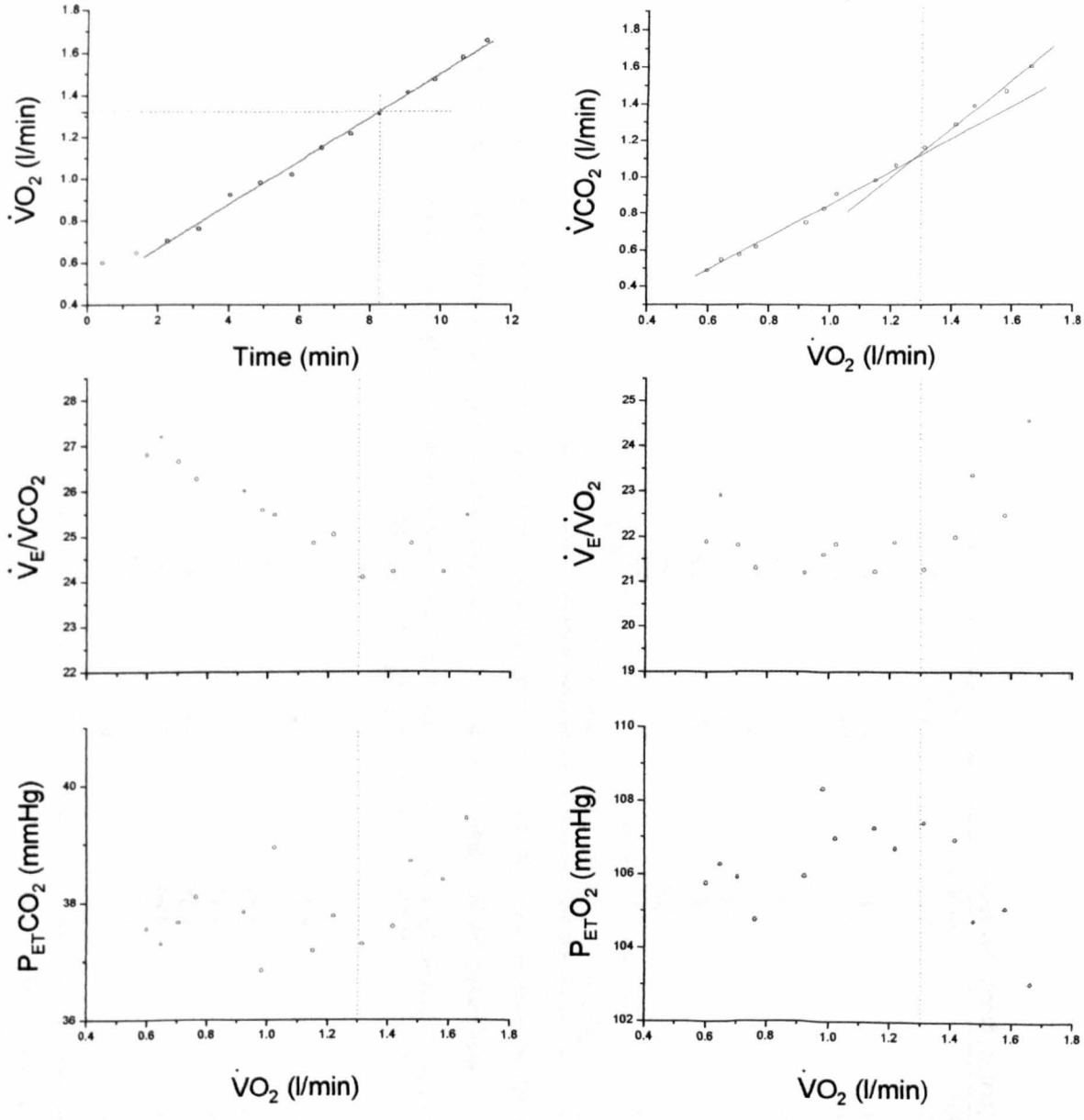


Figure 5.15: The cluster of responses used to non-invasively estimate the lactate threshold from an incremental exercise test (subject number 7). The lines on the top left hand panel show the transformation from a non-steady-state $\dot{V}O_2$ to a time and thence to a work rate. In all other panels the vertical lines indicate the lactate threshold.

