The dynamic hypoxic ventilatory response in normal subjects and its contribution to the severity of hypoxaemia in chronic obstructive pulmonary disease.

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CONTENTS

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I, Jane Elizabeth Hill, declare that the research within and the composition of this thesis are my own work.

ABSTRACT

The inherited intensity of the hypoxic ventilatory response may be important in determining the severity of hypoxaemia in chronic obstructive pulmonary disease (COPD). However, existing methodology does not allow adequate quantification of peripheral chemoreceptor sensitivity in conscious humans. The aim of this project was to validate a new mathematical model and apply this model to investigate the hypoxic ventilatory response of healthy offspring of patients with COPD. The mathematical model (consisting of two linear differential equations in parallel, each with either a fast or slow time constant component, both using the fall in SaO_2 as the stimulus and with the equation outputs summed to give the rise in ventilation above the normoxic baseline value) was used to analyse the ventilatory response to transient and step change hypoxic stimuli in normal conscious human volunteers.

Ten repeated measurements of the hypoxic ventilatory response in four subjects showed that the model parameter estimation was reproducible within a subject, and capable of distinguishing between subjects. Studies at rest and increasing levels of exercise showed that the hypoxic ventilatory response expressed both as the $\dot{V}_E inst/SaO_2$ relationship and as the model gain parameters increased with increasing exercise level.

No significant effect could be demonstrated upon the ventilatory response of varying the rate of onset of the

hypoxic stimulus to give a time course for the fall in SaO2 from 17 to 50 seconds. The central adenosine blocker theophylline given as a slow-release oral preparation did not consistently affect the hypoxic ventilatory response expressed either as the $\dot{V}_E$inst/SaO_2 relationship or as the model gain parameters of six normal subjects.

Thus, the mathematical model appears to quantitate the hypoxic ventilatory response in normal subjects and is capable of reflecting true physiological changes. It may also provide further information regarding the components of the response than conventional methods of analysis.

Studies in 35 offspring of patients with COPD showed a significant relationship between the PaO_2 of a group of patients with COPD and the hypoxic ventilatory response of their offspring in response to step change hypoxia. However the PaO_2 of the patients was best predicted by a combination of the model gain parameters of their offspring (where gain 1 may represent the peripheral chemoreceptor response , and gain 2, the central or combined hypoxic response). Hence the PaO_2 of the patients may be determined by hereditary characteristics of the peripheral chemoreceptor and central components of the hypoxic ventilatory response. The hypoxic ventilatory response of the offspring group was no different from that of a group of 26 age and sex-matched control subjects whose parents did not have

COPD, suggesting that the offspring group were within the wide range of the normal hypoxic ventilatory response. The studies in the offspring of patients with COPD suggest that the PaO_2 in COPD seems to depend on a genetically determined normal variation of both peripheral stimulation and central depression of ventilation by hypoxia.

CHAPTER 1: INTRODUCTION

In order to adapt to the respiratory requirements during exercise or at altitude, the respiratory control system is capable of regulating arterial O_2, CO_2 and pH concentrations. In man, the increase in ventilation due to a fall in arterial oxygen saturation is mediated mainly by the peripheral carotid chemoreceptors (Lugliani et al 1971). The ventilatory response to a rise in arterial PCO_2 or a fall in pH is mediated by peripheral and central chemoreceptors on the surface of the ventral medulla.

In man, the hypoxic and hypercapnic ventilatory drives to breathing vary between healthy individuals (Hirshman et al 1975) and the intensity of the drive may be important in the pathogenesis of complications of chronic obstructive pulmonary disease (COPD). It has been suggested by Dornhorst (1955) that patients suffering from COPD may be divided into two groups: the "pink puffers" who have relatively well preserved arterial blood gases, and the "blue bloaters" who have low PaO_2, high $PaCO_2$, cor pulmonale and pulmonary hypertension. However it was later shown that the two groups could not be distinguished on the basis of the pathology or the degree of airways obstruction (Burrows et al 1966). Flenley et al (1970) showed that patients with the "blue and bloated" form of COPD had a low hypoxic ventilatory drive and this has been shown to be at least partly inherited (Hudgel et al 1974; Scoggin et al 1978;

Kawakami et al 1982a). An accurate method of assessing the carotid body mediated hypoxic ventilatory drive in conscious humans may enable quantification of the mechanisms responsible for the regulation of ventilation by hypoxia.

The adaptation of ventilation to meet the respiratory requirements during exercise or at altitude is controlled by both neural and chemical mechanisms.

I Neural control of breathing

Spontaneous respiration is produced by rhythmic discharge of the motor neurons that innervate the respiratory muscles (intercostal muscles and diaphragm). This discharge is totally dependent on nerve impulses from the brain and breathing stops if the spinal cord is transected above the origin of the phrenic nerves. Respiration is regulated by two separate neural mechanisms, one being responsible for voluntary control and the other for autonomic control (Berger 1977). The voluntary system is located in the cerebral cortex and sends impulses to the respiratory motor neurons via the corticospinal tracts. The autonomic system is located in the pons and medulla, and the motor outflow from this system to the respiratory motor neurons is located in the lateral and ventral portions of the spinal cord. The motor neurons to the expiratory muscles are inhibited when those supplying the inspiratory muscles are active and vice versa.

i) Medullary centres

Rhythmic discharge of neurons in the medulla oblongata produces autonomic respiration. Respiratory neurons are of 2 types: those that discharge during inspiration (I neurons) and those that discharge during expiration (E neurons). The I neurons are actively inhibited during expiration and E neurons during inspiration.

The area in the medulla that is concerned with respiration, classically known as the respiratory centre, consists of two groups of respiratory neurons. The dorsal group of neurons in and near the nucleus of the tractus solitarius is the source of rhythmic drive to the contralateral phrenic motor nerves and hence the muscles of the diaphragm (Berger 1979). The ventral group has two divisions: the cranial division is made up of neurons in the nucleus ambiguus that innervate the ipsilateral accessory muscles of respiration, principally via the vagus nerves. The caudal division is made up of neurons in the nucleus retroambigualis that provide the inspiratory and expiratory drive to the motor neurons supplying the intercostal muscles. The carotid sinus nerve afferents project to the nucleus tractus solitarius (Berger 1979) which is near the dorsal surface of the medulla, and is likely to be the first synapse of the carotid chemoreceptor afferents. Superimposed on this basic neural control of respiration, other afferents provide the "fine adjustments" that affect breathing in particular

situations eg. afferents from pons, hypothalamus and limbic system; afferents from proprioceptors; afferents from pharynx, trachea and bronchi for sneezing, coughing and swallowing; vagal afferents from inflation and deflation receptors in the lungs; afferents from baroreceptors: arterial, atrial, ventricular and pulmonary.

ii) Pontine and vagal influences

The rhythmic discharge of the neurons in the respiratory centre is spontaneous, but it is modified by centres in the pons and by afferents in the vagus nerves from receptors in the lungs (Larrabee and Hodes 1948). Studies of nerve and brain section in anaesthetised cats have shown that complete transection of the brain stem below the medulla stops all respiration. When all of the cranial nerves (including the vagi) are cut and the brain stem is transected above the pons, regular breathing continues. However, when an additional transection is made in the inferior portion of the pons, the inspiratory neurons discharge continuously and there is a sustained contraction of the inspiratory muscles. The area of the pons that prevents the arrest of respiration in inspiration (apneusis) is known as the pneumotaxic centre and is located in the nucleus parabrachialis and the Kolliker Fuse nucleus. The area in the cauda pons responsible for apneusis is called the apneustic centre. When the brain stem is transected in the inferior portion of the pons and the vagus nerves

are left intact, regular respiration continues. In an apneustic animal, stimulation of the proximal stump of one of the cut vagi produces a relatively prolonged inhibition of inspiratory neuron discharge (Pitts 1942). Stretch receptors situated in the lung parenchyma relay to the medulla via afferents in the vagi and rapid inflation of the lung inhibits inspiratory discharge (Hering-Breuer inflation reflex). Therefore stretching of the lungs during inspiration reflexly inhibits inspiratory drive, reinforcing the action of the pneumotaxic centre in producing intermittency of inspiratory neuron discharge. Respiration continues even when all pontine tissue is separated from the medulla, whether or not the vagi are intact. This respiration is rhythmic although irregular and gasping and its occurrence demonstrates that the respiratory centre neurons are capable of spontaneous rhythmic discharge. The precise physiologic role of the pontine respiratory areas is uncertain, but they apparently make the rhythmic discharge of the medullary neurons smooth and regular. It appears that there are tonically discharging neurons in the apneustic centre which drive inspiratory neurons in the medulla, and these neurons are intermittently inhibited by impulses in afferents from the pneumotaxic centre and vagal afferents.

iii) Hypothalamic influences

The reticular activating system within the hypothalamus determines the state of wakefulness of an individual and as the carotid chemoreceptors have projections to the hypothalamus, hypothalamic influences may cause the fall in hypoxic ventilatory drive observed during sleep (Berthon-Jones and Sullivan 1982).

In humans the hypoxic ventilatory drive to breathing has been shown to increase due to a rise in body temperature (Natalino et al 1977). The carotid chemoreceptors have been shown to react to temperature changes in cats (McQueen and Eyzaguirre 1974) and afferent fibres project from the carotid chemoreceptors to the hypothalamus (Caleresu and Ciriello 1980) which is the temperature regulating centre of the body.

iv) Cortical influences

Cortical influences regulate the voluntary control of ventilation. Saunders et al (1972) showed that personality may affect hypercapnic ventilatory drive in normal man with more extrovert people having higher hypercapnic ventilatory drives. The effects of personality, mood, anxiety or fatigue upon hypoxic ventilatory drive have not been studied.

II Chemical control of breathing

In normal subjects, a rise in the PCO_2 or H^+ concentration of arterial blood or a fall in its PO_2 result in an increase in the level of respiratory centre activity and changes in the opposite direction have a slight inhibitory effect. Respiratory chemoreceptors, receptor cells in the medulla and carotid and aortic bodies sensitive to changes in blood chemistry initiate impulses to stimulate the respiratory centre producing a change in ventilation. The regulatory mechanisms adjust ventilation in such a way that the arterial PCO_2 is normally held constant, the effects of a fall in blood pH are combated and the PaO_2 is raised when it falls to a potentially dangerous level.

i) Carotid chemoreceptors

Chemoreceptors sensitive to changes in the PCO_2 and PO_2 of arterial blood are found in the carotid bodies which are ellipsoidal structures situated at the bifurcation of the common carotid artery on each side. The carotid bodies were first described anatomically by Winslow in 1732, and in 1928 DeCastro suggested that they may be sensors for chemicals in the blood (DeCastro 1928). Each carotid body has a volume of about one cubic millimetre and consists of islands of two types of cells, type I (glomus) cells and type II (sustenacular) cells. Each carotid body receives an extremely high blood flow of about two litres per minute per 100 grammes of tissue (Daly et al 1954) via the glomic artery which can be

controlled by a sphincter of smooth muscle in the glomic artery. Both afferent and efferent nerves supply the carotid bodies with the afferent pathway being mainly via fibres in the ganglioglomerular nerve which synapse with the type I cells (DeCastro 1928; Hess and Zapata 1972; and McDonald and Mitchell 1975). The type I cells may also be innervated by ganglion cells within the carotid body, by projections from the retrofacial nucleus and the nucleus ambiguus (Hess and Cassady 1983), by preganglionic sympathetic fibres (McDonald and Mitchell 1975) or by postganglionic sympathetic fibres (Verna 1981). The efferent nerve supply consists of pre- and postganglionic sympathetic fibres (O'Regan 1977 and 1981) and vagal parasympathetic fibres (Neil and O'Regan 1971; Sampson 1971b). The sympathetic nerve endings and the afferent synapses on the type I cells are situated very near one another (Verna 1971) and therefore the type I cells may be inactivated by uptake of catecholamines by the sympathetic nerve cells. The sympathetic efferents may modify the activity of the type I cells due to the release of other chemicals. Their sensitivity was shown by Heymans et al (1930, 1931; Heymans and Neil 1958).

Interaction of hypoxia and hypercapnia at the carotid chemoreceptors has been demonstrated in whole nerve and single fibre preparations in animals (Eyzaguirre and Lewin 1961; Lahiri and Delaney 1975). In human subjects, Nielson and Smith first recognised that a combination of

hypoxia and hypercapnia produced an increase in ventilation which was greater than that caused by either stimulus alone (1951).

The catecholamines contained in the carotid body have been considered to be putative neurotransmitters although this is currently a point of contention (Alfes et al 1977). The type I (glomus) cells of the carotid body are known to contain dopamine which may have both excitatory and inhibitory effects on the carotid chemoreceptors. Bisgard et al (1979) showed that a large dose of dopamine had an excitatory effect upon carotid chemoreceptor activity, whereas a small dose resulted in chemosensory inhibition.

Sampson demonstrated that intra-carotid injection of adrenaline, dopamine or noradrenaline caused inhibition of spontaneous discharge of the carotid chemoreceptors (1971a), whereas O'Regan observed an increase of carotid chemoreceptor activity after injection of adrenaline or noradrenaline (1981). Thus any "flight or fright" situation which causes a release of catecholamines from the adrenal glands into the bloodstream could therefore affect the measurement of hypoxic ventilatory drive.

In man, the aortic bodies situated in the arch of the aorta seem to contribute little to the hypoxic ventilatory drive to breathing as removal or denervation of the carotid chemoreceptors abolished the ventilatory response to both steady state hypoxia (Guz et al 1966; and Lugliani et al 1971) and to transient hypoxia

(Wasserman et al 1975; and Whipp and Wasserman 1980). The aortic bodies may however contribute to the ventilatory response to hypercapnia (Swanson et al 1978).

ii) Central chemoreceptors

An area within the ventro-lateral medulla, thought previously to be sensitive only to pH (Mitchell et al 1963 ; Loeschke 1982), has recently been shown to cause changes in ventilation in response to pH or CO_2 (Teppema et al 1983; Eldridge et al 1985). In both animals and man a multiplicative interaction of the chemoreceptor input and the medullary response to CO_2 and pH has been described (Lee et al 1975; Kao and Mei 1978) although it is assumed by most that the interaction of CO_2 and O_2 in humans can be entirely accounted for by the carotid chemoreceptors (Whipp et al 1976) . There is no evidence for direct central stimulation of ventilation by hypoxia and hypoxia may act centrally to depress ventilation (Weiskopf and Gabel 1975; Easton et al 1986, 1988). This depression of ventilation may be an indirect effect due to increasing cerebral blood flow during hypoxia leading to central hypocapnia (Weiskopf and Gabel 1975). However, Easton et al (1986, 1988) have shown that during sustained hypoxia, the decline in ventilation which occurs in normal adults may be related to central accumulation of a neurochemical, possibly adenosine, with a net inhibitory effect on ventilation.

III Measurement of the chemoreceptor response to hypoxia

Several methods may be used to assess the carotid chemoreceptor response to various time courses of hypoxic stimuli:

In animal preparations it is possible to make direct recordings of the chemoreceptor afferent activity in the carotid nerve. This method provides a direct measurement of carotid chemoreceptor activity but of course cannot be used in conscious human subjects. The significant trauma involved in the procedure and the anaesthetics used in these animal studies may themselves affect the chemoreceptor discharge (Biscoe and Millar 1968).

The second method is by measurement of the ventilatory response to an hypoxic stimulus in conscious healthy human subjects. Several methods have been used to demonstrate and attempt to quantitate the hypoxic drive to breathing expressed as a change in minute ventilation for a fall in arterial oxygen saturation, including steady state, progressive and transient hypoxia techniques.

i) steady state hypoxia

The first method was described by Nielson and Smith (1952). They measured the steady state ventilatory response to CO_2 at two levels of PO_2 (40 and 150mmHg) and expressed hypoxic sensitivity as the ratio of the slopes of the CO_2 responses at the two levels of oxygenation (S40/S150). Severinghaus et al (1966) used a similar experimental technique, constructing two CO_2

response lines, one at a steady state PaO_2 of 200mmHg and the other at 40mmHg. They then defined their measure of hypoxic sensitivity, $\triangle\dot{V}40$ as the rise in ventilation produced from a fall in PaO_2 from 200 to 40mmHg at a standardised "normal" $PaCO_2$ for each subject (the $PaCO_2$ at which the hyperoxic response line intersected a ventilation of 4 $litres min^{-1}$, the normal resting ventilation). A similar method was used by Flenley and Millar (1967) who compared the ratio of the slopes of the CO_2 response lines at a PO_2 of 70mmHg and 120mmHg, giving the ratio S70/S120. Lloyd et al (1958) used a similar experimental method involving at least four different levels of PAO_2 but analysed the results in a different manner. They expresed the linear part of the $\dot{V}/PACO_2$ relationship by the equation

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

where S is the slope of the $\dot{V}/P_ACO_2$ line and B its intercept on the x axis. They noted that the intercept did not change with a change in P_AO_2 but the slope, S did ie. the lower the P_AO_2, the greater the slope. They derived the following equation to describe the relationship between S and P_ACO_2

$$S = D \left\{ 1 + \frac{A}{P_AO_2 - C} \right\}$$

where D is the slope of the $\dot{V}/P_ACO_2$ line at infinite P_AO_2, A is the shape of the hyperbola and C is the P_AO_2 at which $\dot{V}$ tends to infinity. They concluded that A can be regarded as a measure of the hypoxic ventilatory drive.

There are several disadvantages of the steady state technique. The method is very time consuming and it may take up to several hours to measure ventilation at several levels of steady state P_ACO_2 against a background of several levels of steady state P_AO_2. The hyperbolic relationship between ventilation and P_AO_2 has limitations as the shape parameter of the hyperbola, A is affected by changes in C, the P_AO_2 at which ventilation tends to infinity. Lloyd et al (1958) used a constant value of 32 to represent C in all their calculations but Cunningham et al (1964) showed that C may vary between individuals, hence the assumption of a constant C will lead to error. The hyperbolae are not always the same shape for the same value of A, hence the other parameters in the equation must be determined for accurate calculation of hypoxic ventilatory drive. Another disadvantage of the steady state technique is that some subjects with a particularly high hypoxic ventilatory drive may find a steady state level of ventilation very uncomfortable due to the multiplicative action of hypoxia and hypercapnia (Nielson and Smith 1951), thus producing few data points at the low P_AO_2 end of the hyperbola.

A major disadvantage of the $\triangle\dot{V}40$ technique is that a PaO2 of 40mmHg lies on the steep part of the $\dot{V}/P_AO_2$ response curve, therefore a small change in PO_2 results in a large change in ventilation. A small experimental inaccuracy could therefore cause a large variation in

the results.

ii) progressive isocapnic hypoxia

Methods using progressive isocapnic hypoxia were introduced by Weil et al (1971) and involved the gradual addition of N_2 to the inspired gas to reduce $P_{ET}O_2$ from 120mmHg to approximately 40 mmHg over a period of 15-20 minutes. Carbon dioxide was also gradually added to the inspired gas mixture in order to keep $P_{ET}CO_2$ constant. The shape of the curve relating $\dot{V}_E$ and PAO_2 could be described by the equation

$$\dot{V}_E = \dot{V}EO + A / (P_AO_2 - 32)$$

where $\dot{V}EO$ is the asymptote for ventilation at infinite P_AO_2, A is the shape parameter of the curve, and 32 is the constant P_AO_2 at which the $\dot{V}_E/P_AO_2$ slope tends to infinity as used by Cunningham et al (1964) in the calculation of the ventilatory response to steady state hypoxia. A similar progressive isocapnic hypoxia method was used by Kronenberg et al (1972) in which the $P_{ET}O_2$ was reduced from 120 to 40 mmHg over a period of 3-4 minutes.

