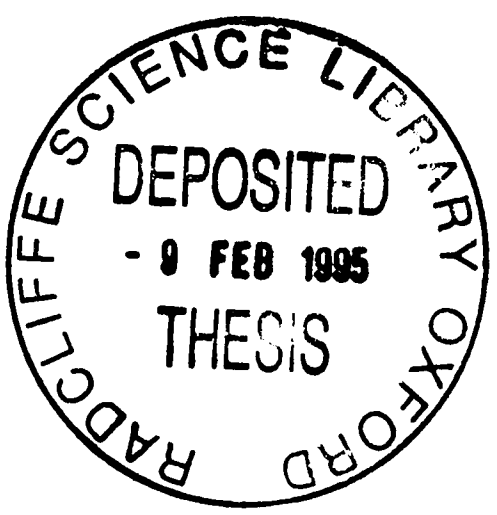
THE EFFECTS OF EXERCISE ON THE CHEMICAL CONTROL OF BREATHING IN MAN

Thesis submitted for the degree of Doctor of Philosophy at the University of Oxford



Jaideep Jagdeesh Pandit
Corpus Christi College
Hilary Term 1993

To My Parents

and, as ever, to Meghana

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Contents

Abstract

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The Effect of Exercise on the Chemical Control of Breathing in Man

This thesis is concerned with the chemical control of breathing during exercise in humans.

Chapter 1 reviews some of the relevant studies in animals and humans.

Chapter 2 describes the experimental apparatus and the technique of dynamic end-tidal forcing performed using a computer-controlled gas-mixing system.

Chapter 3 describes a study of the effects of sustained hypoxia on ventilation during steady exercise. The acute ventilatory response to hypoxia (AHR) was increased during exercise as compared with rest, but the magnitude of the subsequent decline in ventilation (HVD), expressed as a fraction of the AHR, was reduced. A simple model of the hypoxic peripheral chemoreflex is proposed, in which the mechanisms underlying AHR and HVD are functionally separate and can be independently modulated by external factors.

Chapter 4 assesses changes in peripheral chemoreflex sensitivity to hypoxia in terms of the degree of decline in AHR measured in the resting periods shortly after prior conditioning periods of hypoxia and/or exercise. At rest, a second AHR measured 6 min after a period of sustained hypoxia had declined by 30% as compared with the initial AHR. In contrast, the AHR measured in the resting period after a period of sustained hypoxic exercise was only 11% smaller in magnitude than the AHR measured after a period of euoxic exercise. The results suggest that the degree to which hypoxic sensitivity declines during sustained hypoxia is genuinely attenuated, rather than masked, by exercise.

Chapter 5 describes the changes in respiration during prolonged exercise breathing air with and without added CO_2. During prolonged poikilocapnic exercise, ventilation remained constant, but metabolic CO_2 production, respiratory quotient and end-tidal P_{CO2} declined; a result which suggests that in man, ventilation can be dissociated from the CO_2 flux. During hypercapnic exercise, ventilation progressively increased; this was interpreted as being due to a correction by end-tidal forcing of the natural tendency for end-tidal CO_2 to decline, together with an independent effect of CO_2 *per se* on the ventilation.

Chapter 6. Electrical muscle stimulation was used as means of inducing non-volitional exercise. Electrically-induced exercise increased the AHR as compared with rest, and with voluntary exercise at matched external work rate. The AHRs during electrical stimulation and voluntary exercise matched to the internal work rate were similar.

Chapter 7. Electrical muscle stimulation was used in paraplegic subjects in whom there would be no neural control of exercise. Electrically-induced exercise increased the AHR as compared with rest. When compared with the data from Chapter 6, the results suggest that the observed increase in AHR during normal voluntary exercise can be wholly accounted for by the increase in metabolic CO_2 production, or closely related factors.

Chapter 8 presents a brief summary of the findings in this thesis.

CHAPTER 1

INTRODUCTION

Those who came before us did much,
but they did not exhaust the study.
Much remains and will remain.

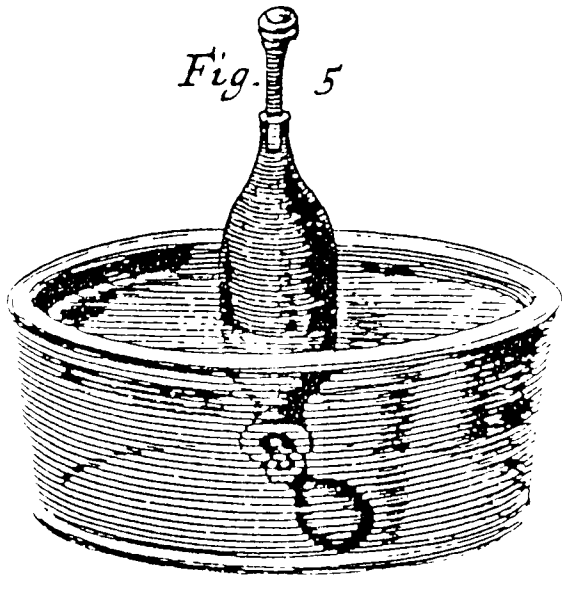
Seneca

The main objectives of this thesis are to investigate the ventilatory effects of sustained hypoxia and hypercapnia during steady exercise and also to examine the role of nervous control of exercise (both reflex and voluntary) in mediating changes in hypoxic chemoreflex sensitivity. This Introduction discusses the context of this work in the light of previous studies.

Early Observations

Before the discovery of oxygen in 1779, very little of practical importance concerning the role and regulation of breathing was, or could be known. It was self-evident that the act of breathing was necessary for life; that during exercise, the depth and frequency of breathing were increased, and that the exercise capacity of people who suffered from difficulties with their breathing was very limited.

However, some of the experiments conducted by Boyle, Lower, Mayow, Willis and others about one hundred years before the discovery of oxygen are worthy of mention for two reasons. First, because their elegant design brought them close to discovering oxygen, and secondly, because much of the work was conducted in Beam Hall on Merton Street in Oxford, where I was housed as an undergraduate. Figure 1.1 shows one set of experiments conducted by Mayow (1674): the original apparatus is now housed in the Museum of the History of Science, a few hundred yards from Beam Hall. When an animal was placed inside the up-turned glass vessel, the water level inside the vessel slowly rose. A similar result was obtained with a burning candle, instead of an animal. If a candle and animal were placed inside together, the animal perished sooner than when placed alone in the vessel, and the water level rose more rapidly. The result owed much to the facts (unknown to Mayow) that the respiratory quotient is less than 1; that carbon dioxide is more soluble in water than oxygen, and that any water vapour produced condensed on the glass. I have also reproduced a contemporary sketch of Beam Hall (Fig. 1.1): my old rooms look out over the garden, at the back.



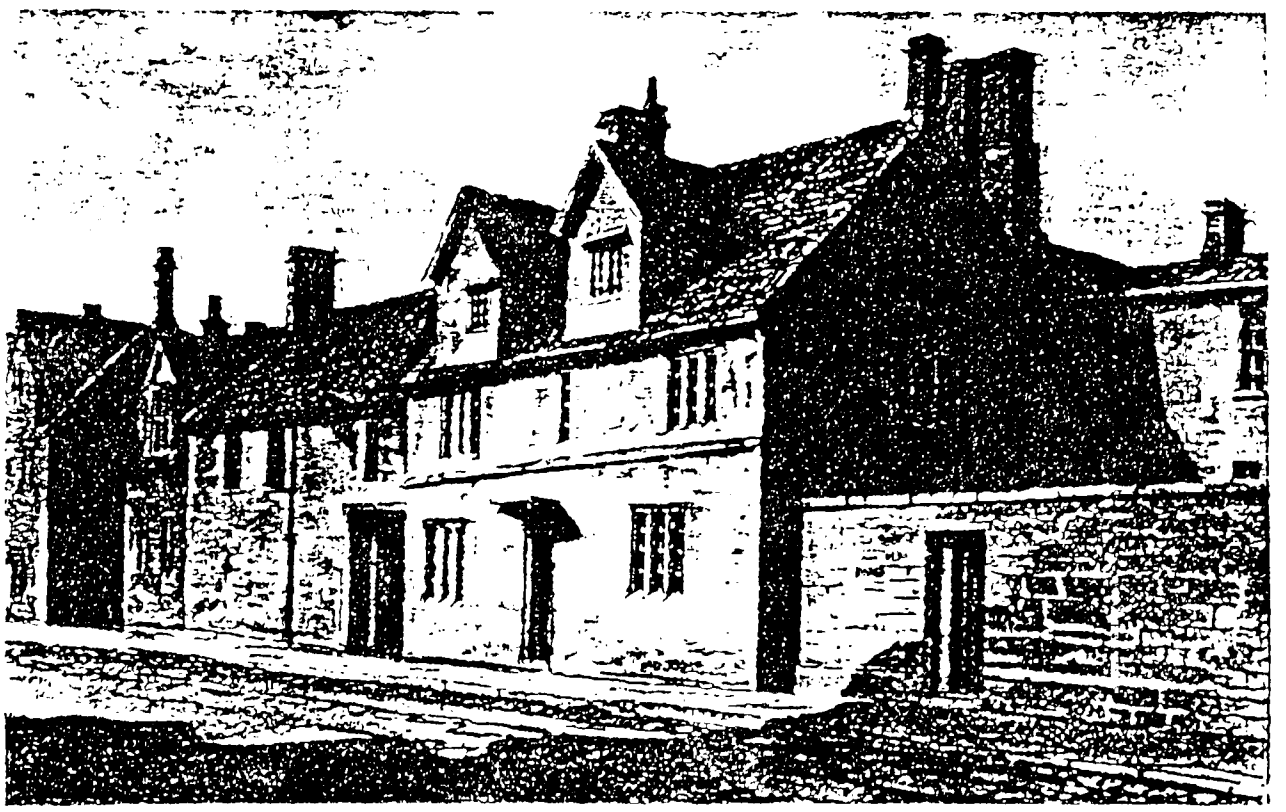


Figure 1.1. *Top panel*: Mayow's apparatus, showing inverted glass vessel with a candle (labelled Fig.5) and a small animal inside (labelled Fig.6). See text for further explanation.
Bottom panel: Beam Hall, on Merton Street, Oxford.

It was Lavoisier who, after noticing that combusted metals actually gained rather than lost weight, conducted a series of experiments which culminated in the discovery, and on 23rd November 1779, the naming of oxygen. Importantly, Lavoisier found that the quantity of heat produced by an animal in the course of an experiment approximated the quantity produced by burning coal, for an equal amount of oxygen consumed (Lavoisier and Laplace, 1780). Essentially, the process of "cellular respiration", or metabolism, was distinguished from "ventilation", or the act of breathing. In his writings he implied that there was a link between the two, documenting an oxygen consumption of about 1 L/min during exercise in man, compared with only 300 ml/min at rest (Lavoisier, 1790).

Lavoisier's work emphasised that the main function of ventilation was gas exchange, but the precise factors which *controlled* the ventilation remained unclear. It was not until 1868 that Pfluger (confirming an earlier suggestion by Lavoisier) found that hypoxia stimulated ventilation. Soon after that, Walter (1877) described fixed acid to be a stimulant. Miescher-Rusch (1885) made one of first attempts to integrate the various chemical factors that had been found to stimulate breathing, but was so impressed by the ventilatory sensitivity to CO_2, that he concluded CO_2 to be the main factor regulating breathing, concluding: "*...so carbonic acid (CO_2) spreads its protective wings over the oxygen needs of the body.*"

Many of these studies were qualitative, however, and it was not until the work of Haldane that the quantitative tradition was revived. Haldane's contribution to *method* was that of accurate measurement: in particular, the accurate measurement of the gas composition of alveolar air in man, which he realised reflected the gas composition of arterial blood. Haldane's important *result* was that, under a variety of conditions, including hypoxia and exercise, it was the alveolar fraction of CO_2, not of O_2, which was remarkably constant (Haldane and Priestley, 1905). Realising that the ventilation is extremely sensitive to a rise in P_{CO2} (Miescher-Rusch, 1885), Haldane concluded that this constancy reflected the fact that ventilation acted to regulate the P_{CO2}. He had, in effect, described a feedback process in respiratory control between ventilation and CO_2. Furthermore, with Douglas *et al.* (1913), he found that during exercise below the level at

which lactate is produced, alveolar ventilation is almost perfectly matched to the metabolic production of CO_2 ($\dot{V}_{CO2}$), so as to keep alveolar P_{CO2} constant. Haldane is said to have remarked to Douglas: "*Here is the link between ventilation and the metabolism*". In other words, if at any time CO_2 production increases and the ventilation does not initially rise proportionally with it, then the P_{CO2} will rise to stimulate the ventilation, which constitutes a control system. A situation in which this relationship may not hold, however, is described in Chapter 5.

The Modern View

Integration of Chemical Stimuli

Clearly it became important to describe quantitatively the relationships between the stimuli discussed above, and how they interacted with each other both at rest and during exercise. It was Cunningham and Lloyd who provided a mathematical description of the steady-state ventilatory responses to hypoxia and hypercapnia in man (Lloyd *et al.*, 1958), according to an equation which has come to be known as the Lloyd-Cunningham equation:

$$V_E = D(P_{CO_2} - B) + DA\frac{(P_{CO_2} - B)}{(P_{O_2} - C)}$$

The value of constants A, B, C and D can be altered by various interventions, such as administration of drugs, raised body temperature, and exercise. The original equations were defined empirically from studies in conscious humans, but taken together with information from animal (Leusen, 1954; Mitchell *et al.*, 1963; Heymans and Heymans, 1927; Heymans *et al.*, 1930; Van Beek *et al.*, 1983) and other human studies (Guz *et al.*, 1966*a* and 1966*b*; Lugliani *et al.*, 1971; Miller *et al.*, 1974), the principles of the equation may be stated in words as:

1. hypoxia only stimulates ventilation, and this is via the peripheral chemoreflex.

2. CO_2 stimulates ventilation both via the central chemoreflex, and via the peripheral chemoreflex.

3. at the peripheral chemoreceptors, CO_2 and hypoxia interact multiplicatively.

4. the drive from the peripheral and central chemoreflexes is additive in its contribution to the total ventilation.

Before turning to the properties of the chemoreflexes during exercise, it is worth considering some to the limitations of this understanding, even for the resting state.

Limitations to Lloyd-Cunningham equation

Increasingly, it has become apparent that the above understanding may be flawed. For example, Robbins (1988) suggested that interaction between peripheral and central chemoreflex drives may be more than additive. Smith *et al.* (1993) have recently demonstrated a stimulation of ventilation in awake goats in response to central hypoxia. Such findings may eventually warrant a re-assessment of some of the four points listed above.

It must also be emphasised that the Lloyd-Cunningham equation is a description of steady-state conditions and as such, cannot be applied to describe the dynamic ventilatory responses to step-changes in P_{CO2} or P_{O2} (although modifications of the equation incorporating time constants and pure delays have been developed; Swanson and Bellville, 1975). Nor does the equation predict that hypoxia can depress the ventilation as well as stimulate it. It is to this phenomenon that we now turn.

The Response to Sustained Hypoxia

In adult man at rest, the response to hypoxia is actually biphasic. Initially, there occurs a rapid rise in ventilation, known as the acute hypoxic response or AHR. Ventilation then declines over the next 20-30 min. This decline is known as hypoxic ventilatory decline, or HVD. The biphasic response to hypoxia is not a new observation, but was previously ascribed to hypocapnia secondary to hyperventilation (D.J.C Cunningham, personal communication). The interesting finding is that HVD still occurs when the end-tidal P_{CO2} (PET_{CO2}) is held constant (Weil and Zwillich, 1976; Easton *et al.*, 1986). The development of the technique of *end-tidal forcing* (Chapter 2) has enabled PET_{CO2} to be held constant with a greater degree of accuracy, and so allowed a more detailed examination of the phenomenon of HVD.

What is the underlying mechanism of HVD?

If it is accepted that the total ventilation is the sum of the various component drives to it, then in general terms, the decline in ventilation with sustained hypoxia may be due to a decline in the activity of the peripheral chemoreflex, or of the central chemoreflex, or due to depression of other central mechanisms controlling breathing. It may also be due to combinations of these processes. Figure 1.2 shows some of the possibilities diagrammatically. The precise mechanism underlying HVD remains unclear, but there do appear to be important differences between animals and humans.

Animal studies

A decline in ventilation with sustained hypoxia occurs in awake (Vizek *et al.*, 1987) and anaesthetised cats (Olievier *et al.*, 1982; van Beek *et al.*, 1984). When the peripheral and central chemoreceptors have been perfused separately, it appears that ventilation declines only with central hypoxia, and not with sustained peripheral hypoxia (van Beek *et al.*, 1984). The discharge from cat carotid bodies does not adapt with sustained hypoxia (Vizek *et al.*, 1987), which also suggests that HVD has a central

mechanism. Lee and Millhorn (1975) using a cross-perfusion technique in dogs, found that cerebral hypoxia resulted in a ventilatory depression which was independent of the peripheral chemoreceptor drive. This depression was also associated with a decrease in the central CO_2 sensitivity.

The results from rabbits and piglets, however, appear to differ from those for cats and dogs. The discharge from single afferent fibres from rabbit carotid bodies declines steadily with sustained hypoxia (Kaiying *et al.*, 1990), and a similar result obtains in piglets (Mulligan and Bhide, 1989). This serves to emphasise the importance of species differences in the mechanisms underlying HVD.

Human studies

Easton *et al.* (1988) proposed that hypoxia acted centrally in humans to generate HVD, as it does in the cat. They felt, intuitively, that the slow recovery of AHR after a period of sustained hypoxia reflected the accumulation of an inhibitory neurotransmitter in the brain with hypoxia, which was slowly washed out or metabolised on return to euoxia. The brain concentrations of the neurotransmitter adenosine increase with hypoxia and administration of exogenous adenosine in animals causes prolonged ventilatory depression (Winn *et al.*, 1981). Georgopoulos *et al.* (1989*b*) found that administration of aminophylline, an adenosine blocker, attenuated the magnitude of HVD in humans, consistent with the suggestion that adenosine may be the chemical involved.

Gamma-aminobutyric acid (GABA) is another inhibitory neurotransmitter to respiration whose brain concentrations increase with hypoxia (Iversen *et al.*, 1983). Dahan and Ward (1991) have shown that administration of midazolam, a drug which potentiates the action of GABA, increases the magnitude of HVD in humans. However, Nagyova *et al.* (1993) have failed to reproduce these results with midazolam.

While such studies show that drugs may modulate HVD in humans, they do not confirm that the site at which HVD is generated is necessarily central. Other studies in man, in which the changes in chemoreflex activity with sustained hypoxia have been assessed (albeit in more indirect manner than is possible in anaesthetised animals), appear

to suggest that HVD in man has a peripheral chemoreflex origin.

Figure 1.2 shows diagrammatically some of the ways in which HVD might be generated by different combinations of changes in chemoreflex activity with sustained hypoxia.

If in humans, HVD is due to a decline in the activity of the central chemoreflex, it might be predicted that the resulting ventilatory response shows a pattern similar to that outlined in Fig. 1.2A. Essentially, the rapid responses, mediated by the peripheral chemoreceptors, into hypoxia (the on-response) and out of hypoxia (the off-response) would be of similar magnitude, and also the initial post-hypoxic ventilation would fall well below that measured prior to any hypoxia (*i.e*, there would be an "undershoot"). This has been observed in the anaesthetised cat (Berkenbosch *et al.*, 1987). Indeed, the occurrence of undershoot would hold for any other mechanism involving a time-dependent decline in central activity (Fig. 1.2C and D).

If, on the other hand, HVD in man was brought about by a decline in peripheral chemoreflex activity, then it might be predicted that the magnitudes of the rapid responses would be asymmetric, and there would be no undershoot (Fig. 1.2B). In other words, the rapid response to a step out of hypoxia is as informative of the sensitivity of the peripheral chemoreflex as that to a step into hypoxia. Khamnei and Robbins (1990) showed that, in fact, the magnitudes of the on and off-responses were unequal and therefore the results were most consistent with the hypothesis that it is the activity of the peripheral chemoreflex which declines with sustained hypoxia (Fig. 1.2B).

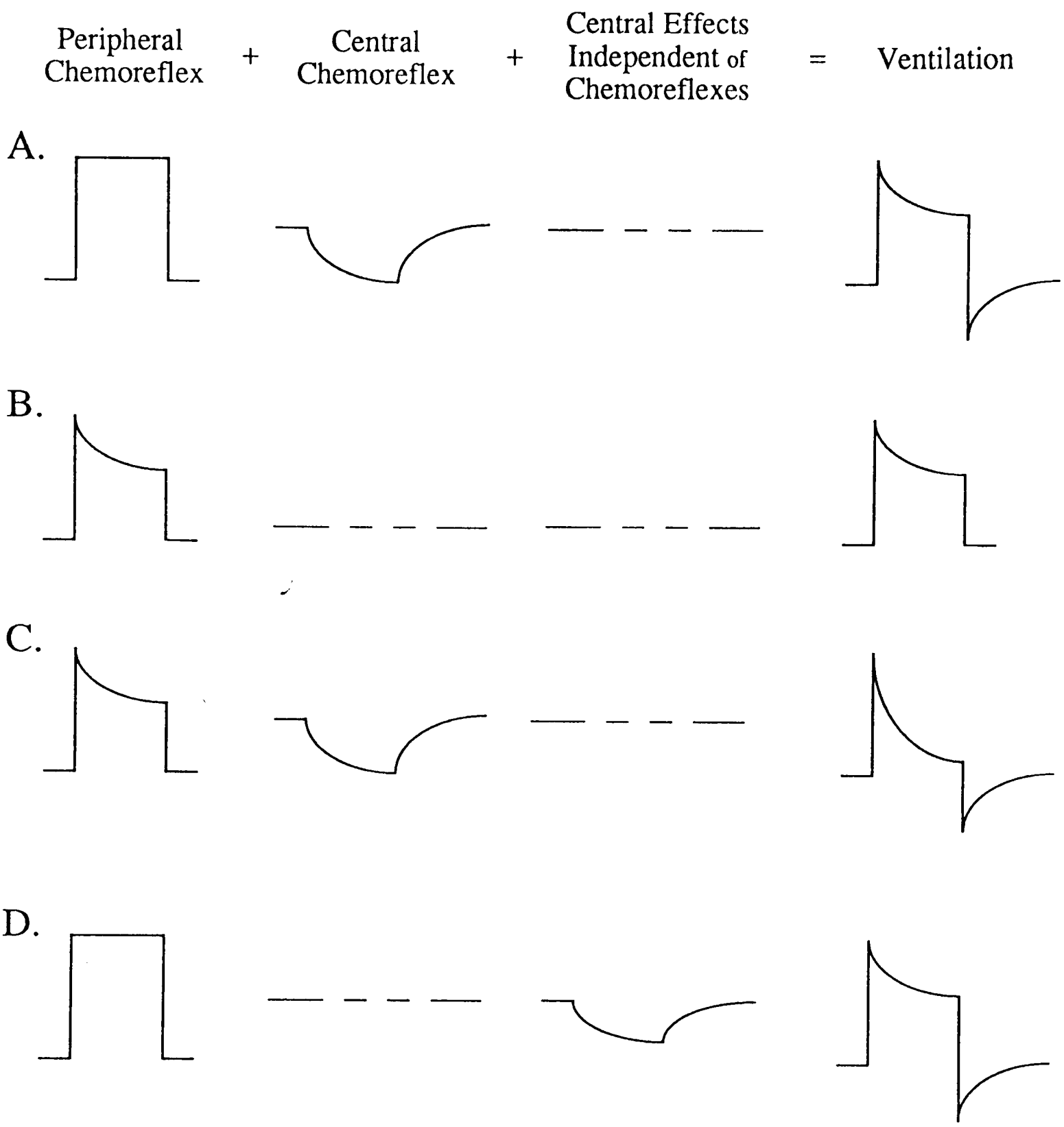


Figure 1.2. Diagrammatic representation of possible changes with time in chemoreflex activity in response to a sustained step-reduction in P_{O2}. Panel A: decline in central chemoreflex activity only; panel B: decline in peripheral chemoreflex activity only; Panel C: decline in both peripheral and central chemoreflex activity; Panel D: decline in other central mechanisms involved in respiratory control. The central assumption is that the contributions of the chemoreflexes are additive in their effects on the total ventilation (*cf.* Lloyd-Cunningham equation). Other combinations, not shown, are also possible.

Easton *et al.*(1988) and Khamnei (1989) observed that the recovery from a period of sustained hypoxia required up to one hour of air-breathing, such that the magnitude of a subsequent response to hypoxia was smaller than the magnitude of the initial response. Bascom *et al.* (1992) and Berkenbosch *et al.* (1992) exploited this observation to assess the degree of decline in hypoxic chemoreflex sensitivity by measuring the AHR 5-6 min after a period of sustained hypoxia. Consistent with the findings of Khamnei and Robbins (1990), the chemoreflex sensitivity measured in this way declined by about 30%.

The change in chemoreflex sensitivity has been documented most directly by Bascom *et al.* (1990), who applied pulses of *extra* hypoxia during a period of sustained hypoxia. The hypothesis was that, since the rapid response to a brief pulse of hypoxia is mediated by the peripheral chemoreflex, then any decline in the magnitude of the response to successive pulses would suggest peripheral, rather than central, chemoreflex modulation. The result of Bascom *et al.* was that the magnitude of response to successive pulses of hypoxia did decline. Furthermore, the response to successive pulses of CO_2 during sustained hypoxia declined to a lesser degree, indicating a differential effect of HVD on the decline in sensitivity to hypoxia and to CO_2. Since the carotid sinus nerve only conveys information to the brainstem about the overall *magnitude* of stimulus to the carotid body, rather than the *type* of stimulus (*e.g*, hypoxia, hypercapnia or both; Lahiri and DeLaney, 1975), it was argued that such a differential effect on hypoxic and CO_2 pulses indicated that the mechanism underlying HVD was situated peripherally in the carotid body itself, before the two stimuli converged, rather than more centrally in the chemoreflex pathway. The finding by Honda (1992) that there was no depression of ventilation by hypoxia in patients with carotid body resection lends further support to this notion.

Correlation of HVD with AHR. A constant relationship between the magnitude of the HVD and the AHR has been suggested, such that individuals with a large AHR also exhibit a large HVD (Easton *et al.*, 1986). Furthermore, interventions which increase the peripheral sensitivity to hypoxia and so increase the AHR such as almitrine (Georgopoulos

et al., 1989*c*), hypercapnia (Georgopoulos *et al.*, 1989*a*; Khamnei and Robbins, 1990) and extra hypoxia (Bascom *et al.*, 1992) also increase the magnitude of the subsequent HVD. When the magnitude of AHR is reduced by somatostatin (Maxwell *et al.*, 1986), the HVD is decreased (Filuk *et al.*, 1988). All these observations lend support to the notion that the mechanism underlying HVD is in some manner related to changes in hypoxic chemoreflex sensitivity, and taken together with the studies outlined above, are consistent with the hypothesis that HVD in humans is brought about by a time-dependent decline in peripheral hypoxic chemoreflex activity.

One agent which has attracted some interest is dopamine (and also its antagonist domperidone). Dopamine D_2 receptors are present in the carotid body (Mir *et al.*, 1984) and dopamine is found as a naturally-occurring substance in the carotid body (Fitzgerald *et al.*, 1983). Exogenous administration of low doses of dopamine has been found to inhibit the responses to hypoxia and hypercapnia in man (Ward and Bellville, 1982, 1983); while domperidone increases the response to hypoxia and hypercapnia (Jahaveri and Guerra, 1990). Bascom *et al.* (1991) found that the results of administration of dopamine and domperidone did not conform to the established pattern of a link between AHR and HVD. Exogenous dopamine reduced the AHR, but the absolute magnitude of HVD was unaffected (increasing the ratio of HVD to AHR); domperidone increased AHR but left HVD unaffected (thus reducing the ratio of HVD to AHR).

Steady exercise is known to increase the acute ventilatory response to hypoxia (see below). In the light of the observations above the question arises of whether exercise increases HVD (*e.g*, like almitrine) or whether HVD is unaffected (*e.g*, like domperidone). Dahan (1990) studied the possible effects of exercise on HVD, performing a total of 11 experiments on six subjects. He found that in 8 out of the 11 experimental periods, HVD did not occur, raising the possibility that exercise, unlike any other modulator of peripheral hypoxic sensitivity, actually abolishes hypoxic ventilatory decline. The effect of steady exercise on HVD, and the interpretation of the results in the light of previous work are examined in Chapters 3 and 4 of this thesis.

The Ventilatory Response to Exercise

Three areas of exercise physiology that are commonly studied are first, the changes which occur during the transition from rest to exercise; secondly, the changes during incremental work tests both below and above the anaerobic threshold; and finally the control of breathing during moderate constant-load exercise in the steady state. It is this last state which has been studied in this thesis.

Properties of chemoreflexes during steady exercise

During moderate exercise, the ventilatory sensitivity to hypoxia is increased as compared with rest (Cunningham *et al.*, 1968; Weil *et al.*, 1972; Masson and Lahiri, 1974). In other words, constants A and C in the Lloyd-Cunningham equation, above, increase. It has been suggested that this represents an augmented drive from the peripheral chemoreceptors during exercise, and this has also been examined by measuring the fall in ventilation using pulses of high inspiratory oxygen during exercise (Dejours, 1957).

The response to added CO_2 during exercise is more controversial: some studies show that there is no greater increase in ventilation during exercise than there is at rest, and that only constant B in exercise is affected (Asmussen and Nielsen, 1957; Bhattacharyya *et al.*, 1970); while others suggest an increase in the CO_2 sensitivity during exercise, and that constant D is increased (Cummin *et al.*, 1986).

A problem arose for Haldane's suggestion that P_{CO2} was the link between metabolism and the control of ventilation: during steady exercise, P_{CO2} in the arterial blood was found to remain constant at the resting value (Wasserman, 1967). There thus appears to be no steady "error signal" to provide a stimulus to the ventilation. This was also true for the arterial P_{O2} (Dejours, 1964). Although slight changes in arterial blood gases have been occasionally reported to occur (Matell, 1973; Dejours, 1964), they remain insufficient to explain the full extent of exercise hyperpnoea, even when taken together with the measured increases in sensitivity to P_{O2} and P_{CO2} during exercise.

Two questions therefore become pertinent. First, what is the "function" of the increased sensitivity to hypoxia during exercise, if not to provide the drive to breathe? Secondly, if not P_{CO2}, what other links are there between the ventilation and the metabolism?

What is the function of the increased sensitivity to hypoxia?

There are a number of possibilities. Wasserman *et al.* (1979) have suggested that the increased hypoxic sensitivity is relatively unimportant in respiratory control since switching from air to 100% O_2 has no ***sustained*** effect on the ventilation during moderate exercise, and because the mean ventilation and arterial P_{CO2} of carotid body-resected subjects is unimpaired during steady exercise (Wasserman *et al.*, 1975; Whipp and Wasserman, 1980).

In contrast, Cunningham (1987) has argued cogently that the peripheral chemoreceptors detect the size of the metabolic load during exercise through their response to blood gas oscillations.

The increased hypoxic sensitivity during exercise may also be viewed, in engineering terms, as an increased sensitivity in a feedback loop at a time of heightened demand. This is advantageous to the system: during exercise, when demands are high, the ventilatory response to a fall in P_{O2} needs to be all the greater.

Whatever the function of the response or its likely advantages, it is true that, like the matching of ventilation to the metabolic CO_2 production or the relative constancy of the blood gases, the increase in hypoxic sensitivity characterises the normal response to moderate exercise in humans.

What are the other links between ventilation and metabolism?

There are several routes by which the metabolic rate may be signalled to the system controlling ventilation. They have been reviewed on numerous occasions (Dejours, 1964; Asmussen, 1967; Wasserman *et al.*, 1986; Cunningham *et al.*, 1986; Cunningham, 1987). In general terms, the routes may be classified as neural or humoral. The neural

pathway includes two separate entities: a cortical component and a reflex component arising from the exercising muscles. Some of these routes are discussed, briefly, below.

Neural drives to breathe in exercise. Krogh and Lindhard (1913) proposed that cortical irradiation to the muscles during exercise also governed the ventilatory response. Eldridge *et al.* (1981) modified this by arguing that although the cerebral cortex was not involved, ventilation was driven by signals originating in the hypothalamus.

A number of studies have shown that stimulation of different types of afferent fibres from the muscles and joints can stimulate ventilation (McCloskey and Mitchell, 1972; Tibes, 1974; Bennett, 1984). In a series of experiments using cross-perfusion techniques, Kao (1963) showed that ventilation increased in a dog whose hindlimbs were electrically-stimulated, even though the blood from the exercising limbs was diverted to a second dog. This ventilatory response was abolished by sectioning the lateral columns of the spinal cord, or by total cordotomy (but not by sectioning the dorsal columns). Furthermore, although ventilation increased in the second dog receiving the diverted blood from the first dog, it did not rise in proportion to the flux of CO_2. When the head and neck of this second dog were vascularly-isolated from the rest of the body, there was no rise in ventilation at all. Kao concluded that the appropriate ventilatory response was achieved primarily through the neural afferent pathway from the limbs, via the spinal cord.

Humoral drives to breathe in exercise. Geppert and Zuntz (1888) reported that stimulation of a dog's hindlimb caused ventilation to rise, even though the vagi and spinal cord were cut. They concluded that the muscles released a substance that was carried to the respiratory centre by the blood. Since then, this unidentified substance has been variously named respiratory x (Haggard and Henderson, 1920), hyperpnein (Henderson, 1938), or the anaerobic work substance (Asmussen and Nielsen, 1946). This last term is somewhat of a misnomer since it appears to be released (by the properties it confers on the system) at intensities of exercise below the anaerobic threshold. Many specific humoral factors

have been proposed to be involved in the matching of ventilation to $\dot{V}_{CO2}$ and the increase in hypoxic sensitivity. Amongst these have been blood-gas oscillations (Yamamoto and Edwards, 1960), catecholamines (Cunningham *et al.*, 1963), arterial plasma potassium (Paterson, 1989), and the CO_2 flux itself (Yamamoto and Edwards, 1960; Phillipson *et al.*, 1981).

Whilst not identifying a specific humoral factor, Asmussen *et al.* (1943), Adams *et al.* (1984*a*) and Brice *et al.* (1988*a*) argued that in normal humans, the cortical drive to exercise was not necessary to achieve a normal ventilatory response to exercise. Using electrical stimulation of the leg muscles to eliminate the influence of cortical drive, they found that the steady-state ventilation in euoxia was still matched to metabolic CO_2 production. Furthermore, the last two groups of investigators repeated the experiments with paraplegic subjects, and concluded that reflex neural inputs from the limbs were also unnecessary for this normal matching of ventilation to $\dot{V}_{CO2}$ (Adams *et al.*, 1984*b*; Brice *et al.*, 1988*b*). These results in humans appear to contrast with those in dogs (Kao, 1963).

However, the role of voluntary control of exercise, or of neural afferents from the limbs in mediating the increase in response to hypoxia normally seen during exercise remains unclear.

Objectives

The main objectives of this thesis are:

1. to investigate the ventilatory response to sustained hypoxia during steady exercise (Chapters 3 and 4).

2. to investigate the ventilatory response to sustained, mild hypercapnia during steady exercise (Chapter 5).

3. to examine the role of voluntary control of exercise in increasing the ventilatory sensitivity to hypoxia (Chapter 6).

4. to examine the role of neural afferents from the working limbs in increasing the ventilatory sensitivity to hypoxia (Chapter 7).

Chapter 2

Experimental Apparatus and Methods

The less that people know about how parliaments pass laws and how factories make sausages, the easier they will sleep at night.

Otto von Bismarck

Introduction

This chapter describes the experimental techniques and apparatus used in the studies presented in subsequent chapters. The bulk of this chapter is concerned with the measurement of respiratory variables and the technique of dynamic end-tidal forcing (DEF). This description is divided into the apparatus used for data acquisition and the apparatus used to supply the appropriate inspired gas mixture to the subject, referred to as the gas-mixing system. The calculation of metabolic gas exchange is also described. A section at the end of the chapter deals with the analysis of blood samples, and another with the apparatus used for electrical leg stimulation. Finally, the stability of some of the measuring apparatus is assessed, and a comment is included on the statistics used in the thesis.

Many of the methods have been described in detail by Khamnei (1990), and are the subject of a number of previous publications (Robbins *et al.*, 1982; Howson *et al.*, 1986; Howson *et al.*, 1987). They have also been reviewed by Bascom (1991) and Clement (1992). The methods and equipment used in this thesis are essentially the same, and to avoid repetition, only the salient points, and aspects not previously covered, will be discussed.

Subjects

Detailed descriptions of the subjects used in these studies are to be found in the appropriate chapters. All subjects gave informed, written consent before each experiment but were unaware of the specific objectives of the experiments. In all experiments, subjects breathed through a mouthpiece with the nose occluded, and were distracted with a radio and/or reading material. A television also became available in some of the later studies. All the studies presented in this thesis had prior approval of the Central Oxford Research Ethics Committee.

Overview of Dynamic End-Tidal Forcing

Dynamic end-tidal forcing is essentially a computer-controlled system which, by appropriate adjustment of the inspired gases, enables the end-tidal (and hence arterial) partial pressures to be held at any set of values desired by the experimenter. When using the system, the subject's ventilation ceases to act to affect the end-tidal gas levels: these are now controlled by the computers and gas-mixing system. This therefore allows the ventilatory responses to be examined in a regulated manner. Dynamic end-tidal forcing might be viewed as a "respiratory physiologist's voltage clamp", or in terms of systems engineering, it may be said to "open the feedback loop" between the ventilation and the blood gases. Experimentally, dynamic end-tidal forcing may be used to expose the subject to precise stimuli, such as, for example, step-changes in P_{O_2}, whilst at the same time keeping P_{CO_2} constant. The resulting ventilation is therefore a response purely to the hypoxia, and is not confounded by any resulting hypocapnia. Alternatively, because the system can effect very rapid changes in end-tidal values, ramp or sine wave stimuli may be generated to study the dynamics of the ventilatory response. In the experiments described in this thesis, the system is used to hold the end-tidal P_{CO_2} constant, usually while reducing the level of P_{O_2}.

Data Acquisition System

This section describes the components, calibration and operation of the data acquisition system. A schematic representation of the dynamic end-tidal forcing system is shown in Fig. 2.1.

Apparatus

The principle elements of the data acquisition system are the mouthpiece assembly, the turbine device and associated ventilation measurement module, the pneumotachograph and associated micromanometer, the mass spectrometer, the multi-channel amplifier, the pen recorder and the data acquisition computer.