Naïve	$\mu\dot{V}O_2$ *(l/min)*	$\mu\dot{V}O_2$ *(ml/kg/min)*	$\dot{V}O_2\,\hat{\theta}_L$ *(l/min)*	*WR* $\hat{\theta}_L$ *(W)*	*Peak Ziggurat WR (W)*
1	1.67	22.1	1.21	68	60
2	1.68	36.1	1.12	65	80
3	1.66	24.1	1.12	58	60
4	1.45	21.9	0.94	53	50
5	3.41	35.5	1.24	68	90
6	2.05	36.8	1.11	70	70
7	2.5	45.9	1.32	83	70
8					70
9	1.77	29.9	0.8	47	50
Mean	2.0	31.5	1.1	64.0	66.7
(SD)	(0.64)	(8.54)	(0.17)	(11.18)	(13.2)

Table 5.15: Subject characteristics (naïve group) detailing both individual and group mean values for absolute peak oxygen uptake ($\mu\dot{V}O_2$ l/min), peak oxygen uptake relative to body weight ($\mu\dot{V}O_2$ ml/kg/min), oxygen uptake at the estimated lactate threshold ($\dot{V}O_2\,\hat{\theta}_L$ l/min), steady-state work-rate equivalent to the lactate threshold ($WR\ \hat{\theta}_L W$) and peak work-rate during a ziggurat trial all functional indices measured during an during an incremental exercise test.

Control	$\mu\dot{V}O_2$ *(l/min)*	$\mu\dot{V}O_2$ *(ml/kg/min)*	$\dot{V}O_2\,\hat{\theta}_L$ *(l/min)*	*WR* $\hat{\theta}_L$ *(W)*	*Peak Ziggurat WR (W)*
1	1.48	31.3	1.11	57	60
2	1.94	39.4	1.23	71	80
3	1.45	21.2	0.87	48	60
4	1.87	28.6	1.03	57	50
5	3.2	28.1	1.61	81	90
6	1.98	39.8	1.2	73	70
7	2.29	37.4	1.27	74	70
8	2.03	32.1	1.13	61	70
9	1.79	32.3	0.9	53	50
Mean	2.0	32.2	1.2	63.9	66.7
(SD)	(0.52)	(6.00)	(0.22)	(11.2)	(13.2)

Table 5.16: Subject characteristics (control group) see above.

5.6 Discussion

The results of this study therefore indicate that the ventilatory response of the naïve subjects to moderate intensity square-wave cycle ergometry is 'normal'; i.e. appropriate with regard to P_aCO_2 regulation, despite the absence of a 'ventilatory memory' for that mode of exercise. Does this show that a learned response is not a required component of the exercise hyperpnoea? If so, does this rule out the potential for LTM in the ventilatory controller?

5.6.1 Rationale for present study

The difficulty experienced by all experimenters in modulating the existing ventilatory response, which has been attributed to this having been formed over years of exercise experience (Moosavi *et al.*, 2002; Schreurs, 1989) pointed away from associative conditioning experiments. Therefore, it was reasoned that it might be more productive to examine a situation where there was an absence of such an 'exercise-memory'. This description was equated to 'cycling naïve subjects', specifically individuals who have never learned to ride a bicycle and have no experience of any form of cycle ergometer. Due to the evident difficulties in locating individuals who met such strict criteria, individuals were also recruited with less than one hour's total experience either learning to ride as a child, or on a stationary cycle ergometer. This, it was reasoned, would be insufficient to create the 'memory bank' applicable to the range of intensities that would be required for LTM to operate as a functional controller of the exercise

hyperpnoea. Therefore, if LTM is primarily responsible for matching $\dot{V}_E$ to the demand created by the exercise intensity, the cycling naïve subjects should not exhibit a 'normal' exercise hyperpnoea.

5.6.2 Difficulties in analysis

This leads to the obvious question of what is a 'normal' exercise hyperpnoea and how do we quantify whether, or not, the cycling naïve subjects exhibit a 'normal' exercise ventilatory response?

