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FEEDBACK CONTROL MECHANISMS REGULATING BREATHING IN HUMANS

by

Claudette Marie St. Croix

Faculty of Kinesiology

Submitted in partial fulfilment
of the requirements for the degree of
Doctor of Philosophy

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ABSTRACT

The respiratory system regulates alveolar ventilation ($\dot{V}_A$) almost exactly to the demands of the body so that the PCO_2 and PO_2 of the arterial blood are hardly altered, even during strenuous exercise or other types of respiratory stress. The feedback control of ventilation was studied in human subjects using the technique of dynamic end-tidal forcings to produce perturbations in end-tidal PCO_2 ($P_{ET}CO_2$) and end-tidal PO_2 ($P_{ET}O_2$) to stimulate the respiratory chemoreceptors.

The purpose of the first study was to investigate the interaction between ventilatory drives from the central (cR_c) and peripheral (pR_c) chemoreceptors using their different speeds of response to enable a temporal separation of their chemical stimulation. It was demonstrated that the chemoreflexes were independent of each other, confirming that hypoxia and the CO_2-H^+ complex interact at the level of the pR_c, and the drives from the periphery and from the central chemosensitive area add together in their effects on ventilation.

The objective of the second study was to examine the contribution of the pR_c to ventilation during the steady state of moderate intensity exercise, using hyperoxic suppression of pR_c drive, while stabilizing the drive at the cR_c by maintaining a constant $P_{ET}CO_2$. The results revealed that the peripheral chemoreceptors were responsible for 15% of the ventilatory drive during moderate intensity exercise. This modest contribution supports the theory that the arterial chemoreceptors function to "fine tune" $\dot{V}_A$ to minimize change in arterial blood gases. Sustained hyperoxia, however, appeared

to lower the set point about which P_aCO_2 was regulated.

The technique of dynamic end-tidal forcings is based on the assumption that changes in $P_{ET}CO_2$ mirror changes in P_aCO_2. The objective of the final study was to compare arterial PCO_2 (P_aCO_2), determined directly in the radial artery, with indirect estimates of P_aCO_2 derived from arterialized-venous blood ($P_{av}CO_2$) and from the respired gases. Mean ($P_{av}CO_2$) agreed most closely with mean P_aCO_2 at rest and in exercise. A significant $P_{ET}CO_2$ to P_aCO_2 difference, positively correlated with the level of inspired PCO_2, was found under both resting and exercise conditions. Of the noninvasive techniques, mean estimates calculated using the regression equation developed by Jones et al. *(J. Appl. Physiol., 1979)* corresponded most closely with P_aCO_2 in exercise.

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CHAPTER 1

INTRODUCTION

The human body has literally thousands of systems that control all life processes. The chemical regulatory mechanisms adjust ventilation in such a way that the arterial partial pressure of CO_2 (P_aCO_2) is normally held constant, the effects of excess hydrogen ion (H^+) in the blood are minimized, and the arterial partial pressure of O_2 (PO_2) is raised when it falls to a potentially dangerous level.

1.1 Thesis Outline

This thesis examines the feedback control of ventilation in human subjects using the technique of dynamic end-tidal forcing to produce perturbations in end-tidal PCO_2 ($P_{ET}CO_2$) and end-tidal PO_2 ($P_{ET}O_2$) to stimulate the respiratory chemoreceptors. Chapter One serves as a general introduction and reviews (1) the classical experiments upon which most of the current theories of respiratory control are based, (2) the chemoreceptors, (3) the feedback loop, (4) the interaction between feedback stimuli and chemoreceptor drives, (5) the peripheral chemoreflex, and (6) the feedback control of exercise ventilation. Chapters Two, Three, and Four are each organized as research studies and include a brief Introduction, Methods, Results and Discussion. References have been grouped at the end of the thesis, and attempts have been made to limit the explanation of methods common to more than one study.

In the first study (Chapter Two), the nature of the interaction between the central

and peripheral ventilatory chemoreflex loops in human subjects will be examined using the differing speeds of response of the central and peripheral chemoreceptors to enable a temporal separation of their chemical stimulation.

In the second study (Chapter Three), the contribution of the peripheral chemoreflex loop to steady state ventilation will be investigated in more detail by the examination of the ventilatory response to a brief period of sustained hyperoxia. The effects of sustained hyperoxia on respiratory control will be discussed with reference to the set point theory of alveolar PCO_2 regulation.

The third study is an examination of the methodologies employed to estimate arterial PCO_2 in studies of respiratory control. The implications of the assumption that changes in end-tidal PCO_2 ($P_{ET}CO_2$) mirror changes in arterial PCO_2 will be discussed.

The findings of the three studies will be summarized in Chapter 5, and recommendations will be made for future research.

1.2 Historical Landmarks: Feedback Control of Ventilation

Haldane and Priestley (1905) were the first to effectively describe a feedback loop between chemical stimuli and ventilation. They noted that alveolar PCO_2 (P_ACO_2) was regulated by lung-ventilation and demonstrated that changes in P_ACO_2, induced by varying the inspired fraction of CO_2, affected alveolar ventilation ($\dot{V}_A$). It had been recognized that chemical stimuli could act on the respiratory system since Pflüger, in 1868, showed that breathing either N_2, or a mixture of CO_2 and O_2, stimulated ventilation in dogs (cited in Cunningham, Robbins and Wolff, 1986). Miescher-Rüsch (1885),

however, was the first to demonstrate quantitatively, in human subjects, that CO_2 was a more important humoral stimulus than oxygen in regulating breathing (cited in Cunningham et al., 1986). The third major chemical stimulus to respiration was identified by Walter (1877), who showed that the administration of intravenous injections of an acid solution caused hyperventilation. Winterstein (1911; 1921; 1956), based on these early discoveries and his work with newborn rabbits, attributed the extent of lung ventilation to the H^+ ion concentration within the respiratory centres, asserting that CO_2 acts to stimulate breathing through its property as an acid in water.

The cross circulation experiments of Frédéricq in 1901 (cited in Bledsoe & Hornbein, 1981) were the first to place the site of chemosensitivity in the head. Initial attempts to localize chemosensor sites in the central nervous system focused on the region of the medullary respiratory centre with little success (Bledsoe and Hornbein, 1981). By the early 1950's, it was felt that the site of central ventilatory chemosensitivity was a separate system from the brainstem respiratory integrating centres. The experiments of Leusen (1954a; 1954b) in anaesthetized dogs, in which respiration was affected by changing the CO_2 and H^+ ion concentrations of bicarbonate solutions perfusing the brain, suggested that the central chemoreceptors might be superficially located. The Böchum group, were the first to localize the site of central chemosensitivity to the ventral surface of the medulla (Mitchell et al., 1963a; Mitchell et al., 1963b).

It was not recognized until the 1930's that there were separate chemoreceptors outside the central nervous system. Heymans and colleagues (1930) found that the carotid body, a structure in the region of the carotid sinus, contained sensors that

transmitted information regarding the chemical composition of the blood, to the respiratory centres of the brain. Comroe (1939) described a second area of peripheral ventilatory chemosensitivity located at the aortic arch. The ventilatory responses to hypoxia were found to be mediated solely via these two sets of peripheral chemosensors (Comroe, 1939; Heymans et al., 1930).

1.3 The Chemoreceptors

The whole reflex arc of a respiratory chemoreflex, as defined by Dejours (1962), is formed by: the chemoreceptors, the afferent fibres to the respiratory centres, the respiratory centres, and the motor pathway to the thorax-lung apparatus. The information from the chemoreceptors provokes a reflex change in ventilation.

The peripheral chemoreceptors are stimulated by metabolic acidosis (Hornbein et al., 1961) and hypercapnia (Heeringa et al., 1979), as well as by decreases in the partial pressure of O_2 in the arterial blood (Heymans, 1951). The gas tensions in the carotid and aortic bodies closely approximate arterial blood gas values because the chemosensors are located near the large arteries, and are perfused at very high flow rates (Fitzgerald and Lahiri, 1986). In humans, most of the ventilatory effects of peripheral chemoreceptor stimulation are attributed to the carotid bodies, and generally little influence is attributed to the aortic bodies, based on the lack of ventilatory response to acute hypoxemia following carotid endarterectomy (Holton and Wood, 1965; Swanson et al., 1978; Wade et al., 1970). The possibility has been raised, however, that a weak component of the hypoxic response may manifest under conditions of simultaneous

hypercapnia (Whipp and Wasserman, 1980).

The central chemoreceptors are not sensitive to decreases in arterial O_2 tension (P_aO_2) and respond more slowly to changes in arterial pH than the peripheral chemoreceptors, but are acutely sensitive to increases in P_aCO_2. The central chemosensitive tissue is separated from blood by the blood-brain barrier. Carbon dioxide readily penetrates the blood-brain barrier, whereas H^+ and HCO_3^- penetrate poorly. It is generally believed that molecular CO_2 has no direct excitatory action on breathing, but that CO_2 acts on the central chemosensitive tissue via the associated H^+ concentration (Bruce and Cherniack, 1987). The stimulus due to changes in P_ACO_2 is therefore often referred to as the CO_2-H^+ complex. When P_aCO_2 rises so too does the CO_2 in the interstitial fluid of the medulla, which immediately reacts with water to form hydrogen ions. When surface electrodes were used in animals to measure pH changes on the ventral medullary surface, it was observed that for a given change in surface H^+ concentration, inhaled CO_2 had a much greater effect on ventilation than did metabolic acidosis or acid perfusion of the cerebrospinal fluid (Eldridge et al., 1984; Shams, 1985). Therefore, because CO_2 crosses the blood-brain barrier more readily than H^+, for a given change in blood H^+ concentration, CO_2 is a more potent ventilatory stimulus than an equivalent acute metabolic acidosis (Bruce and Cherniack, 1987).

1.4 The Feedback Loop

In the chemical regulation of ventilation, the input to the respiratory system is measured as alveolar gas partial pressures and the output as ventilation. There is an

effectively linear relation between ventilation ($\dot{V}$) and P_ACO_2 over a range that extends some 20 Torr upwards from the resting value of 40 Torr. At higher P_ACO_2, $\dot{V}$ tails off and narcosis intervenes. In hypocapnia, the slope of the relation is not different from zero. Over the linear range, the relation is expressed by the open-loop control equation:

$$\dot{V} = S(P_ACO_2 - B) \qquad (1)$$

where S is the sensitivity of the system and B, the intercept of the line produced to the PCO_2 axis (Lloyd et al., 1958). The curve is displaced to the left in acidemia and to the right in alkalemia, with no change in slope (Lloyd, 1963).

The ventilatory response to hypoxia is an inverse function of P_AO_2. Lloyd, Jukes and Cunningham (1958) expressed the relationship with the hyperbolic function:

$$R = R_0 [1 + A/(P_AO_2 - C)] \qquad (2)$$

where R_0 is the response in the absence of hypoxia, A is a hypoxic sensitivity parameter, and C is an asymptotic value of PO_2. Changing the alveolar PO_2 from the normal value of slightly more than 100 Torr down to 60 Torr has little effect on ventilation, but as the PO_2 falls below this alveolar ventilation begins to increase dramatically.

The feedback loop is formed by the controller relations (the effects of the feedback stimuli on ventilation, or equations 1 and 2) and the effect ventilation itself has on the feedback stimuli. In this context, $\dot{V}_A$ is the independent variable, and the gas pressures the dependent variables. The effect is determined by the conservation of matter resulting in the following equations:

$$P_ACO_2 - P_iCO_2 = \dot{V}CO_2/\dot{V}_A \qquad (3)$$

and

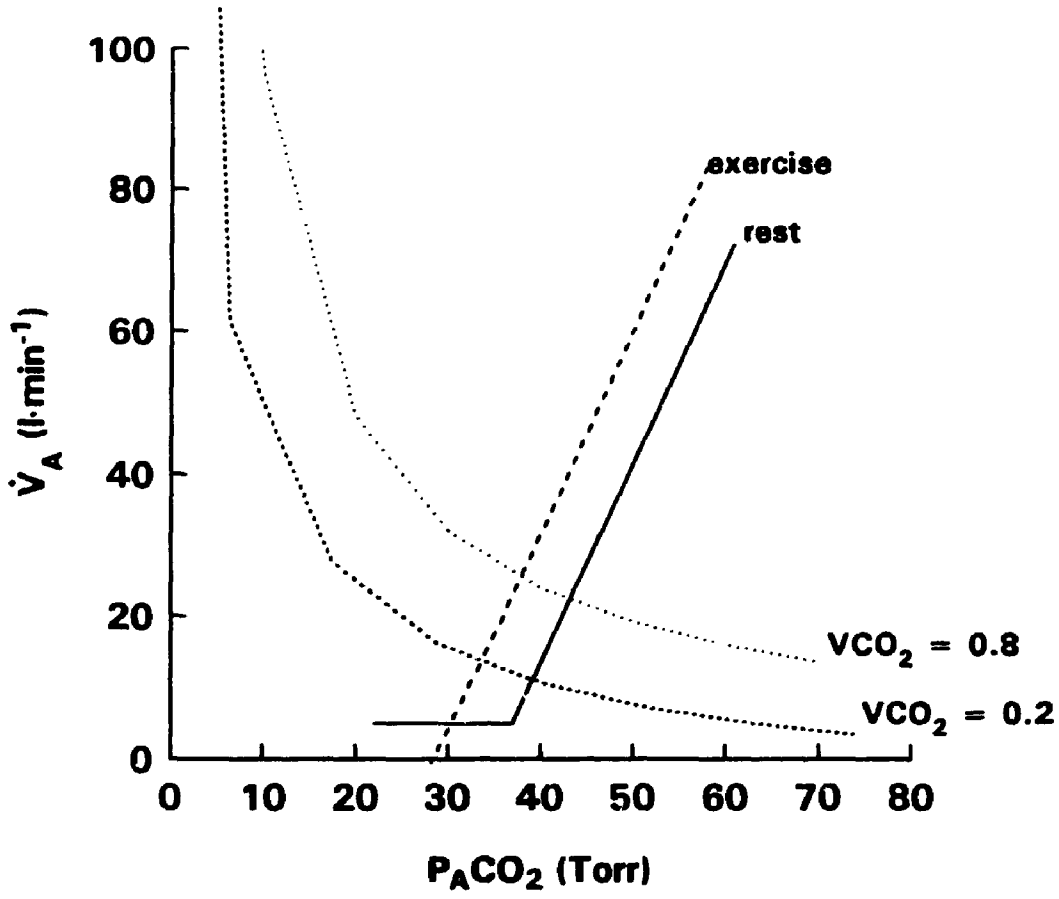
$$P_AO_2 - P_iO_2 = \dot{V}O_2/\dot{V}_A \quad (4)$$

where P_ACO_2 and P_AO_2 are the alveolar partial pressures of CO_2 and O_2 respectively, P_iCO_2 and P_iO_2 are the inspiratory CO_2 and O_2 partial pressures (P_iCO_2 is negligible unless CO_2 is inhaled), $\dot{V}CO_2$ is the rate of CO_2 production, $\dot{V}O_2$ is the rate of oxygen consumption, and $\dot{V}_A$ is the alveolar ventilation. This relation is known as the metabolic hyperbola. At any constant $\dot{V}CO_2$ or $\dot{V}O_2$, $\dot{V}_A$ and (P_ACO_2 - P_iCO_2) or $\dot{V}_A$ and (P_AO_2 - P_iO_2) are inversely related and the relationship represents the effects that $\dot{V}_A$ has on the stimuli (Cunningham et al., 1986).

In the steady state, both the controller equations and the metabolic hyperbola must be satisfied simultaneously (Cunningham et al., 1986). The hyperbola corresponding to the current $\dot{V}CO_2$, or $\dot{V}O_2$, expresses all possible pairs of values of $\dot{V}_A$ and P_ACO_2, or $\dot{V}_A$ and P_AO_2, and the appropriate controller line determines which pair of values satisfies the stimulus-response characteristics. The intersection of the two lines gives the unique stable point for the given conditions, therefore determining the set point for CO_2 regulation (Figure 1).

Figure 1

The feedback loop relating $\dot{V}_A$ and P_ACO_2. The controller relation (the effect of changing P_ACO_2 on $\dot{V}_A$) is shown at rest (solid line) and in exercise (dashed line). The metabolic hyperbola for CO_2, which describes the effect of varying $\dot{V}_A$ on P_ACO_2, is shown at a resting $\dot{V}CO_2$ of 0.2 $l \cdot min^{-1}$ and an exercise $\dot{V}CO_2$ of 0.8 $l \cdot min^{-1}$. The intersections of the metabolic hyperbolas with the controller relations are the points that uniquely satisfy both relations (Cunningham et al., 1986).


100
80
60
40
20
0
$\dot{V}_A$ (l·min^{-1})
exercise
rest
VCO_2 = 0.8
VCO_2 = 0.2
0
10
20
30
40
50
60
70
80
P_ACO_2 (Torr)

1.5 Interaction of Drives

It has been recognized since the work done by Neilsen and Smith (1952) that the drives due to hypoxia and hypercapnia interact multiplicatively in their effects on breathing. The slope of the curve relating PCO_2 to ventilation increases markedly as the PO_2 decreases, with no change in the threshold value of CO_2 at various degrees of hypoxia (Lloyd et al., 1958; Miller et al., 1974; Nielsen and Smith, 1952). Gabel and Weiskopf (1975) demonstrated that the ventilatory interaction between acute hypoxia and hypercapnia is mediated through increased H^+ concentration in man by systematically varying the PCO_2 and H^+ concentration at the peripheral and at the central chemoreceptors.

Early studies by Hornbein and colleagues (1961), which examined the magnitude of the electrical activity in the carotid chemoreceptors, in the cat, in response to changes in arterial PCO_2 and PO_2, established that multiplicative interaction between hypoxic and hypercapnic drives occurred peripherally. The possibility that there was also a degree of central interaction, however could not be eliminated.

Lahiri and Delaney (1975a) simultaneously recorded the mean activity of carotid chemoreceptors (single and multi-fibre preparations) and ventilation, in response to the combined stimuli of hypoxia and hypercapnia in anaesthetized cats. The results confirmed previous observations that the stimulus interaction at the carotid chemoreceptors consisted of multiplicative interaction in the activity of a single afferent fibre (Lahiri and DeLaney, 1975b). The interaction at the carotid bodies, however, did not account for all of the ventilatory effect of changing P_aCO_2 and P_aO_2, leading to the

suggestion that a multiplicative interaction between the activity of peripheral chemoreceptors and central CO_2 excitation might play a role in the regulation of ventilation. In further support for a degree of central interaction, Schlaefke and colleagues (1979) reported that blocking the activity of the central chemosensitive tissue by thermocoagulation of the intermediate area (area S) of the ventral medullary surface, was followed by an approximate halving of the response to hypoxia, and the loss of all interaction between hypoxia and hypercapnia, in the cat. The elimination of the S area did not eliminate direct peripheral chemoreceptor pathways, however central chemosensitivity seemed to be essential to amplify responses to hypoxia and hypercapnia mediated by peripheral chemoreceptors.

Kao and Mei (1978) separated the environments of the central and peripheral chemoreceptors in anaesthetized dogs, by perfusing the isolated carotid region with blood from a donor dog. They found that the $\dot{V}_E$-P_ACO_2 response lines at high and low P_AO_2 were in parallel, whereas in anaesthetized control animals, the response patterns formed a fan of lines. These results suggested that, in anaesthetized dogs, the full multiplicative interaction between hypercapnic and hypoxic stimuli occurs centrally, with no peripheral interaction.

Researchers at the University of Leiden used the artificial brainstem perfusion technique to physically separate the drives from the central and peripheral chemosensitive systems in the anaesthetized cat (Berkenbosch et al., 1979; DeGoede et al., 1985; Van Beek et al., 1983). The technique made possible an experimental analysis of the relations between the overall output ($\dot{V}$) and the four input variables, peripheral P_aCO_2,

peripheral P_aO_2, central P_aCO_2, and central P_aO_2. The studies demonstrated conclusively that the interaction between peripheral and central inputs are additive, and that the interaction between hypoxia and CO_2 originates at the arterial chemoreceptors in cats. Inferences regarding stimulus interactions in the awake human, based on these studies, are complicated by the possible effects of species differences and anaesthesia on the pattern of response.

The main problem in determining the exact nature of the interaction between respiratory feedback stimuli in humans subjects, is the difficulty in changing the peripheral CO_2-H^+ drive without affecting the drive due to CO_2-H^+ at the central chemoreceptor. The peripheral and central responses to changes in P_ACO_2 can be separated on the basis of response latency (Bernards et al., 1966; Dejours, 1962). The central drive lags behind the peripheral drive because of the time taken by blood to reach the two chemosensitive sites, and the time it takes to obtain a new CO_2 equilibrium in the brainstem (Bernards et al., 1966; Cunningham, 1974; Farhi and Rahn, 1960). The results of experiments in human subjects, in which the ventilatory response to the sudden withdrawal of CO_2 was examined (Bernards et al., 1966; Miller et al., 1974), have supported the peripheral multiplicative interaction model in humans. These studies showed a decrease in $\dot{V}_E$ with peripheral timing, only if the subjects were euoxic or hypoxic. There was no early response when the subjects were hyperoxic. These studies cannot exclude the possible occurrence of multiplicative interaction between the peripheral input and a second component of the central CO_2-H^+ sensitivity (Drysdale, Jensen and Cunningham, 1981). Plum and Brown (1963) compared the ventilatory

resp< .ise to hypoxia, and the combination of hypoxia and hypercapnia, in patients with bilateral pyramidal tract disease to the responses in normal subjects. They found that cerebral dysfunction facilitated the ventilatory response to hypoxic-hypercapnia but not to hypoxia alone and concluded that the two respiratory stimuli interact centrally.

Edelman and colleagues (1973) measured the ventilatory response to transient hypoxia and hypercapnia in unanesthetized man. They assumed that the ventilatory responses to transient stimuli reflect the activity of the arterial chemoreceptors, while responses to steady-state stimuli reflect the activity of both the carotid bodies and the central nervous system. They found that hypoxia had a greater effect on the ventilatory response to steady state hypercapnia, than on the response to transient hypercapnia, and concluded that the phenomenon of stimulus interaction occurs at both the peripheral chemoreceptors and within the central nervous system, but that the central effect is the predominant one.

The noninvasive technique of dynamic end-tidal forcing and the feedback method introduced by Swanson and Bellville (1975) and modified by Robbins et al. (1982a; 1982b) can be used to produce perturbations in end-tidal PCO_2 ($P_{ET}CO_2$) and end-tidal PO_2 ($P_{ET}O_2$) that are independent of the $\dot{V}_E$ response to mixed venous blood composition (Swanson and Bellville, 1975) thus opening the feedback loop from $\dot{V}_E$ to $P_{ET}CO_2$ and $P_{ET}O_2$. This technique enables the determination of the time courses of the ventilatory responses to hypercapnia and hypoxia. Good correspondence has been reported between the dynamic forcing technique and the artificial brainstem perfusion technique used in cats to isolate the dynamic responses of the chemoreceptors (DeGoede et al., 1985).

This technique has been used by several groups to study the characteristics of the peripheral and central components of the response to CO_2, to determine the nature of the interaction between the chemoreceptor drives (Bellville et al., 1979; Clement et al., 1992; Dahan et al., 1990; Robbins, 1988; Swanson and Bellville, 1974; Swanson et al., 1978).

Swanson and Bellville (1974) assessed the ventilatory response to a CO_2 sinusoidal perturbation during euoxia and hypoxia and showed that the ratio of the ventilatory response in hypoxia, to that in euoxia, increased as the frequency of the sine wave increased. They concluded that peripheral CO_2-O_2 interaction at the carotid body was sufficient to explain hypoxic-hypercapnic interaction, without the need for postulating hypoxic enhancement of the central chemoreceptor response.

Bellville et al. (1979) used end-tidal step forcings to administer step increases in $P_{ET}CO_2$, while $P_{ET}O_2$ was maintained constant under euoxic and hypoxic conditions, in normal and carotid body resected subjects (CBR). The ventilatory response was studied by fitting a two-compartment model that included peripheral and central gains, time delays, and time constants to the data. Hypoxia increased the speed and the magnitude of the response to hypercapnia in normal subjects, consistent with CO_2-O_2 interaction at the peripheral chemoreceptor. The CBR subjects had a significantly longer central time constant and time delay than the normal subjects, and the central chemoreflex loop gain was half that measured in normal subjects. It was suggested that if the peripheral chemoreceptors are intact they might influence the effect on $\dot{V}_E$ of a given central chemoreceptor input to the respiratory centre, and that removal of the peripheral input

would reduce apparent central response.

Robbins (1988) used the differing speeds of response of the central and peripheral chemoreceptors to assess whether any interaction occurs between the peripheral and central chemoreceptor contributions to $\dot{V}_E$. The same chemoreceptor stimulus (an isocapnic step into hypoxia) was given under conditions of either eucapnia or residual hypercapnia at the central chemoreceptors. It was reported that, in two of the three subjects studied, the ventilatory response to hypoxia was augmented when central PCO_2 was high, supporting the idea that there is an interaction between central hypercapnia and hypoxic stimulation, and consequently an interaction between the central and peripheral chemoreceptors in man.

Robbins (1988) proposed that the debate, as to whether there is a component of the ventilatory response that depends on an interaction between the outputs of the peripheral and central chemoreceptors, may be specified as two competing models. The steady-state response characteristics of the additive model (no interaction) is written as:

$$\dot{V}_E = g_c i_c + g_p i_p \qquad (5)$$

where i_c and i_p are the outputs of the central and peripheral chemoreceptors and g_c and g_p their gain terms. The steady-state response characteristics of the multiplicative model are written as:

$$\dot{V}_E = g_c i_c + g_p i_p + g_m i_c i_p \qquad (6)$$

where g_m is the gain of the interactive term.

Robbins tested the ability of both models to reproduce the ventilatory responses to hypoxia and hypercapnia as described by the Lloyd equation (Lloyd, 1963):

$$\dot{V} = D[1 + A/(P_AO_2)](P_ACO_2 - B) \quad (7)$$

Both models were shown to produce exactly the same $\dot{V}_E$-$P_{ET}CO_2$ and $\dot{V}_E$-$P_{ET}O_2$ responses, as described by the Lloyd equation. However the CO_2 response curves were linear in the additive model but curvilinear, with the convexity upward, for the multiplicative model. This analysis demonstrated that the degree of interaction between CO_2 and O_2 is weaker at the peripheral chemoreceptor in the multiplicative model, than in the additive model. At maximum stimulation, half the drive to breathe in the multiplicative model came from the interactive term.

The results of further studies by the research group at Oxford failed to support the finding of a significant central-peripheral chemoreflex interaction reported by Robbins (1988). Clement et al. (1992) studied the independence of the peripheral and central chemoreflexes in humans using metabolic acidosis, generated by a brief bout of hard exercise, to selectively stimulate the peripheral chemoreceptors, and CO_2 inhalation as a stimulus common to both sets of chemoreceptors. They reported that the ventilatory sensitivity to hypoxia at matched arterial pH values was not significantly different between conditions of high (CO_2 inhalation) and low (metabolic acidosis) central chemoreceptor activity. The authors offered no explanation to account for the differences between the results of their study and that of Robbins (Robbins, 1988) which was published by the same laboratory four years previously. The point was made, however, that the use of exercise in the later study, would introduce a separate set of variables to the system that could affect respiratory control.

Dahan et al. (1990) compared the ventilatory response to CO_2 during euoxia, mild

hypoxia and hyperoxia in resting man using the dynamic end-tidal forcing technique. To gain insight into the existence of interaction, they analyzed all euoxic curves using the two compartment model in which the equation was extended to incorporate an interaction parameter (Robbins, 1988). They obtained convergence in only 70% of the curves, which suggested "overparameterization" of the interaction model with regard to the information content of the experimental data. The interaction was not significantly different from zero. Therefore, they could not demonstrate any significant central-peripheral interaction in the data sets of their subjects. While they could not demonstrate any significant central-peripheral interaction, they found that central CO_2 sensitivity decreased by 15% in hyperoxia (500 Torr), as compared to euoxia, and suggested that this was evidence for central CO_2-O_2 interaction in humans.

In summary, human studies support the presence of peripheral multiplicative interaction between hypoxic and hypercapnic drives (Bellville et al., 1979; Bernards et al., 1966; Clement et al., 1992; Miller et al., 1974; Swanson and Bellville, 1974). There is also evidence, however, that suggests a component of multiplicative interaction between the incoming activity from the periphery and central stimulation from CO_2-H^+ (Bellville et al., 1979; Dahan et al., 1990; Plum and Brown, 1963; Robbins, 1988). The possibility of a degree of central-peripheral chemoreflex interaction, challenges the most widely accepted model describing the interactions between chemical respiratory feedback stimuli, in which, hypoxia and the CO_2-H^+ complex interact multiplicatively at the level of the peripheral chemoreceptor, and the drives from the periphery and from the central chemosensitive area add together in their

effects on ventilation (Cunningham et al., 1986). The independence of the peripheral and central contributions to ventilation has been assumed by many in the field of respiratory control.

1.6 Structure of the Carotid Body

While the physiological role of the carotid body in respiratory control was not described until the work done by Heymans in the early 1930's (Heymans, 1951), the structure was described as early as 1762 by Albrecht von Haller (cited in Fitzgerald and Lahiri, 1986). The carotid bodies are paired organs, located at the bifurcations of the common carotid arteries, and innervated by the carotid sinus and the glossopharyngeal (sympathetic) nerves. They are richly innervated organs consisting of an association of islands of cells and capillaries called glomeruli (Fidone and Gonzalez, 1986). Two types of cells have been distinguished within the carotid body glomeruli based on nuclear shape and density (Gomez, 1908). Type I cells are the primary site of sensation and make up about 80% of the carotid body's volume. They are located centrally within a glomerulus, enveloped within Type II cells. The Type I cells make synaptic contact with the sensory nerves that run with the carotid sinus nerve (Fidone and Gonzalez, 1986; Nye, 1994). The Type II cells have been generally accorded a function like Schwann cells within the glomerulus (Fidone and Gonzalez, 1986).

The blood supply to the carotid body arises from one or more branches of the internal or external carotid arteries, or from the occipital arteries. The arterial supply divides to arterioles that further branch within the stroma to form complex capillary nets

that completely surround each glomerulus (Fidone and Gonzalez, 1986). The carotid body tissue has the largest blood flow per gram of any tissue in the body (Nye, 1994).

Neurochemicals are essential for sensory transmission. The carotid body, which weighs less than 1 mg, contains as many transmitters as the brain tissue (Prabhakar, 1994). The general consensus is that, in response to low PO_2, the Type I cells release neurochemicals which act on the afferent nerve ending to increase sensory discharge (Fidone and Gonzalez, 1986; Prabhakar, 1994). The transmitter that excites the discharge of the sensory nerves is not known. Biogenic amines, neuropeptides, nitrous oxide (NO) and carbon monoxide (CO) are all present in the Type I cells (Prabhakar, 1994).

1.7 The Peripheral Chemoreflex

The early works of Haldane and Priestley (1905) and Frédéricq (1901) would suggest that the carotid bodies have no role in the day-to-day life at sea level. More recently, the assumption that peripheral chemoreflex drive merely adds to the more potent central drive to produce the required ventilation, has been used to support this argument (Forster and Pan, 1994; Weil and Swanson, 1991). In comparison to the powerful drive due to CO_2-H^+ at the central chemoreceptor, the peripheral chemoreceptor drive has been suggested to be unimportant under normal, resting conditions, except in its role in error correction or fine tuning of the homeostasis of blood gases (Weil and Swanson, 1991). The destruction of the arterial chemoreceptor afferent pathway, however, depresses the slope of the $\dot{V}$-P_ACO_2 line, measured during

CO_2 inhalation, by about 15% from its control value at normal O_2 tension (Cunningham, 1974).

Cunningham (1987; 1974) proposed a more active role for the carotid bodies in respiratory control, stating that hypoxia, acting through the arterial chemoreceptors, has a long-term role in determining the set point of the central chemoreceptors (Cunningham, 1974). This assertion is supported by the results of carotid chemoreceptor denervation studies (Bouverot et al., 1965; Mitchell, 1966; Wade et al., 1970). Wade et al. (1970) reported that, in the absence of any arterial chemoreceptor drive, carotid endarterectomy patients showed increases in resting P_aCO_2 from 38.9 to 44.7 Torr. The results indicated that, assuming no change in the contribution of the medullary chemoreflex, the 13% to 24% post-operative decrease in resting $\dot{V}_E$, represents the contribution of the peripheral chemoreflex to the original respiratory drive. Denervated dogs also show increases in resting P_aCO_2 in the order of 20% (Bouverot et al., 1965; Mitchell, 1966). The results of denervation studies do not, however, fully reveal the normal physiological role of the carotid bodies because of the potential central depressant effects of decreased P_aO_2 (Fitzgerald and Lahiri, 1986).

The carotid bodies are the primary site of hypoxic responsiveness, and thus studies that attempt to measure the peripheral chemoreceptor contribution to ventilation typically involve manipulations of the inspired O_2 fraction. The difference in latency times of the response to step increase in CO_2 during euoxia and hyperoxia, defined as the period between the step in $P_{ET}CO_2$ and the first significant change in ventilation (Miller et al., 1974; Ward and Bellville, 1983), has been interpreted as evidence that the carotid

bodies do not contribute to the drive to breathe in hyperoxia (Cunningham et al., 1986). The magnitude of the decline in ventilation, in response to a switch to hyperoxic breathing, can be used as an index of the level of preexisting carotid chemoreflex drive (Dejours, 1962). The decrease is transient, however, and $\dot{V}_E$ subsequently increases to, or even slightly above, air breathing values, despite the continued breathing of O_2 (Becker et al., 1995; Dejours, 1962). The steady state method does not reveal a significant contribution of the peripheral chemoreceptor because of the secondary effects of O_2, including the Haldane effect, and the decrease in cerebral blood flow, which raise the PCO_2 in the CNS and stimulate $\dot{V}_E$ (Fitzgerald and Lahiri, 1986).

The secondary increase in $\dot{V}_E$,observed in prolonged hyperoxic breathing, illustrates the "disequilibrium theory" outlined by Dejours (1962, p.341) which states:

" any new factor imposed upon an organism changes its equilibrium; in the early phase of the period of action of the disturbing agent, at the phase of maximal disruption, the variations observed in the organism can be related directly to it; but when the disturbing agent is applied upon the organism for a prolonged time, many secondary reactions occur, generally tending to cancel the disturbing effects of the factor of disequilibrium, these secondary reactions terminating eventually in a new state of equilibrium, the analysis of which is very complex".

Dejours (1962) argued that it is possible to obtain a change in O_2 drive that is virtually free of secondary reactions, if the O_2 inhalation is restricted to one or two breaths (Dejours test). In euoxia, the transient O_2 switching technique revealed that the peripheral chemoreceptors provided 10% of the total drive to breathe, but it was suggested that this was less than the true carotid chemoreceptor total contribution because the secondary stimulating actions of hyperoxia may oppose the fall in ventilation.

More recently, Ward (1994b) cautioned that the O_2 switching technique may still underestimate the total peripheral contribution if $\dot{V}_E$ starts to increase before the full suppression of the carotid chemoreflex is expressed. While the nadir of the ventilatory decline in response to a hyperoxic switch occurs some 25-30 s after the transition to hyperoxic breathing, the transit time from the lungs to the central chemoreceptors has been estimated to be about 12 s (Miller et al., 1974), which would confine the inactivation process to a period of about 13 s (Ward, 1994b).

In summary, the results of denervation and hyperoxic studies estimate that, under euoxic resting conditions, the carotid body accounts for less than 20% of the total ventilatory drive (Bouverot et al., 1965; Dejours, 1962; Wade et al., 1970). The wide range of estimates can be partly accounted for by the different methodologies. In addition, resting ventilation averages 6 $l \cdot min^{-1}$ in humans, confining the contribution from the peripheral chemoreceptor to about 1 $l \cdot min^{-1}$. A change of this magnitude is difficult to distinguish from methodological or biological variability in response patterns.

1.8 Feedback Control of Exercise Ventilation

Haldane, having established the near constancy of the alveolar PCO_2 in any individual (Haldane and Priestley, 1905), noted that there was a link between ventilation and metabolism (cited in Cunningham, 1987). This link is now expressed quantitatively as the metabolic hyperbola for CO_2 (Equation 3). Exercise provides an excellent means of studying the function and capacity of the respiratory control system. The ventilatory response to exercise illustrates the most fundamental function of the system: the matching

of metabolic demands to gas exchange (Weil and Swanson, 1991). The nervous system adjusts the rate of alveolar ventilation almost exactly to the demands of the body so that P_aCO_2 and P_aO_2 are hardly altered, even during strenuous exercise or other types of respiratory stress.

Something more than the conventional stimuli due to mean CO_2-H^+ and hypoxia are required to maintain the precise concentrations usually observed in the blood in exercise (Cunningham et al., 1986). Feedforward mechanisms, related to the neural drive activating the muscles (Duffin, 1994; Krogh and Lindhard, 1913), and the reflexes originating in the exercising limbs (Duffin, 1994) and the central circulation (Wasserman et al., 1974), allow the system to anticipate, and therefore, minimize the considerable changes in arterial blood gas concentrations that would occur during the transition from rest to exercise, if the feedback components had to cope unaided. Errors in gas tensions and pH stimulate the chemoreceptors and provide feedback correction of any inappropriate feedforward response. It has been suggested that when the primary exercise-ventilatory response coupling is good, the extent of the error, and consequently, the participation of the chemoreceptors in ventilatory control, is small (Weil and Swanson, 1991).

Moderate exercise is considered to represent the range of work rates at which there is no sustained elevation of arterial blood lactate concentration. Heavy exercise is that range in which there is a sustained elevation of arterial lactate which is maintained, or decreased with time. Severe exercise represents those work rates for which arterial lactate concentration continues to increase throughout the duration of the work (Whipp,

1987). The ventilatory response to constant-load exercise imposed from a resting background can be divided into three distinct temporal phases (Whipp, 1987). Phase I ($\phi 1$) describes the initial rapid increase in the gas exchange variables ($\dot{V}_E$, $\dot{V}O_2$, and $\dot{V}CO_2$) which occurs usually within the first breath, and because of its rapidity is generally recognized to be under feedforward control (Ward, 1994a). Phase 2 ($\phi 2$) is the more prominent dynamic phase of the response, in which both altered pulmonary blood flow and changing mixed venous blood composition dictate the rates of pulmonary gas exchange (Whipp, 1987). If exercise is performed below the anaerobic threshold, ventilation reaches a steady-state, or phase 3 ($\phi 3$) within four minutes. Above the anaerobic threshold, $\dot{V}_E$ slowly increases during $\phi 3$.

The mathematical modelling approach to the study of physiology has been applied to the investigation of respiratory control mechanisms in exercise (Oren et al., 1982; Ward et al., 1987; Griffiths et al., 1986). The kinetic behaviour of ventilation can be described by fitting the response data to a first order (single exponential) model of the form:

$$\triangle\dot{V}_E(t) = \triangle\dot{V}_E(ss)[1 - e^{-[(t \pm T)/\tau]}] \qquad (8)$$

where $\triangle\dot{V}_E(t)$ is the increase in $\dot{V}_E$ above the prior steady state value (rest or loadless pedalling) at time t; $\triangle\dot{V}_E(ss)$ is the steady state increase in $\dot{V}_E$; and τ and T are the time constant and time delay of the response (Whipp et al., 1982). This model ignores $\phi 1$ of the $\dot{V}_E$ response. The time constant (τ) of the response to a loadless pedalling to 100 W exercise transition is typically approximately 55 - 65 s with a delay of about 20 s (Wasserman et al., 1986). The ventilatory response is considerably slower than the $\dot{V}O_2$

response (i.e. τ = 30-40 s). There is a close dynamic coupling of $\dot{V}_E$ to $\dot{V}CO_2$ with $\dot{V}CO_2$ (τ = 50-60 s) leading the $\dot{V}_E$ response (Wasserman et al., 1986).

The immediacy of the ϕ1 hyperpnoea is inconsistent with a humorally mediated drive from the chemosensory sites on the ventral medullary surface. The best estimates of the lung to central chemoreceptors latency are in the order of 12 s (Miller et al., 1974; Ward and Bellville, 1983). It is also unlikely that the peripheral chemoreceptors play a role in the ϕ1 response, based on the transit delay between the lungs and the carotid body, as well as the normal ϕ1 response in hyperoxia, or in carotid body resected subjects (Ward, 1994a).