Rebuck and Campbell (1974) developed a rebreathing method based on that of Read (1967) in which subjects rebreathed from a six litre bag containing 7% CO_2 and 24% O_2. A rapid fall in $P_{ET}O_2$ from 140-160mmHg to 30-40mmHg was produced within four minutes. Rebuck maintained isocapnia, keeping $P_{ET}CO_2$ at mixed venous level by drawing a stream of the gas through a soda lime CO_2 absorber. Ear oxygen saturation was measured

continuously during the procedure and the hypoxic ventilatory drive was expressed as the slope of the linear regression relationship between minute ventilation and ear oxygen saturation. The major criticisms of this technique relate to the use of ear oximetry in that the delay between the change in PaO_2 and the response of the oximeter at that time may affect the results and that SaO_2 is probably not the actual chemoreceptor stimulus (Chiodi et al 1941 and Hatcher et al 1973). A disadvantage of both the steady state and progressive isocapnic hypoxia is that the prolonged hypoxia may result in a central depression of ventilation in some subjects even if the duration of the hypoxic stimulus is only 3-4 minutes (Weiskopf and Gabel 1975; Easton et al 1986, 1988), thus causing an underestimation of the peripheral chemoreceptor response.

iii) transient hypoxia

Transient hypoxic stimuli lasting only a few breaths have been used to avoid central hypoxic depression seen in some subjects during steady state or progressive isocapnic hypoxia experiments. It was suggested that the short duration of the stimulus involved would avoid central depression of ventilation (Weiskopf and Gabel 1975; Easton et al 1986, 1988) and that the ventilatory response would therefore more accurately reflect chemoreceptor activity alone. The transient method of assessing chemoreceptor drive was first used by Dejours

et al, in which the subject inhaled one or two breaths of either an hypoxic or hyperoxic gas (1957b, 1958; Dejours 1953, 1963). In order to reduce adequately the PaO_2 at rest, the subject was required to take a vital capacity breath of the gas mixture, and this manoeuvre in itself affected the subjects' breathing pattern and made them aware of the measurement being made (Kronenberg et al 1972; Gabel et al 1973). This technique was modified by Lahiri and Edelman (1969) who gave the subjects 100% O_2 to breathe for 3-5 breaths without their knowledge. Flenley et al (1973) used a similar test during exercise, giving 2 or 3 tidal breaths of 100% O_2. In the studies of Kronenberg et al (1972) only the change in ventilation was measured hence the stimulus of O_2 or N_2 at the chemoreceptors was not taken into account. Edelman et al (1973) used an ear oximeter to measure oxygen saturation as the hypoxic stimulus and expressed the hypoxic ventilatory drive as the slope of the linear regression relationship between $\dot{V}_E$ and SaO_2. The $\dot{V}_E$ value used in this case was the mean of the two breaths with the largest $\dot{V}_E$ after each hypoxic stimulus. Calverley et al (1974) and DeCort et al (1988) have shown that there was no significant difference in the ventilatory response to transient hypoxia whether or not the P_ACO_2 was controlled. The absolute changes measured during a transient hypoxic stimulus can be increased by giving it during exercise, just as exercise will potentiate the increase in

ventilation observed during steady state hypoxia (Flenley et al 1973).

The method of analysis of the transient hypoxic stimuli was improved by Flenley et al (1979). Subjects were studied walking at a moderate speed on a level treadmill breathing 14% O_2 as the background gas and given five tidal breaths of 30% O_2 on six occasions. Hypoxic ventilatory drive was expressed as the slope of the $\dot{V}_E/P_{ET}O_2$ relationship for the pooled data and this analysis included all breaths during the transient relief of hypoxia and the return to the hypoxic gas mixture. The analysis used an iterative process thus avoided any subjectivity in selecting the peak $\dot{V}_E$, included more data points and therefore gave improved statistical power compared with the method of Edelman et al (1973). One criticism of this technique is that $P_{ET}O_2$ was used in the analysis as a measure of PaO_2. However this may not be true due to an increase in the arterial-alveolar difference upon exercise (Whipp and Wasserman 1969). Gould et al (1985) modified the data analysis method of Flenley et al (1979) and demonstrated that analysis of the $\dot{V}_E/P_{ET}O_2$ relationship using all data points for the increase in minute ventilation with hypoxia and the return to normoxic baseline $\dot{V}_E$ gave different results to a method using only the points during the rise in $\dot{V}_E$.

The ventilatory response to hypoxia may be measured as the pressure generated by the insiratory muscles ($P_{0.1}$)

in response to airway obstruction 100msec after the onset of inspiration from residual capacity (Whitelaw et al 1975). This method is simple and non-invasive and is not affected by lung mechanics. However in some subjects, even a very short occlusion time (eg. 150msec) may influence the breathing pattern. The $P_{0.1}$ may be measured during the first 60msec of every breath even during sleep (Gugger et al, in press).

Comparisons between the three methods of quantitating the hypoxic ventilatory drive to breathing have been made by several investigators. In normal subjects at rest, Kronenberg et al (1972) found a significant correlation between the response to the steady state and progressive methods and also between the steady state and transient tests but no correlation between the progressive and transient methods. Edelman et al (1973) compared the transient and steady state hypoxia methods at rest. They found that the ventilatory responses to transient hypoxia were qualitatively similar to the responses to steady state hypoxia although they were quantitatively significantly greater by an average of 18%. Shaw et al (1982) compared progressive isocapnic and transient hypoxia at rest and found a significant correlation between the two techniques in a group of 18 normal subjects (1982). Warren et al (1984) compared the three methods in normal subjects and found a significant correlation between progressive isocapnic and transient hypoxia techniques. However absolute

comparisons could not be made as steady state and progressive isocapnic hypoxia were studied at rest whereas transient hypoxia was studied during exercise.

Airlie et al (1989) have recently shown that the specific peripheral chemoreceptor stimulant almitrine bismesylate significantly increased the hypoxic ventilatory drive (expressed as the $\dot{V}_E$inst/SaO_2 relationship) in six out of seven normal men at rest for progressive isocapnic hypoxia. However there was no consistent change in the ventilatory responses to transient hypoxia measured during exercise. They therefore concluded that the response to transient hypoxic stimuli did not accurately reflect peripheral chemoreceptor activity alone. This group also found that the $\dot{V}_E$inst/SaO_2 relationship in response to rapid onset, short duration transient stimuli was less than that to slower onset, more prolonged hypoxia, and the two responses could not be described adequately by a single linear system (Warren et al 1987). The smaller ventilatory response to transient hypoxic stimuli may be due to the time course of the ventilatory response to hypoxia (air to 9% O_2) shown by Reynolds and Millhorn (1973) to have a mean half-time of 78 seconds ie. significantly greater than the time course of the transient hypoxic stimuli.

As the ventilatory response to transient hypoxia appears to depend upon the rate of onset and the duration of the stimulus, Kirby et al have developed a

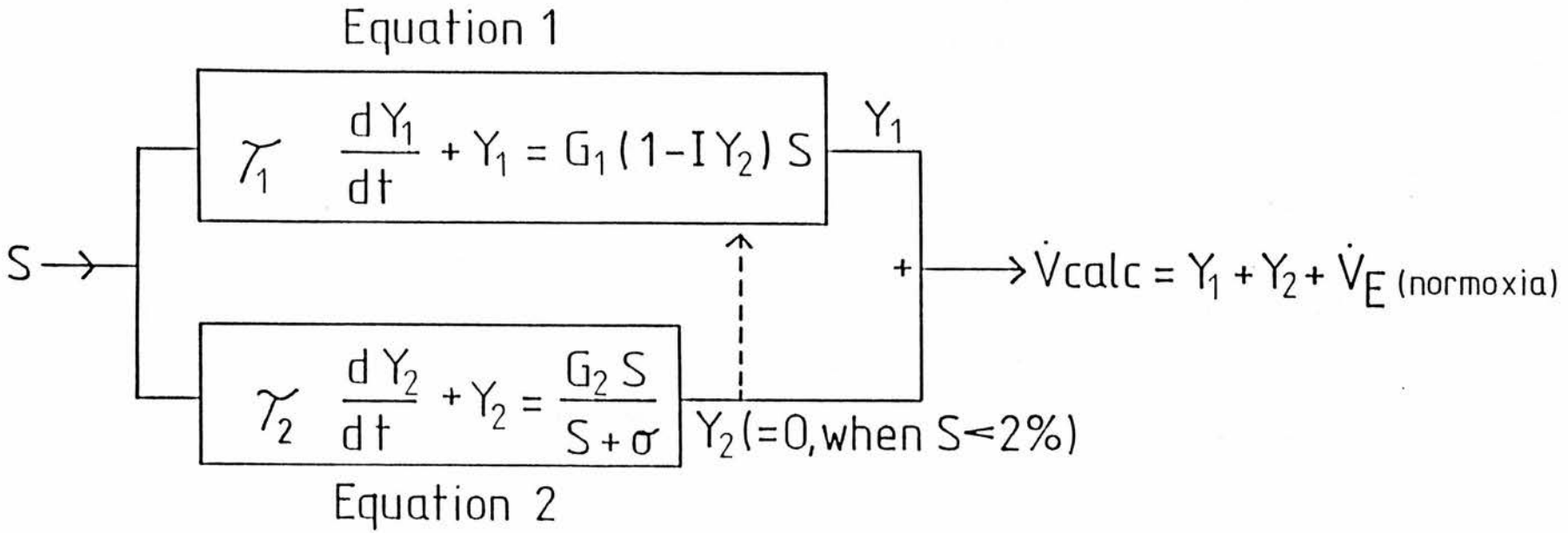
mathematical model (Appendix A; Warren et al 1989) which allows a dynamic analysis of the ventilatory response to both transient and sustained hypoxia.

CHAPTER 2: A MATHEMATICAL MODEL FOR ANALYSIS OF THE DYNAMIC VENTLATORY RESPONSE TO HYPOXIA

Mathematical models have been used to describe the dynamic ventilatory response to O_2 (Sarhan et al 1988) and CO_2 (Belville et al 1979; Grodins et al 1967; Saunders et al 1980). However modelling techniques have not previously been used to investigate the dynamics of the peripheral and central chemoreceptor controllers of the ventilatory response to hypoxia. Kirby et al therefore developed a mathematical model which described the dynamic ventilatory response to both transient (inspired gas changed to 2 breaths N_2) and step change hypoxia (inspired gas changed abruptly to 15% or 12% O_2 for a 3 minute period; Appendix A, Warren et al 1989). The model was systematically developed to fit the observed ventilatory responses both to transient and to step change hypoxia in 10 normal subjects. The simplest model relating $\dot{V}_E$inst to SaO_2 which used the minimum number of parameters, and which adequately described the experimental data obtained in the 10 subjects was derived empirically. The final model structure, shown in Fig 2.1 consisted of two linear differential equations (1 and 2) in parallel. The fall in SaO_2 during hypoxia (S) was taken as the input to both equations. The outputs of both equations (Y_1 and Y_2) were summed to give the rise in ventilation above the normoxic baseline value ($\dot{V}$) and added to the ventilation during normoxia to give $\dot{V}$calc.

The time constant of equation 1 (tau 1) was found to be fast ie. less than 3 sec, and was therefore set to 0.1 sec since the time constant of the ear oximeter (1.6 sec, Douglas et al 1979) prevented this parameter from being measurable. The non-linear terms included in the final model were an inhibition parameter and a saturating effect parameter. The output of equation 1 may be "inhibited" (positive I), or "potentiated" (negative I) by the output of equation 2 (Fig 2.1). The saturating effect parameter allowed the gain to "saturate" in a way analogous to the Michaelis-Menten equation by replacing the gain G with the term G/(sigma + S). Equation 2 has a longer time constant (tau 2) and its gain is allowed to be saturated.

Fig 2.1 : Two compartment mathematical model incorporating non-linear terms.



Schematic representation of the model where:
G_1 = gain of equation 1, τ_1 = time constant of equation 1, Y_1 = output of equation 1
I = inhibition, G_2 = gain of equation 2, τ_2 = time constant of equation 2,
Y_2 = output of equation 2, σ = saturating effect.

The model depends on three assumptions: arterial oxygen saturation was taken as a measure of the stimulus and $\dot{V}_E$inst as the output. The PO_2 within the carotid glomus was considered to be the stimulus to the peripheral carotid chemoreceptors (Eyzaguirre and Zapata 1984) and while SaO_2 was not the actual chemoreceptor stimulant, it is related to arterial PO_2 and provides the only non-invasive measure of arterial oxygenation. The final assumption was that arterial PCO_2 was constant and equal to end-tidal PCO_2 although some evidence suggests that as tidal volume increases during exercise, a small difference develops between end-tidal and arterial PCO_2 values (Cummins et al 1986; Jones et al 1979). The program was written in Fortran IV and assembler on a DEC PDP 11/73 computer and allowed for any arrangement of one or two differential equations, with or without the non-linear terms described above. An input file contained a sequence of values for an individual of SaO_2, time and $\dot{V}_E$inst, including both transient and step change events. To reduce processing time, only 15 normoxic breaths before and after each period of hypoxia were included in the file. A standard Runge-Kutta integration routine (a standard mathematical minimisation process, Press et al 1986) was used to calculate values of ventilation for a given set of equation parameters, and the parameters were adjusted to minimise the sum of weighted squared differences between the calculated and observed ventilation using the

"Simplex" method (a standard stepwise multidimensional minimisation method, Press et al 1986). The weighting prevented the fitting process being biased towards the step changes (due to their greater number of experimental points contributing to the sum of squares) by multiplying the squared differences during step changes by the factor W, where

$$W = \frac{\text{sum of durations of transients}}{\text{sum of durations of steps}}$$

To allow the use of delays within the model and compensate for the delay in response of the ear oximeter (about 2 sec), the SaO_2 was interpolated to one second intervals within the program using the method of Lagrange (a classical polynomial interpolation formula, Margenau et al 1956).

At each stage of the model parameter estimation, the best fit to the data was found, and the process stopped at the level when the addition of further terms did not give a statistically significant decrease in the residual deviations of $\dot{V}_E inst$ from the values for ventilation calculated by the model. A decrease on addition of one new parameter was considered to be significant if the quantity:

$$F = \frac{(RSS_{before} - RSS_{after})}{(RSS_{after}/(n-(p+1)}$$

was greater than the 5% level of Snedecor's F distributed with 1 and (n-(p+1)) degrees of freedom,

where RSS_{before} and RSS_{after} were the residual sum of squares before and after inclusion of additional parameters, p was the original number of parameters, and n was the number of data points being fitted. This is a form of Analysis of Variance.

The starting points for the iterative process were arbitrarily chosen as tau 1: 0.1 sec; gain 1: 2 $lmin^{-1}\%^{-1}$; and gain 2: 2 $lmin^{-1}\%^{-1}$ and the parameters for inhibition (0.1 $lmin^{-1}$) and sigma (0.1 %) were added individually and then in combination until the model of best fit was derived. The repeatibility of the minimisation procedures were tested by starting the parameter estimation from four different sets of points in ten subjects. The parameter estimation generally began with only values for the two time constants and the two gains, then the values for inhibition and saturating effect were introduced individually and then in combination in a stepwise manner. Repeatibility of the model was assessed by starting parameter estimation at the following points, I, II, III and IV

Starting Point	I	II	III	IV
tau 1	0.1	0.1	0.1	0.1
gain 1	2	0	5	10
inhibition	0.1	0	1	2
tau 2	50	0	50	100
gain 2	2	0	5	10
sigma	0.1	0	1	2

In all ten cases tested the final parameter values were identical irrespective of the starting points used . The set I starting points were therefore used for all subsequent estimations. Chapter 4 describes in detail the reproducibility of both the hypoxic ventilatory response and the mathematical model parameters for four subjects and suggests that the model gives a quantitative and reproducible measure of the hypoxic ventilatory response in normal subjects.

The form of the model was developed on a purely mathematical basis, and physiological interpretation therefore needs validation. It can be postulated that the components of the fast differential equation (1) represent the activity of the carotid chemoreceptors and that of equation (2) with the longer time constant represent a central component. The studies described in this thesis aimed to assess the repeatability of the model parameter values obtained in an individual, to investigate the physiological interpretation of the model structure and to use the model in a large familial study. The ventilatory stimulant theophylline, considered to act centrally, has been used to assess and attempt to separate out the peripheral and central components of the model. Several different levels of exercise have been used to investigate the potentiation of the hypoxic ventilatory response and its effect upon the model parameters. The effect of different rates of onset of the hypoxic stimulus upon the model analysis of

the ventlatory response have also been studied. The relationship between the ventilatory response to hypoxia and the model parameters of a group of offspring with the PaO_2 of their parents with COPD has been used to investigate the suitability of the model for use in an epidemiological study.

CHAPTER 3: GENERAL METHODS AND EQUIPMENT USED TO MEASURE THE HYPOXIC VENTILATORY RESPONSE IN NORMAL MAN

I EQUIPMENT AND DATA ACQUISITION

Fig 3.1 shows the breathing circuit used for transient and step change hypoxia throughout the following studies. Subjects breathed through a mouthpiece connected to a low-resistance custom-built valve (range 0-4.9 cmH_2O over a flow range 0-110 $lmin^{-1}$, Fig 3.2) with a dead space of 90ml. The valve had two inlet ports although only one was used in the following studies. Mouth pressure was measured via a port between the inspiratory and expiratory flaps of the valve and the mass spectrometer probe was positioned 7cm from the mouth for continuous inspired and expired gas analysis. In order to provide more than two inspiratory ports, a five-way valve (Hans-Rudolph 2440 series, five-way Gatlin shape valve) with a dead space of 95ml was connected to one inspiratory port of the custom-built valve (Fig 3.3). The Hans-Rudolph valve had a similar pressure-flow relationship to the custom-built valve (Fig 3.2). Each of the ports of the five-way valve was closed by a pneumatically operated silicone rubber balloon which could be inflated or deflated very rapidly by means of a remote controller (Hans-Rudolph series 2430), operated from behind the subject. The combination of the custom-built and five-way valves allowed very abrupt changes in inspired gas. Addition of the five-way valve to the custom-built valve did not affect the mouth

pressure measured over a flow range of 0-110 $lmin^{-1}$ hence inspiratory resistance was not increased (Fig 3.4).

Gas mixtures of 10, 12 or 15% O_2 used for step change hypoxia were made from room air and 100% N_2 supplied from a cylinder of primary gas. The mixtures were made up using a system of rotameters (Rotameter Manufacturing Co. ltd.) connected to a 10.5 litre mixing box. Room air was supplied by a pump through a 0 to 200 $lmin^{-1}$ rotameter, 100% N_2 through a 0 to 50 $lmin^{-1}$ rotameter and 100% CO_2 through a 0 to 10 $lmin^{-1}$ rotameter. CO_2 was then supplied directly to the inspiratory tube close to the Hans-Rudolph valve. Gas mixtures from the rotameters were supplied at a total flow rate of 100 $lmin^{-1}$. The gas from the rotameters passed through a T-piece connected to one inspiratory port of the Hans-Rudolph valve (figs 3.1 and 3.3). Excess gas passed via a T-piece along a 2 metre tube of 5cm diameter (fig 3.3), ensuring that the gas supply from the rotameters exceeded respiratory requirements, that positive pressure was not applied to the inspiratory side of the circuit and that room air was not entrained into the inspiratory mixture.

To produce transient hypoxic stimuli, either 100% N_2 or 1% O_2 in N_2 was supplied from a Douglas bag connected to one port of the Hans-Rudolph valve (Fig 3.1). A second valve, a mechanical foot pedal was positioned between the Douglas bag and the Hans-Rudolph valve.

Simultaneous operation of both the foot pedal and the remote controller of the Hans-Rudolph valve was required before the subject could inhale from the Douglas bag, thus preventing accidental inhalation of 100% N_2.

Expired gas passed from the respiratory valve along a straight metal tube via a pneumotachograph (Fleisch No. 2) to a 3.2 litre mixing chamber. It was then dried and passed through a dry gas meter (Parkinson-Cowan CD4). Mixed expired gas was sampled from the mixing chamber at 500mlmin^{-1} and analysed for O_2 (Sybron-Taylor Servomex Oxygen Analyser 570A) and CO_2 (Gould-Godart Capnograph Mark III) concentrations as required for calculation of steady state gas exchange.

i) Recording Devices

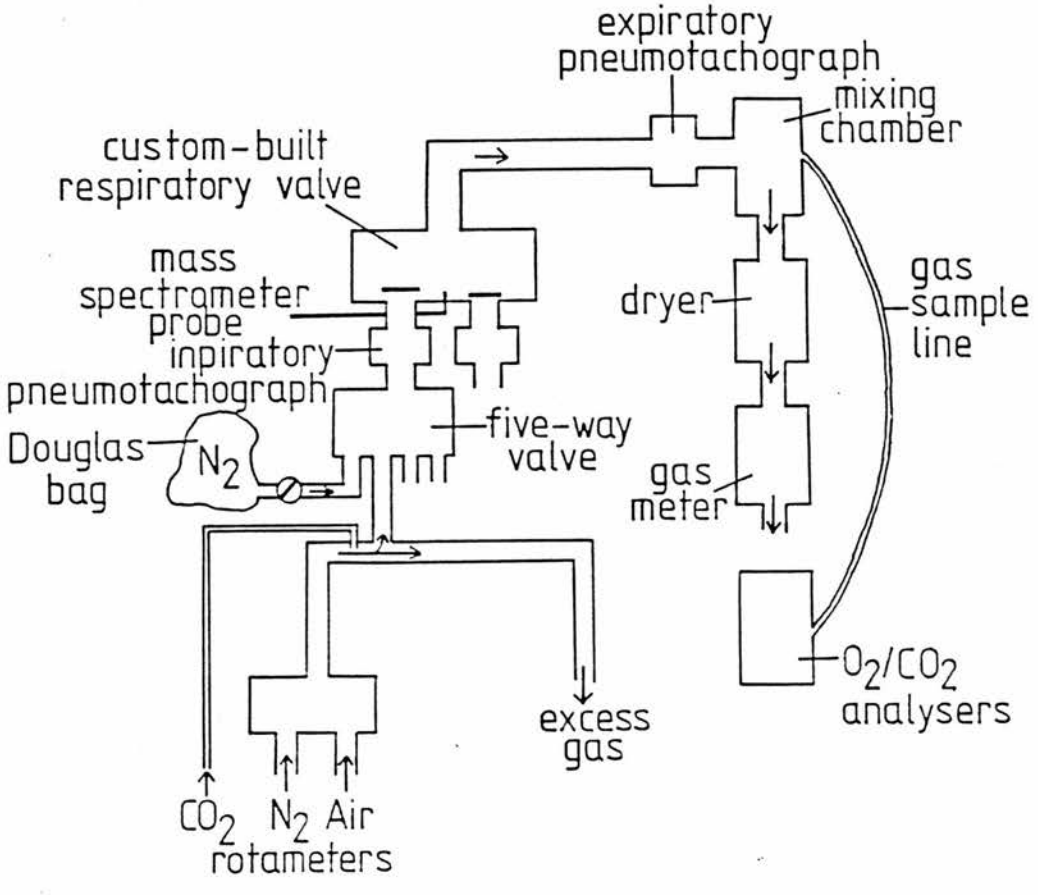
Inspired and expired gas concentration was sampled continuously 7cm from the mouth by a heated mass spectrometer probe (VG-Medical Spectralab M) with a sampling rate of 20-25mlmin^{-1} and a typical transit time of 100-200ms. The 10-90% response time was 65ms (calculated as a mean of ten measurements of the response to abrupt changes from air to gas mixtures containing 8% CO_2 and 5% CO_2). Before each study the mass spectrometer was calibrated for N_2, O_2, CO_2 and Argon with six gases of known composition. The gas mixtures used were 100% N_2, cylinder air and four O_2/CO_2 mixtures (O_2 range 5-21%, CO_2 range 2-8%). The O_2/CO_2 mixtures used were obtained from the British Oxygen Company with certificates of analysis and analysed to a

tolerance within the range ±2.5% of the analysed value. The software operating the mass spectrometer calculated the average difference between the measured and expected concentrations for each gas and the calibration was accepted if the average error was less than 1%. The mass spectrometer calibration was stable over a period of three hours which was the duration of the longest study (table 3.1).