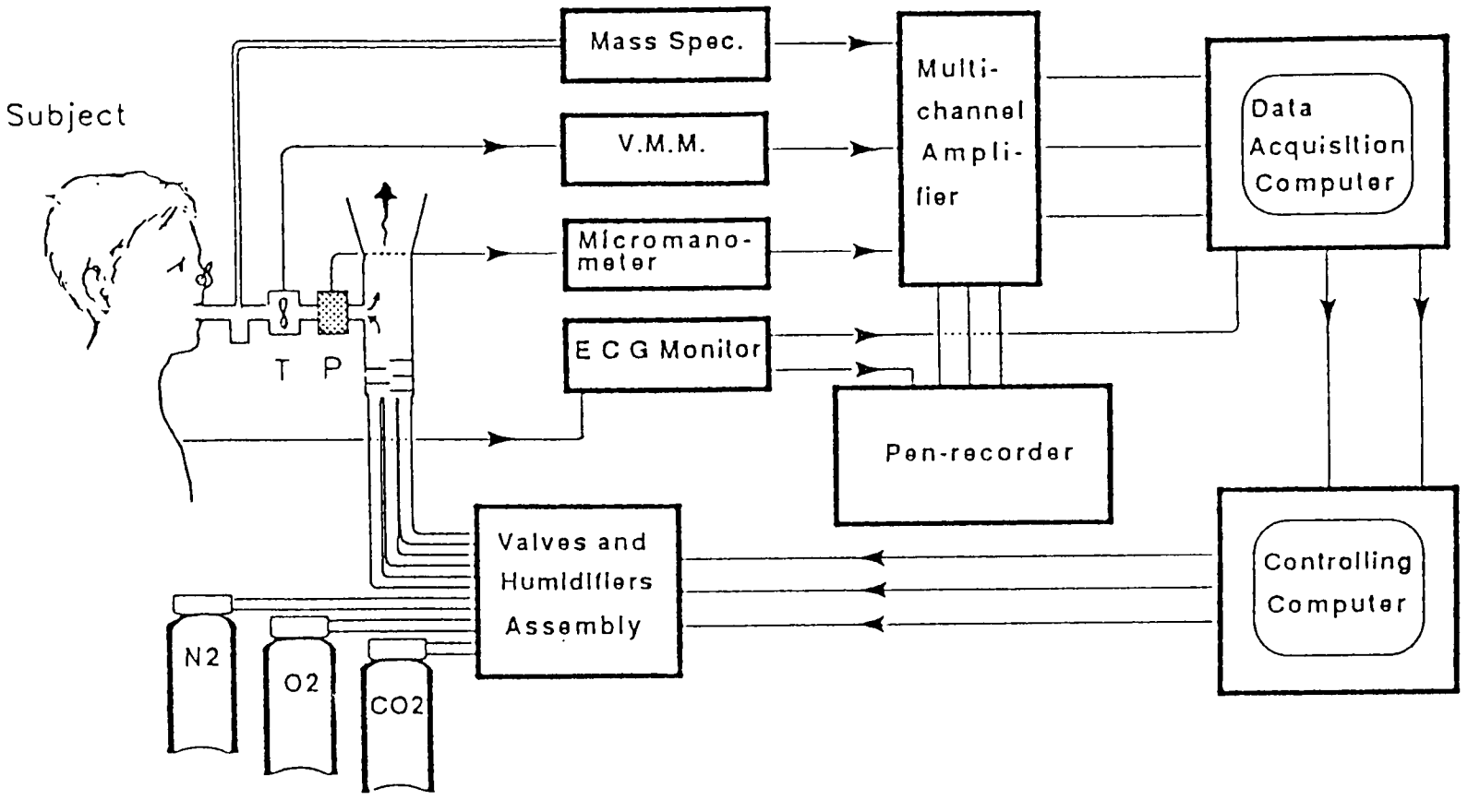


Figure 2.1. Schematic representation of the dynamic end-tidal forcing system. The upper part of the figure shows the elements of the data acquisition system, and the lower part shows the computer-controlled gas mixing system. V.M.M., ventilation measurement module. T, turbine device. P, pneumotachograph (after Khamnei, 1990).

Mouthpiece assembly

The mouthpiece assembly is the apparatus through which the subjects breathe, and forms a T-junction with the chamber through which newly mixed gas flows continuously. The assembly consists of a flexible plastic mouthpiece, a perspex tube with a saliva trap and a port for the sampling catheter from the mass spectrometer, a turbine flow cartridge, and a pneumotachograph. The perspex tube has a 60° bend in it so that the bulk was of the assembly is moved from the subject's line of sight. During experiments the saliva trap was filled with cotton wool to reduce dead space and absorb saliva. The dead space of the mouthpiece assembly was shown by Khamnei (1990) to be 90 ml. Khamnei also showed that over a flow range of 5 L/min to 100 L/min the mouthpiece assembly had a small, flow-independent resistance (0.1 $mmH_2O.min/L$).

Turbine volume device

Volume flows through the mouthpiece assembly are measured by a turbine device (Cardiokinetics Ltd, U.K.) which consists of a volume cartridge, a photodetector pick-up assembly and an electronic processing module (Howson *et al.*, 1986). The volume cartridge is a transparent plastic tube incorporated into the mouthpiece assembly, in the centre of which is a light-weight plastic impeller mounted on virtually frictionless sapphire bearings. The impeller makes approximately one revolution for every 2 ml of gas flow. The pick-up assembly surrounds the impeller and consists of 4 infra red light-emitting diodes and four photodetectors. As the impeller rotates the optical signal to each of the detectors is broken in sequence. The electronic processing module receives the output from the pick-up assembly and from this is able to derive the direction and speed of rotation of the impeller. The output of the electronic processing module is an analogue signal for inspiratory or expiratory volume which is reset at the end of each phase of the respiratory cycle. The volume signal is corrected for the effect of friction at low flows.

Khamnei (1990) studied the behaviour of the turbine device using a mechanical pump which could be made to "breathe" sinusoidally with different tidal volumes (range 0.5 to 3.0 L/min), frequencies (10 to 30 cycles/min) and gas compositions. The response of the turbine to different volumes was highly linear. When the frequency of the pump was changed from 10 to 30 cycles/min there was a mean drop in the measured volume of 1.7% suggesting a small degree of frequency dependence. The measured volumes were virtually independent of gas composition, showing a change of less than 0.5% from pure oxygen to pure nitrogen.

Khamnei (1990) also compared the timings for respiratory phase switches measured by the turbine device with those measured by the pneumotachograph. The turbine reversal times lagged those of the pneumotachograph by up to 360 msec, presumably because of the inertia of the impeller. Phase lags such as these make the turbine device inappropriate for determining phase switching times and instantaneous flow.

Pneumotachograph

The pneumotachograph is used to provide a measurement of flow and to sense respiratory timings (from the reversal of airflow). The two pneumotachographs (Fleisch, Switzerland; UK distributor, P.K Morgan, Chatham, Kent) used are 27 mm in length by 27 mm in diameter, and 60 mm in length by 45 mm in diameter. They both consist of a cylinder filled with a fine honeycomb of small tubes which provides a small but measurable resistance to flow (of the order of 3 $mmH_2O.sec/L$), such that a flow gives a small pressure drop across the resistance. For a given gas, this pressure drop is linear against flow (Poiseuille's Law), provided that flow is laminar. However, Macfarlane (1985) reported that if flow exceeded 130 L/min when using the small pneumotachograph, flow became turbulent. For this reason, the larger pneumotachograph, which provided less resistance, was used when high ventilations were expected, such as occurred during hypoxic exercise. This pneumotachograph increased total dead space by 80 ml.

The pressure difference across the pneumotachograph is transduced into an analogue voltage by a fast-responding micromanometer (Validyne Pressure Transducer, Model No. MP45-14-871; Validyne Engineering Corp., California, USA), set to ± 20 mm Hg full deflection.

Mass spectrometer

Gas close to the subject's mouth is sampled continuously by a fast responding quadrupole mass spectrometer (Airspec MGA3000, U.K.), at a rate of 20 ml/min via a capillary tube seated in a port in the mouthpiece assembly. The mass spectrometer output is updated every 20 msec and returns values for O_2, N_2, CO_2 and Argon. The total output of the four channels of the mass spectrometer is constrained to give a total gas composition of 100%, to prevent drift arising from factors such as partial blockade of the sampling catheter.

At the start of each experimental day, helium was introduced into the mass spectrometer in order to ascertain background levels of current for O_2, N_2, and CO_2 mass numbers in the complete absence of these gases (the zeroing procedure). At this time, the

prevailing ion currents for O_2, N_2, and CO_2 channels were stored and thereafter used as background signals to be subtracted for the total signals for each gas. Prior to each experimental period, the mass spectrometer was tuned and calibrated with air and a standard gas, whose composition had been verified by the Lloyd-Haldane procedure (composition approx. 6.5% CO_2 and 7.5% O_2 in N_2). Tuning involves scanning the specified peaks for the gases and finding the position where the signal is maximal.

On each experimental day, the mass spectrometer delay was measured by suddenly switching from air to the standard gas mixture using a fast-responding solenoid valve. The mass spectrometer response to the sudden change in the composition of the input gas was displayed on an oscilloscope screen (CO_2 channel only). This display enabled the half-time of the response to the change in gas composition to be assessed. This delay was usually 150-200 msec. Delay times of greater than 220 msec suggested an increased resistance in the sampling catheter, possibly due to partial obstruction, in which case the catheter was replaced.

Eight-channel amplifier

The analogue voltage outputs from the equipment described above were passed through a laboratory-built eight-channel amplifier. This served to condition each of the analogue signals to a range of ± 5 Volts which is the input range required by the analogue-to-digital converters (Dash-8, Metrabyte; Keithley Instruments Ltd., U.K.) used by the data acquisition computer.

Pen recorder

A second set of outputs from the eight-channel amplifier was passed to a hot stylus chart recorder (Lectromed Devices M19, U.K.). The chart recorder displayed the outputs of the CO_2 and O_2 channels of the mass spectrometer and the inspired and expired volumes from the ventilation measurement module.

Heart rate monitor

A heart rate monitor (Model 302 Rigel Research Ltd., U.K.) provided continuous visual monitoring of the electrocardiogram measured from chest leads placed on the subject. The timing of the QRS complexes was recorded by the data acquisition computer to allow analysis of the heart rate.

Calibration

Before each experimental trial the complete data acquisition system was calibrated by performing a dummy experiment using a mechanical pump to "breathe" on the apparatus. The calibration pump was set to breathe at a tidal volume of 1 L and a frequency of 20 cycles/min. Data were collected for 2-3 min with the mass spectrometer first sensing air and then standard gas. After the end of the data collection period the calibration program, CLDATA, was run. This calculates the appropriate calibration functions for each of the measured variables using the data collected together with user specified values for standard gas composition, pump volume and frequency, and the room temperature and barometric pressure. The CO_2 and O_2 calibration functions are unconstrained straight lines (2 point calibration) but the inspired and expired tidal volume calibration functions are straight lines constrained to pass through zero. The conversion factors are stored in a DOS file (*.CAL). This file is used by the gas mixing system controlling computer during the experiment and also in the analysis of the data after the experiment (see below).

Operation

Data acquisition during an experiment is performed by the RTDATA program written by P.A. Robbins and K.J. Stratford which runs continuously on the data acquisition computer while an experiment is in progress. The program performs a number of functions which include collecting and storing data from the apparatus described above, and detecting the end-expiratory values of P_{O2} and P_{CO2}.

Data concerning experimental time, gas flow and gas composition are stored every 20 msec by the RTDATA program in a *.RAW file, where * is a user defined file root

name. The speed of data acquisition makes it necessary for the *.RAW file to be written to a virtual disk. RTDATA receives information concerning experimental time from a 1 kHz pulse generator which drives an internal counter in the computer.

During the process of dynamic end-tidal forcing RTDATA passes the uncalibrated end-expiratory values of P_{o2} and P_{co2} on to the gas mixing system computer to allow the appropriate changes in inspiratory gas composition to be made (see below). For this to work efficiently RTDATA needs to identify accurately the time at which the end-expiratory values occur. First the program detects the current respiratory phase from a flow direction signal from the pneumotachograph. The cue for the end of expiration comes when the turbine device indicates a phase switch. RTDATA then allows for the inertia of the turbine (see above) by looking back in time at the pneumotachograph record to see when the last flow reversal occurred. The time associated with this is defined as the time at which expiration ended. The advantage of this procedure is that the inertia of the turbine ensures that a genuine phase switch has indeed occurred whereas the pneumotachograph may show multiple flow reversals during a phase switch. Having determined the end-expiratory time, RTDATA then makes some allowance for the mass spectrometer delay by looking forward in time through the CO_2 and O_2 data record by 140 msec (this value will always be slightly less than the mass spectrometer's pure delay). The CO_2 and O_2 values at this time are recorded as end-expiratory values and can be passed on to the gas mixing system computer.

RTDATA receives input from a manual event marker through a digital input/output channel and records the experimental time of any event marks. At the end of the experiment RTDATA writes a *.PK and a *.HRT file on the computer's hard disk. The *.PK file contains time, volume and gas data associated with the end-inspiratory and end-expiratory point of each breath. The *.HRT file contains a record of the time of each QRS complex during the experiment.

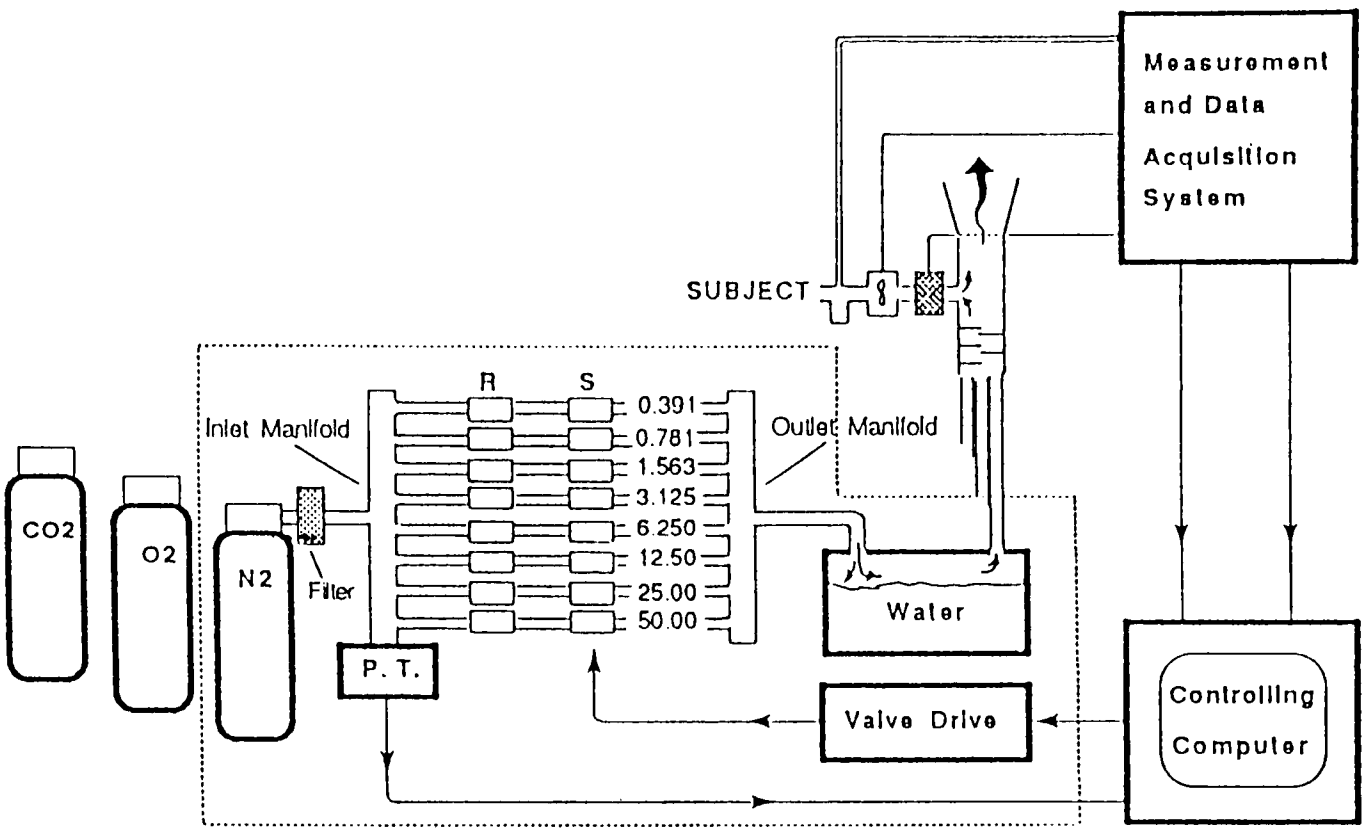


Figure 2.2. Schematic illustration of the layout of the gas mixing system shown completely for one gas species (N_2). The flows through each channel (L/min) at the standard supply pressure of 20 p.s.i. are shown. The resistance of each successive channel halves going from top to bottom in the illustration. P.T., pressure transducer. R, flow resistor (needle valve). S, solenoid valve.

Gas-Mixing System

Accurate breath-by-breath control of end-tidal P_{CO_2} and P_{O_2} is effected by means of a computer-controlled gas-mixing system. The layout of the gas-mixing system is shown schematically in Fig. 2.2. The hardware of the system comprises two main elements, the gas-mixing unit and the controlling computer.

Gas-Mixing Unit

Gas supply

O_2, N_2 and CO_2 are supplied from gas cylinders (British Oxygen Company), O_2 and N_2 as compressed gas and CO_2 as liquid. The supply pressure from each cylinder is controlled by a two-stage pressure regulator (M30, British Industrial Gas). A heating element is included in the CO_2 supply to vaporise the gas. The supply pressures were set to 20 p.s.i. for O_2 and N_2 and 10 p.s.i. for CO_2.

Valve assembly

The valve assembly consists of three similar units, one for each of the gases. Each unit consists of a gas filter (LF-3/8-5, Festo Ltd, U.K.) to remove any particles, an inlet manifold incorporating a pressure transducer, eight channels for gas flow running in parallel, and an outlet manifold.

The pressure transducer served to provide the controlling computer with information on the supply pressure, since the pressure regulators were unable to maintain a perfectly constant supply pressure.

Each of the eight channels connecting the inlet and outlet manifolds consists of a needle valve flow resistor (GRO-1/80 and GRO-M5, Festo Ltd., U.K.) and a solenoid valve connected in series by 4 mm polyethylene tubing. The eight parallel resistors were arranged so that the resistance of successively adjacent channels was halved (Fig. 2.2). The actual values of the resistances were set when the apparatus was built, such that the maximum flow through the valve assembly would be 100 L/min for N_2, 50 L/min for O_2 and 12.5 L/min for CO_2, at the respective supply pressures of 20, 20 and 10 p.s.i..

Downstream of the flow resistor in each of the eight gas channels is a solenoid valve (MFH-2-M5, Festo Ltd for CO_2; MC-2-1/8, Festo Ltd for O_2 and N_2). Each valve is either open or closed and the combination of valves opened and closed is under the control of the gas mixing system controlling computer. The number of combinations possible is therefore 2^8 or 256, giving the controlling computer the ability to select, for each of the gas species, any one of 256 possible gas flows ranging from zero to maximum. In order to open, each of the solenoid valves required a 12 Volt d.c. input, provided from a laboratory built power supply (valve driver). In the absence of this input, the solenoid valve is kept closed by a spring.

Expansion Bags

One problem with the design of the valve assembly is that when any solenoid valve closes, the gas pressure immediately upstream of it builds up towards that of the supply pressure in the inlet manifold. When the valve then opens again a burst of pressure

travels downstream. In an attempt to dampen this out anaesthetic bags (M289M, Leymed Ltd, U.K.) were incorporated into the gas circuits down-stream of the outlet manifold, to act as compliant expansion chambers.

Humidifiers

To supply the subject with a fully-humidified inspirate, heated humidifiers are incorporated into the O_2 and N_2 circuits. The humidifiers are laboratory built perspex boxes (10 L volume) with gas inlet and outlet holes in the top. The boxes are kept about 90% filled with distilled water which is heated to ~30° C by a thermostatically controlled 1 kW element (Elmatic Ltd, U.K.).

Mixing chamber

The inspirate is finally mixed when the O_2, N_2 and CO_2 gas circuits converge in the mixing chamber. The mixing chamber is a perspex cylinder with a volume of 0.12 L upstream of the T-junction with the mouthpiece assembly, from which the subject draws the inspiratory gas. The gas is thoroughly mixed by a number of perspex fins. By mixing the inspirate so close to the subject's mouth the functional dead space of the system is kept very small, giving the gas-mixing system a rapid response time. During experiments the total gas flow was designed to exceed the requirements of the subject so that all of the inspirate was supplied from freshly-mixed gas and all of the expirate washed out. If the subject's inspiratory flow happened to exceed the gas mixing system flow at any moment, the outlet passage downstream of the T-junction with the mouthpiece assembly acted as a reserve of gas to prevent inspiration of outside air.

Controlling computer

The gas flow through each of the circuits of the gas mixing unit is controlled by a computer (IBM PC AT) running the DEF program written by P.A. Robbins. The computer controls the solenoid valves by means of an 8 bit port which communicates with the valve driver box for each gas circuit (Fig. 2.3).

Dynamic End-Tidal Forcing in Action

When an experiment is in progress, the process by which the gas mixing system controlling computer successfully manipulates the inspiratory gas composition so as to force the alveolar gas composition to follow the desired time-course can be considered in two stages. First, the controlling computer decides what the inspired gas tensions should be, based on information received from the data acquisition computer (feedback stage). From this it decides what the flow for each gas should be and what combination of solenoid valve openings and closings will give these flows (output stage) and effects the necessary changes. A flow diagram representing this procedure is shown in Fig. 2.3.

Feedback stage

Following the end of expiration the data acquisition computer identifies the measured end-tidal P_{CO_2} and P_{O_2} values in the way described above. These uncalibrated values are passed on to the gas-mixing system controlling computer (running the DEF program) which converts them to real values using the information in the *.CAL file. In order to calculate the appropriate inspiratory P_{O_2} and P_{CO_2} a feedforward/feedback procedure is used which takes three factors into account: i) a predicted inspiratory partial pressure based on an estimate of the ventilatory response to the forcing function (feedforward); ii) the difference between the actual and desired end-tidal gas value for the previous breath (proportional feedback); iii) the sum of all previous differences (integral feedback). The calculation of the inspiratory gas partial pressure (P_I) can be represented as follows:

$$P_I = P_{I_p} + g_p . (P_{ET_d}^{(n)} - P_{ET_m}^{(n)}) + g_i . (\sum_{j=1}^{n} (P_{ET_d}^{(j)} - P_{ET_m}^{(j)}))$$

where P_{Ip} is the predicted inspiratory gas tension, P_{ETd} and P_{ETm} are the desired and measured end-tidal gas tensions, and g_p and g_i are the proportional and integral feedback gains. The DEF program allows the values of these gains to be varied around a default value during the experiment by means of a potentiometer box. The value of g_i is set to

be one-tenth that of g_p. The accuracy with which the desired forcing function is followed depends on the accuracy of the initial prediction and the values of g_i and g_p. If the gains are too low the control is slack and if the gains are two high the system can become unstable and oscillate. The integral control element serves to remove steady-state errors in the prediction.

Output Stage

Having decided on the appropriate inspiratory gas partial pressures the gas mixing system controlling computer calculates the fractions of the total inspirate required to give these gas pressures, which depend on the barometric pressure. It then calculates the flow of each gas required, which depends on the total gas flow specified by the experimenter. After calculation of the CO_2 and O_2 gas flows the balance is made up by N_2. The final stage is to open the correct sequence of solenoid valves in the valve assembly to give the desired flow for each gas. The computer receives information about the supply pressure in the inlet manifold of the valve assembly from pressure transducers. The DEF program then selects the appropriate combination of valve openings/closings from a previously determined pressure-flow table for each gas. The open/close instruction is then sent to the valve driver box for each gas as an 8 bit binary word. The valve driver then opens and closes the solenoid valves as instructed.

Speed of system

The inspiratory gas composition is updated (i.e. one complete circuit of the loop in Fig. 2.3 is completed) every second and every time a new end-tidal gas value is detected. The speed of response of the system is limited by dead space, which is minimal (see above), and by the delay in detecting the end-tidal gas composition after the end of expiration. This will be the longer of either the mass spectrometer delay or the turbine reversal delay. The system is usually sufficiently fast to modify the gas composition of some of the subsequent inspiration following an expiration.

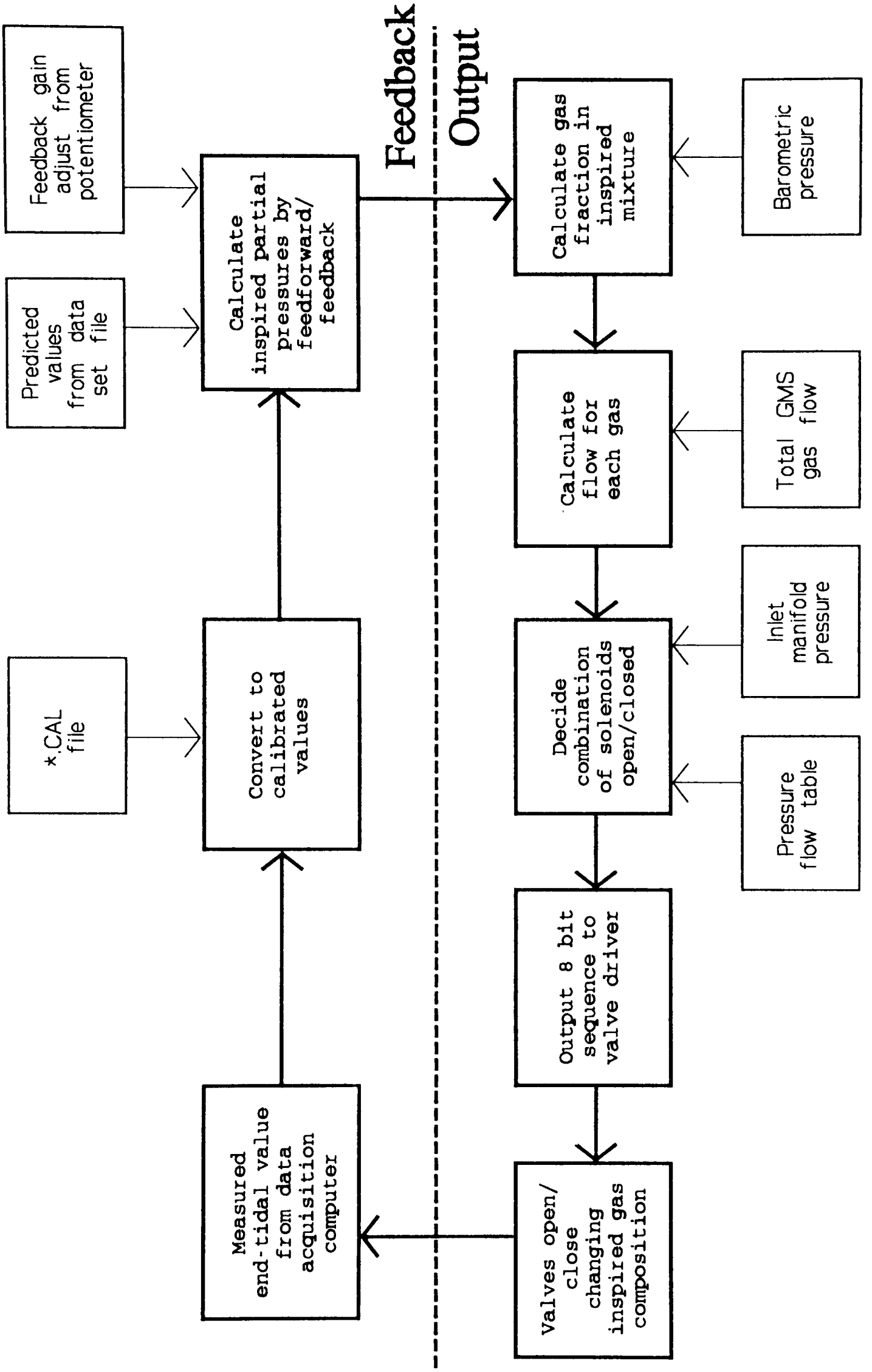


Figure 2.3. Schematic flow diagram illustrating the operation of the dynamic end-tidal forcing system.

Preliminary Data Analysis

After the end of each experiment the uncalibrated data in the *.RAW and *.PK files were converted into meaningful respiratory data using the calibration factors in the *.CAL file. A flow diagram showing the preliminary data analysis performed at the end of each experiment is shown in Fig. 2.4.

BRDATA Program

The BRDATA program written by P.A. Robbins and K.J. Stratford reads the *.RAW, *.PK, *.HRT and *.CAL files and writes the results to four files named *.D1, *.D2, *.D3 and *.D4. These files contain a variety of respiratory parameters described breath by breath. The *.D1 file contains inspiratory and expiratory volumes, times and gas tensions, together with breath-by-breath expiratory ventilation calculated as expired volume divided by breath duration measured from the start of successive inspirations. The *.D2 and *.D3 files contain information regarding oscillations in alveolar P_{CO_2} and P_{O_2}, and metabolic production and consumption of CO_2 and O_2 respectively. The *.D4 file contains a record of the times of any marked events.

Calculation of Metabolic Gas Exchange

The simplest method of calculating O_2 consumption ($\dot{V}_{O_2}$) and CO_2 production ($\dot{V}_{CO_2}$) is by the timed collection of expired air using a Douglas bag. However, this method does not allow estimation of trends in gas exchange which may occur over shorter periods of time, and it cannot deal with situations when the inspired gas composition is changing. A breath-by-breath calculation is therefore desirable. Beaver *et al.* (1973) described an on-line computer application, where O_2 consumption and CO_2 output were calculated from the integral of the product of expired flow from the pneumotachograph and the output from the O_2 analyser and CO_2 analyser for the sample interval respectively. Later developments also took into account the inspired flow and inspired P_{CO_2} and P_{O_2}. However, signals using this method are extremely noisy. The main reason for this is the

breath-by-breath variation between the volume of air inspired and the volume expired, and the consequent breath-by-breath changes in pulmonary gas stores. A calculation of gas exchange at the capillary level which takes these changes in gas stores into account, would therefore result in signals which are less noisy. It is also important to ensure that the net nitrogen exchange over the whole experimental period, as calculated by the method being used, is equal to zero.

A subroutine in the program BRDATA calculated breath-by-breath gas exchange (STPD). This subroutine essentially (a) exploited the better absolute volume-measuring capabilities of the turbine device (for both inspired and expired volume), (b) retained the better dynamic response characteristics of the pneumotachograph in determining the profile of respiratory flow, (c) ensured the net nitrogen exchange over the experimental period *as a whole* was equal to zero, and (d) used the algorithm of Swanson (1980), which minimises the effects of changes in pulmonary stores to obtain an estimate of breath-by-breath gas exchange at the pulmonary capillary.

The steps in the subroutine were as follows:

First, the records of the CO_2 and O_2 composition at the mouth were aligned in time with the record of respiratory flow, as measured by the pneumotachograph, using the measured delay of the mass spectrometer. Secondly, the as yet uncalibrated values of flow from the pneumotachograph were adjusted for changes in viscosity due to changes in gas composition. Thirdly, for each half breath, the respiratory flow from the pneumotachograph was calibrated using the volume measurement from the turbine device. Fourthly, for each gas species, the difference between the amount breathed in and the amount breathed out was calculated, using an assumed expired temperature. This assumed expired temperature was then adjusted until it resulted in the net nitrogen exchange over the experimental period being equal to zero. Finally, the algorithm of Swanson (1980) was used to obtain an estimate of breath-by-breath gas exchange at the pulmonary capillary.

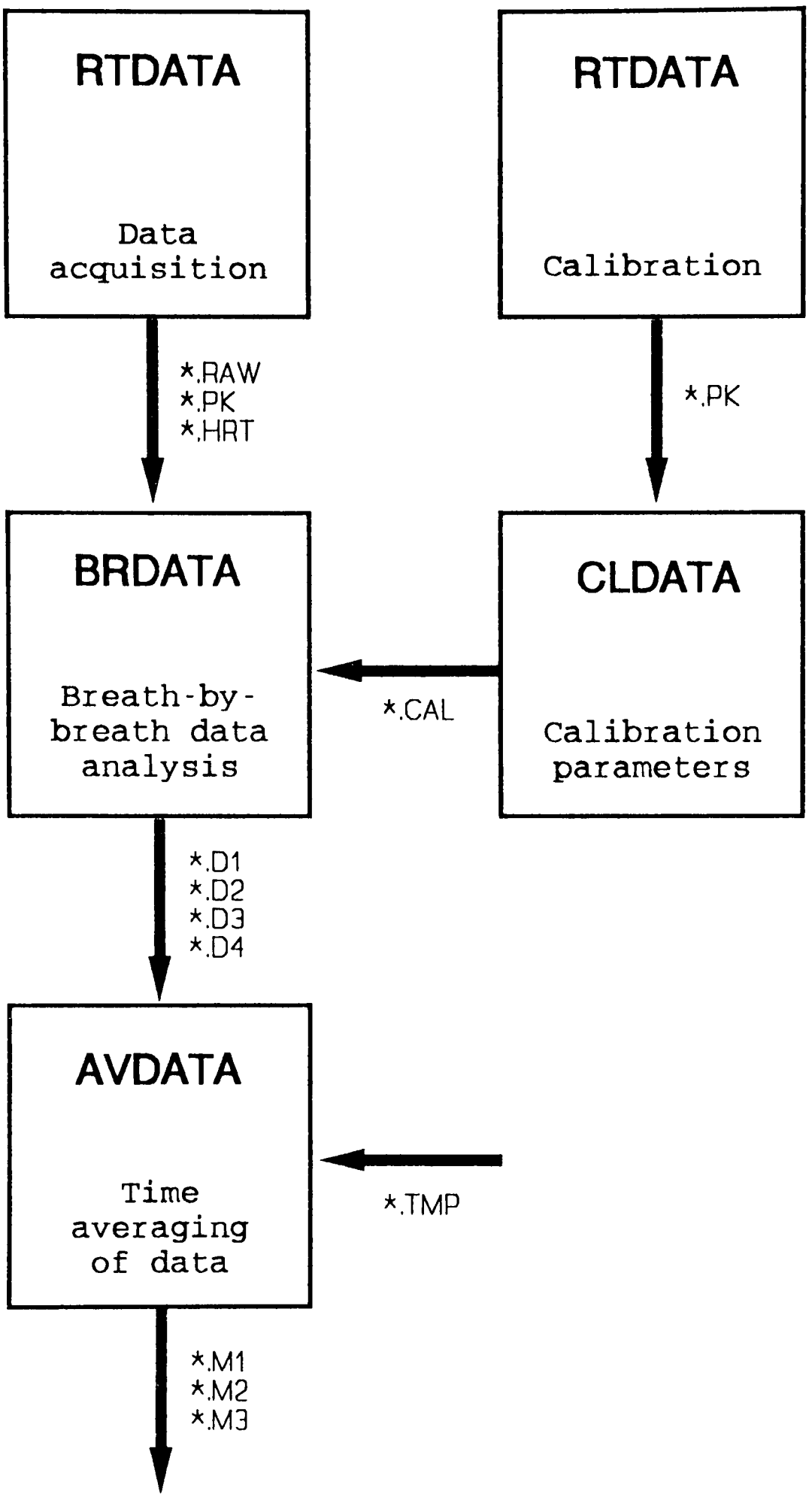


Figure 2.4. Schematic flow diagram showing collection and preliminary analysis of data.

AVDATA Program

The AVDATA program is used to bin-average the breath-by-breath data recorded in the *.D files, over user defined intervals. The program reads in a *.TMP file which gives the start and end points for each averaging period. The results of the averaging are written into three files of the form *.M1, *.M2 and *.M3. For the experiments presented in this thesis, all data were averaged over one min periods.

Other Equipment

Cycle Ergometer

A Mijnhardt KEM-3 electromagnetically-braked cycle ergometer was used for the exercise studies (Howse *et al.*, 1989). The ergometer was set in the constant power mode, thus ensuring a uniform work rate despite fluctuations in pedal rate. A series of coloured lights on the cycle indicated the pedal rate, and subjects were asked to maintain a rate of 60 rpm (the "green range"), since work by Follinsbee *et al.* (1983) suggests that 60 rpm is a suitable frequency for cycle ergometry exercise.

The torque associated with a given work rate setting on the ergometer was measured over a sustained period of use, as described by the method of Russell and Dale (1986). This was found to be accurate and also to remain constant (Fig. 2.5).

Electrical Muscle Stimulator

The electrical muscle stimulator used in Chapters 6 and 7 was kindly lent to us by Professor Guz and his team at Charing Cross Hospital, London, and was originally constructed by their Department of Clinical Engineering. It was used by Adams *et al.* (1984*a*) and (1984*b*) in studies assessing the voluntary control of ventilation during exercise. In this thesis, it was used to achieve electrically-induced exercise in able-bodied and paraplegic subjects. Essentially, two pairs of carbonised rubber electrodes (Slendertone, UK), were strapped to the anterior surfaces of each thigh. A layer of electrode paste was first applied to facilitate even stimulation of the quadriceps muscle

group. The stimulus wave form consisted of a pulse width of 60 μs with a repetition frequency of 40 Hz, modulated by a rectified sawtooth envelope. A repetition frequency of 40 Hz was chosen as this produces the maximum tension in the quadriceps group, whilst at the same time minimising the rapid loss in force which occurs with more sustained stimulus at higher frequencies (Edwards *et al.*, 1977). The amplitude of the stimulus could be varied from 0 - 100 V. Stimuli were delivered to each leg simultaneously at a rate of about 40/min (range 38-50/min, altered by the "envelope" to suit the comfort of individual subjects). The overall shape of each stimulus (*i.e*, the "on" and "off" ramp times) were also varied to suit individual subjects. In the paraplegic subjects, discomfort from electrical stimulation was not a problem: generally higher voltages were used, and the frequency of stimulation was increased to 50/min. This form of electrical muscle stimulation is presumed to be transmitted via a local reflex arc mediated by peripheral nerve fibres, since it is difficult to elicit in patients with very low spinal cord lesions, in whom there is presumable loss of function of anterior horn cells due to injury to the vascular supply to the lower cord segment (Dr H.L Frankel, personal communication).

A chair for use in these experiments was specially constructed. This is described in Chapter 6.

Lactate Analyzer

Lactate concentrations in blood samples were measured using a glucose/lactate analyzer (YSI23, YSI Inc., U.S.A.). The analysis depends on the enzymatic oxidation of lactate to pyruvate by the lactate dehydrogenase enzyme mounted on a membrane. Hydrogen peroxide is generated as a by-product of this reaction and is then oxidised at a platinum anode, generating a current which can be related to the lactate concentration of the sample.

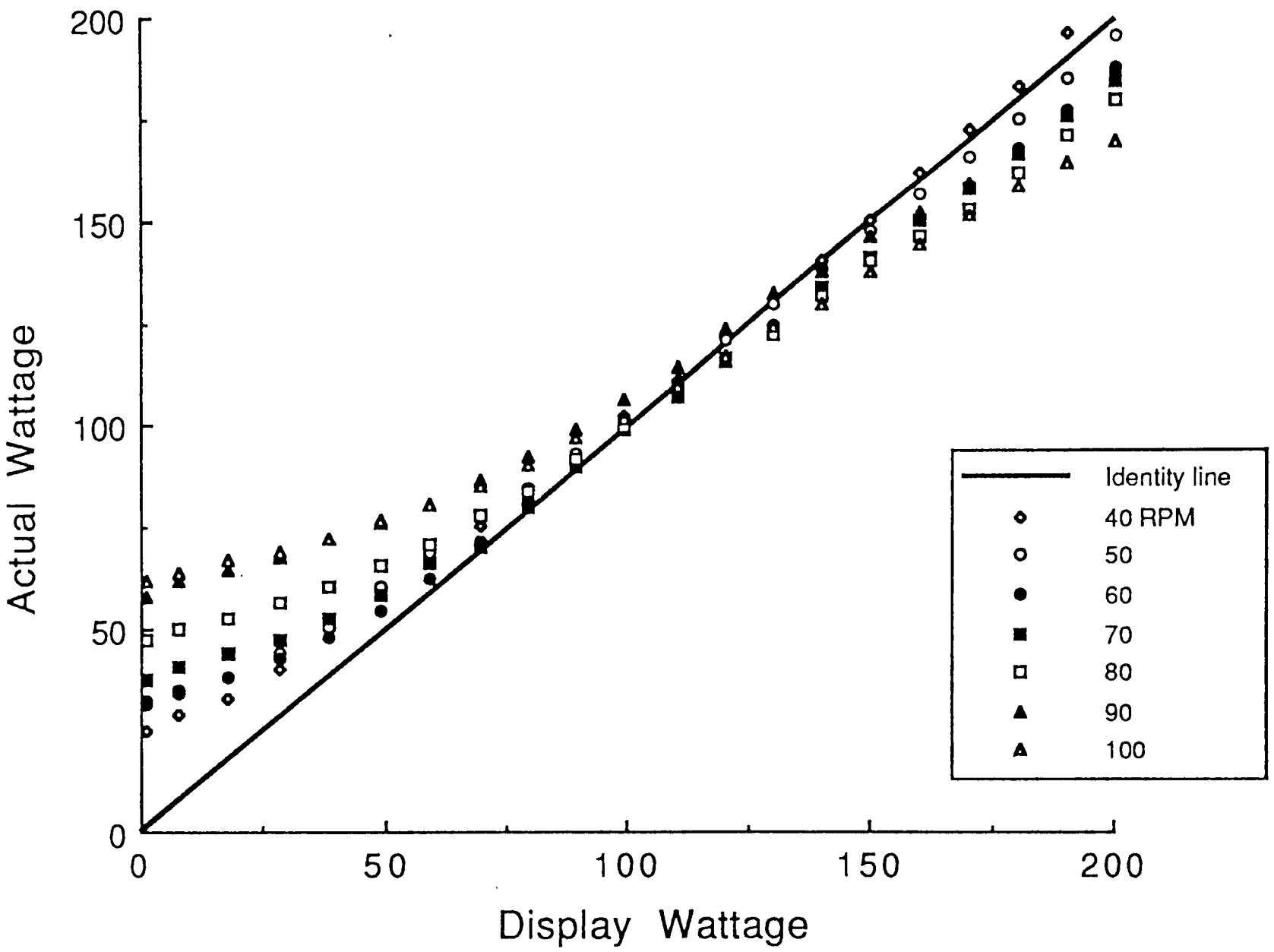


Figure 2.5. Plot of actual wattage yielded by the cycle ergometer (as calculated by the method of Russell and Dale (1986), against the display wattage, over a given range of pedal frequencies (key in inset). The actual wattage of 70 W at 60 rpm, as used in all the experiments, lies on the line of identity and this was found to be constant over a sustained period of use.