The ϕ2 response has been thought to involve humoral mediation because it begins with a time delay consistent with the vascular transit delay between the exercising limbs and the lungs (Whipp et al., 1982). There is considerable evidence which suggests that the central chemoreceptors do not have a role in ϕ2 hyperpnoea. Ward et al. (1987) found that the ϕ2 τ for $\dot{V}_E$ was not affected when exercise was performed against a hypercapnic background during hyperoxia, which would selectively stimulate the central chemoreceptors. This finding is further supported by the work of Shea et al. (1993) with patients who have congenital central hypoventilation syndrome (CCHS). These individuals have no discernable central chemosensitivity, yet show ϕ2 ventilatory kinetics that are not distinguishable from control subjects.

In contrast to ϕ1, the carotid bodies appear to exert an important modulating influence in the ϕ2 ventilatory control. Griffiths et al. (1986) had subjects perform a series of square wave exercise tests with inspired O_2 fractions (F_IO_2) of 0.12 (hypoxia),

0.21 (euoxia) and 1.0 (hyperoxia). They observed that the $\dot{V}_E$ kinetics were speeded by increasing carotid body responsiveness with hypoxia, and slowed when peripheral chemosensitivity was abolished with hyperoxia. Oren et al. (1982) showed that $\dot{V}_E$ kinetics were speeded after carotid body responsiveness was increased by ingestion of ammonium chloride to induce metabolic acidosis. $\dot{V}_E$ kinetics were slowed when the peripheral chemosensitivity was reduced by inducing metabolic alkalosis through sodium bicarbonate ingestion. Exactly how the carotid bodies modulate $\dot{V}_E$ in $\phi 2$ is unknown. It is unlikely that a transient asphyxic condition in $\phi 2$ would be sufficient to influence $\dot{V}_E$, based on the modest degree of hypercapnia involved, and the slow development of the full $\dot{V}_E$ response kinetics to increased PCO_2, as well as the relatively low ventilatory responsiveness to mild hypoxia (Ward, 1994a).

There is no evidence to suggest that the central chemoreceptors play a role in the humoral mediation of ventilatory control during $\phi 3$ of moderate exercise. There appears to be no change in the conventional stimuli to the central chemoreceptors, based on the work of Bisgard et al. (1978), which showed that cerebrospinal fluid pH, in ponies, remained relatively stable over a wide range of work rates. It is also doubtful that there is any exercise-induced change in central chemosensitivity to CO_2 (Duffin et al., 1980). In addition, CCHS subjects show normal $\phi 3$ responses to moderate intensity exercise (Shea et al., 1993), though they regulate P_aCO_2 at an elevated set point (Paton et al., 1993).

The classical experiments of Dejours and colleagues (Dejours, 1962; Dejours et al., 1957a; Dejours et al., 1957b) and Perret (cited in Cunningham, 1987) confirmed that

the peripheral chemoreceptors contribute to ventilatory drive in the steady state of moderate intensity exercise. The normal magnitude of the $\dot{V}_E$ response in carotid body resected (CBR) subjects, however, suggests that its function is one that can be subserved by the central chemoreceptors (Whipp and Davis, 1979). The results of hyperoxic testing suggest that the carotid bodies account for about 15% to 20% of the $\phi 3$ hyperpnoea (Dejours, 1962; MacDonald et al., 1990; Masuda et al., 1988). Concerns have been raised, however, that there is insufficient time for the initial hypoventilatory phase to be fully expressed prior to the induction of the secondary stimulatory effects of hyperoxia on central mechanisms (MacDonald et al., 1990; Ward, 1994b).

The exact nature of the stimulus to the carotid bodies during moderate intensity exercise has been the subject of much debate in the literature. While it has been demonstrated that they do serve a role in $\phi 3$ ventilatory control, it is not clear how the control is mediated. The mean arterial PCO_2 and $[H^+]$ are not systematically increased, nor is arterial PO_2 decreased during moderate exercise (Murphy et al., 1987; Whipp and Wasserman, 1980). Thus, the conventional PO_2 and O_2 response curves can not be applied to characterize the response. Several CO_2-linked mechanisms have been proposed, that are argued to operate independently of the mean level of P_aCO_2, including intra-breath oscillations of P_aCO_2, P_aO_2 and pH (Cross et al., 1982), and CO_2 flux through the lung (from the pulmonary artery to the pulmonary vein) (Wasserman et al., 1977). Murphy et al. (1987) showed that oscillations of arterial pH, recorded from arteriovenous shunts in renal patients with normal ventilatory responses to exercise, usually disappeared in exercise, thus questioning the importance of such oscillations in

respiratory control. Evidence against an important role for CO_2 flux mechanisms has also been presented by Huszczuk et al. (1990). They investigated the ventilatory response to treadmill exercise in calves with artificial hearts, and reported that the $\dot{V}_E$ responses were adequate for the metabolic demands of the exercise, resulting in the regulation of P_aCO_2 and pH, despite the absence of an increase in cardiac output.

The increased potassium (K^+) that is released from contracting muscle cells during exercise has been demonstrated to stimulate ventilation through an action on the carotid bodies (Paterson, 1992). Most of the work in this area examines only the extremes of rest or heavy exercise. It has been suggested, however, that the relationship between modest rises in arterial K^+ and chemoreceptor discharge is not linear, but curves upward, only becoming steep when K^+ rises by 1 or 2 mM (Nye, 1994). Thus small rises in arterial K^+ probably have little effect on breathing. Increases in adenosine, body temperature, and catecholamines may also act to modulate the carotid body drive during exercise (Wasserman et al., 1986; Whipp, 1994).

When exercise is performed above the anaerobic threshold, the control characteristics of the exercise hyperpnoea become nonlinear and steady states are not attained. The ventilatory response to heavy exercise is attenuated in CBR subjects (Wasserman et al., 1975) leading to the conclusion that the carotid bodies are the dominant, or even exclusive, mediators of the respiratory compensation for the metabolic acidosis of heavy exercise (Ward, 1994a). Rausch et al. (1991) reported that subjects performing high-intensity constant-load exercise while inhaling 12% O_2 (hypoxia), showed a more rapid restoration of arterial pH toward normal, than while breathing air.

Thus the arterial chemoreceptors appeared to play an important role in constraining the fall in arterial pH, however, the manifestation of a slow acid-base compensatory component, when carotid body sensitivity was suppressed by hyperoxia, suggested that central chemoreceptor mechanisms may also play a role in the ventilatory compensation for the metabolic acidosis of heavy exercise.

While Masuda et al. (1989) found that the administration of the Dejours test depressed $\dot{V}_E$ to a greater extent with increasing exercise intensity, the relative contribution of the chemoreceptor activity remained the same, at 10-20%. MacDonald et al. (1980) found that the average magnitude of the $\dot{V}_E$ nadir in hyperoxia was actually 3% less in heavy exercise (20.5%), than during constant-load exercise below anaerobic threshold (23.3%). Jeyaranjan et al. (1987) reported a 15% drop in $\dot{V}_E$ following O_2 administration and suggested that the peripheral chemoreceptors are not the sole mediators of the hyperventilation of heavy exercise.

Ward (1994a) noted that the implementation of the Dejours test above anaerobic threshold is hampered by the lack of a ventilatory steady state. Evidence also suggests that, at work rates that induce lactic acidemia, hyperoxia may not effect a full suppression of carotid chemosensitivity (McLoughlin et al., 1993). Therefore hyperoxic testing may underestimate the contribution of peripheral chemoreceptor drive in heavy exercise.

In summary, there is considerable debate regarding the role of the carotid bodies during steady state conditions either at rest or in moderate exercise. In humans the primary exercise drive is tightly matched to metabolic CO_2 production; thus P_aCO_2

changes minimally when metabolic rate changes. The carotid bodies have been suggested by some research groups to provide a fine-tuning or compensatory function when a specific condition (ventilatory loading, asthma, COPD) causes the exercise response to become hypercapnic (Forster and Pan, 1994; Weil and Swanson, 1991).

1.9 Statement of Purpose

The thesis will examine the feedback control mechanisms that regulate ventilation in human subjects. Hypoxic and hypercapnic feedback stimuli interact multiplicatively in their effects on ventilation (Lloyd et al., 1958; Nielsen and Smith, 1952). In anaesthetized cats, the literature supports the peripheral chemoreceptor as the exclusive site of multiplicative interaction between CO_2 and hypoxia (Hornbein et al., 1961; Van Beek et al., 1983) and indicates that the interaction between peripheral and central inputs is strictly additive (Cunningham et al., 1986; Van Beek et al., 1983). In humans, dynamic studies demonstrate the presence of peripheral interaction between hypercapnic and hypoxic stimuli (Bernards et al., 1966; Dahan et al., 1990; Miller et al., 1974; Swanson and Bellville, 1974). While it is commonly accepted that the central and peripheral chemoreflexes contribute independently to the total ventilatory drive in human subjects (Clement et al., 1992; Dahan et al., 1990; Swanson and Bellville, 1974), evidence also exists which supports the possibility of a degree of central interaction between peripheral chemoreceptor afferent signals and signals from the chemosensitive areas of the ventrolateral medulla (Bellville et al., 1979; Edelman et al., 1973; Plum and Brown, 1963; Robbins, 1988). The purpose of the study presented in Chapter 2 is to investigate the nature of the interaction between the central and peripheral chemoreceptors in human subjects. It is hypothesized that the afferent signals from the peripheral chemoreceptors do interact with those from the central chemoreceptors, rather than adding together in their effects on ventilation. The dynamic end-tidal forcing technique can be used to give a period of time when the central chemoreceptors are

exposed to residual hypercapnia, while the peripheral chemoreceptors are exposed to eucapnia. The presence or absence of chemoreflex interaction will be determined by comparing of hypoxic sensitivities measured against this background, with hypoxic sensitivities measured when both environments are eucapnic. If hypoxic sensitivity is increased by central hypercapnia, then interaction is present.

The studies presented in Chapter 3 are designed to examine the contribution of the peripheral chemoreceptor drive to ventilation and study the effects of hyperoxia on respiratory control. The carotid bodies are the primary site of hypoxic responsiveness (Heymans, 1951), and thus studies that attempt to measure the peripheral chemoreceptor contribution to $\dot{V}_E$ typically involve manipulations of the inspired O_2 fraction (Griffiths et al., 1986; Heymans, 1951; Jeyaranjan et al., 1987; Rausch et al., 1991; Whipp and Davis, 1979). The inhalation of hyperoxic gases (>300 Torr) is known to suppress peripheral chemoreceptor drive, and the magnitude of the resulting transient decline in $\dot{V}_E$ has been used as an index of the level of preexisting carotid chemoreflex drive (Dejours, 1962; Ward, 1994b). Sustained hyperoxia is complicated by 1) the increase in $P_{ET}CO_2$ due to the relative hypoventilation and 2) the acidifying effects of cerebral hypofusion, leading to a centrally mediated stimulation of $\dot{V}_E$. This could result in the underestimation of the contribution of the peripheral chemoreflex if $\dot{V}_E$ starts to increase before a true nadir is reached. The first objective of these studies is to measure the contribution of the peripheral chemoreflex to $\dot{V}_E$ during the steady state of moderate intensity exercise, using continuous hyperoxic suppression of carotid body drive, while stabilizing the drive from the central chemoreceptor by maintaining a constant $P_{ET}CO_2$.

In young adults, the contribution of the peripheral chemoreceptor to exercise ventilation has been estimated to be about 20% (Whipp, 1994). It is hypothesized that past studies may have underestimated the role played by the carotid bodies in exercise, and that our estimates will be higher than previously reported. A second objective of this study is to investigate the effect of sustained hyperoxia on the central and peripheral chemoreflex loops, under conditions of constant and varying $P_{ET}CO_2$, by examining the time course of the ventilatory response to hyperoxia, as well as the response to the removal of hyperoxia. Respiratory control could be altered by the sustained removal of carotid body drive. There is an acknowledged redundancy of ventilatory drives (Wasserman et al., 1986), one of which could potentially assume the role of the carotid body in its absence. It has been suggested that the carotid bodies determine the set point about which P_aCO_2 is regulated (Cunningham, 1974). The removal of the peripheral chemoreceptor could possibly change the setpoint about which P_aCO_2 is regulated, as is the case in carotid body resected subjects (Wade et al., 1970).

The studies presented in Chapter 4 are methodological, and are designed to examine the precision of the techniques used to estimate P_aCO_2. The arterial partial pressure of carbon dioxide (P_aCO_2) is an important contributor to the control of breathing, and its accurate measurement critical to studies of ventilatory control. Indwelling catheters can be used for the direct sampling of arterial blood, however, this is an invasive procedure that is not always convenient or acceptable in the research laboratory setting. Several techniques have been developed to approximate P_aCO_2 based on the measurement of the PCO_2 of arterialized venous blood and in the expired gases.

These techniques have been validated for use in young subjects (Forster et al., 1972; Robbins et al., 1990). The appropriateness of these methods has not been investigated in older subjects. The increase in physiological deadspace with aging (Tenney and Miller, 1956), and the greater nonuniformity of the ventilation-perfusion distribution in healthy older aduits relative to young adults (Holland et al., 1968) may result in an altered relationship between $P_{ET}CO_2$ and P_aCO_2, making these estimates of questionable use in this subject group.

CHAPTER 2

THE NATURE OF THE INTERACTION BETWEEN CENTRAL AND PERIPHERAL CHEMORECEPTOR DRIVES

2.1 Abstract

This study examined the nature of the interaction between the ventilatory drives from the central and from the peripheral chemoreceptors in humans. Our experiments were modelled after those of Robbins (J. Physiol., 1988) in which the differing speeds of response of the central and peripheral chemoreceptors were used to enable a temporal separation of their chemical stimulation. Robbins demonstrated a multiplicative interaction between the central and peripheral chemoreflexes in two of three subjects. In these experiments, three protocols were employed. In protocol A, subjects were exposed to an end-tidal PCO_2 of 8-10 Torr above resting PCO_2, with $P_{ET}O_2$ = 100 Torr, for 8 minutes. Thirty seconds after the hypercapnic stimulus was withdrawn, a five minute hypoxic stimulus ($P_{ET}O_2$ = 50 Torr) was introduced. The thirty second interval was believed to be sufficient time for the peripheral chemoreceptors to adapt to the new level of carbon dioxide, however, the central chemoreceptor environment changes more slowly. Over the subsequent five minutes of hypoxia, therefore, the central chemoreceptors were exposed to diminishing hypercapnia. Protocol B was the same as A without the hypoxic step. The effect of hypoxia on ventilation was examined by subtracting the ventilatory response to B from the ventilatory response to A. The response to the hypoxic step in A was then compared to the effect of the same hypoxic

step without the preceding period of hypercapnia in protocol C. If the hypoxic response was affected by relative hypercapnia at the central chemoreceptor, then the ventilation in A should initially have been greater than in C, only becoming the same as central eucapnia was restored. Five subjects were studied, each contributing six sets of data to each of the three protocols. In four of the subjects, the ventilatory response to hypoxia was unaffected by relative hypercapnia at the central chemoreceptor. These results indicate that the occurrence of multiplicative interaction between the central and peripheral chemoreceptors in man is rare and that for the most part the central and peripheral chemoreflexes are independent of each other. The difference between the results of the current study and that by Robbins (1988) might be explained in part by modifications in the administration of the protocols. These changes were made to ensure that there was sufficient time between each protocol to eliminate the possibility that the stimulus in one protocol might potentiate or diminish the ventilatory response in a succeeding protocol.

2.2 Introduction

The slope of the $\dot{V}_E$-P_ACO_2 relation is increased when P_AO_2 is below euoxic values (Nielsen and Smith, 1952). When P_AO_2 rises above euoxic values the slope and intercept are reduced. This suggests that hypoxic and hypercapnic feedback stimuli interact multiplicatively in their effects on ventilation (Cunningham et al., 1986). In anaesthetized cats, the literature supports the peripheral chemoreceptor as the exclusive site of multiplicative interaction between CO_2 and hypoxia (Hornbein et al., 1961; Van Beek et al., 1983) and indicates that the interaction between peripheral and central inputs is strictly additive (Cunningham et al., 1986; Van Beek et al., 1983).

It is commonly accepted that the central and peripheral chemoreflexes contribute independently to the total ventilatory drive in human subjects (Berkenbosch et al., 1992). The possibility remains, however, that interaction between peripheral chemoreceptor afferent signals and signals from the chemosensitive areas of the ventrolateral medulla takes place within the central nervous system.

The main problem in determining the nature of the peripheral-central chemoreflex interaction in human subjects is the difficulty in changing the peripheral CO_2-H^+ drive without affecting the central CO_2-H^+ drive. Robbins (1988) used the differing speeds of response of the central and peripheral chemoreceptors to enable a temporal separation of their chemical stimulation. The dynamic end-tidal forcing technique was used to give a period of time when the central chemoreceptors were exposed to residual hypercapnia while the peripheral chemoreceptors were exposed to eucapnia. Hypoxic sensitivity was measured against this background and when both environments were eucapnic. In two

of the three subjects studied, the ventilatory response to hypoxia was augmented when central PCO_2 was high. These results provided some evidence for a degree of multiplicative interaction between the central and peripheral chemoreceptors in humans.

The purpose of this study was to investigate the interaction between the central and peripheral ventilatory chemoreflex loops in humans. The experiments were modelled after those of Robbins (1988) with modifications to the administration of the experimental protocols. It was hypothesized that the results would strengthen the evidence for interaction between the central and peripheral chemoreflex loops.

2.3 Methods

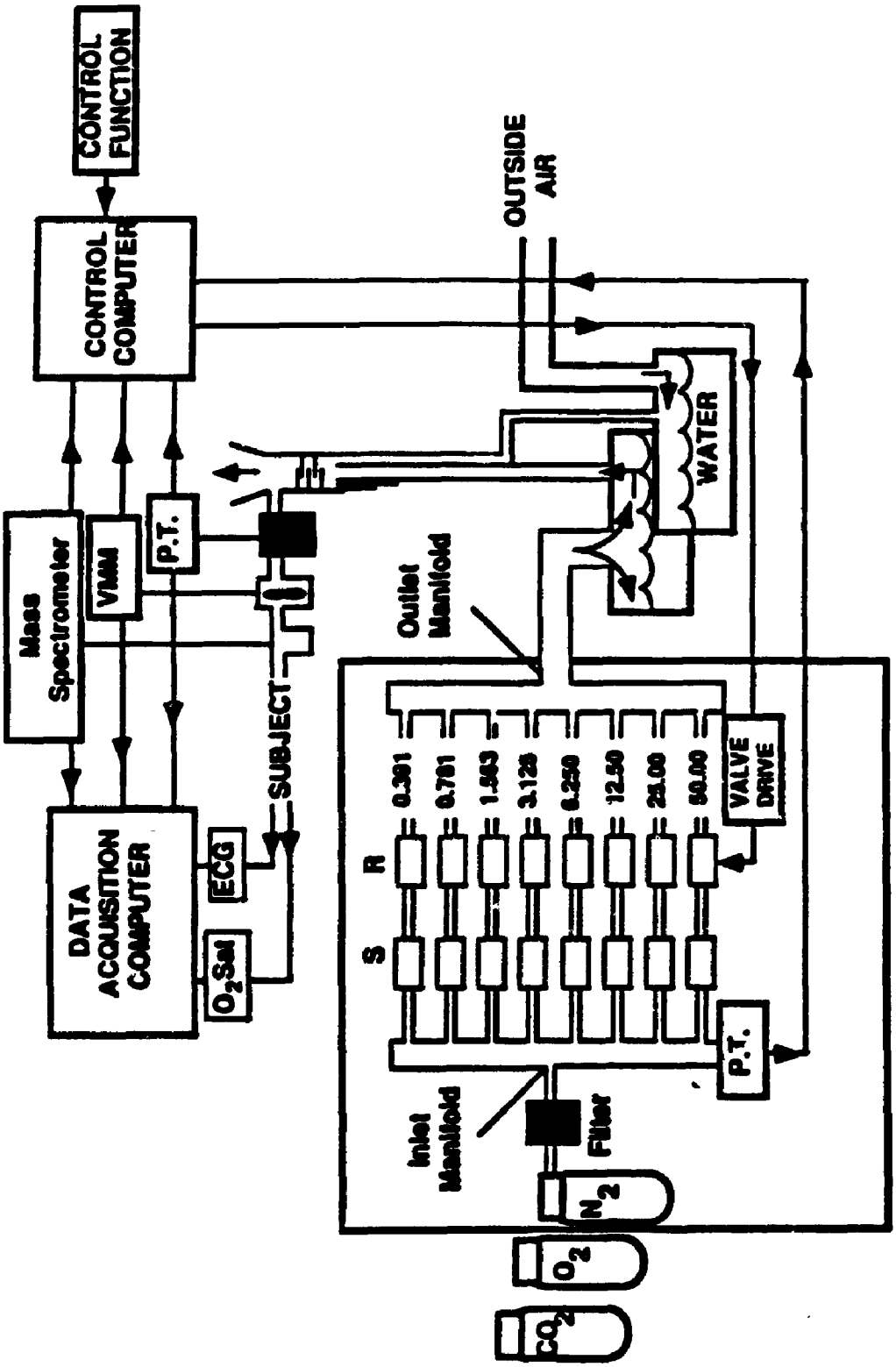
Respiratory apparatus and gas analysis. The experimental set up is similar to that described by Poulin et al. (1993). Subjects were seated and breathed through a mouthpiece with the nose occluded. Inspired and expired ventilation flow rates were measured using a low resistance bi-directional turbine (Alpha Technologies, VMM 110) and volume transducer (Sensor Medics VMM-2A) calibrated with a syringe of known volume (3.01 l). Respiratory flows and timing information were measured using a pneumotachograph (Hans Rudolph, Inc. Model 3800) and differential pressure transducer (Validyne MP45-871). Inspired and expired gases were sampled continuously (20 ml/sec) at the mouth and analyzed by a mass spectrometer (AIRSPEC MGA 2000) calibrated with precision-analyzed gas mixtures. Analog signals were sampled and digitized every 20 ms by computer. Gas concentration signals were aligned with the inspired and expired volumes after correcting for the time delay appropriate for the

instrument.

Two microcomputers were used. The data acquisition computer collected the experimental variables every 20 ms and stored them for later analysis. Accurate control of end-tidal gases was achieved using a computer controlled fast gas-mixing system similar to that described in more detail by Howson et al. (1987) and Robbins et al. (1982b) and presented schematically in Figure 2. The control computer compared the measured end-tidal gas tensions with the target end-tidal tensions (entered into the control computer before the experiment according to the protocol). The variables used for feedback control were $P_{ET}CO_2$ and $P_{ET}O_2$. The inspired PCO_2 and PO_2 required were converted by an algorithm into appropriate values for flows of CO_2, O_2, and N_2. The sensing process for $P_{ET}CO_2$ and $P_{ET}O_2$ was repeated at the end of each breath and the control computer adjusted the gas mixture to force the end-tidal PCO_2 and PO_2 towards the desired values.

Figure 2

Schematic representation of the computer-controlled fast gas mixing system used to administer dynamic end-tidal forcing functions. Respiratory volumes were measured witha turbine and volume transducer (VMM). Respiratory flows and timing were obtained using a pneumotachograph and differential pressure transducer (P.T.). Gas was sampled at the mouth and analyzed by mass spectrometer for fractional concentrations of CO_2, O_2 and N_2. Two microcomputers wre used; one functioned as a data acquisition computer (DAC) and the other functioned as a control computer (CC). The DAC collected data from the the mass spectrometer, VMM, P.T., electrocardiogram (ECG), ear oximeter (O_2Sat), and stored them for later analysis. At the start of the experiment, the control computer (CC) found the end of the first expiration (sensed by the P.T.). The CC compared the measured end-tidal tensions with the target end-tidal tensions (entered into the CC before the experiment according to the protocol). The variables used for feedback control were $P_{ET}CO_2$ and $P_{ET}O_2$. The inspired PCO_2 and PO_2 required (predicted inspired likely to achieve the desired end-tidal pressures) were converted by an algorithm into appropriate values for flows of CO_2, O_2 and N_2, provided by the opening and closing of several solenoid valves (S). The sensing process for $P_{ET}CO_2$ and $P_{ET}O_2$ was repeated at the end of each breath and the CC adjusted the gas mixtures to force the $P_{ET}CO_2$ and $P_{ET}O_2$ towards the desired values. Thus, the control of the new inspiratory mixture delivered was on the next breath.

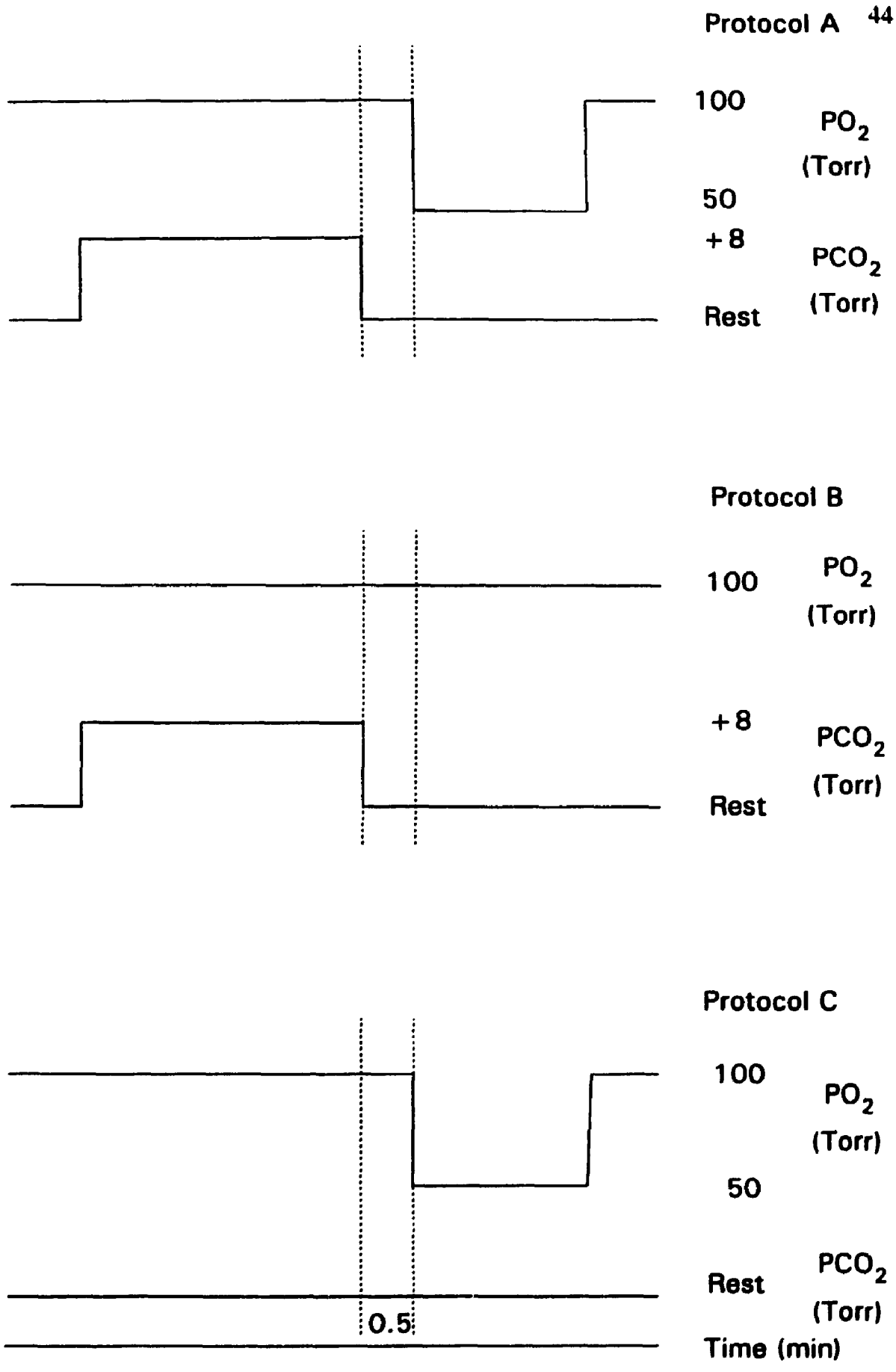

CONTROL FUNCTION
CONTROL COMPUTER
OUTSIDE AIR
WATER
Mass Spectrometer
VMM
P.T.
Outlet Manifold
SUBJECT
DATA ACQUISITION COMPUTER
ECG
O_2Sat
S
R
0.391
0.781
1.563
3.125
6.250
12.50
25.00
50.00
VALVE DRIVE
P.T.
Inlet Manifold
Filter
N_2
O_2
CO_2

Subjects and Protocol. Sample size calculations were made based on the advice of a statistician (see Appendix V). Five young males ranging in age from 22 to 35 years (mean age = 28 years) acted as subjects for the experiments. All subjects were non-smokers with no history of cardiovascular or respiratory disease. The study requirements were fully explained (in written and verbal forms; Appendix II) to all participants, with each subject giving informed consent prior to volunteering to participate in the study . The research was approved by the University's Committee on Human Research.

The experimental protocols were modelled after Robbins (1988), which used the differing speeds of response of the central and peripheral chemoreceptors to enable a temporal separation of their chemical stimulation. The three different protocols that were required are illustrated schematically in Figure 3. In Protocol A, the subject was exposed to a $P_{ET}CO_2$ 8 Torr above resting, with $P_{ET}O_2$ = 100 Torr, for 8 minutes. 30 seconds after the hypercapnic stimulus was withdrawn, a 5 minute hypoxic stimulus ($P_{ET}O_2$ = 50 Torr) was introduced. It was assumed that the 30 s interval would be sufficient for the peripheral chemoreceptor to adapt to the new level of PCO_2. The central chemoreceptor environment changes more slowly, however, and over the subsequent 5 minutes of hypoxia, the central chemoreceptors were exposed to diminishing hypercapnia. The other two protocols were controls. Protocol B was similar to A, but without the hypoxic step. In Protocol C, a five minute step down in $P_{ET}O_2$ from 100 Torr to 50 Torr was administered at the resting level of $P_{ET}CO_2$, and without the preceding period of hypercapnia.

Figure 3

Schematic diagram describing the time related changes in $P_{ET}CO_2$ and $P_{ET}O_2$ forcing functions in each of the three experimental protocols employed.


Protocol A
100
PO_2
(Torr)
50
+8
PCO_2
(Torr)
Rest
Protocol B
100
PO_2
(Torr)
+8
PCO_2
(Torr)
Rest
Protocol C
100
PO_2
(Torr)
50
Rest
PCO_2
(Torr)
0.5
Time (min)

Strict adherence to the protocol outlined by Robbins (1988), wherein protocols A and B were administered in one breathing period, resulted in the ventilatory response to the second hypercapnic stimulus being 9.2 $l \cdot min^{-1}$ (31%) higher than the ventilatory response to the first hypercapnic stimulus (Appendix VI). When two type C protocols were administered in the same breathing period, the ventilatory response to the second hypoxic stimulus was 8.3 $l \cdot min^{-1}$ (30%) lower than the response to the first hypoxic stimulus (Appendix VI). Therefore, on each visit, three periods of breathing on the apparatus were planned, corresponding to one of the three protocols. Each breathing session was separated by at least 30 minutes. Each of the five subjects contributed six sets of data to each of the three protocols.

Data Analysis. The data was analyzed in a similar manner as Robbins (1988). For each protocol, a mean of the respiratory variables for the two minute steady state period prior to the first step was calculated along with the means for each 30 s period following the step. The results were then combined to yield an average response for each subject to each step type. Six individual responses contributed to each average response. The effect of hypoxia on ventilation was examined by subtracting the ventilatory response to B from the ventilatory response to A. The effect of hypoxia in protocol C was measured by subtracting each 30 s data point from the 2 minute control point. The results of these calculations gave the ventilatory response to hypoxia under two sets of conditions. The response to the hypoxic step in A was then compared to the effect of the same hypoxic step without the preceding period of hypercapnia in protocol C using a one-sided paired t-test. The null hypothesis was that the ventilatory response in step type A was the same

(or smaller) than the ventilatory response in step type C. If the hypoxic response was affected by relative hypercapnia at the central chemoreceptor, then the ventilation in A should initially have been greater than in C, only becoming the same as central eucapnia was restored. The comparisons were also made using a Wilcoxon signed-rank test, the nonparametric analogue to the paired t-test (Rosner, 1986).

A two component exponential model (Bellville et al., 1979) was used to estimate the temporal parameters of the ventilatory response to the step decrease in CO_2 in Protocol B. For each individual, the breath-by-breath data for $\dot{V}_E$, $P_{ET}CO_2$ and $P_{ET}O_2$ from each test were interpolated over one second intervals and all tests for a given protocol were ensemble-averaged to increase the signal to noise ratio. The total ventilatory response was made up of the sum of contributions of the peripheral ($\dot{V}_p(t)$) and central ($\dot{V}_c(t)$) chemoreflex loops and a drift term (Drift(t)):

$$\dot{V}_E(t) = \dot{V}_b + \dot{V}_c(t) + \dot{V}_p(t) + \text{Drift}(t)$$

where,

$$\dot{V}_c(t) = G_c(1 - e^{-(t-Tc)/\tau c})$$

and,

$$\dot{V}_p(t) = G_p(1 - e^{-(t-Tp)/\tau p})$$

$\dot{V}_b$ is the baseline ventilation and $\dot{V}_E(t)$ is the time-dependent variation in $\dot{V}_E$. The parameters G_c, τ_c and T_c are the gain, time constant of the response, and time delay of the central chemoreflex loop, respectively. The parameters G_p, τ_p and T_p are the gain, time constant of the response, and time delay of the peripheral chemoreflex loop, respectively.

To obtain optimal parameter estimation, a computerized optimization routine was applied. All combinations between 1 and 25 s, with increments of 0.1 s and with the constraint $T_c \geq T_p$ were used. The minimum time delays were chosen to be 1 s and τ_p was constrained to be at least 0.3 s based on previous studies (Bellville et al., 1979; Dahan et al., 1990).

2.4 Results

The results for each subject are shown in Figure 4. The quality of the end-tidal profiles was the same for the hypercapnic steps in protocols A and B and for the hypoxic steps in protocols A and C.

Table 1 lists the results of subtracting the ventilatory response to hypoxia in protocol C from the difference in ventilation between protocols A and B for each subject. In subject 1569, at the four points indicated by the asterisks, the ventilatory response to hypoxia was significantly ($P < 0.05$) greater when the central chemoreceptor was exposed to diminishing hypercapnia than when both the central and peripheral chemoreceptor environments were eucapnic (Figure 5). There were no significant differences between the ventilatory responses to hypoxia under the two conditions in the other four subjects. Statistical analyses performed by using either a paired t-test, or by using the nonparametric Wilcoxon signed-rank test showed the same results in all five subjects.

The time constants for the fast and slow components ($\pm$ SD) of the ventilatory response to a step down in $P_{ET}CO_2$ averaged 11.5 $\pm$ 4.3 s and 149.6 $\pm$ 34.8 s

respectively (Table 2).

Statistical Power. The power (probability of rejecting the null hypothesis when it is false) of the t-test was estimated by:

$$\Phi(z_\alpha + | \mu_1 - \mu_0 | \sqrt{n}/\sigma)$$

where $\mu_0 = 0$ (Rosner, 1986). μ_1 and σ were estimated from the sample means and standard deviations. In subject 1569, the study had a 98% chance of detecting a significant difference between the hypoxic responses under the two conditions. In subjects 2097, 1643, 2374 and 2402, the chance of finding a significant difference was only 2.9% to 5.6%. Therefore, in these four subjects, an average of 260 repeats, of each of the three protocols, would have to be performed in order to detect a significant difference at a power of 84%. These numbers support the argument that no true difference exists between the $\dot{V}_E$ responses to hypoxia, in these four subjects.

Figure 4

The experimental results of protocols A (closed circle), B (open inverted triangles), and C (closed inverted triangles) for each of the five subjects. Top, $P_{ET}O_2$ (Torr); middle, $P_{ET}CO_2$ (Torr); bottom, $\dot{V}_E$ ($l \cdot min^{-1}$). Dashed lines mark the start and end of the hypoxic step.

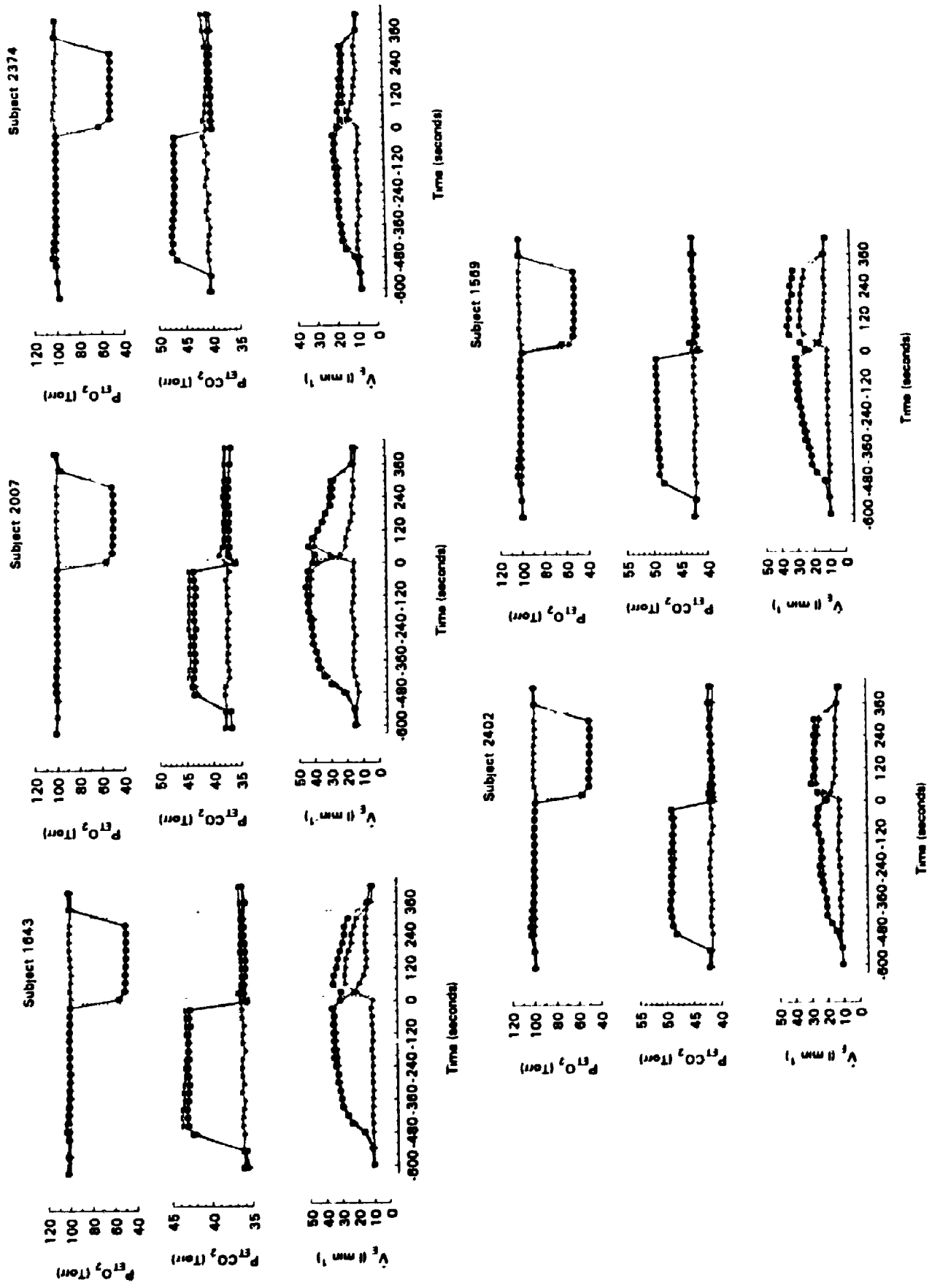

Subject 2374
Subject 2007
Subject 1643
Subject 1569
Subject 2402
$P_{ET}O_2$ (Torr)
$P_{ET}CO_2$ (Torr)
$\dot{V}_E$ (l min^{-1})
Time (seconds)
-600 -480 -360 -240 -120 0 120 240 360

Table 1. The ventilatory response to hypoxia when the central chemoreceptor is exposed to diminishing hypercapnia minus the hypoxic response under control conditions at successive time periods.