Mouth pressure was recorded using a micromanometer (Furness Controls FCO/4) from a port between the inspiratory and expiratory flaps of the custom-made respiratory valve.

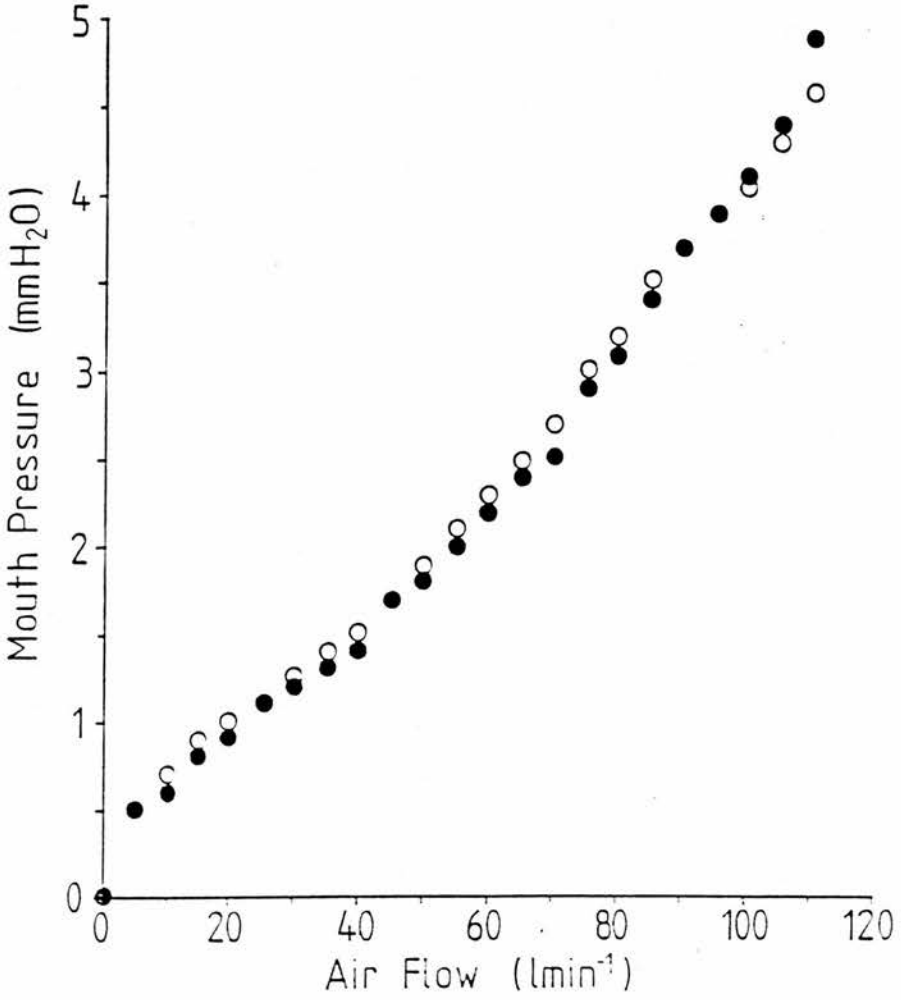
Expiratory gas flow was recorded using an heated pneumotachograph (Fleisch No. 2), the differential pressure across which was measured using a Furness Controls ltd FCO/4 micromanometer and the pressure/flow relationship of the expiratory pneumotachograph is shown in Fig 3.5. Ear oxgen saturation was recorded throughout all studies using an Hewlett-Packard 47201A ear oximeter with fibreoptic earprobe. The oximeter was always used in the fast mode with a delay time of 0.34 sec and a time constant of 1.61 sec (Douglas et al 1979). The electrocardiogram (ECG) and heart rate (measured from the r-r interval) were also recorded throughout all studies using an Hewlett-Packard 78351A Electrocardiogram monitor.

Fig 3.1 : Breathing circuit for transient and step change hypoxia.



Schematic representation of the breathing circuit used for all studies.

Fig 3.2 : Pressure-flow relationships for the custom-made and Hans-Rudolph five-way respiratory valves.

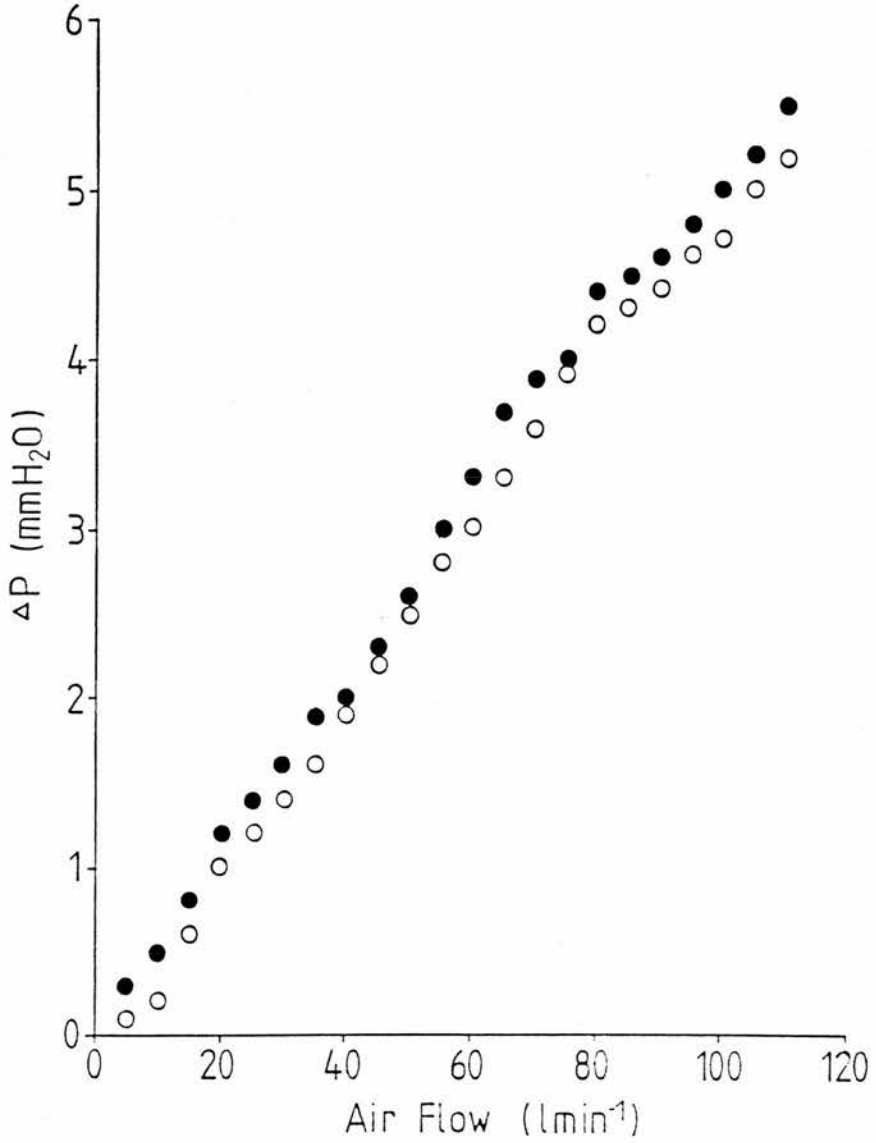


Pressure-flow relationships were determined by passing known constant flows of room air supplied from the rotometers at 20°C through the valves and measuring the pressure difference between the inlet and outlet parts using a Furness Controls FCO/4 micromanometer. Open circles (O) represent pressure-flow relationship for the custom-made valve, and closed circles (●) that for the Hans-Rudolph five-way valve.

Fig 3.3 : Apparatus

Subjects walked on a level treadmill and breathed through the custom-made and Hans-Rudolph five-way valves in combination.

Fig 3.4 : Pressure-flow relationships of the respiratory valves alone and in combination.



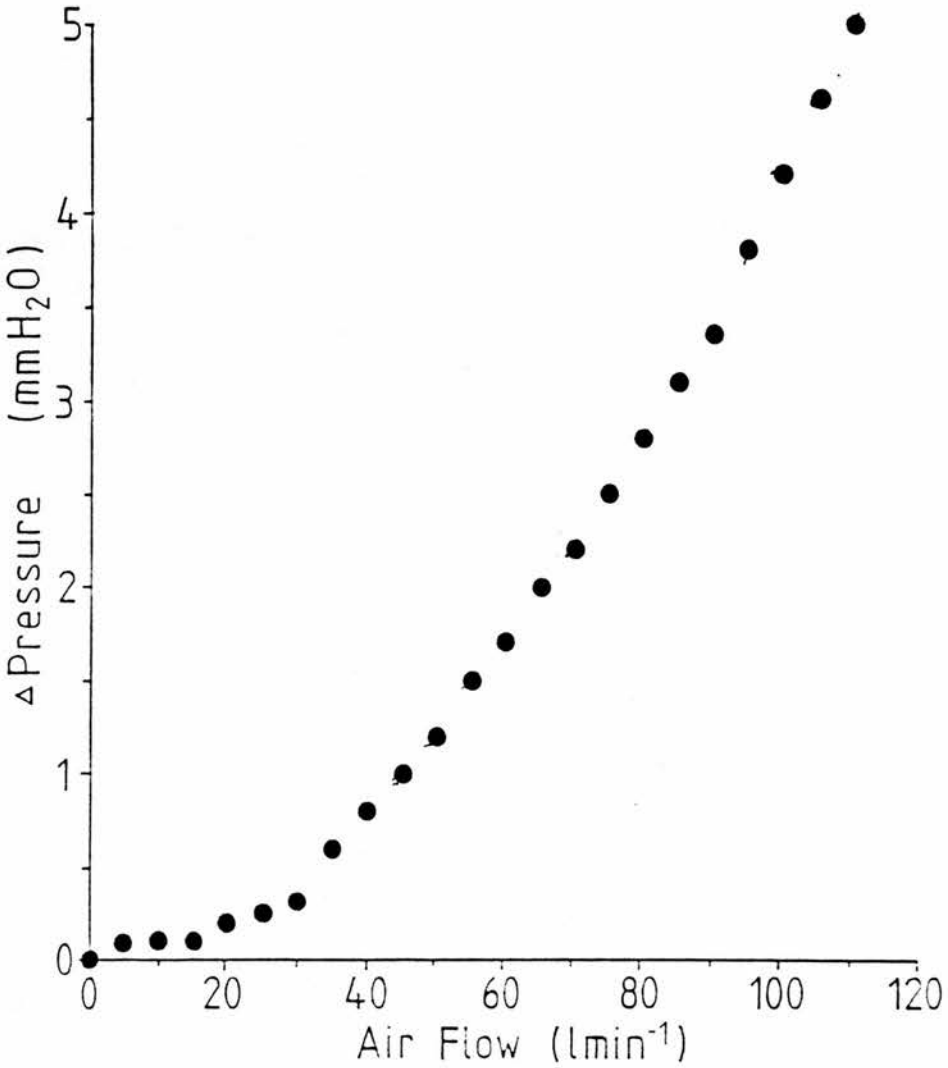
Pressure-flow relationships were measured by passing constant flows of room air at 20°C through the custom-made valve alone, and then in combination with the Hans-Rudolph five-way valve, and measuring the pressure difference across the valves using a Furness Controls FCO/4 micromanometer. The open circles (O) represent the pressure-flow relationship of the custom-made valve and inspiratory pneumotachograph, and the closed circles (●) represent that of the Hans-Rudolph five-way valve, the custom-built valve plus the pneumotachograph.

Table 3.1 Stability of the mass spectrometer

Gas	Concentration (%) time 0 min	Concentration (%) time 180 min
N_2	78.08	78.06
O_2	20.95	20.98
Argon	0.93	0.93
CO_2	0.04	0.03

Composition of room air measured by the mass spectrometer immediately after calibration (time 0 min) and after three hours (time 180 min).

Fig 3.5 : Pressure-flow relationship of the expiratory pneumotachograph.



The pressure-flow relationship was calculated by passing known constant flows of room air from the rotameters through the pneumotachograph and measuring the differential pressure using a Furness Controls FCO/4 micromanometer. Each point represents the change in pressure at a constant air flow.

ii) On-line Data Acquisition

Analogue signals from the recording devices were sampled every 16.67 milliseconds by a PDP 11/73 computer (Digital Equipment Corporation) using custom-written programs.

The beginning and end of the respiratory phase of each breath was identified by the computer using the mouth pressure signal. High frequency noise generated by movement was reduced in the mouth pressure signal by adding a resistance (a needle, the diameter of which was empirically chosen) in the sampling line close to the custom-built valve. The beginning of inspiration was determined as the time at which the mouth pressure fell below a negative threshold, and the beginning of expiration as the time at which mouth pressure rose above a positive threshold equal in size to the negative threshold. Inspiratory and expiratory times (T_I and T_E) were derived for each breath and respiratory frequency (f_R) was then calculated as $60/(T_I+T_E)$.

The output of the expiratory pneumotachograph was integrated to give expired volume which was then corrected using a calibration factor derived every 10 litres from the output of the dry gas meter. The flow integrator was reset every breath (using the mouth pressure signal as a marker), thus giving a breath-by-breath measurement of tidal volume (V_t). Instantaneous minute ventilation ($\dot{V}_E$ inst) was then calculated for each breath from respiratory frequency multiplied by

tidal volume.

The analogue signal from the mass spectrometer was calibrated for N_2, O_2, CO_2 and Argon onto the computer on each study day using five gas mixtures (100% N_2, cylinder air, and three O_2/CO_2 gas mixtures, O_2 range 5-21%, CO_2 range 2-8%). Each was sampled by the mass spectrometer and the least squares linear regression relationship was calculated between the analogue signal and the expected gas concentration. The residual root sum squared for N_2, O_2 and CO_2, the largest individual variation in N_2, O_2 and CO_2 and the gas mixture for which it occurred, the delay time of the mass spectrometer probe and the measured composition of room air were displayed. The calibration was accepted only if the delay time was less than 800ms and the largest individual variation was less than 0.25% for N_2, O_2 and CO_2. The delay time was used to offset all other variables to synchronise the measurements.

The ear oximeter analogue signal was also calibrated onto the computer using the electrical signals for 0 and 100% saturation, and assumed linearity of the relationship between ear oxygen saturation and the analogue signal between these two extremes. Ear oxygen saturation (SaO_2) was recorded as a mean over each breath. Mean heart rate of the previous 8 breaths was recorded breath-by-breath at the end-tidal point.

Inspired gas composition was calculated as an average over 150 ms, 750ms after the onset of inspiration (to

avoid analysis of dead space gas). End-tidal PCO_2 ($P_{ET}CO_2$) was determined as the maximal value during each expiration and end-tidal PO_2 was recorded simultaneously.

At the end of each study, data was archived onto floppy disks for subsequent off-line analysis.

iii) Analogue and Digital Displays

On the computer screen were displayed five breath mean values for inspired and end-tidal $\%O_2$, $\%CO_2$, tidal volume, minute ventilation, respiratory frequency, ear oxygen saturation, heart rate, breath number and stored time. A four channel time based recorder (Watenabe Linear Corder Mark VII) gave a continuous analogue display of PCO_2, tidal volume, expiratory gas flow and mouth pressure. The same information was also displayed on an oscilloscope (Lan-Electronics Ltd.) throughout each study. Ear oxygen saturation, heart rate (in digital form) and ECG were also displayed throughout all studies.

II GENERAL PROCEDURES

i) Subjects and lung function measurements

The subjects studied included laboratory personnel, the offspring of patients with chronic obstructive pulmonary disease and volunteers drawn from the general public. All subjects were healthy and taking no medication at the time of the studies and gave informed consent to the procedures. The studies were approved by the Lothian

Health Board Medicine and Clinical Oncology Ethics of Medical Research Sub-Committee. Anthropomorphic and lung function measurements were made in all subjects. Forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1) were measured using a Vitalograph spirometer (model s, number 20.400). Total lung capacity (TLC), functional residual capacity (FRC), residual volume (RV) and transfer factor (diffusing capacity;TLCO) were all measured using a Gould 2400 Pulmonary Function Laboratory system driven by an IBM PC/AT computer. Total lung capacity and its subdivisions were measured by the closed circuit helium dilution method (Cotes 1979). The TLCO was measured by the single breath method (Cotes 1979) using an 8 second breath holding time.

For men, the predicted normal values for FEV_1, FVC, and FEV_1/FVC% were taken from Crapo et al (1981) and FRC, RV, TLC and RV/TLC% predicted values were taken from Crapo et al (1982). Predicted normal values for TLCO were those given by Cotes (1965).

For women, the predicted normal values for FEV_1, FVC, FEV_1/FVC%, RV, TLC and RV/TLC% were taken from Hall et al (1979). Predicted normal values for TLCO were taken from Billiet et al (1963). Predicted body surface area values for all subjects were taken from Documenta Geigy (1962).

All subjects completed the Medical Research Council's questionnaire on respiratory symptoms, 1986 (a revised

and updated version of the 1965 questionnaire on respiratory symptoms approved by the Medical Research Council's committee on research into chronic bronchitis).

ii) Measurement of gas exchange

Expired gas volume was measured over a period of 2 minutes from the digital output of the Parkinson Cowan CD4 dry gas meter and minute ventilation ($\dot{V}_E$; $lmin^{-1}$ BTPS) calculated. During the same 2 minute period, oxygen and carbon dioxide concentrations were measured in the mixed expired gas drawn from the mixing chamber at 500 $mlmin^{-1}$. Oxygen concentration was analysed with a Servomex O_2 analyser (model 570A) and recorded as an average of three digital measurements taken at time 45, 75 and 105 seconds after the start of the mixed expired gas collection. Carbon dioxide concentration was measured using a Gould Capnograph (Mark III). The concentration was recorded as millimetres deflection of the trace on the chart paper during the last 15 seconds of the gas collection and read off the calibration curve drawn that day for the analyser. Both analysers were calibrated on each study day with four gas mixtures of known O_2 and CO_2 concentrations. Oxygen consumption ($\dot{V}O_2$; $lmin^{-1}$ STPD) and carbon dioxide output ($\dot{V}CO_2$; $lmin^{-1}$ STPD) were calculated off-line using standard steady state equations (Rahn et al 1955). The respiratory exchange ratio (RQ) was also calculated.