5.6.2.1 Steady-state $\dot{V}O_2$

There have been many demonstrations that the oxygen cost per watt of work is unchanged by age, sex, physical characteristics or fitness level (Wasserman & Whipp, 1975; Hansen *et al.*, 1987). Therefore, that no deviation from this relationship of 10ml/watt was found for either experimental or control group was not unexpected. Subject 5 from the control group whose oxygen cost lies well above the regression line is substantially heavier than all the other controls (figure 5.4). This additional weight should give rise to an increased $\dot{V}O_2$-intercept (i.e. the O_2 cost of unloaded pedaling) but no change in slope. From figure 5.4 it is clear that his O_2 cost for 90W and for the off-transition to 50W actually lie on a similar slope to the rest of the group, but with an increased $\dot{V}O_2$-intercept of ~600ml. As the O_2 cost of the exercise is essentially 'normal', the

underlying metabolic drive for $\dot{V}_E$ during the exercise period is expected also to be 'normal'.

5.6.2.2 Steady-state $\dot{V}CO_2$

Despite an unchanging $\dot{V}O_2$ during the steady-state, $\dot{V}CO_2$ may be altered from transition to transition or from trial to trial either due to the influence of the body's capacity to store CO_2 or from shifts in the substrate mixture being metabolised. However, during all performances of a given work-rate all subjects exhibited a relatively consistent level of $\dot{V}CO_2$. Therefore, one facet of the determinants of ventilation (see page 25) remained consistent throughout the trials; i.e. the requirement for ventilation in terms of CO_2 clearance was unchanged across both trials at a given work-rate.

5.6.2.3 Steady-state RER

As there was no evidence of differences between $\dot{V}O_2$ and $\dot{V}CO_2$ across transitions or trials it is safe to conclude that storage of CO_2 or hyperventilation unloading those stores did not differently affect any transition or trial. However, it is possible that continual hyperventilation at all work-rates across all transitions and trials increased the CO_2 output consistently at each measurement period, thus disguising the hyperventilation from discovery, through inspection of either $\dot{V}O_2$ or $\dot{V}CO_2$. Analysis of steady-state RER however, prevents mis-

interpretation, as hyperventilation should be evident through a high RER (e.g. RER ≥ 0.9). At no point during any trial, for either group, except when the work-rate was above $\hat{\theta}_L$ was RER elevated above the expected level (table 5.5, table 5.6). Therefore, there was no evidence of non-specific hyperventilation during the steady-state on the basis of pulmonary gas exchange variables.

Additionally, despite the lack of apparent changes in substrate utilisation across a trial or between trials, from the absence of change in $\dot{V}O_2$ and $\dot{V}CO_2$, it is possible RER may show evidence of such changes. This is because small changes in $\dot{V}O_2$ and $\dot{V}CO_2$ which may lack statistical significance are amplified in RER because their directionally opposite effects are summated. However, despite this there was no evidence of changes in substrate utilisation across an exercise trial or between trials. This is not unexpected as each exercise trial lasted less that 30 minutes and therefore shifts in substrate utilisation would only be expected to be beginning around that time. Furthermore, as the subjects were instructed to come to the laboratory following 2 hours abstinence from food and 4 hours without caffeine (see chapter 2) they should be operating on similar substrate mixtures during both trials.

5.6.2.4 Steady-state $\dot{V}_E$

Due to the design of the experiment there are many possible comparisons of steady-state $\dot{V}_E$ that can be made. Of most interest are the outcomes of three comparisons, firstly that $\dot{V}_E$ is unchanged from the on-transition to the off-

transition of a given work-rate during any test. Secondly, that $\dot{V}_E$ for a given work-rate does vary from test to test. Finally, that two different subjects performing the same work-rate can exhibit markedly different or almost identical ventilatory responses. These facts should be treated carefully, lest they be mis-interpreted. Firstly, that $\dot{V}_E$ is the same for an on- and an off-transition, this could be interpreted to show that $\dot{V}_E$ is being accurately matched to the absolute work-rate, and therefore is under normal control. Secondly, paradoxically, that $\dot{V}_E$ is not the same for a given work-rate between tests, suggesting that in fact $\dot{V}_E$ is not being accurately matched to the absolute work-rate, and therefore not under 'normal' control. Finally, that despite the same underlying drive, from $\dot{V}O_2$, ventilation can either be the same between subjects at a given work-rate, suggesting matching to work-rate, or markedly different, on face value suggesting an error in $\dot{V}_E$ control.