Time Period (s) (relative to hypoxic step)	Ventilatory Difference ($l \cdot min^{-1}$)				
	Subject 2007	Subject 1643	Subject 2374	Subject 2402	Subject 1569
-60	1.87	0.19	1.69	-1.23	1.72
-15	4.22	0.65	1.39	-1.85	2.97
15	1.36	-1.78	-0.08	-1.24	4.95*
45	-1.30	-0.33	0.47	-0.51	5.32*
75	-3.86	1.49	-0.32	-1.42	4.90
105	-3.03	2.09	0.32	-2.60	5.02
135	-1.65	1.64	-0.42	-1.02	4.73
165	-0.97	1.60	0.37	-2.31	5.91*
195	-2.21	1.48	0.40	-2.06	3.63
225	-1.86	0.35	-0.12	0.67	6.36*
255	-0.94	2.11	0.02	-0.51	4.70
285	-1.81	1.58	-0.03	1.21	5.47
+15	0.23	-0.36	-0.72	-1.19	-1.10

* Values are significantly different from zero ($P < 0.05$).

Figure 5

The differences between the hypoxic response 30 s after a step decrease in $P_{ET}CO_2$ and the hypoxic response during steady-state eucapnic breathing. Error bars show the 95% confidence intervals. Dashed lines mark the start and end of the hypoxic period. * $P < 0.05$.

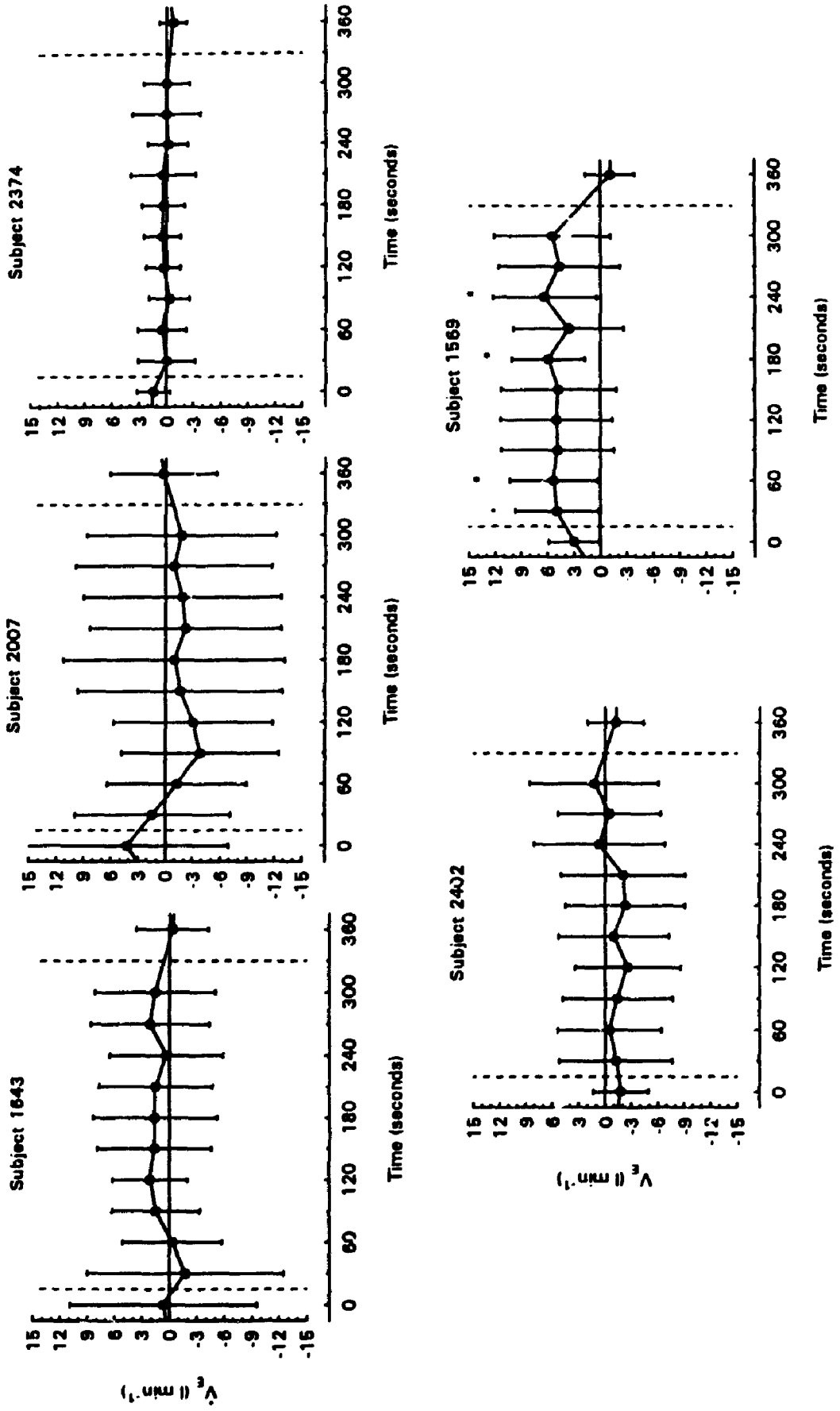

Subject 1643
Subject 2007
Subject 2374
Subject 2432
Subject 1569
Time (seconds)
$\dot{V}_E$ (l·min^{-1})

Table 2. Values for the estimated temporal parameters of the ventilatory response to a step down from hypercapnia.

Subject	G_c	G_p	τ_c	τ_p	T_c	T_p	Drift	RSS
1643	-2.9	-1.1	101.4	18.5	4.9	3.0	.002	258.0
2374	-1.7	-0.5	134.9	10.1	5.8	3.0	.001	80.0
2007	-2.9	-2.2	192.9	11.6	13.4	12.3	.002	347.5
2402	-1.4	-1.0	148.5	6.6	12.7	5.4	.001	267.9
1569	-1.9	-1.5	170.1	10.7	3.5	3.0	.002	62.3
Mean	-2.2	-1.3	149.6	11.5	8.0	5.3	.002	
SD	(0.7)	(0.6)	(34.8)	(4.3)	(4.6)	(4.0)	(.0004)	

Note: G_c and G_p, the central and peripheral gain terms in $l \cdot min^{-1} \cdot torr^{-1}$; τ_c and τ_p, the central and peripheral time constants in s; T_c and T_p, the time delays of the central and peripheral chemoreflex loops in s; Drift term in $l \cdot min^{-1}$.

2.5 Discussion

In four of the five subjects studied, the drives from the central and peripheral chemoreceptors were independent. The differences between the results of the current study and those of Robbins (1988) might be explained in part by the modifications to the administration of the protocols. The literature supports the findings that a five minute hypoxic exposure potentiates a subsequent hypercapnic test (Davidson and Cameron, 1985) and depresses hypoxic sensitivity (Easton et al., 1988) for up to one hour. Administering two protocols in the same breathing session had the effect of lowering the average response to hypoxia in C and increasing the average ventilatory response to hypercapnia in A or B, thus yielding a larger difference between A and C.

While subject 1569 showed some evidence for multiplicative interaction between the chemoreflexes, the ventilation remained higher throughout the hypoxic period in protocol A, rather than decreasing as central eucapnia was restored, as Robbins (1988) observed in his two subjects. It was likely, therefore, that some mechanism other than central-peripheral chemoreflex interaction was responsible for the augmented ventilatory response in protocol A.

One alternative explanation was described by Michel, Lloyd and Cunningnam (1966). They reported that the in vitro relationship between the pH and bicarbonate in true plasma does not apply in vivo when the P_ACO_2 is altered by CO_2 inhalation. CO_2 breathing gave way to a small metabolic as well as a respiratory acidosis due to distribution of bicarbonate across the extracellular fluid, and this persisted long after the inspired PCO_2 had been lowered. The acidemia was reduced by 20-40% during the first

10 min of the recovery period, but further recovery was a very slow process. Blood samples taken 20 to 30 min after the CO_2 had been lowered, revealed that the blood had not yet returned to its former acid-base condition. Due to the time course of the recovery period, Robbins (1988) concluded that this effect could not have influenced his results. If the metabolic acidemia was significant, the augmentation of hypoxic sensitivity at the peripheral chemoreceptors would last throughout the hypoxic, period as seen in subject 1569.

A major assumption of this study is that the 30 s interval was sufficient time for the discharge from the peripheral chemoreceptor to return to its resting level. If the CO_2 at the carotid bodies remained high for the duration of the hypoxic step, then the results would be complicated by the interaction between hypoxia and CO_2-H^+ at the peripheral chemoreceptor (Gabel and Weiskopf, 1975). The results in subjects 1643, 2007, 2374 and 2402 argue against such interaction. When a two component exponential model (Bellville et al., 1979) was used to estimate the temporal parameters of the ventilatory response to the step decrease in CO_2 in Protocol B, however, the average ($n = 5$) time constant of the fast ventilatory component was 11.5 ± 4.3 s, indicating that the peripheral chemoreceptor response to a step down in $P_{ET}CO_2$ would not be complete until approximately 16 s (4τ x 11.5 s = 46 s less the 30 s time interval between the hypercapnic and hypoxic steps) into the hypoxic step, in the experimental protocol A. It is also doubtful that peripheral interaction between hypercapnia and hypoxia could account for the increased hypoxic response to protocol A in subject 1569, as the time constant of the peripheral chemoreceptor response to a step down from hypercapnia was

10.7 s with a time delay of 3.0 s. Any interaction between hypoxia and H^+ would have decayed by 12 s into the hypoxic step in protocol A, when the carotid body response to a step down in $P_{ET}CO_2$ was complete.

A second critical assumption of this experimental design is that the PCO_2 at the central chemoreceptors remains high for some time after the hypoxic stimulus is introduced. The time constant of the central chemoreceptor response to a step down from hypercapnia averaged 150 s. These results are similar to those of previous studies (Dahan et al., 1990; Swanson and Bellville, 1975) and would suggest that the discharge from the central chemosensitive tissue was still significant for at least 2 min into the hypoxic step. In addition, the ventilation at 5 min post-hypercapnia in protocol B was 2.59 ± 1.56 l·min^{-1} higher ($P < 0.05$) than the pre-hypercapnia baseline, indicating that full recovery from the ventilatory response to hypercapnia had not yet occurred. The maintained increase in ventilation could be attributed either to slow changes in brain tissue or cerebrospinal fluid pH or to a continued neural afterdischarge (Eldridge and Gill-Kumar, 1980; Millhorn et al., 1980). The literature does not support the possibility that afterdischarge following the removal of hypercapnia in protocol A would be attenuated by central hypoxia (Engwall et al., 1994).

The effects of changes in P_aCO_2 and P_aO_2 on cerebral blood flow (CBF) complicate the interpretation of results of whole body studies of respiratory control. Ventilation and cerebral blood flow are intimately related because of the central role played by the circulation in controlling the chemical environment of the brain. Ventrolateral medullary surface blood flow is CO_2 sensitive (Feustal et al., 1984).

Increases and decreases in PCO_2 cause vasodilation and vasoconstriction of the arteriolar channels, respectively, and the associated changes in peripheral vascular resistance are responsible for the changes in cerebrovascular circulation time and the velocity of flow (Markwalder et al., 1984). The CBF response to step changes in PCO_2 are rapid with time constants in the order of 20 s (Severinghaus and Lassen, 1967). The faster ventilatory responses to a step increase in PCO_2 than to a step decrease in PCO_2 (Bellville et al., 1979; DeGoede et al., 1985) have been attributed to a lower blood flow in the recovery phase than in the hypercapnic phase (Bellville et al., 1979; Feustal et al., 1984). Cerebral blood flow has also been reported to be sensitive to changes in PO_2 in unanesthetized humans (Ellingsen et al., 1987; Kety and Schmidt, 1948). In this study, the increase in cerebral blood flow associated with a step down in PO_2 could facilitate the washout of CO_2 from the central chemosensitive area in the experimental protocol A, reducing the amount of time the central chemoreceptors would be exposed to high CO_2. In contrast to the rapid CBF response to changes in PCO_2, however, the response to hypoxia has a slow time course. While the time constant in experimental animals has been described to be 35 to 40 s (Doblar et al., 1979; Van Beek et al., 1986), the time constant in humans has been estimated to be approximately 6 min (Ellingsen et al., 1987). Therefore changes in CBF are unlikely to mask the presence of central-peripheral interaction in this study.

While the results of this study are contrary to those hypothesized, they are consistent with the most widely accepted model describing the interactions between chemical respiratory feedback stimuli (Bellville et al., 1979; Berkenbosch et al., 1992;

Dahan et al., 1990; Cunningham et al., 1986). In this model, hypoxia and the CO_2-H^+ complex interact at the level of the peripheral chemoreceptor and the drives from the periphery and from the central chemosensitive area add together in their effects on ventilation. The appropriateness of this model has been demonstrated in cats using the artificial brainstem perfusion technique (Van Beek et al., 1983). The evidence in humans is not as definitive due to the difficulty in isolating respiratory stimuli to a single chemosensitive site. The results of experiments using the technique of dynamic end-tidal forcing (Swanson and Bellville, 1974), and attempts to fit the ventilatory response to CO_2 during euoxia, using a two compartment exponential model, in which the equation was extended to incorporate the interaction parameter (Dahan et al., 1990) introduced by Robbins (1988), have failed to demonstrate any significant central-peripheral interaction.

The results of this study were contrary to those hypothesized based on the Robbins (1988) data, which appeared to advance the possibility of interaction between the central and peripheral chemoreceptors in man. Further work done in the Oxford laboratory, by Clement et al. (1992), using metabolic acidosis, generated by a brief bout of hard exercise, to selectively stimulate the peripheral chemoreceptors, and CO_2 inhalation as a stimulus common to both sets of chemoreceptors, also failed to support this theory. It was reported that the ventilatory sensitivity to hypoxia at matched arterial pH values was not significantly different between conditions of high (CO_2 inhalation) and low (metabolic acidosis) central chemoreceptor activity.

In conclusion, this study demonstrated that the central and peripheral chemoreflexes are independent of each other.

CHAPTER 3

PERIPHERAL CHEMOREFLEX DRIVE IN MODERATE INTENSITY EXERCISE

3.1 Abstract

The carotid bodies are effectively silenced by 100% O_2. Sustained hyperoxia (HO) is associated with a slight hyperventilation, attributed to a centrally mediated stimulation of ventilation ($\dot{V}_E$) by the hypercapnia that results from the primary fall in $\dot{V}_E$, and the acidifying effects of cerebral hypofusion on cerebral fluids. Increases in PO_2 have no effect on middle cerebral artery blood velocity when end-tidal CO_2 ($P_{ET}CO_2$) is kept constant. The purpose of this study was to examine the effect of sustained HO on the central (cR_c) and peripheral (pR_c) chemoreflex loops, during moderate intensity exercise, under conditions of constant and varying $P_{ET}CO_2$. Exercise was performed on a cycle ergometer at a work rate corresponding to 80% of ventilatory threshold. In protocol A, $P_{ET}CO_2$ was allowed to vary naturally. A 4 min HO step ($P_{ET}O_2$ = 600 Torr) was initiated after $\dot{V}_E$ had reached a steady state at the given work rate. In protocol B, the same HO step was given while $P_{ET}CO_2$ was clamped at the peak level measured during HO in A. Five subjects completed 4 repetitions of each of A and B . The step in to HO was characterized by a 15% decrease in $\dot{V}_E$, in both A and B, due to the silencing of the pR_c. In A, HO potentiated the output from the cR_c by 4.5 $l \cdot min^{-1}$, with a τ of 106.8 s, however the initial decline in $\dot{V}_E$ was maintained in B. The step out of HO, in A, was characterized by a rapid increase in $\dot{V}_E$ to a steady-state, at a level higher (1.2 $l \cdot min^{-1}$, P = 0.02) than pre-HO $\dot{V}_E$, despite a decreasing $P_{ET}CO_2$. The average $\dot{V}_E$ response was

best fit by a monoexponential model with a τ of 21.6 s, attributed to the pR_c. In B, the average post-HO $\dot{V}_E$ response, was best fit to a two-compartment model. The fast component, having a τ of 15.0 s, was attributed to the pR_c, and the slow component, with a τ of 345.2 s, was regarded to be due to the slow activation of the cR_c. The post-HO $\dot{V}_E$ in B, was not significantly different from the pre-HO $\dot{V}_E$ (1.5 $l \cdot min^{-1}$ greater, $P = 0.29$), however the τ indicated that $\dot{V}_E$ was still increasing, despite a constant $P_{ET}CO_2$, when the work load was reduced. The carotid bodies were found to provide about 15% of the drive to breathe in moderate intensity exercise, suggesting that the arterial chemoreceptors function to "fine tune" alveolar $\dot{V}$ to minimize change in arterial blood gases. It is suggested that even short periods of sustained hyperoxia alter respiratory control. Though isocapnia appeared to stabilize the cR_c drive during HO, the post-HO response indicated that the either the conventional stimuli, or the actual control mechanisms (CO_2 set-point or the sensitivity to CO_2) had been modified.

3.2 Introduction

The peripheral chemoreceptor is said to exert considerable influence on the kinetics of the ventilatory response to moderate exercise (Griffiths et al., 1986) and on the compensatory hyperventilation for the lactic acidosis of heavy exercise (Rausch et al., 1991), but serve a less important role during the steady-state of moderate exercise (Whipp, 1994). The best known stimuli to the carotid bodies (i.e. hypoxia, hypercapnia and acidity) remain essentially unchanged in light to moderate exercise, yet the peripheral chemoreceptors are said to account for up to 20% of the steady state drive (Ward, 1994b) which is the same or slightly less than above threshold estimates (Jeyaranjan et al., 1987; Rausch et al., 1991).

The Dejours O_2 test (1962) is the most widely utilized noninvasive method of measuring the contribution of the peripheral chemoreceptor to ventilation. This test is based on the assumption that the carotid bodies are effectively silenced by 100% O_2 (Miller et al., 1974). The magnitude of the transient ventilatory decline following the hyperoxic challenge is said to provide an index of previously existing carotid chemoreflex drive. The latency of the ventilatory decline is typically 2 to 3 breaths and the nadir is reached about 20 s later (Dejours, 1962; Whipp and Wasserman, 1980). The relative hypoventilation is said to be mediated by the carotid bodies based on the latency of the response (8 to 10 s at rest, 5 s in exercise), which corresponds with the lung-to-carotid body transit delay (as measured by ear oximetry as the latency of the S_aO_2 increase) (Dejours et al., 1957; Jeyaranjan et al., 1987), and the absence of any change in ventilation in response to hyperoxia in carotid body resected subjects (Whipp and

Wasserman, 1980).

Sustained hyperoxia, at rest, is associated with a slight hyperventilation compared with air breathing (Dejours et al., 1958), attributed to a centrally mediated stimulation of ventilation (Dejours, 1962) by the hypercapnia that results from the primary fall in $\dot{V}_E$, and the acidifying effects of cerebral hypofusion on cerebral fluids (Kety and Schmidt, 1948). A critical premise of hyperoxic tests, used to measure peripheral chemoreceptor drive, is that the true nadir of the initial ventilatory decline is expressed prior to the secondary central stimulating actions of hyperoxia on ventilation. If $\dot{V}_E$ begins to increase before the initial hypoventilatory response is complete, then the carotid body contribution to ventilatory drive will be underestimated.

Recent studies that measured cerebral blood flow noninvasively using transcranial Doppler have shown that increases in PO_2 had no effect on middle cerebral artery blood velocity when the end-tidal CO_2 was kept constant (Bew et al., 1994). The noninvasive technique of dynamic end-tidal forcings and the feedback method introduced by Swanson and Bellville (1975) and modified by Robbins et al. (1982b) can be used to clamp the end-tidal PCO_2 ($P_{ET}CO_2$) at a constant level.

The first objective of this study was to measure the gain of the peripheral chemoreflex, during moderate intensity exercise, using hyperoxic suppression of carotid body drive, while clamping the $P_{ET}CO_2$ at a constant level high enough to ensure that the hypoventilation associated with hyperoxia would not cause any further hypercapnia. The end-tidal clamp was instituted in an effort to stabilize the ventilatory drive from the central chemosensitive tissue by circumventing both the increase in $P_{ET}CO_2$, and the

changes in cerebral blood flow, associated with breathing hyperoxic gas mixtures.

The breathing of hyperoxic gas mixtures has been used to silence the carotid bodies, in order to isolate the central chemoreceptor (Dahan et al., 1990; Poulin et al., 1993), or gain an estimate of pre-existing peripheral chemoreflex drive (Jeyaranjan et al., 1987; Masuda et al., 1988; McLoughlin et al., 1993). The effect of hyperoxia itself on ventilatory control is rarely addressed. Dejours (1962) introduced the idea of studying the initial responses that follow the rupture of a physiological equilibrium. The variations in the organism's response to the disturbing agent, at the phase of maximal disruption, can be related directly to that disturbing agent. The secondary reactions that occur with prolonged application of the agent, however, will eventually terminate in a new state of equilibrium. Respiratory control could be altered by the sustained removal of carotid body drive. There is an acknowledged redundancy of ventilatory drives (Wasserman et al., 1986), one of which could potentially assume the role of the carotid body in its absence. The removal of the peripheral chemoreceptor could possibly change the setpoint about which P_aCO_2 is regulated, as is the case in carotid body resected subjects (Wade et al., 1970). A second objective of this study, therefore, was to investigate the effect of sustained hyperoxia on the central and peripheral chemoreflex loops, under conditions of constant and varying $P_{ET}CO_2$, by examining the time course of the ventilatory response to hyperoxia, as well as the response to the removal of hyperoxia.

3.3 Methods

Respiratory apparatus and gas analysis. The experimental set up and the technique used for accurate control of end-tidal gases was described in Chapter Two (pp.38-39).

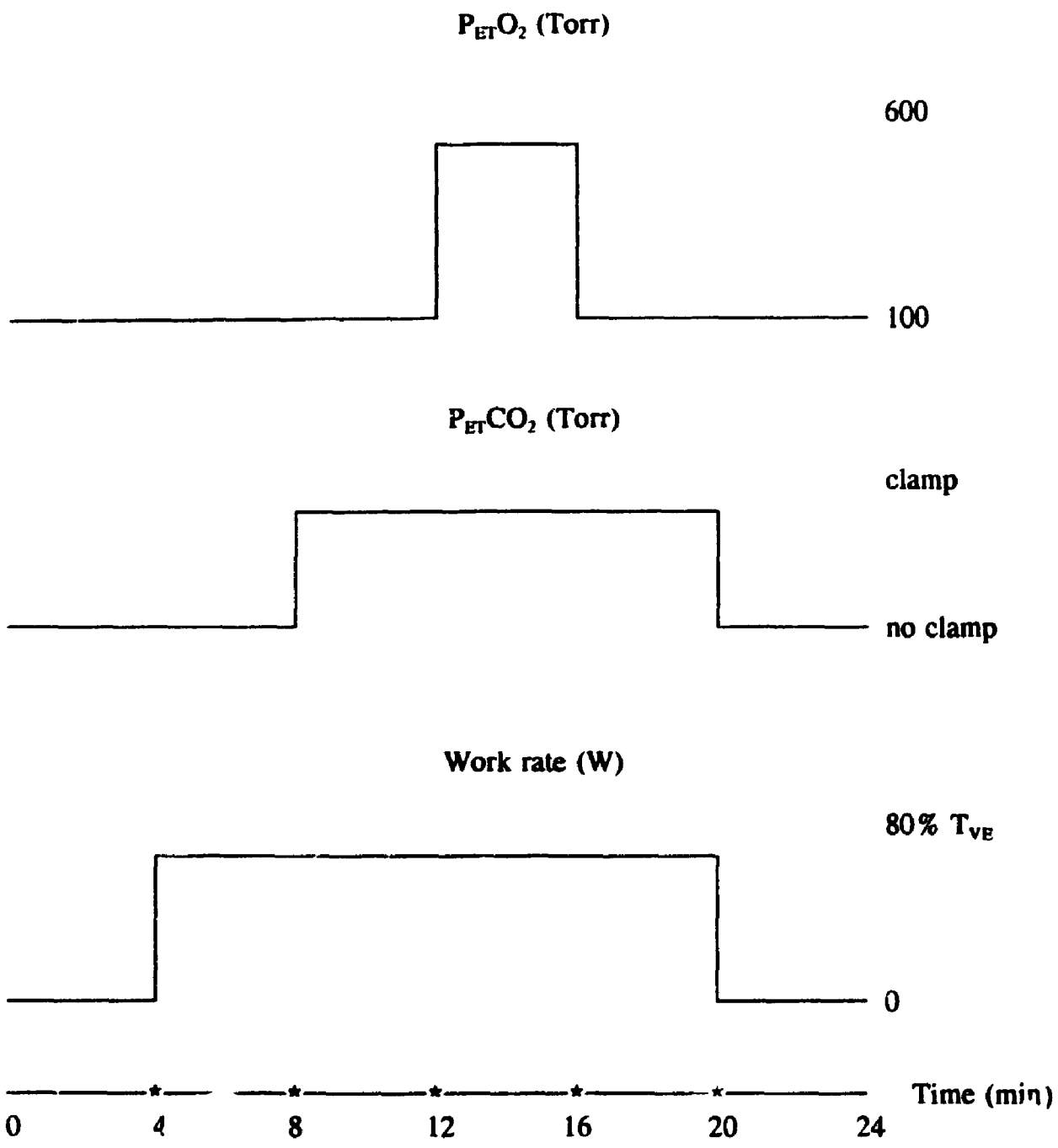
Subjects and Protocol. Five subjects ranging in age from 22 to 35 years were studied. All subjects were non-smokers with no history of cardiovascular or respiratory disease. The study requirements were fully explained (in written and verbal forms; Appendix III) to all participants, with each subject giving informed consent prior to volunteering to participate in the study. The research was approved by the University's Committee on Human Research.

Each participant underwent a ramp test on an electrically braked cycle ergometer to determine maximal oxygen uptake ($\dot{V}O_2max$) and ventilatory threshold (T_{VE}). T_{VE} was established as the $\dot{V}O_2$ at which the ventilatory equivalent for O_2 ($\dot{V}_E/\dot{V}O_2$) and $P_{ET}O_2$ began to increase systematically without the ventilatory equivalent for CO_2 ($\dot{V}_E/\dot{V}CO_2$) increasing and $P_{ET}CO_2$ decreasing simultaneously (Wasserman et al., 1973). The subjects performed a series of square wave exercise tests of 16 min duration from a background of loadless pedalling to a work rate corresponding to 80% of their T_{VE} in order to avoid the complexities associated with sustained lactic acidosis.

The study required four exercise protocols (one experimental and three control). A schematic of the main experimental protocol (B) is presented in Figure 6. In Protocol B, the $P_{ET}CO_2$ clamp was instituted four minutes into the workload step, at the peak $P_{ET}CO_2$ achieved during the hyperoxic period in Protocol A. $P_{ET}O_2$ was clamped at a level corresponding to 100 Torr. A 4 minute hyperoxic (600 Torr) step was initiated at

Figure 6

Schematic representation of the main experimental protocol (B). A step increase in workrate occurred from a baseline of loadless pedalling to a work rate corresponding to 80% of ventilatory threshold for 16 minutes. At minute 4 of the increased work rate, the $P_{ET}CO_2$ was clamped at the peak $P_{ET}CO_2$ achieved during the hyperoxic period in the control Protocol A. $P_{ET}O_2$ was clamped at 100 Torr. A 4 minute hyperoxic (600 Torr) step was initiated at minute 8 of the exercise step. The end-tidal clamping was removed at the same time as the step down in work rate to loadless pedalling.



PROTOCOL A: Same as B but without the $P_{ET}CO_2$ clamp.

PROTOCOL B: Experimental protocol (as illustrated).

PROTOCOL C: Exercise only, no end-tidal clamping.

PROTOCOL D: Same as A but without the hyperoxic step.

minute 8 of the exercise step. The end-tidal clamping was removed at the same time as the step down in work rate to loadless pedalling. Protocol A was the same as B except $P_{ET}CO_2$ was not clamped, but allowed to vary naturally. In Protocol C, $P_{ET}CO_2$ and $P_{ET}O_2$ were not clamped and the subject was breathing room air. The purpose of this protocol was to establish the normal ventilatory response to the exercise load, and to ensure that a steady state was maintained. In Protocol D, $P_{ET}CO_2$ was clamped as in Protocol A and $P_{ET}O_2$ was maintained at 100 Torr. The purpose of this protocol was to ensure there is no drift in ventilation due to the $P_{ET}CO_2$ clamp.

Data Analysis. Each subject contributed 4 sets of data to the hyperoxic protocols A and B, and 2 sets of data to the control protocols C and D. For each subject, the breath-by-breath data from each test were interpolated over one second intervals and all tests for a given protocol were ensemble-averaged to increase the signal to noise ratio. For each of the unclamped (A) and clamped (B) protocols, the pre-hyperoxic $\dot{V}_E$ was an average taken over the last 30 seconds prior to the hyperoxic step. The data was then averaged over 5 s intervals and the nadir was observed in all subjects in the 20-30 s interval after the step up into hyperoxia. The average relative gain of the peripheral chemoreceptor response to hyperoxia in protocol A was compared to the average relative gain of the response in protocol B using Student's paired t-tests. The level of significance was $P < 0.05$. The comparisons were also made using a Wilcoxon signed-rank test, the nonparametric analogue to the paired t-test (Rosner, 1986).

The temporal profiles of the ventilatory responses to the step in to hyperoxia, and the step out of hyperoxia, were examined by fitting mathematical models to the averaged

data for each subject. Comparisons were made using paired t-tests ($P < 0.05$) and the nonparametric Wilcoxon signed-rank test.

Mathematical Modelling. The data for each subject were examined to determine whether the nadir of the hypoventilatory response occurred prior to the subsequent stimulation of $\dot{V}_p$, by estimating the magnitude of the predicted $\dot{V}_E$ nadir from the best fit to the corresponding averaged $\dot{V}_E$ response (Ward, 1994b), using a single exponentially declining model of the form:

$$\dot{V}_p(t) = G_p(1 - e^{-(t-Tp)/\tau p}),$$

where $\dot{V}_p$ is the output of the peripheral chemoreflex loop. G_p is the gain of the peripheral chemoreceptor. T_p and τ_p are the time delay and the time constant of the best-fit response respectively (Whipp et al., 1982). To obtain optimal parameter estimation, a computerized optimization routine was applied. All combinations between 1 and 25 s, with increments of 0.1 s were used. The minimum time delays were chosen to be 1 s and τ_p was constrained to be at least 0.3 s based on previous studies (Bellville et al., 1979; Dahan et al., 1990).

The secondary increase in $\dot{V}_E$ in response to hyperoxia, in the poikilocapnic protocol (A), was best fit to a monoexponential model of the form:

$$\dot{V}_c(t) = G_c(1 - e^{-(t-Tc)/\tau c}),$$

where $\dot{V}_c$ is the output of the central chemoreflex loop. G_c is the gain of the central chemoreceptor. T_c and τ_c are the time delay and the time constant of the best-fit response respectively. The model was fit from the time corresponding to the nadir of the initial $\dot{V}_E$ decline.

The average $\dot{V}_E$ response to the step out of hyperoxia in protocol A (poikilocapnia) was best fit to a monoexponential model of the form:

$$\dot{V}(t) = G(1 - e^{-(t-T)/\tau}).$$

The average post-hyperoxic $\dot{V}_E$ response in the isocapnic protocol (B), was best fit to a two-compartment model of the form:

$$\dot{V}_E(t) = \dot{V}_b + \dot{V}_s(t) + \dot{V}_f(t)$$

where,

$$\dot{V}_s(t) = G_s(1 - e^{-(t-Ts)/\tau s})$$

and,

$$\dot{V}_f(t) = G_f(1 - e^{-(t-Tf)/\tau f})$$

$\dot{V}_b$ is the baseline ventilation and $\dot{V}_E(t)$ is the time-dependent variation in $\dot{V}_E$. The parameters G_s, τ_s and T_s are the gain, time constant, and time delay of the slow component of the response respectively. The parameters G_f, τ_f and T_f are the gain, time constant, and time delay of the fast component, respectively.

3.4 Results

The physical characteristics of the subjects and the results of the ramp exercise tests are presented in Table 3. The work rate for the square wave exercise tests corresponded to 80% of T_{VE} and ranged from 60 W to 100 W in the five subjects.

Group means of the ventilatory and $P_{ET}CO_2$ responses to protocols A and B are shown in Figure 7, and to protocols C and D in Figure 8. The mean responses were interpolated over 1 s intervals and represent an ensemble average of the mean responses of each subject, each of which is an ensemble average of 4 repetitions in protocols A and B, and 2 repetitions in protocols C and D. All subjects achieved a steady state $\dot{V}_E$ in response to the work rate (control protocol C) within 4 min. The average $\dot{V}_E$ in the 30 s interval surrounding minute 4 (40.98 $l \cdot min^{-1}$) was not significantly different from the $\dot{V}_E$ in the last 30 s interval of the work rate function (41.36 $l \cdot min^{-1}$). There was no drift in ventilation due to the $P_{ET}CO_2$ clamp in control protocol D. The average $\dot{V}_E$ in the 30 s interval surrounding minute 4 of the clamping function (54.50 $l \cdot min^{-1}$) was not significantly different from the $\dot{V}_E$ in the last 30 s of the $P_{ET}CO_2$ clamp (53.90 $l \cdot min^{-1}$).

In protocol A (poikilocapnia), $\dot{V}_E$ declined following the step into hyperoxia and then began to drift upward, whereas the decline in $\dot{V}_E$ was maintained in protocol B (isocapnia). The mean decrease in $\dot{V}_E$ was not significantly different between protocols A (16.08 $\pm$ 5.02%) and B (14.90 $\pm$ 4.41%) (Table 4). Statistical analyses performed by using either a paired t-test, or by using the nonparametric Wilcoxon signed-rank test showed the same results. The nadir of the individual $\dot{V}_E$ response to hyperoxia determined using the 5 s averages, were not different from the nadirs in the second-by

second data ($P < 0.05$) in both protocols (A and B). The mean difference between the $\dot{V}_E$ nadir in the 5 s data and the $\dot{V}_E$ nadir in the 1 s data was 0.81 $l \cdot min^{-1}$ in the poikilocapnic protocol (A), and 0.52 $l \cdot min^{-1}$ in the isocapnic protocol (B).

The step out of hyperoxia, in the unclamped protocol (A) was characterized by a rapid increase in $\dot{V}_E$ to a steady-state, at a level higher (1.15 $l \cdot min^{-1}$) than the pre-hyperoxic $\dot{V}_E$, despite a decreasing $P_{ET}CO_2$ (Figure 9). The post-hyperoxic $\dot{V}_E$ in protocol B, was not significantly different from the pre-hyperoxic $\dot{V}_E$ (1.51 $l \cdot min^{-1}$), however the $\dot{V}_E$ was still increasing, despite a constant $P_{ET}CO_2$, when the work load was reduced (Figure 9).

TABLE 3. Subject characteristics

Subject	Sex	Age (years)	Height (cm)	Weight (kg)	$\dot{V}O_{2max}$ (l/min)	T_{VE} (%$\dot{V}O_{max}$)	Work rate (W)
2410	F	25	160	50	2.1	72	60
2464	F	24	170	57	2.8	75	100
1643	M	35	170	78	3.6	67	100
2374	M	22	170	64	2.4	62	60
2375	M	22	182	89	4.5	50	80
Mean		25.6	170.4	67.6	3.1	65.2	80
± SD		5.4	7.8	15.8	1.0	9.8	20

Figure 7

Average ventilatory, $P_{ET}CO_2$ and $P_{ET}O_2$ responses to the poikilocapnic protocol, A (left) and to the isocapnic protocol, B (right). The mean responses were interpolated over 1 s intervals and represent the mean responses of each subject, each of which is an ensemble average of 4 repetitions. The dashed lines mark the start and end of the 4 min hyperoxic period.

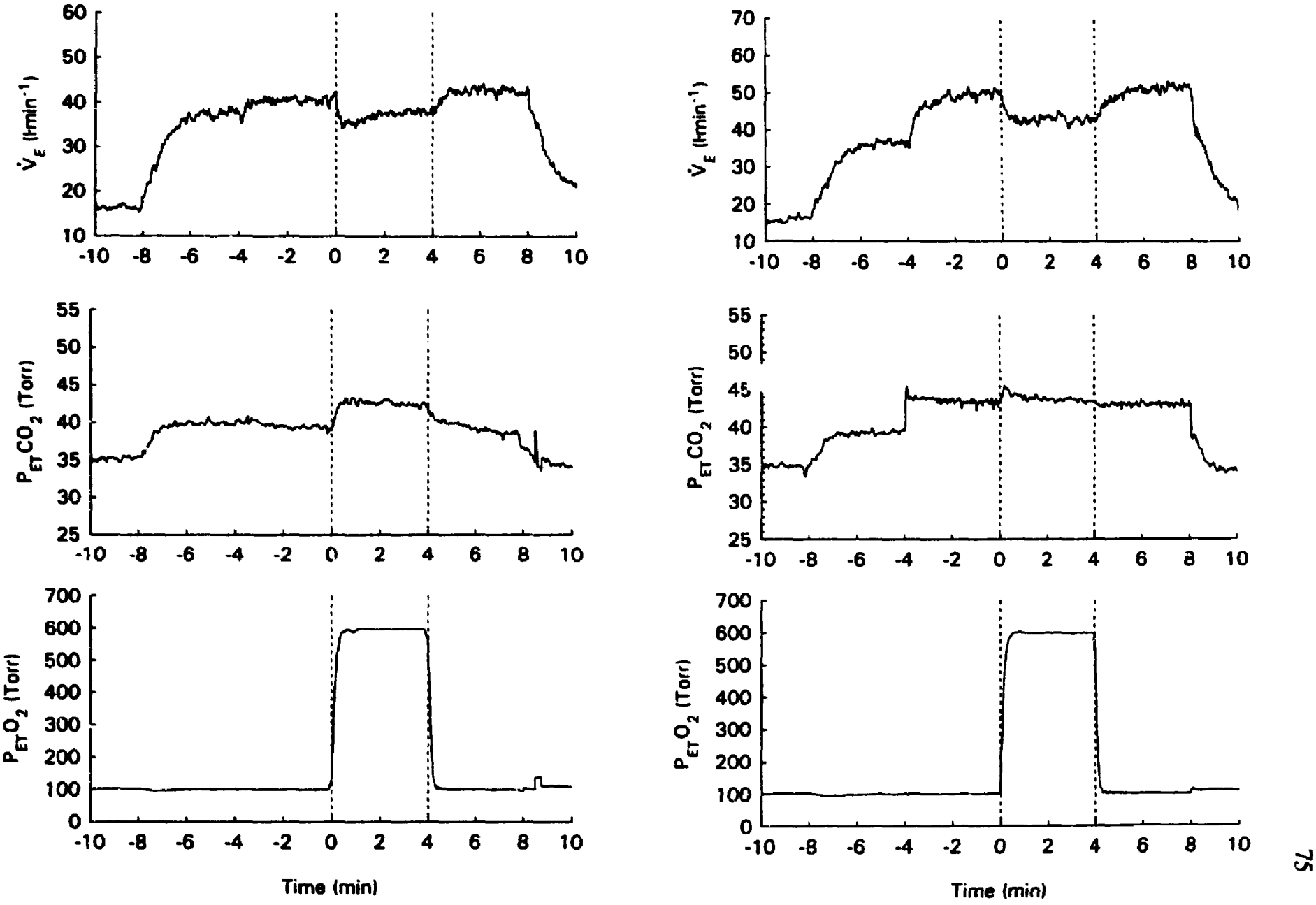
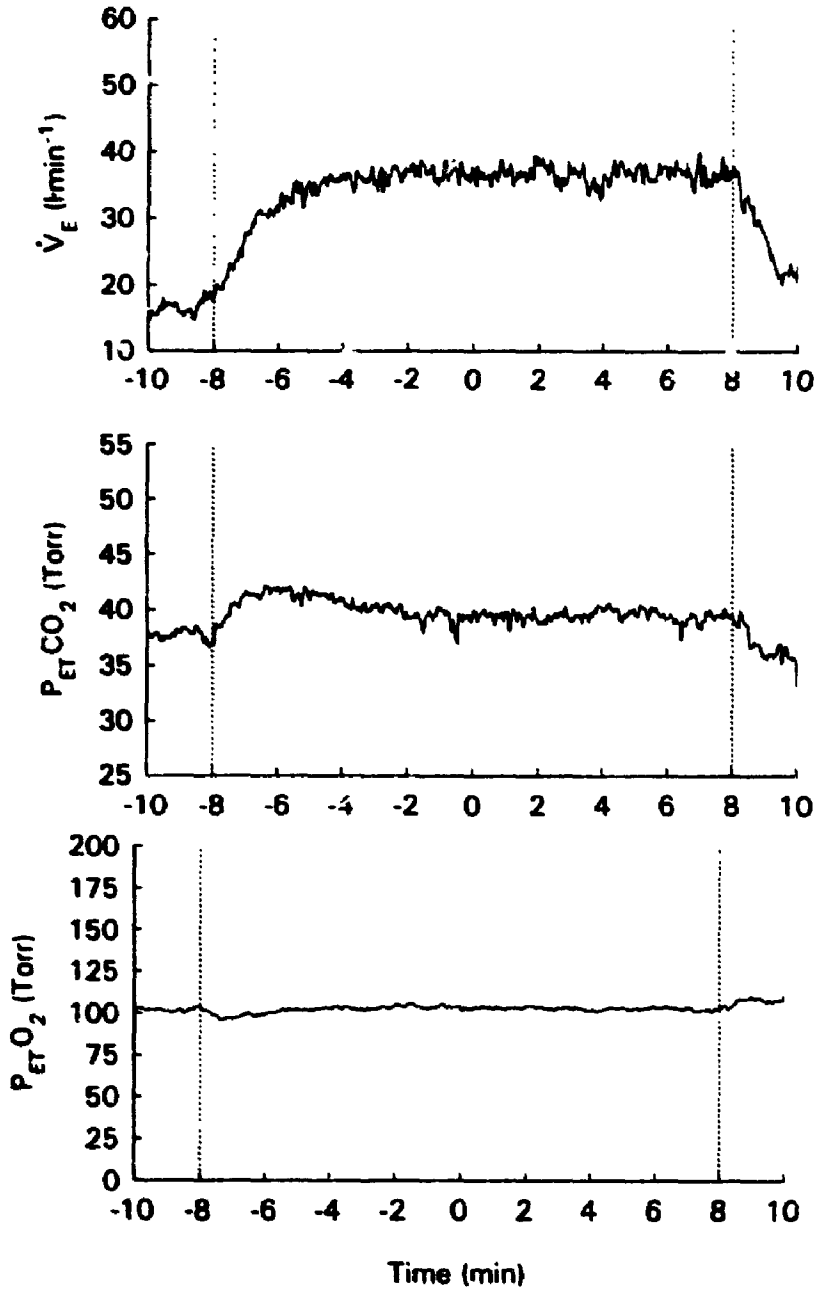

$\dot{V}_E$ (lmin^{-1})
$P_{ET}CO_2$ (Torr)
$P_{ET}O_2$ (Torr)
Time (min)
$\dot{V}_E$ (lmin^{-1})
$P_{ET}CO_2$ (Torr)
$P_{ET}O_2$ (Torr)
Time (min)

Figure 8

Average ventilatory, $P_{ET}CO_2$ and $P_{ET}O_2$ responses to the control protocols C (left) and D (right). The mean responses were interpolated over 1 s intervals and represent the mean responses of each subject, each of which is an ensemble average of 2 repetitions. The vertical dotted lines in protocol C indicate the start and end of the work load function. The vertical dotted lines in control protocol D mark the start and end of the $P_{ET}CO_2$ clamp.