The $\dot{V}O_2$, $\dot{V}CO_2$ and $\dot{V}_E$ were measured between 5-7 and

7.15-9.15 minutes after the onset of either rest or exercise periods and between 5-7 minutes after the last hypoxic episode to confirm that steady state gas exchange had been achieved. Steady state gas exchange was considered to have been achieved if the initial two measurements of $\dot{V}O_2$ were within 100ml. If this was not achieved, then further two minute collections of mixed expired gas were made until two consecutive values of $\dot{V}O_2$ were within 100ml.

iii) Measurement of the hypoxic ventilatory response

Hypoxic ventilatory drive was determined either at rest (Chapter 6) or during exercise (Chapters 4,5,6,7,8), under conditions of steady state gas exchange using either transient or step change hypoxic stimuli.

iv) Transient hypoxia

The inspired gas was changed abruptly during an expiration from room air to either 100% N_2 or 1% O_2 in N_2 for between 1 and 3 breaths (depending on the tidal volume) to cause a fall in ear oxygen saturation (SaO_2) to 80-90%. Inspired gas was then returned to room air during an expiration. The transient hypoxic stimulus was repeated at intervals of at least 40 breaths until at least three acceptable stimuli were given (causing a fall in SaO_2 to 80-90%) with a maximum of 6 transient stimuli being given. Isocapnia was maintained by the addition of CO_2 close to the Hans Rudolph valve in the inspiratory limb of the circuit to keep end-tidal CO_2

constant.

v) Step change hypoxia

The inspired gas was abruptly changed during an expiration to an hypoxic mixture which was sustained for three minutes. The first inspired oxygen concentration was selected to reduce SaO_2 to 90-93% (usually F_IO_2 0.12 at rest, 0.15 on exercise). The inspired gas was then returned to room air, again during an expiration. The subject breathed room air for at least 5 minutes before the inspired gas was changed abruptly to a second hypoxic mixture selected to reduce SaO_2 to 85-88% (usually F_IO_2 0.10 at rest, 0.12 on exercise) for three minutes. Inspired gas was then returned to room air during an expiration. Isocapnia was maintained during the periods of hypoxia by the addition of CO_2 close to the Hans Rudolph valve to keep end-tidal CO_2 constant.

III OFF-LINE DATA ANALYSIS

Analysis of data recorded breath-by-breath

Data was analysed off-line using custom-written programs with a PDP 11/73 computer.

i) Elimination of spurious breaths

Breaths during which the subject swallowed, coughed or sighed produced spurious values for inspiratory and expiratory timing, and thus unusually large or small values for $\dot{V}_E$ inst. Such breaths were therefore eliminated using a custom-written computer program if

they did not conform to the following criteria:

a) $2kPa < P_{ET}CO_2 < 10kPa$

b) $P_{ET}O_2 > 2kPa$

c) $V_t > 0.15l$

d) $T_E > 300ms$

e) $T_I > 500ms$

However, not all spurious breaths could be identified by this program especially those associated with swallowing. The analogue trace of mouth pressure and expiratory flow (simultaneously zero during a swallow) were therefore used to identify a swallow which was then deleted from the computer file along with the previous and following breath (Fig 3.6).

ii) Calculation of hypoxic ventilatory response

Hypoxic ventilatory response was expressed as the slope of the linear regression relationship between $\dot{V}_E$inst and SaO_2.

For transient hypoxic stimuli, the data from the breath before the onset of hypoxia and all breaths up to and including the breath with the lowest SaO_2 (ie. the "on-phase" of the ventilatory response) were included in the calculation. Data from acceptable transient hypoxic stimuli (no spurious breaths for 10 breaths either side of the onset of hypoxia and a fall in SaO_2 to between 80 and 90%) were pooled. To take into account physiological and instrumental lag, an iterative process was used to calculate the best relationship between $\dot{V}_E$inst and SaO_2 during the response to transient hypoxia, using the

equation:

$$\dot{V}_E inst \text{ (breath } n+z) = a+b \text{ } (SaO_2, \text{ breath } n)$$

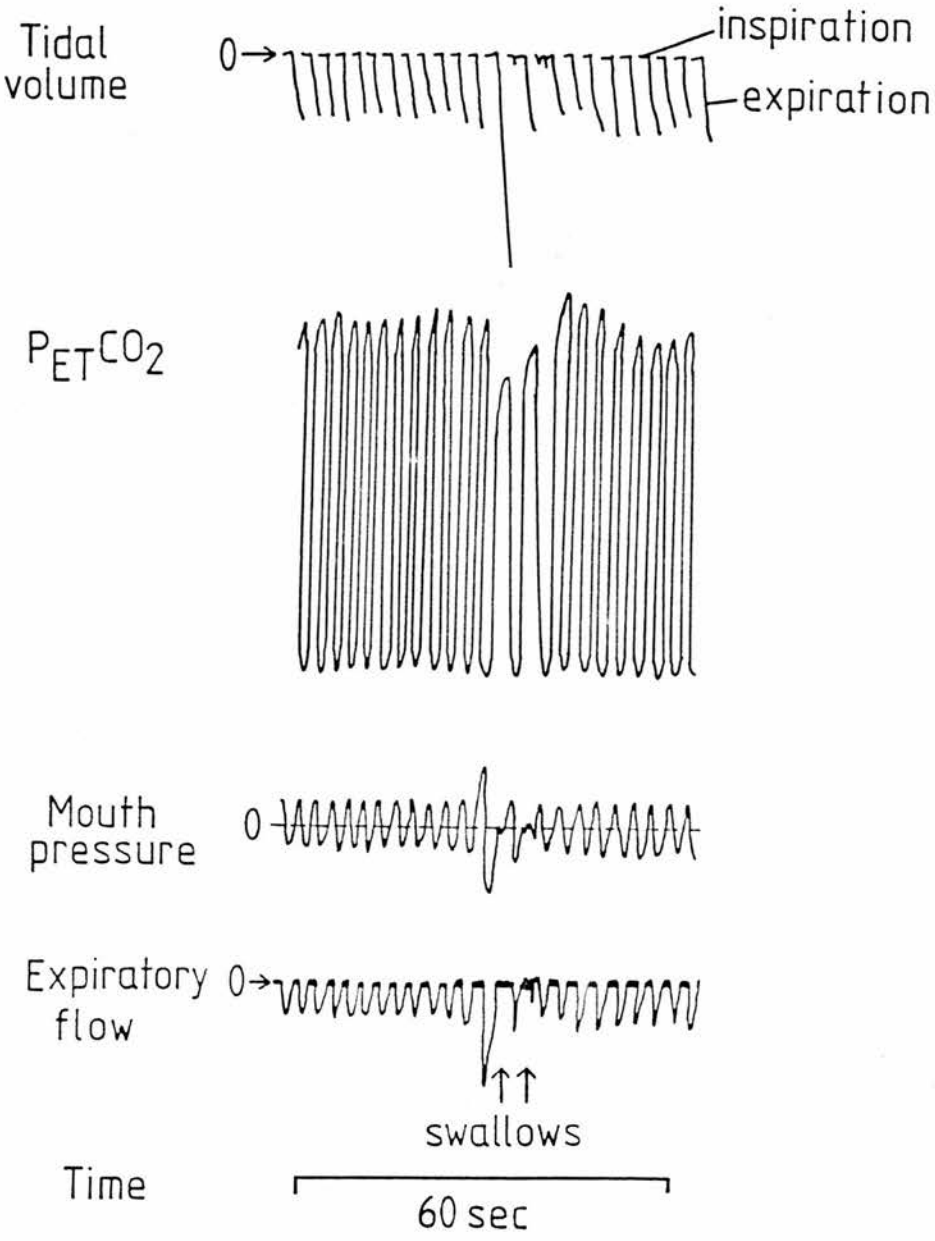
where z was allowed to vary from -5 to +5. Correlation coefficients (r) of these relationships were calculated and the slope of the $\dot{V}_E inst/SaO_2$ relationship at the most negative correlation was used to describe hypoxic ventilatory drive to transient hypoxia (Fig 3.7).

The ventilatory response to step change hypoxia was calculated from data ten breaths before the onset of each hypoxic period and all breaths during each three minute period of step change hypoxia. Data were then analysed by least squares linear regression to produce a slope for the $\dot{V}_E inst/SaO_2$ relationship which were generally negative (Fig 3.8).

iii) Calculation of baseline and hypoxic $P_{ET}CO_2$ values

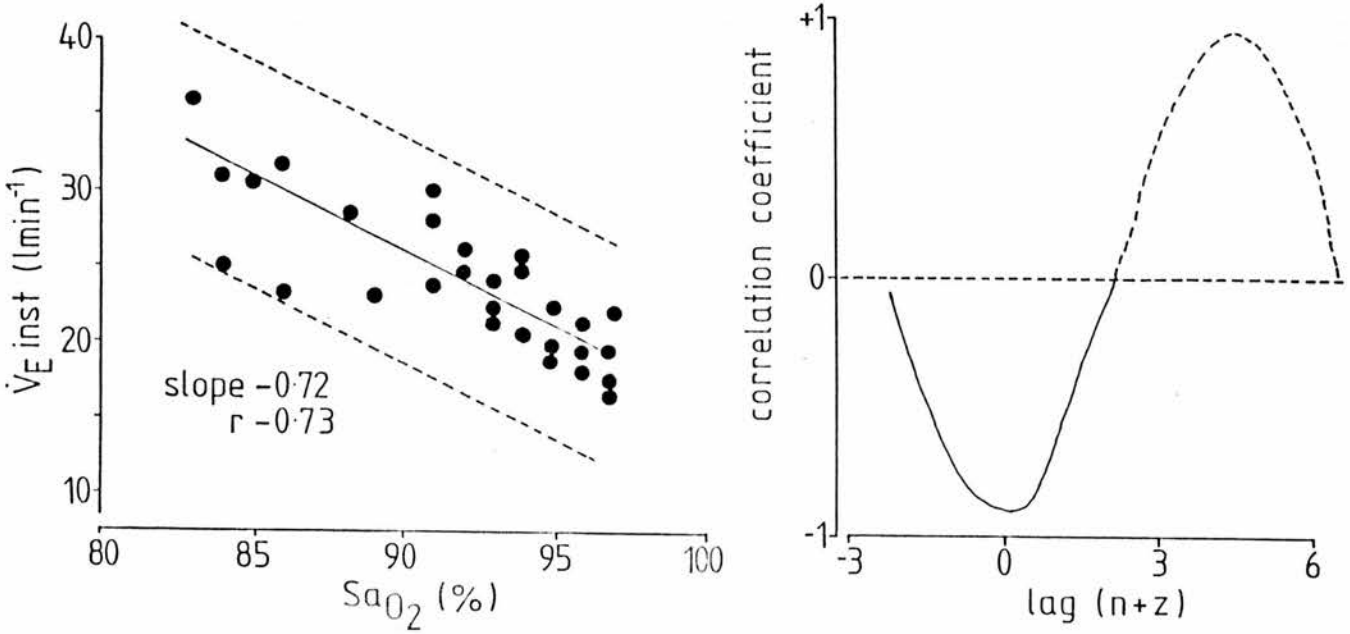
Baseline $P_{ET}CO_2$ was calculated as the mean of 20 breaths before each hypoxic stimulus and 20 breaths five minutes after the end of the last period of hypoxia. Step change hypoxia $P_{ET}CO_2$ was calculated as the mean of 10 breaths before and all breaths during the step change periods of hypoxia. Transient hypoxia $P_{ET}CO_2$ was calculated as the mean of all breaths included in the calculation of transient hypoxic ventilatory response.

Fig 3.6 : Identification of swallows



Analogue traces drawing tidal volume, $P_{ET}CO_2$, mouth pressure and expiratory flow. Mouth pressure and expiratory flow are simultaneously zero during a swallow.

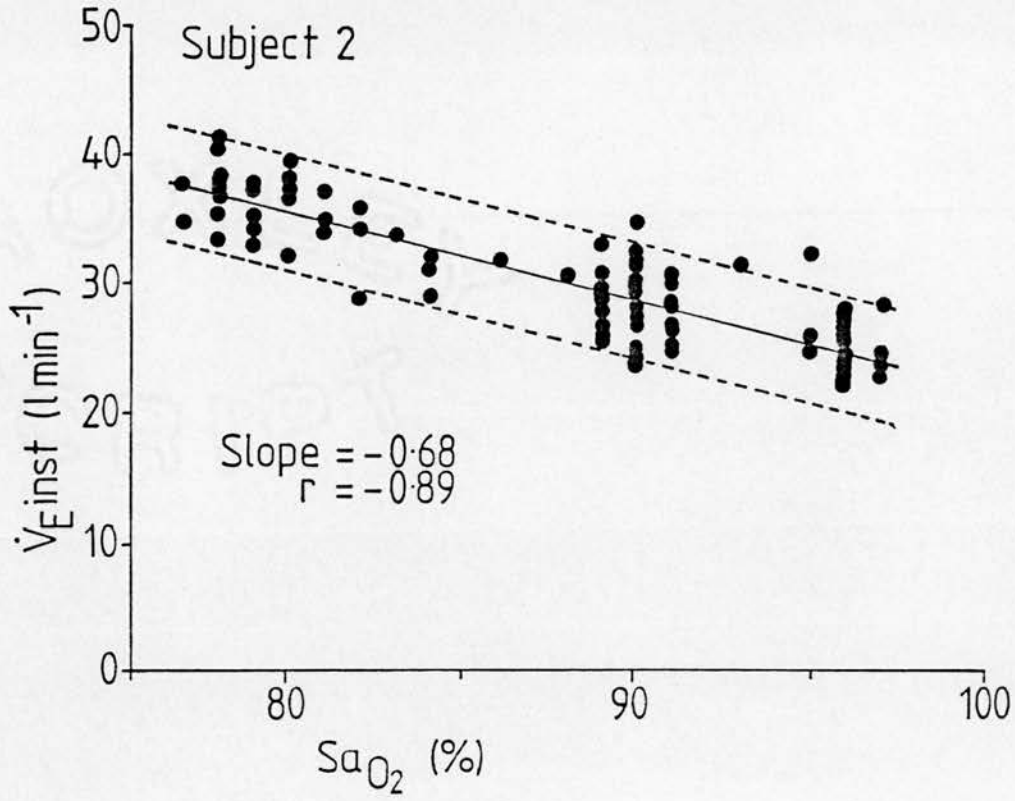
Fig 3.7 : Calculation of the slope of the $\dot{V}_E inst/SaO_2$ relationship following transient hypoxia.



Left hand side: hypoxic ventilatory response expressed as the slope of the linear regression line (——), and 95% confidence limits (---), of the $\dot{V}_E inst/SaO_2$ relationship at the most significant negative correlation at lag 0, as shown in the right hand panel.

Right hand side: the correlation coefficient (r) for the linear regression relationship between the SaO_2 and $\dot{V}_E inst$ for each breath calculated by the equation $\dot{V}_E inst$ (breath n + z) = a + b (SaO_2 of breath n) for the pooled data, from three breaths before, up to and including the breath with the lowest recorded SaO_2. The lag z includes both physiological and instrumental response time.

Fig 3.8 : Calculation for the ventilatory response to step change hypoxic stimuli.



The hypoxic ventilatory response to step change hypoxia expressed as the slope of the linear regression line (——). Each point represents the corresponding values for $\dot{V}_E$inst and SaO_2 for 10 breaths before and all breaths during each three minute period of step change hypoxia in subject 2. The 95% confidence limits are given by the broken lines (---).

CHAPTER 4: REPRODUCIBILITY OF THE HYPOXIC VENTILATORY RESPONSE AND MATHEMATICAL MODEL PARAMETER ESTIMATION

I INTRODUCTION

The ventilatory response to hypoxia varies widely between individual normal subjects (Forster et al 1971, Hirshman et al 1975, Kronenberg et al 1973, Rebuck et al 1973, Weil et al 1972, Anderton et al 1964). This may be partly due to differences in body size since the hypoxic ventilatory drive measured using progressive isocapnic hypoxia has been shown to correlate with height, weight, and body surface area (Hirshman et al, 1975). However, studies in cats suggest that there is a between individual difference in carotid body chemoreceptor sensitivity to oxygen (Black et al 1971, Eyzaguirre et al 1961). This difference in peripheral chemosensitivity may be genetically determined (Moore et al 1976, Collins et al 1978, Kawakami et al 1982a), and the inherited intensity of the drive may be important in the pathogenesis of some respiratory diseases (Flenley et al 1970, Mountain et al 1978, Fleetham et al 1984).

However, comparisons between individuals is complicated by both the within day and between day variability in the hypoxic ventilatory drive in an individual. Both Anderton et al (1964) and Kronenberg et al (1972) measured the ventilatory response to hypoxia on two occasions within twenty minutes. Anderton et al noted a greater increase in minute ventilation for the same alveolar oxygen tension, whereas Kronenberg et al

found no significant difference between the two measurements in each subject. It has been suggested that prior exposure to hypoxia potentiates the hypoxic ventilatory drive (Davidson et al 1983, Jennett et al 1984), and this may therefore influence measurements of hypoxic ventilatory drive repeated within thirty minutes. However, a diurnal variation in the sensitivity to CO_2 and the CO_2 threshold has been reported (Koepchen et al 1953, 1954) and a similar variation to O_2 sensitivity may affect measurements of hypoxic ventilatory drive. This diurnal variation in hypercapnic ventilatory drive may be due to the diurnal variation in adrenocorticotrophic hormone (ACTH) which has been shown to potentiate the ventilatory response to CO_2 (Koepchen et al 1954). Within day variability will also be affected by meals as Zwillich et al (1977) have shown that ingestion of carbohydrates potentiates the hypoxic ventilatory drive.

The only study of between day variability in the hypoxic ventilatory drive (Sahn et al 1977) showed a greater between day than within day variability in the ventilatory response to hypoxia. Sahn et al recorded the ventilatory response to progressive isocapnic hypoxia in eight normal subjects on 5 occasions 30 minutes apart on the same day, and over a period of seven months. They reported a mean co-efficient of variation of 19.4% for measurements made on the same day, but found that the between day variability was more

than 4 times as variable as the within day variability in 5 of the 8 subjects. Thus there is a wide variability in the measured hypoxic ventilatory response between days even within an individual. Many physiological changes may account for this variability. Sahn et al noted that the between day variability could be partially accounted for by variations in arterial pH measured prior to each study. In women, hypoxic ventilatory drive has been shown to vary with the menstrual cycle, with an increased drive in the luteal phase compared to the follicular phase (White et al 1983), probably due to the increased level of progesterone at this time, which is known to increase ventilatory responsiveness (Sutton et al 1975). Between day variations in metabolic rate may also affect the ventilatory response to hypoxia. Factors such as exercise (Weil et al 1972), hyperthermia (Natalino et al 1977), hyperthyroidism (Zwillich et al 1976) and ingestion of food (Zwillich et al 1977) which increase metabolic rate have been shown to cause a rise in hypoxic ventilatory drive, whereas myxoedema (Zwillich et al 1975) or semi-starvation (Doekel et al 1976) which decrease metabolism are associated with a fall in hypoxic ventilatory responsiveness.

Thus if comparisons are to be made between individuals, some estimate of the variability of the method for assessing hypoxic ventilatory drive must be known. The aim of this study therefore was to determine

the between day variability of the ventilatory response to transient and step-change hypoxic stimuli expressed both conventionally as the minute ventilation/oxygen saturation relationship, and by parameter estimation using the mathematical model.

II METHODS

i) Subjects

The subjects were two male (2 and 4) and two female (1 and 3), aged 23-40 years. Their anthropomorphic and lung function measurements are given in Appendix table 1. Subjects 1,2 and 3 were non-smokers but subject 4 occasionally smoked cigarettes. None were taking any medication at the time of the study.

ii) Protocol

Subjects 1,2, and 4 were studied at the same time of day on ten consecutive weeks. Subject 3 was studied at the same time of day on ten occasions over a period of 34 weeks, with an interval of at least a week between studies. The total duration and day of the current menstrual cycle was recorded for each study for the female subjects. All fasted for at least four hours before each study.

The subjects walked on a level treadmill at speeds of 1.35-1.55msec^{-1} which had previously been selected for each individual and the same speed was used for the ten studies. The subjects initially walked for 10 minutes breathing room air to establish steady state gas exchange. Mixed expired gas was collected between 5-7 and 7.15-9.15 minutes after the start of exercise, and from 5-7 minutes after the last step change hypoxic stimulus for calculation of $\dot{V}_E$, $\dot{V}O_2$, $\dot{V}CO_2$ and RQ to confirm steady state conditions.

The ventilatory response was measured first to

transient hypoxia and then to step change hypoxia.

iii) Transient hypoxia

Inspired gas was abruptly changed during an expiration from room air to 1% O_2 in N_2 for 1-3 breaths to cause a fall in SaO_2 to between 80-90%. Inspired gas was then returned to room air during an expiration. The transient hypoxic stimulus was repeated at 2 minute, or at least 40 breath, intervals until at least three acceptable stimuli were given (SaO_2 falling to between 80-90%). A maximum of 6 transient hypoxic stimuli were given during each study. Isocapnia was maintained by the addition of CO_2 close to the Hans-Rudolph valve in the inspiratory limb of the circuit to keep $P_{ET}CO_2$ constant.

iv) Step change hypoxia

Following the last transient hypoxic stimulus the subjects breathed room air for at least 3 minutes.