Pulse Oximeter

Arterial oxygen saturations were monitored using a finger probe connected to a pulse oximeter (Lifestat 1600, Physio-Control Corp., U.S.A.). This works by measuring the absorbence of the finger at two wavelengths. The data is processed to remove all the non-pulsatile elements of the signal so that the absorbence of the remaining signal is related to the arterial blood, and the arterial oxygen saturation can then be estimated. In practice the machine was suitable for measuring oxygen saturation in the steady state, but was slow to respond following sudden changes in alveolar oxygen tension.

Blood Gas Analyzer

Arterial blood from only four of the subjects taking part in experiments described in Chapter 6 was analysed for P_{CO_2} using a blood gas analyzer (IL1306, Instrumentation Laboratory, Italy). This instrument also measures P_{O_2} and pH.

Arterial pH is sensed by a pH electrode which consists of a pH sensitive glass membrane separating two solutions, one being the control solution of known pH and the other being the test solution. A potential difference, which is proportional to the pH difference between the two solutions, develops across the glass membrane. Arterial P_{CO_2} is measured using a modified form of pH electrode. The P_{CO_2} electrode consists of a pH electrode in contact with a solution contained within a CO_2 gas permeable membrane. When this electrode is in contact with a test solution, CO_2 diffuses across the gas permeable membrane until in partial pressure equilibrium with the test solution. A CO_2/H^+ equilibrium is established in the solution contained within the gas permeable membrane so that the pH of this solution is related to the P_{CO_2} of the test solution. The pH of the solution contained within the gas permeable membrane is then measured by the pH electrode.

The P_{O_2} of the test solution is measured using a Clark electrode. An electrical potential is generated by the reduction of oxygen at a platinum wire cathode, with electrons supplied from a silver/silver chloride anode.

Finapres Blood Pressure Measurement

Continuous recording of finger arterial blood pressure was made in six paraplegic subjects using the Finapres as described in Chapter 7. The Finapres (Ohmeda, Denver, USA) works by using the principle that if an externally-applied pressure (*e.g*, via the bladder on the finger cuff) is maintained at all times equal to the intra-arterial pressure, then the transmural pressure across the vessel walls will be zero and the arteries will not change in size. The Finapres essentially measures the constantly-changing pressure in the finger cuff, required to maintain the arterial volume constant on a within-beat basis. The volume of the vessels is estimated by a photoplethysmographic technique, using an infrared light source in the finger cuff. The set-point at which the system operates is first chosen by a "servo start-up adjustment" which consists of stepping the cuff pressure through various levels and interpreting the magnitude of the photoplethysmogram at each of these levels. When cuff pressure is below diastolic pressure, the resulting photoplethysmogram has a small pulsatile component; when cuff pressure is above systolic pressure, there is virtually no pulsatile component. The Finapres selects a point between these limits as the set-point for operation. In addition, a similar operation of stepping cuff pressure through various levels occurs every 10 beats ("servo self-adjust"), and the set-point is either increased, decreased or is maintained.

Stability of Gas Analysis

In order to ensure that there were no underlying drifts over periods of time in the measuring apparatus, a number of experiments were performed to assess its stability. The stability of the cycle ergometer has been discussed, above.

To exclude a drift in the mass spectrometer measurement of P_{CO_2}, the composition of a standard gas was measured on a number of occasions both before and after repeated experimental periods. In total, this was done on 18 occasions and there was found to be no significant change in the mass spectrometer estimates of P_{CO_2} before and after an experimental period (mean change ± SE = 0.01 ± 0.06 Torr, n = 18).

Finally, in order to exclude the possibility that increasing the inspired level of CO_2, (as occurs during dynamic end-tidal forcing) might interfere with the method of calculation of metabolic gas exchange, experiments were performed using the calibration pump as a "subject", both with and without added CO_2. Figure 2.6 confirms that no significant error arises in assessing gas exchange when this is done.

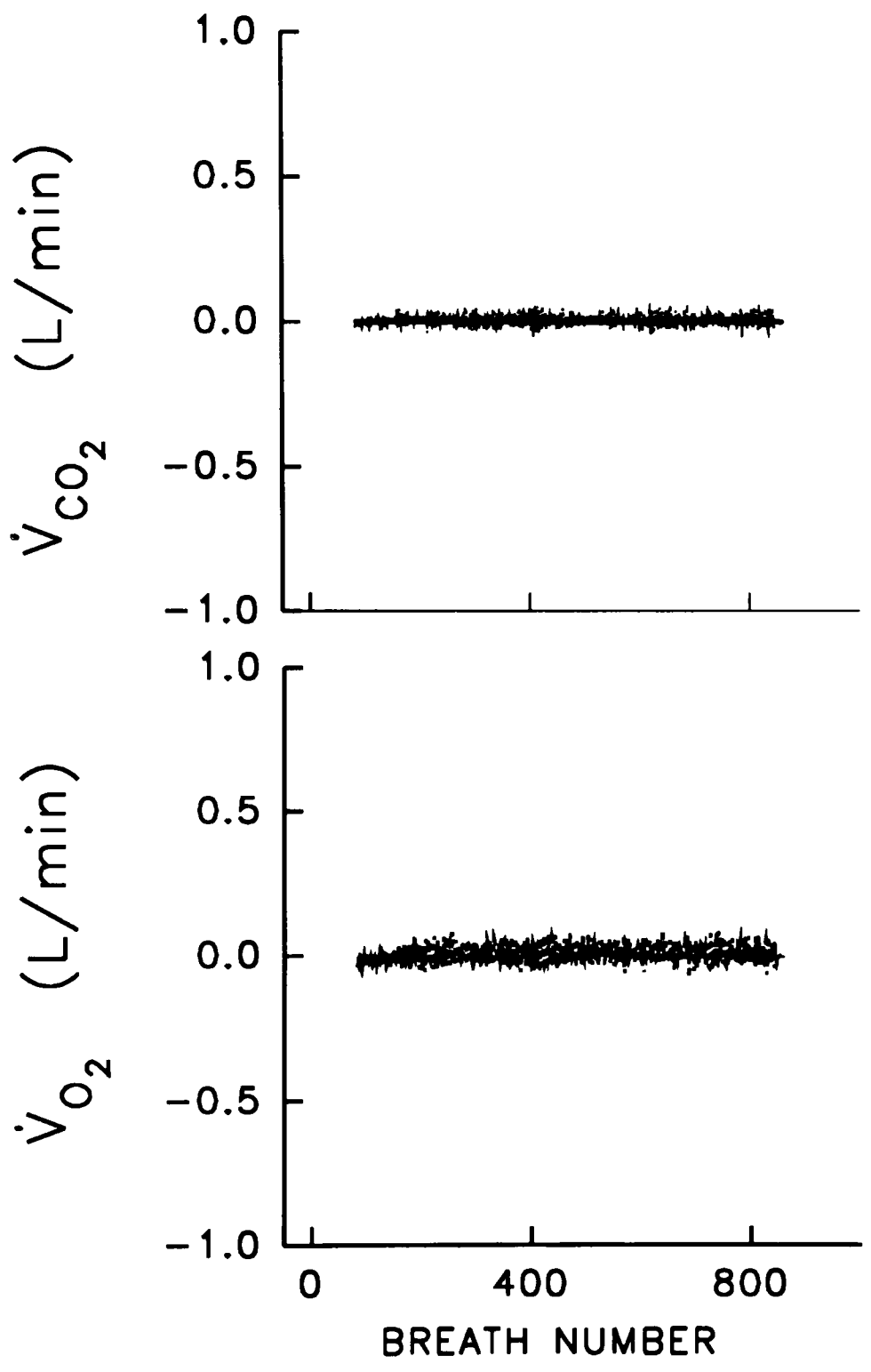


Figure 2.6. Calculation of $\dot{V}_{CO2}$ (top panel) and $\dot{V}_{O2}$ (bottom panel) when the calibration pump "breathes" (a) room air (dotted lines), and (b) through the gas-mixing system with P_{O2} held at 100 Torr and P_{CO2} held at 42 Torr (solid lines). For both $\dot{V}_{CO2}$ and $\dot{V}_{O2}$, the lines lie very close to zero and are superimposed.

Statistics

The use of complicated statistics is not a substitute for good physiology. In this thesis, the general forms of the ventilatory responses to the various interventions constitute the main results, and statistical tests have been used simply to analyse these responses in a more quantitative manner.

The main statistical test which has been used for the studies presented here is Student's t-test, which is a valid method of comparing the means of relatively small samples which are assumed to have a normal distribution (Bailey, 1985). Furthermore, since in these studies, individual subjects have effectively been their own controls (*i.e*, measurements have been made before and after an intervention such as hypoxia in the same subject; or the same subject has been studied in different situations such as rest and exercise), the method of paired comparisons has been used.

Problems with the use of the t-test, however (or indeed, any statistical test) include the risk of making a "type 1" or a "type 2" error. A type 1 error is made when the null hypothesis is rejected by the test when, in fact, it is true: in simple terms, the test indicates that two samples are significantly different when, in fact they are not. A type 2 error is made when the null hypothesis is accepted when, in fact it is untrue: in simple terms, the test indicates that two samples are not different when, in fact, they are. In general terms, the risk of making a type 1 error using the t-test increases when using a one-tailed test, as opposed to a two-tailed test, and when making multiple comparisons.

One-tailed vs. two-tailed test. Whether a one-tailed or a two-tailed test should be used depends on the scientific question being asked in the experiment. Generally, a one-tailed test should be used when there is interest only in one tail of the comparison between two samples, *e.g*, whether the first sample mean is significantly *larger* than the second (when the null hypothesis can be stated as: "the first sample mean is not significantly larger than the second). In this case, there is usually a working hypothesis, or some prior knowledge of the likely outcome of the experiment (for example, that ventilation is higher during

exercise than it is at rest). A two-tailed test should be used when there is interest in both tails of the comparison, *i.e*, whether the first sample mean differs (is *either* larger *or* smaller) than the second. This would be appropriate where no prior knowledge of the outcome exists (for example, the effects of an entirely new drug or gas on the ventilation).

In some respects, a one-tailed test is, if valid, preferable to the two-tailed test (Krauth, 1988), since it allows a more precise interpretation of the results (the statement: "A is larger than B" contains more information than: "A and B differ"). The increased risk of making a type 1 error using the one-tailed test should, however, be borne in mind.

Multiple comparisons. The chance of making a type 1 error increases with the number of t-tests performed. Thus, if a total of twenty comparisons is made using the t-test, then it becomes likely that of these one will yield a significant result, by chance alone, at the $P < 0.05$ level. Some form of statistical correction to the t-test is therefore required. This general statement, however, needs some qualification. First, the total number of t-tests, referred to above, usually means the total number for a given variable, or for a specific hypothesis being tested, and not simply the total number of tests in a given set of experiments or in a thesis (Parker, 1979). Secondly, correction may not be needed if t-tests are used to compare multiple data points along a time-series: since these can be near-infinite, corrections applied to t-tests will often guarantee that none of the comparisons within a time-series will yield a significant result.

In this thesis, for studies in which a number of t-tests have been used, the following procedure has been applied. First, the data have been subjected to an analysis of variance (Parker, 1979). The limitation of the analysis of variance, however, is that it only indicates whether any significant differences exist within a group, and it does not indicate which specific comparisons are significant and which are not. Therefore, if the analysis of variance has indicated there to be significant differences, the data have been subsequently assessed using a corrected form of the t-test. The correction that has been used is the Bonferroni procedure, in which the level of significance is taken to be 0.05/k, where k is the number of comparisons made for the given variable (Krauth, 1988).

The proper use of statistics to analyse results in a quantitative manner can sometimes be difficult. The principles outlined above have been used as a guide in this thesis. Ultimately, however statistically significant a particular comparison might be, whether the result is believed to be important or useful remains wholly subjective.

Appendix 2.1.

This appendix summarises the main equations used in the calculation of metabolic gas exchange at the level of the pulmonary capillary.

The net transfer of gas, g, at the alveolar level can be calculated if both gas exchange at the mouth and the change in pulmonary gas stores are determined on a breath-by-breath basis:

$$V_{g,alv,i} = V_{g,m,i} - \Delta V_{g,s,i} \qquad (1)$$

where $V_{g,\,alv,\,i}$ and $V_{g,\,m,\,i}$ represent the volume of gas transferred at alveolar and mouth level respectively, and $\Delta V_{g,\,s,\,i}$ is the change in pulmonary stores, throughout the ith breath. The volume of gas transfer at the mouth, $V_{g,\,m,\,i}$ can be calculated as the difference between the amount breathed in and the amount breathed out during the current breath:

$$V_{g,m,i} = (F_{I,g} \times V_I)_i - (F_{E,g} \times V_E)_i \qquad (2)$$

where $F_{I,\,g}$ and $F_{E,\,g}$ are the inspiratory and expiratory gas fractions, and V_I and V_E the corresponding volumes.

Auchincloss *et al.* (1966) described the breath-by-breath changes of the pulmonary stores as:

$$\Delta V_{g,s,i} = V_{A,i-1}(F_{A,g,i} - F_{A,g,i-1}) + F_{A,g,i}(V_I - V_E)_i \qquad (3)$$

where $V_{A,\,i\text{-}1}$ is the end-expiratory volume at the end of the previous breath, and $F_{A,g,\,i}$ and $F_{A,\,g,\,i\text{-}1}$ are the alveolar gas fractions at the end of the current and of the previous breath, respectively. The changes in lung volume (ΔV_L) over the current breath ($(V_I - V_E)_i$)

can be estimated from the nitrogen balance equation (Swanson, 1980):

$$\Delta V_{L,i} = (V_I - V_E)_i = V_{N_2,m,i} - [(F_{A,N_2,i} - F_{A,N_2,i-1}) V_{A,i-1}] / F_{A,N_2,i} \qquad (4)$$

By substituting equation (3) into equation (1) and using the term $\Delta V_{L,i}$, the following algorithm for the breath-by-breath calculation of alveolar gas exchange can be obtained:

$$V_{g,alv,i} = V_{g,m,i} - [V_{A,i-1}(F_{A,g,i} - F_{A,g,i-1}) + F_{A,g,i}\Delta V_{L,i}] \qquad (5)$$

The only quantity in equation (5) not directly measurable on a breath-by-breath basis is $V_{A,i-1}$. Attempts to update this value breath-by-breath have been unsuccessful, due to a large cumulative error (Wessel *et al.*, 1979). The options are (a) to omit the term completely, (b) to assign to it a fixed, arbitrary value, usually the functional residual capacity (Beaver *et al.*, 1981), or (c) to assign to it a value which minimises the variation in breath-to-breath gas exchange, *i.e*, the concept of "effective lung volume" (Swanson, 1980). Effective lung volume is defined as "that portion of the end-expiratory volume which *effectively* participates in gas exchange" (by analogy with effective blood volume or tissue volume in pharmacology). The method is therefore one which essentially smooths the data: the effective lung volume is calculated to minimise breath-by-breath variation in pulmonary stores, and hence its use also provides data for gas exchange at the pulmonary capillary which have the least variation in terms of noise. Previous studies have shown that of these, option (c), that of effective lung volume, yields the least variation in gas exchange (Hughson and Swanson, 1989). Although this method is well-validated for steady-state measurements, the lack of a "gold standard" for breath-by-breath measurements still means that the extent to which the observed variation is a real, physiological phenomenon, particularly in the study of transients, remains unknown (as discussed by di Prampero and Lafortuna, 1989).

Appendix 2.2.

This appendix shows samples of .D1, .D2 and .D3 files.

Below is a partial listing of a .D1 file. The first ten lines are header lines which provide information of file name and conditions of the experiment. The eleventh line consists of the variables, and below this, the calibrated data values. The third column (E12345678) marks any events. Minute ventilation was calculated as:

$$V_{E,n} = 60,000 \times \frac{V_{TE,n}}{(T_{I,n} + T_{E,n})}$$

where $V_{T\,E,\,n}$ is the expired volume in breath *n* (litres) and $T_{I,\,n}$ and $T_{E,\,n}$ are the inspired and expired breath durations, respectively (millisec).

Data File written as j9210516.d1
DATE 21:12:1992
EXPERIMENTER jjp
SUBJECT NO 921
EXPERIMENT NO 5
RUN NO 16
NO.OF BREATHS 174
MODE OF THIS EXPERIMENT 1 1 1 1
INSPIRED GAS TEMPERATURE USED 24.0
EXPIRED GAS TEMPERATURE USED 31.3

BRNO	TIME	E12345678	VTI	TI	PIC2	PIO2	VTE	TE	PAC2	PAO2	VENT	W	HR
1	1665	00000000	0.405	1440	-2.3	180.2	0.395	2360	37.3	105.5	6.2	0	38
3	10645	00000000	0.445	1320	28.5	127.1	0.472	2680	39.7	98.5	7.1	0	47
4	14645	00000000	0.568	1600	66.2	121.8	0.545	3200	40.2	99.6	6.8	0	47
5	19445	00000000	0.548	1460	21.0	146.7	0.524	2140	42.9	102.3	8.7	0	32
6	23045	00000000	0.517	1420	17.7	138.7	0.551	2980	43.4	103.9	7.5	0	46
7	27445	00000000	0.485	1500	11.0	136.6	0.478	2380	42.2	105.0	7.4	0	46
8	31325	00000000	0.570	1280	17.2	138.4	0.620	2400	42.4	104.9	10.1	0	47
9	35005	00000000	0.638	1780	15.5	139.8	0.595	1740	39.7	106.9	10.1	0	48
10	38525	00000000	0.608	1220	25.4	134.6	0.663	1980	42.2	107.4	12.4	0	48
11	41725	00000000	0.636	1260	12.6	135.7	0.638	2200	44.9	104.6	11.1	0	49
12	45185	00000000	0.566	1280	15.5	134.6	0.590	3260	44.0	104.5	7.8	0	49
13	49725	00000000	0.723	1500	6.1	140.2	0.699	2300	41.5	106.5	11.0	0	54
14	53525	00000000	0.680	1360	20.5	132.7	0.746	3000	41.1	104.0	10.3	0	52
15	57885	00000000	0.640	1520	22.2	136.4	0.630	2360	41.8	106.8	9.7	0	50
16	61765	00000000	0.592	1340	12.2	129.1	0.628	2380	41.3	107.4	10.1	0	49
17	65485	00000000	0.584	1380	16.2	123.1	0.609	2220	41.6	105.3	10.2	0	48
18	69085	00000000	0.604	1420	18.8	125.9	0.649	2660	41.6	103.4	9.5	0	64
19	73165	00000000	0.626	1400	23.8	134.0	0.663	3120	41.8	102.5	8.4	0	49
21	81605	00000000	0.630	1240	14.9	130.8	0.667	1840	41.2	108.1	13.0	0	50
22	84685	00000000	0.600	1220	17.8	122.1	0.694	3260	44.6	104.7	9.3	0	48
23	89165	00000000	0.644	1480	13.0	122.6	0.632	1560	41.8	104.0	12.5	0	50
24	92205	00000000	0.640	1220	36.0	119.3	0.736	2400	40.6	102.2	12.2	0	61
26	100785	00000000	0.666	1320	11.6	138.4	0.667	2720	42.3	102.8	9.9	0	53
27	104825	00000000	0.711	1480	42.0	122.0	0.703	2520	41.9	104.4	10.5	0	54
28	108825	00000000	0.751	1380	21.7	120.8	0.728	2260	40.3	100.5	12.0	0	56
29	112465	00000000	0.694	1260	25.0	133.7	0.796	2920	41.0	104.3	11.4	0	53
30	116645	00000000	0.717	1620	19.3	121.6	0.597	1580	41.1	106.0	11.2	0	53
31	119845	00000000	0.638	1120	20.2	124.3	0.757	2900	42.6	104.0	11.3	0	52

Below is a partial listing of a .D2 file. Data from these files were not used in this thesis. The first eight lines are headers, and the ninth and tenth list the apparatus and anatomical deadspace. The columns refer to calculations made to reconstruct the alveolar values for P_{CO2} and P_{O2} from the expired gas profiles. Thus COSLO and O2SLO are the calculated slopes for CO_2 and O_2 respectively; COMIN and O2MAX are the minimum calculated values for CO_2 and maximum values for O_2 respectively; COMN and O2MN are the mean values for CO_2 and O_2 respectively.

Data File written as j9210516.d2
DATE 21:12:1992
EXPERIMENTER jjp
SUBJECT NO 921
EXPERIMENT NO 5
RUN NO 16
NO.OF BREATHS 174
MODE OF THIS EXPERIMENT 1 1 1 1
APPARATUS DEADSPACE USED 0.100
ANATOMICAL DEADSPACE USED 0.150

BRNO	COMIN	COSLO	COMN	O2MAX	O2SLO	O2MN
1	37.3	0.000	37.3	105.5	0.000	105.5
3	39.7	0.000	39.6	98.5	0.000	99.6
4	38.3	0.768	39.4	103.3	1.563	100.4
5	43.4	-0.362	42.5	108.2	3.730	102.7
6	43.5	-0.172	43.1	106.0	1.165	103.1
7	42.2	0.000	42.4	105.0	0.000	104.2
8	41.6	0.069	41.8	104.3	-0.121	104.6
9	39.1	0.657	40.4	104.1	-2.023	105.2
10	40.8	0.969	41.3	109.2	1.942	107.8
11	44.0	0.555	44.1	112.3	4.212	107.4
12	42.7	0.590	43.9	107.7	1.269	105.0
13	41.8	-0.011	42.6	106.8	-0.208	105.9
14	40.6	0.216	41.0	106.3	0.424	106.1
15	40.4	0.910	41.3	110.8	2.856	107.1
16	39.9	1.040	41.4	113.3	3.285	108.1
17	40.1	0.637	41.3	110.0	3.022	106.3
18	39.8	0.794	40.9	108.8	2.763	104.7
19	41.5	0.239	41.9	107.2	1.729	103.8
21	41.7	-0.242	41.7	108.1	-0.076	106.3
22	44.6	0.028	44.0	102.2	-1.036	104.6
23	40.9	0.516	42.5	102.9	-0.973	104.5
24	39.9	0.203	40.6	100.5	-0.625	102.0
26	43.3	-0.398	43.4	101.1	-0.777	101.0
27	41.4	0.217	41.7	106.6	1.441	104.7
28	37.9	1.855	40.3	109.7	4.787	104.5
29	40.4	0.159	41.1	104.4	0.566	102.3
30	42.2	-1.005	41.3	107.1	0.990	105.0
31	41.8	0.261	41.7	102.1	-0.841	103.6
32	41.6	1.190	42.5	107.5	4.946	104.5
33	39.8	1.596	41.8	104.1	1.404	101.4
34	37.5	2.159	40.3	109.2	4.726	104.0
35	39.7	0.707	41.0	103.7	0.756	101.5
36	41.5	0.534	41.9	103.1	1.685	101.4
37	41.6	0.074	42.0	100.6	-0.006	99.9
38	38.3	2.054	40.4	105.2	3.155	102.2
39	41.2	0.235	41.7	101.6	0.151	100.7
40	41.5	0.954	42.2	101.2	1.108	100.6
41	40.2	1.107	41.8	102.1	2.018	100.0

Below is a partial listing of .D3 file. The first eight lines are header lines, as in the .D1 file. The next two lines contain information about the inspired and expired temperatures. Note that the expired temperature (calculated so that net nitrogen balance is zero) is close to the value expected of a subject breathing room air. The "estimated expiratory flow error" in the next line is the error if the process of adjusting temperature fails to yield a net nitrogen exchange of zero: the value here should always be zero. Note that the calculated effective lung volume for CO_2 is larger than that for O_2, which Swanson (1980) has argued is consistent with the observation that pulmonary CO_2 stores are larger than O_2 stores. The fifteenth line lists column headers for the calculated variables for gas exchange. The first column is the breath number. The next two columns list the mean inspired and expired fractional concentration at the mouth for CO_2 and O_2 respectively; followed by the mean expired fractions at the mouth for these gases in the succeeding two columns. FAC2 and FAO2 refer to the end-tidal concentrations for CO_2 and O_2 respectively. The next three columns list, in order, the net flows at the mouth (in L/min) for CO_2, O_2 and N_2. It is this last value, whose mean is calculated to be equal to zero over the whole experimental period, by adjusting the expired temperature. The last two columns show the calculated $\dot{V}_{CO2}$ and $\dot{V}_{O2}$ at the pulmonary capillary, using the method of Swanson (1980).

Data File written as j9210516.d3
DATE 21:12:1992
EXPERIMENTER jjp
SUBJECT NO 921
EXPERIMENT NO 5
RUN NO 16
NO.OF BREATHS 174
MODE OF THIS EXPERIMENT 1 1 1 1
INSPIRED GAS TEMPERATURE USED 24.0
EXPIRED GAS TEMPERATURE USED 31.3
ESTIMATED EXPIRATORY FLOW ERROR 0.0
ALVEOLAR GAS EXCHANGE COMPUTED USING METHOD PER SWANSON
EFFECTIVE LUNG VOLUME CO2 2.449
EFFECTIVE LUNG VOLUME O2 1.070

BRNO	FMIC2	FMIO2	FMEC2	FMEO2	FAC2	FAO2	VMCO2	VMO2	VMN2	VCCO2	VCO2
1	.0061	.1862	.0394	.1656	.0519	.1468	0.177	0.180	0.367	0.201	0.113
3	.0328	.1696	.0517	.1470	.0552	.1370	0.119	0.120	-0.110	0.149	0.223
4	.0258	.1818	.0528	.1489	.0559	.1386	0.145	0.285	0.367	0.199	0.195
5	.0526	.1947	.0527	.1586	.0596	.1423	-0.035	0.404	0.238	0.157	0.271
6	.0381	.1908	.0534	.1553	.0604	.1445	0.105	0.201	-0.250	0.118	0.208
7	.0325	.1851	.0511	.1543	.0587	.1461	0.108	0.263	0.201	0.058	0.200
8	.0284	.1883	.0510	.1549	.0589	.1460	0.207	0.224	-0.379	0.188	0.296
9	.0230	.1889	.0472	.1588	.0552	.1486	0.188	0.457	0.755	0.082	0.271
10	.0466	.1814	.0541	.1575	.0587	.1495	0.104	0.168	-0.551	0.238	0.239

CHAPTER 3

THE VENTILATORY EFFECTS OF SUSTAINED ISOCAPNIC HYPOXIA DURING EXERCISE IN HUMANS

I never wear a brassiere because I believe in
giving free play to my respiratory system.

Marilyn Monroe

Introduction

In the Introduction, some of the studies investigating the mechanisms underlying the genesis of HVD in man were reviewed. In particular, it was noted that the magnitude of the HVD appears to be related to the magnitude of the acute ventilatory response to hypoxia (AHR), both between subjects and within an individual subject, when the subject's AHR is modified by a number of interventions. Steady exercise is known to increase the acute ventilatory response to hypoxia. The objective of this study was to investigate whether exercise increases HVD (*e.g*, like almitrine; Georgopoulos *et al.*, 1989*c*) or whether HVD is unaffected (*e.g*, like domperidone; Bascom *et al.*, 1991).

Dahan (1990) in his doctoral thesis studied the possible effects of exercise on HVD. He looked at six subjects: five were studied twice, one was studied once so that a total of 11 experiments at rest and 11 in exercise were obtained. At rest, HVD was observed in all 11 experiments. However in exercise, HVD occurred in only 3 experiments: in 8 of the 11 experiments it was not evident. His findings suggest that unlike all other modulators of peripheral hypoxic sensitivity, exercise actually reduces, or possibly even abolishes hypoxic ventilatory decline.

The purpose of this investigation was to see if we could confirm the findings of Dahan (1990), and at the same time overcome some of the technical problems associated with his study. First, the protocols were repeated several times on each subject. Secondly, we wished to ensure that a steady-state of exercise had been achieved, and so performed control experiments involving exercise in which no hypoxia was administered. Finally, the possibility that lactic acidosis occurred during hypoxic exercise also needed to be excluded.

Methods

Subjects

We studied five normal young adult males. Their individual characteristics are listed in Table 3.1.

Experimental technique

During experiments, the subjects breathed through a mouthpiece and wore a noseclip. The end-tidal gases were controlled by dynamic end-tidal forcing, as described in Chapter 2. For the protocols at rest, subjects were seated in a chair. Exercise was performed on an electromagnetically-loaded cycle ergometer and subjects were cooled by fans. A heparinised venous blood sample was taken before the start of exercise and when exercise was complete for the measurement of plasma lactate. During all protocols, an ECG was monitored continuously and a pulse oximeter was used to record arterial oxygen saturation: readings were noted at one min intervals (Powers *et al.*, 1989).

TABLE 3.1.

Physical characteristics of subjects.

Subject	Sex	Height(m)	Weight(kg)	Age(yrs)
797	M	1.70	68	26
799	M	1.80	76	26
800	M	1.70	67	24
807	M	1.82	81	24
811	M	1.84	80	25

Protocols

The five subjects undertook each of the following four protocols in random order six times, giving a total of 120 experimental periods.

Protocol A: with subject at rest, PET_{O2} was held at 100 Torr for 10 min, then at 50 Torr for 25 min, then returned to 100 Torr for the final 8 min.

Protocol B: with subject at rest, PET_{O2} was held at 100 Torr for 43 min. This served as a control for protocol A.

Protocol C: with subject performing constant 70 Watt exercise, PET_{O2} was held at 100 Torr for 10 min. Then, PET_{O2} was brought down to a value that approximated the arterial saturation achieved during the hypoxic period at rest: in four subjects, PET_{O2} was held at 55 Torr for 25 min; in one subject (800), PET_{O2} was held at 60 Torr for 25 min. Then, PET_{O2} was restored to 100 Torr for the final 8 min.

Protocol D: with subject exercising, PET_{O2} was held at 100 Torr for 43 min. This served as a control for protocol C.

End-tidal PET_{CO2} was held constant at 2-3 Torr above end-tidal values both at rest and in exercise for all protocols. In one subject (807), the breathing was somewhat erratic, as was consequently the end-tidal P_{CO2}. We therefore held his PET_{CO2} 4-5 Torr above his average end-tidal value which helped to achieve better control during hypoxia.

Data analysis

Results from the first 5 min from each protocol were excluded from the analysis. Data from each trial were averaged over one min periods. Four of the one min periods from each individual hypoxic experiment were used for calculation of the fast components of the ventilatory response to hypoxia (on- and off-responses) and the HVD (Fig. 3.1.).

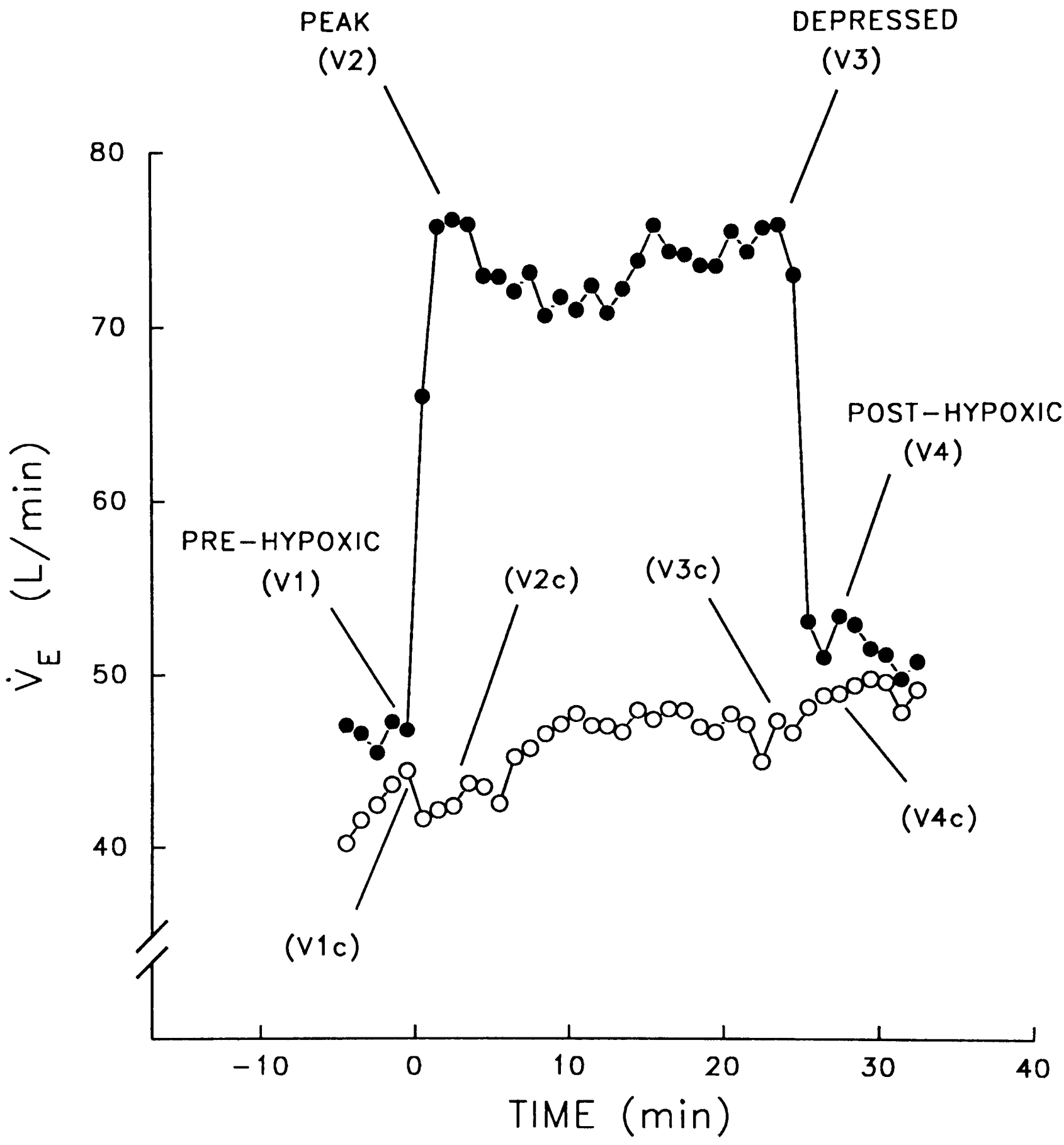


Figure 3.1. Illustration of the points used to calculate the magnitude of the acute hypoxic response (AHR), HVD and off-response. One single representative hypoxic experimental period in exercise (●), and the average of six euoxic control experimental periods combined, during exercise (○) in one subject. Hypoxic period from time = 0 to 25 min.

These four periods were: 1) the last min of the euoxic period (the "pre-hypoxic" point, V1), 2) the peak ventilation reached in the first 5 min of hypoxia (the "peak" point, V2), 3) the last min of hypoxia (the "depressed" point, V3), 4) the minimum ventilation reached in the first 5 min of euoxia (the "post-hypoxic" point, V4). Values for the four points (V1, V2, V3 and V4) were taken from each of the individual experiments of protocols A and C for each subject. Values for the corresponding ventilations in the control protocols (V1c, V2c, V3c, V4c) were taken from the mean results of protocols B and D for each subject, (Fig. 3.1).

Thus, for each experimental period at rest and exercise,

On-response (AHR) = (V2 - V2c) - (V1 - V1c)

HVD = (V2 - V3) - (V2c - V3c)

Off-response = (V3 - V3c) - (V4 - V4c)

For each subject, six values of HVD, AHR and off-response at rest and six in exercise were thus obtained. These values were averaged to give the mean value of AHR, HVD and off-response for each subject (the subject mean). These subject means were then averaged to obtain the mean for all five subjects. The absolute values for AHR, HVD and off-response for each experiment were divided by the fall in saturation during hypoxia for that experiment, so that values for ventilatory sensitivities per unit desaturation were also obtained.

The statistical significance of the differences between these parameters for rest and exercise were assessed using a one-tailed, paired t-test (Bailey, 1985).

Results

The values for the end-tidal gas input stimuli for each of the 5 subjects and the averages for all subjects combined are shown in Fig. 3.2. End-tidal P_{O2} reached the desired levels rapidly in both rest and exercise and remained constant throughout the hypoxic period. End-tidal P_{CO2} values were constant throughout all protocols, apart from a few imperfections in some subjects during transitions into and out of hypoxia.

Figure 3.3 shows the hypoxic input in terms of the arterial saturation. Levels of desaturation were very similar in two subjects in rest and exercise. In the three subjects in whom the saturations were dissimilar, the saturations reached in exercise were always lower than those reached at rest. The saturations remained essentially constant throughout the hypoxic period.

The general form of the ventilatory response to hypoxia at rest and in exercise both for the euoxic and hypoxic protocols is shown in Fig. 3.4. All subjects showed a biphasic response to hypoxia at rest. During exercise, the size of the acute response to hypoxia was increased, but the HVD appeared to be attenuated. However, there appeared to be a gradual rise in baseline ventilation in all subjects during exercise, such that the post-hypoxic ventilation was slightly greater than the pre-hypoxic value. This gradual rise in ventilation was also observed in the data from all control protocols in exercise in which there was no hypoxia, and in the data from subjects 797, 807 and to some extent, 800 at rest. The data analysis took this rising baseline into account and so avoided an underestimation of the HVD (Fig. 3.1).