$\dot{V}_E$ (lmin^{-1})
60
50
40
30
20
10
-10 -8 -6 -4 -2 0 2 4 6 8 10
$P_{ET}CO_2$ (Torr)
55
50
45
40
35
30
25
-10 -8 -6 -4 -2 0 2 4 6 8 10
$P_{ET}O_2$ (Torr)
200
175
150
125
100
75
50
25
0
-10 -8 -6 -4 -2 0 2 4 6 8 10
Time (min)

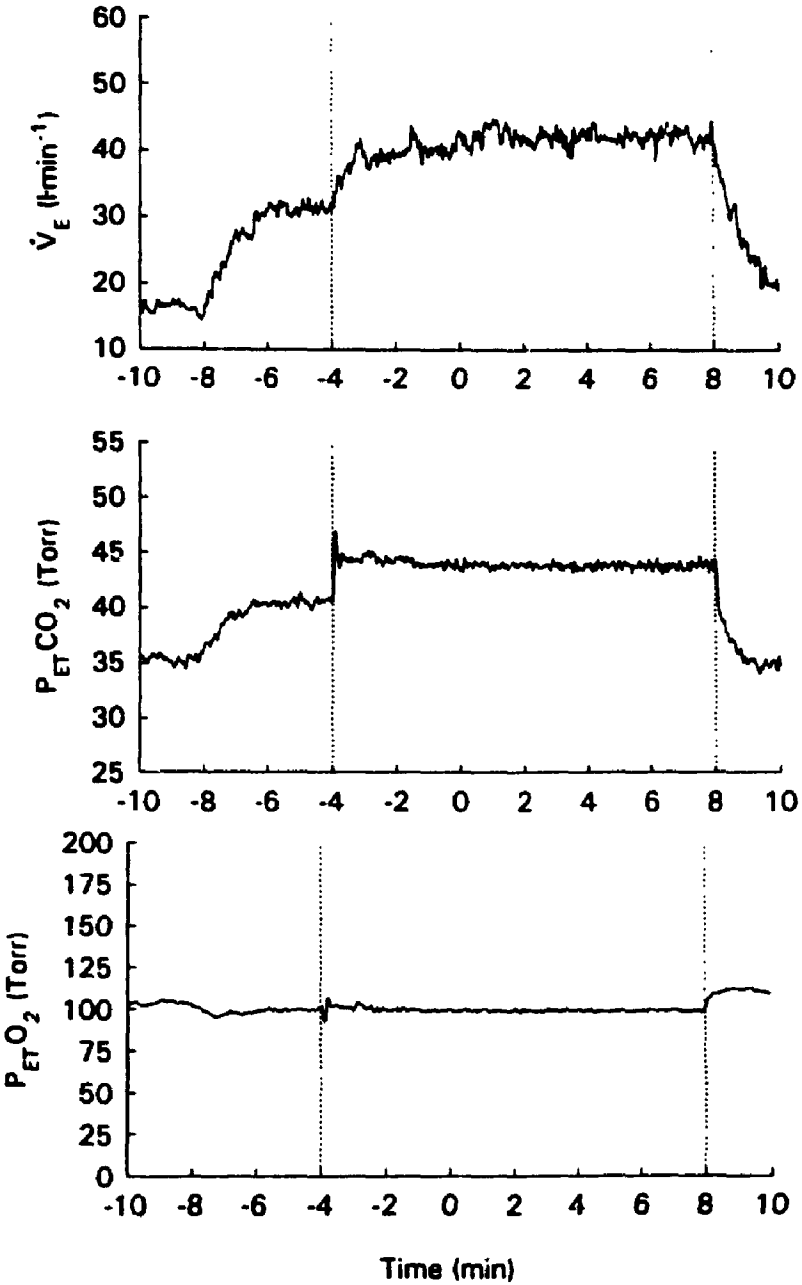
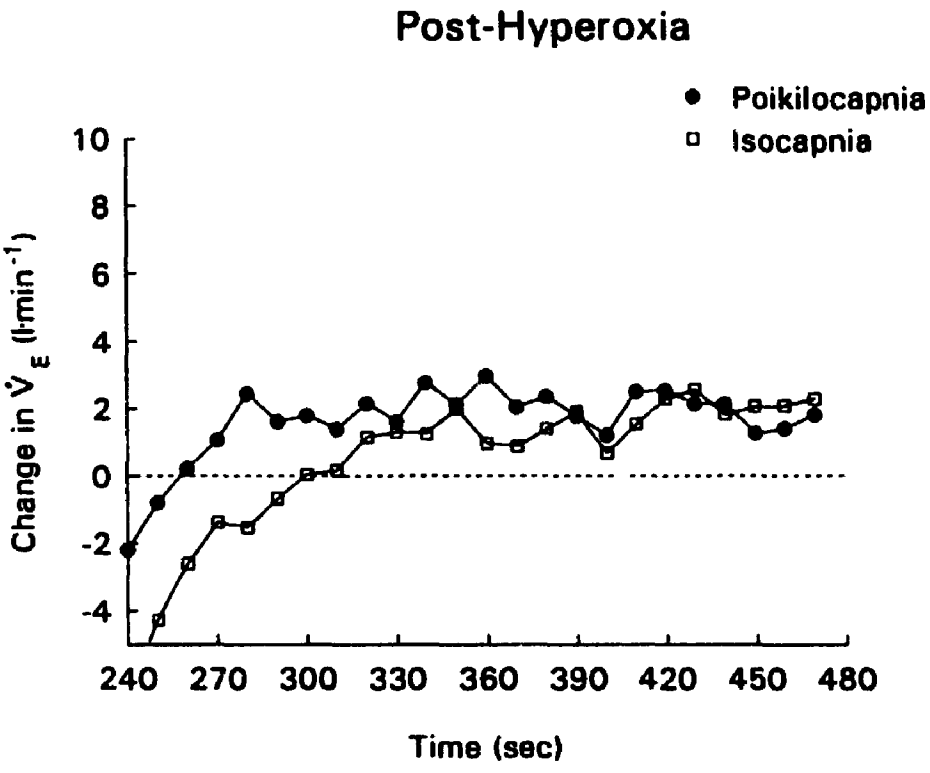

$\dot{V}_E$ (lmin^{-1})
60
50
40
30
20
10
-10 -8 -6 -4 -2 0 2 4 6 8 10
$P_{ET}CO_2$ (Torr)
55
50
45
40
35
30
25
-10 -8 -6 -4 -2 0 2 4 6 8 10
$P_{ET}O_2$ (Torr)
200
175
150
125
100
75
50
25
0
-10 -8 -6 -4 -2 0 2 4 6 8 10
Time (min)

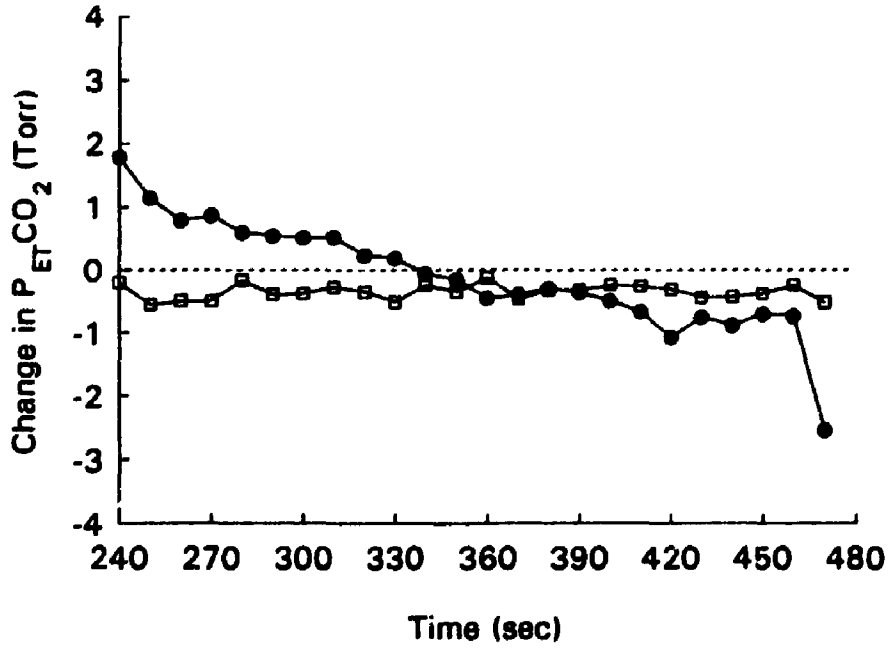
Table 4. Magnitude of the ventilatory decline in response to hyperoxia

ID#	Poikilocapnia			Isocapnia		
	$\dot{V}_E$ pre $l \cdot min^{-1}$	$\dot{V}_E$ nadir $l \cdot min^{-1}$	% change	$\dot{V}_E$ pre $l \cdot min^{-1}$	$\dot{V}_E$ nadir $l \cdot min^{-1}$	% change
2410	33.83	30.66	9.37	39.75	35.35	11.00
2464	57.43	44.82	21.95	74.01	57.55	22.24
2375	34.89	30.21	13.41	37.91	33.41	11.87
2374	25.93	21.83	15.81	34.96	29.87	14.56
1643	51.66	41.41	19.84	61.72	52.63	14.73
Mean	40.75	33.79	16.08	49.67	41.76	14.90
± SD	13.22	9.30	5.02	17.25	12.45	4.41

Figure 9

The average (n = 5) difference between the post-hyperoxic $\dot{V}_E$ and the steady-state pre-hyperoxic $\dot{V}_E$ (upper panel) and between the post-hyperoxic $P_{ET}CO_2$ and the steady state pre-hyperoxic $P_{ET}CO_2$ (lower panel) in the poikilocapnic (A) and isocapnic (B) protocols.


Post-Hyperoxia
Poikilocapnia
Isocapnia
Change in $\dot{V}_E$ (l·min^{-1})
10
8
6
4
2
0
-2
-4
240
270
300
330
360
390
420
450
480
Time (sec)


Change in $P_{ET}CO_2$ (Torr)
4
3
2
1
0
-1
-2
-3
-4
240
270
300
330
360
390
420
450
480
Time (sec)

Mathematical Modelling. The average relative gains in protocol A (15.8 ± 5.8 %) and protocol B (14.7 ± 5.2 %) listed in Table 5, were not different from those visually determined (Table 4). If an inappropriately high value of $\dot{V}_E$ was selected for the predicted nadir, the $\dot{V}_E$ response would not conform to a monoexponential but would yield a downwardly curving decline when expressed semilogarithmically with respect to time (Ward, 1994b). In all subjects, the averaged data, in both protocols A and B, conformed to the monoexponential model, yielding a linear semilogarithmic decline with respect to time (Figure 10), providing support for the finding that in both the unclamped and clamped procedures a true nadir in $\dot{V}_E$ was achieved prior to the central stimulation of ventilation. Typical ventilatory responses with model fits and residuals are shown for subject 1643 in the poikilocapnic and isocapnic protocols (Figure 11). The estimated parameters are listed in Table 5. The remainder of the individual ventilatory responses, model fits and residuals are shown in Appendix VII.

In the unclamped protocol (A), the initial hypoventilatory response to hyperoxia was followed by a slowly developing potentiation of the output from the central chemoreceptor with an average τ of 106.8 s (Table 6). A typical ventilatory response with model fit and residuals is shown for subject 2464 (Figure 12). The remainder of the individual ventilatory responses, model fits and residuals are shown in Appendix VIII. The decline in $\dot{V}_E$ was maintained in B in four of the five subjects. In subject 2464 the $\dot{V}_E$ drifted up by 2.40 $l \cdot min^{-1}$ from the predicted nadir over the 4 minute period of hyperoxia (see Appendix VII).

The average $\dot{V}_E$ response, in all 5 subjects, to the step out of hyperoxia in

protocol A was best fit to a monoexponential model with a τ of 21.6 s (Table 7). The average post-hyperoxic $\dot{V}_E$ response in the clamped protocol (B), was best fit to a two-compartment model. Typical ventilatory responses with model fits and residuals are shown for subject 1643 in the poikilocapnic and isocapnic protocols (Figure 13). The estimated parameters are listed in Tables 7 and 8. The remainder of the individual ventilatory responses, model fits and residuals are shown in Appendix IX. The τ_1 indicated that $\dot{V}_E$ was still increasing when the work load was reduced.

Statistical Power. The power (probability of rejecting the null hypothesis when it is false) of the t-test was estimated by:

$$\Phi(z_{\alpha/2} + | \mu_1 - \mu_0 | \sqrt{n}/\sigma)$$

where $\mu_0 = 0$ (Rosner, 1986). μ_1 and σ were estimated from the sample means and standard deviations. The study had an 18% chance of detecting a significant difference between the poikilocapnic and isocapnic protocols, in the magnitude of the change in $\dot{V}_E$ in response to hyperoxia (Table 4). The chance of finding a significant difference between the poikilocapnic and isocapnic protocols, in the relative gain of the peripheral chemoreflex (Table 5), was 23%. It is not surprising, given the low power, that the test did not find a significant difference between the two conditions. If the sample mean and standard deviation are appropriate estimates of the population μ and σ, the sample size would have to be increased to 40 to detect a significant difference at a power of 84%. These numbers support the argument that no true difference exists between the poikilocapnic and isocapnic $\dot{V}_E$ responses to hyperoxia.

Table 5. Values for the estimated temporal parameters of the ventilatory response to hyperoxia.

	Poikilocapnia					Isocapnia				
ID#	V_B	G_p	%	τ_p	T_p	V_B	G_p	%	τ_p	T_p
	$l \cdot min^{-1}$	$l \cdot min^{-1}$	G_p/V_B	s	s	$l \cdot min^{-1}$	$l \cdot min^{-1}$	G_p/V_B	s	s
2410	33.8	3.1	9.1	8.5	1.5	40.8	4.0	9.9	3.0	1.3
2464	56.0	13.0	23.2	4.2	1.0	72.8	16.8	23.0	13.0	1.1
2375	34.3	3.9	11.3	16.5	1.6	38.0	4.0	10.5	3.9	1.0
2374	26.1	4.1	15.8	12.0	5.6	35.5	5.3	15.0	6.6	1.0
1643	50.2	9.9	19.8	27.0	1.0	61.7	9.2	15.0	8.6	1.1
Mean	40.1	6.8	15.8	13.6	2.1	49.8	7.9	14.7	7.0	1.1
± SD	12.5	4.4	5.8	8.7	1.9	16.6	5.4	5.2	4.0	0.1

Note: V_B, the baseline V_E; G_p, the peripheral gain term; % = $(G_p/V_B) \times 100$; τ_p, the peripheral time constant; T_p, the time delay of the peripheral chemoreflex loop.

Figure 10

The best fit $\dot{V}_E$ response to poikilocapnic hyperoxia expressed semilogarithmically with respect to time. In all five subjects, the predicted nadir (from the best fit model) was not different from the observed nadir.

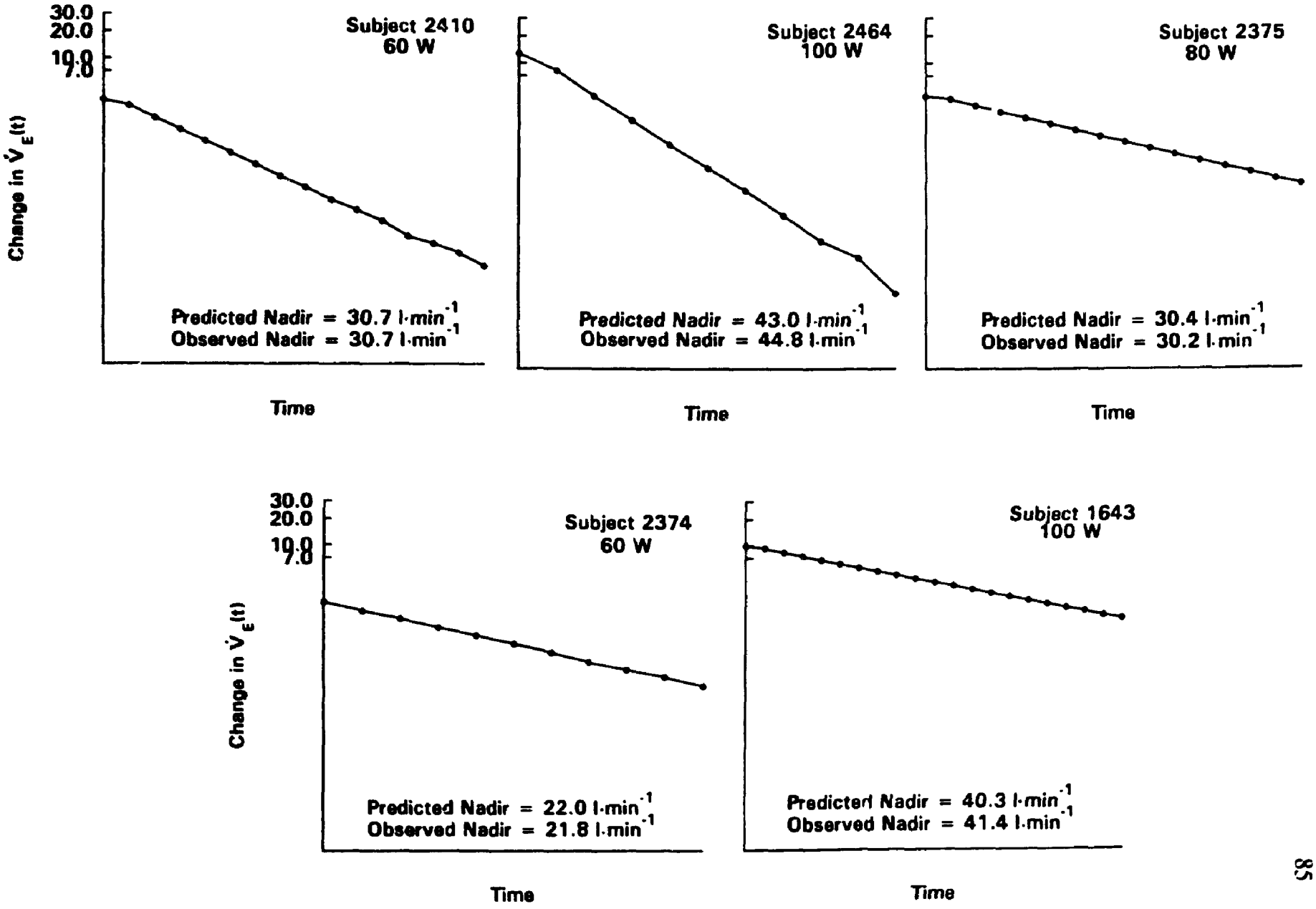
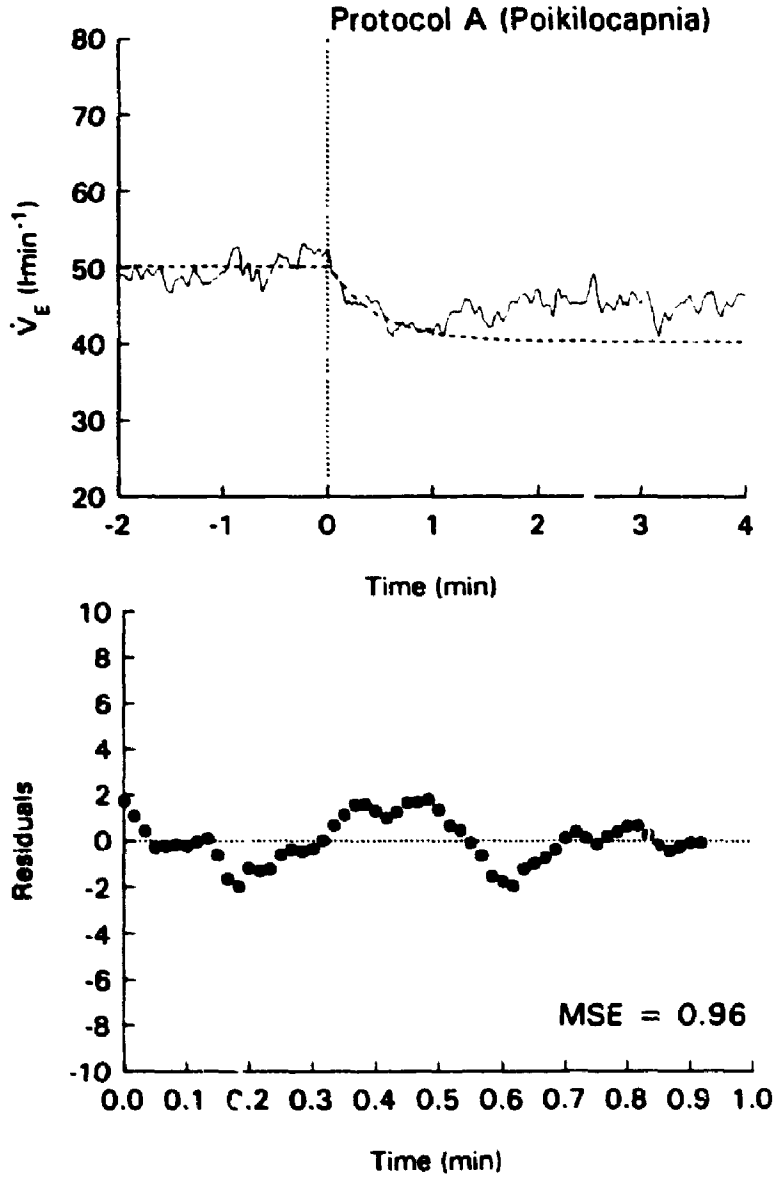

30.0
20.0
10.0
7.0
Change in $\dot{V}_E(t)$
Subject 2410
60 W
Predicted Nadir = 30.7 l·min^{-1}
Observed Nadir = 30.7 l·min^{-1}
Time
Subject 2464
100 W
Predicted Nadir = 43.0 l·min^{-1}
Observed Nadir = 44.8 l·min^{-1}
Time
Subject 2375
80 W
Predicted Nadir = 30.4 l·min^{-1}
Observed Nadir = 30.2 l·min^{-1}
Time
30.0
20.0
10.0
7.0
Change in $\dot{V}_E(t)$
Subject 2374
60 W
Predicted Nadir = 22.0 l·min^{-1}
Observed Nadir = 21.8 l·min^{-1}
Time
Subject 1643
100 W
Predicted Nadir = 40.3 l·min^{-1}
Observed Nadir = 41.4 l·min^{-1}
Time

Figure 11

The average ventilatory response (solid line) to the hyperoxic challenge in the protocol A and protocol B (upper panel) in subject 1643. The vertical dotted line marks the start of the 4 min hyperoxic step. The best fit single exponentially declining model (dashed line) is superimposed on the corresponding averaged $\dot{V}_E$ response. The residuals are graphed in the lower panel.

SUBJECT1643



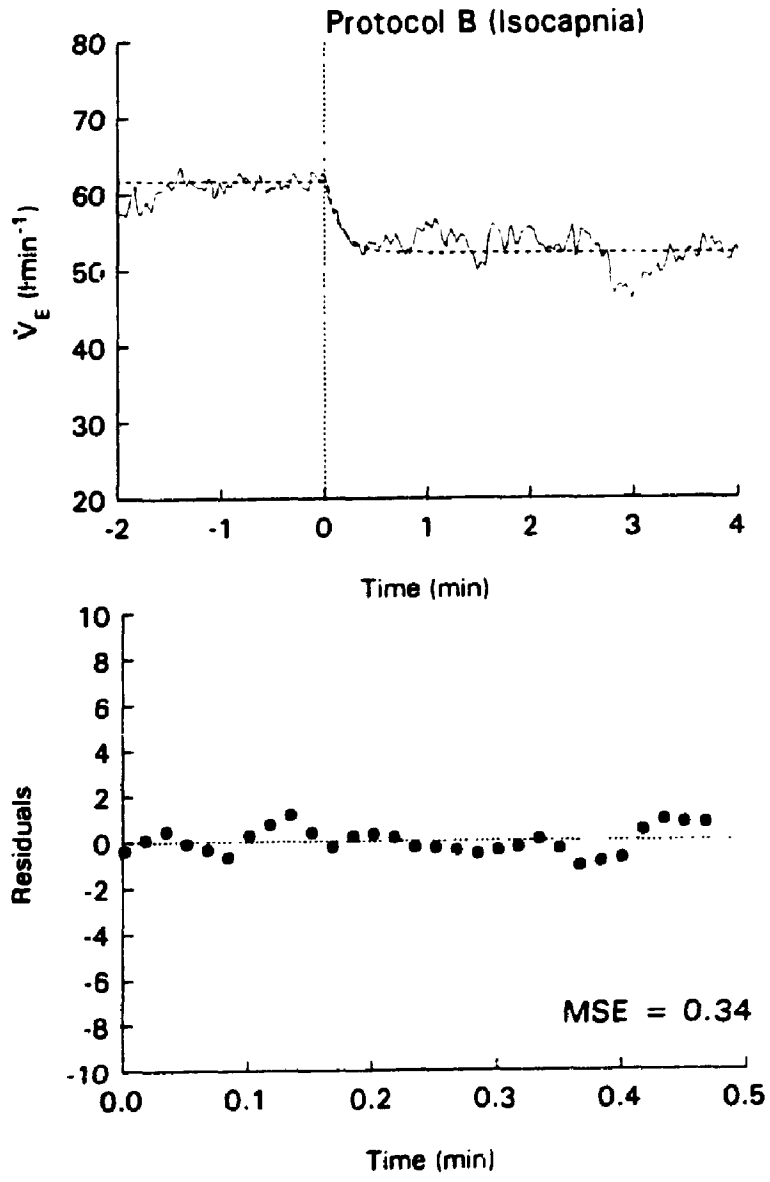


Table 6. Estimated temporal parameters of the secondary increase in $\dot{V}_E$ during poikilocapnic hyperoxia.

ID#	V_B ($l \cdot min^{-1}$)	Gain ($l \cdot min^{-1}$)	% change	τ (s)	T (s)
2410	31.0	4.0	12.9	299.4	9.0
2464	44.4	9.0	20.2	33.4	7.3
2375	30.7	1.7	5.5	54.0	4.4
2374	22.9	3.9	16.9	94.9	3.3
1643	41.8	3.8	9.0	52.2	6.1
Mean	34.2	4.5	12.9	106.8	6.0
± SD	8.8	2.7	5.9	110.0	2.2

Note: The response was modelled from the nadir of the initial drop in $\dot{V}_E$ in hyperoxia. V_B, baseline $\dot{V}_E$; % change = (Gain/V_B) x 100; τ and T, the time constant and time delay of the response respectively.

Figure 12

The best-fit single exponential model fit (dashed line) to the secondary increase in $\dot{V}_E$ in subject 2464. The response was modelled from the nadir in the initial decline in $\dot{V}_E$ (t = 0.35 s). The residuals are graphed in the lower panel (RSS = 53.72).

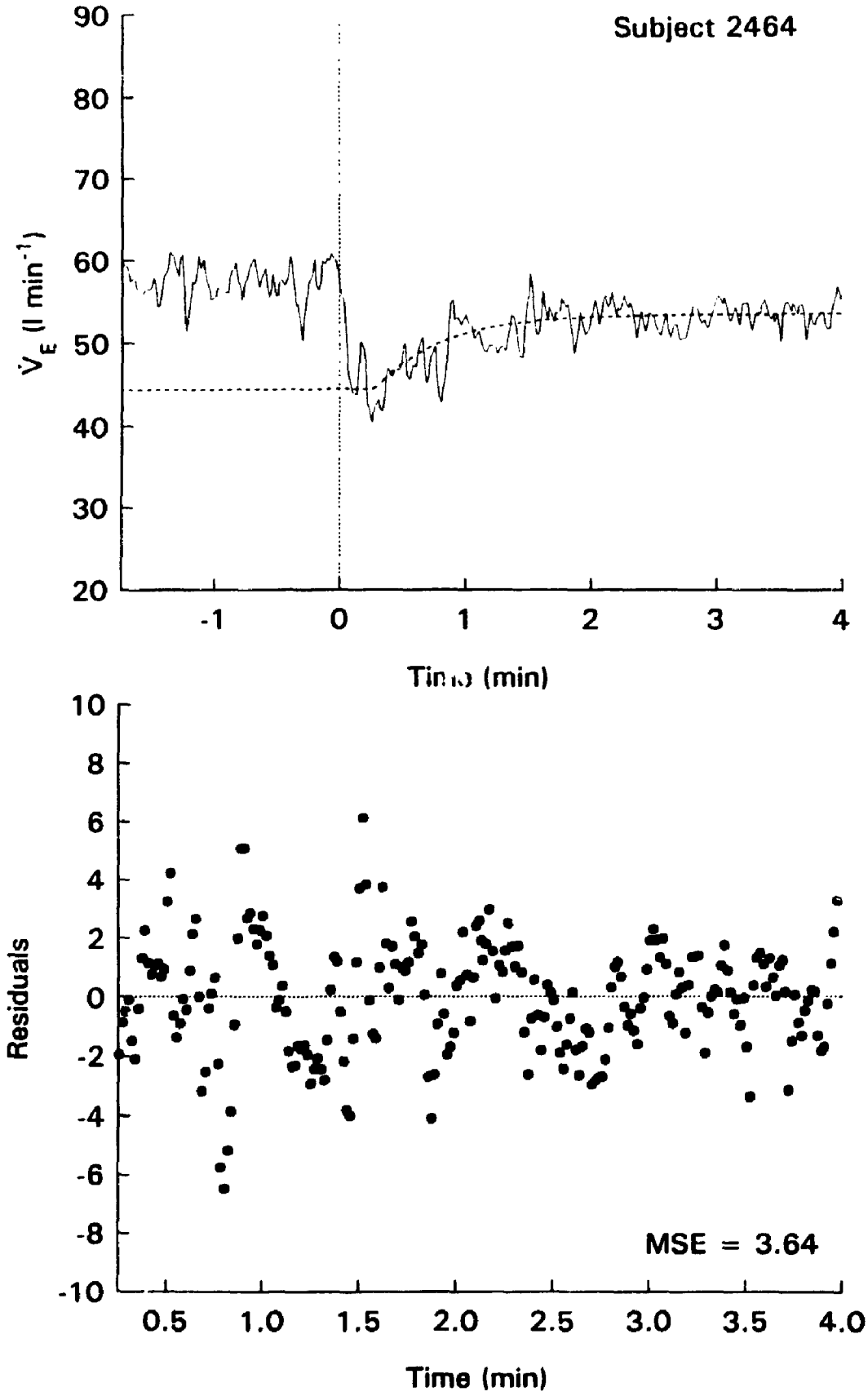

Subject 2464
$\dot{V}_E$ (l min^{-1})
90
80
70
60
50
40
30
20
-1
0
1
2
3
4
Time (min)
Residuals
10
8
6
4
2
0
-2
-4
-6
-8
-10
MSE = 3.64
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
Time (min)

Table 7. Estimated temporal parameters of the ventilatory response to the step out of poikilocapnic hyperoxia.

ID#	V_B $l \cdot min^{-1}$	Gain $l \cdot min^{-1}$	% change	τ_{on} s	T s
2410	33.3	1.2	5.1	17.5	6.0
2464	54.1	6.6	12.3	13.1	3.3
2375	31.4	5.9	18.6	17.5	3.7
2374	25.5	2.2	8.6	29.7	4.8
1643	46.8	6.1	13.0	30.0	7.2
Mean	38.2	4.5	11.5	21.6	5.0
± SD	11.8	2.3	5.1	7.8	1.6

Note: V_B, baseline $\dot{V}_E$; % change = (Gain/V_B) x 100; τ_{on} and T, the time constant and time delay of the ventilatory response to a step out of poikilocapnic hyperoxia.

Figure 13

The average ventilatory response (solid line) to the step out of hyperoxia in protocol A (poikilocapnia) and protocol B (isocapnia) in subject 1643 (upper panel). The vertical dotted line marks the end of the 4 min hyperoxic step. The best fit model to the data (dashed line) is superimposed on the corresponding averaged $\dot{V}_E$ response. The residuals are graphed in the lower panel.

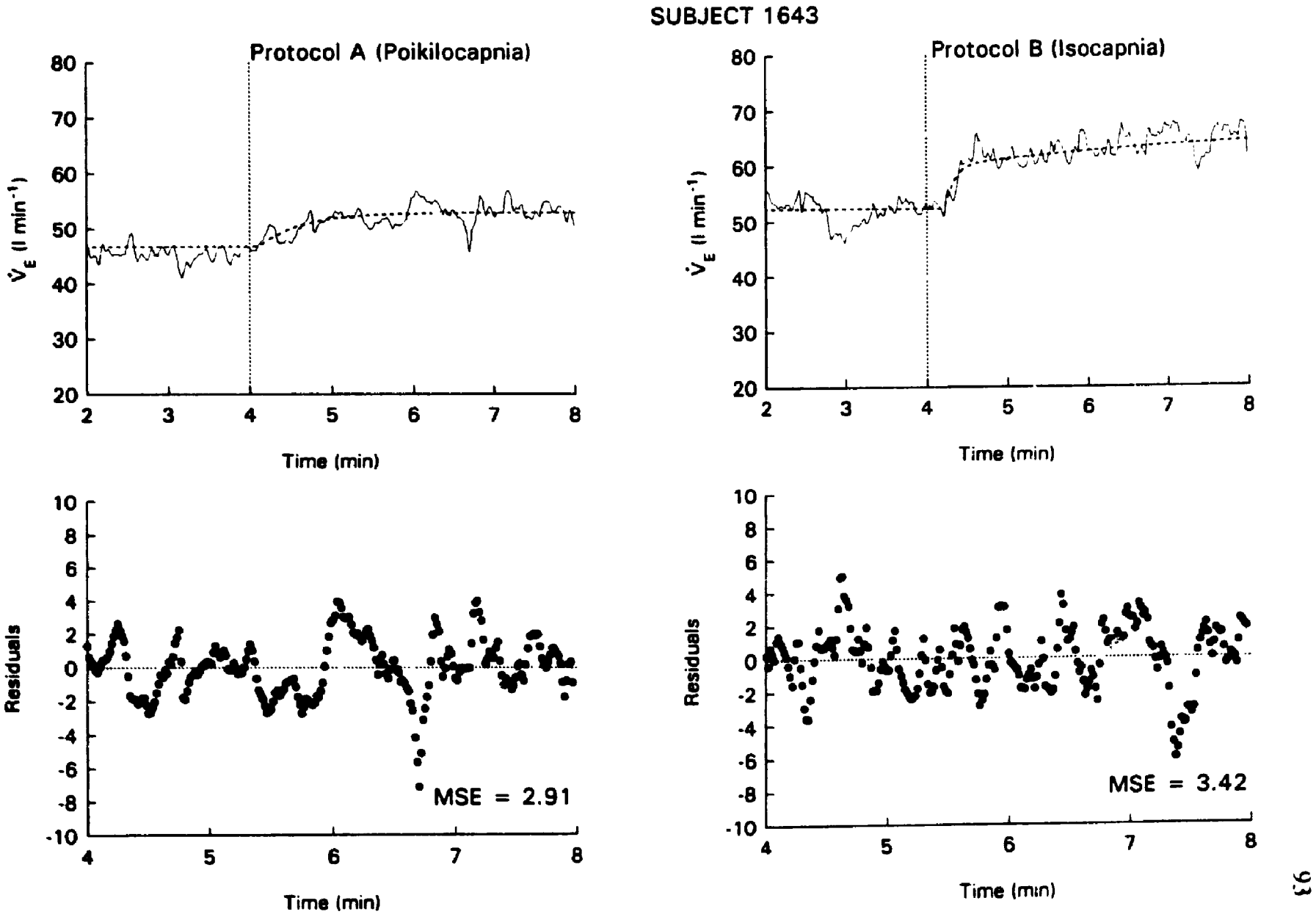

SUBJECT 1643
Protocol A (Poikilocapnia)
Protocol B (Isocapnia)
$\dot{V}_E$ (l min^{-1})
Time (min)
Residuals
MSE = 2.91
MSE = 3.42

Table 8. Estimated temporal parameters of the ventilatory response to the step out of isocapnic hyperoxia.