The ventilatory responses to two levels of step change hypoxia (F_IO_2 0.15 and 0.12) were then studied. Inspired gas was abruptly changed during an expiration from room air to 1% O_2 in N_2 for one breath. In the subsequent expiration, inspired gas was changed to 15% O_2 for the remainder of the 3 minute period. The subjects then breathed room air for at least 5 minutes. Inspired gas was then abruptly changed during an expiration to 1% O_2 in N_2 for two breaths followed by 12% O_2 for the remainder of the 3 minute period. Inspired gas was then returned to room air during an expiration. Isocapnia

was maintained by the addition of CO_2 close to the Hans-Rudolph valve in the inspiratory limb of the circuit to keep $P_{ET}CO_2$ constant.

v) Analysis

The results of the transient hypoxic stimuli were pooled and the response expressed as the slope of the $\dot{V}_E$ inst/SaO_2 relationship as described in Chapter 3. The results for the step change hypoxic stimuli were also pooled and the response expressed as the slope of the linear regression relationship between $\dot{V}_E$inst and SaO_2 for 10 breaths before and all breaths during each 3 minute hypoxic period.

Baseline normoxic end-tidal CO_2 ($P_{ET}CO_2$) was calculated as the mean of the 20 breaths before the first transient stimulus, 20 breaths before each of the step change hypoxic stimuli and 20 breaths five minutes after the end of the last hypoxic period. The mean $P_{ET}CO_2$ during transient hypoxia was calculated for all breaths used in the analysis of the ventilatory response. The mean $P_{ET}CO_2$ during step change hypoxia was calculated for 10 breaths before each, and all breaths during both 3 minute periods of hypoxia. Baseline respiratory frequency (f_R) and tidal volume (V_t) values were calculated for each study as the mean of 20 breaths before the first transient stimulus, 20 breaths before each of the step change hypoxic stimuli and 20 breaths five minutes after the end of the last hypoxic period.

The ventilatory response to both transient and step

change hypoxia was also analysed using the mathematical model and the parameters derived which best described the ventilatory response on each study day.

The Kruskal-Wallis one-way analysis of variance was used to compare each set of ten baseline values for $\dot{V}_E$, $\dot{V}O_2$, $\dot{V}CO_2$ and $P_{ET}CO_2$ for each subject between study days, and also the $P_{ET}CO_2$ values during transient and step change hypoxia with the corresponding baseline normoxic values for each subject. Simple least squares linear regression analysis was used to assess the effect of baseline values for $\dot{V}_E$, $\dot{V}O_2$, $\dot{V}CO_2$ and $P_{ET}CO_2$ upon transient $\dot{V}_E inst/SaO_2$ slope, step change $\dot{V}_E inst/SaO_2$ slope, gain 1 or gain 2. The coefficients of variation were calculated for transient and step change $\dot{V}_E inst/SaO_2$ slopes, gain 1, gain 2 and tau 2 for the group.

III RESULTS

The values for baseline normoxic $\dot{V}E$, $\dot{V}O_2$ and $\dot{V}CO_2$ for each subject on the ten study days are given in Appendix table 2. Although there was between day variability in all three variables within a subject, none of these differences were significant. The RQ value remained below 1.0 for all studies in all subjects.

There was no significant difference in the mean baseline $P_{ET}CO_2$ on the ten occasions in the four subjects, although the differences between the means in any one subject ranged from 0.10 kPa to 0.37 kPa (Appendix table 3). Breath-by-breath variation in $P_{ET}CO_2$, as indicated by the standard deviation, varied from 0.07 to 0.32 kPa during normoxia. There was no significant difference in any subject on any occasion between mean $P_{ET}CO_2$ during transient or step change hypoxia and the mean baseline normoxic value (Appendix table 3). Breath by breath variation in $P_{ET}CO_2$ during transient (sd 0.15 to 0.34 kPa) and step change (sd 0.09 to 0.23 kPa) hypoxia was similar to that seen during baseline normoxic ventilation. There was a significant correlation between baseline $P_{ET}CO_2$ and V_t or f_R ($p<0.02$ and $p<0.001$, respectively).

Similar maximal falls in SaO_2 were achieved on the ten occasions in each individual subject in response to either transient hypoxia, a step change hypoxic stimulus using 15% O_2, or a step change hypoxic stimulus using 12% O_2 (Appendix table 4). There was also no significant

difference between the mean lowest SaO_2 achieved in response to transient hypoxia or in response to a 12% O_2 step change stimulus in an individual on any occasion.

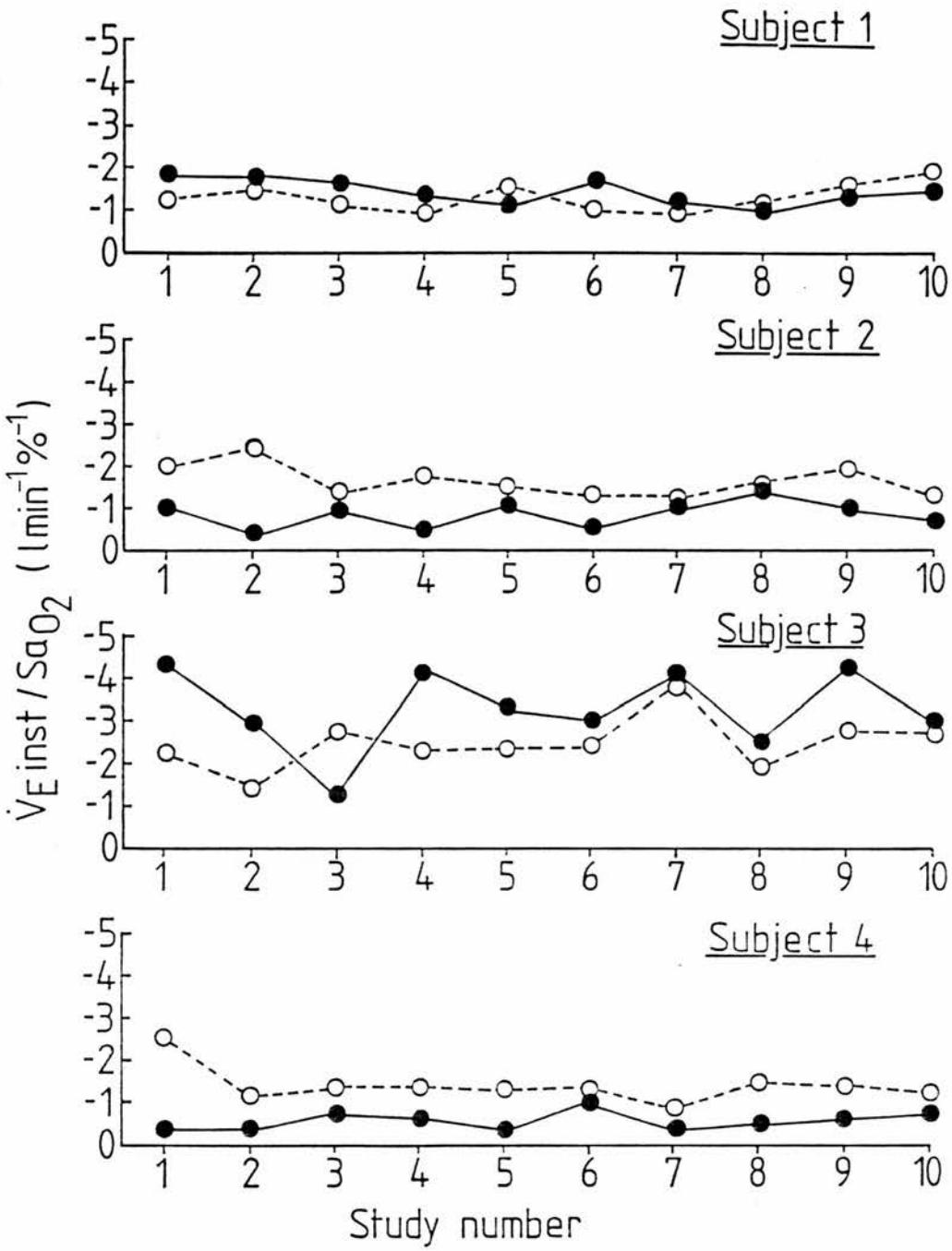
Day to day variability in the ventilatory response to transient and to step change hypoxia expressed as the $\dot{V}_E inst/SaO_2$ relationship is illustrated in Fig 4.1, with the results summarised in Appendix tables 5 and 6. The coefficient of variation for the response to transient hypoxia varied from 22 to 32%, and for the response to step change hypoxic stimuli from 19 to 37% (Table 4.1).

The between day variability in the parameters derived using the mathematical model for the four subjects is illustrated in Fig 4.2 with the results summarised in Appendix tables 7 to 11 and the model of best fit for subject 1 on three study days is given in Fig 4.3. The co-efficients of variation ranged from 19 to 34% for gain 1 and from 20 to 45% for gain 2. There was a significant difference ($p<0.001$) between subjects for gain 1 and gain 2 but not for tau 2. A positive value for the inhibition parameter (I) was consistently needed to obtain the best fit in subjects 2 and 4, although the absolute value varied between days (Fig 4.2, Appendix table 8). In subjects 1 and 3, a negative value for I (ie. potentiation) was generally needed to obtain the best fit, with between day variation in the absolute value for I. However, in subject 1 a positive value for I was needed on study day 8, and in subject 3, the parameter was not needed to obtain the best fit on study

day 1 whereas a positive value for I was needed on study day 3. The use of parameter sigma (the "saturating" effect acting on gain 2) was less consistent. In subject 1, sigma was consistently needed to obtain the best fit, but the absolute value for sigma was substantially lower on study days 8, 9 and 10 compared to days 1 to 7. In subject 4, sigma was only needed on one occasion (day eight). In subjects 2 and 3, varying values for sigma were required on six and four days respectively (Fig 4.2, Appendix table 11). The slow time constant, tau 2, showed the greatest between day variation (Fig 4.2, Appendix table 9), with co-efficients of variation ranging from 75 to 121% in the four subjects (table 4.1). There was no correlation between the $\dot{V}_E inst/SaO_2$ slope measured in response to transient or to step change hypoxia or parameters gain 1 and gain 2 with baseline normoxic $\dot{V}_E$, $\dot{V}O_2$, $\dot{V}CO_2$, or $P_{ET}CO_2$.

Subject 1 had a regular 28 day menstrual cycle and there appears to be a trend of a lower ventilatory response in the follicular phase of the menstrual cycle than in the luteal phase (Fig 4.4). No similar trend could be demonstrated in subject 3 but she did not have a regular 28 day menstrual cycle. However, both male and female subjects studied showed a similar variability in either the $\dot{V}_E inst/SaO_2$ slopes or the model gain parameters.

Fig 4.1 : $\dot{V}_E inst/SaO_2$ slopes for transient and step change hypoxia.



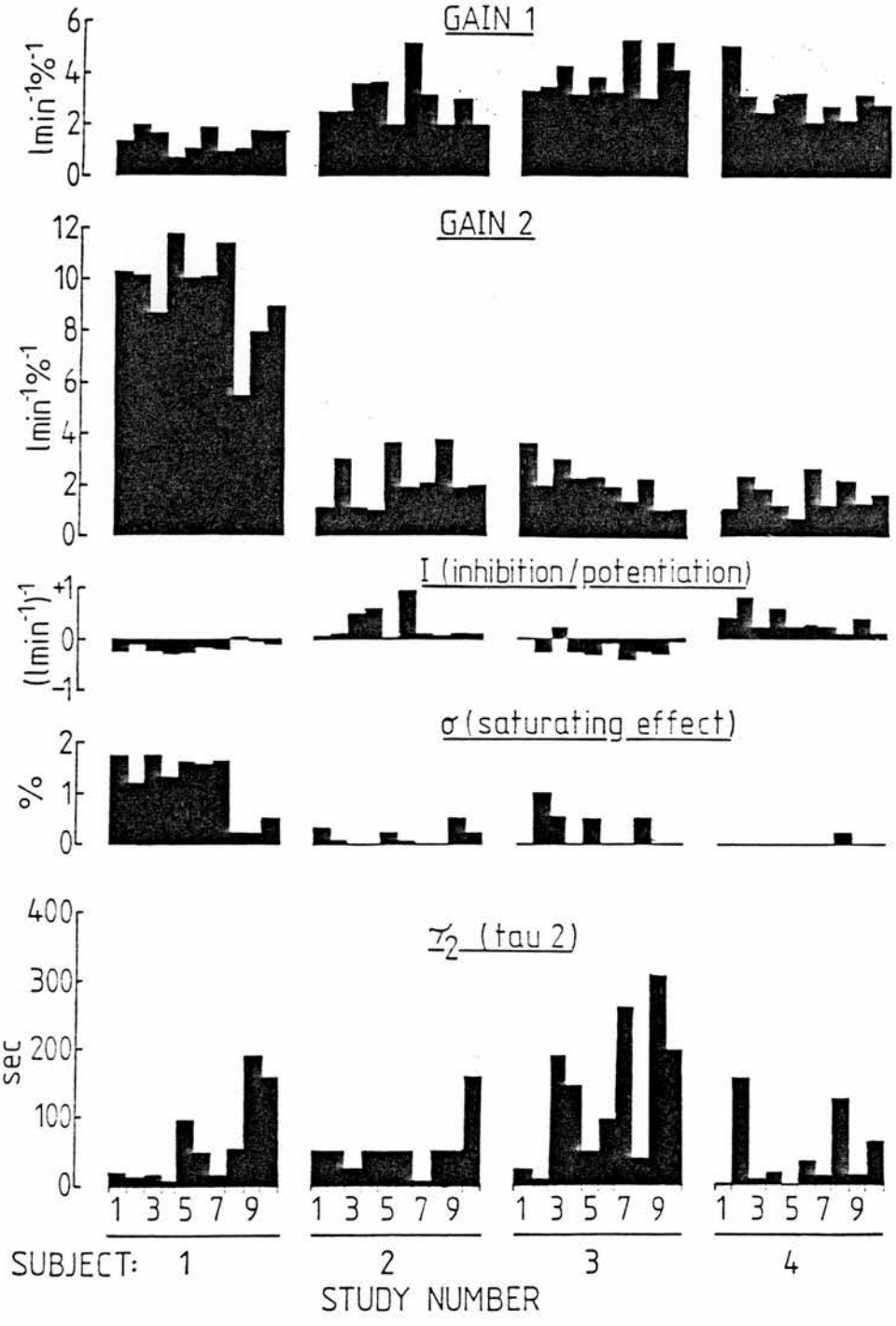
Each point represents the $\dot{V}_E inst/SaO_2$ slopes to transient (--O--) and step change (—●—) hypoxia, given for each subject on each study day.

Table 4.1 Coefficient of variation values for transient and step change hypoxia $\dot{V}_E inst/SaO_2$ slopes, gain 1, gain 2 and tau 2 (%)

	Subject			
	1	2	3	4
transient $\dot{V}_E inst/SaO_2$	23	22	25	32
step change $\dot{V}_E inst/SaO_2$	19	37	30	37
gain 1	29	34	19	29
gain 2	20	45	40	40
tau 2	90	75	79	121

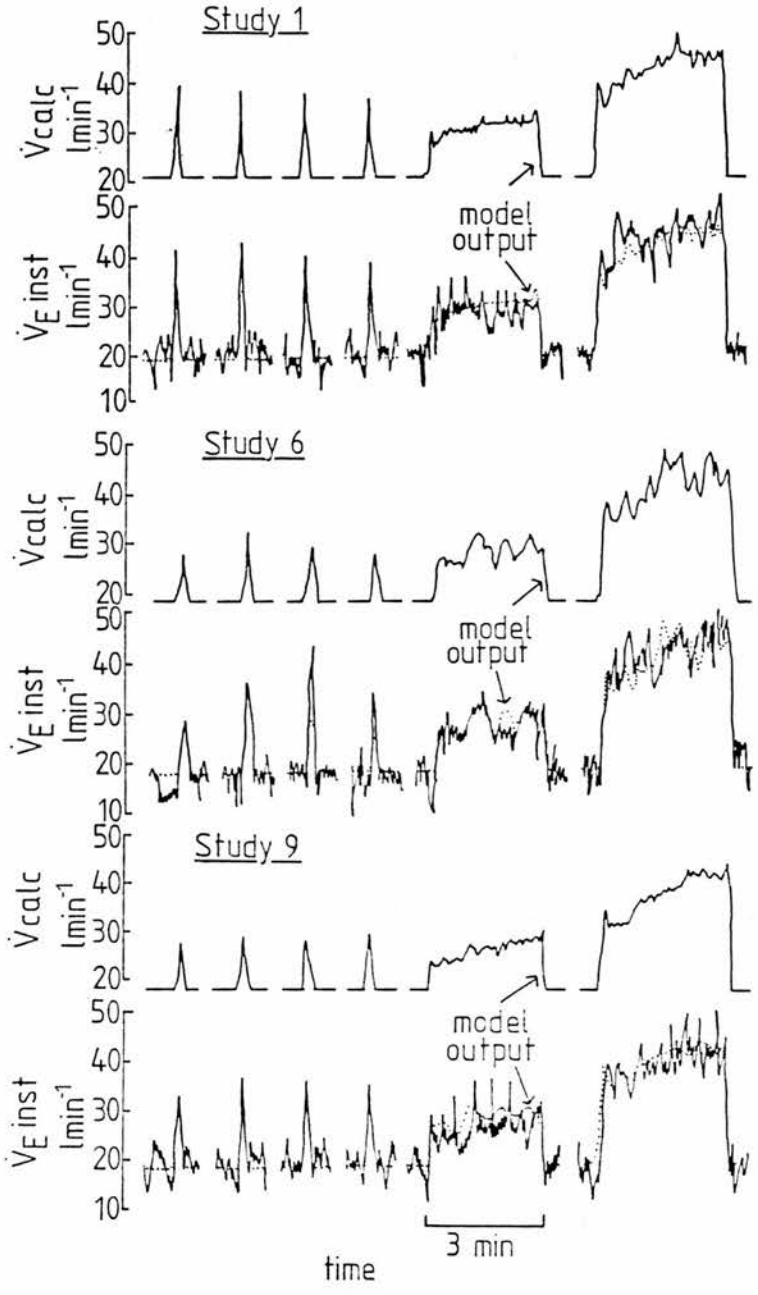
Coefficient of variation calculated as (standard deviation / mean) x 100. Transient and step change hypoxia $\dot{V}_E inst/SaO_2$ slopes and gain 1 expressed as $lmin^{-1}\%^{-1}$, gain 2 as $lmin^{-1}$ if sigma also required or as $lmin^{-1}\%^{-1}$ if sigma not included and tau 2 in sec.

Fig 4.2 : Between-day variability using parameter estimation.



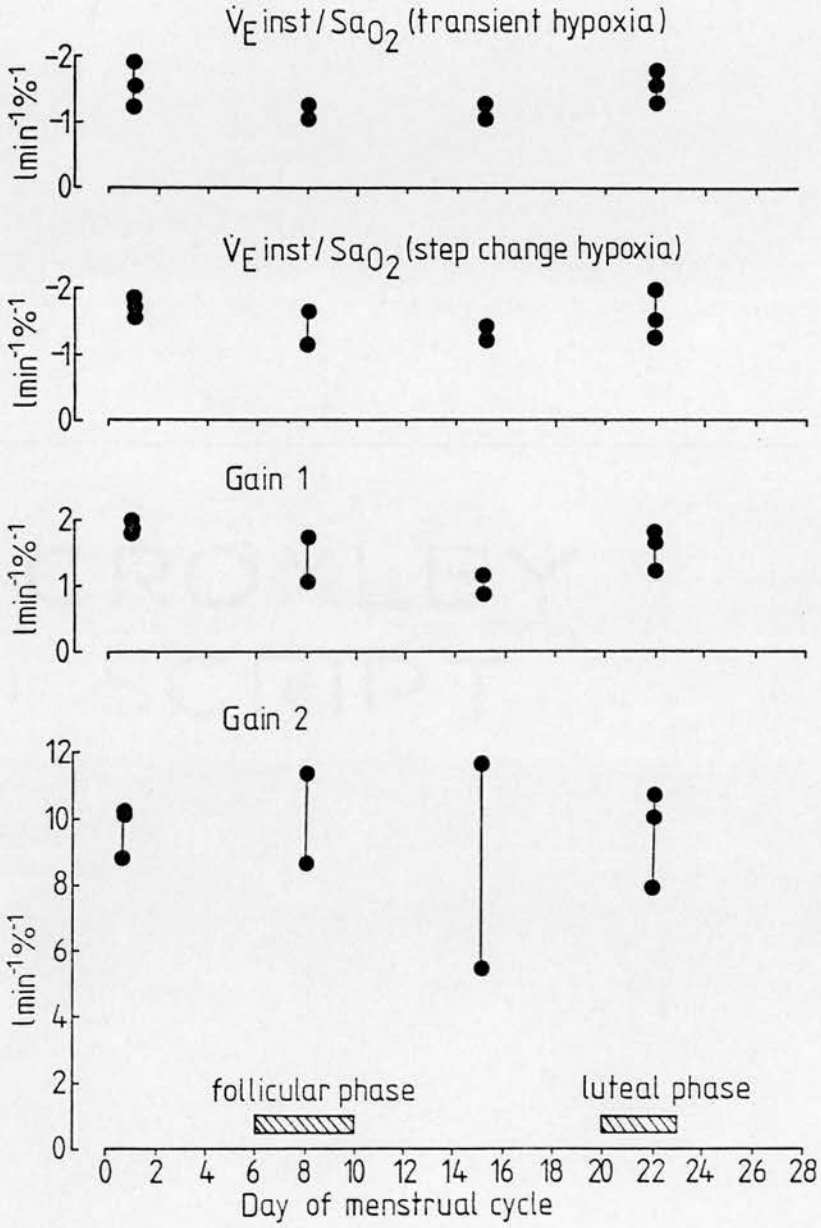
Between-day variability of the model parameters for each subject on each of ten study days. Gain 1 expressed as $lmin^{-1}\%^{-1}$; gain 2 as $lmin^{-1}$ if sigma also required or as $lmin^{-1}\%^{-1}$ if sigma not required; I as $(lmin^{-1})^{-1}$; sigma as % and tau 2 as sec.