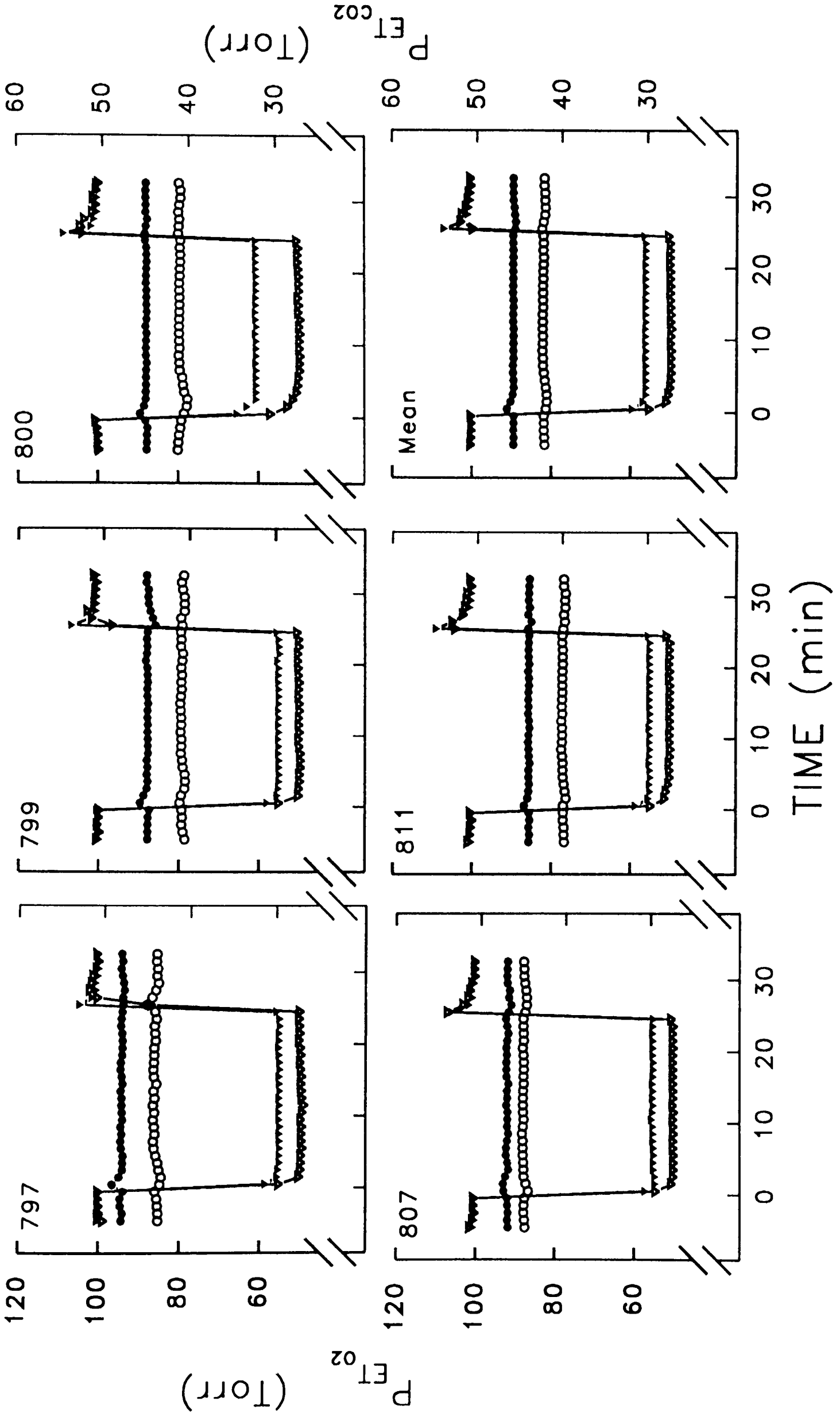


Figure 3.2. Mean end-tidal gas profiles for each of the five subjects and the average for all five combined. For clarity, only gas profiles for the hypoxic protocols (A and C) are plotted. $P_{ET_{O_2}}$ at rest (▽); $P_{ET_{O_2}}$ during exercise (▼); $P_{ET_{CO_2}}$ at rest (○); $P_{ET_{CO_2}}$ during exercise (●).

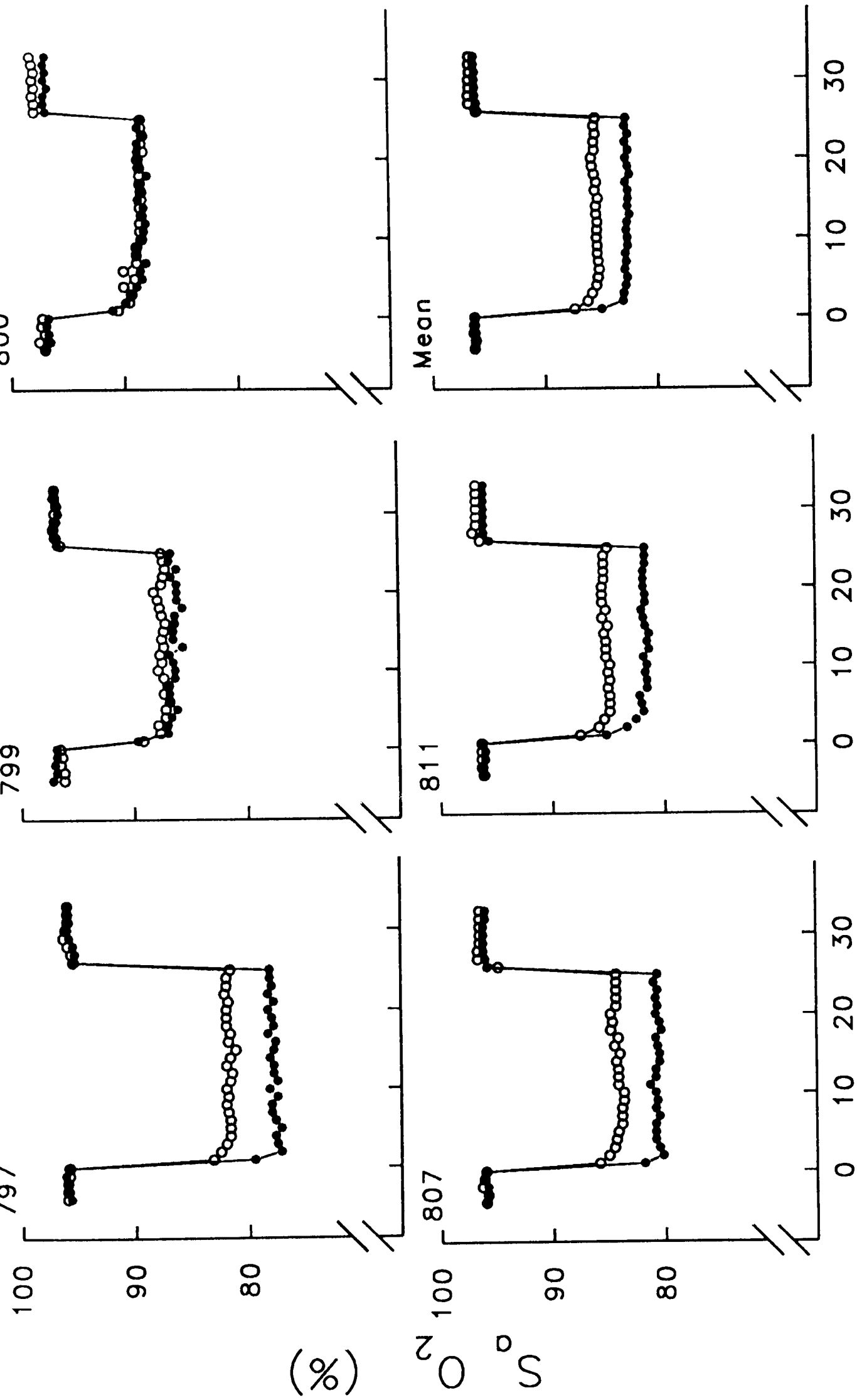


Figure 3.3. Mean arterial saturations for each of the five subjects and the average for all five combined. For clarity, only gas profiles for the hypoxic protocols (A and C) are plotted. Saturation at rest (○); saturation during exercise (●).

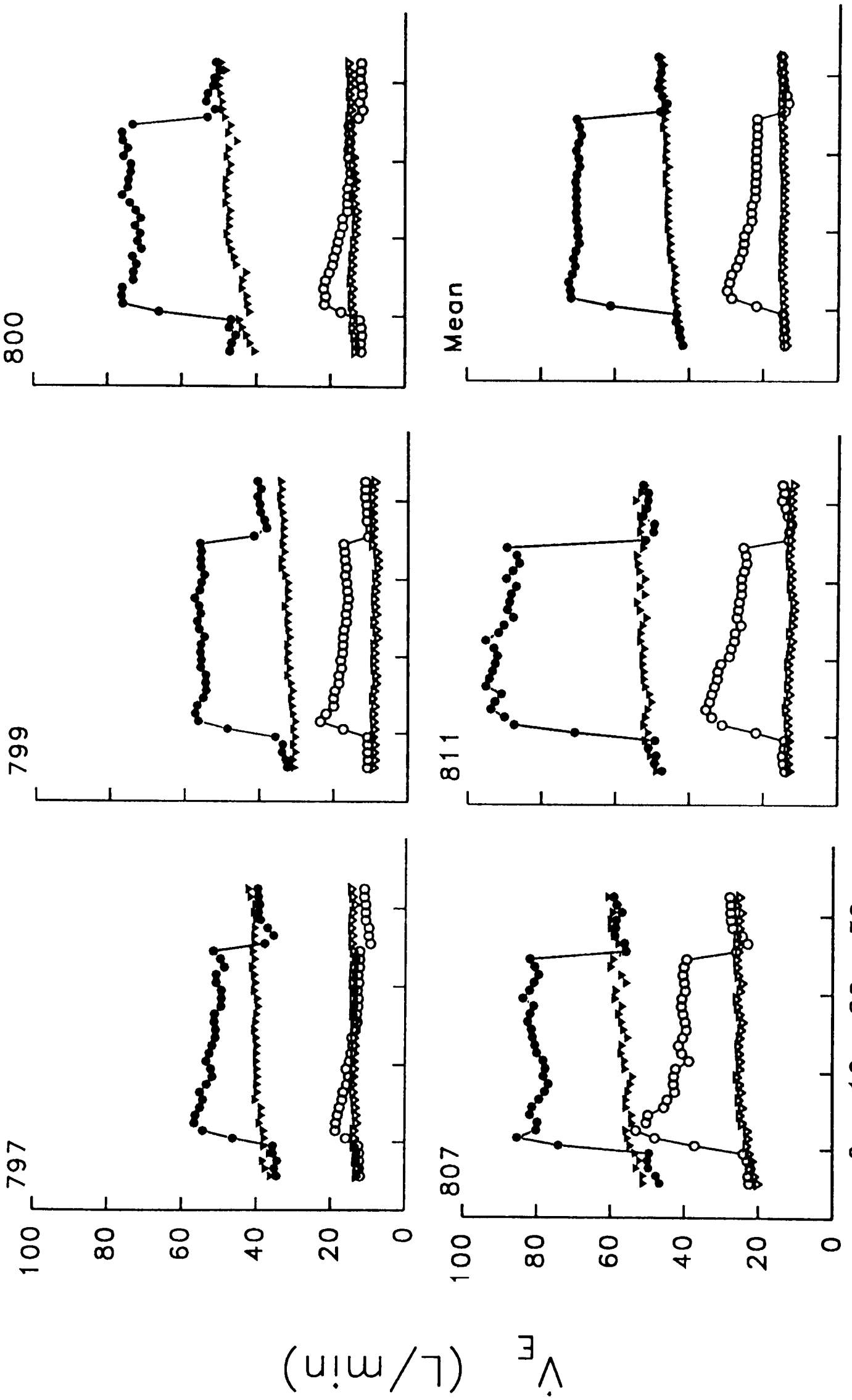


Figure 3.4. Mean ventilations against time for each of the five subjects and the average for all five combined, for all four protocols. Hypoxic period from time = 0 to 25 min. Hypoxia at rest, protocol A (○); euoxic control, protocol B (▽); hypoxia during exercise, protocol C (●); euoxic control during exercise, protocol D (▼).

Table 3.2 shows the average values for the on-response (AHR), HVD and off-response for the individual subjects. The absolute values for both the on-response and off-response were greater during exercise than at rest for all subjects. These values expressed per unit desaturation, the ventilatory sensitivities, were also greater during exercise than at rest for all subjects, except in subject 807. Hypoxic ventilatory decline, either expressed in absolute terms or as relative to arterial desaturation, was decreased during exercise in all subjects.

Figure 3.5A shows the absolute magnitude of the on-response, HVD and off-response for all 5 subjects combined. The on-response is significantly increased by exercise ($P < 0.01$) and the absolute magnitude of HVD appears reduced, although this latter observation does not reach statistical significance. Since the saturations did not match exactly between conditions of hypoxia at rest and during exercise, the absolute values were divided by the change in saturation and Fig. 3.5B shows the result. The AHR per unit desaturation is still significantly increased by exercise ($P < 0.05$), but the HVD is actually significantly reduced ($P < 0.05$). Thus, for an equal hypoxic stimulus, HVD is reduced in exercise when compared with rest. Figure 3.5C illustrates the change in the ratio of HVD to AHR from rest to exercise.

There was no significant increase in venous lactate after hypoxic exercise, the mean values (± SE) being 0.81 ± 0.06 mmol/L before exercise and 0.81 ± 0.13 mmol/L after exercise.

TABLE 3.2

(a) Absolute ventilatory responses (L/min ± SE)

	On-Response		HVD		Off-Response		HVD/On-Response	
Subject	Rest	Exercise	Rest	Exercise	Rest	Exercise	Rest	Exercise
797	6.21	19.7	6.71	6.70	3.38	15.8	1.11	0.27
	0.59	2.36	0.50	3.61	0.72	2.13	0.09	0.16
799	12.9	20.8	6.60	4.10	6.93	17.5	0.50	0.18
	1.40	2.07	1.70	1.20	1.08	0.93	0.09	0.04
800	9.70	31.4	7.57	7.38	3.53	24.2	0.85	0.24
	1.78	1.72	1.12	3.18	1.14	2.26	0.10	0.10
807	28.6	33.7	16.6	7.27	16.5	22.9	0.56	0.22
	2.30	2.51	2.57	1.53	1.14	2.65	0.05	0.06
811	20.2	45.3	9.02	5.77	12.7	40.3	0.45	0.10
	2.06	6.03	0.75	3.51	1.95	4.11	0.03	0.10

(b) Ventilatory sensitivities (L/min/% ± SE)

	On-Response		HVD		Off-Response	
Subject	Rest	Exercise	Rest	Exercise	Rest	Exercise
797	0.44	1.11	0.47	0.38	0.24	0.89
	0.04	0.13	0.03	0.20	0.05	0.12
799	1.40	2.06	0.69	0.41	0.74	1.70
	0.18	0.25	0.22	0.12	0.12	0.09
800	1.15	3.47	0.91	0.82	0.42	2.61
	0.19	0.32	0.13	0.36	0.14	0.24
807	2.37	2.20	1.37	0.48	1.37	1.49
	0.19	0.18	0.21	0.12	0.09	0.17
811	1.81	3.19	0.81	0.41	1.12	2.83
	0.21	0.43	0.09	0.20	0.17	0.29

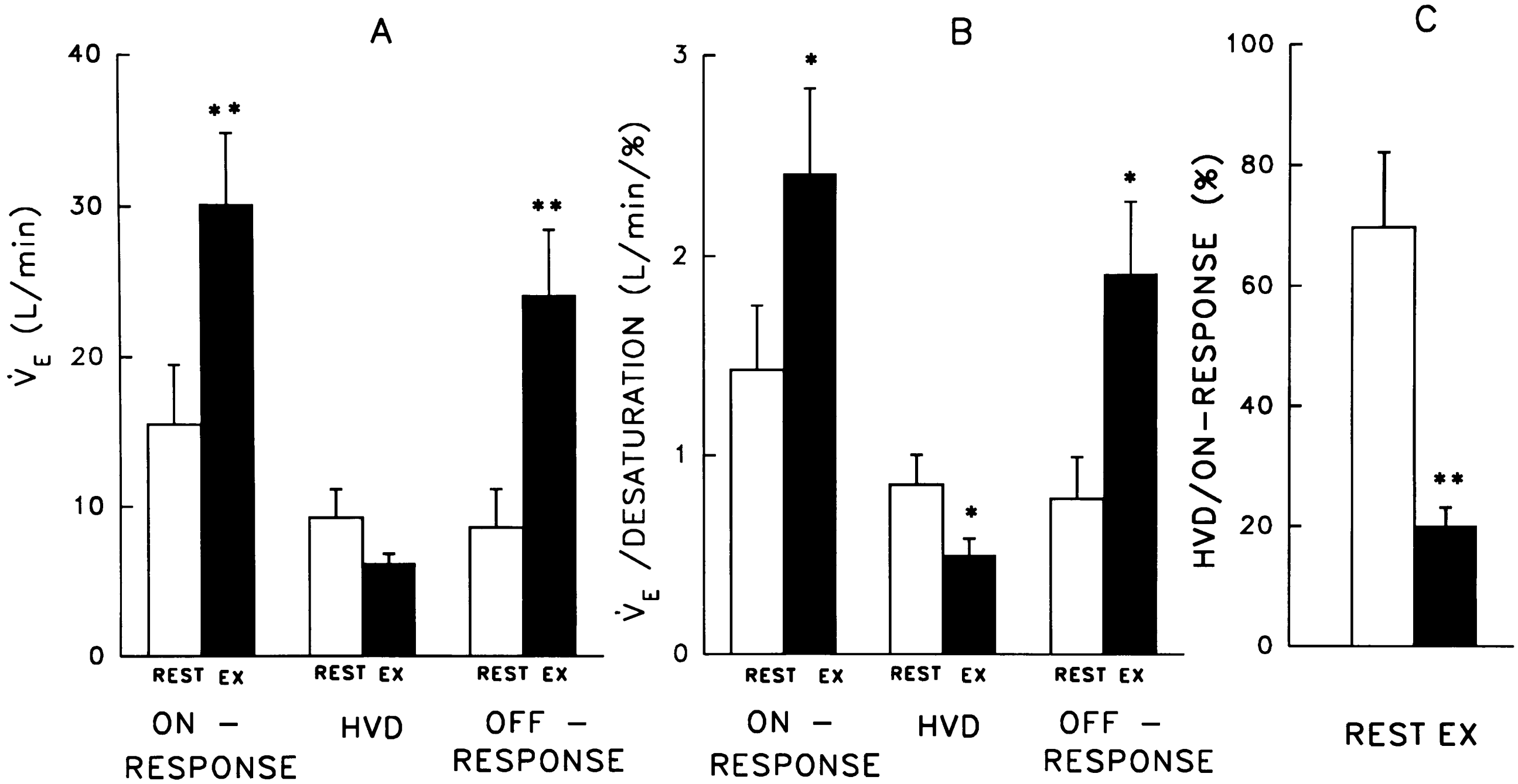


Figure 3.5. Mean values (± SE of all five subjects combined, for the On-response, HVD, and Off-response. Panel A: the absolute ventilations. Panel B: the ventilatory sensitivities. Panel C: HVD expressed as a percentage of the On-response. Rest: hollow bars; exercise: filled bars. ($^{*}P < 0.05$, $^{**}P < 0.01$, for comparisons between rest and exercise. All values are significantly different from zero ($P < 0.05$).

Discussion

The major finding in this study is that exercise reduces the hypoxic ventilatory decline expressed both relative to the acute hypoxic response and as absolute HVD per unit of desaturation.

Possible problems with experimental technique

1) Steady-state exercise not achieved. The subjects undertook at least 5 min of exercise before data collection began to allow a steady-state to be reached. There was a further 5 min of exercise before hypoxia was administered. However, a gradual rise in baseline ventilation was observed in hypoxic studies both at rest and during exercise such that the ventilation after the hypoxic period was higher than that before. This was also seen in the control, euoxic experiments: ventilation was continuing to rise even after 40 min of exercise. A steady rise in ventilation in response to a constant hypercapnic stimulus has been reported previously (Reynolds *et al.*, 1972; Georgopoulos *et al.*, 1989*a*; Khamnei and Robbins, 1990). The mechanism of the effect is unclear, but it is possible a similar phenomenon occurred in the current study. Since the dynamic end-tidal forcing technique requires end-tidal CO_2 to be held a little higher than the subject's own value to achieve good control, there was a mild hypercapnic stimulus in our subjects. However, in this study, the mean ventilations of the control experiments were subtracted from the ventilations of individual hypoxic exposures to allow for any progressive effect of sustained hypercapnia and avoid underestimating hypoxic ventilatory decline (Fig. 3.1).

2) *Development of lactic acidosis*. Lactate levels did not rise after hypoxic exercise.

3) *Control of hypoxia*. Hypoxia was well-controlled and there was no trend in either $P_{ET_{O2}}$ or saturation.

4) *Matching of arterial P_{CO2} between rest and exercise*. This is always difficult by estimation of end-tidal values alone: while end-tidal values give an acceptable estimate of arterial values at rest, during 70 Watt exercise they may overestimate arterial levels by 1-2 Torr (Robbins *et al.*, 1990). We assumed that, during air-breathing, there would be arterial isocapnia from rest to steady-state exercise. We then held end-tidal P_{CO2} at 2-3 Torr above the air-breathing value observed at rest and 2-3 Torr above the air-breathing value observed in exercise. The hypercapnic stimulus in the two states would thus have been similar. While some differences in the actual arterial values between rest and exercise may have occurred, they would have been small and insufficient to have accounted for the striking effect of exercise on the magnitude of HVD.

Physiological Significance of Results

Khamnei and Robbins (1990) argue that the sensitivity of the peripheral chemoreflex declines during exposure to prolonged hypoxia. This argument is based on the observation that the magnitude of the rapid fall in ventilation at the relief of hypoxia, the "off-response", is smaller than the rapid rise in ventilation at the start of hypoxia, the "on-response". This decline in sensitivity of the peripheral chemoreflex loop during sustained hypoxia has been documented directly by applying pulses of extra hypoxia during the development of hypoxic depression of ventilation: the ventilatory response to successive pulses declines (Bascom *et al.*, 1990). The mechanism by which sustained hypoxia causes a decline in peripheral chemoreflex sensitivity is essentially unknown. This mechanism may lie at the level of the peripheral chemoreceptor, or more centrally in the chemoreflex pathway, or at both sites.

However, the hypothesis that it is peripheral chemoreflex sensitivity that is affected by sustained hypoxia does lead to a prediction. If an additional factor independently alters peripheral chemosensitivity, without directly modulating the mechanism itself by which sustained hypoxia causes a decline in sensitivity, then factors which increase peripheral chemosensitivity should also increase the absolute magnitude of HVD, and factors which decrease the peripheral chemosensitvity should decrease the magnitude of the subsequent HVD. The requirement for this may be seen by considering the simple model in Fig. 3.6.

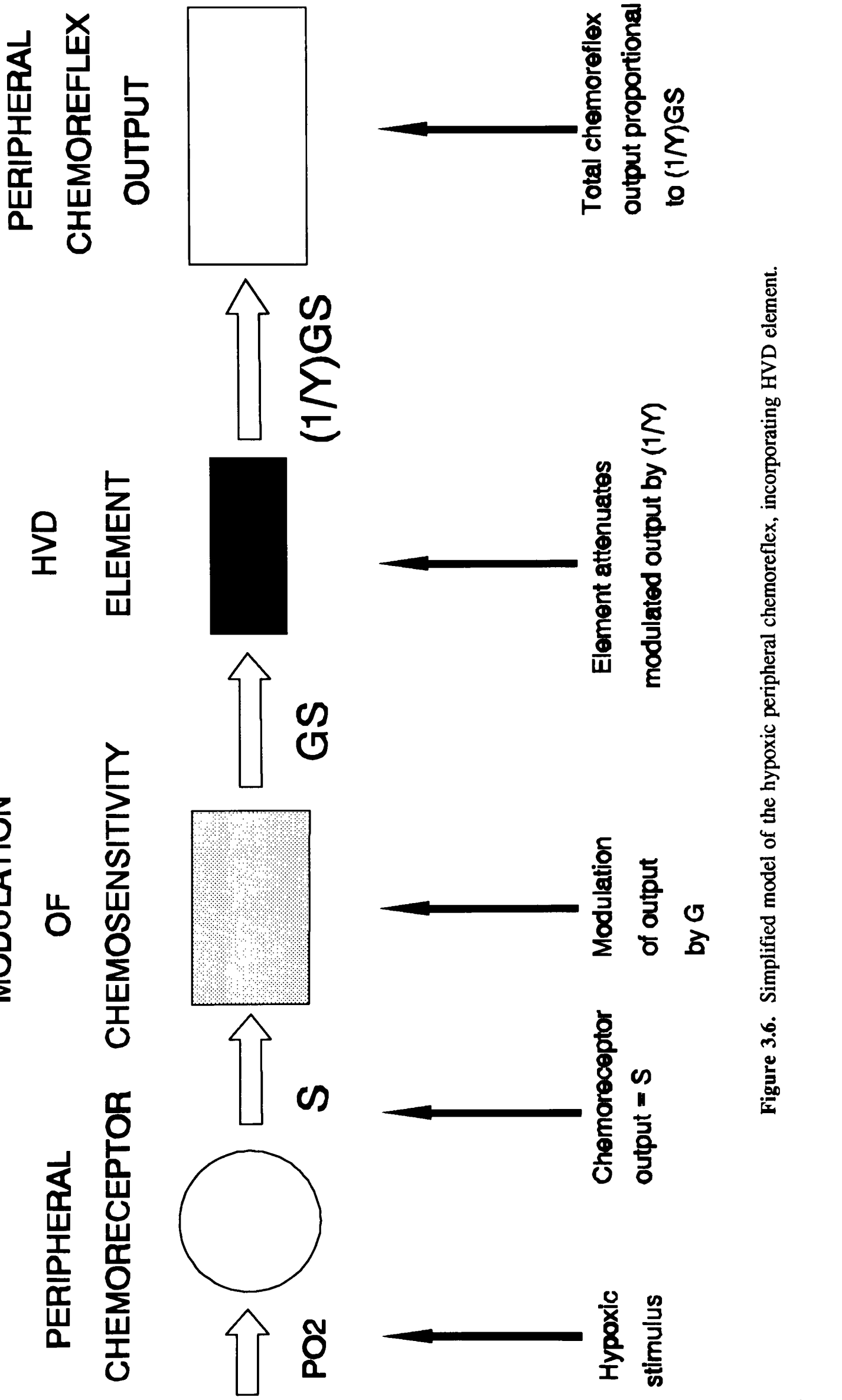


Figure 3.6. Simplified model of the hypoxic peripheral chemoreflex, incorporating HVD element.

HVD is represented as an element in series with the peripheral hypoxic chemosensor. The element is essentially activated by prolonged hypoxia in a time-dependent manner and its net effect over a given period of time is to reduce the magnitude of the response (S) to a given hypoxic stimulus by a constant fraction (1/Y).

If an additional factor acts to modulate the response to hypoxia by some multiple (G), but does not also interact independently with the HVD element, its effect on the output will always be reduced by the constant fraction (1/Y). The greater the stimulus in terms of the acute response to hypoxia, the greater will be the absolute magnitude of the subsequent decline in ventilation.

This prediction appears to be realised by almitrine (Georgopoulos *et al.*, 1989*c*), somatostatin (Maxwell *et al.*, 1986; Filuk *et al.*, 1988), and to some extent by carbon dioxide (Georgopoulos *et al.*, 1989*a*; Khamnei and Robbins, 1990) and hypoxia itself (Bascom *et al.*, 1992).

The striking observation with exercise is that this prediction is far from realised. This raises two possibilities. Either hypoxic depression of ventilation is not brought about by a modulation of peripheral chemoreflex sensitivity (a proposition that seems unlikely in the light of previous experimental results) or exercise itself has a direct effect on the mechanism by which sustained hypoxia depresses peripheral chemosensitivity (i.e, a direct effect on the HVD element in Fig. 3.6).

This conclusion does not help to determine whether the modulation of peripheral chemosensitivity by sustained hypoxia is a peripheral chemoreceptor event or whether it is located in the medulla, as it is clear that exercise could affect hypoxic sensitivity at either or both sites (Masson and Lahiri, 1974). However, the suggestion that exercise directly interacts with the mechanism generating HVD does raise the possibility that the mechanisms by which exercise increases peripheral sensitivity to hypoxia may themselves be involved in the processes by which HVD is generated.

CHAPTER 4

HYPOXIC VENTILATORY DECLINE: IS THE UNDERLYING PROCESS GENUINELY ATTENUATED BY EXERCISE ?

Up to the present, more concern has been given to enlarging the building
than to giving proper strength to the foundations

Jean d'Alembert

Introduction

In the previous chapter, some of the ventilatory effects of sustained hypoxia during exercise were examined. In particular, it was observed that while the magnitude of the acute ventilatory response to hypoxia (AHR) was increased during exercise as compared with rest, the magnitude of the subsequent decline in ventilation (HVD) was reduced. This response appears to differ from that in many other instances, when in general, the magnitude of the HVD is related to the magnitude of the AHR.

The precise mechanism underlying HVD is unkown, but a number of studies suggest that in conscious man it is, at least in part, due to a time-dependent decline in the sensitivity of the peripheral chemoreflex to hypoxia (Khamnei and Robbins, 1990; Bascom *et al.*, 1990). In the light of this, one interpretation of the observation that exercise differs from many other interventions is that exercise, by some mechanism, directly attenuates the time-dependent decline in peripheral hypoxic sensitivity. A simple model describing how exercise may achiece this has been discussed in the previous chapter (Chapter 3; Pandit and Robbins, 1991).

Another interpretation of the observation, however, is that the underlying decline in chemoreflex sensitivity occurs to the same degree during hypoxic exercise as it does at rest, but that exercise simply masks it. This interpretation is supported by the observation that while sustained hypoxia during exercise does indeed attenuate the decline in total minute ventilation, the magnitude of the decline in tidal volume (V_T) is unaffected, and V_T declines to the same extent during hypoxic exercise as it does at rest (Ward and Nguyen, 1991).

It is important to establish whether or not the ventilatory sensitivity to hypoxia genuinely adapts during sustained hypoxic exercise. If, as suggested by the study of Ward and Nguyen (1991), the true degree of decline in hypoxic sensitivity remains unaffected by exercise, it follows that the measured HVD during hypoxic exercise cannot simply be assumed to reflect accurately changes in peripheral chemoreflex activity. This result would have important implications for the interpretation of results regarding the mechanism of

HVD, both at rest and during exercise, and might suggest that modulation of the peripheral chemoreflex is not the only mechanism involved in the genesis of HVD.

The purpose of this study was to assess changes in the ventilatory sensitivity to hypoxia in a manner more direct than by inference from changes in the pattern of breathing. It has been shown that complete recovery from a sustained exposure to hypoxia requires up to one hour of air-breathing (Easton *et al.*, 1988; Khamnei, 1989). Bascom *et al.* (1992) and Berkenbosch *et al.* (1992) have exploited this observation to assess the degree of decline in the hypoxic chemoreflex sensitivity by measuring the AHR 5-6 min after a period of sustained hypoxia, and comparing its magnitude with that of the AHR on or before administration of hypoxia. This method has been used in the study presented here. To assess the degree of decline in hypoxic sensitivity during exercise, the AHR in the resting period following a prior period of hypoxic exercise was measured, and compared with the AHR following a period of control, euoxic exercise.

Methods

Subjects

We studied seven normal young adult males. Their physical characteristics are shown in Table 4.1. Their end-tidal values for P_{CO2} were obtained as the mean over a 20 min period breathing air at rest, after familiarisation with the laboratory. Similarly, their end-tidal P_{CO2} values during exercise were obtained over a 20 min period performing 70 W exercise breathing air.

TABLE 4.1

Physical characteristics of subjects

Subject	Sex	Height (m)	Weight (kg)	Age (years)
802	M	1.79	70	21
835	M	1.78	69	21
836	M	1.82	74	23
842	M	1.77	75	22
846	M	1.81	74	21
851	M	1.80	70	22
903	M	1.65	75	19

Experimental technique

The subjects breathed through a mouthpiece and wore a noseclip during the experiments. The end-tidal gases were controlled by dynamic end-tidal forcing, as described in Chapter 2. Exercise was performed on an electromagnetically-loaded cycle ergometer. For resting studies, subjects were seated on the ergometer, and they remained seated on this also after the exercise periods in protocols B and C (see below). Subjects were cooled by fans. A heparinised venous blood sample was taken before and after the exercise protocols for the measurement of plasma lactate (YSI Model 23L Lactate Analyser). An ECG was monitored continuously during all protocols, and a pulse oximeter was used to record arterial oxygen saturation, with readings noted at one min intervals.

Protocols

Six subjects undertook each of the following three protocols in random order six times, and one subject (903) undertook each protocol four times, giving a total of 120 separate experimental periods. The protocols are summarised in Fig. 4.1.

Protocol A: with the subject at rest, $P_{ET_{O2}}$ was held at 100 Torr for 10 min, at 50 Torr for 20 min, then returned to 100 Torr for 6 min. There was then a second exposure to 50 Torr for a final 6 min. The $P_{ET_{CO2}}$ was held 2-3 Torr above the subject's resting end-tidal value throughout this protocol.

Protocol B: the subject performed constant 70 Watt exercise for 30 min. During this period, $P_{ET_{O2}}$ was held at 100 Torr for the first 10 min and then reduced for 20 min to a value which approximated the arterial saturation during the hypoxic period at rest. In all subjects, this was a value of 55 Torr. The $P_{ET_{CO2}}$ was held 2-3 Torr above the subject's exercising end-tidal value throughout the exercise period. After 30 min of exercise (20 min hypoxia), there were three interventions: (a) the subject suddenly stopped exercise, (b) at the same time, the $P_{ET_{CO2}}$ was held at 2-3 Torr above the subject's now resting end-tidal value, and (c) the $P_{ET_{O2}}$ was returned to 100 Torr for 6 min. Finally, with the subject still at rest, $P_{ET_{O2}}$ was reduced to 50 Torr for the last 6 min.

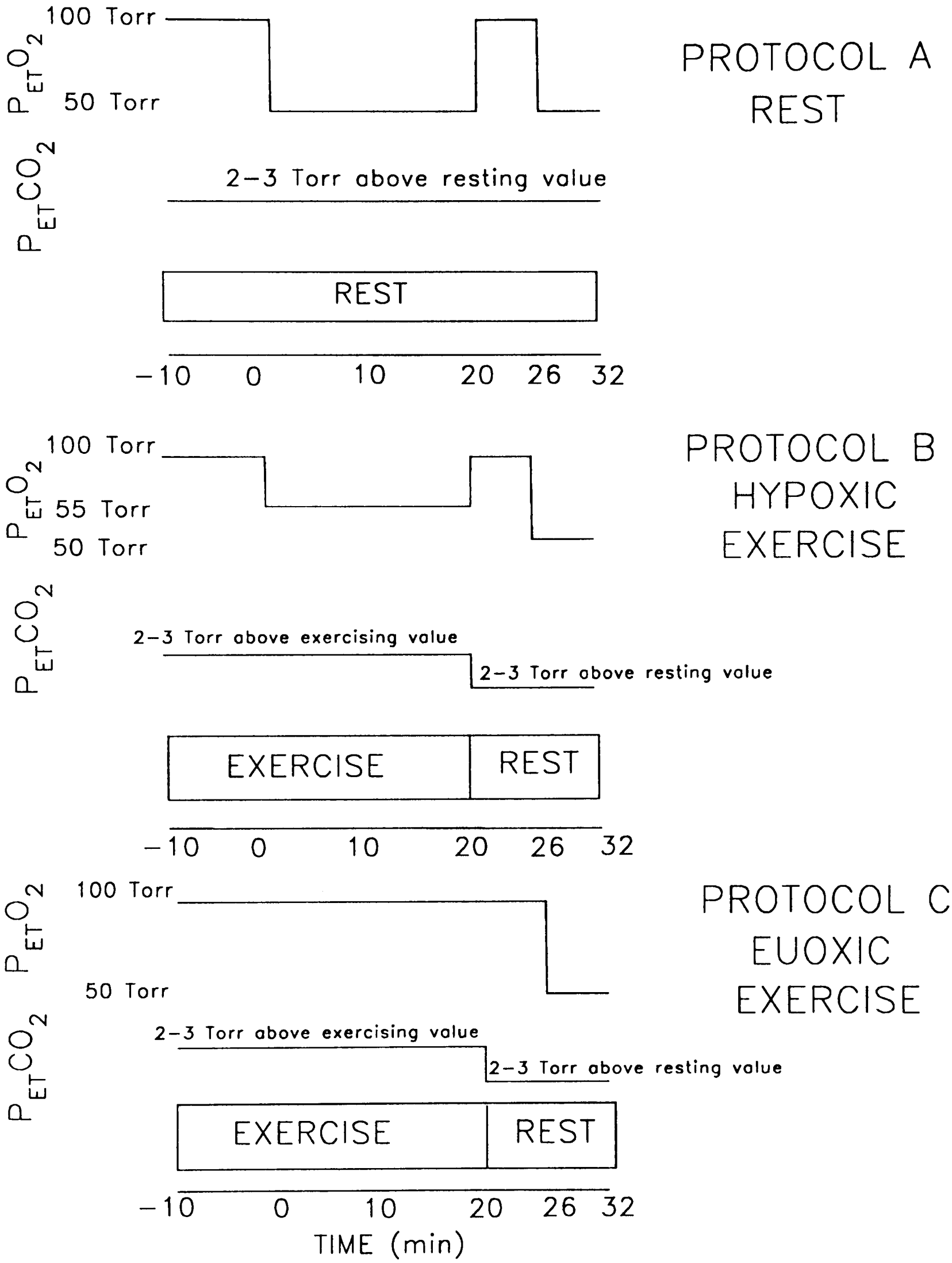


Figure 4.1. Diagrammatic illustration of the three protocols, A, B and C, showing end-tidal gas values for P_{O2}, P_{CO2} and work rate with relation to time.

Protocol C: the subject performed constant 70 Watt exercise for 30 min, with the $P_{ET}O_2$ held at 100 Torr. During this period, the $P_{ET}CO_2$ was held 2-3 Torr above the subject's exercising end-tidal value. At 30 min, (a) the subject suddenly stopped exercise, (b) the $P_{ET}CO_2$ was held 2-3 Torr above the subject's now resting end-tidal value, and (c) the $P_{ET}O_2$ was maintained at 100 Torr for a further 6 min at rest. Finally, with the subject still at rest, $P_{ET}O_2$ was reduced to 50 Torr for the last 6 min. This protocol served as a euoxic control for the hypoxic exercise protocol B.

Data Analysis

Results from the first 5 min from each protocol were excluded from the analysis. Data from the remaining 37 min in each experimental period were averaged over one min periods.

Up to five of these one min periods, depending on whether the protocols involved hypoxia or not, were used to calculate the acute ventilatory responses to hypoxia and the HVD (Fig. 4.2). These five periods were: 1) the last min of the first euoxic period (point V1), 2) the peak ventilation reached in the first 5 min of the first hypoxic exposure (point V2), 3) the last min of sustained hypoxia (point V3), 4) the last min of the second euoxic period (point V4), 5) the peak ventilation reached in the first 5 min of the second exposure to hypoxia (point V5).

In protocol A, two acute hypoxic responses at rest were obtained. The first was the AHR at the onset of the period of sustained hypoxia: AHR*(rest,control)*. The second was the AHR after the period of sustained hypoxia: AHR*(rest,post-hypoxia)*. Also obtained was the value for HVD at rest;

$$AHR(rest,control) = V2 - V1$$

$$AHR(rest,post\text{-}hypoxia) = V5 - V4$$

$$HVD(rest) = V2 - V3$$

In protocol B, two acute hypoxic responses were also obtained. The first was the AHR during exercise: AHR*(exercise)*. The second was the AHR after the period of sustained hypoxic exercise: AHR*(post-hypoxic exercise)*;

AHR*(exercise)* = V2 - V1

AHR*(post-hypoxic exercise)* = V5 - V4

The HVD during exercise was also calculated for protocol B: HVD*(exercise)*. But, in order to avoid underestimating this value, the corresponding rise in ventilation which occurs during euoxic exercise was added to this value, from data points in protocol C. This calculation was the same as in the previous chapter;

HVD*(exercise)* = (V2 - V3) - (V2c - V3c)

where V2 and V3 are points from protocol A and V2c and V3c are the corresponding points from protocol C (Fig. 4.2).

For protocol C, only one acute hypoxic response was calculated. This was the AHR after the euoxic, control exercise period: AHR*(post-euoxic exercise)*;

AHR*(post-euoxic exercise)* = V5 - V4

For each subject, six values of each of these variables were obtained for each protocol (four values in subject 903). These values were averaged to give the mean values of AHR and HVD for each subject (the subject means). These subject means were then averaged to obtain the mean for all seven subjects (the group means).