Therein lies the reason why interpreting absolute $\dot{V}_E$ must be approached with caution. For a given $\dot{V}O_2$ the requirement for $\dot{V}_E$ is based on several factors. Firstly, the amount of CO_2 to be cleared, which differs from $\dot{V}O_2$ in the non-steady state due to transient storage of CO_2 and in the steady-state depending on the substrate being metabolised. Secondly, the set-point at which P_aCO_2 is to be regulated, which is likely to be different above and below the lactate threshold. Finally by the efficiency of the lung, i.e. the dead space fraction of the breath (V_D/V_T), the larger the subject the larger the dead space. Therefore, during any given test it is possible that for the same $\dot{V}O_2$ there may be a different requirement to clear CO_2 from one transition to the next, meaning that $\dot{V}_E$ being

unchanged from on- to off-transient may not indicate 'normal' control of $\dot{V}_E$. Equally, a shift in V_D/V_T between tests could mean that $\dot{V}_E$ was required to be different for the same work-rate, therefore the differences in $\dot{V}_E$ for a given intensity from test to test could indicate 'normal' control of $\dot{V}_E$. Furthermore, due to different substrate mixtures, dead-space volumes and P_aCO_2 set points comparisons of absolute ventilation between subjects at a given work-rate are essentially meaningless; without knowledge of all the above factors. So analysis of absolute $\dot{V}_E$ is of little use in determining whether the cycling naïve subjects are able to 'normally' control the exercise hyperpnoea.

5.6.2.5 $\dot{V}_E$ - $\dot{V}CO_2$

If the naïve subjects were not able to match $\dot{V}_E$ to the requirement to clear CO_2 across each work-rate transition there should be no specific relationship between the two variables. However, as with a 'normal' exercise hyperpnoea the naïve subjects exhibited a tight linear relationship between $\dot{V}_E$ and $\dot{V}CO_2$ across the duration of each test (figure 5.8). Also, if the naïve subjects were learning the required response as the tests proceeded, the $\dot{V}_E$ - $\dot{V}CO_2$ relationship should tighten as they became more accurate in tracking $\dot{V}_E$ to the requirement. Figure 5.9 and figures 5.10 and 5.12 illustrate that there was no significant change in the $\dot{V}_E$ - $\dot{V}CO_2$ relationship from the first transition of the first test to the last transition of the second test. The range of slopes and $\dot{V}_E$-intercepts found for both the naïve subjects and their controls also lies within the range for most healthy and clinical populations (Neder *et al.*, 2001).

5.6.2.6 Steady-state $P_{ET}CO_2$ and P_ACO_2

More important, even than the $\dot{V}_E$ - $\dot{V}CO_2$ relationship, in quantifying the naïve subjects as having a 'normal' exercise hyperpnoea or not is the actual outcome of the hyperpnoea, i.e. the regulation of P_aO_2, P_aCO_2 and pH_a (Asmussen & Nielsen, 1958; McIlroy, 1964; Jones, 1975; Lamb *et al.*, 1965; Lugliani *et al.*, 1971; Masson & Lahiri, 1974; Sutton *et al.*, 1976; Wasserman *et al.*, 1975; Whipp & Wasserman, 1969). This is because, as has already been discussed, the requirement to clear CO_2 is not the sole determinant of the $\dot{V}_E$ requirement. For example, an increase in physiological dead space between tests would increase the required $\dot{V}_E$ to maintain $\dot{V}_A$ and clear the same level of CO_2. Thus meaning that the $\dot{V}_E$ - $\dot{V}CO_2$ relationship would operate at an increased $\dot{V}_E$-intercept, for 'normal' control of the exercise hyperpnoea to be in operation.