Fig 4.3 : Best fit model traces for subject 1 on three study days.



For each study (1, 6 and 9) the upper trace shows the model output ($\dot{V}calc$) and the lower trace, the experimental $\dot{V}_E inst$ for subject 1. The model output, the dotted line, is also superimposed on the experimental data in the lower trace for each study day.

Fig 4.4 : Change of hypoxic ventilatory response with phase of the menstrual cycle in subject 1.



The hypoxic ventilatory response expressed as the slope of the $\dot{V}_E$inst/SaO_2 relationship for transient and step change hypoxia, and as the model gain parameters are plotted against the day of the menstrual cycle. The cross-hatched areas indicate the predicted (Best and Taylor 1985) follicular and luteal phases of the cycle. Each point represents the value obtained on a single study day. Units for gain 2 are $l min^{-1}$ if sigma also required or $l min^{-1}\%^{-1}$ if sigma not required to produce the model of best fit.

IV DISCUSSION

This study has shown that in four subjects studied at the same metabolic rate on 10 occasions over a period of 10-34 weeks, the within-subject variability of the gain parameters derived using the mathematical model was similar to that of the $\dot{V}_E inst/SaO_2$ relationships for the responses to both transient and step change hypoxia. In addition, there was a significant between-subject difference in the model gain parameters.

The coefficients of variation for between-day variability in the $\dot{V}_E inst/SaO_2$ relationships of 22-32% for transient hypoxic stimuli and 19-37% for step change stimuli are similar to those reported by Sahn et al (1977). They found that the mean coefficient of variation in response to progressive isocapnic hypoxia was 19% for each subject over a two hour period, but the between-day variability of this measurement was 1-15 times greater than the within-day variability.

The values for gain 1 showed a similar variability as the $\dot{V}_E inst/SaO_2$ relationships with the coefficients of variation ranging 19-35%. Values for gain 2 showed a similar variability with a range of coefficients of variation of 19-45%. The time constant of equation 2, tau 2 , was highly variable within each subject, with a range of coefficients of variation from 75 to 121%. The "inhibition" and "saturating effect" parameters of the model showed some within-subject variability but were generally either required or not required to produce the

model of best fit for each subject. The similar variability of the $\dot{V}_E inst/SaO_2$ slopes and the model gain parameters suggests that the variability of the model gains is probably due to daily physiological changes rather than random error of the parameter estimation.

The experimental values for $\dot{V}_E inst/SaO_2$ found in this study are comparable with a normal range of values demonstrated by Rebuck and Campbell (1974) by a similar method. The mathematical model showed significant differences between the gain parameters of the four subjects studied and may therefore be used to study individual differences within a population.

This study did not aim to determine the causes of the day-to-day variability of the hypoxic ventilatory response. However, the effect of some factors were minimised by the study design. The hypoxic ventilatory response has been shown to increase with the level of exercise (Weil et al 1972, Martin et al 1978). In this study, the $\dot{V}O_2$ did not vary significantly between the ten studies in an individual, and in all studies the RQ remained below 1.0 suggesting that the subjects exercised below their anaerobic threshold (Buchfuhrer et al 1983). Thus it is unlikely that day-to-day differences in the level of exercise were responsible for the variations in the hypoxic ventilatory response. Potentiation of the ventilatory response to hypoxia by ingestion of carbohydrates (Zwillich et al 1977) was avoided because all subjects fasted for at least four

hours before each study. For all subjects, the fall in SaO_2 produced by either transient or step change hypoxic stimuli were consistent over the ten studies. Thus the day-to-day variability in the hypoxic ventilatory response cannot be attributed to variation in the size of the stimuli.

Diurnal variation in the hypercapnic ventilatory response due to a change in ACTH has been demonstrated (Koepchen et al 1954), and may therefore also contribute to the variability of the hypoxic ventilatory response. Diurnal variation in the response was avoided by studying all subjects at the same time of day (to within one hour) on each occasion. A circadian rhythm of wakefulness has been demonstrated (McFadden 1988), with dips in wakefulness at around 2pm and 10pm. The periods of increased drowsiness during mid-afternoon (when all studies took place), would therefore have had a similar effect on each study.

Both pH and PCO_2 interact with hypoxia at the peripheral chemoreceptors to potentiate the hypoxic ventilatory response (Eyzaguirre and Lewin 1961). Sahn et al (1977) reported that the between-day variability in the hypoxic ventilatory response in an individual was at least partly due to a change in arterial pH. However, they measured the pH of arterialised venous blood which may not represent a true arterial pH. Arterial pH was not measured in this study.

Although the differences between baseline normoxic

$P_{ET}CO_2$ between study days were not statistically significant for each subject, there was some variation and this may partly account for the variability of the response. For all four subjects studied, there was a significant correlation between baseline $P_{ET}CO_2$ and either f_R or V_t. This suggests that between-study changes in the baseline $P_{ET}CO_2$ were due to changes in breathing pattern, which varies considerably between days (Tobin et al 1988). Furthermore, throughout this study, $P_{ET}CO_2$ is assumed to equal $PaCO_2$. End-tidal PCO_2 has been directly compared with simultaneously measured $PaCO_2$ in normal subjects at rest and during exercise and a small positive difference has been shown in $P_{ET}CO_2$ - $PaCO_2$ as tidal volume and respiratory frequency increase during exercise (Jones et al 1979, Cummins et al 1986). Thus manual addition of CO_2 into the apparatus to keep $P_{ET}CO_2$ constant may have introduced the possibility of a slight variable degree of hypercapnia. However, several studies indicate that at mild to moderate exercise levels (as in this study), (A-a) PCO_2 differences are either unchanged or only very slightly changed from resting values (Jones et al 1966, Whipp and Wasserman 1969). Therefore the difference between $P_{ET}CO_2$ and $PaCO_2$ was probably small and contributed little to the between-day variability of the hypoxic ventilatory response within an individual. Between-day changes in normoxic baseline $P_{ET}CO_2$ probably therefore reflect small changes in arterial PCO_2 initiated by a

fluctuation in pH (Gabel and Weiskopf 1975).

Two of the four subjects studied (1 and 3) were female. Progesterone, which is known to potentiate the hypoxic ventilatory response (Sutton et al 1975), is considered to be the cause of the increased response during the luteal phase of the menstrual cycle (White et al 1983, Regensteiner et al 1989). Subject 1 had a regular 28 day menstrual cycle and demonstrated a trend of a lower hypoxic ventilatory response during the follicular phase of the menstrual cycle than in the luteal phase. Subject 3 did not demonstrate such a trend but did not have a regular 28 day menstrual cycle. Since only two complete cycles were studied and the progesterone levels in the blood were not measured, no conclusions can be drawn from a trend in only one subject. Nevertheless, changes in progesterone levels may have contributed to the variability of the response in the female subjects. However, it is unlikely that these hormonal changes are the sole cause of the variability, as the hypoxic ventilatory responses of the male subjects (2 and 4) were no less variable than those of the females.

Several other factors may be responsible for the between-day variability in the hypoxic ventilatory response. A variation in sympathetic activity may alter peripheral chemoreceptor sensitivity (Briggs 1920, Hickham et al 1951, Asmussen and Nielson 1957, 1958, Biscoe and Purves 1967, Bhattacharyya et al 1970,

Lugliani et al 1971, Weil et al 1972, Masson and Lahiri 1974, Leitch et al 1976, Martin et al 1978, Flenley et al 1979). A decrease in CNS activity during sleep has been shown to cause a fall in the hypoxic ventilatory reponse (Douglas et al 1982, Berthon-Jones and Sullivan 1982, Hedemark and Kronenberg 1982). The effect of personality (Saunders et al 1972) and mental stimuli (Morgan and Cameron 1984) upon the hypercapnic ventilatory response in normal subjects has been demonstrated and may also influence the hypoxic ventilatory response.

In spite of a wide between-day variability of the hypoxic ventilatory response and model parameters, there were distinct differences between subjects, suggesting that the model is capable of differentiating between individuals and reflects true between-day physiological variation in the ventilatory response. The mathematical model therefore appears to be a reliable method for estimation of the hypoxic ventilatory response in normal subjects and may allow further investigation of factors such as mood, central nervous system or sympathetic activity which may be responsible for day-to-day variability in the response.

CHAPTER 5: THE EFFECT OF RATE OF ONSET OF HYPOXIA UPON THE HYPOXIC VENTILATORY RESPONSE

I INTRODUCTION

In order to avoid central hypoxic depression of ventilation (Weiskopf and Gabel 1975, Easton et al 1986), the transient hypoxic stimulus has often been used specifically to measure peripheral chemoreceptor mediated ventilatory drive (Dejours et al 1953, 1957b, 1958, 1963). However, the ventilatory response to transient hypoxia may be attenuated as the time to reach stable ventilation during step change hypoxia exceeds the duration of the transient stimulus (Reynolds and Millhorn 1973). A close correlation has been reported between the ventilatory response to transient and either steady state hypoxia (Kronenberg et al 1972, Edelman et al 1973), or progressive isocapnic hypoxia (Shaw et al 1982, Warren et al 1984). However, Shaw et al (1982) found the ventilatory response (expressed as the $\dot{V}_E inst/SaO_2$ slope) to transient hypoxia was approximately half that to progressive isocapnic hypoxia. Furthermore, Warren et al (1987) found that, during moderate exercise, the slope of the $\dot{V}_E inst/SaO_2$ relationship in response to transient hypoxic stimuli was significantly lower than to 3 minute isocapnic step change hypoxic stimuli produced by abruptly changing inspired gas from air to 15% O_2 and air to 12% O_2. They found that the half time of the response to 12% O_2 was on average three times the duration of the transient

stimulus and therefore concluded that this contributed to the underestimation of the hypoxic ventilatory drive since insufficient time was available for the response to develop. However, they also found that Fourier deconvolution of the $\dot{V}_E$inst/SaO_2 relationship showed a difference between the amplitude/frequency plot of the convolution function derived from the transient data, and that derived from the step change data. These results suggest that the response to transient or step change hypoxia may not adequately be described by a single linear system. They therefore concluded that the ventilatory response was dependent on the rate of onset of the stimulus.

Only two groups have studied the effects of differing time courses of hypoxic stimuli upon the ventilatory response. Dutton et al (1973) investigated the effects of different perfusion rates of hypoxic blood into the carotid bodies of 24 anaesthetised dogs using step, ramp, pulse and pulse train hypoxic forcing functions. In contrast to the abrupt increase in ventilation observed during step forcing with hypoxic blood at the carotid bodies, ramp forcing resulted in a ventilatory response which developed more slowly than the decrease in PO_2, especially during the first second of perfusion. They observed that the large increase in tidal volume within the first second occurred too rapidly to be explained by even the fastest increase in neural activity observed by Black et al (1971). They therefore

concluded that at some site in the respiratory control system central to the chemoreceptor, the initial change in chemoreceptor activity triggered a signal for a substantial ventilatory response, followed by little further rise in ventilation despite a subsequent increase in sinus nerve input. Hence an element of rate sensitivity appeared to be present during step hypoxia, even though there was no overshoot in ventilation. Dutton et al also analysed the hypoxic ventilatory response using a mathematical model which consisted of two parallel pathways, each containing linear second-order dynamics, one with fast pathway dynamics (time constant 2.9 sec), and the second, a slow pathway (time constant 13.9 sec). The input for the model was the fall in PO_2, and the output, the ventilation ratio (hypoxic ventilation divided by control ventilation). They found that an additional feed forward pathway with linear dynamics and a time constant of 1 sec was required to account for the initial rapid change in ventilation but which was not responsive to subsequent rapid changes in arterial oxygen tension. They concluded that the abrupt increase in respiratory centre output at the onset of severe hypoxia could provide an emergency mechanism for rapidly raising alveolar oxygen tension in an intact animal.

Bertholon et al (1989) are the only group to have studied the effect of the rate of onset of an hypoxic stimulus upon the ventilatory response in humans. They

investigated stepwise increases and decreases of four different levels of hypoxia with varying time courses in 7 healthy subjects. They found that the maximum ventilatory responses were found at times 8 and 24 minutes after the start of hypoxia and these results were similar to those obtained using a similar protocol and CO_2 stimulation by the same group (Bertholon et al 1988). Measuring the difference between normoxic resting $\dot{V}_E$ and that at a P_AO_2 of 50 mmHg for each subject ($\angle\dot{V}_{50}$), they demonstrated that hypoxic sensitivity was rate dependent with two preferential rates of rise for the stimulus. They also used a mathematical model to describe the ventilatory response which included a central neural controller with no dynamics of its own and a linear response to chemoreceptor inputs. However, they found that the single compartment model could not describe the two maxima of the ventilatory response produced at times 8 and 24 minutes after the start of hypoxia. They therefore suggested the existence of an hypoxic threshold of activation for the regulatory system, the value of which depended on the rate of rise of the stimulus. They also hypothesised that the central neural controller displays differential sensitivity properties.

Bertholon et al (1989) investigated the ventilatory response to hypoxic stimuli with longer time courses than those studied by Warren et al (1987). Therefore, the aim of this study was to investigate the effect of

the rate of onset of an hypoxic stimulus when varied between that of transient and step change hypoxic stimuli (giving a mean time course of the fall in SaO_2 varying from 17 to 50 sec).

II METHODS

i) Subjects

The subjects were six healthy volunteers (3 male, 3 female), aged 22-39 years drawn from laboratory staff. Their anthropomorphic and normal lung function details are given in Appendix table 12. All subjects were non-smokers apart from subject 3 who occasionally smoked cigarettes and none were taking any medication at the time of the study.

ii) Protocol

The subjects exercised for four 40 minute periods, each separated by 15 minutes rest. They walked on a level treadmill at a speed of between 1.35-1.60 $msec^{-1}$ which had previously been selected to produce a $\dot{V}O_2$ of 10-12$mlmin^{-1}kg^{-1}$, and the same speed was used for all four exercise periods in an individual. The subjects initially walked for 12 minutes breathing room air to establish steady state gas exchange conditions. Mixed expired gas was collected between 7-9 and 9.15-11.15 minutes after the start of exercise and from 5-7 minutes after the end of the last hypoxic stimulus for calculation of $\dot{V}_E$, $\dot{V}O_2$, $\dot{V}CO_2$ and RQ to confirm steady state conditions.

The ventilatory response was measured either to transient hypoxia or to one of three rates of onset of step change hypoxia during exercise. The order of the types of hypoxic stimuli were randomised.

iii) Transient hypoxia

Inspired gas was abruptly changed from air to 100% N_2 for two breaths to cause a fall in SaO_2 to between 80-90%. Inspired gas was then returned to room air during an expiration. The transient hypoxic stimulus was repeated six times at 60 breath intervals. Isocapnia was maintained by the addition of CO_2 close to the Hans-Rudolph valve in the inspiratory limb of the circuit to keep $P_{ET}CO_2$ constant.

iv) Step change hypoxia

Three forms of step change hypoxia were given:

a) **1%+12% O_2** Inspired gas was abruptly changed during an expiration from room air to 1% O_2 in N_2 for two breaths followed by 12% O_2 for the remainder of a three minute period. Inspired gas was then returned to room air during an expiration and the procedure was repeated five minutes later.

b) **6%+12% O_2** Inspired gas was abruptly changed during an expiration to 6% O_2 in N_2 for two breaths followed by 12% O_2 for the remainder of the three minute period. Inspired gas was then returned to room air during an expiration and the procedure was repeated five minutes later.

c) **12% O_2** Inspired gas was abruptly changed during an expiration to 12% O_2 for three minutes. Inspired gas was then returned to room air during an expiration and the procedure was repeated five minutes later.

Isocapnia was maintained during the onset and duration

of all step change hypoxic stimuli by the addition of CO_2 close to the Hans-Rudolph valve in the inspiratory limb of the circuit.

v) Analysis

Baseline normoxic $P_{ET}CO_2$ was calculated for each section of the study as the mean of 20 breaths before each hypoxic stimulus and 20 breaths five minutes after the end of the last hypoxic stimulus. The mean $P_{ET}CO_2$ during transient hypoxia was calculated for all breaths used in the analysis of the ventilatory response. The mean $P_{ET}CO_2$ for each type of step change hypoxia was calculated for 10 breaths before each and all the breaths during both 3 minute periods of step change hypoxia. The rate of onset of each type of stimulus was calculated as the mean value for the SaO_2 to fall to a minimum for transient stimuli, or to the mean value for step change hypoxic stimuli from the onset of hypoxia, for each subject.

The results of the acceptable transient hypoxic stimuli (SaO_2 falling to between 80-90%) were pooled and the response expressed as the slope of the $\dot{V}_E inst/SaO_2$ relationship as described in Chapter 3. The data from the two periods of step change hypoxia for each onset type were also pooled for calculation of the hypoxic ventilatory drive expressed as the negative $\dot{V}_E inst/SaO_2$ slope.

The half-time of the ventilatory response for step change hypoxia was calculated as the time from the onset

of hypoxia for half the mean final minute $\dot{V}_E$inst to be reached. The duplicate measurements at each rate of onset of step change hypoxia were pooled with the responses to transient stimuli for each subject. The mathematical model was then used to derive a set of unique parameters which best described the ventilatory response for each rate of onset of step change hypoxia for each subject.

Variables were compared using the Kruskall-Wallis one way analysis of variance test (Colquhoun 1971).

III RESULTS

There was no significant difference between baseline gas collection values for $\dot{V}_E$, $\dot{V}O_2$ and $\dot{V}CO_2$ for each section of the study for each subject (Appendix table 13). The respiratory quotient (RQ) was less than 1.0 throughout the study for all subjects. Baseline $P_{ET}CO_2$ was not significantly different for any subject during the four exercise periods, and the maximal standard deviation about the mean in $P_{ET}CO_2$ during hypoxia was 0.21kPa (Appendix table 14).

Transient hypoxia caused a rapid fall in SaO_2 accompanied by a brief rise in $\dot{V}_E$inst (fig 5.1). Step change hypoxic stimuli of 1% +12% O_2, 6%+12% O_2 and 12% O_2 caused progressively slower rates of onset of hypoxia with sustained rises in $\dot{V}_E$inst (fig 5.1).

The times for SaO_2 to fall to a minimum value for transient stimuli and to the mean value for step change hypoxic stimuli are given in table 5.1. The rate of onset of the transient hypoxic stimuli was significantly shorter than that of the 6%+12% O_2 or 12% O_2 step change hypoxic stimuli ($p<0.05$) but was similar to that of the 1%+12% O_2 step change hypoxic stimuli. There was no significant difference between the lowest SaO_2 values reached during transient hypoxia and any of the types of step change hypoxia for each individual subject (table 5.2).

To determine whether a steady state of ventilation had been achieved for each type of step change hypoxia for

each subject, mean values of $\dot{V}_E$inst were calculated for each 30 second block during the hypoxia. There was no significant difference between mean $\dot{V}_E$inst values for the periods 2-2.5 and 2.5-3 minutes after the start of hypoxia for any subject during any type of step change hypoxia.

The $\dot{V}_E$inst/SaO_2 relationship ranged -0.70 to -4.29 $lmin^{-1}\%^{-1}$ for transient hypoxia, -0.60 to -3.49$lmin^{-1}\%^{-1}$ for 1%+12% O_2 step change hypoxia, -0.47 to -3.60 $lmin^{-1}\%^{-1}$ for 6%+12% O_2 step change hypoxia, and -0.72 to -3.48$lmin^{-1}\%^{-1}$ for 12% O_2 step change hypoxia (table 5.3). The $\dot{V}_E$inst/SaO_2 slope in response to transient hypoxia was consistently greater than that for any rate of onset of step change hypoxia in 3 subjects (2, 3 and 5), less in 2 subjects (1 and 6), and in one subject (4) the responses to all four types of stimuli were similar (figs 5.2 and 5.3). There was no significant difference between the $\dot{V}_E$inst/SaO_2 slope for transient hypoxia and any rate of onset step change hypoxia for the group as a whole (table 5.3, fig 5.2). There was no systematic order effect upon the $\dot{V}_E$inst/SaO_2 responses measured.