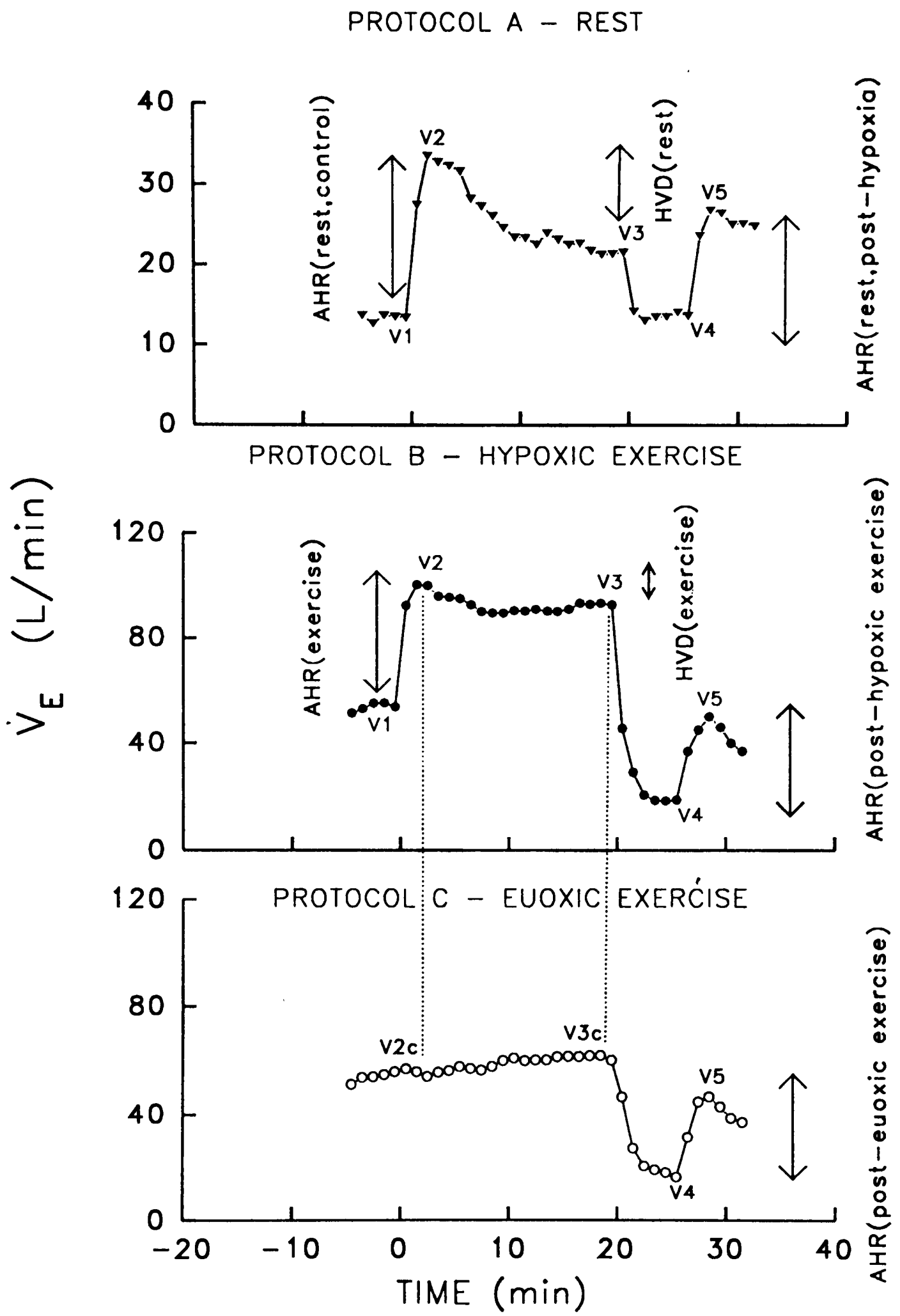


Figure 4.2. Illustration of the points used to calculate the magnitudes of the AHR and the HVD. Representative experimental periods of (from top panel to bottom panel): hypoxia at rest (▼); hypoxic exercise (●); euoxic exercise (○). The periods of hypoxia in the upper two panels are from time = 0 to 20 min; the periods of exercise in the lower two panels are from time = -5 to 20 min. See text for further explanation.

Using the individual subject means, two quantities were calculated. The first was the HVD expressed as a percentage of the corresponding AHR (HVD/AHR), for rest and exercise respectively;

$$\frac{HVD}{AHR}\ Rest = \frac{HVD(rest)}{AHR(rest,control)}$$

$$\frac{HVD}{AHR}\ Exercise = \frac{HVD(exercise)}{AHR(exercise)}$$

The second was the percentage decline in the AHR after a period of sustained hypoxia. This is a measure of the degree to which hypoxic chemoreflex sensitivity declines with sustained hypoxia. For rest, the magnitude of AHR*(rest,post-hypoxia)* for each subject was compared with its control, AHR*(rest,control)*; for exercise, the magnitude of AHR*(post-hypoxic exercise)* was compared with its proper control, AHR*(post-euoxic exercise)*, and the degree of decline expressed as a percentage.

The significance of the difference between the mean value of HVD/AHR at rest and during exercise for the group as a whole was assessed using a one-tailed, paired t-test (Bailey, 1985). The same statistical analysis was used to assess the significance of the difference between the percentage decline in AHR after sustained hypoxia at rest and that after exercise.

Results

The end-tidal gas partial pressures for the two hypoxic protocols (protocol A, at rest, and protocol B, in exercise) for each of the subjects and the means for the group are shown in Fig. 4.3. End-tidal P_{O2} reached the desired levels rapidly in both protocols and remained constant throughout the periods of hypoxia. End-tidal P_{CO2} values were essentially constant throughout the experimental periods at rest. During the exercise protocols, there were a few imperfections in the control of PET_{CO2} in the transition into hypoxia in some subjects. When exercise ceased, there was also a transition back into euoxia, but the PET_{CO2} settled to its new, lower, resting value within two min in all subjects and remained constant thereafter.

Figure 4.4 shows the hypoxic input in terms of arterial saturation for rest and exercise. Levels of arterial desaturation during hypoxia were reasonably well-matched between rest and exercise for the individual subjects and for the mean as a whole.

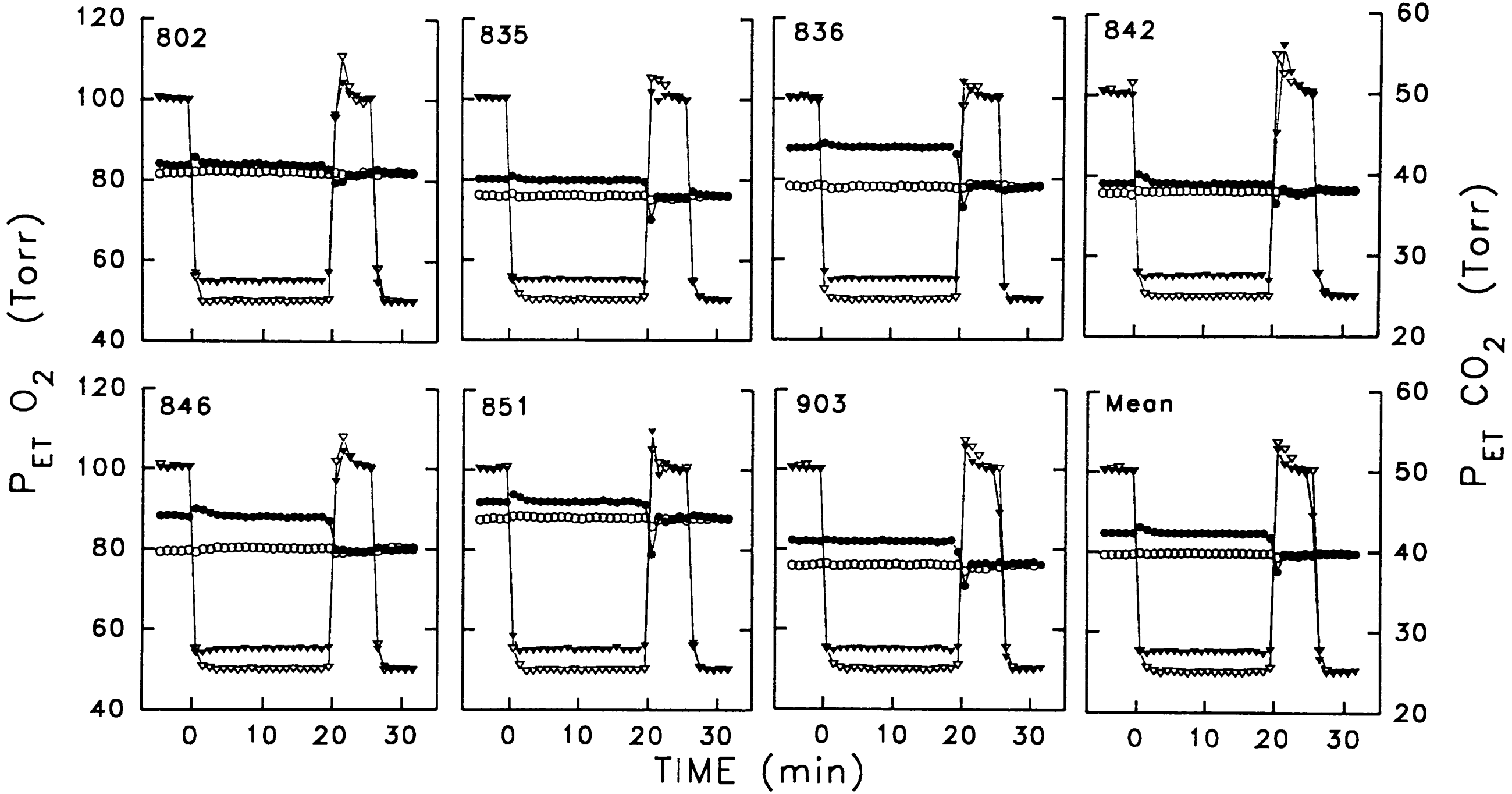


Figure 4.3. Mean end-tidal gas profiles for each of the seven subjects, and the mean for the group. For clarity, only gas profiles for protocol A (at rest) and protocol B (hypoxic exercise) are plotted. PET_{O2} at rest (▽); PET_{O2} during hypoxic exercise (▼); PET_{CO2} at rest (○); PET_{CO2} during hypoxic exercise (●).

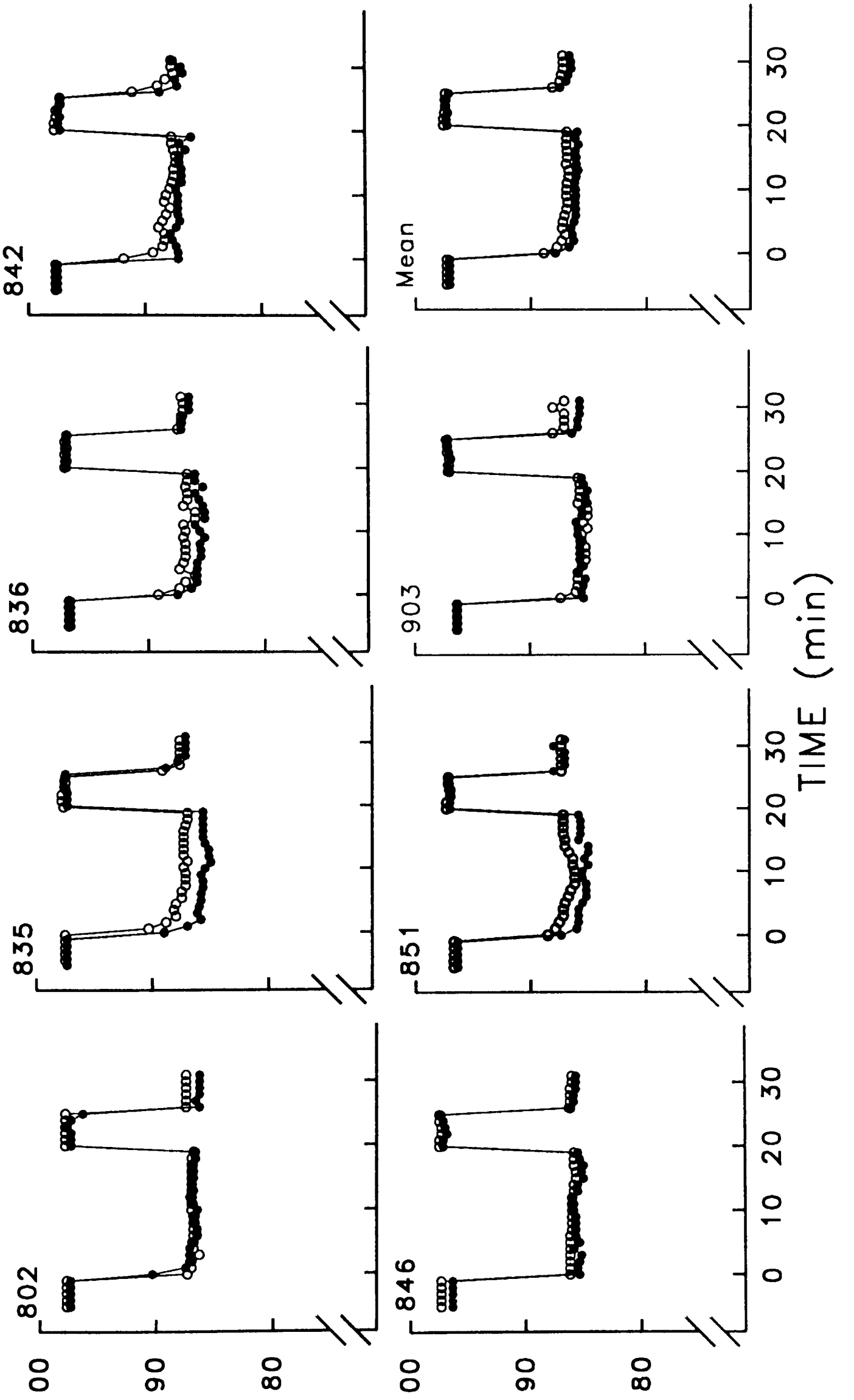


Figure 4.4. Mean saturations against time for each of the seven subjects, for all three protocols, and the means for the group. For clarity, only saturations from the two protocols involving sustained hypoxia are shown. Protocol A, at rest (▼); protocol B, hypoxic exercise (●). First hypoxic exposure from time = 0 to 20 min; second hypoxic exposure from time = 26 to 32 min.

The general form of the ventilatory responses for all three protocols in each subject, and the group means, is shown in Fig. 4.5. All subjects showed a biphasic response to hypoxia at rest. The size of the response to the second hypoxic exposure at rest was smaller in magnitude than the response to the first exposure. During exercise, the size of the acute response to hypoxia was generally increased as compared with rest, although in two subjects (842 and 851), the magnitudes of the two responses were similar. The magnitude of the subsequent HVD during exercise appeared to be attenuated as compared with rest in all subjects. However, the results from the control protocol suggested that there was a gradual rise in the baseline ventilation during sustained hypercapnia. This was particularly marked in subjects 836 and 851. Our data analysis using the control protocol C took this rising baseline into account, and so avoided an underestimation of HVD during hypoxic exercise (Fig. 4.2 and Chapter 3).

On cessation of exercise, new baseline ventilations were attained at rest. The baselines after the two exercise protocols were generally somewhat higher than at rest. The euoxic ventilation at rest after the period of sustained hypoxia appeared to be a little lower than the initial baseline at rest.

With regard to the magnitudes of the AHR after exercise and/or hypoxia, it appears that for the group mean, the AHR after hypoxic exercise is similar to the AHR after euoxic, control exercise. In contrast, the AHR after a period of hypoxia at rest appears much smaller than the first AHR at rest.

Table 4.2 shows the values for AHR and HVD, for each of the protocols. Two well-established observations are confirmed. The first is that the AHR during exercise is increased, as compared with rest. Secondly, the AHR after a period of hypoxia at rest is smaller than the control AHR at rest.

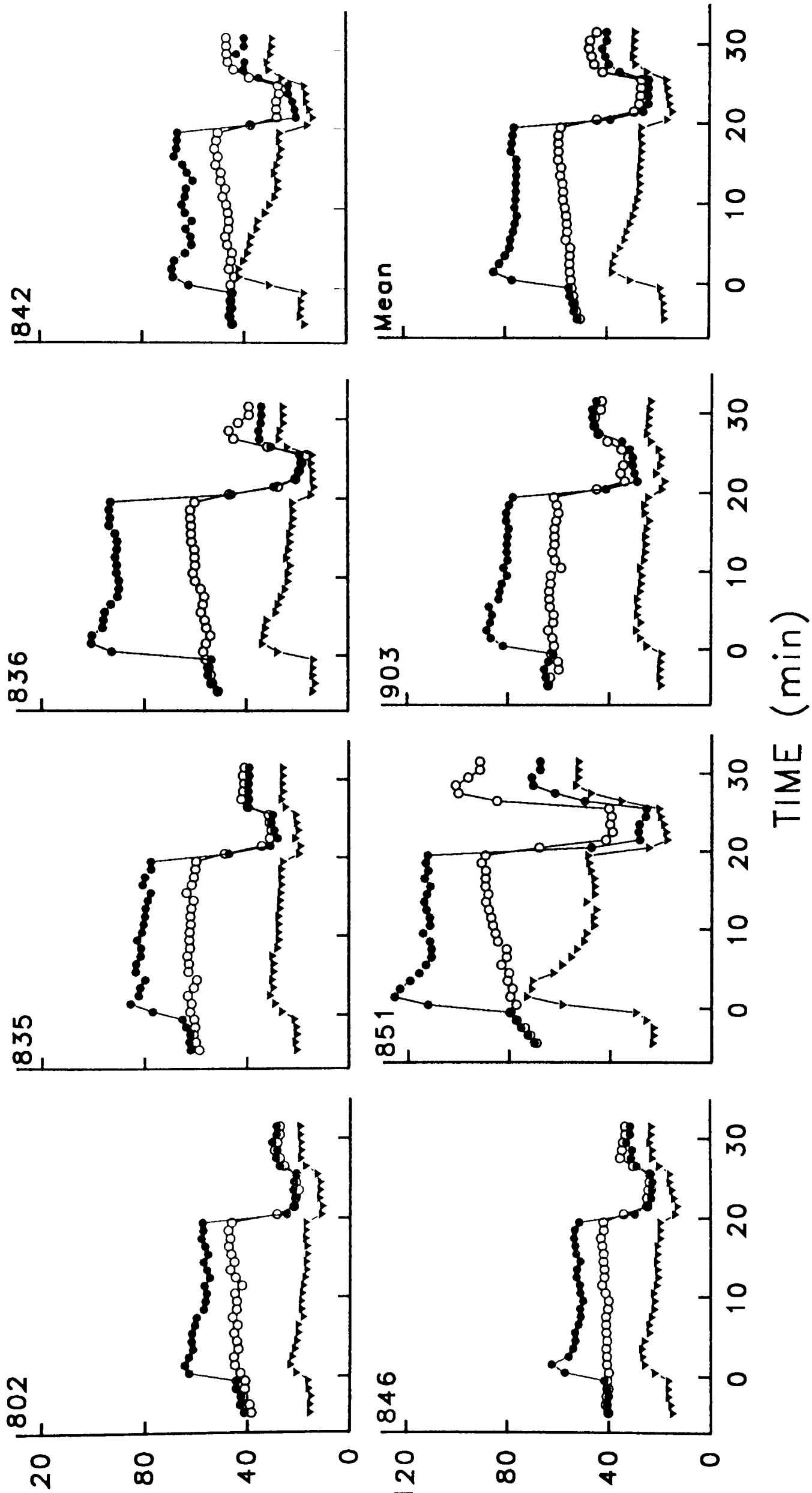


Figure 4.5. Mean ventilations against time for each of the seven subjects, for all three protocols, and the means for the group. Protocol A, at rest (▼); protocol B, hypoxic exercise (●); protocol C, euoxic exercise (○). First hypoxic exposure from time = 0 to 20 min; second hypoxic exposure from time = 26 to 32 min.

TABLE 4.2

Average values ± SE (L/min) for the ventilatory responses (AHR and HVD) for individual subjects and the mean for all seven combined

	Protocol A			Protocol C	Protocol B		
Subjects	AHR *(rest, control)*	AHR *(rest, post-hypoxia)*	HVD *(rest)*	AHR *(post-euoxic exercise)*	AHR *(post-hypoxic exercise)*	AHR *(exercise)*	HVD *(exercise)*
802	6.93 0.81	6.68 1.11	6.13 1.30	8.63 0.99	9.37 1.44	20.0 1.59	8.21 3.34
835	9.75 1.66	5.75 1.49	5.23 1.59	10.7 2.25	9.50 1.66	20.2 2.68	4.50 3.66
836	20.2 0.95	13.1 1.54	12.0 1.83	30.3 2.34	16.0 2.56	46.9 3.32	10.9 4.62
842	25.4 2.89	14.6 1.32	16.1 2.28	20.1 2.30	20.2 1.46	25.8 3.49	6.67 2.71
846	10.5 0.64	7.90 1.02	7.15 0.60	11.7 1.07	9.63 0.74	20.7 1.33	12.0 1.04
851	43.2 3.81	32.9 5.32	24.3 4.77	60.8 4.18	45.4 4.48	46.3 5.25	21.9 3.28
903	10.8 2.34	6.50 1.66	6.58 1.57	14.2 4.45	16.3 5.55	22.3 7.66	10.4 2.43
Mean	18.1 4.50	12.5 3.37	11.1 2.45	22.3 6.47	18.1 4.47	28.9 4.29	10.7 1.96

Figure 4.6A shows the HVD expressed as a fraction of the AHR, for both rest and exercise. Exercise greatly reduces the ratio of HVD to AHR, as compared with rest. Figure 4.6B shows the percentage decline in the AHR after a period of sustained hypoxia. At rest, the subsequent AHR declines by 30% ± 5% (mean ± SE). After hypoxic exercise, however, the subsequent AHR declines by only 11% ± 7%. The degree of decline in hypoxic chemoreflex sensitivity, as measured by this method, is therefore less during hypoxic exercise than it is during hypoxia at rest ($P < 0.05$).

Lactic acidosis did not occur during either hypoxic or euoxic exercise. The mean values for lactate (± SE) before exercise were 0.95 ± 0.10 mmol/L (hypoxic exercise) and 0.92 ± 0.12 mmol/L (euoxic control); after exercise, the values were 0.97 ± 0.15 mmol/L (hypoxic exercise) and 0.79 ± 0.06 mmol/L (euoxic control).

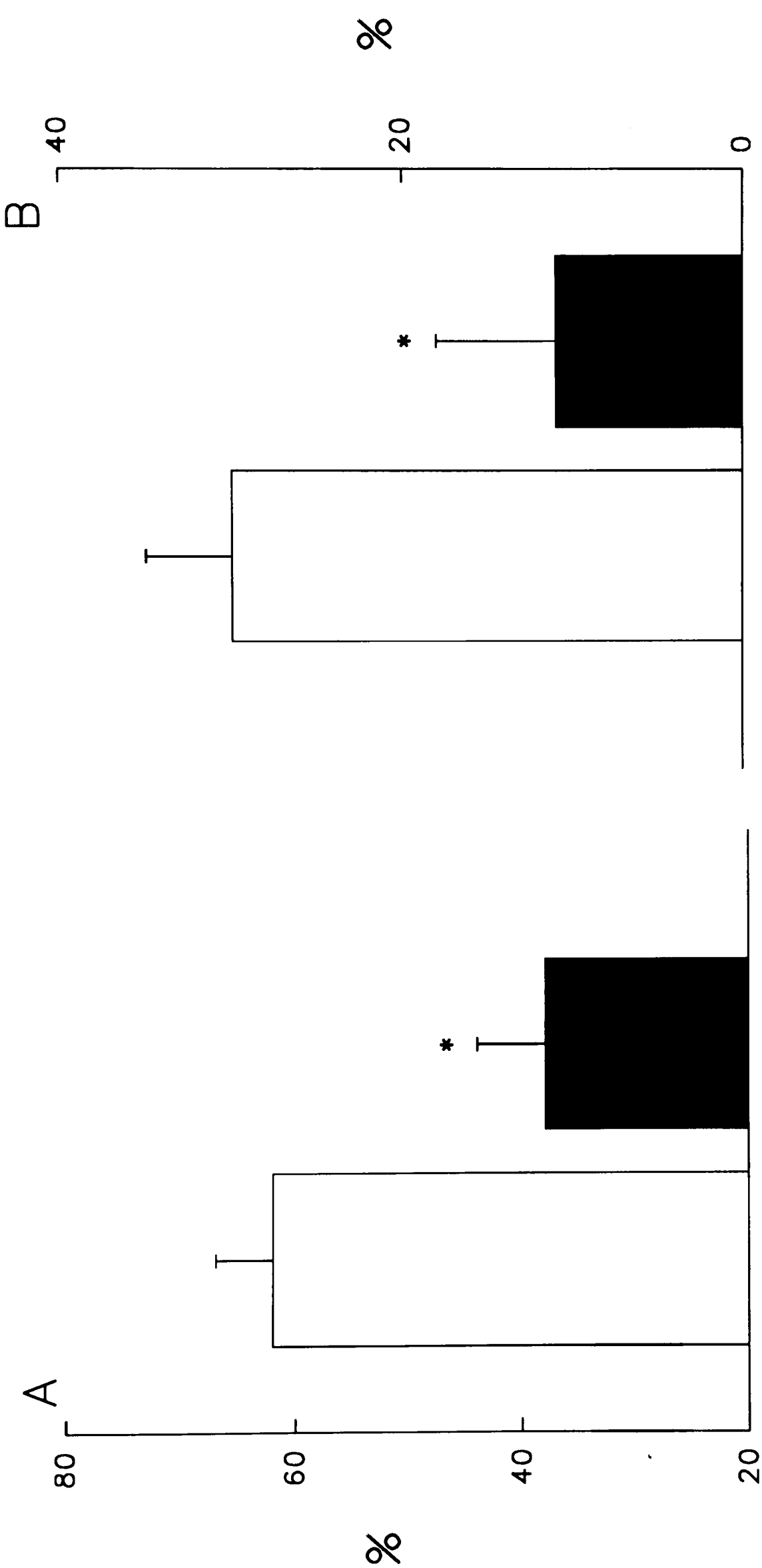


Figure 4.6. Mean values ± SE of all seven subjects combined. Panel A: HVD expressed as a percentage of the AHR for rest (hollow bar) and exercise (filled bar), respectively. Panel B: percentage decline in the AHR after a period of hypoxia at rest (hollow bar), and exercise (filled bar). Figures are calculated from those presented in Table 1: see text for further details. (* indicates $P < 0.05$; comparisons between rest and exercise).

Discussion

The major finding in this study is that exercise genuinely attenuates the decline in hypoxic peripheral chemosensitivity during sustained hypoxia, as compared with the resting state.

Possible problems with experimental technique

1) Control of P_{CO2} in rest and exercise. End-tidal forcing held PET_{CO2} steady during the protocols (Fig. 4.3), and good control was achieved during hypoxia. All measurements of the AHR relevant to this study were undertaken at rest, with the same PET_{CO2} stimulus held 2-3 Torr above air-breathing values in all protocols (Fig. 4.1). The exercise protocols did not generate a lactic acidosis, so we can also be confident that the lactic acid stimulus was constant between protocols.

It was only *during* hypoxic exercise, therefore, that the hypercapnic stimulus might have differed from rest, and so affected the measurement of AHR*(exercise)*. End-tidal values give an acceptable estimate of arterial values at rest, but during 70 Watt exercise, they may overestimate arterial levels by 1-2 Torr (Robbins *et al.*, 1990). Matching of arterial P_{CO2} between rest and exercise is thus difficult by the estimation of end-tidal values alone, but the method of the previous study was used, and PET_{CO2} was held at 2-3 Torr above the air-breathing value at rest, and 2-3 Torr above the air-breathing value observed during exercise, in an attempt to overcome this problem (Chapter 3; Pandit and Robbins, 1991). While some small differences in the actual arterial values between rest and exercise may have occurred, the overall pattern of ventilatory responses in rest and exercise remained consistent with those reported in previous studies (Fig. 4.5).

2) The ventilatory effects of CO_2 breathing during exercise. Sustained CO_2 breathing during exercise causes the ventilation to rise progressively throughout the exercise period. The phenomenon does not occur at these levels of P_{CO2} at rest (Fig. 4.5). The possible mechanisms of this at levels of P_{CO2} similar to those used in this study are examined in detail in the following chapter (Chapter 5). The underlying mechanism during exercise may, in part, be related to gradual changes in the respiratory quotient during exercise (Pandit and Robbins, 1992). One effect of this progressive rise in ventilation in this study is that it constitutes a rising baseline which may lead to an underestimation of the HVD during hypoxic exercise. Care has therefore been taken to avoid underestimating HVD by subtracting the mean ventilations of the control experiments during exercise from the ventilations of individual hypoxic experiments (Fig. 4.2).

It is interesting that after hypoxic and euoxic exercise has ceased, the baseline ventilation remains higher than the euoxic ventilation at rest (Fig. 4.5; Appendix 4.1). This effect may be related to prior exercise: Clement *et al.* (1993) have reported that, at matched PET_{CO2}, PET_{O2} and pH, the ventilation may remain elevated for up to 30 min after the cessation of exercise, as compared with rest. The mechanism of this is unknown, but it does not appear to affect the magnitude of hypoxic ventilatory responses measured after exercise has ceased (Clement, 1992; Clement *et al.*, 1993; Table 4.2).

Comparison with previous studies

In the study reported in the previous chapter, HVD constituted about 60% of the AHR at rest (Chapter 3; Pandit and Robbins, 1991). This is in good agreement with the value obtained in this study of 64% (Fig. 4.6A). In the previous study, the mean absolute magnitude of HVD during exercise was 6.2 L/min. In this study, the figure is slightly higher (10.7 L/min). The reasons for this may be individual subject differences and differences in protocol and the duration of hypoxic exposure between the two studies. However, the finding that HVD, expressed as a fraction of the AHR, is much smaller during exercise than at rest (Fig. 4.6A), remains consistent with the previous result.

Is the process underlying HVD genuinely attenuated by exercise?

The major purpose of this investigation was to determine whether the decline in hypoxic sensitivity during sustained hypoxia is genuinely attenuated during exercise, or whether it occurs to a significant degree and is simply masked. We conclude that it is genuinely attenuated.

The degree of decline in the magnitude of AHR following a period of sustained hypoxia provides a measure of the decline in hypoxic sensitivity. Figure 4.6B shows that at rest, this decline is 30%. This value agrees well with the result of Bascom *et al.* (1992) who found that the magnitude of the AHR measured 5 min after a 20 min period of hypoxia declined by 27% relative to the first AHR, and with those of Berkenbosch *et al.* (1992) who found the the percentage decline to be 24%.

Figure 4.6A confirms that exercise reduces the ratio of the HVD to the AHR (Pandit and Robbins, 1991; Ward and Nguyen, 1991). If the underlying decline in hypoxic sensitivity was similar during hypoxic exercise as at rest, we would expect to observe a similar reduction in the magnitudes of the AHR after a period of hypoxic exercise as after a period of hypoxia at rest (i.e, about 30%). In fact, the degree of decline in the AHR following sustained hypoxic exercise is significantly smaller than after sustained hypoxia at rest (i.e, 11%; Fig. 4.6B). The conclusion is, therefore, that the decline in chemoreflex sensitivity has been genuinely attenuated by exercise.

Physiological significance of the results

A number of interventions which increase the ventilatory sensitivity to hypoxia in man, such as almitrine (Georgopoulos et al., 1989*c*), hypercapnia (Georgopoulos et al., 1989*a*) and extra hypoxia (Bascom et al., 1992) also increase the magnitude of the subsequent HVD. The results from exercise, however, do not conform to this pattern. This might suggest that some mechanism is present during exercise which modifies the process underlying HVD in a manner which is *independent* of any effects on the acute ventilatory response to hypoxia. We have previously described how such a mechanism might operate in the hypoxic chemoreflex (Chapter 3; Pandit and Robbins, 1991).

The results presented in this paper lend no support to the notion that the underlying decline in hypoxic chemoreflex sensitivity during sustained hypoxic exercise occurs to the same extent as it does at rest, but that exercise simply masks it (Ward and Nguyen, 1991). The conclusion that this decline in sensitivity is genuinely attenuated during exercise suggests that factors involved in exercise act in a manner which is qualitatively different from many other interventions which affect the hypoxic chemoreflex. Our study does not indicate which factors may be involved, since many neural and humoral stimuli are thought to operate during exercise and to mediate ventilatory and chemoreflex responses. In this respect, however, it is interesting that the drug domperidone (a dopamine antagonist), increases the AHR but leaves HVD unaffected, and so like exercise, reduces the ratio of HVD to AHR; while exogenous dopamine reduces AHR, but leaves HVD unaffected (Bascom *et al.*, 1991). These results taken together raise the possibility that dopaminergic processes may be involved in peripheral chemoreflex modulation during exercise.

Appendix 4.1.

This appendix (Table 4.3) shows the mean individual subject values for the euoxic baseline ventilations for each of the three protocols.

TABLE 4.3

Average values (L/min ± SE) for the baseline euoxic ventilation ($\dot{V}E$) for individual subjects, and the mean for all seven subjects combined

Subjects	Protocol A		Protocols B and C	
	$\dot{V}E$ *(rest)*	$\dot{V}E$ *(post-hypoxia)*	$\dot{V}E$ *(post-euoxic exercise*	$\dot{V}E$ *(post-hypoxic exercise)*
802	16.2 2.36	12.6 1.67	21.1 5.12	21.4 4.06
835	21.1 1.46	20.5 2.31	31.6 3.9	30.0 4.63
836	13.4 2.72	13.7 2.26	16.4 2.02	19.0 0.98
842	16.5 2.42	16.1 3.57	26.7 5.88	22.6 1.28
846	16.1 2.63	15.7 2.35	23.8 2.33	23.4 2.24
851	29.5 5.88	20.4 2.37	40.4 8.83	25.6 2.41
903	19.1 1.66	19.6 1.66	29.9 2.26	32.0 4.22
Mean	18.8 2.00	16.9 1.23	27.1 3.00	24.9 1.77

Chapter 5

Ventilation and Gas Exchange During Sustained Exercise at Normal and Raised Co_2 in Man

Everything is in a state of flux

Heraclitus

Introduction

In adult man at rest, prolonged steady hypercapnia leads to a gradual rise in ventilation for periods of over 30 min (Reynolds *et al.*, 1972; Easton and Anthonisen, 1988; Georgopoulos *et al.*, 1989*a*; Khamnei and Robbins, 1990). This progressive rise occurs with relatively high levels of hypercapnia of about 8-10 Torr above resting end-tidal values. In our studies concerning the effects of sustained hypoxia during exercise presented in the preceding chapters, we noted that a similarly rising trend in baseline ventilation may be present during hypercapnic exercise (particularly evident in the control protocols), but at levels of hypercapnia considerably lower than those used in the resting studies previously reported by others. The primary purpose of this study was to investigate whether there is indeed a progressive rise in ventilation during exercise at these low levels of hypercapnia. We also wished to perform control experiments to examine whether there are any trends in ventilation during prolonged exercise when no carbon dioxide is administered.

Methods

Subjects

We studied five normal young adult males. Their physical characteristics are shown in Table 3.1 (Chapter 3). Their end-tidal values for P_{CO2} were obtained as the mean over a 20 min period breathing air at rest, after familiarisation with the laboratory. Similarly, their end-tidal P_{CO2} values during exercise were obtained over a 20 min period performing 70 W exercise breathing air.

Experimental technique

The subjects breathed through a mouthpiece and wore a noseclip during the experiments. The end-tidal gases were controlled by dynamic end-tidal forcing, as described in Chapter 2. For protocols at rest, subjects were seated in a chair. Exercise was performed on an electromagnetically-loaded cycle ergometer and subjects were cooled by fans. A heparinised venous blood sample was taken before the start of exercise and when exercise was complete for the measurement of lactate. During all protocols, an ECG was monitored continuously and a pulse oximeter was used to record arterial oxygen saturation. Sublingual temperature was taken using a mercury thermometer before and after each period of exercise.

Protocols

The five subjects undertook each of the following three protocols in random order six times, giving a total of ninety experimental periods.

Protocol A: with the subject at rest, $P_{ET_{O2}}$ was held constant at 100 Torr for 43 min. The $P_{ET_{CO2}}$ was held constant at 2-3 Torr above end-tidal values. Since the breathing of one subject (807) was somewhat erratic, we held his $P_{ET_{CO2}}$ 4-5 Torr above his average end-tidal value and this helped to achieve better control.

Protocol B: with the subject performing constant 70 Watt exercise, PET_{O2} was held at 100 Torr for 43 min. The PET_{CO2} was not controlled (poikilocapnic exercise).

Protocol C: with the subject performing constant 70 Watt exercise, PET_{O2} was held at 100 Torr for 43 min. The PET_{CO2} was held 2-3 Torr (4-5 Torr in subject 807) above the normal exercising value (hypercapnic exercise).

Data analysis

Data collection began five min after the start of each protocol and data from the remaining 38 min were averaged over one min periods. For each experimental period in each subject, data for ventilation, PET_{CO2}, tidal volume, inspiratory and expiratory times, O_2 consumption, CO_2 production and respiratory quotient were averaged for the first 5 min and for the last 5 min to obtain the "subject means". The subject means for each variable were then averaged to obtain the mean and standard error for all five subjects combined, the "group means". To assess whether the mean values differed significantly between the first and last five min of each protocol, a two-tailed, paired t-test was used (Bailey, 1985), using the subject means as the individual observations.

Stability of gas analysis and work rate

In order to ensure that there were no underlying trends in our measuring apparatus, we performed two additional experiments. First, to exclude a drift in our mass spectrometer measurement of P_{CO2}, we measured the composition of a standard gas both before and after a number of exercise periods. The results of this are discussed in Chapter 2: there was essentially no significant change in the mass spectrometer estimates of P_{CO2}. Secondly, to confirm that the work rate was constant over the period of exercise, we measured the torque associated with a given work rate setting on the electromagnetically-braked cycle ergometer over a sustained period of use using the dynamic torquemeter as descibed by Russell and Dale (1986). As discussed in Chapter 2, this was also found to remain constant.

Results

Figure 5.1 shows the trends in ventilation for each of the three protocols. The ventilation appears to have remained constant during poikilocapnic exercise, but showed a steadily rising trend in all subjects during hypercapnic exercise. Ventilation at rest was also essentially constant for all subjects, except 807, in whom there appeared to be a rise to a steady-state over the initial 10 min.

Figure 5.2 confirms that the gas-mixing system held the end-tidal CO_2 levels constant at both rest and during hypercapnic exercise. However, during poikilocapnic exercise $P_{ET_{CO2}}$ showed a downward trend over the exercise period in all subjects.