ID#	V_B	G_s	%	τ_s	T_s	G_f	%	τ_f	T_f	Drift
	$l \cdot min^{-1}$	$l \cdot min^{-1}$	(slow)	s	s	$l \cdot min^{-1}$	(fast)	s	s	$l \cdot min^{-1}$
2410	39.1	1.5	3.7	392.6	16.2	2.6	6.7	18.8	8.8	0.001
2464	60.3	10.8	17.9	305.1	19.9	8.5	14.1	24.9	2.4	0.033
2375	32.0	5.1	15.9	393.1	8.3	4.4	13.8	12.1	5.7	0.002
2374	29.4	8.2	27.9	387.8	16.2	4.5	15.4	12.5	2.6	0.542
1643	52.4	7.4	14.1	247.3	14.1	7.7	14.7	6.9	13.7	0.260
Mean	42.6	6.6	15.9	345.2	14.1	5.5	12.9	15.0	6.7	0.168
± SD	13.3	3.5	8.6	66.3	4.4	2.5	3.6	6.9	4.7	0.236

Note: G_s and G_f, the gain terms of the slow and fast components of the response; τ_s and τ_f, the time constants of the slow and fast components; T_s and T_f, the time delays of the slow and fast components.

3.5 Discussion

The peripheral chemoreflex loop provided about 15% of the drive to breathe in moderate intensity exercise. The true nadir of the hypoventilatory response to a hyperoxic step was expressed prior to the secondary stimulating actions of hyperoxia on the central chemosensitive tissue. These results validated the use of hyperoxic testing to silence the carotid bodies and thereby estimate the pre-existing peripheral drive. The ventilatory response to the step out of hyperoxia suggested, however, that even short periods of sustained hyperoxia alter respiratory control. Though isocapnia appeared to prevent the increase in central chemoreflex drive during hyperoxia, the post-hyperoxic $\dot{V}_E$ response was different from that when the $P_{ET}CO_2$ was allowed to vary naturally, indicating that either the control mechanisms had been modified.

Hyperoxic Response. Our estimate of the peripheral chemoreceptor contribution to $\dot{V}_E$ during moderate intensity exercise is in agreement with previous reports (Masuda et al., 1988; Ward et al., 1987; Whipp, 1994). While there was a large degree of variability between subjects, the range (9.37% to 21.95%) was similar to that described by Masuda et al. (1988). The two subjects (1643 and 2464) who exercised at the greatest workload had the greatest peripheral chemoreceptor drive. It has been suggested that the carotid body contribution to the drive to breathe is greater during the steady state of moderate intensity exercise than at rest, and greater the greater the work rate (Whipp, 1994). We found a strong positive correlation between work rate and the % change in ventilation with hyperoxia ($r = 0.77$), indicating that an absolute work rate below $T_{V\dot{E}}$ may have been more appropriate than the relative work rate corresponding to 80% $T_{V\dot{E}}$. However,

Masuda et al. (1988) found that the relative contribution of peripheral chemoreceptor activity remained the same (10 to 20%) at exercise levels ranging from 0 to 80 W. In addition, the carotid body contribution to $\dot{V}_E$ in heavy exercise (above T_{VE}) has also been estimated to be 15% (Jeyaranjan et al., 1987) which does not support a relationship between absolute work rate and peripheral chemoreceptor drive.

A fundamental assumption of this methodology is that the peripheral chemoreflex drive is effectively suppressed by hyperoxia. The difference in latency times of the response to step increase in CO_2 during euoxia and hyperoxia, defined as the period between the step in $P_{ET}CO_2$ and the first significant change in ventilation (Miller et al., 1974; Ward and Bellville, 1983), has been interpreted as evidence that the carotid bodies do not contribute to the drive to breathe in hyperoxia (Cunningham et al., 1986). Dahan et al. (1990), however, raised the possibility that, at rest, the inspiration of 100% O_2 does not silence, but rather reduces, the peripheral chemoreceptor response to inhaled carbon dioxide. It has also been suggested that 100% O_2 does not abolish the carotid body response to metabolic acidosis during heavy exercise (McLoughlin et al., 1993). While this does not appear to be true in moderate intensity exercise (McLoughlin et al., 1993; Ward and Bellville, 1983), the possibility remains that the peripheral chemoreceptors may still be providing some drive to breathe in hyperoxia and that this method would thereby underestimate the total carotid body drive.

The breathing of hyperoxic gas mixtures is associated with a transient increase in alveolar and arterial PCO_2 due to the fall in $\dot{V}_E$ under conditions of constant metabolic CO_2 production. In addition, the cerebral vessels constrict in response to increases in

the arterial O_2 content, resulting in a decrease in brain blood flow (Kety and Schmidt, 1948; Brown et al., 1985), and the acidification of the cerebral fluids (Dejours, 1962; Ward, 1994b). These two mechanisms cause the stimulation of the central chemosensitive tissue and a consequent increase in $\dot{V}_E$. The average τ (106.8 s) of the secondary increase in $\dot{V}_E$, following the initial decline with the administration of the hyperoxic switch, in the unclamped protocol (A), was consistent with central chemoreceptor stimulation by hypercapnia (Dahan et al., 1990).

The clamping of $P_{ET}CO_2$ (protocol B) appeared to prevent the potentiation of the output from the central chemoreflex in four of the five subjects. The $P_{ET}CO_2$ clamp was not well maintained in subject 2464, who showed a secondary increase in $\dot{V}_E$ of 2.40 l min^{-1} by the end of the hyperoxic step, and an average transient overshoot in $P_{ET}CO_2$ of 2.5 Torr above the clamp when hyperoxia was introduced. The end-tidal PCO_2 was clamped at the peak level attained during the hyperoxic control ride (averaging about 4 Torr above normal values). Bew (1994), using transcranial doppler to measure blood velocity in the middle cerebral artery, observed no change in response to hyperoxia when the $P_{ET}CO_2$ level was kept constant. These findings appear to be in agreement with the protocol B data. Ventrolateral medullary surface blood flow is CO_2 sensitive (Feustal et al., 1984). The vasodilatory response of the cerebral vessels, in response to hypercapnia, is in the order of 6% per Torr (Kety and Schmidt, 1948). The competing vasomotor effects of CO_2 and hyperoxia in protocol B may have resulted in little or no change in cerebral blood flow.

In protocol B, the end-tidal clamp prevented the $P_{ET}CO_2$ from increasing further

during the hyperoxic step, and there was no evidence of a secondary increase in $\dot{V}_E$. Recently, Becker et al. (1995) found that the prolonged breathing of hyperoxic gas mixtures (30 min), at rest, under isocapnic conditions, stimulated respiration markedly. It was remarked that while there was no change in $P_{ET}CO_2$, the actual change in PCO_2 at the level of the central chemoreceptor could not be determined. Aside from changes in CSF acidity induced by changes in cerebral blood flow, the increase in HbO_2 with hyperoxic breathing reduces the buffering of CO_2 by haemoglobin (Lambertsen et al., 1953) resulting in a further decrease in the pH of the CSF. Becker et al. (1995) attributed the $\dot{V}_E$ response to impaired ability to remove CO_2, and possibly a direct stimulation of the respiratory centres by O_2. They reported a significant increase in $\dot{V}_E$ at 7 min into the hyperoxic period, but did not indicate if $\dot{V}_E$ had increased significantly during the earlier phase. On visual examination of the temporal profile of the average ventilation, it appears that the response developed slowly, and that the 4 min period of hyperoxia induced in the present study would not be long enough to observe a significant change.

It has been suggested that hyperoxic testing underestimates the full magnitude of the contribution of the peripheral chemoreceptor to the total ventilatory drive (Dejours, 1962; Ward, 1994b; Whipp, 1994). Dejours (1962) cautioned that $P_{ET}O_2$ does not rise sufficiently rapidly to attain the high values capable of effecting suppression, prior to the expression of the secondary stimulating effects of hyperoxia on $\dot{V}_E$. The dynamic end-tidal forcings technique used $P_{ET}CO_2$ and $P_{ET}O_2$ as the variables for feedback control. The sensing process for $P_{ET}CO_2$ and $P_{ET}O_2$ were repeated at the end of each breath and

the required inspired gas mixture was delivered on the next breath. The hyperoxic step of 600 Torr used in this study, ensured that the $P_{ET}O_2$ was greater than 300 Torr within the first high-O_2 breath. This degree of hyperoxia is considered to be adequate to silence the carotid bodies (Ward, 1994b; Dejours, 1962).

Ward (1994b) noted that, while the nadir of the ventilatory decline in response to a hyperoxic switch occurs some 25-30 s after the transition to hyperoxic breathing, the transit time from the lungs to the central chemoreceptors has been estimated to be about 12 s (Miller et al., 1974), confining the inactivation process to a period of about 13 s. This would translate into a carotid body τ_{off} of 3 s if first-order kinetics are assumed for the chemoreflex inactivation. The kinetics of the ventilatory response to a step increase in $P_{ET}CO_2$ in hyperoxia are long ($\tau \geq 2$ min), (Bellville et al., 1979; Dahan et al., 1990), however, suggesting that the CO_2 effect in the 25-30 s interval should be insignificant (Whipp, 1994).

Mathematical modelling was used to examine whether the nadir of the $\dot{V}_E$ decline represented the full suppression of carotid body drive prior to the secondary increase in $\dot{V}_E$. If an inappropriately high value of $\dot{V}_E$ had been selected for the predicted nadir, the $\dot{V}_E$ response would not conform to a monoexponential but would yield a downwardly curving decline when expressed semilogarithmically with respect to time (Ward, 1994b). This was not the case, providing additional support for the finding that in both the unclamped (A) and clamped (B) protocols a true nadir in $\dot{V}_E$ was achieved prior to any central stimulation of ventilation.

Post-hyperoxic Response. Sustained periods of hyperoxia are rarely used because of the

complicating influences of changes in arterial PCO_2 and cerebral hypofusion. The transient O_2-switching technique, in which 100% O_2 is abruptly and surreptitiously substituted for the normal inspirate for a few breaths, is thought to circumvent these influences (Dejours, 1962; Ward, 1994b). Thus, there is very little data in the literature which examines the ventilatory response to a step out of hyperoxia. This study illustrates the complexity of the respiratory control mechanisms involved in resetting the equilibrium after a period of sustained hyperoxia during the steady state of moderate intensity exercise.

The removal of hyperoxia, in protocol A, was characterized by a rapid increase to a steady state $\dot{V}_E$ that was higher than the pre-hyperoxic $\dot{V}_E$. The latency of the response was consistent with the reactivation of the peripheral chemoreceptor. The steady state $\dot{V}_E$ was maintained despite the decrease in $P_{ET}CO_2$. Hypocapnia causes a rapid decrease in cerebral blood flow in the order of about 4.5 % per Torr CO_2 (Severinghaus and Lassen, 1967). While the $P_{ET}CO_2$, and presumably the P_aCO_2 at the level of the peripheral chemoreceptor, decreased, the associated changes in cerebral blood flow could cause the PCO_2 of the brain tissue to increase. The temporal profile of the post-hyperoxic response, however, was not consistent with central mediation. Mathematical modelling of the ventilatory response to the step out of hyperoxia revealed that the response was characterized by single exponential phase. Expanding the model to incorporate a central component increased the sum of squares, and the comparison of the model fits using a F-test (Motulsky and Ransnas, 1987), indicated that the simpler model fit the data significantly better than the two compartment model.

The maintenance of a relatively constant level of arterial PCO_2 at rest, and through moderate exercise suggests the presence of a control mechanism that holds P_aCO_2 at its operative resting level (Oren et al., 1981). The "set point" theory allows the prediction of the $\dot{V}_E$ response to exercise, at, or below the anaerobic threshold, based on the metabolic rate, the P_aCO_2, and the deadspace $\dot{V}_E$. As predicted by the alveolar air equation

$$(\dot{V}_A = \dot{V}CO_2 \times 1/k \cdot P_aCO_2),$$

where k is a dimensional constant and $\dot{V}CO_2$ is the metabolic CO_2 output. The increment of $\dot{V}_A$ is greater, for a given change in $\dot{V}CO_2$, if the regulated level of P_aCO_2 is lower than normal. The increase in the level of the steady state $\dot{V}_E$ in the post-hyperoxic phase of protocol A (poikilocapnia), is consistent with the either a lowering of the P_aCO_2 set point, or an increase in the sensitivity to CO_2, either by hyperoxia or by the combination of hyperoxia and exercise. Exercise itself does not appear to have any effect on the resting central and peripheral chemoreceptor thresholds to CO_2 (Casey et al., 1987; Duffin and McAvoy, 1988). However, Oren et al. (1981) demonstrated that when resting P_aCO_2 was lowered by a chronically induced metabolic acidosis, the P_aCO_2 was regulated at a reduced level, with no significant change during the exercise. The ventilatory response to the same metabolic increment was therefore larger when PCO_2 was reduced. In this study, if the CO_2 set point was lowered by the decrease in pH, due to the reduced buffering power of haemoglobin during hyperoxic breathing, then the ventilatory response to the same work rate would be greater post-hyperoxia. Oren et al. (1981) found no change in the sensitivity to CO_2 ($d\dot{V}_E/dPCO_2$) during metabolic acidosis.

It must be considered that the $P_{ET}CO_2$ profile may not reflect the actual P_aCO_2. $P_{ET}CO_2$ has been shown to be an acceptable estimate of P_aCO_2 at rest, but not during exercise (Jones et al., 1972; Jones et al., 1979; Matell, 1963; Robbins et al., 1990; Whipp and Wasserman, 1969), when the variation in PCO_2 during a respiratory cycle is amplified by the increase in tidal volume and metabolic CO_2 production. Jones et al. (1979) studied this problem and developed a regression equation to estimate P_aCO_2 which incorporates both $P_{ET}CO_2$ and tidal volume (P_TCO_2). This technique has been shown to give reliable estimates of mean P_aCO_2 in exercise in young subjects (Jones et al., 1979; Robbins et al., 1990). The use of P_TCO_2 to estimate arterial PCO_2 lowered the values of P_aCO_2 by about 2 Torr, however, the response profile remained the same.

Cummin et al. (1986) examined the ventilatory response to CO_2 at rest, and at various levels of exercise, concentrating on the area around the physiological control point. Their results showed that, at the lower end of the response, there is a progressive increase in the slope with exercise, and that, at higher levels of exercise, CO_2 sensitivity close to the control point may be very high. The results in the control experiments (protocols C and D) argue against exercise alone effecting a change in CO_2 sensitivity during either hyperoxic protocol (A and B). In protocol D, both $\dot{V}_E$ and $P_{ET}CO_2$ achieved a steady state by 4 min and remained constant for the duration of the workload function, though there was an initial overshoot in $P_{ET}CO_2$, with the step up in work rate, which stabilized in the first 2 min. The mechanism through which hyperoxia could cause a change in CO_2 sensitivity is unknown.

The $\dot{V}_E$ response to the step out of hyperoxia, in protocol B, was best fit to a two-

compartment model. The fast component, having an average τ of 15.0 s, was attributed to the peripheral chemoreceptor. The slow component, with an average τ of 345.2 s, was regarded to be due to the slow activation of the central chemoreceptor. The dynamic end-tidal forcing technique controlled the end-tidal value of PCO_2 and could have provided a hypercapnic stimulus. In the poikilocapnic exercise test (A), the $P_{ET}CO_2$ decreased in the post-hyperoxic phase. The end-tidal forcing, in protocol B, could produce a progressively increasing hypercapnic stimulus, relative to the natural poikilocapnic value over this time period, as inspired CO_2 is added to maintain a constant $P_{ET}CO_2$. This theory was introduced by Pandit and Robbins (1992) to explain the progressive rise in $\dot{V}_E$, observed during a 43 min hypercapnic exercise test. We did not observe this drift upward in $\dot{V}_E$, however, in the control protocol D, when the $P_{ET}CO_2$ was clamped an average of 4 Torr above resting values.

The lowering of the CO_2 set-point by hyperoxia would also be consistent with the results of the isocapnic protocol (B), where ventilation continues to increase in the face of a constant $P_{ET}CO_2$. Becker et al. (1995) reported an acute decrease in $\dot{V}_E$, after the step down from hyperoxia, but found that respiration was still increased above resting euoxic levels after 15 min of breathing room air with $P_{ET}CO_2$ kept constant. This may reflect slow changes in brain tissue pH or a continued neural afterdischarge (Millhorn et al., 1980), but could also be argued to support the resetting of the P_aCO_2 set point by hyperoxia.

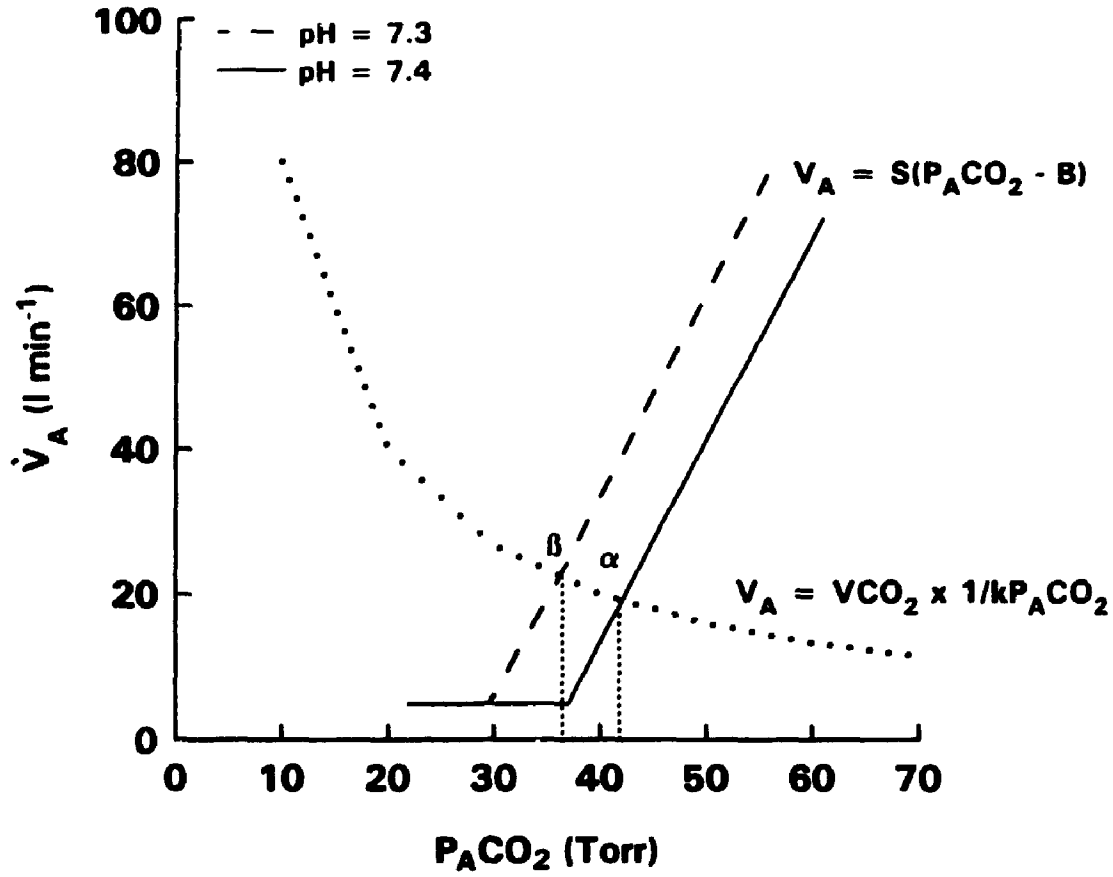
The potential means by which the CO_2-setpoint might be lowered is presented graphically in Figure 14. The intersection of the metabolic hyperbola for CO_2,

corresponding to the steady state exercise $\dot{V}CO_2$, with the appropriate controller line, gives the unique stable point for the given set of conditions. α indicates the set-point for P_ACO_2, at the normal pH of 7.4. The breathing of hyperoxic gas mixtures causes a diminution of the Haldane effect and a resultant decrease in pH (Cunningham et al., 1986). Acidosis shifts the $\dot{V}_A$ - P_ACO_2 controller relation to the left, with no change in slope (Lloyd, 1963). ß represents the consequent lowering of the regulated level of P_ACO_2. In the isocapnic protocol, the sustained increase in PCO_2 might also provoke a metabolic acidosis, which would shift the controller relation to the left, decreasing the CO_2 set-point. In the study presented in Chapter 4, a strong negative correlation was found between inspired PCO_2 and arterial pH both at rest and in exercise (Appendix X).

There is no evidence to suggest that other ventilatory control mechanisms operating in exercise would be affected by either the hyperoxic or exercise perturbations induced in this study. The $\dot{V}_E$ had achieved a steady state prior to the hyperoxic step, therefore, the nonrespiratory peripheral neural feedback from the working muscles and exercising limbs, transmitted via group III and group IV afferents (Eldridge and Waldrop, 1991), should remain constant. The K^+ released from working muscle has been suggested to drive $\dot{V}_E$ in exercise by direct stimulation of the carotid bodies (Paterson, 1992). Hyperoxia removes the K^+-induced ventilatory drive (Paterson and Nye, 1991), but should not affect the release or uptake of K^+. The modulating effects of circulating catecholamines, high body temperature, and increased blood osmolarity could supplement $\dot{V}_E$ drive during above $T_{\dot{V}E}$ exercise (Whipp, 1987), but are unlikely to play a role in driving $\dot{V}_E$ at the moderate exercise intensities employed in this study.

Figure 14

Graphical representation of the lowering of the P_ACO_2 set-point by metabolic acidosis. α represents the CO_2 set-point at a pH of 7.4. ß represents the regulated level of P_ACO_2 when the CO_2 response curve is shifted to the left by metabolic acidosis.


100
80
60
40
20
0
0
10
20
30
40
50
60
70
pH = 7.3
pH = 7.4
$V_A = S(P_ACO_2 - B)$
$V_A = VCO_2 \times 1/kP_ACO_2$
ß
α
$\dot{V}_A$ (l min^{-1})
P_ACO_2 (Torr)

In summary, this study examined the ventilatory response to sustained hyperoxia in moderate intensity exercise. It was demonstrated that hyperoxic testing provides an accurate estimate of the contribution of peripheral chemoreflex loop to the total ventilatory drive. The carotid bodies were found to provide about 15% of the drive to breathe in moderate intensity exercise. This modest contribution supports the theory that the arterial chemoreceptors function to "fine tune" alveolar $\dot{V}$ to minimize change in arterial blood gases (Forster and Pan, 1994; Weil and Swanson, 1991). Sustained hyperoxia, however, appeared to lower the set point about which P_aCO_2 was regulated. The concept of the carotid bodies as regulators, or gain controllers of the CO_2 set point, is also supported by the 15% rise in the CO_2 set point observed in chemoreceptor-denervated asthmatics (Cunningham, 1974; Wade et al., 1970). The time course of the ventilatory response in protocol B (τ = 345 s) would suggest that the central chemoreceptors may also play a role in the regulation of P_aCO_2.

CHAPTER 4

THE ESTIMATION OF ARTERIAL PCO_2 IN THE ELDERLY

4.1 Abstract

Arterial PCO_2, determined directly in the radial artery, was compared to indirect estimates of PCO_2 in six el 'erly men (mean age 73.8 yrs). Estimates of arterial PCO_2 included arterialized-venous ($P_{av}CO_2$); end-tidal ($P_{ET}CO_2$); mean alveolar PCO_2, calculated using a reconstruction of the alveolar oscillation in PCO_2, and accounting for the presence of deadspace ($P_{AD}CO_2$); and values calculated using the empirical formula developed by Jones et al. (J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 47(5): 954-960, 1979) which incorporates $P_{ET}CO_2$ and tidal volume (P_TCO_2). Measurements were made at rest and during cycle ergometry at 25 and 50 Watts, while breathing various gas mixtures (euoxic/eucapnic; hypoxic/eucapnic; hyperoxic/eucapnic; and hyperoxic/hypercapnic). The mean differences between the estimates and the actual P_aCO_2 $\pm$ standard deviations, at rest and in 25 W and 50 W exercise were as follows: $P_{av}CO_2$, 0.3 $\pm$ 0.7, -0.1 $\pm$ 0.7, and 1.8 $\pm$ 1.2 Torr; $P_{ET}CO_2$, 2.9 $\pm$ 1.7, 4.0 $\pm$ 3.1, and 3.7 $\pm$ 3.2 Torr; $P_{AD}CO_2$, 2.6 $\pm$ 1.9, 3.3 $\pm$ 3.1, and 3.6 $\pm$ 3.8 Torr; and P_TCO_2, 2.4 $\pm$ 1.3, 1.3 $\pm$ 3.0, and 0.6 $\pm$ 2.9 Torr. It is concluded that mean $P_{av}CO_2$ agreed most closely with mean P_aCO_2 both at rest and in exercise. All methods of deriving P_aCO_2 using measurements from the respired gases overestimated arterial values at rest. Of the noninvasive techniques, mean estimates calculated using the regression equation developed by Jones et al. (1979) corresponded most closely with P_aCO_2 in exercise.

4.2 Introduction

The arterial partial pressure of carbon dioxide (P_aCO_2) is an important contributor to the control of breathing, and its accurate measurement critical to studies of ventilatory control. Currently, several studies of respiratory control (Clement et al., 1992; Poulin et al., 1993) have utilized the noninvasive technique of dynamic end-tidal forcings and the feedback method introduced by Swanson and Bellville (1975) and modified by Robbins et al. (1982b) to produce perturbations in end-tidal PCO_2 ($P_{ET}CO_2$) and end-tidal PO_2 ($P_{ET}O_2$) to stimulate the respiratory control system. The interpretation of the results in these studies is based on the assumption that changes in the $P_{ET}CO_2$ mirror changes in the PCO_2 of the arterial blood supply to the respiratory chemoreceptors. In young subjects, however, $P_{ET}CO_2$ has been reported to underestimate P_aCO_2 at rest and overestimate P_aCO_2 in exercise (Robbins et al., 1990) or when CO_2 is added to the inspirate (Matell, 1963).

Indwelling catheters can be used for the direct sampling of arterial blood, however, this is an invasive procedure, associated with potential health risks, that is not always convenient or acceptable in the research laboratory setting. Several techniques have been developed to approximate P_aCO_2. The measurement of the PCO_2 of arterialized venous blood ($P_{av}CO_2$) was established as a substitute for arterial blood both at rest and during graded exercise (Forster et al., 1972), and while just as invasive, does not have the potential health risks attributed to sampling arterial blood directly. Techniques have also been developed for the estimation of P_aCO_2 from the measurement of PCO_2 in the expired gases. $P_{ET}CO_2$ has been shown to be an acceptable estimate of

P_aCO_2 at rest, but not during exercise (Robbins et al., 1990; Jones et al., 1979), when the variation in PCO_2 during a respiratory cycle is amplified by the increase in tidal volume and metabolic CO_2 production. Jones et al. (1979) studied this problem and developed a regression equation to estimate P_aCO_2 which incorporates both $P_{ET}CO_2$ and tidal volume (P_JCO_2). A final technique used to estimate P_aCO_2 from the respired gases uses a time weighted mean of the alveolar PCO_2 throughout the respiratory cycle ($P_{AD}CO_2$). This method uses the end-tidal plateau in PCO_2 to reconstruct the alveolar oscillation in PCO_2 and incorporates the effects of respiratory and apparatus deadspace. Robbins et al. (1990) assessed the validity of using these techniques for estimating P_aCO_2 from the PCO_2 of the expired gases and found that, in six young males, $P_{ET}CO_2$, $P_{AD}CO_2$ and P_JCO_2 underestimated P_aCO_2 at rest. In exercise, Robbins (1990) found that $P_{ET}CO_2$ overestimated P_aCO_2, but $P_{AD}CO_2$ and P_JCO_2 were not significantly different from P_aCO_2. The appropriateness of these methods has not been investigated in older subjects. The increase in physiological deadspace with aging (Tenney and Miller, 1956), and the greater nonuniformity of the ventilation-perfusion distribution in healthy older adults relative to young adults (Holland et al., 1968) may result in an altered relationship between end-tidal PCO_2 and P_aCO_2, making these estimates of questionable use in this subject group. Data published previously from our laboratory (Overend et al., 1992) reported a significantly lower P_aCO_2 in a group of elderly, as compared to young adults, as estimated using the regression equation developed by Jones et al. (1979). This raised the question of whether the P_aCO_2 was actually lower in older individuals, or whether the technique used to estimate P_aCO_2 was inappropriate for the older subject group.

The purpose of this study was to compare measured P_aCO_2 values to the estimates obtained from arterialized venous blood, end-tidal PCO_2, mean alveolar PCO_2 (corrected for respiratory and apparatus deadspace), and the regression equation developed by Jones et al. (1979), in healthy elderly subjects, while breathing gas mixtures that are frequently used in studies of ventilatory control (euoxic/eucapnic; hypoxic/eucapnic; hyperoxic/eucapnic; and hyperoxic/hypercapnic) as a means of stimulating the respiratory chemoreceptors.

4.3 Methods

Respiratory apparatus and gas analysis. Subjects breathed through a mouthpiece with the nose occluded. Inspired and expired ventilation flow rates were measured using a low resistance bi-directional turbine (Alpha Technologies, VMM 110) and volume transducer (Sensor Medics VMM-2A) calibrated with a syringe of known volume (3.01 l). Respiratory flows and timing information were measured using a pneumotachograph (Hans Rudolph, Inc. Model 3800) and differential pressure transducer (Validyne MP45-871). Inspired and expired gases were sampled continuously (20 ml/s) at the mouth and analyzed by a mass spectrometer (AIRSPEC MGA 2000) calibrated with commercially available certified precision (± 0.02 %) gas mixtures analyzed gravimetrically (Scott Medical). The delay due to the transit time for the gas in the capillary and the response speed of the instrument were measured before each experiment. Analog signals were sampled and digitized every 20 ms by computer. Gas concentration signals were aligned with the inspired and expired volumes after correcting for the time delay appropriate for

the instrument.

The experimental set up and the technique used for accurate control of end-tidal gases was described in Chapter Two (pp.38-39).

Subjects and Protocol. Six elderly males (71-80 yr) acted as subjects for the experiments. Two younger subjects (mean age 39.5 yr) were included in the study for comparison with results previously published by Robbins et al. (1990). The study requirements were fully explained (in written and verbal forms; Appendix IV) to all participants, with each subject giving informed consent prior to volunteering to participate in the study . The research was approved by the University's Committee on Human Research.

The protocol required two visits to the laboratory. In the first session, the participant underwent a medical examination including a medical history and physical examination, a twelve-lead electrocardiogram (ECG), blood pressure measurements, pulmonary function tests (MVV and $FEV_{1.0}$), and a ramp test on the cycle ergometer to determine maximal oxygen uptake ($\dot{V}O_2max$). $\dot{V}O_2max$ and the stress ECG were measured as part of the screening process to ensure that the subjects were free of any clinical symptoms of underlying cardiorespiratory disease and able to tolerate the exercise levels chosen for study. In order to maintain adequate control of the end-tidal gases using the dynamic end-tidal forcing method, the $P_{ET}CO_2$ was clamped 1.5 to 2.0 Torr above normal levels, necessitating the addition of CO_2 to the inspirate. The $P_{ET}CO_2$ was examined during the ramp test to establish the baseline for end-tidal clamping during the experimental protocol. In addition, during this visit the subjects were accommodated to

the breathing apparatus and introduced to the sensation of breathing hypoxic and hypercapnic gas mixtures.

At the start of the second session, a catheter was positioned in the radial artery for drawing samples of arterial blood. Prior to catheterization, subjects were examined to ensure that the ulnar artery was capable of supplying the total blood flow to the hand. Arterialized-venous blood was obtained from a catheter placed in a superficial vein on the back of the opposite hand. Venous blood was "arterialized" by wrapping the hand and forearm in an electric heating pad (temperature set on high). Blood was sampled anaerobically and analyzed immediately by a blood gas-electrolyte analyzer (Nova StatProfile 5, Nova Biomedical Canada Ltd., Mississauga, Ontario) calibrated at regular intervals with precision-analyzed ($\pm$ 0.03%) gas mixtures and verified by tonometry.

The experimental procedures were the same as those of Robbins et al. (1990). The subjects were seated comfortably in a chair for the study at rest. Haemoglobin saturation was monitored by ear oximetry (Radiometer). Exercise studies were performed on an electrically braked cycle ergometer. The subjects were studied during 25 and 50 W exercise. A schematic of the experimental protocols is presented in Figure 15. For the study at rest, the gas collection series was initiated with a five minute accommodation to the system in euoxia and eucapnia followed by a series of three minute step changes in the end-tidal gases. Either O_2 or N_2 was added to the inspirate to achieve end-tidal partial pressures of O_2 of 70, 55 and 200 Torr. While still in hyperoxia, CO_2 was added to achieve end-tidal PCO_2's of +4 and +8 Torr above resting (mean resting $P_{ET}CO_2$ = 37.3 $\pm$ 2.4 Torr).

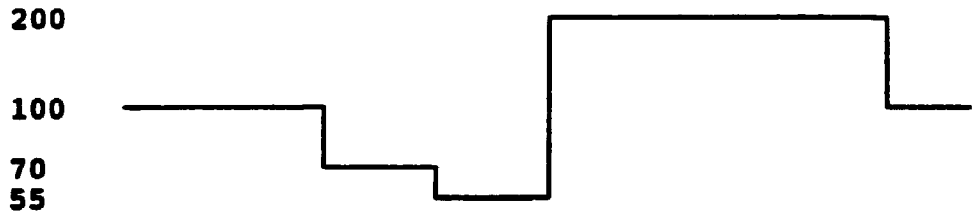
Figure 15

Experimental protocols describing the time related changes in gas forcing functions. A five minute accommodation to the system in euoxia ($P_{ET}O_2$ = 100 Torr), eucapnia, was followed by a series of three minute step changes in the end-tidal gases. At rest, two hypoxic periods (70 Torr and 55 Torr) were followed by a period of hyperoxia (200 Torr) with the $P_{ET}CO_2$ held near eucapnia (1-2 Torr above resting $P_{ET}CO_2$). Hyperoxia was then maintained for two periods of hypercapnia (+4 and +8 above resting $P_{ET}CO_2$). Single periods of hypoxia ($P_{ET}O_2$ = 55 Torr) and hypercapnia ($P_{ET}CO_2$ = +8 above resting) were studied at both exercise levels (25 and 50 W) following a five minute accommodation to each exercise level.

EXPERIMENTAL PROTOCOL

REST

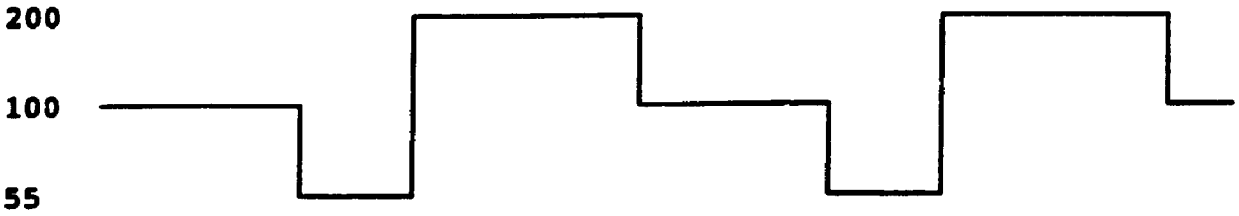
Oxygen (Torr)



Carbon Dioxide (Torr)

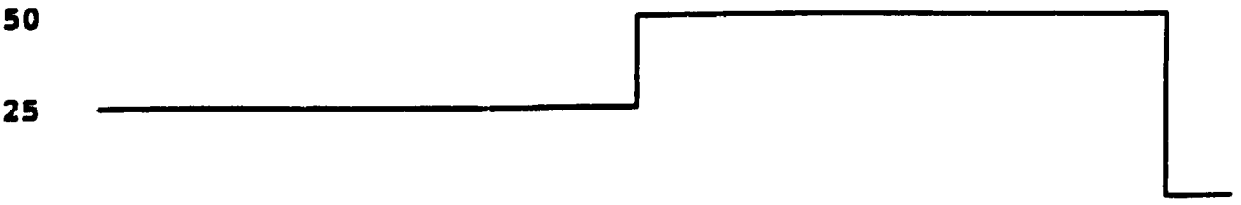
EXERCISE

Oxygen (Torr)



Carbon Dioxide (Torr)

Workload (W)



For the exercise studies, the gas collection began with a five minute accommodation to each exercise level in euoxia and eucapnia followed by a series of three minute step changes in end-tidal gases. At each exercise level, either N_2 or O_2 was added to the inspirate to achieve end-tidal PO_2's of 55 and 200 Torr. Finally CO_2 was added to the hyperoxic mixture to achieve a $P_{ET}CO_2$ of +8 Torr above resting.

Blood samples were drawn simultaneously from the arterial and venous catheters over a 20 s period once the $P_{ET}CO_2$ had been stable for two minutes. The sampling period was then aligned with the appropriate portion of the respiratory data allowing for the transit time of the blood from the lung to the hand. There was sufficient time between samples to enable each blood gas determination to be done immediately. The arterial sample was analyzed first, followed directly by the arterialized venous sample. The maximum delay between sampling and measurement of arterialized venous blood was two minutes.

Calculation of P_aCO_2 estimates. $P_{av}CO_2$ was analyzed directly in the blood gas electrolyte analyzer by selective electrode.

$P_{ET}CO_2$ was taken as the PCO_2 of the respired gas at the mouth at the end of expiration (after allowing for the pure delay of the mass spectrometer).

P_ICO_2 was calculated using the regression equation developed by Jones et al. (1979):

$$P_ICO_2 = 5.5 + 0.9(P_{ET}CO_2) - 2.1(V_T)$$

where V_T is tidal volume (liters).

$P_{AD}CO_2$ was calculated using the method developed by Cochrane et al. (1982) and

refined by Robbins et al. (1990). The alveolar oscillation in PCO_2 was reconstructed using respiratory flow data and a value for deadspace to account for the time at which each PCO_2 value recorded at the mouth actually left the alveoli. The slope of the expiratory phase of the PCO_2 record was used to estimate the rate of rise of PCO_2 in the alveoli during expiration. $P_{AD}CO_2$ was calculated as the time-weighted mean of this segment. This technique is illustrated in Robbins et al. (1990). An assumed deadspace value of 180 ml at rest and 300 ml in exercise was used for the young subjects (Jones, 1988). Respiratory deadspace was estimated to be 235 ml at rest (Richards, 1986) and 400 ml in exercise for the elderly subjects. In the elderly subjects, an average of 5 breaths (6 in the hypercapnic segments of the protocol) represented each $P_{ET}CO_2$ measurement. In 25 W exercise $P_{ET}CO_2$ was represented by an average of 7 breaths (8 in the hypercapnic segment) and by 10 breaths (12 in the hypoxic and hypercapnic segments) in 50 W exercise.

Tonometry. The accuracy of the blood gas analyzer was verified with blood tonometered (RNA Medical EQUILibrator) using three precision-analyzed gas mixtures (RNA Medical). Overall, 48 analyses of tonometered blood were performed. The mean difference between the standard and measured PCO_2 was -0.23 Torr $\pm$ 1.14 (SD) Torr and the 95% confidence interval was -2.50 to 2.10 Torr.

Statistical treatment. The data for each subject was combined so that each subject contributed one data point to each of the resting, 25 W and 50 W exercise protocols. Mean values were calculated for $P_{\bar{v}}CO_2$, $P_{ET}CO_2$, $P_{AD}CO_2$ and P_iCO_2 and compared to directly measured mean P_aCO_2 at rest, and in 25 W and 50 W exercise using Student's

paired t-tests. The level of significance was $P < 0.05$. n = 6 for each of the $P_{ET}CO_2$, $P_{A(I)}CO_2$, and P_ICO_2 comparisons with directly measured P_aCO_2, at rest and at both exercise levels. n = 5 for the $P_{a\bar{v}}CO_2$ resting comparison, and n = 4 for each of the exercise comparisons. The comparisons were also made using a Wilcoxon signed-rank test, the nonparametric analogue to the paired t-test (Rosner, 1986). The different estimation techniques were compared using repeated measures analysis.

4.4 Results

Subjects. Six elderly subjects ranging in age from 70 to 80 years (mean age 73.8 yrs), and having maximal oxygen uptakes ranging from 1.94 to 2.60 $l \cdot min^{-1}$ (mean $\dot{V}O_{2max}$ = 2.23 $l \cdot min^{-1}$) completed the rest and 25 W (17% maximal power output) exercise protocols, however two subjects were prevented from completing the 50 W (35% maximal power output) exercise protocol when their haemoglobin saturation dropped below 75% in the hypoxic challenge. This value was set as the lower limit for haemoglobin saturation (a safety procedure for the elderly subjects). In addition, it was only possible to obtain arterialized-venous samples on two of the remaining four subjects at this workload. Two younger individuals (mean age 39.5 yrs, $\dot{V}O_{2max}$ = 4.44 $l \cdot min^{-1}$) were studied at rest and in 50 W exercise (19% maximal power output) in order to compare our values with previous reports. The physical characteristics of the subjects are listed in Table 9.