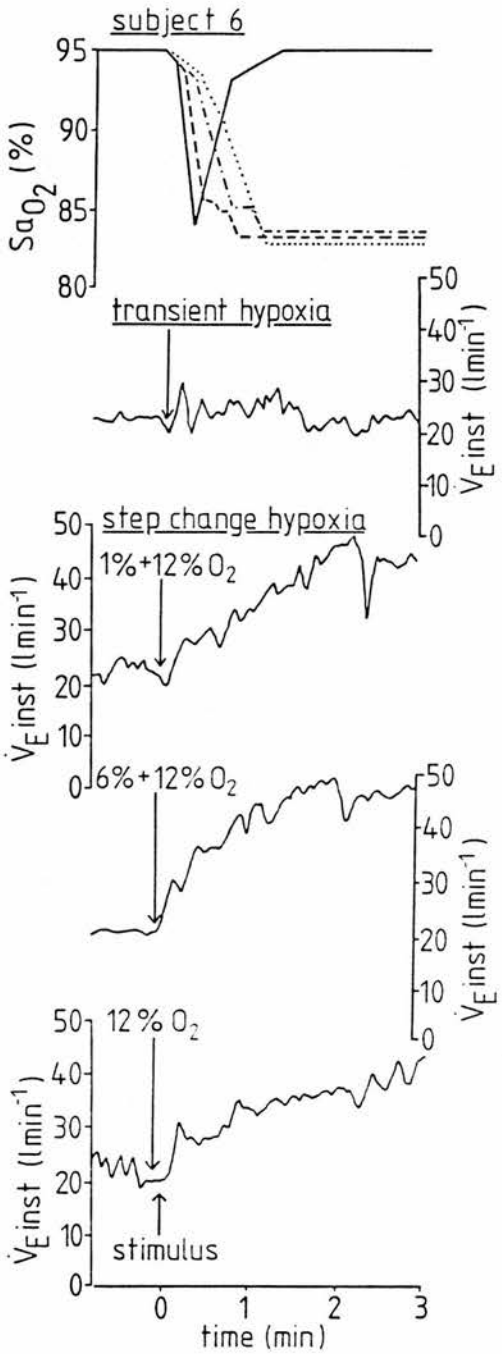
Mean half-times for the ventilatory response were 29 sec (range 14-44 sec) for 1%+12% O_2 step change hypoxia, 28 sec (range 21-44 sec) for 6%+12% O_2 step change hypoxia and 28 sec (range 17-42 sec) for 12% O_2 step change hypoxia (table 5.4). There were no significant differences between the half-times for the ventilatory response to any of the rates of onset of

hypoxia. Due to large breath-to-breath variability in the hypoxic ventilatory response, the mean $\dot{V}_E$inst values were calculated for each 30 second block throughout the three minute period following the onset of each type of hypoxia for each subject (fig 5.4). There was no significant difference between the corresponding mean 30 sec values for each type of hypoxia for each subject. However, calculating the 30 sec mean of $\dot{V}_E$inst values may smooth the transient ventilatory response, therefore the $\dot{V}_E$inst/SaO_2 slope in response to the first 7 breaths of each type of hypoxia was calculated for each subject. There was no significant difference between the initial 7 breath $\dot{V}_E$inst/SaO_2 slopes for each type of hypoxia.

The values for the mathematical model parameters at each rate of onset of step change hypoxia are summarised in table 5.5. In an individual, no consistent change in gain 1 or gain 2 due to a change in the rate of onset of hypoxia could be detected (table 5.5, fig 5.5). A wide variation in tau 2 was demonstrated (table 5.5). There was no systemmatic effect of the rate of onset of the hypoxic stimulus upon the non-linear terms of the model. Positive values for the inhibition parameter I were consistently required by four subjects (1, 3, 4 and 5). Negative values for I were consistently required by subject 6. It was not always necessary to incorporate an inhibition parameter to produce the model of best fit in subject 2 (table 5.5). The saturating effect parameter sigma was always required by subject 6 and never

required by subjects 2 and 3. For the remaining subjects, the sigma parameter was sometimes necessary to produce the model of best fit. Subjects 3 and 5 who showed a greater slope for transient than for any type of step change hypoxia also had a consistently greater value for gain 1 than for gain 2. Subjects 1 and 6 who showed a greater slope for step change than for transient hypoxia also had consistently greater values for gain 2 than for gain 1.

Fig 5.1 : Effect of the four types of hypoxic stimuli on Sa_{O_2} and $\dot{V}_E$inst in one subject.



Breath-by-breath values are plotted against time for Sa_{O_2} and $\dot{V}_E$inst. The upper trace shows the fall in Sa_{O_2} in response to transient hypoxia (——) and step change stimuli of 1% + 12%O_2 (---), 6% + 12%O_2 (—·—·), and 12%C_2 (....). The lower four traces show the ventilatory response to a single stimulus of each type.

Table 5.1 Onset time for hypoxic stimuli

Subject number	Time for SaO_2 to fall (s) Stimulus type: Transient	1%+12% O_2	6%+12% O_2	12% O_2
1	16.7	21.5	32.4	34.4
2	14.0	14.8	30.5	30.3
3	16.4	17.4	42.9	46.6
4	17.3	26.8	35.2	49.4
5	20.3	48.5	47.9	73.7
6	18.7	27.7	36.4	63.0
mean	17.2	26.1	37.6 *	49.6 *
s.d.	2.1	12.1	6.6	16.6

Mean duration for SaO_2 to fall to a minimum value for transient stimuli and to the mean value for step change hypoxic stimuli. * represents a significant difference ($p<0.05$) between the time for SaO_2 to fall for the different rates of onset of hypoxia.

Table 5.2 Mean SaO_2 values for transient hypoxic stimuli and the minimum values during each type of step change hypoxia for each subject

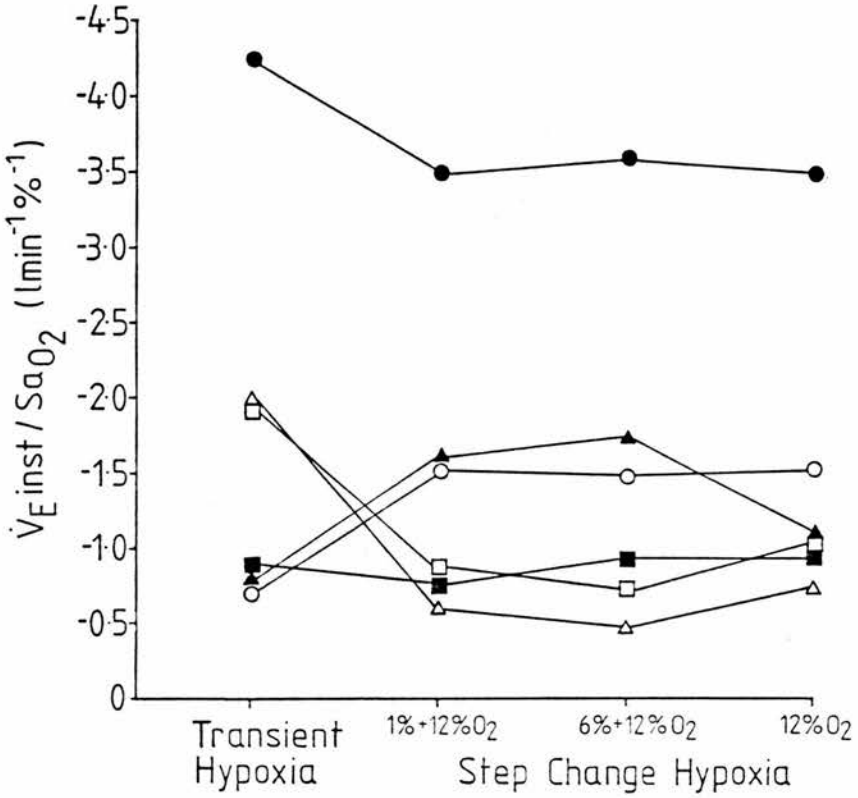
Subject number	Mean transient SaO_2	Minimum step change SaO_2 (%)		
		1%+12% O_2	6%+12% O_2	12% O_2
1	80	86	85	85
2	87	87	85	88
3	84	82	82	85
4	86	83	83	85
5	87	82	81	82
6	86	84	82	81

There was no statistical difference between the fall in SaO_2 due to each rate of onset of hypoxia for any subject.

Table 5.3 $\dot{V}_E$ inst/SaO_2 slopes for each section of the study

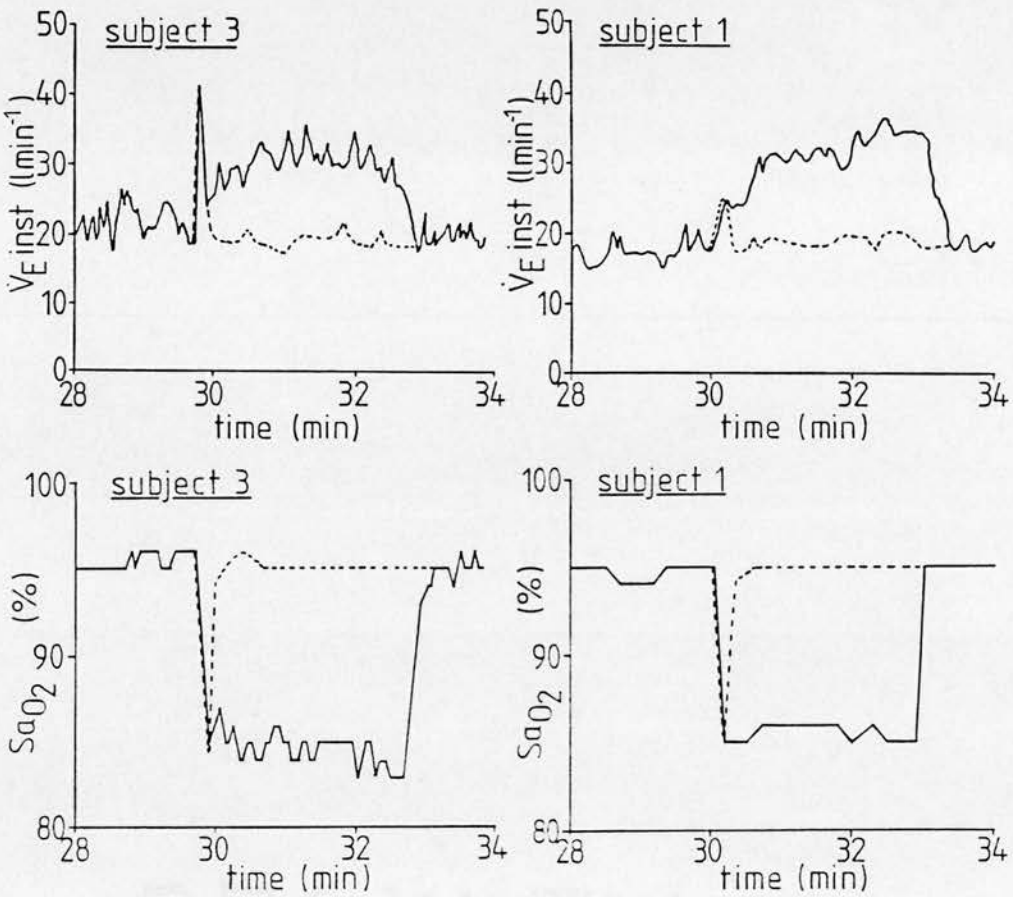
Subject Number	$\dot{V}_E$ inst/SaO_2 ($lmin^{-1}$ $\%^{-1}$) Transient Hypoxia	1% + 12% O_2	Step change hypoxia 6% + 12% O_2	12% O_2
1	-0.70	-1.52	-1.47	-1.52
2	-4.29	-3.49	-3.60	-3.48
3	-1.93	-0.87	-0.72	-1.00
4	-0.85	-0.76	-0.91	-0.98
5	-1.98	-0.60	-0.47	-0.72
6	-0.83	-1.60	-1.73	-1.03

Fig 5.2 : Slope of $\dot{V}_E inst/SaO_2$ for each subject at each rate of onset of hypoxia.



Each point represents the $\dot{V}_E inst/SaO_2$ slope derived in an individual at each rate of onset of hypoxia and the symbols represent different subjects (1○; 2●; 3□; 4■; 5△; 6▲). There was no statistical difference between the ventilatory responses at any rate of onset of hypoxia.

Fig 5.3 : Comparison of the ventilatory responses to transient and 1% + 12%O_2 step change hypoxic stimuli in two subjects.



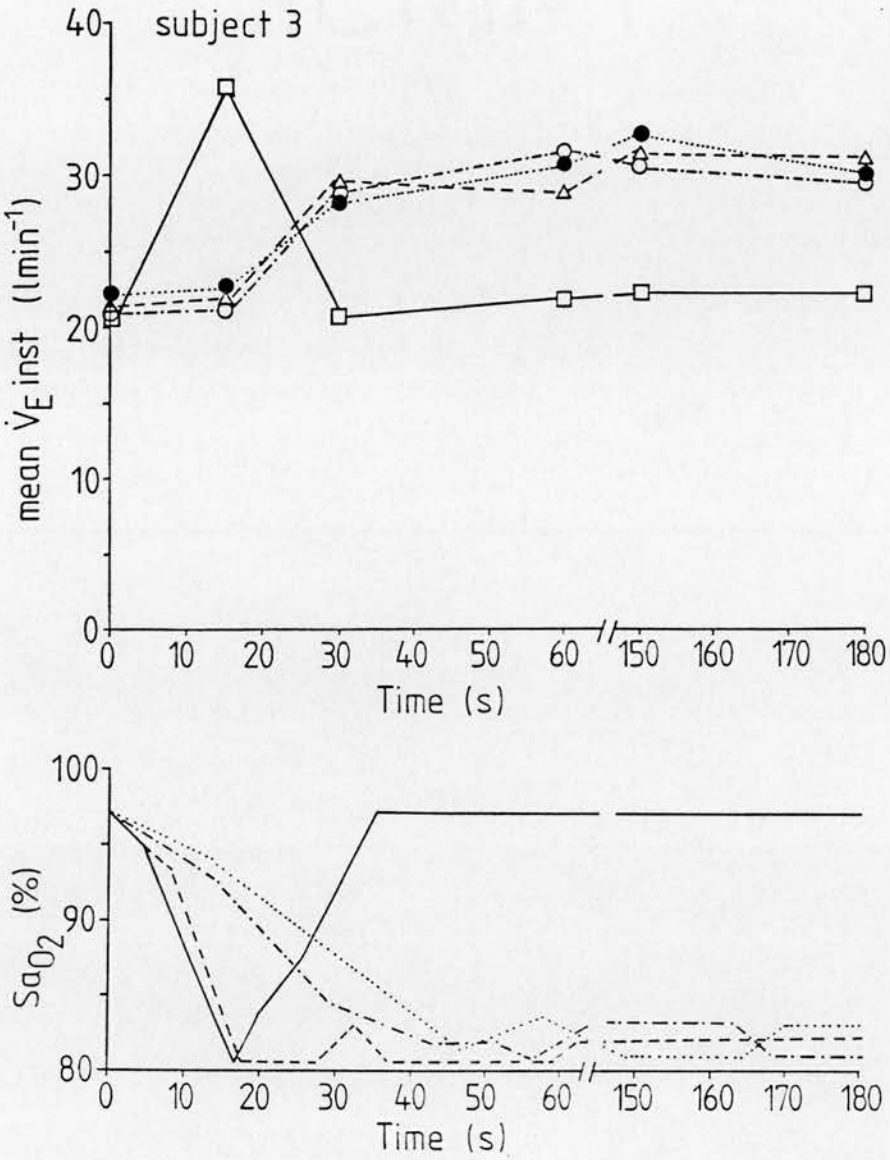
Breath-by-breath values for $\dot{V}_E$inst (upper traces) and Sa_{O_2} (lower traces) are plotted against time for the responses to transient hypoxia (---) and 1% + 12%O_2 step change hypoxia (——) for subjects 3 and 1. In both subjects the initial abrupt fall in Sa_{O_2} was similar, however the ventilatory responses differed. Subject 3 showed an initial large rise in $\dot{V}_E$inst in response to 1% + 12%O_2 which then fell to a lower steady state level. Subject 1 showed a small initial rise in $\dot{V}_E$inst in response to 1% + 12%O_2 which then increased to a steady state level.

Table 5.4 Mean half-times for the ventilatory response to each type of step change hypoxia

Subject number	Half-time (s) 1%+12% O_2	6%+12% O_2	12% O_2
1	22	21	24
2	19	22	24
3	44	23	17
4	34	34	34
5	14	25	29
6	42	44	42
mean	29	28	28
s.d.	13	11	9

The half-time of the ventilatory response for each type of step change hypoxia was calculated as the time in seconds from the onset of hypoxia for half the mean final minute $\dot{V}_E$inst to be reached.

Fig 5.4 : Mean $\dot{V}_E$inst and SaO_2 responses to the four types of hypoxic stimuli in one subject.



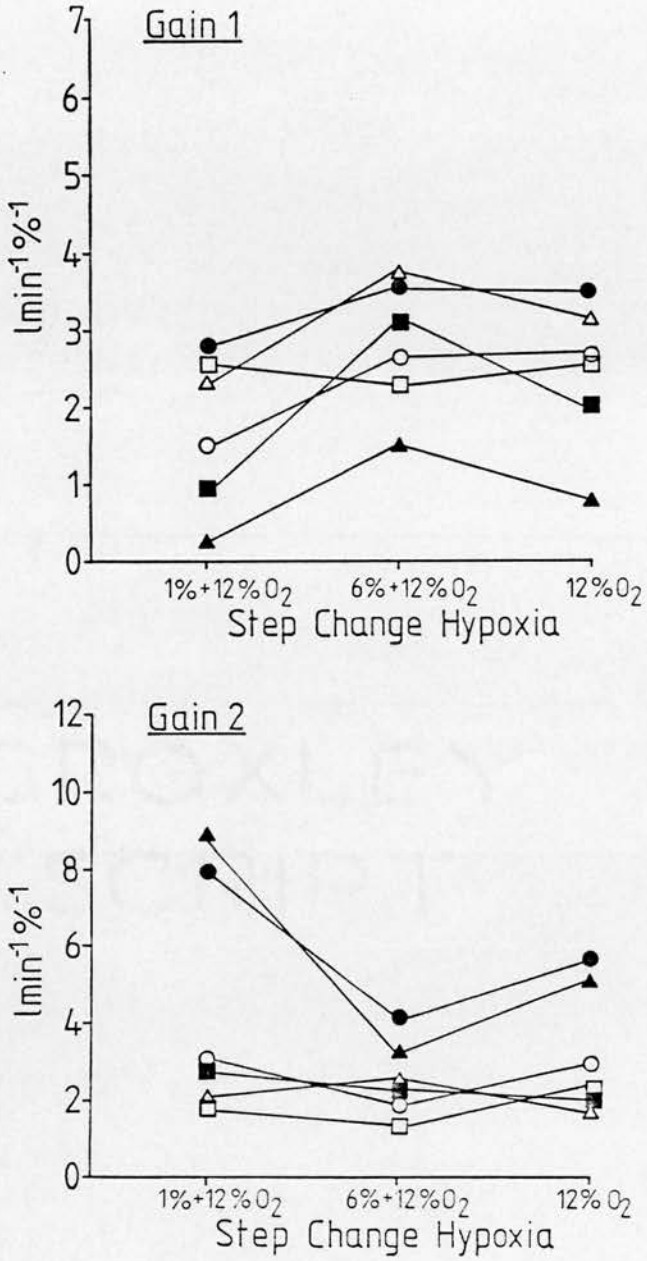
Mean values for $\dot{V}_E$inst and SaO_2 calculated over consecutive 30 sec blocks are plotted against time for the responses to transient (□—□), and step change hypoxic stimuli of 1% + 12%O_2 (△--△), 6% + 12%O_2 (O-·-·O), and 12%O_2 (●····●). The ventilatory response to the transient stimulus was greater than to any of the step change stimuli in spite of similar falls in SaO_2. However, there was no difference in the ventilatory response to the 3 types of step change stimuli despite different rates of onset.