As discussed earlier it is P_aCO_2 rather than P_aO_2 or pH_a (though pH_a is intrinsically linked to P_aCO_2) that is the variable most directly 'regulated'. Due to considerations of subject recruitment no direct measurement of P_aCO_2 was made, however breath-by-breath measurements of $P_{ET}CO_2$ were made. Again, caution must be employed when viewing the response of $P_{ET}CO_2$ to exercise, especially with regard to the behaviour of P_aCO_2. Too often assumptions about P_aCO_2 are drawn directly from $P_{ET}CO_2$, this can be dangerous as not only is $P_{ET}CO_2$ likely to, usually, overestimate P_aCO_2 it is unlikely to do so consistently. This is because $P_{ET}CO_2$ is the product of several factors (see page 226 for discussion).

Therefore any conclusion on the behaviour of P_aCO_2, based solely on $P_{ET}CO_2$ should be viewed with considerable caution.

However, while no quantitative assumptions can be made about P_aCO_2 based on $P_{ET}CO_2$, as long as the intensity domain in which the subject is exercising is known the response profile for $P_{ET}CO_2$ across a test can be used to infer whether or not it is possible P_aCO_2 is being correctly regulated. This can be equated to whether or not $\dot{V}_E$ is being controlled normally. From the three components of $P_{ET}CO_2$ it is possible to relate its behaviour to P_aCO_2 or vice versa. If P_aCO_2 does not change, the 'normal' situation during moderate exercise, then component 1 (see page 226) is likely to have the same influence regardless of the work-rate, as long as it is $<\hat{\theta}_L$. As the work-rate is increased the alveolar slope (phase II of the capnogram) should increase (component 2 page 226) leading to an increased $P_{ET}CO_2$, if breathing frequency does not increase substantially. For the moderate range of work-rates ($<\hat{\theta}_L$) breathing frequency is unlikely to change substantially. Therefore, for increases in work-rate from one moderate work-rate to another where P_aCO_2 remains unaltered $P_{ET}CO_2$ should increase. Or more importantly, during the transition from one sub-$\hat{\theta}_L$ work-rate to a higher one where $P_{ET}CO_2$ increases, this would not be inconsistent with P_aCO_2 having been maintained at or close to resting levels. The reverse also being true, decreases in work-rate accompanied by a small decrease in $P_{ET}CO_2$ may be indicative of an unaltered P_aCO_2.

From figure 5.13 the pattern of $P_{ET}CO_2$ response to the ziggurat protocol appears very similar in nature to that described above. The trend for the mean response

being a stepwise increase of 1-2mmHg for each on-transition, and a similar decrease for each off-transition, although there was actually no significant difference in $P_{ET}CO_2$ across the duration of the ziggurat tests. This is not inconsistent with P_aCO_2 being 'normally' regulated close to resting levels. The variation from test 1 to test 2, which is actually more marked in the controls, is still consistent with the regulation of P_aCO_2 as a day to day variability in $P_{ET}CO_2$ of 1.1 mmHg would be expected (Crosby & Robbins, 2001).

Furthermore, while it is likely that P_aCO_2 was not being regulated around resting levels in the tests where $P_{ET}CO_2$ fell during work-rate 3, these were all supra-$\hat{\theta}_L$ work-rates. Therefore, the expected pattern of response would have been hyperventilation as an attempt to lower P_aCO_2 and constrain the fall in pH_a (Rausch *et al.*, 1991; Sutton & Jones, 1979; Wasserman & Casaburi, 1991; Wasserman & Whipp, 1975; Wasserman *et al.*, 1967). The outcome of this in terms of $P_{ET}CO_2$ would be a reduction, as P_aCO_2 is reduced and breathing frequency is increased. So the response of these individuals (subjects 2 and 5) is still consistent with a 'normal' control of the exercise hyperpnoea.

However, while the response pattern of $P_{ET}CO_2$ is not inconsistent with regulation of P_aCO_2, and hence with a 'normal' control of the exercise hyperpnoea, it does not allow any formal conclusion to be drawn. A more robust indicator of the behaviour of P_aCO_2 is P_ACO_2, as an equilibrium is reached between P_aCO_2 and P_ACO_2 during the steady-state of moderate intensity ($<\hat{\theta}_L$) exercise. From a continuous on-line recording of respired PCO_2 it is possible to reconstruct the alveolar PCO_2 profile, and hence calculate P_ACO_2 (DuBois *et al.*,

1952). Using P_ACO_2 as a surrogate for P_aCO_2 it is clear from figure 5.14 that P_aCO_2 has been maintained at a constant level throughout the moderate exercise performed during the ziggurat tests. However, as it is not possible to calculate P_ACO_2 in this way at rest it is not possible to say that P_aCO_2 has been regulated at its resting level (DuBois *et al.*, 1952). Therefore, on the basis of P_ACO_2, the naïve subjects appear able to control $\dot{V}_E$ accurately enough to maintain P_aCO_2 throughout a range of work-rates.