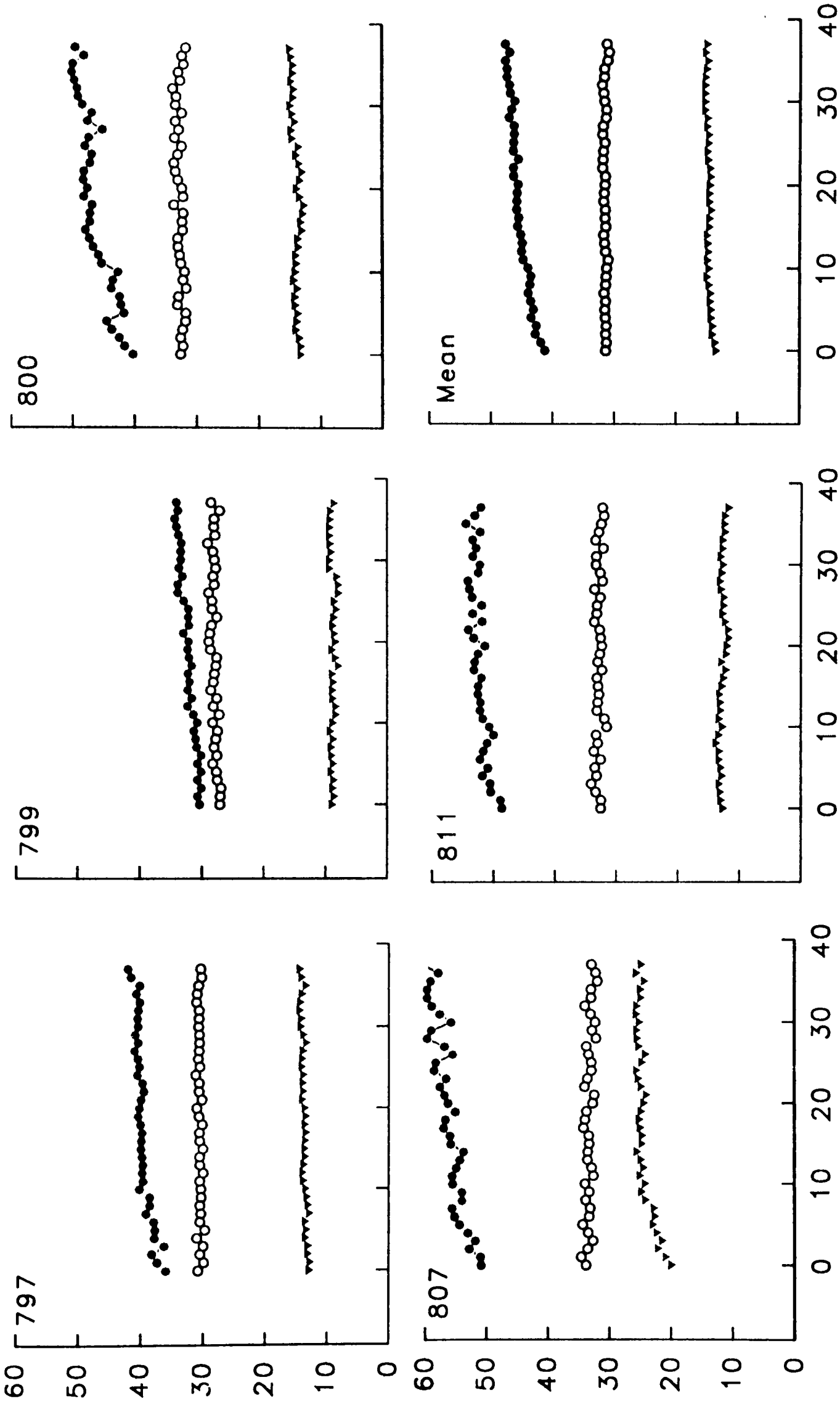


Figure 5.1. Mean ventilations against time for each of the five subjects and the average for all five combined, for all three protocols. Hypercapnia at rest, protocol A (▼); poikilocapnic exercise, protocol B (○); hypercapnic exercise, protocol C (●).

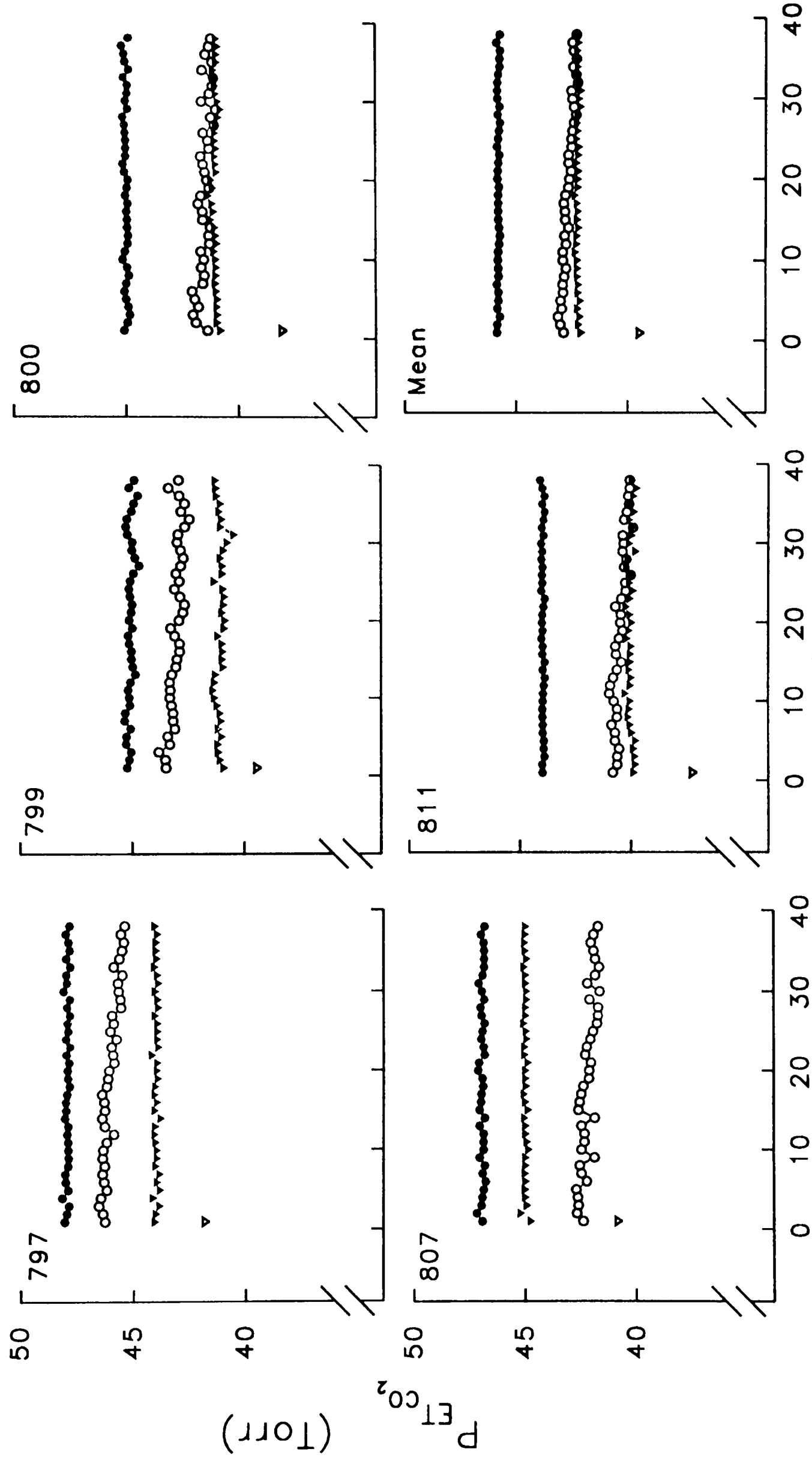


Figure 5.2. Mean end-tidal P_{CO_2} profiles for each of the five subjects and the average for all five combined. Hypercapnia at rest, protocol A (▼); poikilocapnic exercise, protocol B (○); hypercapnic exercise, protocol C (●). Single point shows the resting end-tidal value of P_{CO_2} for each subject and for all five combined (▽).

Apart from subject 811, the oxygen consumption during the steady level of exercise (Fig. 5.3) remained essentially constant and was of a similar magnitude for both poikilocapnic and hypercapnic exercise. Subject 811, however, appeared to show a downward trend in O_2 consumption during the first part of the exercise period, and his O_2 consumption during poikilocapnic exercise was slightly lower than during hypercapnic exercise.

Figure 5.4 shows in contrast that there is a downward trend in CO_2 production during both exercise protocols in all subjects, except perhaps subject 799. It also appears that the magnitude of CO_2 production is always lower during hypercapnic than during poikilocapnic exercise, this being marked in subjects 800 and 807.

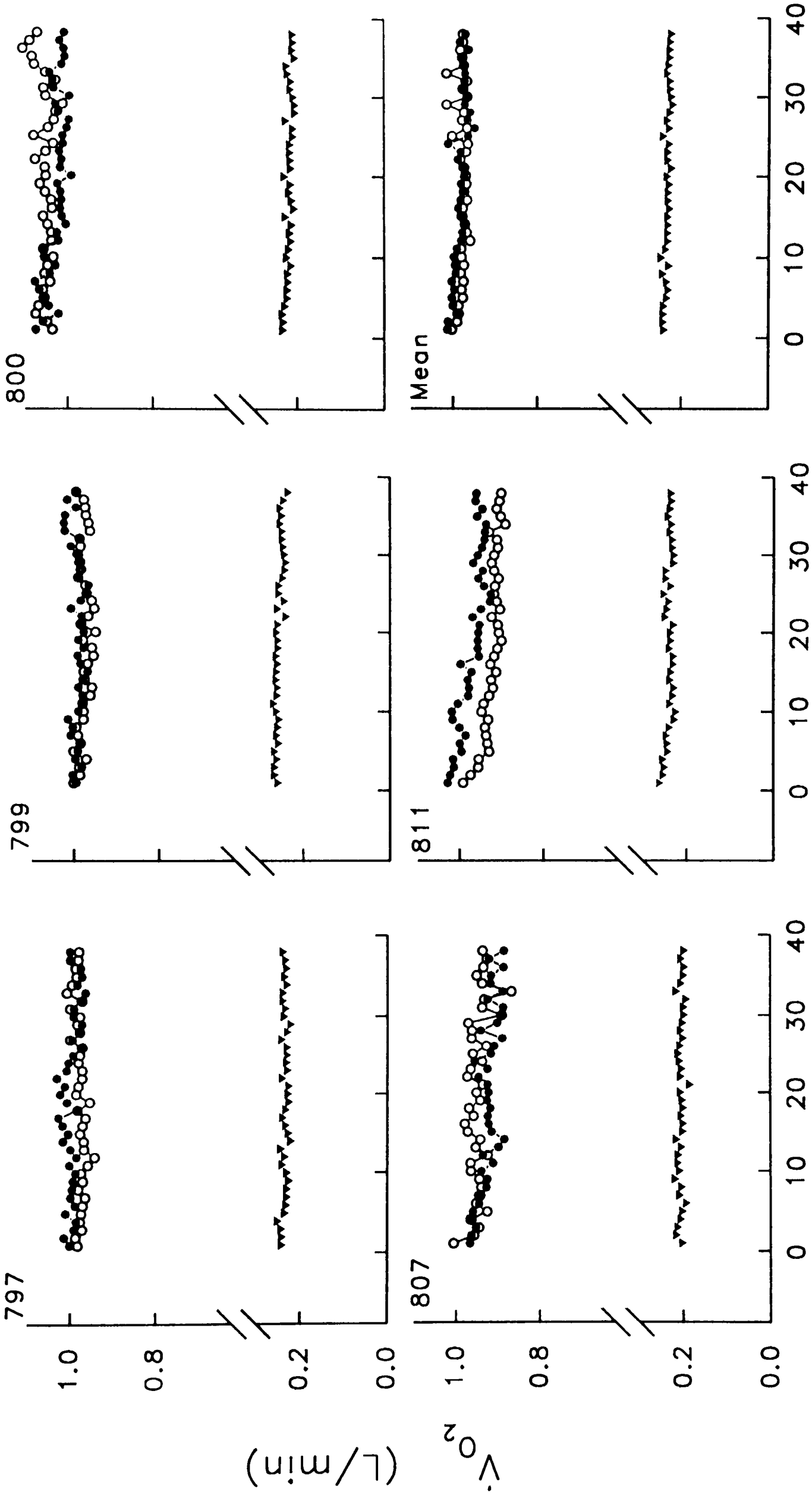


Figure 5.3. Mean values of oxygen consumption against time for each of the five subjects and the average for all five combined, for all three protocols. Hypercapnia at rest, protocol A (▼); poikilocapnic exercise, protocol B (○); hypercapnic exercise, protocol C (●).

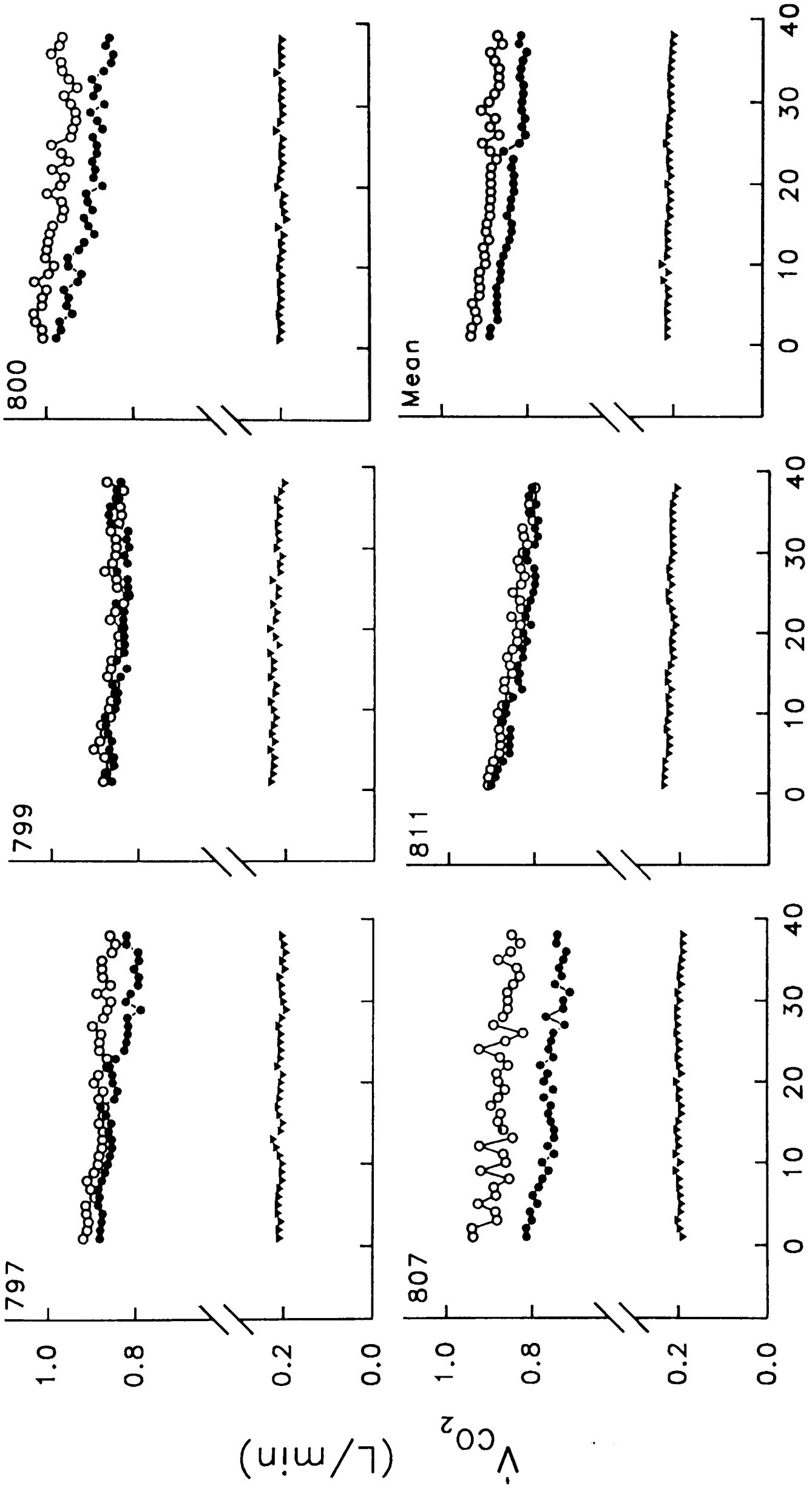


Figure 5.4. Mean values of CO_2 production against time for each of the five subjects and the average for all five combined, for all three protocols. Hypercapnia at rest, protocol A (▼); poikilocapnic exercise, protocol B (○); hypercapnic exercise, protocol C (●).

Table 5.1 shows the numerical values for the trends in protocol A. There were few significant changes in the variables during hypercapnia at rest. The values for the changes in PET_{CO2} and O_2 consumption did reach significance, but these mean values were very small (0.07 Torr and 10 ml/min respectively).

Table 5.2 shows the trend values for poikilocapnic exercise. Ventilation and the pattern of breathing were essentially unchanged, but there was a significant fall in end-tidal P_{CO2}. Carbon dioxide production also fell significantly, and with no change in oxygen consumption, the respiratory quotient was significantly reduced.

Table 5.3 shows the trend values for hypercapnic exercise. Ventilation rose significantly and appeared to be largely due to a shortening of expired time, T_E. End-tidal P_{CO2} was unchanged, as expected. Carbon dioxide production was again significantly reduced over the course of exercise, as was the respiratory quotient, with O_2 consumption remaining constant.

Lactic acidosis did not occur during either poikilocapnic or hypercapnic exercise. The mean values ± SE (mmol/L) before exercise were 0.82 ± 0.07 (poikilocapnic exercise) and 0.83 ± 0.11 (hypercapnic exercise); after exercise the values were 0.81 ± 0.08 (poikilocapnic exercise) and 0.86 ± 0.13 (hypercapnic exercise). There was no rise in body temperature with mean ± SE being 37.0 ± 0.1 °C before exercise and 37.1 ± 0.1 °C after exercise.

TABLE 5.1

Mean values (± SE) of all five subjects combined for protocol A (rest, hypercapnia). Probabilities that values from the first and last 5 min are the same: * = $P < 0.05$, ** = $P < 0.01$ (two-tailed, paired t-test)

	$\dot{V}E$ (L/min)	VTE (L)	TI (msec)	TE (msec)	PET_{CO2} (Torr)	$\dot{V}CO_2$ (L/min)	$\dot{V}O_2$ (L/min)	R
Mean of first	13.9	0.80	1375	2188	42.1	0.21	0.24	0.89
5 min	1.80	0.08	50	119	0.98	0.01	0.01	0.02
Mean of last	15.0	0.82	1385	2074	42.2	0.20	0.23	0.89
5 min	2.40	0.09	42	83	0.98	0.00	0.01	0.02
	(NS)	(NS)	(NS)	(NS)	(**)	(NS)	(**)	(NS)

TABLE 5.2

Mean values (± SE) of all five subjects combined for protocol B (poikilocapnic exercise). Probabilities that values from the first and last 5 min are the same: * = $P < 0.05$, ** = $P < 0.01$ (two-tailed, paired t-test)

	$\dot{V}E$ (L/min)	VTE (L)	TI (msec)	TE (msec)	PET_{CO2} (Torr)	$\dot{V}CO_2$ (L/min)	$\dot{V}O_2$ (L/min)	R
Mean of first	31.3	1.42	1171	1673	43.0	0.93	0.99	0.94
5 min	1.03	0.04	54	69	0.97	0.02	0.02	0.01
Mean of last	31.3	1.34	1123	1621	42.3	0.87	0.98	0.89
5 min	0.80	0.03	39	73	0.93	0.02	0.02	0.01
	(NS)	(NS)	(NS)	(NS)	(**)	(**)	(NS)	(**)

TABLE 5.3

Mean values (± SE) of all five subjects combined for protocol C (hypercapnic exercise). Probabilities that values from the first and last 5 min are the same: * = $P < 0.05$, ** = $P < 0.01$ (two-tailed, paired t-test)

	$\dot{V}E$ (L/min)	VTE (L)	TI (msec)	TE (msec)	PET_{CO2} (Torr)	$\dot{V}CO_2$ (L/min)	$\dot{V}O_2$ (L/min)	R
Mean of first	42.4	1.75	1105	1442	45.8	0.88	1.01	0.87
5 min	3.58	0.10	47	106	0.74	0.02	0.01	0.01
Mean of last	47.3	1.79	1050	1318	45.7	0.81	0.97	0.83
5 mins	4.02	0.08	58	138	0.73	0.02	0.02	0.01
	(**)	(NS)	(NS)	(*)	(NS)	(**)	(NS)	(**)

Discussion

The main results of this study are first, that during prolonged, light exercise, ventilation remains constant while the end-tidal P_{CO2} falls steadily by a small amount and secondly, that when PET_{CO2} is held 2-5 Torr above eucapnic levels during exercise, ventilation rises steadily over the exercise period. At this mild level of hypercapnia, there is no progressive change in the ventilation at rest.

Comparison With Other Studies

While the effects of mild hypercapnia during prolonged exercise have not been previously examined, the results of our poikilocapnic exercise protocol B are broadly consistent with those from previous studies. For example, Kalis et al. (1988) found that ventilation and oxygen consumption were unchanged after 40 min of air-breathing exercise at a slightly higher work rate (O_2 consumption of 1.68 L/min) than used in our study. The respiratory quotient in their study also showed a significant downward trend, but the values of CO_2 production and end-tidal P_{CO2} were not reported. Similarly, Kearon *et al.* (1991) found ventilation and oxygen consumption to remain constant during one hour of 93 W exercise, while CO_2 output and respiratory quotient fell. They did not report on changes in either end-tidal or arterial P_{CO2}, but found that the pattern of breathing during the exercise was unchanged, and speculated that P_{CO2} would decline.

It has been suggested that a "ventilatory drift" occurs during prolonged poikilocapnic exercise, such that both ventilation and O_2 consumption rise progressively. However, such a drift has been observed only when exercise is at work intensities greater than 50% of maximal O_2 consumption or sustained for periods over 60 min (Forster and Pan, 1991). The factors contributing to this ventilatory drift are variously suggested to be lactic acidosis (Casaburi et al, 1987), a rise in body temperature (Hagberg et al, 1978) and a progressive rise in catecholamine levels (Kalis et al, 1988). We have used a much lower work rate which avoided lactic acidosis and a rise in body temperature, and consequently we have not seen any rising drift in ventilation or O_2 consumption during our poikilocapnic protocol. Indeed, ventilatory drift during sustained exercise is not a constant

finding even at higher work loads: Ekelund (1967) found no drift in subjects exercising for one hour at 57% of their maximal O_2 consumption and Jansson (1982) reported no rise in ventilation or O_2 consumption in subjects exercising at 65% of their maximal O_2 consumption for 25 min.

Although many of the factors introduced by hard exercise are avoided in our study, one consequence of the low work rate we have chosen is that the changes that we report are relatively modest. However, they appeared to be consistent between the subjects and the results do raise some interesting questions.

Why Does End-Tidal P_{CO2} Fall During Prolonged Exercise?

It is unlikely that this fall in PET_{CO2} was a measurement artefact since we confirmed the stability of both the mass spectrometer measurements and the cycle ergometer work rate calibration. It is possible that the mean alveolar/arterial P_{CO2} remained unchanged over the exercise period, and that a change in the depth or frequency of breathing affected the measured end-tidal value of P_{CO2}. However, there was no consistent or significant change in either the tidal volume or frequency over the course of exercise (Table 5.2). The most likely explanation that remains for the modest fall in PET_{CO2} is therefore that it reflects a genuine fall in alveolar/arterial P_{CO2}, which itself occurs as a result of a constant ventilation in the face of a falling metabolic CO_2 production (Fig. 5.1 and 5.2 and Table 5.2).

Why Does Ventilation Rise Progressively During Hypercapnic Exercise?

The dynamic end-tidal forcing system controls the end-tidal value of P_{CO2}. If this end-tidal value shows a natural downward trend over the exercise period when the inspiratory P_{CO2} is zero, then end-tidal forcing will effectively ensure a progressively greater hypercapnic stimulus relative to the natural poikilocapnic value over the course of the exercise. If the ventilation is constant during poikilocapnic exercise when the end-tidal P_{CO2} is falling, then it might be expected that ventilation will rise during exercise when inspired CO_2 is added to maintain end-tidal CO_2 constant.

The mean fall in $P_{ET}CO_2$ over the period of poikilocapnic exercise was 0.7 Torr and therefore we might predict that ventilation would rise during the course of hypercapnic exercise by 0.7 multiplied by the CO_2 sensitivity. An estimate may be made from Table 5.2 and 5.3 of the mean CO_2 sensitivity of the subjects during exercise: at the start of exercise, the mean hypercapnic stimulus is 2.9 Torr above the natural value, with the mean difference in ventilation between poikilocapnic and hypercapnic exercise being 11.1 L/min. The CO_2 sensitivity is therefore about 3.8 $L.min^{-1}.Torr^{-1}$. Thus the predicted increase in ventilation would be 2.7 L/min (i.e, 0.7 multiplied by 3.8). This falls short of the observed mean increase of 4.9 L/min (Table 5.3). The remainder of the rise in ventilation is unaccounted for, but it is possible that it represents the progressive effect of CO_2 on ventilation previously observed at rest (Reynolds *et al.*, 1972; Easton and Anthonisen, 1988; Georgopoulos *et al.*, 1989*a*; Khamnei and Robbins, 1990) but now operating at lower levels of hypercapnia during exercise.

In conclusion, the mechanism of the progressive rise in ventilation during hypercapnic exercise is probably a combination of the correction by the gas-mixing system of an underlying trend downwards in $P_{ET}CO_2$, added to the progressive effect on ventilation of CO_2 *per se*.

Why Does The Respiratory Quotient Fall Progressively During Exercise?

It is well-established that during prolonged exercise in man, there is a change in utilization of substrate from carbohydrate to fatty acids over the course of exercise, and that this change occurs gradually and continuously (Ahlborg *et al.*, 1974; Felig and Wahren, 1975). This results in a progressively reduced CO_2 production for a given O_2 consumption. Our result in this study of a reduced respiratory quotient towards the end of the exercise period reflects this change in metabolic substrate. Of itself, this finding is not new, and our results are consistent with those of Hermansen *et al.* (1967) who used a higher exercise level ($\dot{V}_{O2}$ of 2.8 L/min) for shorter periods (five periods of 20 min each, separated by 15 min of rest) and observed a fall in the respiratory quotient of about 0.04 - 0.08. Kearon *et al.* (1991) found a mean fall of 0.08 after one hour of 93 W exercise.

We have noted in our study that the values for $\dot{V}_{CO2}$ and respiratory quotient are consistently lower during exercise in which there was hypercapnia (protocol C) than during the poikilocapnic exercise (protocol B). The precise reason for this is unclear, particularly since the protocols were performed in random order and on different days. One possibility, however, is that since the body stores of CO_2 equilibrate at a very slow rate (Farhi and Rahn, 1960), carbon dioxide is still absorbed into the body stores for some time after an increase in the alveolar P_{CO2}. This may therefore attenuate the measured net CO_2 output at the mouth and contribute to an apparently reduced respiratory quotient. In other words, the total measured respiratory quotient is actually dependent upon both the metabolic exchange ratio and the exchange ratio of the stores (Farhi and Rahn, 1960).

Relationship to CO_2 Flux Experiments

During exercise, it has long been argued that the ventilation is closely linked to the body's metabolic CO_2 production, so that alveolar/arterial P_{CO2} is maintained at a constant level (Haldane and Priestley, 1905). More recently, dynamic studies and studies of incremental exercise have suggested that the ventilation is more closely coupled to CO_2 production than to O_2 consumption (Diamond *et al.*, 1977). Furthermore, a number of experiments involving addition of CO_2 to, or removal of CO_2 from the venous return have suggested that CO_2 flux to the lungs is an important respiratory stimulus, possibly independent of the arterial P_{CO2}, and Phillipson *et al.* (1981) in studies on conscious sheep in which CO_2 was removed from the venous return have even suggested that CO_2 flux is a necessary stimulus for the ventilation: when the CO_2 flux was removed entirely, apnoea resulted.

Our study using prolonged exercise effectively constitutes a "CO_2 removal experiment": metabolic production of CO_2, and hence the flux to the lungs falls as the substrate and respiratory quotient change. However, in contrast to previous studies, ventilation in our subjects remained constant. Figure 5.5 is a plot of the ventilation against the CO_2 production and confirms this independence of ventilation from the CO_2 flux in the range of $\dot{V}_{CO2}$ observed in our study.

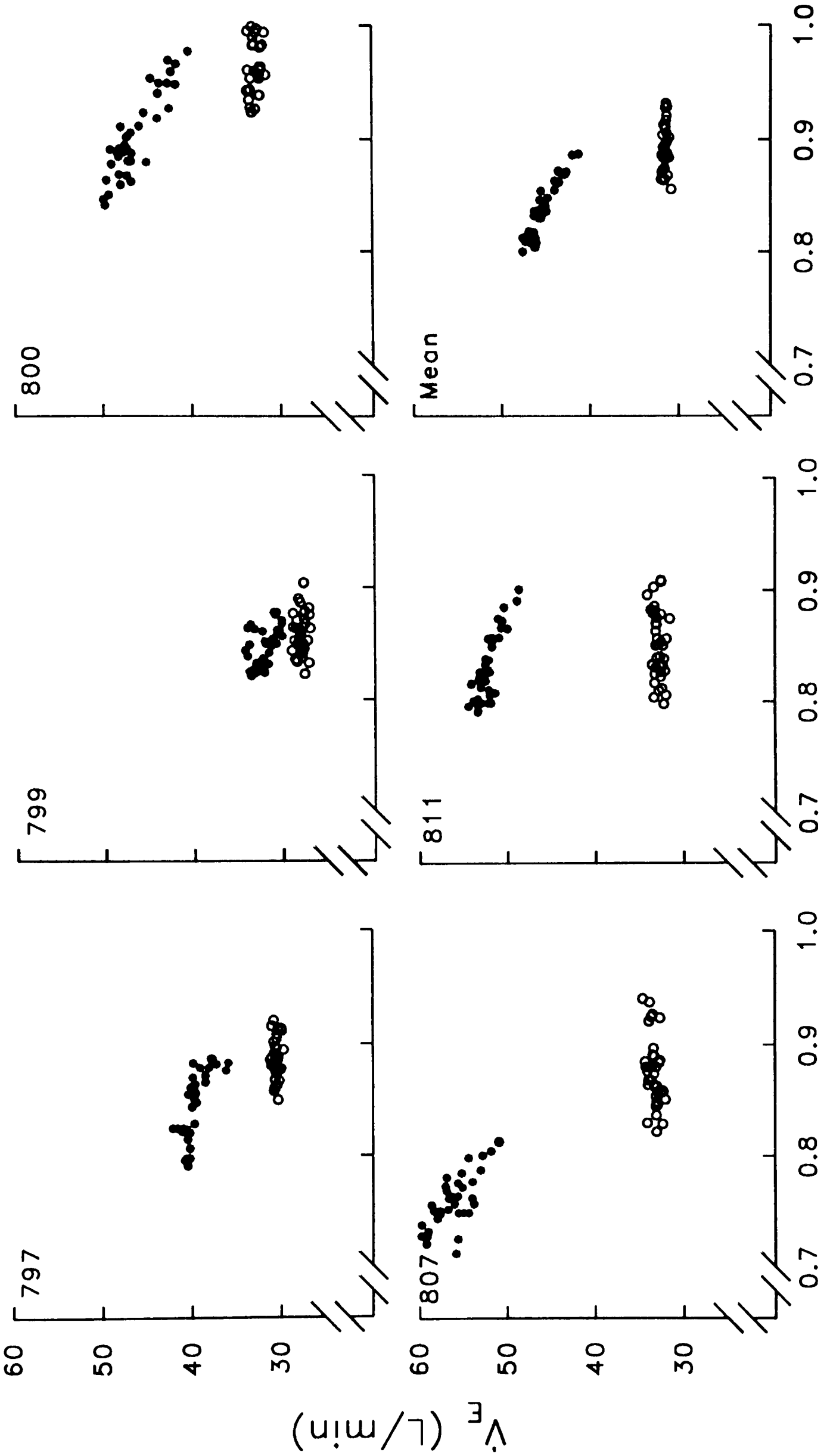


Figure 5.5. Mean values of ventilations plotted against the mean values of CO_2 production for each of the five subjects and the average for all five combined, for the two protocols in exercise. Poikilocapnic exercise, protocol B (○); hypercapnic exercise, protocol C (●).

Indeed, during exercise in hypercapnia, there is an apparently negative relationship between $\dot{V}E$ and $\dot{V}_{CO2}$, probably brought about by the correction of the falling end-tidal P_{CO2}. A similar dissociation of $\dot{V}E$ from $\dot{V}_{CO2}$ after a change in substrate was reported by Heigenhauser *et al* (1983), who used a 24 hour diet to deplete glycogen stores and so reduce the respiratory quotient. When their subjects undertook incremental exercise tests to exhaustion, they found that ventilation was higher and PET_{CO2} lower after glycogen depletion, at any given level of $\dot{V}_{CO2}$ during the test, in spite of lower levels of lactate after glycogen depletion.

Our result raises some interesting questions. First, if the ventilation during prolonged exercise is independent of CO_2 output, what other drives are involved? Exercise was performed well below the anaerobic threshold and lactic acidosis was not a factor. There are, of course, numerous other drives that operate in exercise, including reflexes from the working muscles and arterial potassium. Any one of these could carry information about how much work is being done. It is interesting however, that as the substrate changes, such factors appear to maintain a constancy of ventilation over and above a constancy of P_{CO2}, and the reasons for this are unclear.

Finally, the study emphasises that genuine steady-state exercise is a condition that is difficult to achieve. A certain period of time is obviously required from the onset of exercise for the system to equilibrate with the level of exercise and with the conditions imposed by the inspired gases. However, if the exercise is too prolonged, gradual changes occur in the substrate and metabolic CO_2 production, leading to conditions that cannot accurately be described as "steady-state".

Appendix 5.1

This appendix (Tables 5.4, 5.5, 5.6) presents the ventilation and gas exchange results for each of the three protocols for each individual subject. The results are shown as the mean change in each variable between the first and last five min of the protocol. Also appended to the foot of each table are the results of using another approach in assessing whether the observed trends were statistically significant. This second approach was to fit straight lines to the minute-averaged data for each measured variable in each experimental period in each subject. The fitted slopes were then averaged to obtain the mean slope and standard error for the group. The probability that these mean slopes did not differ from zero was assessed using a two-tailed t-test (Bailey, 1985).

When assessed in this way, the statistical comparisons differ from those presented in Tables 5.1, 5.2 and 5.3 in only three ways. The trends at rest in $P_{ET_{CO2}}$ and in $\dot{V}_{O2}$ are no longer significant; and thirdly, the reduction in T_E during hypercapnic exercise no longer reaches statistical significance.

TABLE 5.4

Mean changes (± SE) in respiratory variables for each of the five subjects for protocol A (rest, hypercapnia). NS indicates that slopes do not significantly differ from zero

Subjects	$\Delta\dot{V}_E$ (L/min)	ΔV_{TE} (L)	ΔT_I (msec)	ΔT_E (msec)	ΔP_{ETCO2} (Torr)	$\Delta\dot{V}_{CO2}$ (L/min)	$\Delta\dot{V}_{O2}$ (L/min)	R
797	1.10	0.05	73	-84	0.06	-0.01	-0.01	0.01
	0.15	0.01	17	13	0.02	0.00	0.00	0.00
799	0.33	0.00	79	-207	0.07	-0.02	-0.02	-0.01
	0.11	0.01	20	24	0.05	0.00	0.00	0.01
800	1.04	0.01	-88	-157	0.07	0.00	-0.02	0.06
	0.10	0.01	13	14	0.03	0.00	0.00	0.01
807	3.80	0.10	-41	-181	0.02	0.00	0.00	0.00
	0.20	0.01	2	18	0.04	0.00	0.00	0.01
811	-0.62	-0.02	25	61	0.07	-0.02	-0.02	-0.02
	0.14	0.01	13	13	0.05	0.00	0.00	0.01
	(NS)	(NS)	(NS)	(NS)	(NS)	(NS)	(NS)	(NS)

TABLE 5.5

Mean changes (± SE) for the respiratory variables during protocol B (poikilocapnic exercise). Probabilities that the slopes do not differ from zero: * = $P < 0.05$; ** = $P < 0.01$; NS indicates no statistical significance from zero

Subjects	$\Delta\dot{V}_E$ (L/min)	ΔV_T (L)	ΔT_I (msec)	ΔT_E (msec)	ΔP_{ETCO2} (Torr)	$\Delta\dot{V}_{CO2}$ (L/min)	$\Delta\dot{V}_{O2}$ (L/min)	R
797	0.29	0.01	-1	-185	-0.76	-0.05	0.01	-0.06
	0.13	0.01	13	11	0.07	0.01	0.00	0.00
799	0.86	-0.01	-32	5	-0.69	-0.04	0.02	-0.02
	0.26	0.01	18	21	0.13	0.01	0.01	0.00
800	0.17	-0.16	-142	10	-0.52	-0.06	0.02	-0.07
	0.30	0.02	26	36	0.08	0.01	0.01	0.00
807	-0.67	-0.10	-42	-20	-0.74	-0.06	0.01	-0.06
	0.29	0.01	9	16	0.06	0.02	0.03	0.03
811	-0.66	-0.11	-19	-70	-0.53	-0.09	-0.05	-0.04
	0.21	0.01	4	8	0.04	0.00	0.00	0.00
	(NS)	(NS)	(NS)	(NS)	(**)	(*)	(NS)	(**)

TABLE 5.6

Mean changes (± SE) for the respiratory variables during protocol C (hypercapnic exercise). Probabilities that the slopes do not differ from zero: * = $P < 0.05$; ** = $P < 0.01$. NS indicates no statistical significance from zero

Subjects	$\Delta\dot{V}_E$ (L/min)	ΔV_T (L)	ΔT_I (msec)	ΔT_E (msec)	ΔP_{ETCO2} (Torr)	$\Delta\dot{V}_{CO2}$ (L/min)	$\Delta\dot{V}_{O2}$ (L/min)	R
797	3.85	0.10	-11	-72	-0.07	-0.07	-0.02	-0.06
	0.33	0.01	7	8	0.03	0.01	0.01	0.00
799	3.43	0.14	19	-25	-0.22	-0.01	0.02	-0.03
	0.15	0.01	9	26	0.07	0.01	0.01	0.00
800	6.66	-0.06	-159	-228	0.03	-0.09	-0.04	-0.06
	0.29	0.01	11	11	0.05	0.01	0.01	0.00
807	7.46	0.03	-82	-164	-0.10	-0.07	-0.06	-0.03
	0.35	0.01	5	10	0.03	0.00	0.01	0.01
811	2.94	-0.03	-38	-129	0.00	-0.08	-0.07	-0.02
	0.36	0.00	6	11	0.03	0.00	0.01	0.00
	(**)	(NS)	(NS)	(NS)	(NS)	(**)	(NS)	(**)

Appendix 5.2

The *P* values reported in Tables 5.1, 5.2 and 5.3 have not been corrected for multiple comparisons, but simply calculated in the standard way. The justification for this is that for each variable in each protocol, only one comparison has been made, and significance has therefore been accepted at the $P < 0.05$ level. In fact, all the comparisons which reach statistical significance do so at the $P < 0.01$ level, except one comparison in Table 5.3. Multiple paired t-tests appear to have been the standard method of assessing changes in numerous variables during the course of prolonged exercise (Hermansen *et al.*, 1967; Ahlborg *et al.*, 1974; Hagberg *et al.*, 1978; Jansson, 1982).

If, however, it is argued that each variable has in fact undergone multiple comparisons, then a correction to the t-test might be used. We have, therefore, also subjected the individual subject values (the means of which are presented in Tables 5.1, 5.2 and 5.3) to an analysis of variance. When this is done, the comparisons which are reported in the tables as significant are unaltered. We then subjected the comparisons to a t-test, using a modified value of *P* using the Bonferroni correction. We assumed that, in general terms, the variables $\dot{V}_E$, V_{TE}, T_I and T_E should be considered as dependent upon each other, and therefore, comparisons for these variables in each protocol were accepted as significant at a value of $P < 0.05/4$. Similarly, $\dot{V}_{CO2}$, $\dot{V}_{O2}$ and R were taken to be dependent, and the modified critical level of *P* for these comparisons was 0.05/3. The variable $P_{ET_{CO2}}$ was assumed to be dependent on all the other variables, except $\dot{V}_{O2}$ ans so the modified level of $P < 0.05/6$ was used for each protocol. When these modified levels of *P* were used, only one comparison reported as significant was altered and ceased to reach significance: this was the change in T_E during hypercapnic exercise (Table 5.3). This does not change the interpretation of the physiology.