P_aCO_2 Estimated in Young Subjects. The two younger subjects showed only small differences between $P_{a\bar{v}}CO_2$ and P_aCO_2 at rest and in 50 W exercise (Table 10).

Table 9. Anthropometric and maximal exercise testing values.

Subject	Age (yrs)	Weight (kg)	Height (cm)	$\dot{V}O_{2max}$ ($l \cdot min^{-1}$)
Elderly Group				
1244	72	78.7	173.0	2.12
1755	71	77.6	175.5	2.10
0189	76	79.0	178.0	2.28
0531	80	87.3	178.0	1.94
1780	70	81.3	173.0	2.60
0308	74	68.8	171.0	2.35
Mean	73.8	78.8	174.8	2.23
Young Group				
0021	55	71.5	189.0	3.91
2007	24	80.5	184.0	4.96
Mean	39.5	76.0	186.5	4.44

Table 10. Directly measured P_aCO_2 and the differences between indirect estimates and measured P_aCO_2 in young subjects

	Rest (Torr)	50 W Exercise (Torr)
P_aCO_2	39.8 ± 1.7	38.5 ± 2.8
$P_{av-a}CO_2$	0.2 ± 0.02	-0.01 ± 0.8
	(-0.05, 0.4)	(-6.9, 6.9)
$P_{ET-a}CO_2$	-0.1 ± 0.6	2.5 ± 0.1
	(-5.5, 5.2)	(2.0, 3.0)
$P_{AD-a}CO_2$	-0.1 ± 0.8	0.6 ± 0.2
	(-7.2, 6.9)	(-0.9, 2.2)
$P_{J-a}CO_2$	-1.3 ± 0.3	-0.8 ± 0.8
	(-4.1, 1.6)	(-7.9, 6.4)

Values are means ± SD with 95% confidence intervals in parentheses.

P_aCO_2 Estimated in Older Subjects. For the elderly subjects, the relationships between the actual P_aCO_2 and the P_aCO_2 estimated using the four different techniques are plotted in Figure 16. The dashed line represents the best-fit regression line of the points. The solid line is the line of equality along which all points would lie if the indirect estimates were in perfect agreement with the actual P_aCO_2 values. The regression slopes are provided in Table 11. Inspection of Figure 16 and Table 11 suggests that the slopes for all estimates against P_aCO_2 were less than unity, which reached significance ($P < 0.05$) only in 25 W exercise for $P_{\bar{a}v}CO_2$ and P_JCO_2.

The $P_{\bar{a}v}CO_2$ agreed closely with the actual P_aCO_2 at rest and in 25 W exercise, with no differences between the two measurements (Table 12). The differences between the three estimates of P_aCO_2 derived from the respired gases and the measured P_aCO_2 in the elderly subjects are presented in Table 13. The results given in the upper panel of Table 14 are a composite of the measurements made during a number of step changes in end-tidal gases. The results of the measurements made during the euoxic/eucapnic segments of the protocol are reported in the lower panel in order to examine the differences between the indirect estimates and the actual P_aCO_2, independent of the effect that perturbations in end-tidal PCO_2 and PO_2 might have on the results. When all the experimental protocols were included in the analysis, the three estimates derived from the respired gases (i.e. $P_{ET}CO_2$, P_JCO_2, $P_{AD}CO_2$) overestimated P_aCO_2 ($P < 0.05$) at rest. When the euoxic/eucapnic data were analyzed separately, however, the differences were no longer significantly different from zero. A significant ($P < 0.001$) correlation ($r = 0.81$) was found between inspired PCO_2 (P_iCO_2) and the $P_{ET}CO_2$-P_aCO_2 difference at rest

and in exercise ($r = 0.53$). The relationship between the two variables is plotted in Figure 17 illustrating that $P_{ET}CO_2$-P_aCO_2 increases as P_iCO_2 increases. The young subjects also showed a strong correlation between the $P_{ET}CO_2$ - P_aCO_2 difference and inspired PCO_2 of 0.89, at rest. The young data were provided in Figure 16 to show the similarity of the slopes of the regression lines at 0.169 for the older subjects and 0.155 for the young subjects. No significant relationship was found between inspired PCO_2 and the end-tidal to arterial PCO_2 difference in exercise in the two younger subjects.

In exercise, $P_{ET}CO_2$ and $P_{AD}CO_2$ continued to overestimate arterial PCO_2 when all the experimental protocols were included in the analysis (Table 13). The Jones predictive equation produced estimates that were not significantly different from P_aCO_2 at either exercise level, however, individual points deviated considerably from the line of equality (Figure 16). The mean differences between the estimates ($P_{ET}CO_2$, $P_{AD}CO_2$ and P_iCO_2) and actual P_aCO_2 were not significantly different from zero when the euoxic/eucapnic data were analyzed separately, with the exception of $P_{ET}CO_2$ in 25 W exercise, though the 95% confidence intervals were large (Table 13).

Statistical analyses performed by using either a paired t-test, or by using the nonparametric Wilcoxon signed-rank test showed the same results. The power (probability of rejecting the null hypothesis when it is false) of the t-test was estimated by:

$$\Phi(z_{\alpha/2} + |\mu_1 - \mu_0|\sqrt{n}/\sigma)$$

where $\mu_0 = 0$ (Rosner, 1986). μ_1 and σ were estimated from the sample mean and standard deviation for $P_{ET}CO_2$ - P_aCO_2 (Table 13). At rest, the study had a 98% chance

of detecting a significant difference. In exercise, the chance of finding a significant difference was 88%.

Figure 16

Relationship between P_aCO_2 and the four different methods of estimating P_aCO_2 in elderly subjects. The regression line fit to the points is dashed. The solid line is the line of equality.

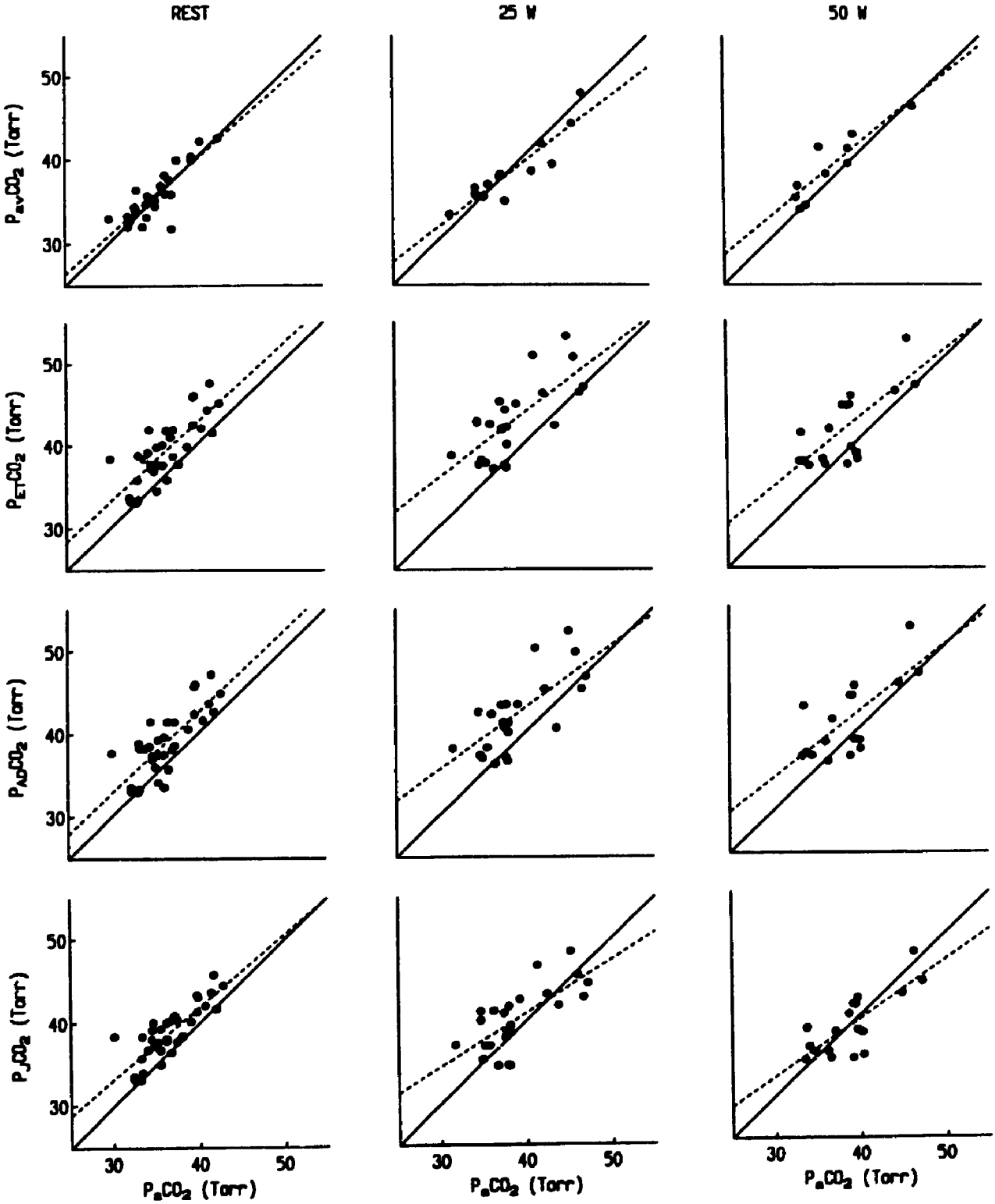

REST
25 W
50 W
$P_{\bar{v}}CO_2$ (Torr)
$P_{ET}CO_2$ (Torr)
$P_{AD}CO_2$ (Torr)
P_JCO_2 (Torr)
P_aCO_2 (Torr)
30
40
50

Table 11. Comparison of regression slopes for the four methods of estimation of P_aCO_2 in elderly males.

		Exercise	
	Rest	25 W	50 W
$P_{av}CO_2$	0.90	0.78*	0.84
	(0.70, 1.11)	(0.59, 0.97)	(0.50, 1.18)
$P_{ET}CO_2$	0.95	0.79	0.83
	(0.70, 1.21)	(0.46, 1.12)	(0.43, 1.23)
$P_{AD}CO_2$	0.97	0.75	0.81
	(0.72, 1.23)	(0.42, 1.08)	(0.38, 1.24)
P_JCO_2	0.87	0.65*	0.72
	(0.67, 1.07)	(0.37, 0.94)	(0.41, 1.03)

95% confidence intervals in parentheses
*Slopes are significantly different from unity ($P < 0.05$)

Table 12. Directly measured P_aCO_2 and the differences between $P_{av}CO_2$ and measured P_aCO_2 in elderly subjects

		Exercise	
	Rest (Torr)	25 W (Torr)	50 W (Torr)
		All experimental protocols†	
P_aCO_2	36.3 ± 2.1	38.9 ± 1.6	38.9 ± 3.1
$P_{av-a}CO_2$	0.3 ± 0.7	-0.1 ± 0.7	1.8 ± 1.2
	(-0.6, 1.2)	(-1.3, 1.0)	(-0.1, 3.7)
		Euoxia, Eucapnia	
P_aCO_2	35.6 ± 2.2	37.3 ± 1.7	37.2 ± 2.6
$P_{av-a}CO_2$	-0.1 ± 0.9	0.7 ± 2.2	1.5 ± 1.3
	(-1.2,1.0)	(-6.1, 4.6)	(-0.6, 3.7)

Values are means ± SD with 95% confidence intervals in parentheses.
† See Figure 15.

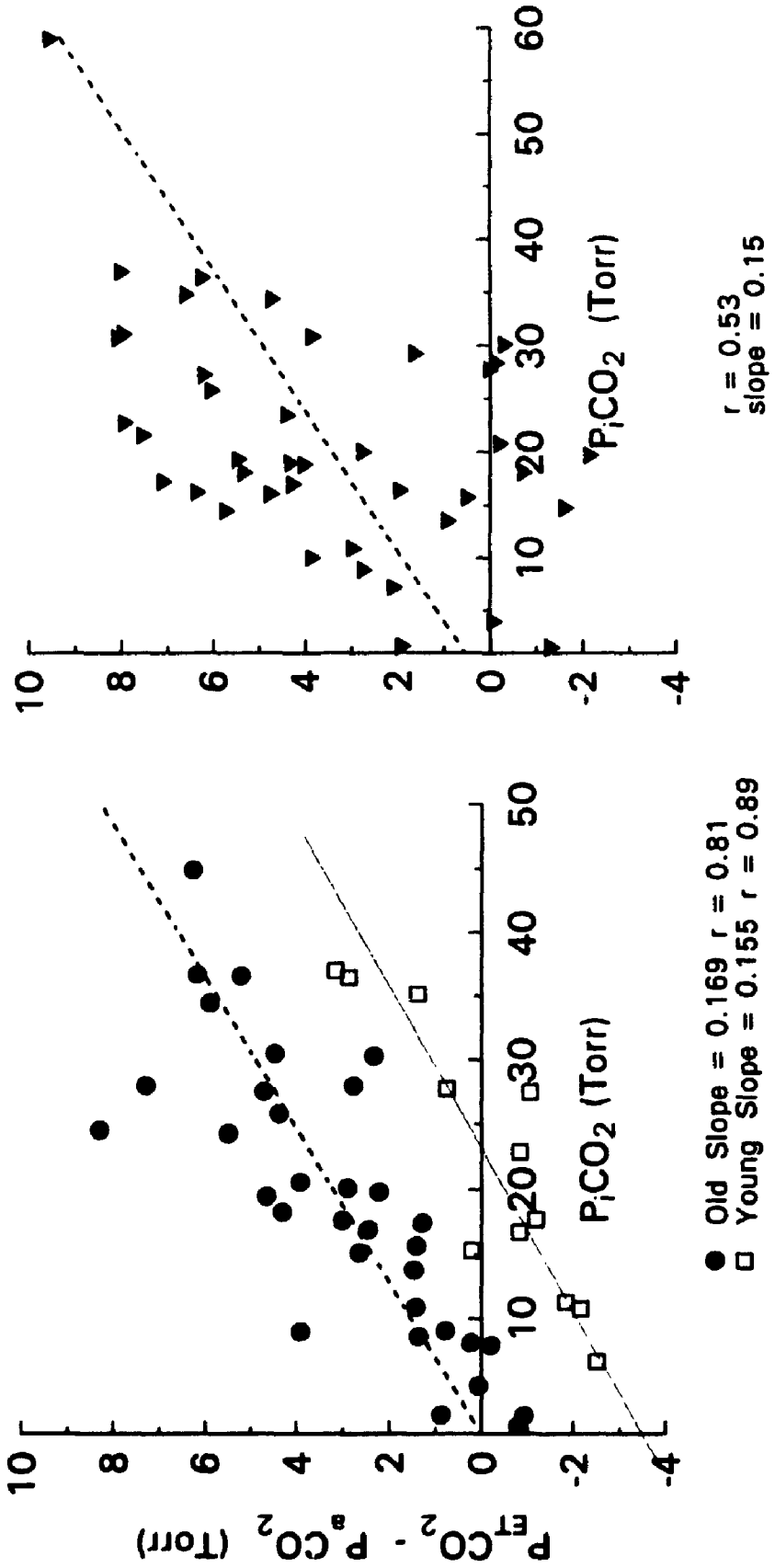
Table 13. Differences between indirect estimates and measured P_aCO_2 in elderly subjects

		Exercise	
	Rest (Torr)	25 W (Torr)	50 W (Torr)
		All experimental protocols[†]	
$P_{ET-a}CO_2$	2.9 ± 1.7*	4.0 ± 3.1*	3.7 ± 3.2*
	(1.1, 4.7)	(0.8, 7.2)	(0.4, 7.0)
$P_{AD-a}CO_2$	2.6 ± 1.9*	3.3 ± 3.1*	3.6 ± 3.8
	(0.6, 4.6)	(0.1, 6.6)	(-0.4, 7.7)
$P_{J-a}CO_2$	2.4 ± 1.3*	1.3 ± 3.0	0.6 ± 2.9
	(1.0, 3.7)	(-1.8, 4.4)	(-2.4, 3.6)
		Euoxia, eucapnia	
$P_{ET-a}CO_2$	1.7 ± 1.9	3.5 ± 3.1*	3.1 ± 3.5
	(-0.3, 3.7)	(0.3, 6.8)	(-0.5, 6.8)
$P_{AD-a}CO_2$	0.9 ± 2.4	3.1 ± 3.0	2.9 ± 4.2
	(-1.6, 3.5)	(-0.1, 6.3)	(-1.5, 7.2)
$P_{J-a}CO_2$	1.7 ± 1.7	1.6 ± 3.3	0.4 ± 3.1
	(-0.1, 3.5)	(-1.9, 5.0)	(-2.8, 3.7)

Values are means ± SD with 95% confidence intervals in parentheses.
* Values are significantly different from zero ($P < 0.05$).
† See Figure 15.

Figure 17

$P_{ET}CO_2$-P_aCO_2 difference related to P_iCO_2 at rest (left) in the elderly (closed circles) and young (open squares) subjects, and in exercise (right) for the elderly subjects only. The dashed line is the regression line fit to the points.


$P_{ET}CO_2 - P_aCO_2$ (Torr)
P_iCO_2 (Torr)
● Old Slope = 0.169 r = 0.81
□ Young Slope = 0.155 r = 0.89
P_iCO_2 (Torr)
r = 0.53
slope = 0.15

4.5 Discussion

This study was undertaken to examine the usefulness of the various methods for indirectly estimating P_aCO_2 in elderly subjects. The results of the experiments on the two younger subjects were consistent with those presented by Robbins et al. (1990) on a group of healthy young males. The older subjects in this study, however, demonstrated a number of significant differences between P_aCO_2 and the indirect estimations. At rest, our results showed that $P_{av}CO_2$ agreed closely with P_aCO_2, while all other indirect techniques overestimated arterial values. In exercise at 25 W and 50 W the $P_{av}CO_2$ and P_ICO_2 provided good mean estimates of P_aCO_2 values. All but two of the indirect techniques at both rest and in exercise, produced estimates that were greater than actual values of P_aCO_2.

Arterialized-venous PCO_2. The $P_{av}CO_2$ measurements provided the best estimates of P_aCO_2 at rest and in light exercise (25 W). These results agree with those previously reported for healthy, young adults in which arterialized-venous blood gave reliable estimates of arterial PCO_2, pH and lactate values (Forster et al., 1972; McEvoy and Jones, 1975; McLoughlin et al., 1992). In 50 W exercise however, the difference between $P_{av}CO_2$ and P_aCO_2 was much larger (1.8 Torr) than the differences reported for the rest and 25 W exercise protocols. This may have been due to the smaller number of samples at this work rate. It has been our experience that the maintenance of a patent venous catheter is difficult in the elderly due to the tendency of the superficial veins to collapse during catheterization. In this study, the vein was catheterized for at least one hour when the subjects began the 50 W exercise protocol, and patency could be

maintained in only two of the six catheters beyond this point. In addition, a reduction of skin or forearm blood flow during exercise can result in poor arterialization and inconsistent blood gases (Henriksen et al., 1984; Linderman et al., 1990; McEvoy and Jones, 1975). In this study, the PO_2 of each of the arterialized-venous samples was greater than 70 Torr, indicating that the venous blood was adequately arterialized (Forster et al., 1972). While this technique gave reasonable estimates of P_aCO_2 its usefulness in studies of respiratory control may be limited, particularly in resting studies when ventilatory drive may by augmented by anxiety or curiosity during venous blood sampling. It is known that ventilation at rest is easily stimulated by such things as discomfort, restlessness, and anxiety (Severinghaus, 1976).

End-tidal PCO_2. $P_{ET}CO_2$ consistently overestimated P_aCO_2 both at rest and in exercise in elderly subjects. At rest in the upright position, $P_{ET}CO_2$ was expected to be less than P_aCO_2 due to dilution by gas from the alveoli at the apices of lungs which are underperfused as a consequence of the effects of gravity on blood flow (Bjurstedt et al., 1962). This was confirmed, in the case of our two young subjects, by the negative 0.1 Torr difference between $P_{ET}CO_2$ and P_aCO_2. Several other authors have reported that $P_{ET}CO_2$ underestimates P_aCO_2 in young subjects at rest (Matell, 1963; Robbins et al., 1990). We anticipated that this difference would be greater in our elderly subjects due to the increase in alveolar deadspace with aging (Tenney and Miller, 1956). However, this was not the case as $P_{ET}CO_2$, $P_{AD}CO_2$ and P_ICO_2 were significantly higher than P_aCO_2 at rest in the elderly subjects (Table 14).

The reversal of the expected relationship between the estimates and P_aCO_2 in the

elderly subjects might be caused in part by the fact that these results are a composite for both air breathing and the inhalation of CO_2-air mixtures. The breathing of hypercapnic gas mixtures may have masked or reversed the diluting effect of the alveolar deadspace on the $P_{ET}CO_2$ measurements (Matell, 1963). This is consistent with our finding of a significant positive correlation of 0.81 between inspired PCO_2 and the end-tidal to arterial PCO_2 difference. Robbins et al. (1990) found that $P_{ET}CO_2$ underestimated P_aCO_2 by 1.7 Torr. The fact that this difference was lower than those previously reported (Whipp and Wasserman, 1969; Bjurstedt et al., 1962) was attributed to the breathing of different CO_2-air mixtures. This possibility may be considered by examining the sections of the protocol that did not involve the manipulation of inspired gases. When the euoxic/eucapnic resting data were analyzed separately, the mean difference between $P_{ET}CO_2$ and P_aCO_2 was lowered from 2.9 Torr to 1.7 Torr in the elderly group. This difference was no longer significant ($P > 0.05$) but remained in the opposite direction to that anticipated.

In addition, the positive end-tidal to arterial PCO_2 differences found in the elderly in this study have been reported in rebreathing equilibrium (Green et al., 1983; Jones et al., 1967), and under conditions of steady-state gas exchange in dogs (Hlastala and Robertson, 1980) and in man (Matell, 1963) under resting hypercapnic conditions. In resting human subjects rebreathing from a bag containing 7.0% to 12.0% CO_2, Jones et al. (1967) showed a small positive difference between the rebreathing alveolar PCO_2 and arterial PCO_2. Matell (1963) reported that in human subjects breathing a mixture of 6.3% CO_2, $P_{ET}CO_2$ increased by an average of 15.4 Torr, whereas the average increase

in mean P_aCO_2 was 12.3 Torr. During the hypercapnic segments of our protocol, the subjects were breathing a gas mixture that was 6.5% to 8.0% CO_2.

The blood-gas equilibration of CO_2 in the lung has been the subject of continuing controversy (Piiper, 1986). Mechanisms proposed to explain the positive gas-blood PCO_2 differences reported in the literature include the charged-membrane hypothesis (Green et al., 1983; Gurtner et al., 1969) and the delayed equilibrium hypothesis (Hlastala and Robertson, 1980), however, to date none of the suggested mechanism can account for the experimental observations quantitatively (Hlastala and Robertson, 1980). The positive gas-blood PCO_2 differences have also been attributed to directional errors that result in the overestimation of gas PCO_2 or in the underestimation of blood PCO2 (Scheid and Piiper, 1980), however, a single error or artifact sufficient to explain all of the reported positive $P_{ET}CO_2$-P_aCO_2 differences cannot be identified. In the present study, great care was taken to reduce the possibility of directional error in measurement techniques.

In spite of the problems associated with the use of hypercapnic gases as shown by the comparisons made in this study, this technique is critical to the examination of chemoreceptor controls in human experimental models. Isolated perfusion of the chemoreceptor sites is not possible in studies with human subjects, thus arterial gas tensions are altered by adjusting inspiratory gas mixtures. The dynamic end-tidal forcing technique produces perturbations in the $P_{ET}CO_2$ and $P_{ET}O_2$ that are independent of ventilation and mixed venous blood composition (Swanson and Bellville, 1975) thus opening the feedback loop from $\dot{V}_E$ to $P_{ET}CO_2$ and $P_{ET}O_2$. This technique enables the

determination of the time courses of the ventilatory responses to hypercapnia and hypoxia. Good correspondence has been reported between the dynamic forcing technique and the artificial brainstem perfusion technique used in cats to isolate the dynamic responses of the chemoreceptors (DeGoede et al., 1985). While recognizing the importance of this technique, this study indicates that caution must be exercised when determining the degree of stimulation at the chemoreceptor based on the measurement of $P_{ET}CO_2$, particularly under hypercapnic conditions.

In exercise, the occurrence of significant positive $P_{ET}CO_2$-P_aCO_2 differences has been well documented (Jones et al., 1979; Whipp and Wasserman, 1969). Our results are consistent with these findings. The slope of the alveolar phase for PCO_2 during a respiratory cycle is magnified in exercise because of the increase in CO_2 production and tidal volume (Allen et al., 1984; DuBois et al., 1952). The PCO_2 at the end of expiration becomes higher than mean P_aCO_2 approximating the maximal alveolar PCO_2 occurring during the respiratory cycle.

Jones Regression Equation. Of the noninvasive techniques for estimating arterial PCO_2, mean values for P_JCO_2 corresponded most closely with P_aCO_2 in exercise. Jones et al. (1979) developed an empirical equation using multiple regression analysis to predict P_aCO_2 from $P_{ET}CO_2$ and tidal volume. This technique has been shown to give reliable estimates of mean P_aCO_2 in exercise in young subjects (Jones et al., 1979; Robbins et al., 1990). Our data for elderly subjects showed no significant difference between mean values of P_JCO_2 and P_aCO_2, however individual differences were often quite large (Figure 16) ranging from - 6.4 to 3.9 Torr at 25 W and -4.7 to 5.3 Torr at 50 W. In exercise,

therefore, P_ICO_2 is useful for estimating mean P_aCO_2 in groups of elderly subjects, but not for individual values. The standard deviation from the mean at both 25 W (3.0 Torr) and 50 W (2.9 Torr) for the elderly subjects was greater than that of our young subjects (0.8 Torr at 50 W) as well as that reported for young males of 1.79 (50 W) and 1.96 (100 W) Torr by Robbins et al. (1990). The present data suggest that our previous findings of lower P_aCO_2 (estimated by P_ICO_2) in elderly subjects compared to young subjects (Overend et al., 1992) do not appear to be the result of the estimation technique employed. The mean P_aCO_2 in the young group (39.8 Torr) was typical of that reported in the literature (Robbins et al., 1990). The mean P_aCO_2 in the elderly group (36.3 Torr) was lower than the mean P_aCO_2 in the young subjects, at rest, though not in 50 W exercise. The aging of the lung results in a progressive reduction in arterial PO_2 (Mahler et al., 1986). However, no consistent change in arterial PCO_2 has previously been reported with age (Levitzky, 1984).

Mean alveolar PCO_2 (corrected for deadspace). In the present study $P_{AD}CO_2$ did not estimate P_aCO_2 accurately, either at rest or during exercise in our elderly subjects. Robbins et al. (1990) reported that there was no significant difference between $P_{AD}CO_2$ and P_aCO_2 (0.25 Torr) in exercise in young subjects. In our elderly subjects, $P_{AD}CO_2$ tended to overestimate P_aCO_2 in exercise.

The method used to calculate $P_{AD}CO_2$, in this study, was the same as that described by Robbins et al. (1990). This technique involves the reconstruction of the alveolar cycle in PCO_2 using respiratory flow data and a value for deadspace to calculate a time at which each PCO_2 value recorded at the mouth actually left the alveoli

(Cochrane et al., 1982). The time-weighted mean of the alveolar PCO_2 throughout the respiratory cycle ($P_{AD}CO_2$) estimates P_aCO_2. The accuracy of our $P_{AD}CO_2$ calculations could be affected by the approximation of deadspace (V_D). However the use of repeated measures analysis to compare $P_{AD}CO_2$ measurements when the V_D estimate was varied, in two of the elderly subjects, revealed that the overestimation or underestimation of V_D by as much as 100 ml had no significant effect on the $P_{AD}CO_2$ calculation. Anatomical deadspace has been reported to increase from about 150 ml in the young adult to 235 $\pm$ 67.5 ml in the elderly (mean age = 78.4 yrs) at rest (Tenney and Miller, 1956). The deadspace to tidal volume ratio (V_D/V_T) falls from between 25% and 35% at rest, to between 5% and 20% in exercise, as a result of the increase in (V_T) (Jones, 1988). Deadspace was approximated for the young and elderly subjects for each activity level using this relationship and an appropriate value for V_T (Jones, 1988). Robbins (1990) used a constant deadspace of 150 ml throughout the respiratory cycle. The $P_{AD}CO_2$ technique, however, is not widely used when compared to the regression equation developed by Jones et al. (1979).

In summary, of all the estimates of P_aCO_2, $P_{av}CO_2$ agreed most closely with true arterial values. All estimates derived from the respired gases overestimated P_aCO_2 at rest. This observation must be considered in studies of respiratory control. In human studies it is not viable to directly access the blood supply to the chemoreceptors, thus manipulations of $P_{ET}CO_2$ are commonly accepted to reflect P_aCO_2 changes at the level of the carotid bodies. It should be recognized that the P_aCO_2 at the chemoreceptor may actually be significantly less than indicated by the $P_{ET}CO_2$. Of the noninvasive

techniques, mean estimates calculated using the regression equation developed by Jones et al. (1979) agreed most closely with mean P_aCO_2 in exercise. However, individual differences between the two measures were often very large, indicating that this method is not useful for deriving individual values.

CHAPTER 5

CONCLUSIONS

5.1 General Summary

In the most widely accepted model describing the interactions between chemical respiratory feedback stimuli (Bellville et al., 1979; Berkenbosch et al., 1992; Cunningham et al., 1986; Dahan et al., 1990), hypoxia and the CO_2-H^+ complex interact at the level of the peripheral chemoreceptor, and the drives from the periphery and from the central chemosensitive area add together in their effects on ventilation. The appropriateness of this model has been demonstrated in cats using the artificial brainstem perfusion technique (Van Beek et al., 1983). The evidence in humans is not as definitive due to the difficulty in isolating respiratory stimuli to a single chemosensitive site. The purpose of the first study was to investigate the nature of the interaction between the central and peripheral chemoreflex loops in human subjects, using the different speeds of response of the central and peripheral chemoreceptors to enable a temporal separation of their chemical stimulation. In four of the five subjects studied, the ventilatory response to hypoxia was unaffected by relative hypercapnia at the central chemoreceptor. The response profile of the single individual that showed an enhanced ventilatory response to hypoxia when the central chemoreceptor was hypercapnic, also was not consistent with a multiplicative interaction between the central and peripheral chemoreceptor drives. The results indicated that the central and peripheral chemoreflexes are independent of each other and simply add together to produce the required

ventilation.

The assumption that peripheral chemoreflex drive merely adds to the more potent drive due to CO_2-H^+ at the central chemoreceptor to produce the required ventilation, has been used to support the argument that the carotid body drive is unimportant under normal, steady state conditions, except in its role in error correction or fine tuning of the homeostasis of blood gases (Forster and Pan, 1994; Weil and Swanson, 1991). The purpose of this study was to measure the contribution of the peripheral chemoreflex to $\dot{V}_E$ during the steady state of moderate intensity exercise using continuous hyperoxic suppression of carotid body drive, while stabilizing the drive from the central chemoreceptor by maintaining a constant $P_{ET}CO_2$. The peripheral chemoreceptors bodies were found to provide about 15% of the drive to breathe in the steady state of moderate intensity exercise. This modest contribution supported the theory that the arterial chemoreceptors function to "fine tune" alveolar $\dot{V}$ to minimize change in arterial blood gases (Forster and Pan, 1994; Weil and Swanson, 1991). Sustained hyperoxia, however, appeared to alter respiratory control by lowering the set point about which P_aCO_2 was regulated.

The purpose of the final study was to compare measured P_aCO_2 values to the estimates obtained from arterialized venous blood ($P_{av}CO_2$), end-tidal PCO_2 ($P_{ET}CO_2$), mean alveolar PCO_2, corrected for respiratory and apparatus deadspace ($P_{AD}CO_2$), and the regression equation developed by Jones et al. (1979) (P_JCO_2), in healthy elderly subjects, while breathing gas mixtures that are frequently used in studies of ventilatory control (euoxic/eucapnic; hypoxic/eucapnic; hyperoxic/eucapnic; and

hyperoxic/hypercapnic) as a means of stimulating the respiratory chemoreceptors. $P_{a\bar{v}}CO_2$ agreed most closely with true arterial PCO_2 values, however the usefulness of this technique in resting studies of respiratory control is limited, particularly in resting studies when ventilatory drive may by augmented by anxiety or curiosity during venous blood sampling. It is known that ventilation at rest is easily stimulated by such things as discomfort, restlessness, and anxiety (Severinghaus, 1976). All estimates derived from the respired gases overestimated P_aCO_2 at rest. This observation must be considered in studies of respiratory control. In human studies it is not viable to directly access the blood supply to the chemoreceptors, thus manipulations of $P_{ET}CO_2$ are commonly accepted to reflect P_aCO_2 changes at the level of the carotid bodies and the medulla. It should be recognized that the P_aCO_2 at the chemoreceptor may actually be significantly less than that indicated by the $P_{ET}CO_2$. Of the noninvasive techniques, mean estimates calculated using the regression equation developed by Jones et al. (1979) agreed most closely with mean P_aCO_2 in exercise. However, individual differences between the two measures were often very large, indicating that this method is not useful for deriving individual values.

5.2 Implications for Future Research

1. One means by which human physiologists study respiratory control, is by altering the inspired partial pressures of CO_2 and O_2 and measuring the resultant change in $\dot{V}_E$. A particular profile of alveolar gas partial pressures leads to a change of arterial gas partial pressures which stimulates the chemoreceptors. It is usually assumed that the measured end-tidal or alveolar partial pressures are in close agreement with the arterial pressures of the gases. The experimental techniques employed in the studies outlined in this thesis use feedback control of the inspired gas tensions to produce perturbations in $P_{ET}CO_2$ and $P_{ET}O_2$. $P_{ET}CO_2$ is commonly used to predict P_aCO_2 in this type of study (Bascom et al., 1992; Becker et al., 1995; Berkenbosch et al., 1992; Dahan et al., 1990; Easton et al., 1988; Paterson et al., 1993). The results of the studies presented in Chapter 4 demonstrated, however, that $P_{ET}CO_2$ is not always a reliable indicator of P_aCO_2, particularly when CO_2 is added to the inspirate. Therefore caution must be exercised regarding the limits of interpretation of the exact stimulus to the chemosensitive tissue. Arterialized-venous PCO_2 was found to be in close agreement with P_aCO_2, however, concerns were raised that anxiety or curiosity during blood sampling may influence ventilatory output, and thus limit the ability to determine the true stimulus to breathe in control studies. In respiratory control studies, subjects typically undergo a period of accommodation to the apparatus, in an effort to prevent such augmentation of ventilatory drive due to the mouthpiece and noseclips. Arterialized-venous sampling may provide a valuable means of estimating P_aCO_2 if the subject can be accommodated to the catheter,

and if great care is taken to ensure minimal disruption during sampling.

2. The effects of changes in inspired PCO_2 and PO_2 on cerebral blood flow (CBF) complicate the interpretation of respiratory control studies. Transcranial ultrasound Doppler recording provides a noninvasive means of assessing the velocity of flow in cerebral arteries (Markwalder et al., 1984). This technique is currently being used in a number of laboratories to study the dynamic responses of CBF to step changes in end-tidal gases. Knowledge of the CBF response to a given stimulus, would enable a more accurate estimate of the intensity of the stimulus, at the level of the central chemoreceptors.

3. The results of the exercise studies in Chapter Three indicate that sustained hyperoxia alters respiratory control. The recovery from hyperoxia has not been studied in any detail, yet the time course of the adjustments in the control mechanisms, appears to be prolonged. Extending the time period of data collection in this recovery phase, would provide valuable information regarding the contributions of the chemoreflexes to the resetting of the equilibrium following a hyperoxic challenge. Resting studies would eliminate any complications due to the exercise. Measurements of CO_2 sensitivity pre- and post-hyperoxia, would provide one means of examining potential mechanisms of change. The sampling of arterial or arterialized-venous blood, at regular intervals throughout the protocol, would allow the direct measurement of the regulated level of P_aCO_2, as well as the changes in pH induced by the Haldane effect.

APPENDIX I

GLOSSARY OF VARIABLES AND UNITS

PARTIAL PRESSURES

Partial Pressure	the pressure required to support a column of mercury 1.0 mm Hg or in Torr (1 Torr = 1 mmHg at 0° C)
Euoxia	a normal amount of oxygen in the air, blood or tissues
Hypercapnia	a greater than normal arterial partial pressure of carbon dioxide
Hypocapnia	a lower than normal arterial partial pressure of carbon dioxide
Hyperoxia	a greater than normal amount of oxygen in the air, blood or tissues
Hypoxia	a lower than normal partial pressure of oxygen or arterial saturation, or both
PCO_2	partial pressure of carbon dioxide (Torr)
PO_2	partial pressure of oxygen (Torr)
P_aCO_2	arterial partial pressure of carbon dioxide (Torr)
P_ACO_2	alveolar partial pressure of carbon dioxide (Torr)
$P_{ET}CO_2$	end-tidal partial pressure of carbon dioxide (Torr)
P_aO_2	arterial partial pressure of oxgen (Torr)
P_AO_2	alveolar partial pressure of oxygen (Torr)
$P_{ET}O_2$	end-tidal partial pressure of oxygen (Torr)

RESPIRATORY VARIABLES

O_2	Oxygen
CO_2	Carbon dioxide
N_2	Nitrogen

H^+	Hydrogen ion
HCO_3^-	Bicarbonate ion
RER	respiratory exchange ratio (carbon dioxide production/oxygen consumption)
T_{VE}	ventilation threshold
$\dot{V}_A$	alveolar ventilation ($l \cdot min^{-1}$)
$\dot{V}_E$	expired ventilation ($l \cdot min^{-1}$)
V_D	deadspace or physiologic deadspace (ml)
$\dot{V}_D$	deadspace ventilation ($l \cdot min^{-1}$)
V_T	tidal volume (ml)
V_D/V_T	ratio of deadspace to tidal volume
$\dot{V}CO_2$	carbon dioxide production ($l \cdot min^{-1}$)
$\dot{V}O_2$	oxygen consumption ($l \cdot min^{-1}$)
$\dot{V}_E/\dot{V}CO_2$	ventilatory equivalent for carbon dioxide
$\dot{V}_E/\dot{V}O_2$	ventilatory equivalent for oxygen
$\dot{V}O_2max$	maximal oxygen consumption ($l \cdot min^{-1}$)

TEMPORAL PARAMETERS, CONTROL OF BREATHING, AND MODELLING

Exponential Function	a function of type e^{At}, where e is the base of the natural logarithm and A is the characteristic time constant of the particular growth or decay process.
τ_c	time constant, central chemoreflex loop (s)
τ_p	time constant, peripheral chemoreflex loop (s)
G_c	gain, central chemoreflex loop ($l \cdot min^{-1} \cdot Torr^{-1}$)

G_p	gain, peripheral chemoreflex loop ($l \cdot min^{-1} \cdot Torr^{-1}$)
T_c	time delay, central chemoreflex loop (s)
T_p	time delay, peripheral chemoreflex loop (s)

STATISTICAL NOTATIONS

μ	mean (population)
σ	standard deviation (population)
SD	standard deviation (sample)
SEM	standard error of the mean (sample)
r	correlation coefficient
n	number of observations
P	probability
$P \leq 0.05$	denoting level of significance
Residuals	the distance from the data point to a regression line. The residuals represent the unexplained variation after fitting the model.
RSS	Residual Sum of Squares
MSE	Mean Square Error (RSS/n)

APPENDIX II
LETTER OF INFORMATION AND CONSENT FORM FOR STUDY DESCRIBED IN CHAPTER 2

LETTER OF INFORMATION

A Study of the Contributions of the Central and Peripheral Chemoreflex Loops to the Control of Ventilation in Humans

Principal Investigator: David A. Cunningham Ph.D.

The process of ageing is accompanied by many changes and adaptations in the way the body reacts to physical and chemical changes. There are alterations in the respiratory system which may affect the way in which an older person responds to breathing air with low oxygen (50-70% of normal) or high carbon dioxide (less than 20% above normal).