5.6.3 LTM as a controller of the exercise hyperpnoea

There is no evidence in terms of absolute $\dot{V}_E$, the linking of $\dot{V}_E$ to $\dot{V}CO_2$ or in the outcome of $\dot{V}_E$ (the regulation of P_aCO_2, based on both $P_{ET}CO_2$ and P_ACO_2) to suggest that the lack of an 'exercise-memory' had any effect on the control of the exercise hyperpnoea in these cycling naïve subjects. Can it be concluded from this that LTM is not a primary controller of the exercise hyperpnoea? No; firstly, while there was no evidence of a role for LTM in the control of phase II or III exercise $\dot{V}_E$ the experimental paradigm employed in this study does not allow for any conclusion to be drawn for phase I. The rapid increase in $\dot{V}_E$ at exercise onset is considered by many to be the most likely to be under central neural control and therefore the most likely to be influenced by LTM. Secondly, and possibly most important, are the repeated demonstrations that in the absence of any one of the classical control mechanisms the exercise hyperpnoea appears essentially 'normal' (e.g. Adams *et al.*, 1984; Banner *et al.*, 1988; Shea *et al.*, 1993; Wasserman *et al.*, 1975). Therefore, this redundancy exhibited by the

ventilatory control system could explain the naïve subjects' ability to control $\dot{V}_E$ accurately and correctly regulate P_aCO_2. Although, it should be asked what benefit a learned control of breathing would provide if in fact these subjects were utilising 'normally' redundant systems so efficiently. Therefore, the most appropriate conclusion seems to be that learning the ventilatory response to a particular mode of exercise is not a required part of the exercise ventilatory control

5.7 Future directions

Two separate ideas stem from this study. Firstly the ziggurat profile for constant load exercise tests, alongside the standard square-wave, should perhaps become a more commonly used tool of the exercise physiologist and clinician. As it potentially contains substantially more information regarding the performance of the cardio-respiratory system during exercise than in the single transition observed from the square-wave. This is without any substantial increase in subject effort or time demands.

Secondly, the issue is raised of how to further knowledge of LTM. A similar group of subjects to those used during this study may provide ideal pupils to teach a new ventilatory response to, possibly specifically for phase I. A potential source of such subjects that was proposed during this study, although subsequently rejected by the university ethics committee, would be those visually impaired from birth. It was proposed that this subset of the population is likely to

show a higher incidence of being generally exercise naïve and specifically cycling naïve individuals. However, the concerns outlined in the previous chapter regarding the conditioning paradigm require to be addressed first.

Finally, it is of course possible that the subjects recruited were simply not naïve enough. Exercise forms such a basic part of day to day life walking, climbing stairs, carrying shopping etc that despite the choice of subject group and mode of exercise it is possible that a learned response had been developed through daily activity. Therefore, how can this question be resolved? Clearly the progressive study of children through their development to look for the emergence of a learned response is not practical. However, groups constrained by situation to have never performed any lower limb physical activity or any physical activity, i.e. individuals who have been paraplegic or tetraplegic from birth, may be capable of providing an insight.

5.8 Conclusions

In conclusion, it has not been possible to demonstrate any role for learning in the control of the exercise hyperpnoea in subjects lacking a specific 'exercise-memory'. On face value this suggests that another mechanism appears to account for the close linking of $\dot{V}_E$ to $\dot{V}CO_2$ which results in accurate regulation of P_aCO_2 at or close to its resting levels. However, due to the redundancy typically exhibited by the ventilatory control system (e.g. Swanson *et al.*, 1992;

Yamamoto, 1980) it is possible that under 'normal' conditions LTM may play an active role somewhere in the control of the exercise hyperpnoea.