CHAPTER 6

ACUTE VENTILATORY RESPONSES TO HYPOXIA DURING ELECTRICALLY-INDUCED LEG EXERCISE IN MAN

We have designed this machine, Dr Feynman,

to test your theory. It costs $ 37 million.

Director of CERN

Don't you trust me?

Richard Feynman

Introduction

As discussed in the Introduction, the studies of Asmussen *et al.*, (1943), Adams *et al.*, (1984*a*) and Brice *et al.*, (1988*a*) have suggested that the voluntary control of exercise is unnecessary to achieve the normal matching of ventilation to metabolic CO_2 production during exercise. Furthermore, the last two groups of investigators repeated their experiments with paraplegic subjects, and concluded that reflex neural inputs from the electrically-stimulated limbs were also unnecessary for this normal ventilatory response to exercise (Adams *et al.*, 1984*b*; Brice *et al.*, 1988*b*). However, the role of voluntary drive in increasing the chemoreflex sensitivity to hypoxia which also normally occurs during steady exercise is unclear.

The purpose of this study was to use the method of Adams *et al.* (1984*a*) and use electrical muscle stimulation in normal human subjects to investigate whether a cortical drive is necessary to augment the acute hypoxic ventilatory response (AHR) during exercise. We compared the magnitude of the AHR during electrical exercise not only with that in the resting state, but also with that during two voluntary exercise protocols: one matched with electrical exercise for external work rate, and one matched for internal work rate (*i.e*, metabolic rate).

METHODS

Subjects

Six normal males were studied. Their physical characteristics are described in Table 6.1.

TABLE 4.1

Physical characteristics of subjects

Subject	Sex	Height (m)	Weight (kg)	Age (yrs)
802	M	1.79	70	21
835	M	1.78	69	21
846	M	1.81	74	21
905	M	1.75	60	21
908	M	1.91	86	26
911	M	1.85	74	29

Determination of End-tidal and Blood Gas Tensions

The end-tidal values for P_{CO2} (PET_{CO2}) for each subject breathing air at rest, and during electrical and voluntary exercise were obtained as the mean over a 15 min period, after familiarisation with the laboratory. End-tidal values for P_{CO2} were also recorded in the few minutes prior to commencing each experimental period of each protocol.

In three subjects (802, 905 and 911), three arterial blood samples from an indwelling radial catheter (20 G Angiocath, Utah, USA) were drawn at 5 min intervals at rest, and during electrical and both the voluntary exercise protocols to determine possible changes in arterial P_{CO2} (Pa_{CO2}) with exercise (IL 1306 Blood Gas Analyser, Warrington, Cheshire). In a fourth subject (835), it was possible only to obtain arterial blood samples at rest.

Experimental Technique

Subjects were seated in a specially-designed chair (Fig. 1). Their feet were strapped to a footplate which, when the leg was extended at the knee, lifted a set of weights (M) via a system of pulleys. The height (h) through which the weights were moved, and the frequency of muscular contraction (f) were recorded during all three exercise protocols. The additional load (I) associated with the weight of the subject's leg and unloaded pulley system was estimated as the force registered on a spring balance pulling the footplate (with a subject in the chair and subject's feet attached) in the horizontal plane over a similar distance achieved during the exercise protocols. This load was used in the estimate of external work rate, as described below.

The apparatus for electrical muscle stimulation was the same as that used by Adams *et al.* (1984*a*), and it has been described in Chapter 2. After familiarisation of the subjects to electrical stimulation, the output current was adjusted to provide the strongest contraction that could be achieved without any discomfort. Relaxation of the muscles was passive. The weight lifted during electrical exercise was adjusted to allow a reasonably free swing of the legs: it was found, for example, that if the weight was too heavy and the legs did not swing, electrical stimulation was painful.

Subjects breathed through a mouthpiece with their nose occluded. The end-tidal gases were controlled by dynamic end-tidal forcing, as described in Chapter 2. Heart rate was monitored by an ECG and arterial oxygen saturation by a finger pulse oximeter.

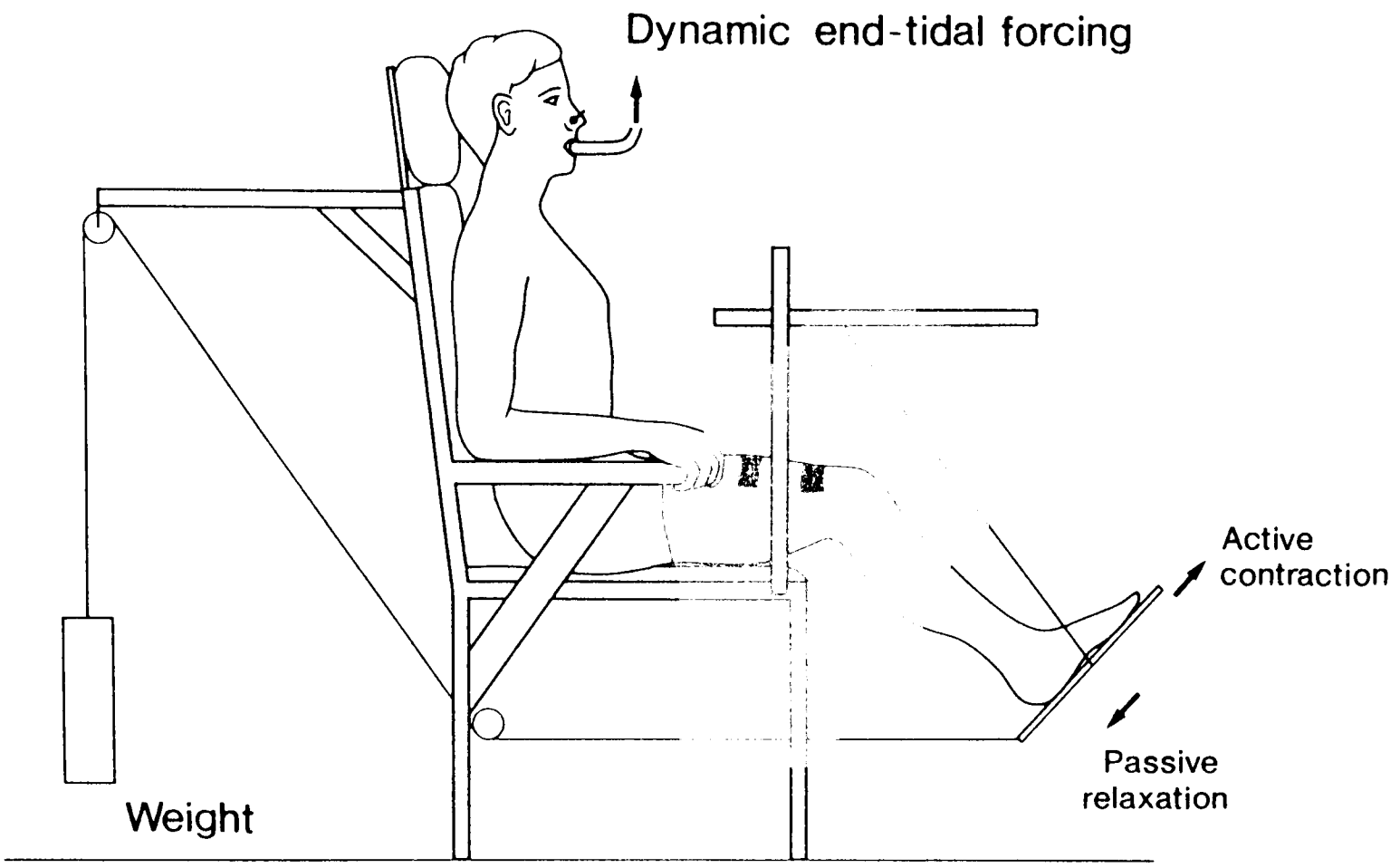


Figure 6.1. Schematic diagram of the experimental set-up, showing the chair and pulley system which allow the external work rate during electrical and voluntary exercise to be estimated.

Protocols

After an initial 5 min to allow a steady-state to be reached, data collection was begun. Each experimental period was of 27 min duration. End-tidal P_{O2} was held at 100 Torr for 5 min, and then there were a total of three separate exposures to 3 min of hypoxia (PET_{O2} 50 Torr), separated by 5 min periods of euoxia (PET_{O2} 100 Torr). After the last of the three hypoxic exposures, there was a final 3 min period of euoxia (Fig. 2). Throughout each protocol, PET_{CO2} was held 1-2 Torr above resting values.

There were four protocols, and each of the six subjects undertook each of these protocols six times, giving a total of 144 separate experimental periods, with a total of 432 separate hypoxic exposures. Protocols A, B and C were undertaken in random order on different days, and protocol D undertaken after the mean gas exchange data from protocol B had been calculated.

In protocol A, the subject was at rest. Protocol B was undertaken during electrical muscle stimulation (E_{EL}). For each experimental period of protocol C, the subject performed voluntary exercise, lifting the same weight through the same height and at the same frequency (using an electrical metronome) as a matched period in protocol B: this protocol was therefore matched to E_{EL} for *external* work rate (E_{V1}). In protocol D, the subject performed voluntary exercise, but this time the weight, height lifted and frequency were adjusted to achieve similar $\dot{V}_{CO2}$ and $\dot{V}_{O2}$ as in protocol B: this protocol was therefore matched to E_{EL} in terms of *internal* work rate (E_{V2}).

A blood sample for determination of venous lactate (YSI 23L Enzymatic Lactate Analyser, Yellow Springs, Ohio) was taken after each experimental period of electrical exercise (E_{EL}) and its matched voluntary exercise period (E_{V2}).

Data Analysis

Data for $\dot{V}_E$, PET_{CO2}, PET_{O2}, $\dot{V}_{CO2}$ and $\dot{V}_{O2}$ for each experimental period were averaged over 1 min periods.

The last min of the initial 5 min of euoxia, before any hypoxia was administered, was taken to represent the "baseline" $\dot{V}_E$. Thus, for each subject, 6 such baseline

ventilations were obtained for each protocol, and these were combined to give the subject means for each protocol.

The AHR for each experimental period was calculated as the difference between the ventilation attained in the second min of hypoxia during each hypoxic exposure, and the last min of euoxia, before the hypoxic exposure. Thus, for each subject, a total of 18 separate values of AHR were obtained for each of the four protocols. These values were then combined to give the mean AHR for each subject for each protocol.

Values of $\dot{V}_{O2}$ and $\dot{V}_{CO2}$ were averaged over each experimental period, and then combined to give the relevant subject means for each protocol. External work rate for the protocols involving exercise was estimated by the equation:

$$\textit{External Work} = ([M + I] \times g \times h \times f)$$

where g = the acceleration due to gravity. The internal work rate during the exercise protocols was calculated using the measured $\dot{V}_{CO2}$ and $\dot{V}_{O2}$ to obtain the respiratory quotient, R, for each subject. The energy production per unit O_2 consumption for this particular value of R was estimated from standard tables (Evans, 1941); and from the measured $\dot{V}_{O2}$, the actual energy used was calculated. An estimate of efficiency was obtained from the ratio of the external work rate to the internal work rate for the protocol.

Statistical Analysis

The individual subject means for each of the variables were first subjected to an analysis of variance. If this indicated significant differences between the protocols, the differences between means of the variables from the group as a whole were assessed using a paired, two-tailed t-test. Statistical significance for each variable was accepted at a value of $P < 0.05/k$ (Bonferroni's correction) where k is the number of comparisons made for that variable (Krauth, 1988).

RESULTS

Table 6.2 shows the mean values of end-tidal P_{CO2}, as calculated from the values obtained in the air-breathing periods in each experiment before dynamic end-tidal forcing was started. Also shown are the arterial P_{CO2} values from the four subjects in whom these were obtained. In each of the subjects, PET_{CO2} did not change appreciably or consistently between the different protocols. Similarly, Pa_{CO2} did not change between protocols in the subjects in whom it was measured.

TABLE 6.2

Mean air-breathing values (Torr ± SE) for end-tidal P_{CO2} (PET_{CO2}, n = 6) and arterial P_{CO2} (Pa_{CO2}, n = 3) for each subject, in each of the four protocols. The end-tidal value at which each subject was held by dynamic end-tidal forcing during the experiments (Clamp, PET_{CO2}) is also shown

	Protocol								
Subject	Rest		E_{V1}		E_{EL}		E_{V2}		Clamp
	PET_{CO2}	Pa_{CO2}	PET_{CO2}	Pa_{CO2}	PET_{CO2}	Pa_{CO2}	PET_{CO2}	Pa_{CO2}	PET_{CO2}
802	38.4 0.1	39.5 0.4	38.2 0.4	39.3 0.6	38.6 0.1	39.7 0.4	38.9 0.2	40.1 0.4	40.0
835	36.9 0.2	38.8 0.4	36.3 0.1		36.3 0.1		36.8 0.3		38.0
846	38.0 0.3		37.8 0.2		38.4 0.3		37.7 0.3		40.0
905	40.0 0.1	42.0 0.6	39.5 0.2	42.8 0.4	40.0 0.3	42.3 0.2	40.4 0.3	42.6 0.3	42.0
908	37.3 0.2		37.5 0.4		37.2 0.3		37.0 0.3		39.0
911	36.5 0.1	37.3 0.1	36.4 0.3	36.6 0.1	36.3 0.2	36.2 0.2	36.6 0.3	36.1 0.2	38.0

Figure 6.2 (upper panel) shows the control of end-tidal gases in one representative subject (911) for the E_{EL} protocol only. The gas-mixing system effected three rapid step reductions in $P_{ET_{O2}}$, while at the same time holding $P_{ET_{CO2}}$ constant.

The ventilatory responses in the same subject are shown in Fig. 6.2 (lower panel), and the mean responses for all six subjects combined are shown in Fig. 6.3. The baseline ventilation was lowest in the protocol at rest, somewhat increased by E_{V1}, and highest during E_{EL} and E_{V2}. Regarding the magnitudes of the acute hypoxic responses, the smallest occurred at rest. The AHR appeared to be slightly increased by E_{V1}. The largest AHR clearly occurred during E_{EL} and E_{V2}, and the magnitudes of these appeared to be similar. For all protocols (Fig. 6.3) the mean peak ventilation during hypoxia was reached in the second min. The magnitudes of the three successive AHRs within each protocol were similar.

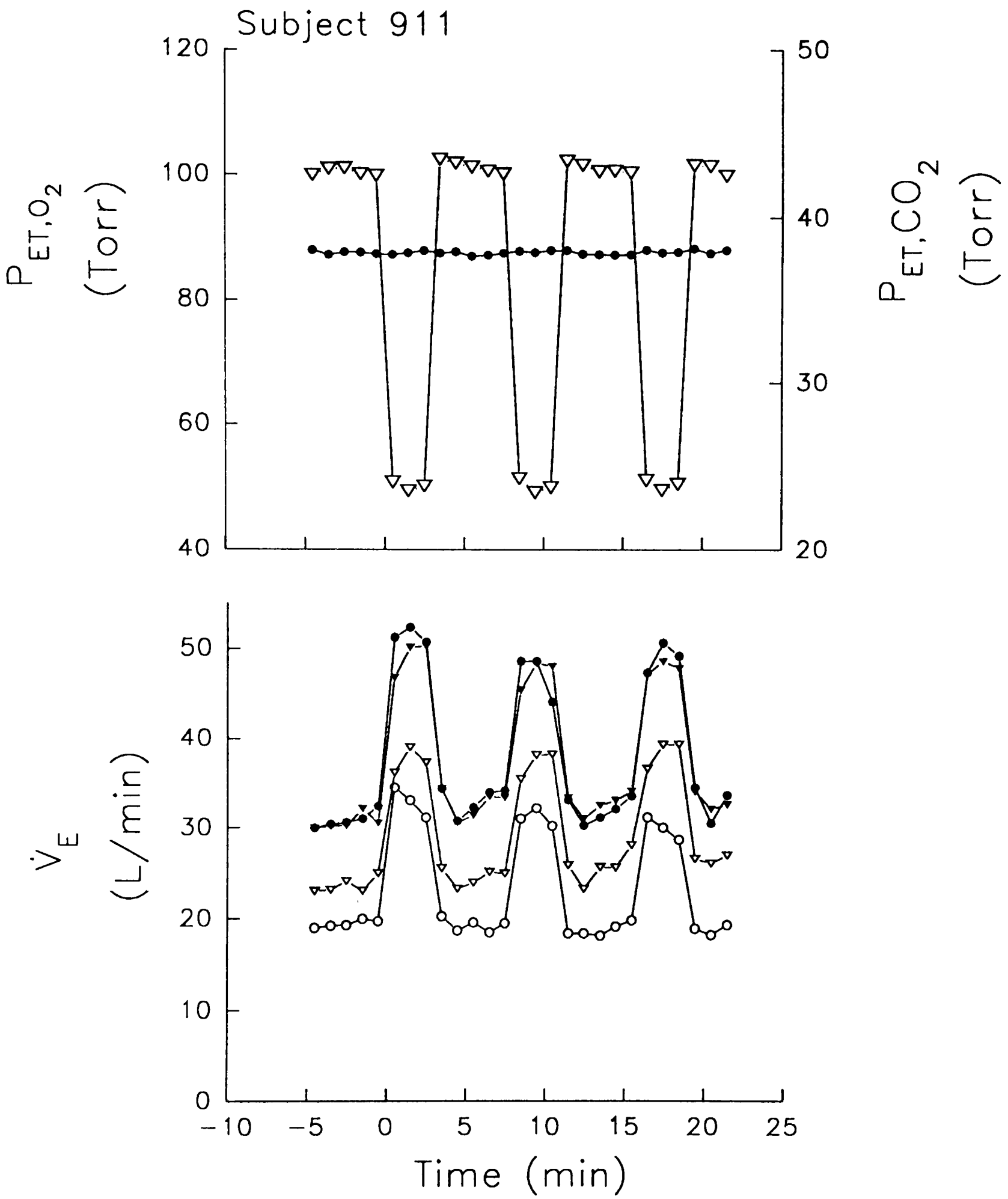


Figure 6.2. *Upper panel*: End-tidal gas profiles for P_{O2} (∇) and P_{CO2} (●) for one subject (911). For clarity, only the mean profiles during E_{EL} are shown. The dynamic end-tidal forcing system effects three successive hypoxic steps of 3 min duration, each separated by 5 min of euoxia. Throughout the protocols, end-tidal P_{CO2} remains constant.

Lower panel: Mean ventilatory responses for all four protocols in the same subject (911). At rest (○); during E_{V1} (∇); during E_{EL} (●); and during E_{V2} (▼).

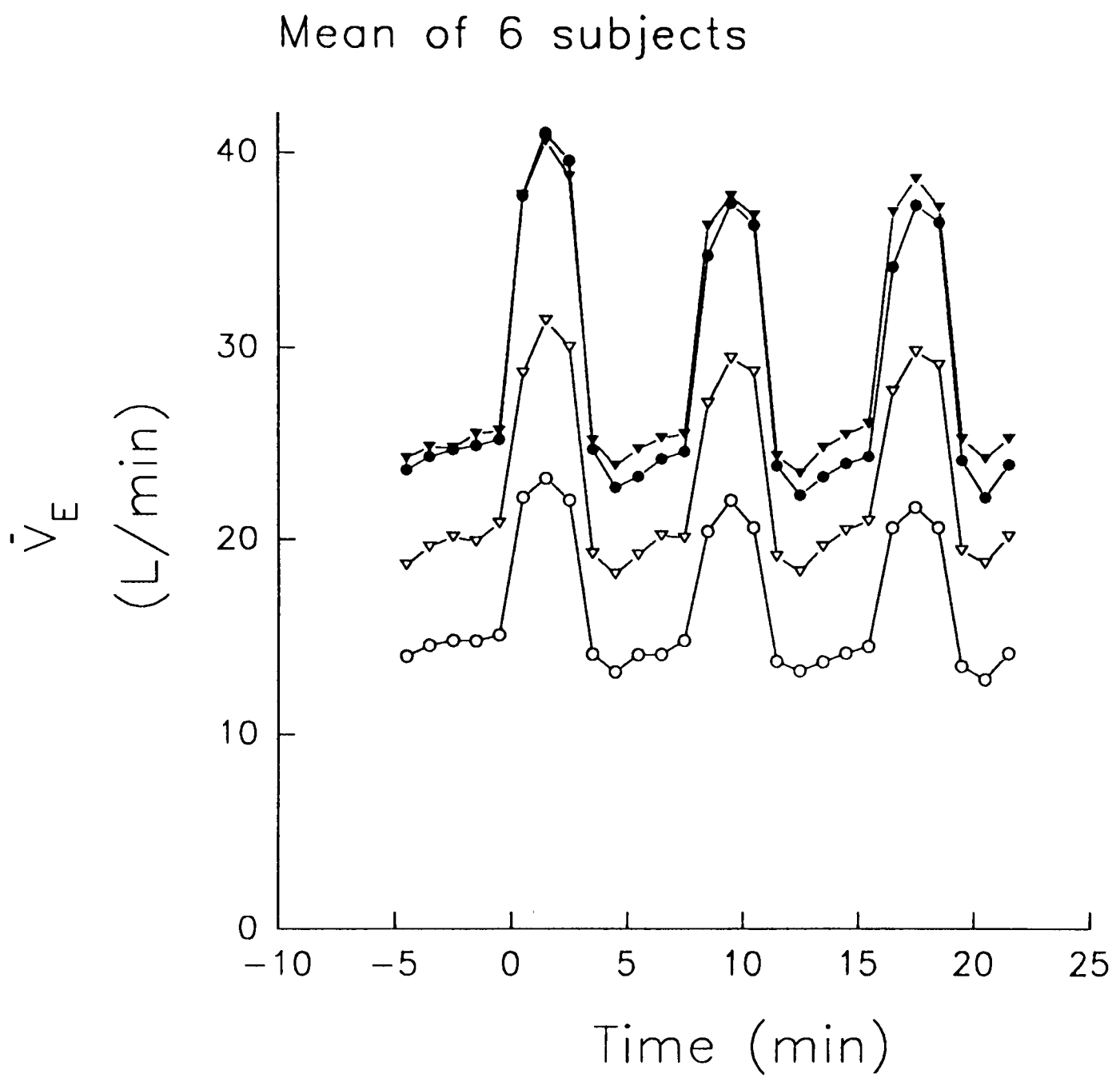


Figure 6.3. Mean ventilatory responses for all four protocols, for all six subjects combined. At rest (○); during E_{V1} (▽); during E_{EL} (●); and during E_{V2} (▼). The three periods of hypoxia are from time = 0 to 3 min, 8 to 11 min and 16 to 19 min.

Table 6.3 shows the calculated values for euoxic baseline ventilation for each subject, and Fig. 6.4 (upper panel) shows the mean values for all six subjects combined, and the statistical comparisons between the protocols. Electrical exercise significantly increased the euoxic ventilation as compared with rest, and the baseline ventilation during E_{V2} was similar to that during E_{EL}. Voluntary exercise matched to the external work rate of electrical stimulation (E_{V1}) also slightly increased the baseline ventilation as compared with rest. The baselines during *both* E_{EL} and E_{V2} were significantly higher than those during *both* Rest and E_{V1}.

TABLE 6.3

Mean values of the euoxic baseline ventilations (L/min ± SE) for each subject in each of the four protocols

	Protocol			
Subject	Rest	E_{V1}	E_{EL}	E_{V2}
802	13.7 ± 1.97	17.7 ± 1.33	20.9 ± 2.07	24.8 ± 1.40
835	15.5 ± 0.97	25.9 ± 0.89	31.5 ± 2.36	32.4 ± 2.72
846	13.4 ± 0.74	16.5 ± 1.23	21.3 ± 0.80	22.3 ± 1.58
905	13.2 ± 1.05	17.8 ± 1.09	21.7 ± 2.18	18.4 ± 1.52
908	14.9 ± 1.21	22.3 ± 2.79	25.3 ± 1.23	26.2 ± 1.83
911	22.1 ± 2.52	25.0 ± 2.34	32.4 ± 1.65	30.6 ± 1.02
MEAN	15.5 ± 1.38	20.8 ± 1.67	25.5 ± 2.14	25.8 ± 2.12

Table 6.4 shows the values of AHR for each of the subjects, and Fig. 4 (lower panel) shows the mean values for all six subjects combined and the statistical comparisons between the protocols. Electrical exercise almost doubled the AHR attained at rest. The AHR during the matched voluntary protocol, E_{V2}, was similar to that during E_{EL}. Voluntary exercise matched to the external work rate of E_{EL} did increase the AHR modestly, as compared with rest (mean = 2.07 L/min), and this value reached statistical significance. *Both* E_{EL} and E_{V2} increased the AHR significantly, as compared with *both* Rest and E_{V1}.

TABLE 6.4

Mean values of the acute hypoxic responses (L/min ± SE) for each subject in each of the four protocols

	Protocol			
Subject	Rest	E_{V1}	E_{EL}	E_{V2}
802	4.82 ± 0.42	6.62 ± 0.49	9.21 ± 0.51	8.84 ± 0.34
835	5.21 ± 0.43	8.32 ± 0.66	14.2 ± 0.64	13.6 ± 0.58
846	7.26 ± 0.45	9.36 ± 0.35	11.0 ± 0.61	10.7 ± 0.40
905	7.14 ± 0.44	9.46 ± 0.48	17.2 ± 1.06	17.2 ± 0.92
908	8.61 ± 0.32	11.1 ± 0.65	15.0 ± 0.86	14.4 ± 0.64
911	12.3 ± 0.76	12.8 ± 0.69	18.2 ± 1.27	16.7 ± 1.05
MEAN	7.55 ± 1.10	9.62 ± 0.88	14.1 ± 1.42	13.6 ± 1.35

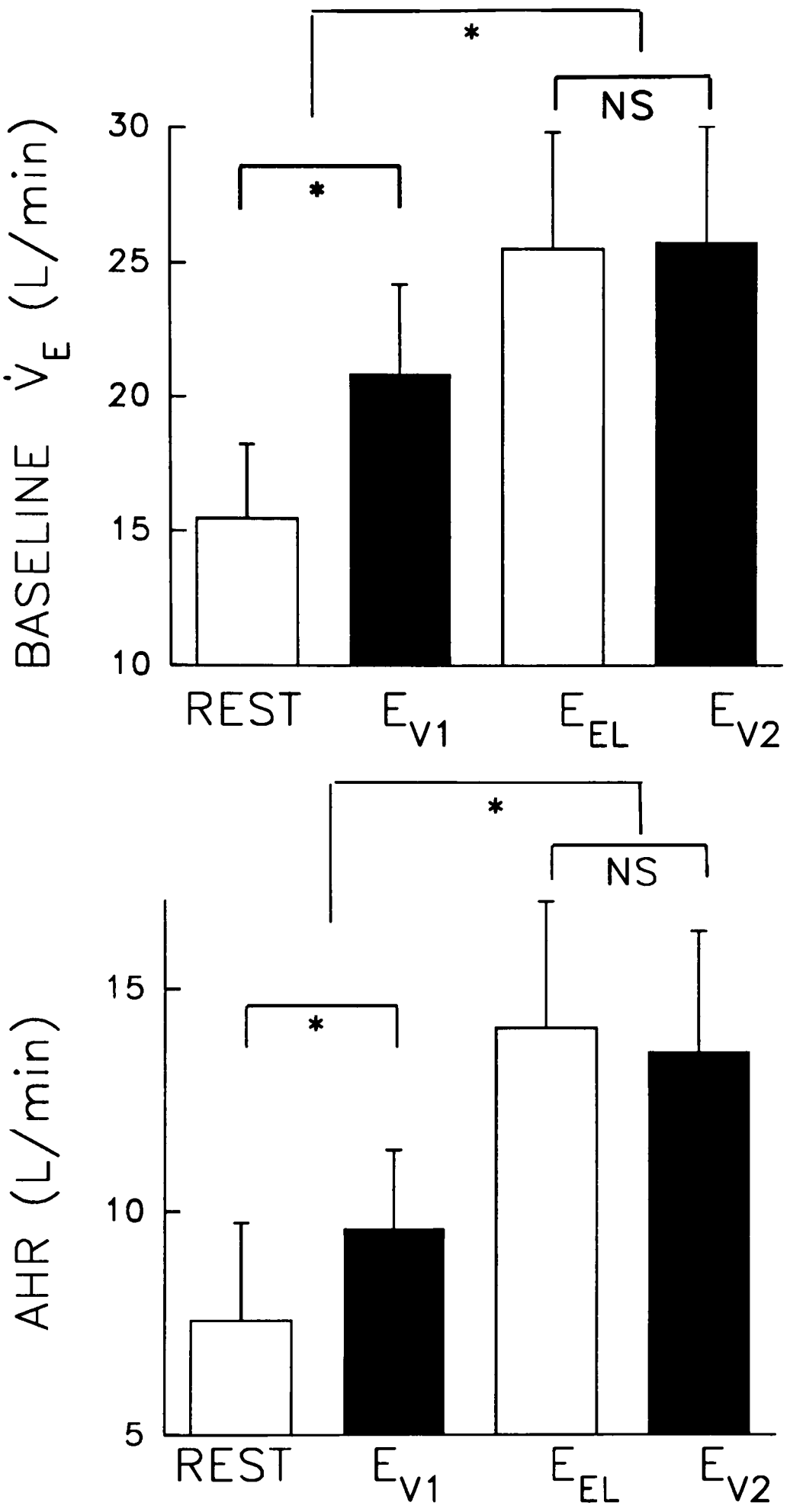


Figure 6.4. *Upper panel*: Mean values (± 2 SE) of euoxic baseline ventilation of all six subjects combined, for each protocol, and the comparisons between the protocols. *NS* indicates no significant difference; * indicates significant difference (Student's paired t-test with Bonferroni correction). Baseline ventilations in *both* E_{EL} and E_{V2} are significantly different from ventilations in *both* Rest and E_{V1}.

Lower panel: Mean values (± 2 SE) of the acute hypoxic responses of all six subjects combined, for each protocol, and the comparisons between the protocols. Statistical comparisons are made as described for the upper panel and in the text. Acute hypoxic responses in *both* E_{EL} and E_{V2} are significantly different from responses in *both* Rest and E_{V1}.

Table 6.5 shows the gas exchange data, work rate and efficiency, and changes in lactate. During E_{V1}, metabolic CO_2 production and O_2 consumption increased significantly, by a small amount (53 ml/min and 71 ml/min respectively), even though external work rate during this protocol was small. Electrical exercise increased metabolism by a much greater amount, and while E_{V2} matched E_{EL} adequately both in terms of $\dot{V}_{CO2}$ and $\dot{V}_{O2}$, overall efficiency of work was much lower during electrical stimulation. There was no significant rise in lactate either after E_{EL} or E_{V2}.

TABLE 6.5

Mean values (± SE) for all six subjects combined, for gas exchange ($\dot{V}_{CO2}$ and $\dot{V}_{O2}$), external work rate, efficiency of work and change in plasma venous lactate before and after exercise (ΔLactate), for each of the four protocols (* between columns indicates significant differences for comparisons between the columns on either side; NS denotes no significant difference; Student's t-test with Bonferroni correction applied)

	Protocol						
	Rest		E_{V1}		E_{EL}		E_{V2}
$\dot{V}_{CO2}$ (ml/min)	245 ± 13	*	298 ± 13	*	388 ± 14	NS	401 ± 15
$\dot{V}_{O2}$ (ml/min)	306 ± 17	*	377 ± 21	*	476 ± 21	NS	482 ± 11
External work rate (W)	——		3.73 ± 0.83	NS	3.73 ± 0.83	*	9.06 ± 1.15
Efficiency (%)	——		13.9 ± 1.78	*	5.3 ± 0.60	*	14.8 ± 1.33
ΔLactate (mmol l^{-1})	——		——		0.07 ± 0.04	NS	0.03 ± 0.05

DISCUSSION

The main conclusion of this study is that the increase in the acute ventilatory response to hypoxia which normally occurs during exercise in man can also occur in the absence of a voluntary drive to exercise from the cortex.

Is electrical exercise involuntary?

A number of methods were used to try and establish whether the subjects voluntarily contracted their leg muscles during electrical stimulation. First, all the subjects were comfortable during electrical stimulation and denied that they had co-operated voluntarily with the electrical stimulus. We stopped the experiment if any discomfort whatsoever was reported. Secondly, we used the method suggested by Adams *et al.* (1984*a*) to assess voluntary involvement by occasionally switching off the current discretely during E_{EL}: a voluntary contraction might have been expected if the subject had been driving his muscles in tune with the stimulator. However, this did not occur. Occasionally, we also told the subject that the experiment and data collection had finished, but continued the muscle stimulation: if any part of the leg movement had been voluntary, we might have expected a change in the height through which the weight was lifted (Fig. 6.1). Again, this did not occur. Indeed, careful observation of the height lifted and the frequency of contractions revealed a remarkable constancy during all experimental periods of electrical stimulation.

In addition, careful observation was kept of the legs themselves. Electrical stimulation produces a characteristic generalised contraction of the quadriceps, very different from that produced during voluntary contraction. Relaxation during electrical stimulation was passive: we might have expected to see an active contraction of the hamstrings had the subject been making voluntary efforts. It was also noticed that on some occasions, the strength of contraction of the right and left quadriceps was slightly unequal during electrical stimulation: such inequality would be unexpected during normal voluntary activity, and was not seen in the same subjects during E_{V1} and E_{V2}.

Observation of the subjects' feet showed that with electrical stimulation, the muscles around the ankle joint were relaxed and flaccid, thus keeping the foot in a natural partial plantar flexion during leg extension; during the voluntary protocols (E_{V1} and E_{V2}), the foot was, in contrast, kept partially dorsiflexed as the leg was raised. Any dorsiflexion of the foot during electrical quadriceps activation, therefore, might have suggested an active component to contraction.

Finally, the overall efficiency of work during E_{EL} was much lower than during E_{V1} or E_{V2} (Table 6.5): a significant voluntary contribution during E_{EL} would have been unlikely to yield such large differences in efficiency.

We are therefore satisfied that exercise during E_{EL} was involuntary. While we cannot entirely exclude all possibility that some volitional reinforcement might have been involved, we feel that, if it occurred, its contribution to the overall results was extremely small.

Control of P_{CO2} and lactic acid stimuli

When comparing the magnitudes of the acute ventilatory hypoxic responses between different protocols, it is important to ensure that the P_{CO2} and lactic acid stimuli between the protocols are similar. In the three exercise protocols in this study, the overall work load was relatively low, and the end-tidal, air-breathing P_{CO2} (and the arterial P_{CO2} in the three subjects in whom it was measured) was not appreciably changed by the level of exercise, as compared with rest (Table 6.2). Since the dynamic end-tidal forcing system held PET_{CO2} at the same level in all the protocols, the level of arterial P_{CO2} should also have been similar between protocols.

The constancy of PET_{CO2} and $P_{a,CO2}$ between rest and electrically-induced exercise is consistent with the finding of Adams *et al.* (1984*a*), who measured end-tidal P_{CO2} in all subjects and $P_{a,CO2}$ in two of their subjects. Brice *et al.* (1988*a*) measured the temporal patterns of $P_{a,CO2}$ during electrical leg exercise, and found a slight tendency to hypocapnia in the first 30 sec of electrical exercise, but found that eucapnia was achieved in the steady state.

There were no significant rise in lactate levels during E_{EL} or E_{V2} (Table 6.5). This result differs somewhat from that of Adams *et al.* (1984*a*), who obtained a mean lactate level of 2.17 mmol l^{-1} after E_{EL}, and with that of Brice *et al.* (1988*a*) who reported a small but significant rise of about 0.5 mmol l^{-1}. These differences may be related to the intensity of electrical stimulation: in both these previous studies, the increase in $\dot{V}_{CO2}$ was of the order of 300 ml/min. Our stimulation achieved a more modest increase in $\dot{V}_{CO2}$ of about 150 ml/min, and so avoided the problem of any lactic acidosis.

Comparison with previous studies

Although we are not aware of any other reports of the acute hypoxic ventilatory response during electrically-induced exercise in humans, it is interesting to compare the nature of the electrical stimulation in our study with that employed by previous studies involving electrically-stimulated exercise.

Adams *et al.* (1984*a*) stimulated both the quadriceps and hamstring muscle groups and achieved a higher $\dot{V}_{CO2}$ during E_{EL} than in our study. Data were collected after only 4 min of stimulation, but nonetheless there was increased production of lactic acid. They also attempted to match voluntary exercise to E_{EL} by asking subjects to copy the tension signal displayed on an oscilloscope, as recorded during their electrically-induced exercise. The protocol of Brice *et al.* (1988*a*) was very similar to this, but they did not ask subjects to copy tension signals.

The overall stimulus strength used in this study appears to have been more modest than used in these previous studies. We have therefore avoided fatigue and lactic acidosis, and been able to achieve electrical exercise for more prolonged periods, taking our measurement of euoxic ventilation after 10 min. While the probable pattern of muscular contractions between E_{EL} and E_{V2} was different in this study, a second voluntary exercise protocol, E_{V1}, was also included, in which the *external* work rate was the same as during E_{EL}. Finally, end-tidal forcing was used to maintain $P_{ET}CO_2$ constant 1-2 Torr above resting values, enabling us to measure the steady-state isocapnic hypoxic ventilatory responses.

Physiological significance of the results

The purpose of this study was to assess whether voluntary control is necessary for the increase in ventilatory sensitivity to hypoxia which occurs during exercise. Our conclusion is that it is not.

Voluntary exercise at the lower work rate (E_{V1}) increased the ventilatory response to hypoxia by a small amount (about 2 L/min). However, the AHR was increased much more by the electrical exercise protocol E_{EL}, although the *external* work rate was the same as during E_{V1} (Fig. 6.4). Furthermore, it is interesting that the AHR obtained during E_{V2} was so similar to that during E_{EL}. Although the metabolic rates in these two protocols were matched, there was in addition voluntary effort during E_{V2} which might be expected to act independently to increase the magnitude of the AHR, as compared with E_{EL}. It appears, therefore, that *internal* work rate is the important variable, rather than external work rate or how the work is achieved. This result suggests that $\dot{V}_{CO2}$ or factors related to it are important in determining the ventilatory sensitivity to hypoxia during exercise in man.

This study does not indicate which precise factors may be involved, since the increased $\dot{V}_{CO2}$ may increase the stimulus from blood-gas oscillations, or the sensation of "electricity" in normal subjects could evoke a sympathetic response and catecholamine release, or electrical stimuli to the muscle might release potassium and increase the levels in arterial blood. Any, or all, of these humoral stimuli could be proportional to the work rate and may increase the chemoreflex sensitivity to hypoxia by a direct action on the carotid bodies. Finally, this study does not exclude the possible involvement of nervous afferents from the muscles: these would also carry information about the amount of internal work being done (whether voluntary or involuntary) and could mediate an increased sensitivity to hypoxia more centrally in the chemoreflex pathway.

Appendix 6.1.

This appendix shows the values for O_2 consumption and CO_2 production for each of the four protocols for each individual subject.

TABLE 6.6

Values for $\dot{V}_{CO2}$ and $\dot{V}_{O2}$ (ml/min ± SE) for each individual subject for each of the four protocols

	Protocol							
	Rest		E_{V1}		E_{EL}		E_{V2}	
Subject	$\dot{V}_{CO2}$	$\dot{V}_{O2}$	$\dot{V}_{CO2}$	$\dot{V}_{O2}$	$\dot{V}_{CO2}$	$\dot{V}_{O2}$	$\dot{V}_{CO2}$	$\dot{V}_{O2}$
802	229	273	275	335	353	409	351	451
	3	19	2	21	3	20	2	23
835	274	332	299	364	454	551	473	508
	2	16	3	15	6	16	3	23
846	252	320	302	400	380	462	404	494
	2	22	2	26	4	26	3	23
905	184	232	249	311	354	432	373	448
	2	20	3	20	6	18	3	19
908	268	350	351	471	400	537	417	523
	4	23	4	22	4	24	4	22
911	261	330	313	379	389	465	385	471
	3	15	6	23	5	20	3	18

CHAPTER 7

ACUTE VENTILATORY RESPONSES TO HYPOXIA DURING ELECTRICALLY-INDUCED LEG EXERCISE IN PARAPLEGIC SUBJECTS

Keep your manuscript on the shelf for nine years.
You can destroy what you haven't published,
but you can't take back what you have.