You are being asked to participate in a study that will investigate the way the nervous system responds to changes in the composition of the gases in the air we breathe. The breathing responses of young individuals (aged 18 to 45 years) will be compared with those of older individuals (60+ years) to examine the effects of the process of ageing on the control of breathing.

The experiments will be conducted using computer controlled equipment which will alter the concentrations of oxygen and carbon dioxide that you breathe. The effects of these changes will be monitored by instruments and observation, and adjustments made to avoid any feelings of discomfort.

The experiments involve your sitting in a chair, breathing through a mouthpiece while wearing a noseclip and ECG leads (to monitor heart rate). You may feel your breathing change during the experiments. This is expected, but it is extremely unlikely that you will experience any faintness. You may indicate that you wish to stop an experiment at any time, and an experiment will be stopped if any signs occur which indicate that there are problems.

Participation in this study involves 10 to 12 visits to the laboratories at the Centre for Activity and Ageing. Each visit will last two to three hours and will involve two or three related experiments (20 minutes each) with breaks in between. Because caffeine can affect the breathing, it is important that you do not consume any tea, coffee, colas or any preparation which contains it for four hours prior to attending the laboratory. You may read during the experiments. While some magazines are provided, you may wish to bring your own reading material.

During the series of experiments blood sampling may be required on a single occasion to measure the exact concentration of oxygen and carbon dioxide in the blood.

On this occasion, the physician at the Centre for Activity and Ageing will place a catheter into a vein on the back of one hand. There may be some pain experienced when the catheter is placed into your vein (no more than when you get a needle in your arm), after which you should feel no pain or discomfort. The amount of blood taken will amount to no more than 5 teaspoons. Your hand will be kept warm using a heating pad. Minor bruising is common following catheterization, but it generally fades in a few days. Precautions will be taken to prevent serious bruising, discomfort and infection.

You may refuse to participate. If you agree to participate, you are free to withdraw from the study at any time. Withdrawal will not affect your relationship with the Centre. Records from the studies are confidential and securely stored. The records are listed according to an identification number rather than your name.

If you have any questions regarding this study, please contact:

Claudette St. Croix
Room 022
Centre for Activity and Ageing
Mount St. Joseph's
London

661-1614

or

David A. Cunningham, Ph.D.
Centre for Activity and Ageing
Mount St. Joseph's
London

661-1603

LETTER OF INFORMED CONSENT

A Study of the Contributions of the Central and Peripheral Chemoreflex Loops to the Control of Breathing in Humans

Principal Investigator: David A. Cunningham, Ph.D.

I have carefully read the accompanying "Letter of Information", and have had the nature of the study and the procedures satisfactorily explained to me. All my questions have been answered to my satisfaction.

By signing below, I agree to participate in this study.

_____

Name (please print)

_____

Signature

_____

Date

APPENDIX III
LETTER OF INFORMATION AND CONSENT FORM FOR STUDY DESCRIBED IN CHAPTER 3

LETTER OF INFORMATION

Chemoreceptor Control of Ventilation During Exercise in Humans

Principal Investigator: David A. Cunningham, Ph.D.

You are being asked to participate in a study that examines way in which the nervous system adjusts our breathing in response to exercise. The process of ageing is accompanied by many changes and adaptations in the way the body reacts to physical and chemical challenges. There are alterations in the respiratory system which may affect the way in which an older person responds to an exercise stress. This study will examine the effect of aging on the mechanisms that control the breathing response to exercise.

Participation will involve 10 to 12 visits to the laboratory at the Centre for Activity and Aging. Each visit will be approximately two hours. During the first visit, you will be asked your family medical history and then undergo a progressive exercise test on an exercise bicycle to voluntary exhaustion under medical supervision.

On the remainder of the visits you will be asked to perform two exercise tests on an exercise bicycle, each lasting 24 minutes with a 30 minute rest in between tests. The test will begin with 4 minutes of loadless pedalling, where there will be no resistance placed on the pedals. The resistance will then increased instantaneously and you will continue pedalling at this workrate for 16 minutes. The resistance will then be removed instantaneously and you will continue pedalling for an additional 4 minutes. The resistances will represent either a moderate or a heavy exercise intensity.

The experiments will be conducted using computer controlled equipment which will alter the concentrations of oxygen and carbon dioxide that you breathe. The effects of these changes will be monitored by instruments and observation, and adjustments made to avoid any feelings of discomfort.

Any intensity of exercise causes a slight risk of heart attack, or may be uncomfortable if you are unfit or not used to exercise. You may experience an increased awareness of breathing, muscle pain and/or fatigue, increased sweating, general feeling of fatigue, nausea. You will be required to wear a noseclip (to prevent you from breathing through your nose) and a mouthpiece (to measure the amount of air you breathe) during the exercise test and these may offer some initial discomfort.

On two of the visits, you will be required to have a catheter placed in a vein in the back of your hand. This will be done by a qualified and experienced individual. There may be some pain experienced when the catheter is placed in your vein (no more than when you get a needle in your arm), after which you should feel no pain or discomfort. The catheter will remain in your vein during the entire test and will be used to sample blood.

The volume of blood taken will amount to no more than 100 ml (25 tsp). Your arm will be kept warm using a heating pad. Minor bruising sometimes occurs following venous catheterization, but generally fades after a few days.

You may refuse to participate. If you agree to participate, you are free to withdraw from the study at any time. Withdrawal will not affect your relationship with the Centre. Records from the studies are confidential and securely stored. The records are listed according to an identification number rather than your name.

If you have any questions regarding this study, please contact:

Claudette St. Croix
Room 022
Centre for Activity and Ageing
Mount St. Joseph's
London

661-1614

or

David A. Cunningham, Ph.D.
Centre for Activity and Ageing
Mount St. Joseph's
London

661-1603

LETTER OF INFORMED CONSENT

Chemoreceptor Control of Ventilation During Exercise in Humans

Principal Investigator: David A. Cunningham, Ph.D.

I have carefully read the accompanying "Letter of Information", and have had the nature of the study and the procedures satisfactorily explained to me. All my questions have been answered to my satisfaction.

By signing below, I agree to participate in this study.

_____

Name (please print)

_____

Signature

_____

Date

APPENDIX IV
LETTER OF INFORMATION AND CONSENT FORM FOR STUDY DESCRIBED IN CHAPTER 4

LETTER OF INFORMATION

An Examination of the Use of End Tidal Carbon Dioxide Tension as a Predictor of Arterial Carbon Dioxide Tension in Elderly Males

Principal Investigator: David A. Cunningham, Ph.D.

The process of ageing is accompanied by many changes and adaptations in the way the body reacts to physical and chemical challenges. The level of carbon dioxide in the blood is one of the most important factors controlling our breathing. When researchers study the regulation of breathing at rest or when we are exercising, it is important for them to understand what is going on in the blood. The levels of blood gases in healthy young people can be estimated from measuring the amounts of carbon dioxide and oxygen in the air we expire. However, in older individuals there may be changes in the lungs that would cause this estimate to be inaccurate.

You are being asked to participate in a study that will investigate the available ways for measuring and estimating the levels of carbon dioxide in the blood, and assess their usefulness in an older population. The results will be of use to future research in the control of breathing in older individuals.

You will be required to come to the laboratory on two occasions. The first session will take approximately two hours. During this visit you will be asked your family medical history and then undergo a progressive exercise test to voluntary exhaustion under medical supervision.

The specific experimental procedures will be performed during your second visit to the laboratory. The tests require the placement of a catheter in an artery in your forearm. The risks associated with this procedure are minimal in healthy individuals. The physician from the Centre for Activity and Ageing will accompany you to St. Joseph's Health Centre where the catheter will be inserted by highly qualified and experienced staff. All the necessary precautions will be taken to prevent any possibility of excess bleeding, discomfort, bruising or infection. The doctor will then escort you back to the Centre where the testing will take place.

A catheter will also be placed into a vein on the back of your opposite hand. This will be done by a physician. There may be some pain experienced when the catheter is placed into your vein (no more than when you get a needle in your arm), after which you should feel no pain or discomfort. The catheters will remain in place during the entire test and will be used to sample blood. The amount of blood taken will amount to no more than 25 ml (7 tsp). Your hand will be kept warm using a heating pad. Minor bruising is common following catheterization, but it generally fades in a few days. Precautions will be taken to prevent serious bruising, discomfort and infection.

The experiments will be conducted using computer controlled equipment which will alter concentrations of oxygen and carbon dioxide that you breathe. The effects of the changes in the gas you breathe will be monitored by instruments and observation, and adjustments made to avoid any feelings of discomfort.

The experiments will involve sitting in a chair or doing light exercise on a stationary bicycle, breathing through a mouthpiece while wearing a noseclip and ECG leads (to monitor heartrate). You may feel your breathing change during the experiments. This is expected, but it is extremely unlikely that you will experience any faintness. You may indicate that you wish to stop an experiment at any time and an experiment will be stopped if any signs occur which indicate that there are problems.

Once the tests have been completed, the catheters will be removed by the Centre's physician.

You may refuse to participate. If you agree to participate, you are free to withdraw from the study at any time. Withdrawl will not affect your relationship with the Centre. Records from the studies are confidential and securely stored. The records are listed according to an identification number rather than your name.

If you have any questions regarding this study, please contact:

Claudette St. Croix
Room 013
Centre for Activity and Ageing
Mount St. Joseph's
London

661-1603

Protocol No. 3498

LETTER OF INFORMED CONSENT

An Examination of the Use of End Tidal Carbon Dioxide Tension as a Predictor of Arterial Carbon Dioxide Tension in Elderly Males

Principal Investigator: Dr. David Cunningham

I have carefully read the accompanying "Letter of Information", and have had the nature of the study and the procedures satisfactorily explained to me. All my questions have been answered to my satisfaction.

By signing below, I agree to participate in this study.

_____ _____

Name (please print) Signature

_____

Date

Protocol No. 3498

APPENDIX V
SAMPLE SIZE CALCULATION FOR THE STUDY DESCRIBED IN CHAPTER 2

Estimations of the mean and variance of the primary outcome measure were obtained from Robbins (1988). The standard formula for calculating sample size was adjusted for the examination of differences within subjects.

$$n = 1 \times (z_{1-\alpha/2} + z_{1-\beta})^2 \times (\text{SD/expected change})^2$$

$$n = 1(1.96 + 0.84)^2 \times (2.78/4.5)^2$$

$$n = 2.99$$

APPENDIX VI

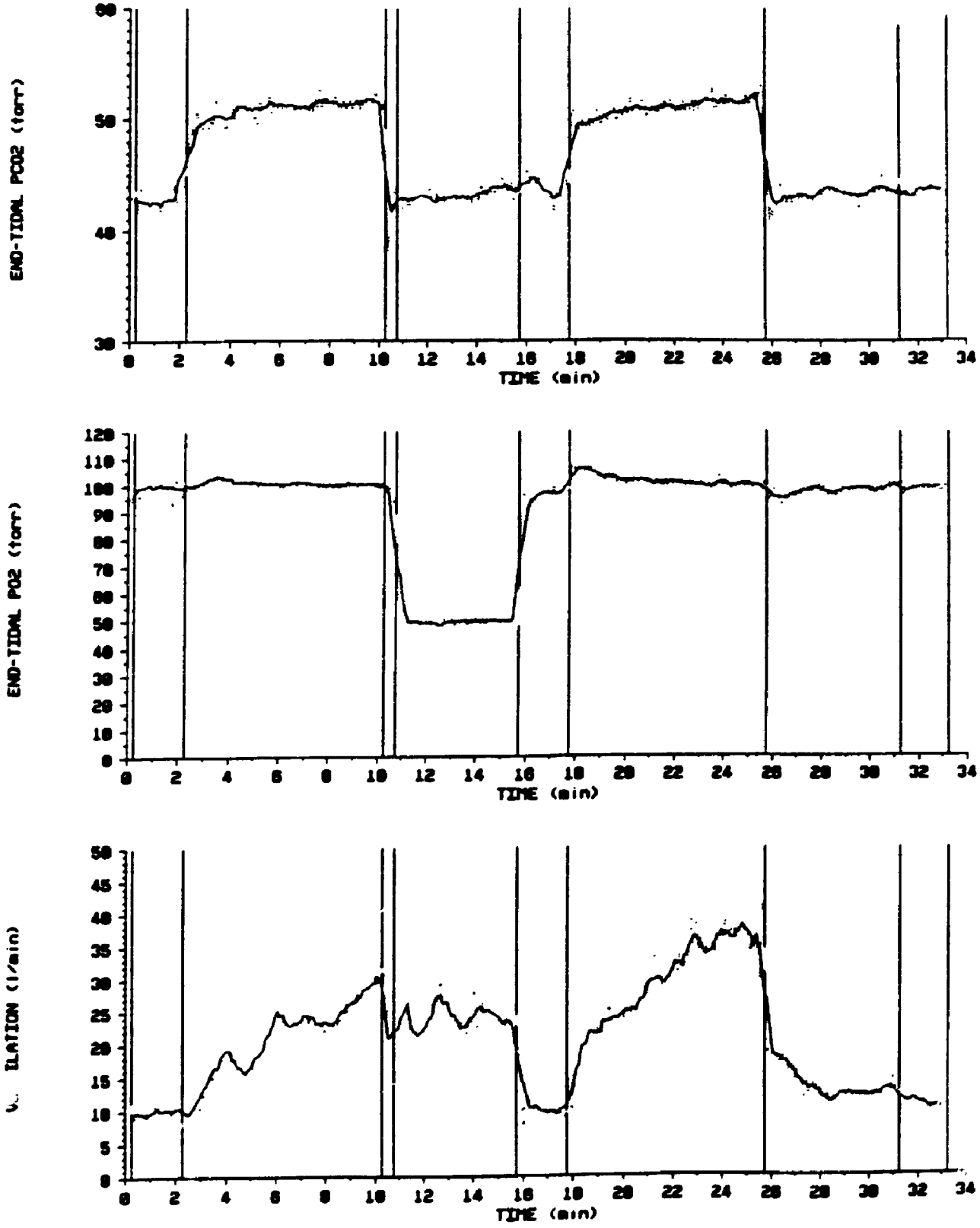
$\dot{V}_E$, $P_{ET}CO_2$, and $P_{ET}O_2$ responses for a single subject to protocols A and B, and two type C protocols admininistered in the same breathing session

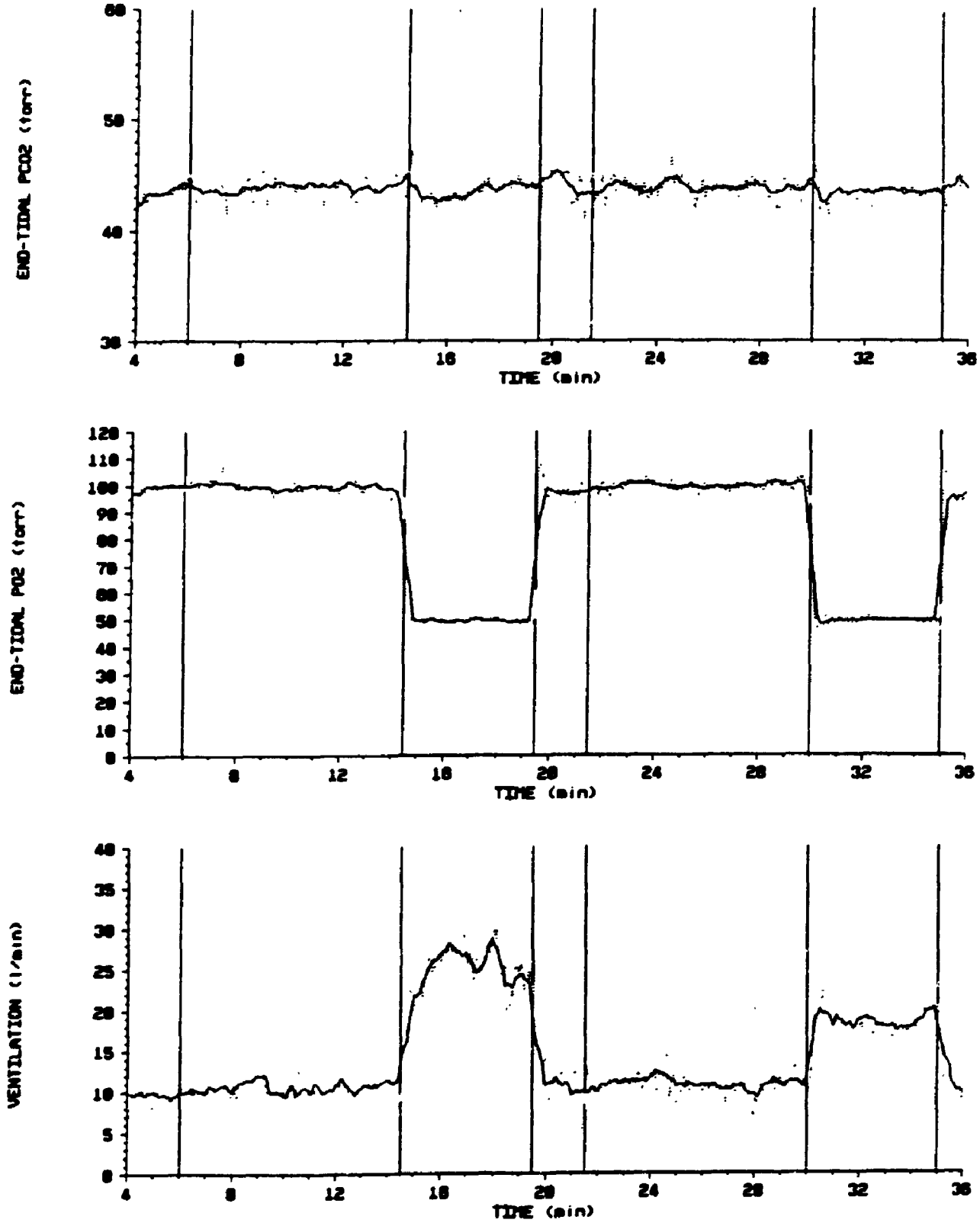
Page 151

$P_{ET}CO_2$, $P_{ET}O_2$, and $\dot{V}_E$ responses of a single subject to a type A experimental protocol immediately followed by a hypercapnia control protocol B as described in Chapter 2.

Page 152

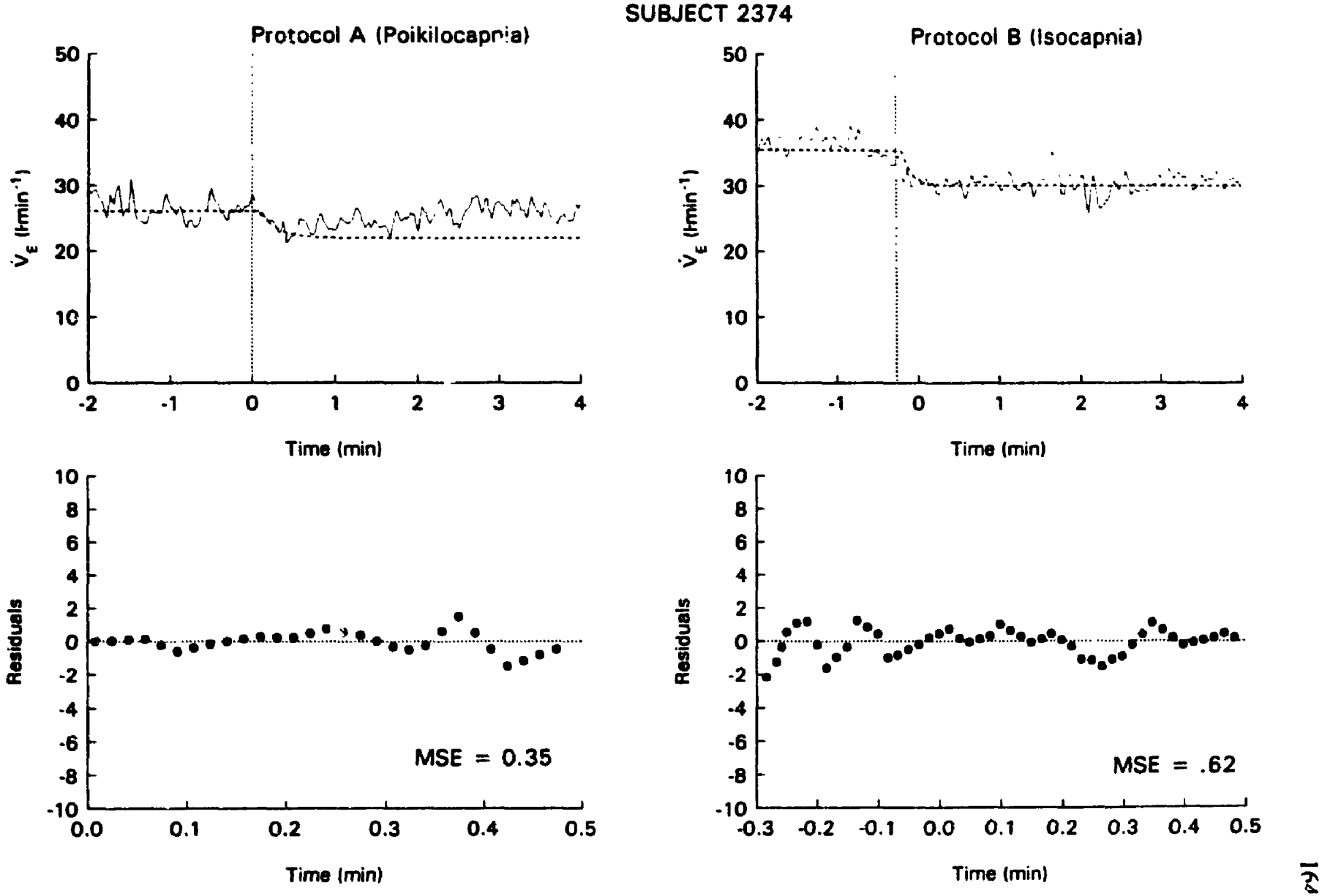
$P_{ET}CO_2$, $P_{ET}O_2$, and $\dot{V}_E$ responses of a single subject to two successive type C hypoxic protocols administered in the same breathing session as described in Chapter 2.

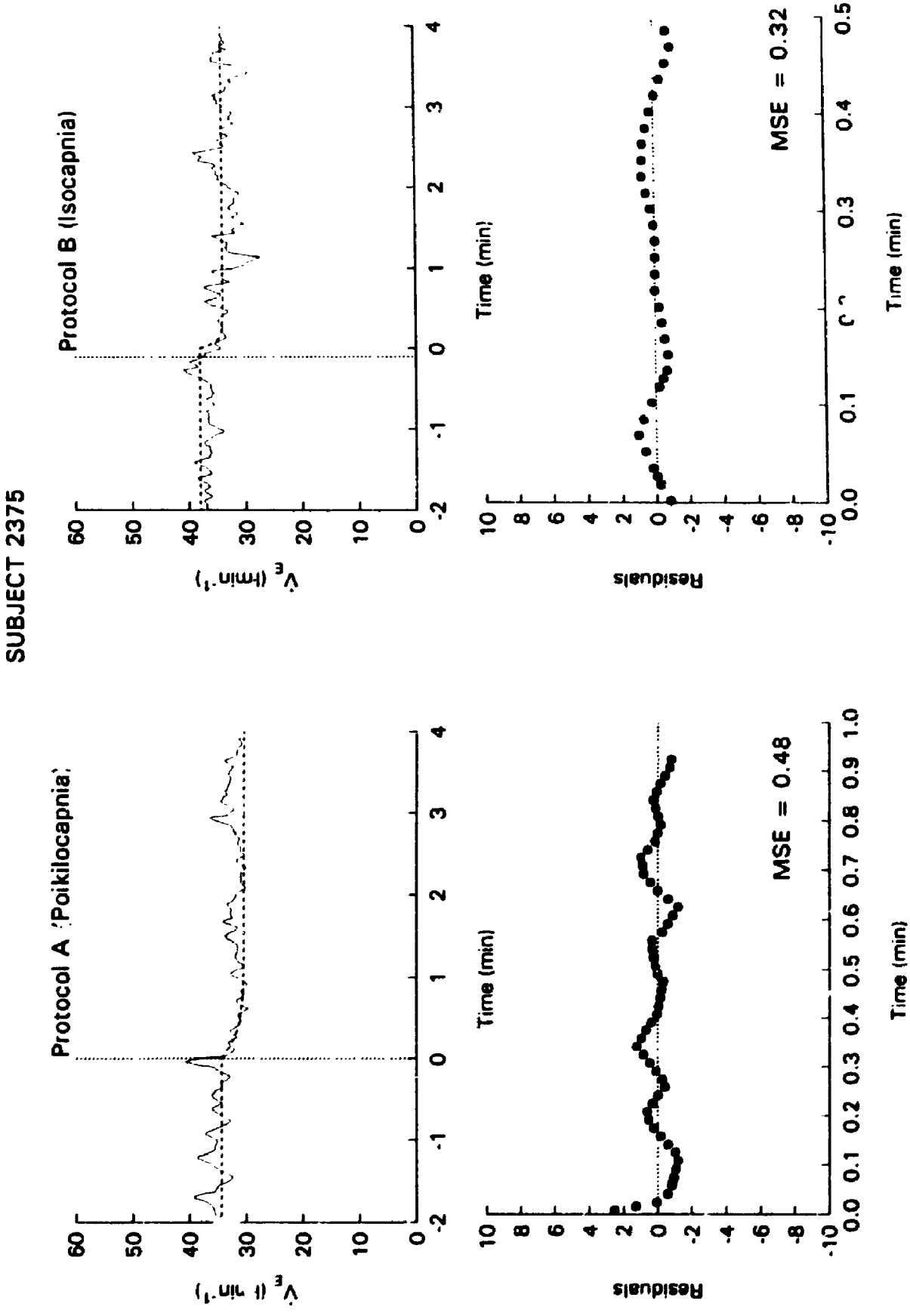

END-TIDAL PCO2 (torr)
TIME (min)
END-TIDAL PO2 (torr)
TIME (min)
TIME (min)


END-TIDAL PCO2 (torr)
60
50
40
30
4
8
12
16
20
24
28
32
36
TIME (min)
END-TIDAL PO2 (torr)
120
110
100
90
80
70
60
50
40
30
20
10
0
4
8
12
16
20
24
28
32
36
TIME (min)
VENTILATION (l/min)
40
35
30
25
20
15
10
5
0
4
8
12
16
20
24
28
32
36
TIME (min)

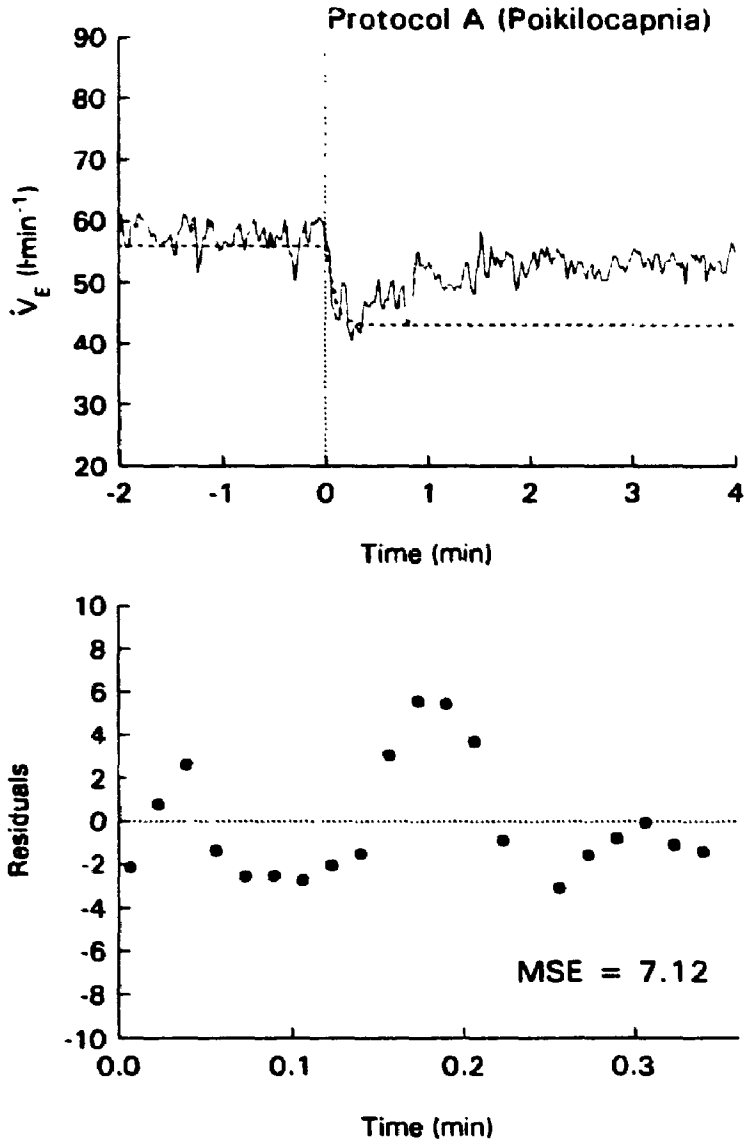
APPENDIX VII

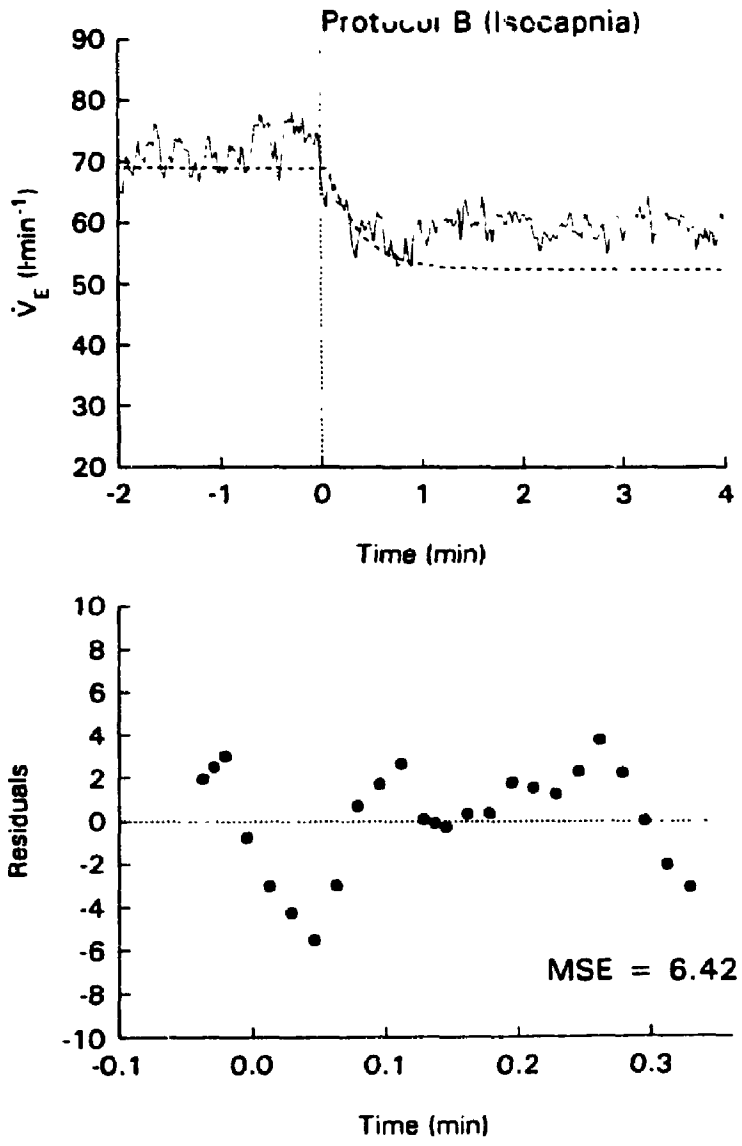
VENTILATORY RESPONSES, MODEL FITS AND RESIDUALS FOR THE STEP UP INTO HYPEROXIA IN INDIVIDUAL SUBJECTS


SUBJECT 2374
Protocol A (Poikilocapnia)
Protocol B (Isocapnia)
$\dot{V}_E$ (lmin^{-1})
Time (min)
Residuals
MSE = 0.35
MSE = .62

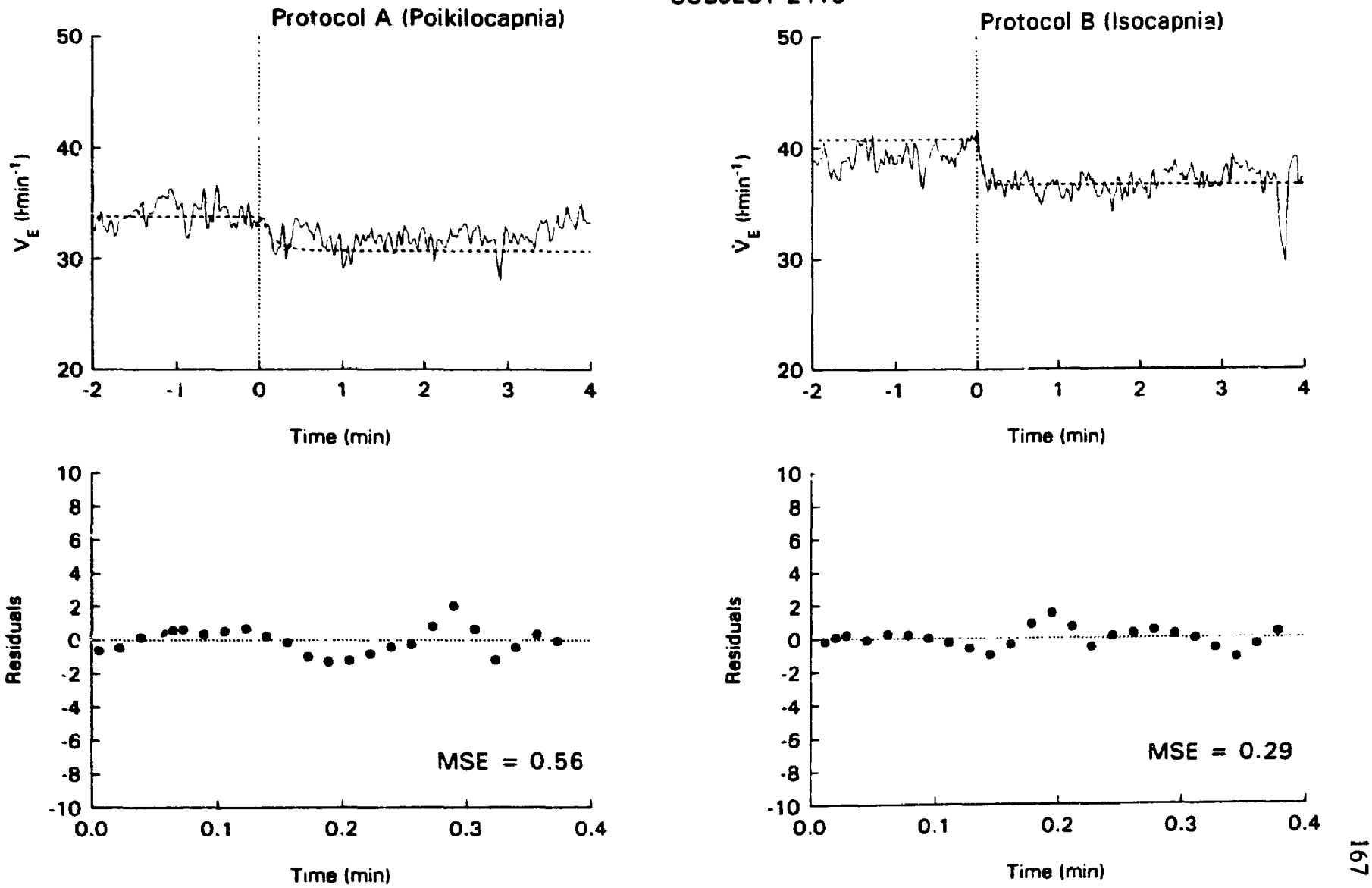

SUBJECT 2375
Protocol A 'Poikilocapnia'
Protocol B (Isocapnia)
$\dot{V}_E$ (l.min^{-1})
Residuals
Time (min)
MSE = 0.48
MSE = 0.32