Horace

Introduction

In the previous chapter, it was argued that the increased acute ventilatory response to hypoxia (AHR), which characterises the normal respiratory response to steady exercise can also occur in the absence of the usual drive to exercise from the cerebral cortex in normal subjects. However, the technique of using electrically-induced leg exercise does not exclude the possible involvement of neural afferents in the respiratory response in these subjects.

The purpose of this study, therefore, was to investigate the effects of electrically-induced muscle stimulation on the AHR in paraplegic subjects, in whom there are no known functional nervous connections between the legs and the brainstem or cerebral cortex. The hypothesis was that electrical muscle stimulation in these subjects would exclude the involvement of both neural afferents and volitional control in the respiratory responses with even more certainty, and therefore, that any resulting responses would be probably mediated by humoral factors from the working limbs.

METHODS

Subjects

Nine subjects with traumatic complete spinal cord transection were studied. Each had been through a full rehabilitation program at the Spinal Injuries Unit of Stoke Mandeville Hospital. All subjects were fully-independent, six were in full-time employment and all were free of respiratory disease. None was taking any medication known to affect the respiratory system. Individual subject characteristics and spirometry is shown in Table 7.1.

TABLE 7.1

Physical characteristics of subjects

Subject	Sex	Age (yrs)	Level of lesion	Duration of paraplegia (yrs)	FEV_1 (L/min)	FVC (L/min)
913	F	40	T5	14	2.0	2.4
914	M	26	T6/7	6	3.0	3.4
915	M	44	T3/4	25	1.8	2.4
916	F	45	T5	11	1.9	2.3
917	M	50	T4	21	2.6	3.9
918	M	52	T5	14	3.5	4.0
919	M	31	T6/7	4	3.1	3.5
921	M	48	T4	40	3.0	3.5
922	M	40	T5	7	2.8	3.2

Stimulation technique

Subjects attended the laboratory for one experimental day. They remained seated in their own wheelchairs for the experimental periods. The footplates of the chair were removed and the legs were allowed to hang freely, but were cushioned against possible

friction with parts of the chair by the use of a foam pad behind the calves. The apparatus for electrical muscle stimulation was the same as that used by Adams *et al.* (1984*a*), and that used in the previous chapter: it has been described in detail in Chapter 2. Two pairs of electrically-conducting pads were applied to the anterior surfaces of both thighs, positioned to stimulate the quadriceps muscles. The output of the stimulator was adjusted until contraction of the muscle belly was evident and produced a movement of the legs.

Although it was possible to elicit vigorous contractions, these were relatively short-lived and the muscles clearly fatigued relatively rapidly at these high stimulus strengths. If subjects were asked to sit in the chair described in the previous chapter, rather than in their own wheelchair, it was possible to obtain a lift of a 0.5 kg weight through a few centimetres, but this level of work could not be sustained for more than a few min. Since the purpose of this study was to obtain measurements of ventilation and acute hypoxic responses after more prolonged stimulation during a steady state, a lower stimulus strength was used, as a result of which the legs (hanging free from the wheelchair) were extended at the knee through a few centimetres against gravity by quadriceps contraction. Clearly, the useful external work acheived by this movement was very small, but it could be sustained.

Although subjects did not report any sensations from their legs or any feelings of "electricity", and although their legs were screened so that they could not see any movement, it became apparent during trial experiments that they could often guess correctly, when asked, that electrical leg stimulation was being employed. It was felt that these paraplegic subjects were very sensitive to vibrations through the wheelchair and to occasional rhythmic movements located to the lower part of the body. In order to control for any effect of this, therefore, a weight on the end of a pendulum was used to knock against and vibrate the wheelchair rhythmically during the protocols at rest. Subjects were than asked at the end of each experimental period whether they felt any electrical stimulation had been employed, and their answers were noted.

Measurement of ECG, blood pressure, O_2 saturation and control of end-tidal gases

Subjects breathed through a mouthpiece with their nose occluded. The end-tidal gases were controlled by dynamic end-tidal forcing, as described in Chapter 2. Heart rate was monitored by an ECG and arterial oxygen saturation by a finger pulse oximeter. In six subjects, blood pressure was monitored using a Finapres (Ohmeda, Denver, USA), and the readings during euoxia for each experimental period were noted.

Protocols

At the start of the experimental day, subjects undertook a preliminary study to determine their natural air-breathing end-tidal gas tensions at rest and during electrically-induced exercise. The end-tidal values for P_{CO2} (PET_{CO2}) for each subject breathing air at rest and during electrical exercise were obtained over a 10-15 min period, after familiarisation with the laboratory.

There were two substantive protocols: at rest, and during electrically-induced exercise (E_{EL}). Repeated experimental periods of each protocol were undertaken throughout the day, in random order, with periods of rest in between.

Each experimental period in each protocol consisted of an initial 2-3 min to allow a steady-state to be reached, after which data collection was begun. Thereafter, for E_{EL} in some subjects, it was possible to achieve electrically-induced leg exercise for 17 min, but in others each experimental period was of only 9 min duration (subjects 913, 916 and 921). It is interesting that this last group who were more prone to muscle fatigue included the two women, and also the subject who had been a paraplegic for forty years (Table 7.1). The duration of experimental periods at rest were matched with the duration achieved during E_{EL}, for each subject.

In the subjects who undertook experimental periods of 17 min, end-tidal P_{O2} was held at 100 Torr for the first 5 min, was reduced to 50 Torr for 3 min, then returned to 100 Torr for 5 min, followed by a second exposure to hypoxia of 50 Torr for 3 min. There was a final 1 min period of euoxia before the experimental period was ended. In the three subjects who undertook periods of 9 min duration, there was first a 5 min period

of euoxia (PET_{O2} 100 Torr), followed by only one 3 min exposure to hypoxia (PET_{O2} 50 Torr; 60 Torr in subject 913) and a final 1 min of euoxia. Throughout each protocol, PET_{CO2} was held 1-2 Torr above resting values in all subjects.

Subjects who undertook experimental periods of 17 min repeated each of the two protocols either 5 or 6 times. Subjects who undertook the 9 min periods completed each protocol 10 times. There were in total 128 separate experimental periods and 196 separate exposures to hypoxia.

A blood sample for determination of venous lactate (YSI 23L Enzymatic Lactate Analyser, Yellow Springs, Ohio) was taken before and after the experimental periods of electrical exercise (E_{EL}).

Data analysis

Data for $\dot{V}_E$, PET_{CO2}, PET_{O2}, $\dot{V}_{CO2}$ and $\dot{V}_{O2}$ for each experimental period were averaged over 1 min periods.

The last min of euoxia, before each hypoxic exposure, was taken as a measure of the "baseline" $\dot{V}_E$. For each subject, these values were combined to give the subject means for each protocol.

The AHR for each experimental period was calculated as the difference between the ventilation attained in the second min of hypoxia during each hypoxic exposure, and the last min of euoxia, before the hypoxic exposure. These values were then combined to give the mean AHR for each subject for each protocol.

Values of $\dot{V}_{O2}$ and $\dot{V}_{CO2}$ were averaged to give the mean for each experimental period, and these values were then averaged to give the relevant subject means for each protocol.

Statistical analysis

The significance of the differences between means of the variables from the group as a whole were assessed using a paired, two-tailed t-test (Bailey, 1985). Statistical significance for each variable was accepted at a value of $P < 0.05$.

RESULTS

From the preliminary protocol to determine end-tidal gas tensions, the mean ± SE end-tidal P_{CO2} at rest for the group as a whole was 37.9 ± 0.6 Torr (range 34.9 - 40.6 Torr). During E_{EL}, the mean PET_{CO2} was 38.3 ± 0.2 Torr (range 35.4 - 40.3 Torr). There was no statistically significant difference in PET_{CO2} between rest and E_{EL}.

Figure 7.1 shows the end-tidal gas profile for P_{CO2} and P_{O2} during dynamic end-tidal forcing. End-tidal values for P_{O2} were held steady in euoxia and reached desired levels rapidly in hypoxia. End-tidal P_{CO2} was held constant throughout the experiments, with occasional imperfections in the switches out of hypoxia.

Figure 7.2 shows the general form of the ventilatory responses during the resting and the E_{EL} protocols. Electrically-induced exercise did not greatly appear to increase the baseline euoxic ventilations as compared with rest, except possibly in one subject (921). However, in all subjects there appeared to be a small but consistent increase in the ventilatory response to hypoxia during E_{EL}, as compared with rest. Table 7.2 confirms these findings quantitatively.

Table 7.2 also shows the mean changes in $\dot{V}_{CO2}$ and $\dot{V}_{O2}$ achieved by E_{EL}. The overall effect of electrical stimulation was modest (mean $\Delta\dot{V}_{CO2}$ of 41 ml/min), but these changes in metabolism were consistent in all subjects and significant.

There was no significant rise in venous lactate after E_{EL} (Table 7.2), although in some subjects there appeared to be a small rise even at this relatively low work rate.

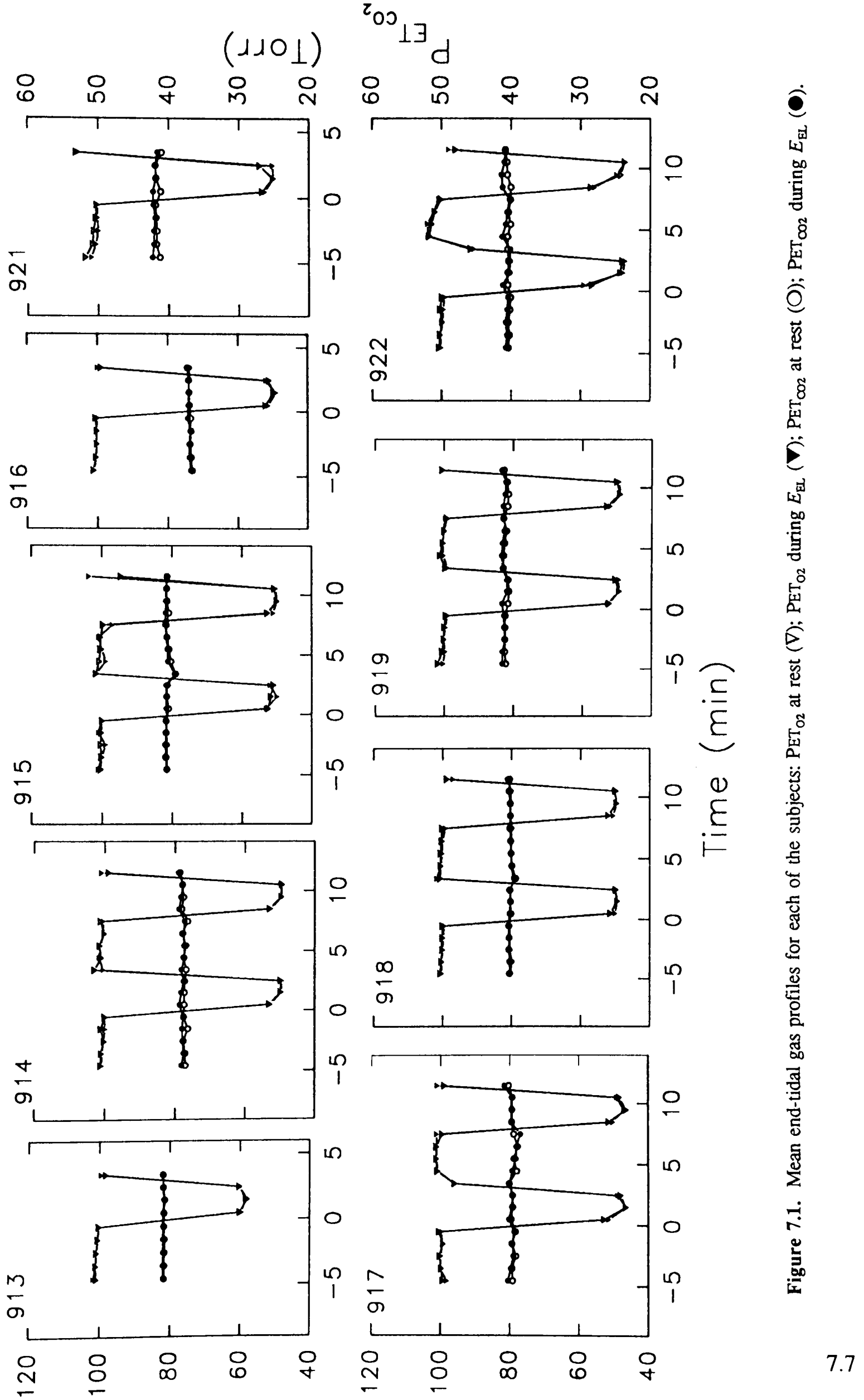


Figure 7.1. Mean end-tidal gas profiles for each of the subjects: $P_{ET_{O2}}$ at rest (▽); $P_{ET_{O2}}$ during E_{EL} (▼); $P_{ET_{CO2}}$ at rest (○); $P_{ET_{CO2}}$ during E_{EL} (●).

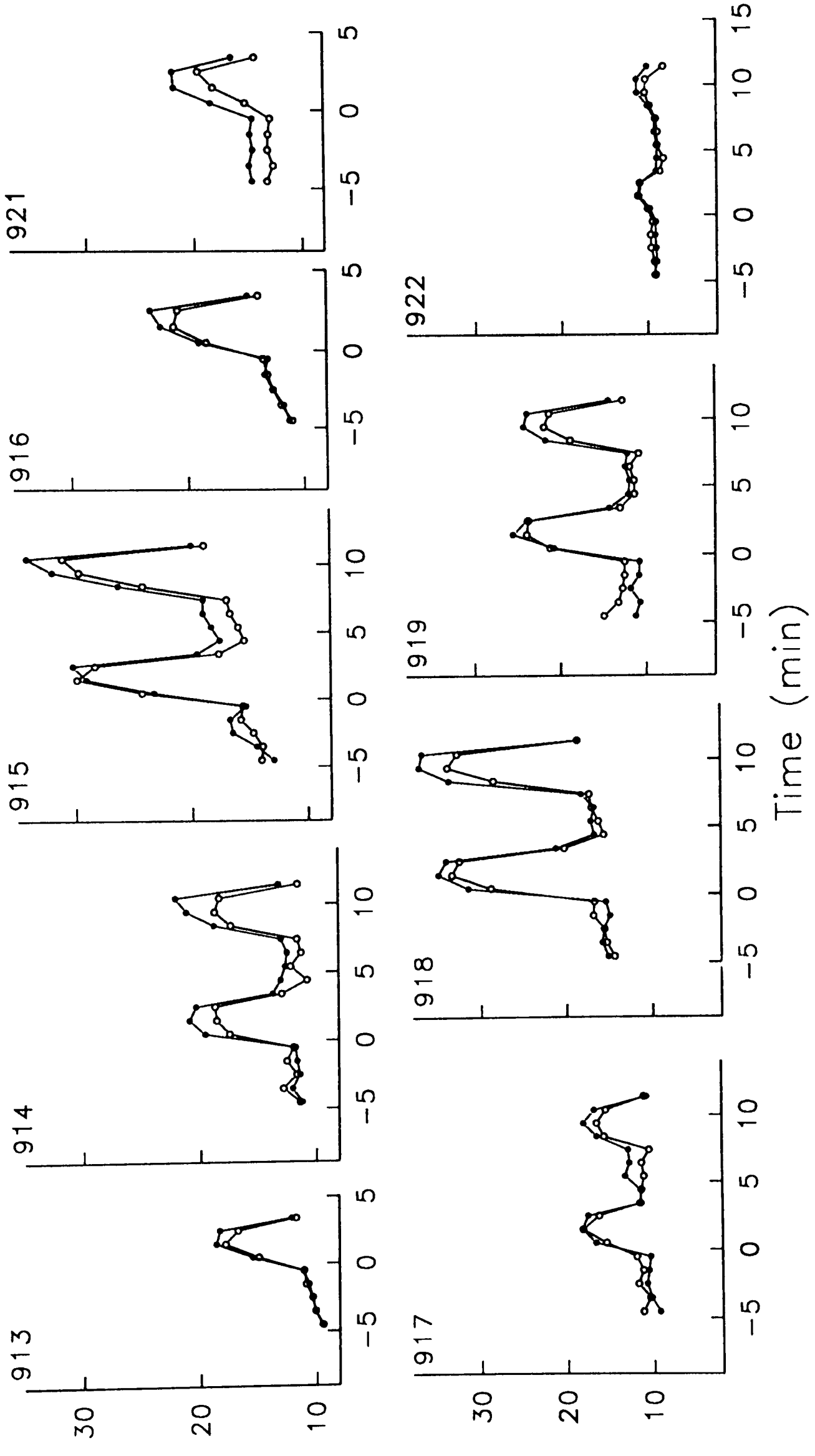


Figure 7.2. Mean ventilations against time for each of the subjects. Hypoxia from time = 0 to 3 min and, for subjects who were exposed to hypoxia again, from time = 8 to 11 min: rest (○); E_{EL} (●).

TABLE 7.2

Respiratory variables and lactate values for individual subjects and the mean for the group as a whole, for (a) rest and (b) the change with electrically-induced exercise (ΔE). Statistical comparisons are between the change with electrical stimulation and zero (* indicates $P < 0.001$; NS denotes no significant difference from zero; Student's paired-test). The number in brackets below each subject number indicates the separate experimental periods undertaken by each subject: subjects undertook either 10 periods of 9 min duration or 5 or 6 periods of 17 min duration.

	$\dot{V}_{CO2}$ (ml/min)		$\dot{V}_{O2}$ (ml/min)		Baseline $\dot{V}_E$ (L/min)		AHR (L/min)		Lactate (mmol/L)	
Subject	Rest	ΔE	Rest	ΔE	Rest	ΔE	Rest	ΔE	Rest	ΔE
913	143	21	187	18	11.0	-0.11	6.77	1.01	0.83	0.08
(10)	3	1	2	2	0.39	0.45	0.76	1.09	0.21	0.32
914	224	61	267	42	11.7	0.65	6.89	1.78	0.98	0.29
(6)	8	7	7	5	0.51	0.71	0.63	0.68	0.04	0.09
915	178	42	236	33	15.6	1.20	13.5	0.77	0.73	0.19
(6)	6	3	9	8	0.14	0.65	1.02	1.03	0.10	0.14
916	130	38	185	19	13.4	0.01	7.41	1.91	0.68	0.25
(10)	5	4	4	5	0.67	0.65	0.40	0.55	0.04	0.04
917	226	34	274	28	11.2	-0.20	6.15	1.47	0.95	-0.22
(5)	8	5	8	11	0.64	0.51	0.76	0.68	0.08	0.05
918	184	58	236	56	15.7	1.12	16.6	2.63	1.03	-0.18
(6)	4	6	5	7	2.16	1.57	0.44	0.79	0.03	0.04
919	183	32	226	25	11.9	-0.43	11.0	2.24	0.75	0.57
(6)	5	7	5	7	1.12	1.06	0.49	0.67	0.04	0.09
921	155	38	199	20	12.9	1.75	5.28	1.93	0.73	0.04
(10)	2	2	3	1	1.26	1.06	0.95	0.83	0.05	0.04
922	177	47	212	34	9.22	-0.16	1.46	0.72	0.80	0.04
(5)	6	5	3	5	0.27	0.35	0.28	0.36	0.10	0.10
Mean	178	41	225	31	12.5	0.31	8.34	1.61	0.83	0.12
	11	5	11	4	0.71	0.24	1.53	0.22	0.04	0.08
		*		*		NS		*		NS

Arterial blood pressure was measured in subjects 913, 914, 915, 918, 919 and 922, and there were no significant changes with E_{EL}. The systolic BP at rest in these six subjects was 132 ± 8 mm Hg (mean ± SE); during E_{EL} it was 132 ± 7 mm Hg. Mean diastolic BP was 93 ± 5 mm Hg at rest and 98 ± 2 mm Hg during E_{EL}.

Heart rate, measured during euoxia in all subjects, showed no change with E_{EL}. At rest, the mean rate was 77 ± 4/min; during E_{EL}, it was 72 ± 5/min.

When asked to guess the type of protocol they had undertaken, subjects found it difficult, and often felt that there might have been a short spell of electrical stimulation during *all* of the experimental periods. There were occasions, however, when they felt certain that a particular protocol had been fully "at rest" or "electrical". Subjects were marked as obtaining a correct answer if (a) they guessed the protocol accurately or (b) they felt there had been only a short spell of electrical stimulation in a protocol which had, in fact, consisted entirely of electrically-induced exercise. The results of the group responses were that, as a whole, subjects guessed correctly after only 43% of the runs: 57% of the guesses were incorrect. These figures show quantitatively that subjects could not distinguish E_{EL} accurately from rest.

DISCUSSION

Adams *et al.* (1984*a* and 1984*b*) and Brice *et al.* (1988*a* and 1988*b*) concluded from their studies that afferent input from the limbs and volitional control are unnecessary for the normal matching of ventilation to the metabolic CO_2 production which occurs during exercise. The main conclusion of this study is that reflex neural input from the limbs and volitional control are also unnecessary for the increase in the acute ventilatory response to hypoxia which occurs during steady exercise in humans.

It is pertinent to examine some of the characteristics of our group of paraplegic patients and compare their respiratory variables and responses with those found by previous studies.

Is E_{EL} in paraplegic subjects isocapnic?

Adams *et al.* (1984*b*) found that, relative to normal subjects, paraplegic subjects exhibited a lower resting $P_{ET_{CO2}}$, and they reported a mean of 35.8 Torr. During electrical stimulation, they observed a mean rise in $P_{ET_{CO2}}$ of 3.2 Torr, which might suggest that steady exercise in these patients was not isocapnic. However, they emphasised that these end-tidal values were extremely variable, and reported a smaller increase in arterial P_{CO2} of 1.7 Torr in three patients in whom it was measured.

In this study, the mean value for $P_{ET_{CO2}}$ of 37.9 Torr obtained at rest during air-breathing as part of the preliminary study cannot be regarded as particularly low, and there was no significant rise in $P_{ET_{CO2}}$ with E_{EL}. Our results are therefore more consistent with those of Brice *et al.* (1988*b*), who obtained a mean arterial P_{CO2} of about 39 Torr in paraplegic subjects; a value which remained constant during E_{EL}. We did not measure arterial P_{CO2} directly, but at the relatively low level of exercise during E_{EL}, and taken together with the results of Brice *et al.* (1988*b*), it is unlikely that large changes in the arterial/end-tidal gradient occurred during E_{EL} in this study. The fact that, during end-tidal forcing in the substantive study, the mean ventilation in euoxia was similar between rest and E_{EL} (Table 7.2), also suggests that the overall hypercapnic stimulus during E_{EL} was unlikely to have been greater than the hypercapnic stimulus at rest.

Resting metabolic rate and changes with E_{EL}

Resting O_2 consumption and CO_2 production in paraplegic subjects is relatively low (Table 7.2). This has been reported previously by Adams *et al.* (1984*b*) and Brice *et al.* (1988*b*). It is interesting that in this study, the lowest values of $\dot{V}_{CO2}$ were obtained in those subjects with the smallest muscle bulk, namely the two female subjects and the male who had been a paraplegic for 40 years (Table 7.2).

The change in metabolism produced by E_{EL} in this study was relatively modest (41 ml/min; Table 7.2). This rise is consistent with previous reports of the increase in $\dot{V}_{CO2}$ obtained in paraplegic subjects who have not undergone prior training with electrical stimulation. Brown *et al.* (1990) found that at the start of a training period, the rise in $\dot{V}_{CO2}$ with stimulation averaged 36 ml/min: after 8 weeks, this value had risen to 118 ml/min, but two of their subjects still showed increases of only 50 ml/min.

Both Adams *et al.* (1984*b*) and Brice *et al.* (1988*b*) used electrical stimulation for a few min at a time and did obtain much larger increases in $\dot{V}_{CO2}$ (mean of 172 and 196 ml/min respectively). However, this degree of exercise produced a moderate acidosis in both of these studies. Adams *et al.* (1984*b*) measured arterial pH in three subjects and found a mean fall of 0.028 units with E_{EL}; Brice *et al.* (1988*b*) reported a fall in pH of 0.025 units and a rise in lactate to about 2.3 mmol/L after E_{EL}. This problem has been avoided to a large extent in our study, and we have been able to achieve more prolonged stimulation and to measure the steady-state hypoxic responses.

Changes in baseline euoxic ventilation with E_{EL}

Even if the degree of acidosis obtained in the study of Adams *et al.* (1984*b*) is ignored, their data predict a mean rise in baseline ventilation of, at most, about 1 L/min for a rise in $\dot{V}_{CO2}$ of 41 ml/min in paraplegic subjects. Although this value was exceeded in three subjects in this study, the mean rise of 0.31 L/min did not reach statistical significance (Table 7.2).

Changes in the acute hypoxic response with E_{EL}: Mechanisms

The main purpose of this study was to assess the effect of E_{EL} on the acute hypoxic response in subjects who have no known functional nervous connections between the working limbs and the brainstem. Table 7.2 confirms that the sensitivity to hypoxia is increased in the absence of such reflex neural input. Furthermore, we are satisfied that in this group of patients there was neither a volitional drive to exercise, nor was there any accurate sensation of electrically-induced leg exercise.

Which other drives are involved? The results are consistent with the notion that humoral factors released by the stimulated muscles increase the sensitivity of the peripheral chemoreflex to hypoxia. The study does not indicate, however, which precise factor is involved.

While it is also possible that the cardiac output might have increased and constituted a "cardiodynamic" drive, large changes in cardiac output would have been relatively unlikely in the absence of any changes in heart rate and blood pressure from rest to E_{EL} in these subjects. Banner *et al.* (1988) have shown that the ventilatory response to electrically-induced leg exercise is normal in patients after heart-lung transplantation, even though increases in cardiac output were minimal compared to normal controls, thus questioning the role of cardiodynamic drive in this type of exercise. Furthermore, while some studies have suggested that increases in cardiac output may stimulate ventilation (Wasserman *et al.*, 1974), we are unaware of any study which shows that cardiodynamic drive increases the ventilatory response to hypoxia.

Changes in the acute hypoxic response with E_{EL}: Comparison with normal subjects

This study extends the work in the previous chapter on the effects of E_{EL} on the AHR in normal, able-bodied subjects. Despite a number of control protocols, the possibility that the sensation of electrical stimulation in normal subjects affects the AHR could not be excluded entirely. While the present study lends further support to the notion that this was not the case, it is also possible to make a more quantitative comparison between the two studies.

In particular, it is interesting to compare the magnitudes of AHR during protocol E_{V1} of the previous chapter with E_{EL} in paraplegic subjects. During E_{V1}, subjects exercised voluntarily at an external work rate of about 4 W (matched to the external work rate of their electrically-induced exercise protocol). This resulted in a mean rise in $\dot{V}_{CO2}$ of 53 ml/min and an associated increase in the AHR of 2 L/min. In this study, E_{EL} in paraplegic subjects increased $\dot{V}_{CO2}$ by 41 ml/min, and the consequent increase in AHR was 1.6 L/min. These figures are very similar. Figure 7.3 shows the result of taking the *change* in AHR as compared with rest obtained for each of the protocols (E_{EL} in paraplegics and normals, and E_{V1} and E_{V2} in normals), and dividing it by the *change* in $\dot{V}_{CO2}$ from rest achieved during the appropriate protocol. It appears that, irrespective of the clinical state of the subject (*e.g*, paraplegic or able-bodied) or the type of exercise undertaken (*e.g*, electrically-induced or voluntary), this ratio remains relatively constant.

If it is accepted that changes in AHR during E_{EL} in paraplegic subjects are mediated only by humoral factors, it appears that the measured increase in hypoxic sensitivity in normal humans can be wholly accounted for by the increase in $\dot{V}_{CO2}$ which occurs during the exercise, or by factors closely and quantitatively related to $\dot{V}_{CO2}$. This result therefore complements the work of Adams *et al.* (1984*a* and 1984*b*) and Brice *et al.* (1988*a* and 1988*b*) who reached a similar conclusion regarding the control of steady-state ventilation during exercise.

While other factors such as volitional drive and neural afferents may play a part during normal exercise, their involvement does not appear to be critical in achieving the observed increase in chemoreflex sensitivity to hypoxia.

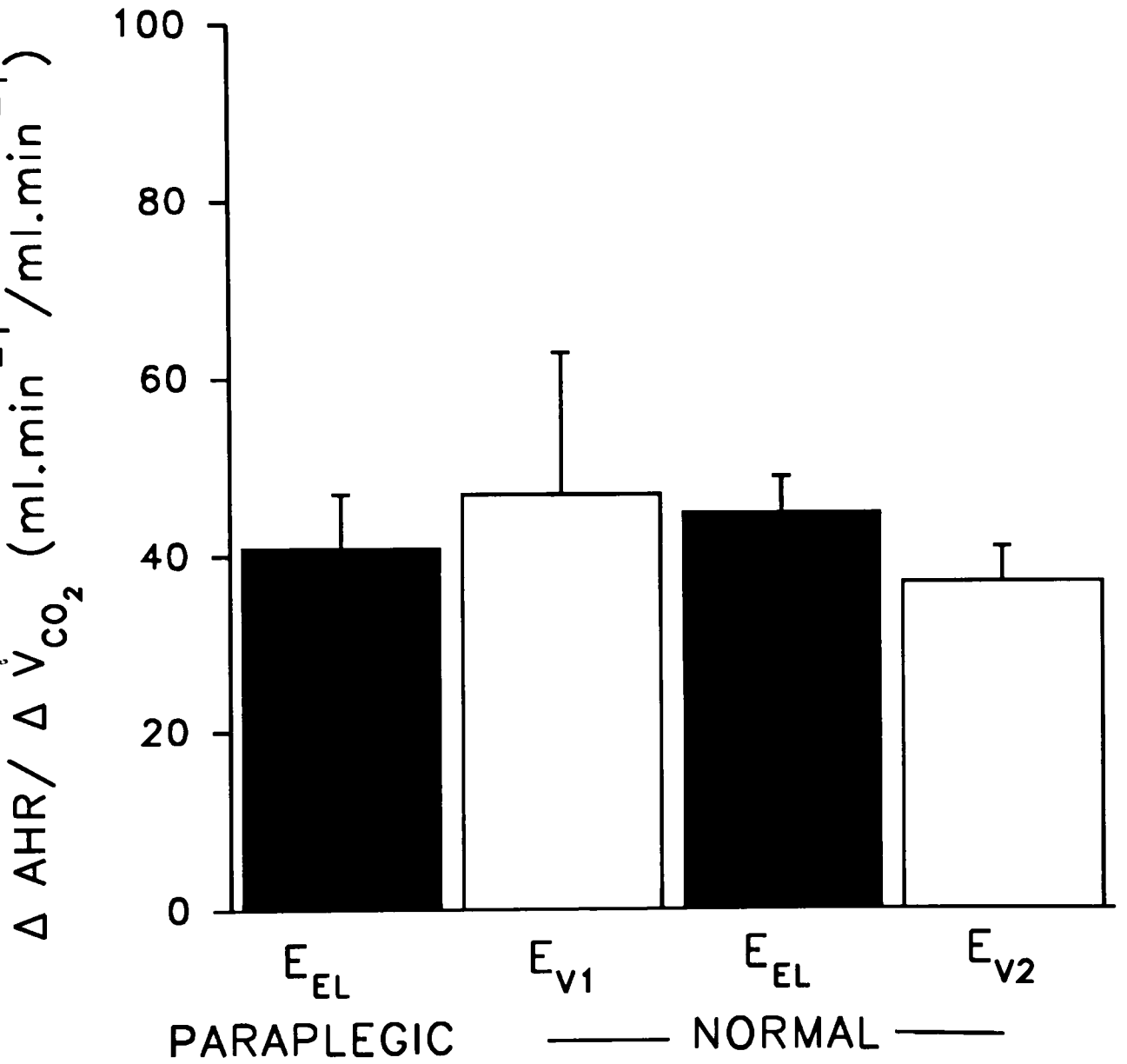


Figure 7.3. The change in AHR (in ml/min) from the AHR at rest divided by the increase in $\dot{V}_{CO2}$ (in ml/min) from rest, for each of the exercise protocols undertaken by (a) paraplegic and (b) normal subjects. Data for paraplegic subjects were obtained from Table 7.2; data for normal subjects were obtained from Tables 6.4 and 6.5 and Appendix 6.1 of the previous chapter. Error bars show + 1 SE, calculated from individual subject ratios. Analysis of variance does not show any significant differences between the mean values for the four protocols.

CHAPTER 8

CONCLUDING REMARKS

Your councils are long.

Your doings are slow.

Consider the end.

Brutus: letter to the Samians,

Plutarch's "Life of Brutus"

This chapter summarises the important results of this thesis and discusses some possibilities for future work.

Hypoxic Ventilatory Decline During Exercise

The results of Chapters 3 and 4 may be summarised as:

1. the magnitude of HVD, in absolute terms, tends to be reduced during hypoxic exercise, as compared with rest.

2. the magnitude of HVD, when expressed as a fraction of the AHR, is attenuated during exercise, as compared with rest.

3. the results are consistent with the notion that in conscious man, HVD arises primarily from a time-dependent decline in peripheral chemoreflex sensitivity to hypoxia.

4. taken together with the results of previous work, a model of the hypoxic chemoreflex is proposed in which the mechanisms underlying HVD are functionally distinct (and so can be modulated independently) from the mechanisms underlying the AHR.

There are a number of possible ways in which future work could follow on from this, but two experiments would, I think, be particularly interesting.

Interaction of HVD and humoral factors involved in exercise hyperpnoea

While the interactions of AHR, HVD and a number of exogenous agents has been investigated, none of these agents to date has been a substance implicated as a natural humoral factor involved in exercise hyperpnoea. The studies may have to be performed in animals (and in animals whose mechanism of HVD is a peripheral one, such as the rabbit or piglet), but it would be of interest (a) to assess whether induced exercise attenuates the magnitude of HVD expressed as a fraction of AHR, as it does in man, and (b) to assess whether infusion of potassium, or catecholamines, or venous CO_2 loading in the absence of exercise achieves a similar result. This may show which factor, if any, is able to resemble the effects of exercise on ventilation, AHR *and* HVD.

Chemically-assisted ventilation in patients with ventilatory failure

Patients with ventilatory failure in whom mechanically-assisted ventilation is contra-indicated are often treated with either almitrine or doxapram. Both these drugs increase the ventilatory response to hypoxia through an action on the peripheral chemoreceptors (Georgopoulos *et al.*, 1989*c*; Honda *et al.*, 1979), and the rationale for their use is that they increase the drive to breathe in hypoxic patients. It is clear, though, from clinical experience that these drugs do not always work. The possible reasons for this include inappropriate commencement of therapy (either too early or too late in the illness), inappropriate dosage (either too high or too low), and the fact that often, patients are simply too severely ill to respond.

However, the phenomenon of HVD might also be an important limiting factor. Almitrine appears to be perceived at the carotid body as "extra hypoxia" (Nye *et al.*, 1990). The consequence of extra hypoxia and of exogenous almitrine, as discussed, is an increase in HVD (Georgopoulos *et al.*, 1989*c*; Bascom *et al.*, 1992). Doxapram also appears to stimulate the carotid body in a manner very similar to extra hypoxia (Mitchell and Herbert, 1975), and a secondary decline in ventilation after doxapram has been reported (Burki, 1984; Okubo *et al.*, 1988).

If HVD is a significant limiting factor in the use of these drugs, it would be desirable to use a drug which, while increasing the response to hypoxia, minimises the HVD: in other words, a drug which acts like exercise. The similarity between exercise and domperidone on the relationship between AHR and HVD was noted in Chapter 4. The problem with domperidone is that, quantitatively, it does not greatly increase the AHR. However, the model described on page 3.15 suggests a solution: if the mechanisms underlying AHR and HVD are truly independent, then a *combination* of almitrine or doxapram *and* domperidone may increase the AHR significantly, while minimising the magnitude of any subsequent decline in ventilation. It would be of interest to assess the effects of this combination first in healthy volunteers, and then if successful, in patients.

Sustained Hypercapnia During Exercise

The results of Chapter 5 may be summarised as:

1. during prolonged air-breathing exercise with no added CO_2, the ventilation remains constant, while the respiratory quotient, $\dot{V}_{CO2}$ and PET_{CO2} decline. This result suggests that situations may arise when the ventilation is independent of CO_2 flux in man.

2. sustained, mild hypercapnia progressively stimulates ventilation during exercise at levels lower than previous studies have shown it to stimulate ventilation progressively at rest.

3. a part of this effect may be explained by the gas-mixing system increasing inspired P_{CO2} to hold PET_{CO2} constant, while the subject's natural PET_{CO2} tends to decline.

Two areas for future work are:

Changes in chemosensitivity during sustained hypercapnia

Whether chemoreflex activity changes during the progressive rise in ventilation is unclear, and it might be informative to assess the ventilatory responses to pulses of hypoxia and extra hypercapnia *during* a sustained exposure to hypercapnia at rest, and during exercise.

Changes in arterial blood-gas and chemical composition during prolonged exercise

It would be important to confirm that the decline in end-tidal P_{CO2} observed during prolonged air-breathing exercise does truly reflect a decline in arterial P_{CO2}. The data of Wasserman *et al.* (1967) appear to show a small but non-significant trend downwards in Pa_{CO2} during prolonged exercise. However, their results were somewhat unusual in that they did not find any changes in the respiratory quotient during prolonged exercise. The use of arterial sampling would also allow changes in humoral factors to be assessed, which might account for an extra stimulus to breathe as the P_{CO2} declines. In particular, any changes occurring in arterial plasma potassium would be of interest.

Electrically-Induced Exercise

The results of Chapters 6 and 7 can be summarised as:

1. the voluntary control of exercise is unnecessary for the increase in hypoxic chemoreflex sensitivity which normally occurs during exercise.

2. reflex neural control of exercise is also unnecessary for the increased acute hypoxic response.

3. quantitatively, the increase in acute hypoxic response which occurs during voluntary exercise in normal subjects can be fully accounted for by the rise which occurs in $\dot{V}_{CO2}$, or by factors closely related to it.

In further studies, arterial sampling for potassium, catecholamines or even blood-gas oscillations during electrically-induced exercise might indicate possible humoral mediators of the ventilatory response. The results of using pressure cuffs around the thighs during electrical muscle stimulation in paraplegics might be useful: the prediction would be that cuff inflation would abolish the ventilatory responses and also attenuate changes in putative humoral factors sampled in the arterial blood. Brown *et al.* (1990) have attempted cuff inflation experiments in paraplegics, but did not sample for chemicals other than arterial P_{CO2} and did not perform control experiments to assess the ventilatory effects of cuff inflation alone.

The interpretation of results from such experiments, however, is often limited by the principle of redundancy. As discussed in the Introduction, experiments have shown that it is possible to eliminate the influence of apparently vital components of the respiratory control system, and yet obtain a normal ventilatory response to exercise. In humans at least, there is no single factor which might be considered the *sine qua non* of the ventilatory response. Further studies will undoubtedly teach us more about the control system, but the overall conclusion may simply be that when an action or a response is necessary for life, the body has evolved several ways to accomplish it properly.

CHAPTER 9

REFERENCES

If we take in our hand any volume, let us ask of it:

Does it contain any abstract reasoning concerning quantity?

Does it contain any experimental reasoning concerning existence?

David Hume

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