SUBJECT 2464



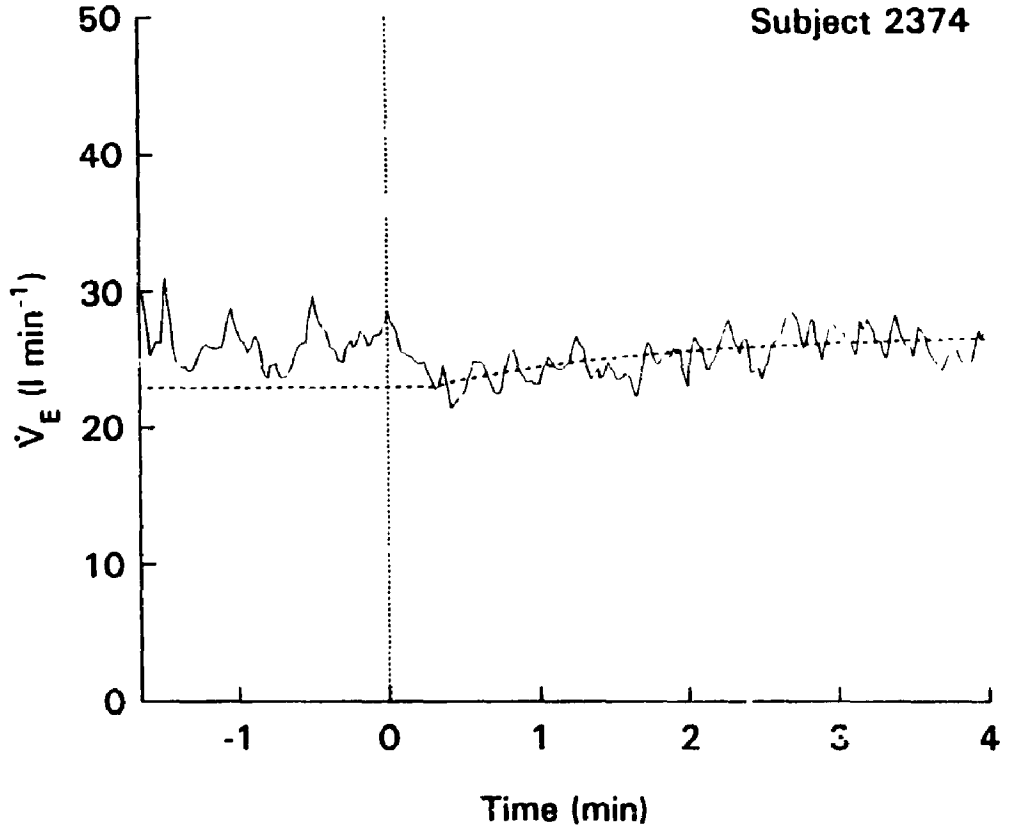


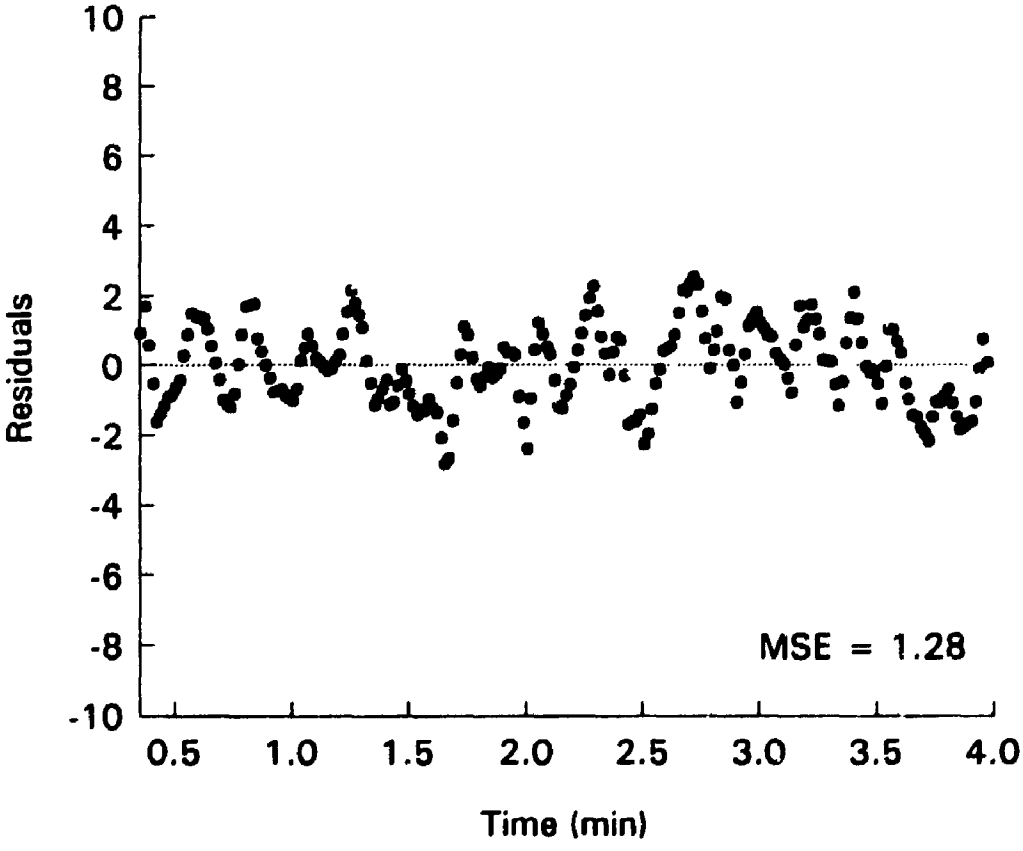
SUBJECT 2410

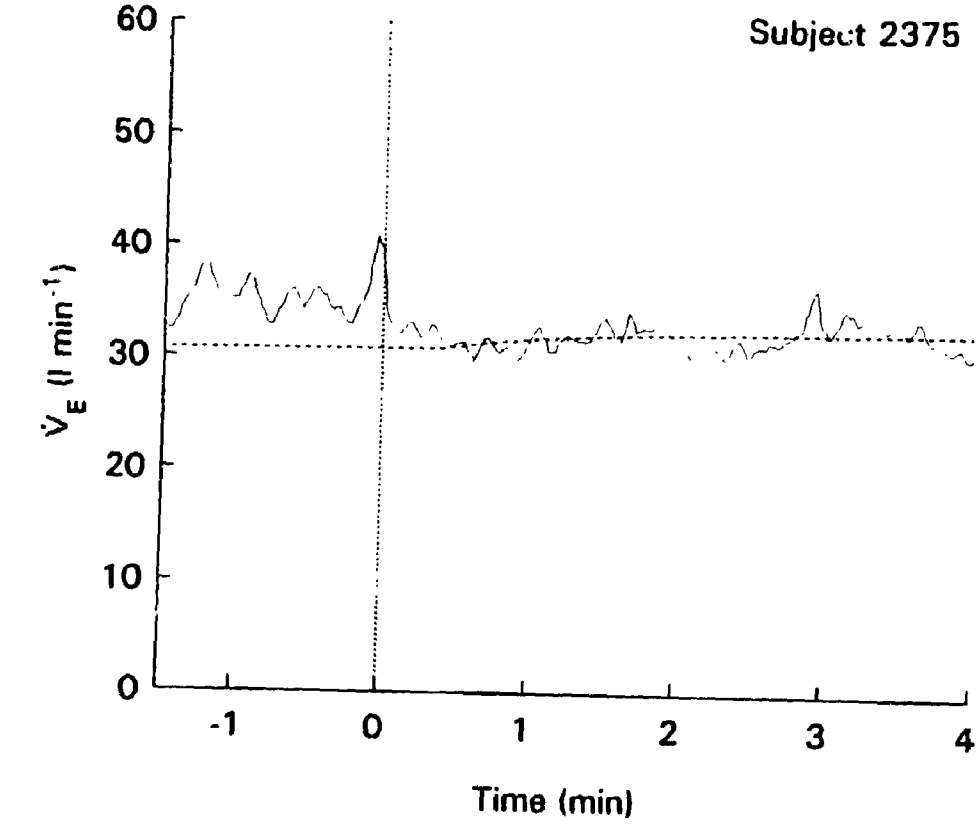


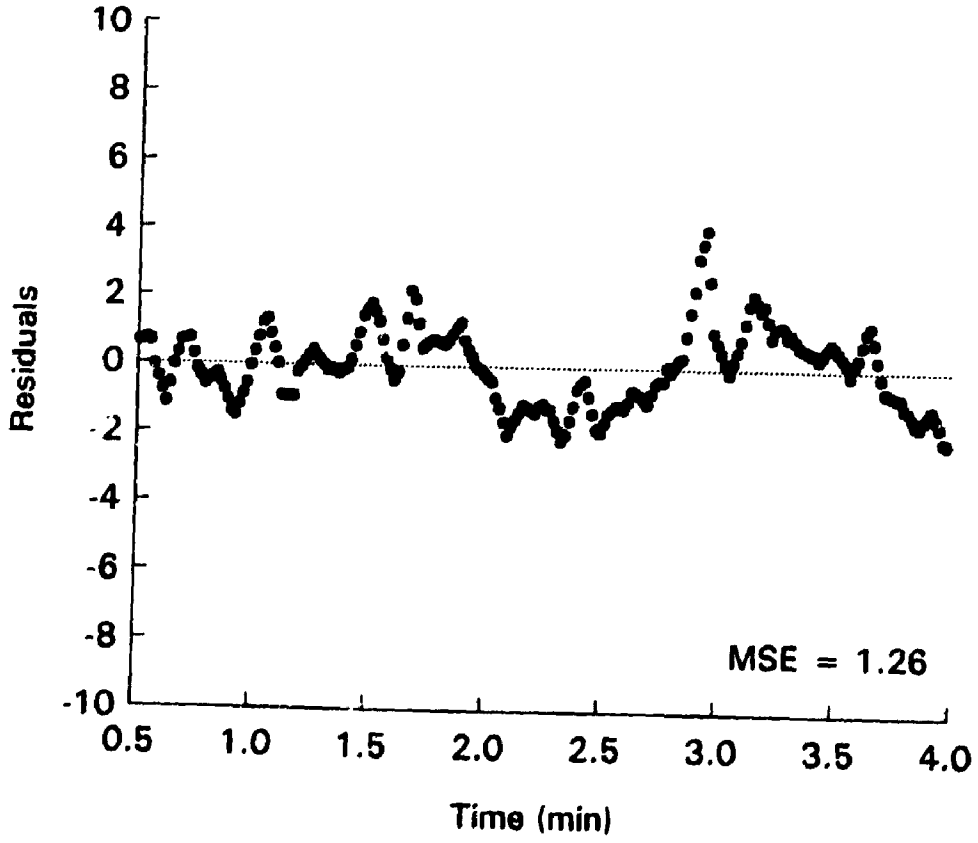
APPENDIX VIII

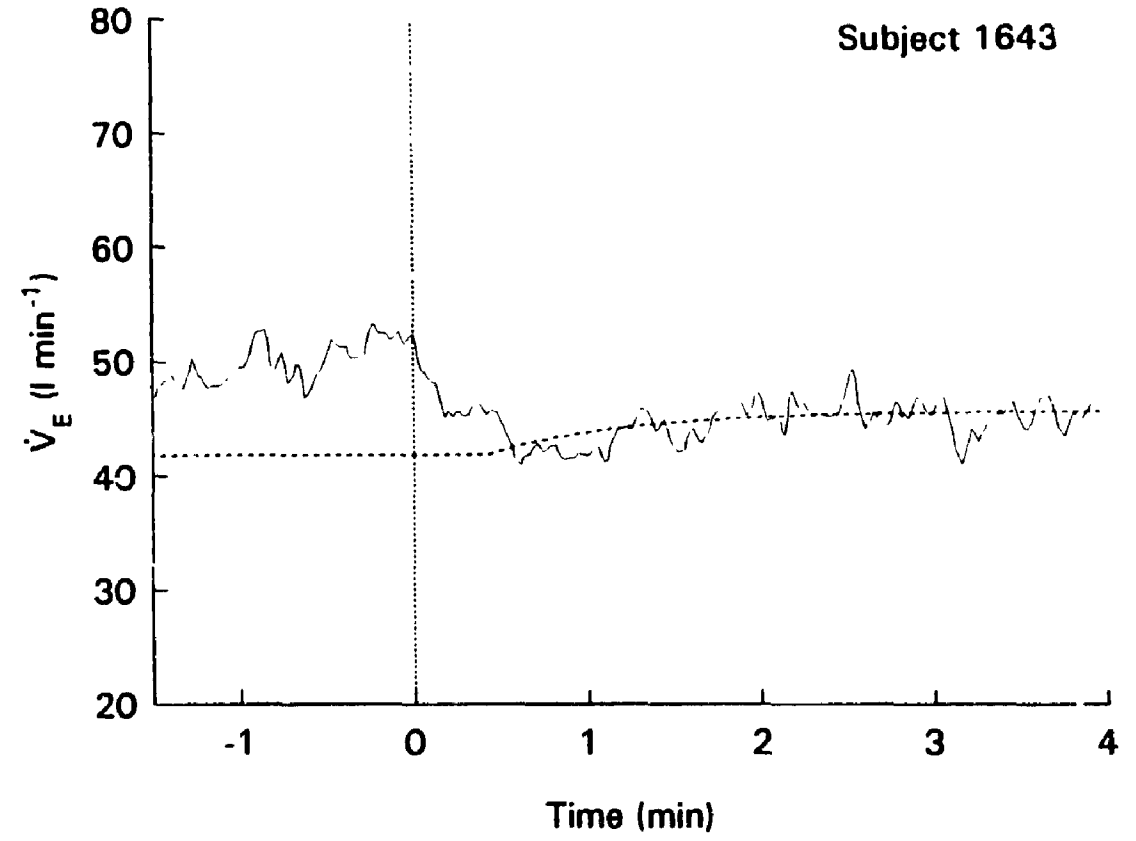
VENTILATORY RESPONSES, MODEL FITS AND RESIDUALS FOR THE SECONDARY INCREASE IN $\dot{V}_E$, IN RESPONSE TO HYPEROXIA

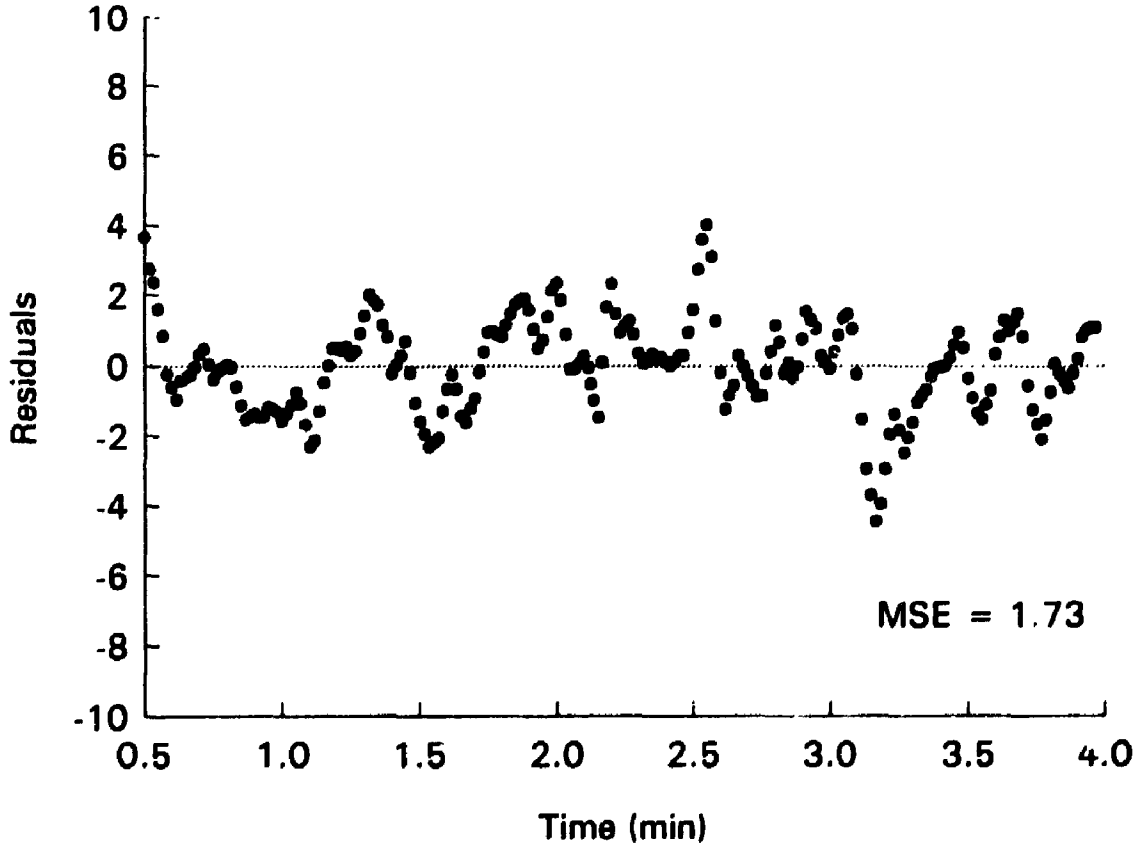

Subject 2374
50
40
30
20
10
0
$\dot{V}_E$ (l min^{-1})
-1
0
1
2
3
4
Time (min)

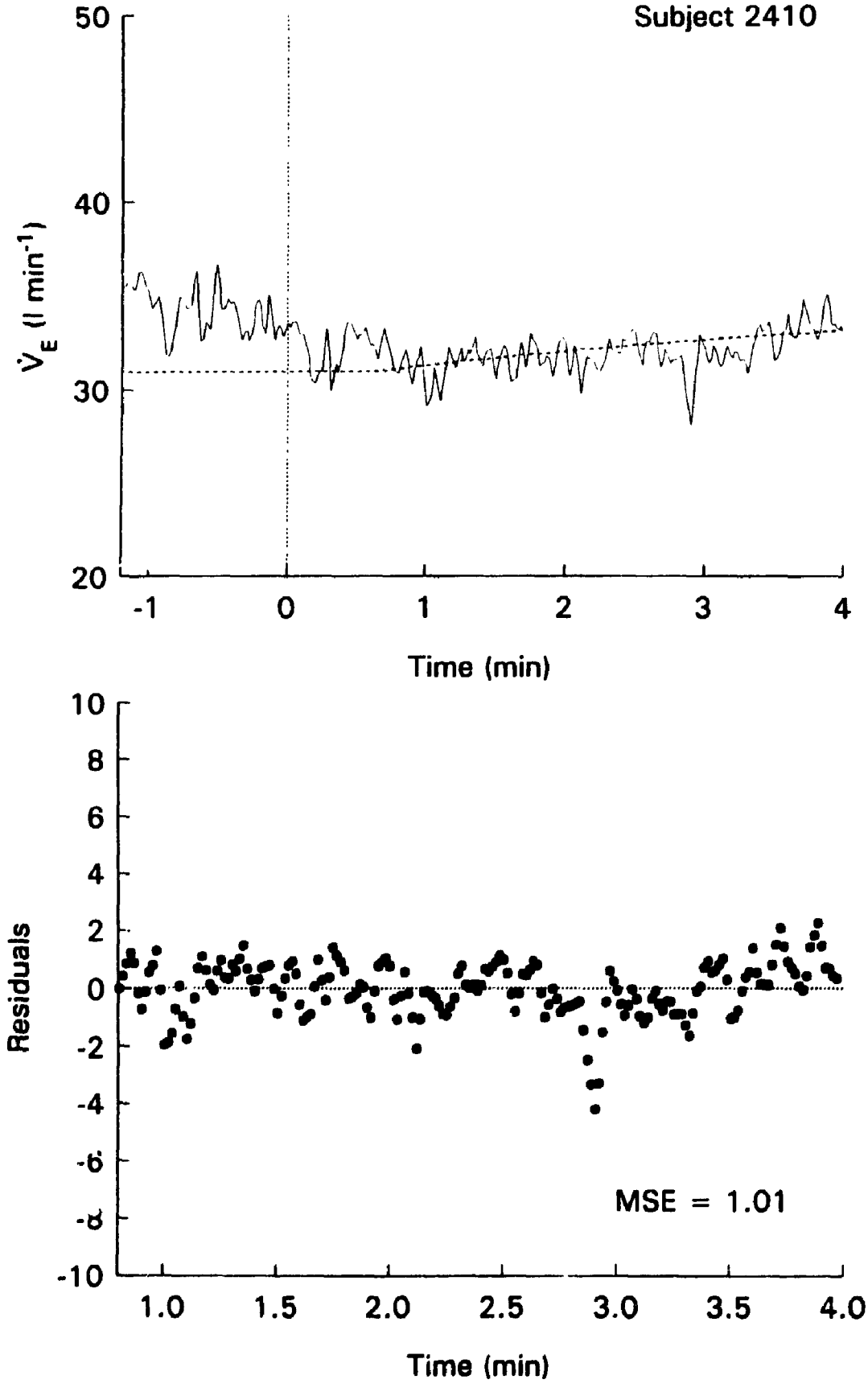

10
8
6
4
2
0
-2
-4
-6
-8
-10
Residuals
MSE = 1.28
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
Time (min)


Subject 2375
$\dot{V}_E$ (l min^{-1})
60
50
40
30
20
10
0
-1
0
1
2
3
4
Time (min)


Residuals
10
8
6
4
2
0
-2
-4
-6
-8
-10
MSE = 1.26
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
Time (min)


Subject 1643
80
70
60
50
40
30
20
$\dot{V}_E$ (l min^{-1})
-1
0
1
2
3
4
Time (min)

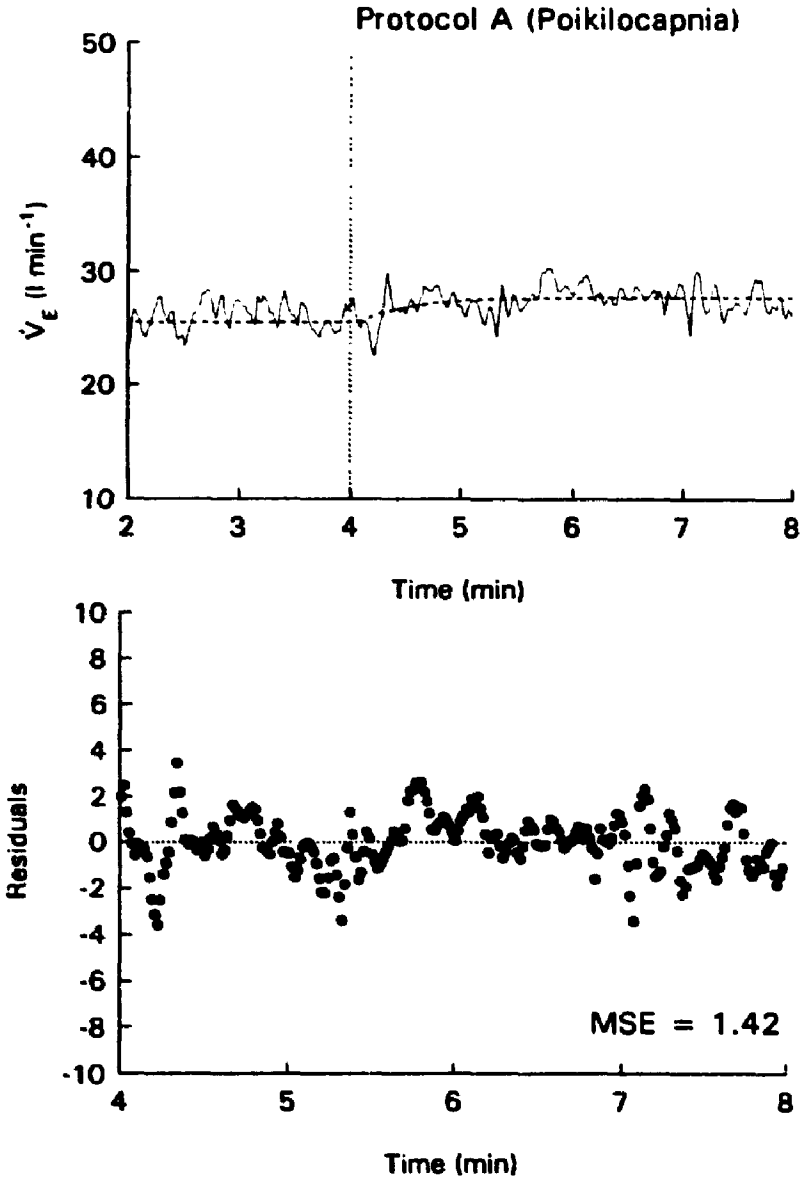

10
8
6
4
2
0
-2
-4
-6
-8
-10
Residuals
MSE = 1.73
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
Time (min)

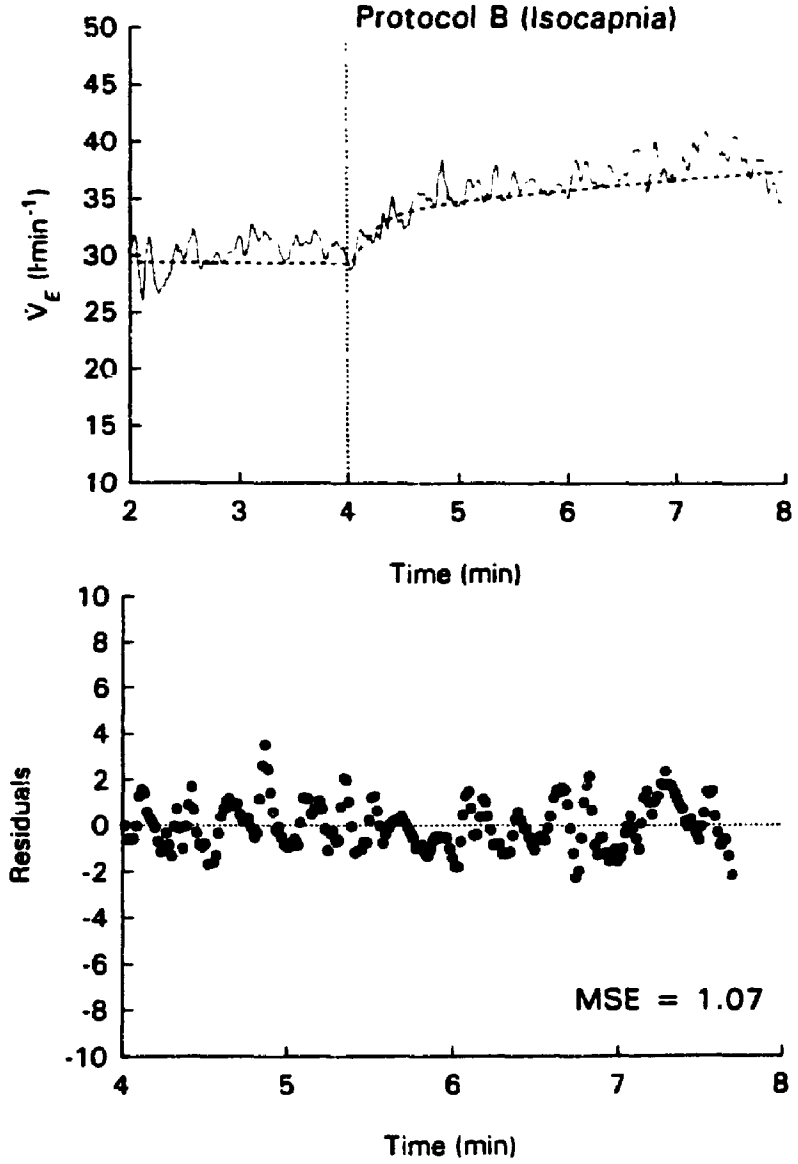

Subject 2410
50
40
30
20
$\dot{V}_E$ (l min^{-1})
-1
0
1
2
3
4
Time (min)
10
8
6
4
2
0
-2
-4
-6
-8
-10
Residuals
MSE = 1.01
1.0
1.5
2.0
2.5
3.0
3.5
4.0
Time (min)

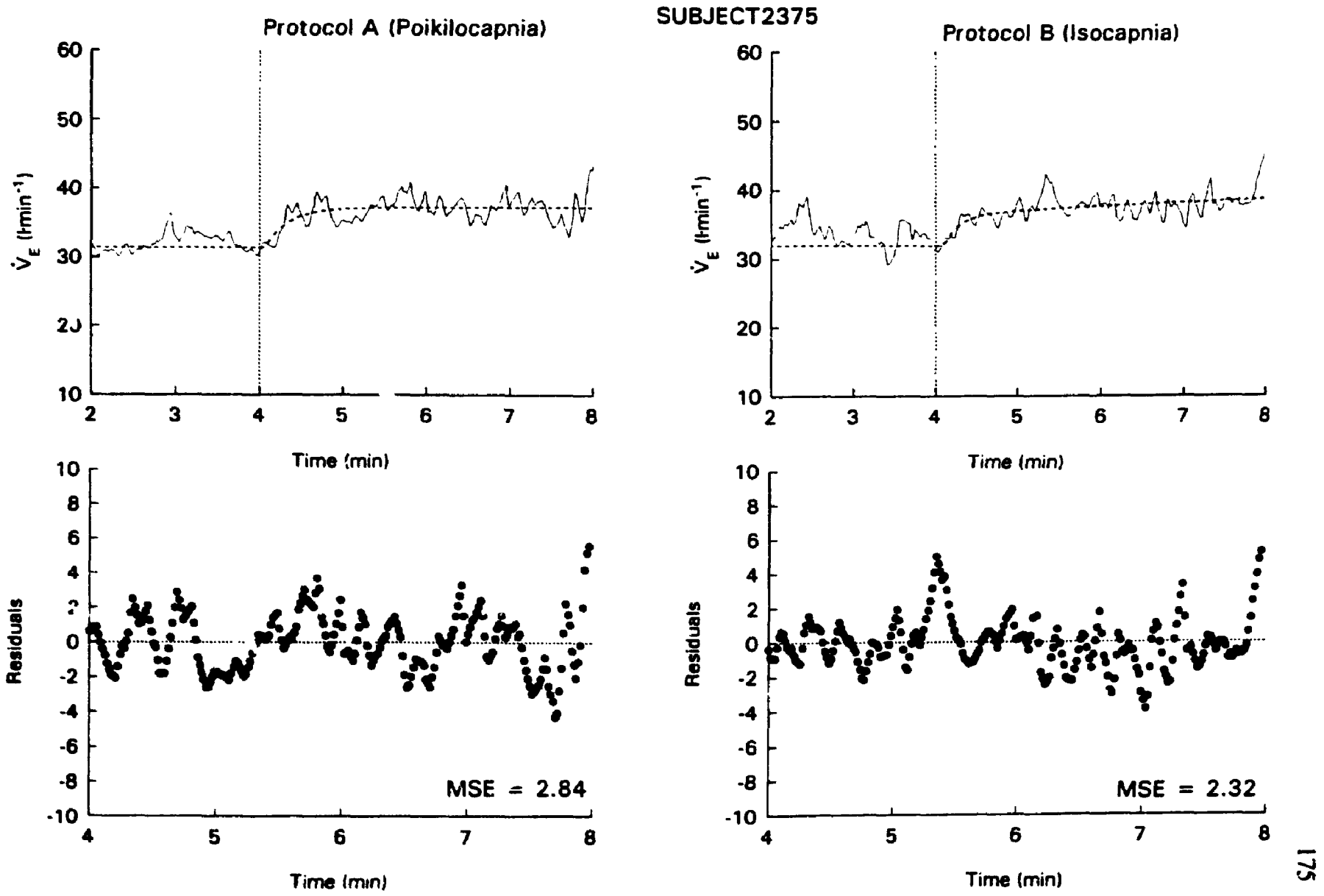
APPENDIX IX

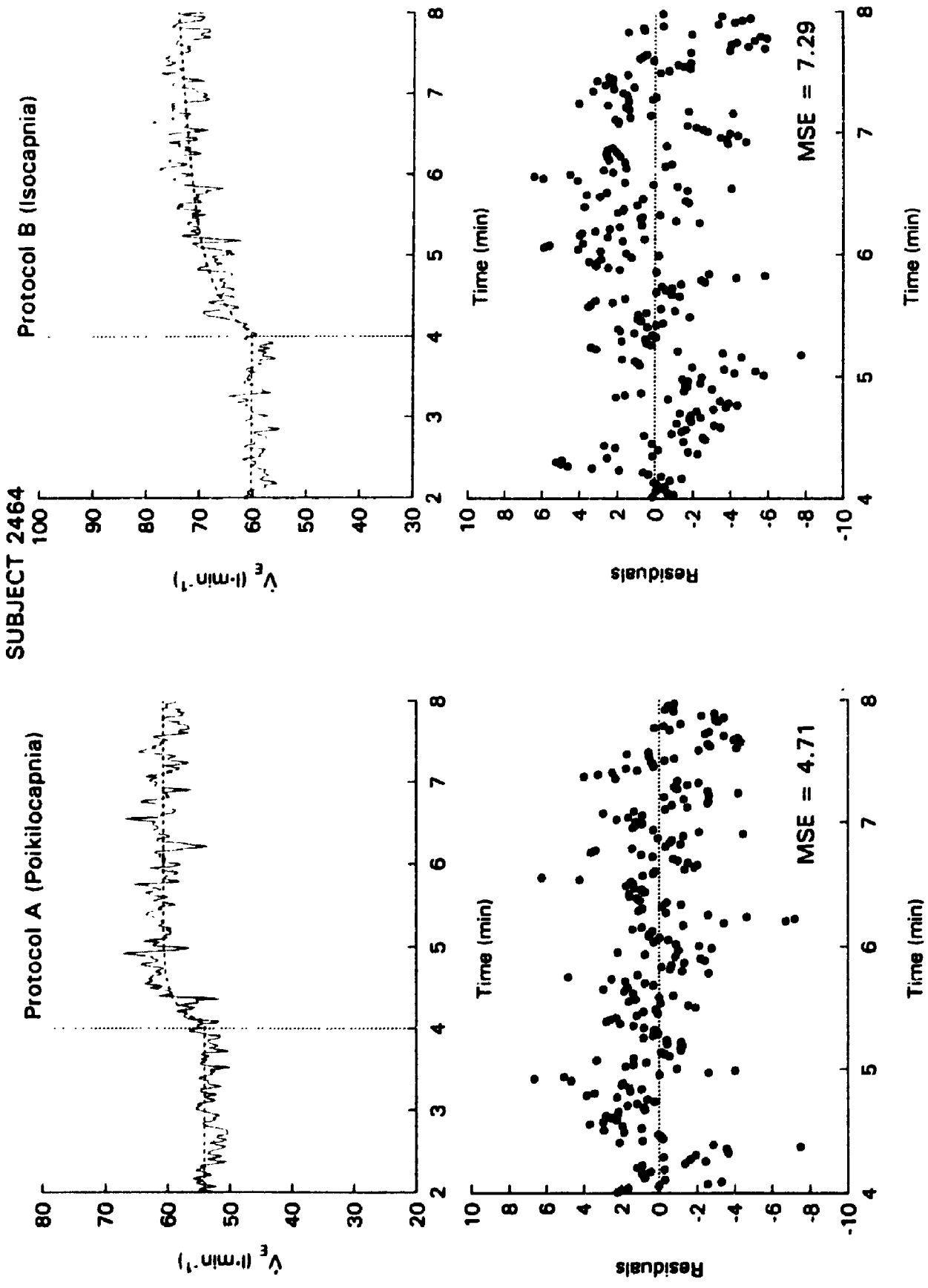
VENTILATORY RESPONSES, MODEL FITS AND RESIDUALS FOR THE STEP OUT OF HYPEROXIA IN INDIVIDUAL SUBJECTS

SUBJECT2374

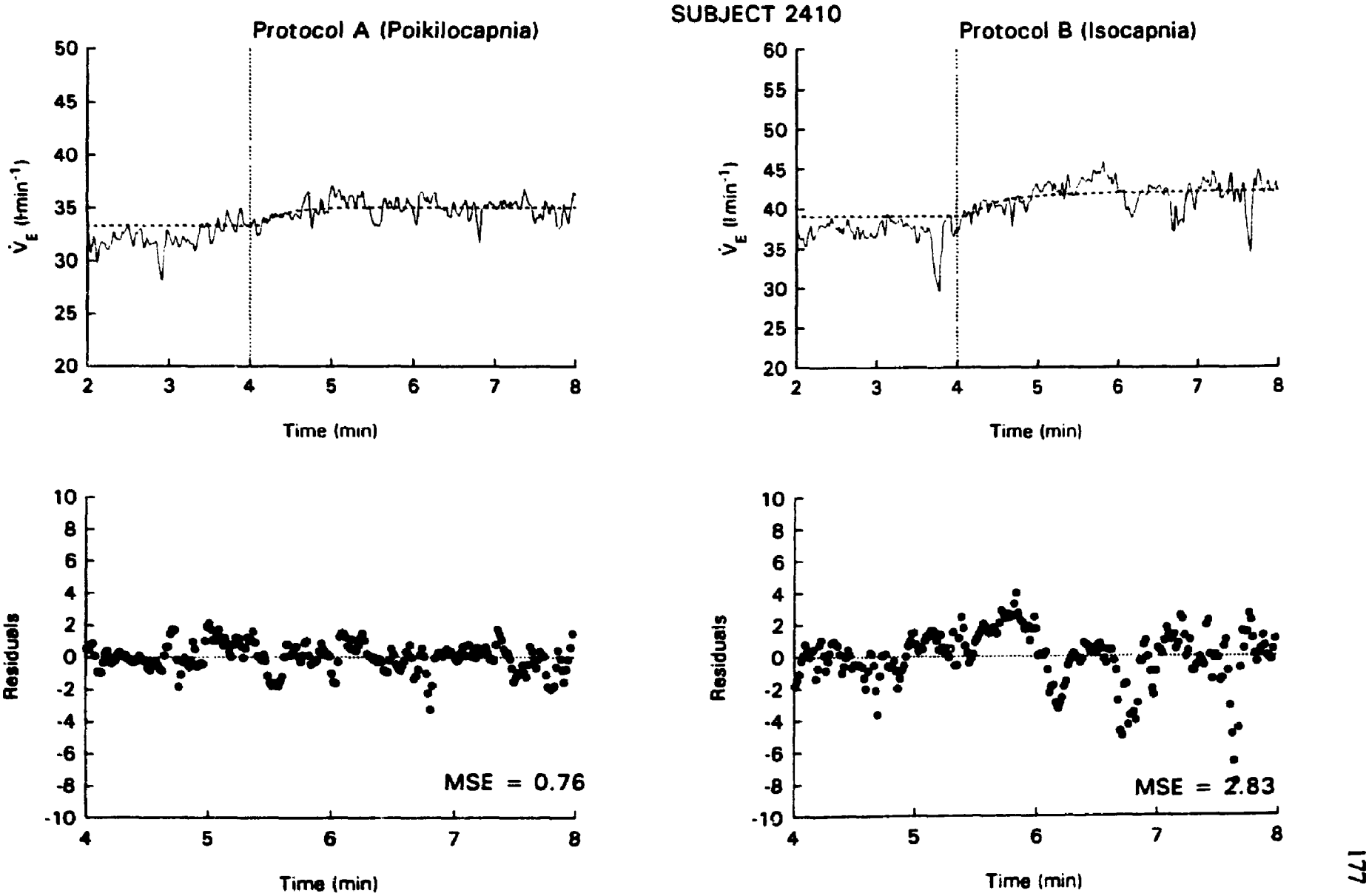





SUBJECT2375
Protocol A (Poikilocapnia)
Protocol B (Isocapnia)
$\dot{V}_E$ (lmin^{-1})
Time (min)
Residuals
MSE = 2.84
MSE = 2.32

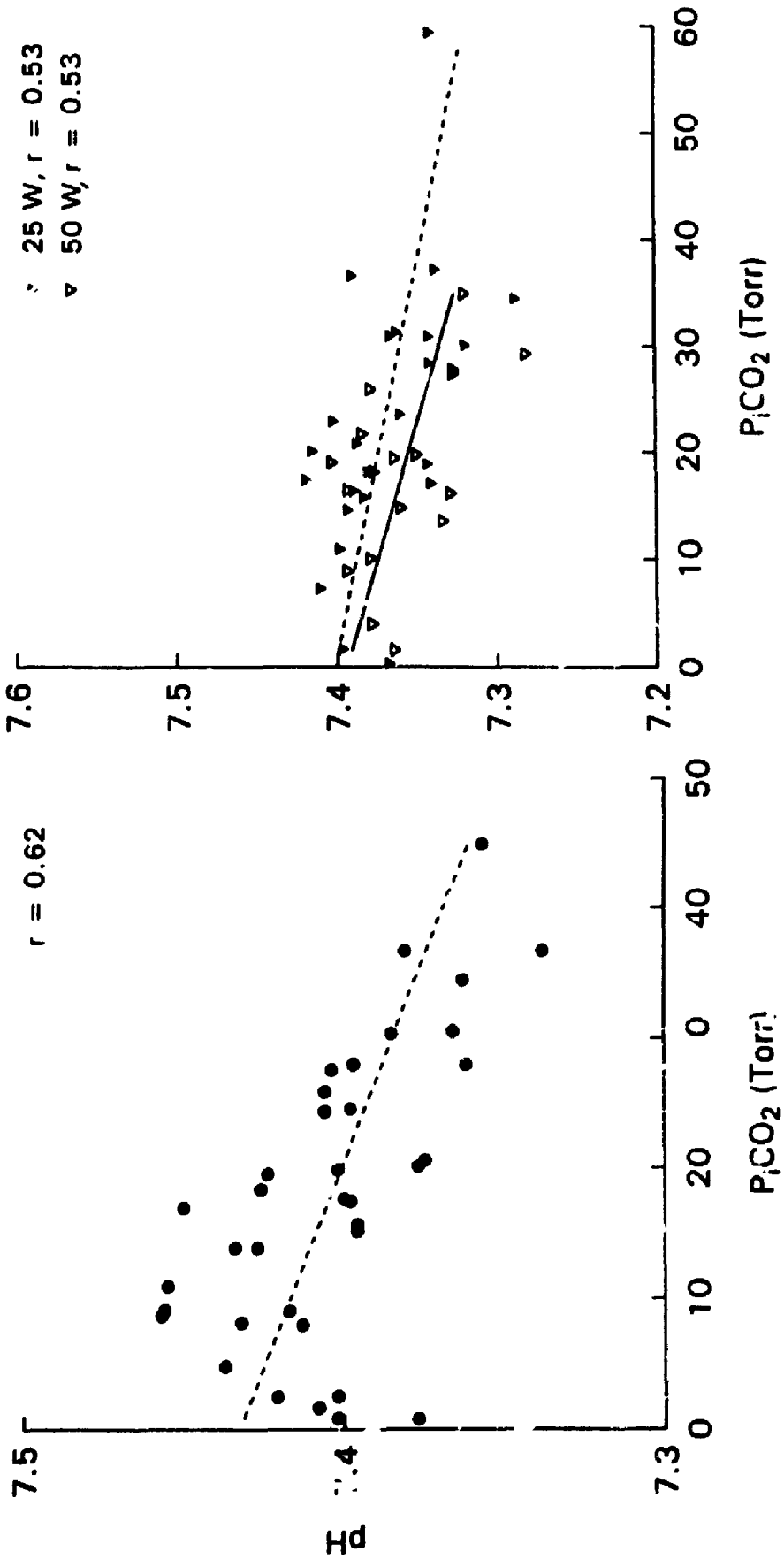

SUBJECT 2464
Protocol A (Poikilocapnia)
Protocol B (Isocapnia)
$\dot{V}_E$ (l·min^{-1})
Residuals
Time (min)
MSE = 4.71
MSE = 7.29

SUBJECT 2410



APPENDIX X
RELATIONSHIP BETWEEN pH AND P_ICO_2 (CHAPTER 4)

Pelationship between P_ICO_2 and pH, at rest (left), and in exercise (right), for the elderly subjects. For the figure on the left, the dashed line is the regression line fit to the points. For the figure on the right, the dashed line is the regression line fit to the points obtained in 25 W exercise, and the solid line is the regression line fit to the points obtained in 50 W exercise.


25 W, r = 0.53
50 W, r = 0.53
7.6
7.5
7.4
7.3
7.2
0
10
20
30
40
50
60
P_iCO_2 (Torr)
r = 0.62
7.5
7.4
7.3
0
10
20
40
50
P_iCO_2 (Torr)
pH

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