Table 5.5 Model parameter values for each subject at each level of step change hypoxia

Subject Number	Step Change Hypoxia	G1	I	tau 2	G2	Sigma	Subject Number	Step Change Hypoxia	G1	I	tau 2	G2	Sigma
1	1% + 12% O_2	1.53	0.17	10	3.06	*	4	1% + 12% O_2	0.98	0.01	22	2.77	0.29
	6% + 12% O_2	2.65	0.04	157	2.00	0.06		6% + 12% O_2	3.10	0.10	4	2.07	*
	12% O_2	2.64	0.16	7	2.92	*		12% O_2	2.00	0.19	15	2.06	*
2	1% + 12% O_2	2.79	0.02	12	7.99	*	5	1% + 12% O_2	2.31	0.06	115	1.80	*
	6% + 12% O_2	3.62	*	14	4.17	*		6% + 12% O_2	3.57	0.09	21	2.26	0.16
	12% O_2	3.51	*	17	5.69	*		12% O_2	3.10	0.06	65	1.77	*
3	1% + 12% O_2	2.56	0.25	9	1.74	*	6	1% + 12% O_2	0.25	-0.26	55	8.84	0.24
	6% + 12% O_2	2.32	0.08	246	1.34	*		6% + 12% O_2	1.52	-0.02	40	3.18	0.07
	12% O_2	2.64	0.08	279	2.21	*		12% O_2	0.77	-0.37	95	5.11	0.75

Model gain parameters for each rate of onset of step change hypoxia for each subject. Units for parameters are gain 1 (G1) $lmin^{-1}\%^{-1}$; gain 2 (G2) $lmin^{-1}$ if sigma also included, or $lmin^{-1}\%^{-1}$ if sigma not required to produce the model of best fit; inhibition (I) $(lmin^{-1})^{-1}$; tau 2 sec; sigma%.

Fig 5.5 : Model gain values for each subject at each rate of onset of hypoxia.



Each point represents the gain 1 or gain 2 parameter derived in one subject at each rate of onset of step change hypoxia (key as for Fig 5.2). There was no statistical difference between either gain 1 or gain 2 at any rate of onset of step change hypoxia. Units for gain 2 are $lmin^{-1}$ if sigma also included or $lmin^{-1}\%^{-1}$ if sigma not required to produce the model of best fit.

IV DISCUSSION

The fall in SaO_2 in response to transient hypoxia was significantly faster than those due to 6%+12% O_2 or 12% O_2 step change hypoxic stimuli but similar in intensity. However, no significant differences could be detected in the ventilatory responses to these stimuli with different rates of onset when expressed as either the $\dot{V}_E inst/SaO_2$ slopes, the final half minute steady state ventilation values, the half-time for the ventilatory response, mean 30 second $\dot{V}_E inst$ values during hypoxia, first 7 breaths of hypoxia $\dot{V}_E inst/SaO_2$ slopes, or as the model parameter values. The response to transient hypoxia and the initial response to 1%+12% O_2 step change hypoxia were also similar for each subject. However, although this study does not show significant differences in the ventilatory responses to different rates of onset of hypoxia for the group as a whole, the results are qualitatively similar to those of Warren et al (1987). They found that 12% O_2 step change hypoxia produced a greater ventilatory response than transient hypoxia in 8 out of 10 subjects (personnal communication), but the reverse in other subjects. In this study, three subjects showed a greater ventilatory response to transient hypoxia than to step change hypoxia, two subjects showed the reverse and one subject showed similar responses to all types of hypoxia studied. A similar trend was seen in the model gain parameters although not for tau 2 or the non-linear

terms inhibition and sigma. This may suggest that the mechanisms of the response may vary between subjects although a statistically significant difference was not possible due to the small number of subjects studied.

The results of this study cannot be compared directly with the only previous study in humans by Bertholon et al (1989), as their rates of onset of hypoxia were generally slower ranging from 77 to 5 $mmHgmin^{-1}$, compared to rates of approximately 165 to 32 $mmHgmin^{-1}$ (calculated using the Severinghaus slide rule [Severinghaus 1966] from the fall in SaO_2 and assuming a pH of 7.38) used in this study. However, they found that the ratio of ($\dot{V}_E$ during hypoxia) / ($\dot{V}_E$ during rest) was greater for the first 3 minute period of steady state hypoxia than for the first 20 second period of step change hypoxia, implying a rate of change of stimulus effect. Their results suggest that the three minute step change hypoxic stimuli used in this study would cause a greater ventilatory response than the transient hypoxic stimuli and this occurred in 2 of the 6 subjects in this study.

The results of this study are similar to those reported in dogs by Dutton et al (1973) who were also unable to demonstrate a statistically significant difference between the ventilatory responses to ramp and pulse hypoxic stimuli. Dutton et al (1973) concluded that an element of rate sensitivity was present during step change hypoxia and at some site in the respiratory

control system central to the chemoreceptors, the initial change in chemoreceptor activity triggered a signal for a substantial ventilatory response, followed by little further increase in ventilation, despite a subsequent rise in sinus nerve input. The initial rapid increase in ventilation due to step change stimuli may suggest some form of unique input to the control system at the initiation of a change from control steady state. Possible sources for this input may be rapidly adapting vagal receptors in the bronchi (Knowlton and Larrabee 1946), or inspiratory neurons in the medulla facilitated by lung inflation (Cohen 1969). Evidence that a rapidly adapting augmentation of ventilation may be operating during the first breath of step change hypoxia is supported by the finding that vagotomy abolishes the initial overshoot in tidal volume (Casaburi 1972). However, reductions in input to the respiratory centre other than by vagotomy may have a similar effect on the hypoxic ventilatory response.

The structure of the mathematical model derived by Kirby et al (Warren et al 1989) which is used in this study is similar to that of Dutton et al (1973), consisting of two linear differential equations with non-linearities although their model also incorporates an additional linear feedforward pathway to account for the rapid change in ventilation with a time constant of 1 second. Kirby et al (personal communication) also attempted to incorporate a feed forward pathway into the

model structure but found that inclusion of two non-linear terms instead produced a more capable model. The model of Dutton et al (1973) also requires a non-linear switching device to model the respiratory control system adequately which may mimic the physiological central facilitation of ventilation. Although the mathematical model did not give any statistically significant differences between the parameters at the different rates of onset of hypoxia, a trend was shown with the two subjects having a larger $\dot{V}_E inst/SaO_2$ slope for transient hypoxia than for step change hypoxia also having consistently larger values for gain 1 than gain 2, and two subjects demonstrated the reverse. If the rate of onset of the hypoxic stimuli were to affect the ventilatory response, then the model parameters expected to be affected are tau 2 and the inhibition parameter, I. This effect could not be demonstrated in this study although several factors may account for this. As discussed in Chapter 4, the non-linear terms and tau 2 values tend to be very variable within a subject between days hence any systematic change in these variables due to a change in the rate of onset of the hypoxic stimulus may be difficult to detect. Also, the model structure was empirically derived to fit the data for transient and 15 and 12% O_2 step change hypoxic stimuli and it may therefore not be applicable to analyse the ventilatory responses to hypoxic stimuli with differing rates of onset. Several experimental factors may have

contributed to the failure to demonstrate changes in the ventilatory responses to hypoxic stimuli with different rates of onset. The duration of each study was at least two hours and thus problems of subject fatigue may have affected the results, particularly for the less fit subjects, although this effect was minimised by administering the tests in random order and allowing all subjects to rest for at least 15 minutes between each section of the study.

The large rise in ventilation in response to transient hypoxia or two breaths of 1% O_2 in N_2 demonstrated especially by subjects 2, 3 and 5 may be due to cortical influences, with the subjects reacting to the sensation of arterial oxygen desaturation, even though they were distracted by music. However, it is equally likely that cortical influences would also affect the response to step change stimuli. Also, the oximeter value for SaO_2 is not the actual chemoreceptor stimulus therefore the exact rate of change of the stimulus at the carotid body is unknown. However SaO_2 is closely related to arterial PO_2 (and therefore to carotid glomus PO_2), and provides the only non-invasive measure of arterial oxygenation.

As only six subjects were studied, type II statistical error may have contributed to the lack of any difference in the ventilatory responses to different rates of onset of hypoxia.

Although this study has not been able to demonstrate an effect of the rate of onset of an hypoxic stimulus

upon the ventilatory response, two patterns of response are apparent. The physiological mechanisms for the transient hypoxic response being less than step change hypoxia response may be due the time course of the transient stimulus being insufficient for the response to develop, or due to central facilitation of ventilation. The mechanism for the step change hypoxia response less than the transient response may be central depression of ventilation or a feed forward pathway as suggested by Dutton et al (1973). A difference in the ventilatory response due to different rates of onset of hypoxia may also be affected by the peripheral chemoreceptor response time. However Black et al (1971) have shown that the peripheral chemoreceptor response to hypoxia (recorded from single or few fibre strands of the carotid sinus nerve) was fast, reaching a maximum within 1-5 seconds. Also, DeCort et al (1987) showed in cats that the chemoreceptor discharge during transient hypoxia was not significantly different from that achieved at the same $Sa0_2$ during step change hypoxia, either before or after treatment with the specific chemoreceptor stimulant almitrine. This may suggest that chemoreceptor discharge is independent of the rate of onset of the hypoxic stimulus.

Evidence of central facilitation or inhibition of the hypoxic ventilatory reponse comes from studies in anaesthetised cats. Tenney and Ou (1977) showed that in decerebrate cats, the ventilatory responses to hypoxia

and hypercapnia were indistinguishable from control studies. However, in decorticate cats, the occurrence of tachypnea and slight hyperventilation on room air suggest the existence of a tonic facilitatory influence from the diencephalon (Redgate and Gellhorn 1958) on the brainstem. As removal of the cortex exposes this effect, there must normally be a tonic descending cortical inhibitory influence which holds the system in balance. The presence of a mechanism of peripheral chemoreceptor excitation and a direct depression of the brain stem respiratory complex by hypoxia was demonstrated by St. John and Wang (1977) in decerebrate cats. They concluded that central chemoreceptor afferent influences are widely distributed throughout the medullary respiratory complex, whereas peripheral chemoreceptor afferents produce only a discrete and unequal excitation of respiratory units. Dutton et al (1973) also found that a non-linear switching device was required to model the respiratory control system and suggested that this may mimic the central facilitation of ventilation.This study has not been able to demonstrate any effect of the rate of onset of an hypoxic stimulus upon the ventilatory response, although previous evidence has suggested that such an effect be important in determining the ventilatory response. Further work is required to investigate the central controller of ventilation, looking at the central depression and facilitation of ventilation and the recovery phase after hypoxia.

CHAPTER 6: THE EFFECT OF EXERCISE LEVEL UPON THE HYPOXIC VENTILATORY RESPONSE

I INTRODUCTION

It has long been recognised that the ventilatory response to hypoxia is increased during exercise in humans. Several groups (Briggs et al 1920, Hickham et al 1951, Asmussen and Nielson 1957, Flenley et al 1979) have shown that the depression of ventilation during inhalation of 100% O_2 was greater during exercise than at rest, and concluded that exercise potentiated the peripheral chemoreceptor mediated hypoxic ventilatory drive. A similar conclusion was drawn by Bhattacharyya et al (1970) and Masson and Lahiri (1974) who demonstrated a left upward shift of the $\dot{V}_E/PACO_2$ relationship at a given level of hypoxia during mild exercise as compared to rest in normal male subjects. A rise in the threshold for the ventilatory response to hypoxia has also been demonstrated by Hornbein and Roos (1962) who found that whilst at rest ventilation did not increase until P_AO_2 fell below 60mmHg (8kPa) but during exercise a reduction in P_AO_2 to only 94mmHg (12.5 kPa) was sufficient to cause a significant increase in ventilation.

The potentiation of the ventilatory response to hypoxia increases with the level of exercise. Two groups (Weil et al 1972, Martin et al 1978), using the progressive isocapnic technique, demonstrated an increase in the shape parameter A with increasing $\dot{V}O_2$

measured at four levels from 0.25 $lmin^{-1}$ (rest) to 1.20 $lmin^{-1}$ in 8 normal men (Weil et al 1972), or when measured at rest, and at 1/3 and 2/3 $\dot{V}O_2max$ in 16 male athletes (Martin et al 1978). However there may not be a simple linear relationship between $\dot{V}O_2$ and the hypoxic ventilatory response. Flenley et al (1979) studied the effect of transient relief of hypoxia (14% O_2) by 30% O_2 at rest and at two levels of exercise in four normal males. Their data shows a greater increase in hypoxic ventilatory sensitivity expressed as the $V_E inst/SaO_2$ relationship, for a rise in $\dot{V}O_2$ from 1 to 2 $lmin^{-1}$ than for an increase in $\dot{V}O_2$ from rest to 1 $lmin^{-1}$. Furthermore, the data of Weil et al (1972), replotted as the slope of the $\dot{V}_E inst/SaO_2$ relationship (Flenley and Warren 1983), shows a curvilinear rise in the hypoxic ventilatory response with increasing $\dot{V}O_2$ suggesting that the degree of potentiation is greater at higher levels of $\dot{V}O_2$.

The mechanism of the potentiation of the hypoxic ventilatory response during exercise remains unclear. Mechanisms which have been proposed include increased sensitivity of the peripheral chemoreceptors, circulating chemical mediators, and muscle afferent activity interacting centrally with chemoreceptor input.

Asmussen and Nielson (1958) first suggested that exercise potentiated the hypoxic ventilatory response in man due to an enhancement of carotid chemoreceptor sensitivity. Other groups have drawn the same conclusion

since they showed that the ventilatory response to transient hypoxia (Leitch et al 1976) or transient relief of hypoxia (Flenley et al 1979), both of which were considered to be measures of peripheral chemoreceptor activity (Dejours et al 1957a), increased with exercise. This hypothesis was supported by the work of Lugliani et al (1971) who found that the ventilatory response to hypoxia during exercise was abolished in carotid body resected subjects, although their steady state ventilatory response to exercise was intact. Thus carotid chemoreceptor input is required for the ventilatory response to hypoxia. However, these receptors are not necessarily the site at which potentiation of the response occurs. The evidence from direct carotid sinus nerve recordings in animals is conflicting. Biscoe and Purves (1967) found that passive exercise of the hindlimbs caused a rapid increase in carotid chemoreceptor activity during normoxia in anaesthetised cats. However, Davies and Lahiri (1973) were unable to demonstrate a rise in chemoreceptor activity in response to passive hindlimb exercise in either anaesthetised or decerebrate cats despite an increase in ventilation. They therefore concluded that exercise and hypoxia interact as ventilatory stimulants at a central location rather than at the carotid chemoreceptors. Hypermetabolism in cats induced by intravenous administration of 2,4-dinitrophenol (DNP) has been shown to have no effect upon peripheral

chemoreceptor activity, however it did enhance the central transduction of the carotid afferent nerve resulting in an increased hypoxic ventilatory response at any given level of hypoxia (Adams et al 1983). This may be due to stimulation of muscle receptors sensitive to metabolic rate. Changes in the concentration of a circulating chemical mediator may be responsible for the potentiation of the hypoxic ventilatory response on exercise. One such possible mediator is the catecholamine noradrenaline. Catecholamines exist within the glomus cells of the carotid bodies and are putative neurotransmitters for the hypoxic stimulus (Alfes et al 1977). Noradrenaline is known to potentiate the hypoxic ventilatory response at rest in normal subjects (Cunningham et al 1963) and circulating levels increase during exercise (Christensen et al 1979). Furthermore plasma noradrenaline levels and ventilation have both been shown to be higher during hypoxic than during normoxic exercise (Clancy et al 1975), and are both reduced during inhalation of 100% O_2 (Hesse et al 1981).

Central interaction may result from an increase in muscle afferent feedback from either muscle stretch receptors or from nociceptors stimulated by an increase in metabolites in the hypoxic exercising muscle. A local build up of anaerobic metabolites in exercising or hypoxic muscle, or an increase in muscle stretch receptor activity may cause an increase in muscle

afferent activity leading to an increase in hypoxic sensitivity. Sergeant et al (1981) showed that the increase in ventilation which accompanied leg exercise was maintained if the muscle venous outflow circulation was occluded using cuffs around the thighs both during and after exercise. This supports the hypothesis that the increase in muscle afferent activity as a result of metabolite build up, rather than release of chemical stimulants from the muscle into the circulation may be responsible for the potentiation of the ventilatory response to hypoxia during exercise, especially since an increase in blood lactate was observed following release of the cuffs.

Since carotid chemoreceptor activity does not appear to increase during hypoxic exercise in anaesthetised cats (Davies and Lahiri 1973), the muscle afferents are likely to interact centrally rather than by causing a reflex increase in peripheral chemosensitivity perhaps via sympathetic efferents. Conflicting evidence regarding the role of muscle afferent activity acting centrally to produce the ventilatory response comes from the work of Duncan et al (1981) and Adams et al (1984a). Both groups demonstrated a normal ventilatory response to isometric arm exercise or electrical muscle stimulation in patients with either sensory neuropathies affecting forearm afferent nerves, or with traumatic spinal cord transection respectively. Adams et al (1984a) concluded that in spite of spinal cord

transection with a presumed loss of muscle afferents there was still a rapid ventilatory response to electrically induced exercise which cannot be explained by classical chemoreception, therefore suggesting an, as yet, undetermined chemical stimulus.

Separation of peripheral and central mechanisms in conscious humans has been difficult with previously existing methodology and hence it has been difficult to elucidate the site of potentiation. The aim of this study, therefore, was to a) investigate further the increase in the hypoxic ventilatory response at several levels of exercise, and b) use the mathematical model to analyse the hypoxic ventilatory response to investigate the peripheral and central mechanisms of potentiation.

II METHODS

i) Subjects

The subjects were nine healthy volunteers (4 male, 5 female), aged 23-40 years (Appendix table 15), drawn from laboratory staff. The subjects were non-smokers apart from subject 3 who occasionally smoked cigarettes. All subjects had normal lung function (Appendix table 15) and none were taking any medication at the time of the study apart from subject 7 who was taking Terfenadine for mild hayfever.

ii) Protocol

Hypoxic ventilatory drive was measured using step change hypoxia at rest and at three levels of exercise, apart from subject 8 who was studied at rest and at two levels of exercise, and subject 7 who was studied at rest and at four levels of exercise. The levels of exercise at which hypoxic ventilatory drive was measured ranged between rest and the fastest speed at which each subject could walk comfortably for at least half an hour (the approximate duration of each section of the study). Hypoxic ventilatory drive was always measured initially at rest with the subject seated comfortably in an armchair and then at the various levels of exercise in random order. The subjects rested for 15 minutes between each level of exercise.

At rest and at each exercise level the subjects initially breathed room air in order to establish conditions of steady state gas exchange. Mixed expired

gas was collected between 7-9 and 9.15-11.15 minutes after the start of either the rest period or each exercise level, and from 5-7 minutes after the last step change hypoxic stimulus for calculation of $\dot{V}_E$, $\dot{V}O_2$, $\dot{V}CO_2$ and RQ.

For the rest measurements an abrupt onset, sustained fall in SaO_2 to about 90% was achieved by changing inspired gas to 1% O_2 in N_2 for two breaths and then to 12% O_2 for the remainder of a three minute period. The subjects then breathed room air for five minutes after which an abrupt onset, sustained fall in SaO_2 to about 80% was achieved by changing inspired gas to 1% O_2 in N_2 for two breaths and then to 10% O_2 for the remainder of three minutes. On exercise, similar falls in SaO_2 were achieved by changing inspired gas to 1% O_2 in N_2 for one breath followed by 15% O_2 for the remainder of three minutes to achieve a stable SaO_2 of about 90% and , five minutes later to 1% O_2 in N_2 for two breaths followed by 12% O_2 for the remainder of three minutes to achieve a SaO_2 of about 80%. All changes in inspired gas were made during an expiration. Isocapnia was maintained by the addition of CO_2 close to the Hans-Rudolph valve in the inspiratory limb of the circuit to keep $P_{ET}CO_2$ constant.

iii) Analysis

Baseline normoxic $P_{ET}CO_2$ at rest and at each exercise level was calculated as the mean of the 20 breaths before each step change hypoxic stimulus and 20 breaths five minutes after the end of the second hypoxic period.

The mean $P_{ET}CO_2$ during step change hypoxia was calculated from 10 breaths before and all breaths during both three minute periods of hypoxia at rest and at each exercise level.

The method for calculation of the slope of the $\dot{V}_E inst/SaO_2$ relationship is described in detail in Chapter 3. The data from the two levels of step change hypoxia at rest and at each exercise level were pooled for calculation of the hypoxic ventilatory drive expressed as the negative $\dot{V}_E inst/SaO_2$ slope.

The ventilatory response to the two periods of step change hypoxia at rest and each level of exercise were also analysed using the mathematical model and the parameters derived which best described the ventilatory response at each exercise level. A double weighting was applied to the start of each period of step change hypoxia in order to compensate for the lack of transient stimuli.

Differences between variables at rest and at the various levels of exercise were tested using the Kruskal-Wallis one-way analysis of variance. Correlations between variables were calculated using least squares linear regression. The increase in the $\dot{V}_E inst/SaO_2$ relationship from rest to light exercise was compared with that from light to moderate exercise for the group using a Wilcoxon signed rank test.

III RESULTS

In all subjects $\dot{V}_E$, $\dot{V}O_2$ and $\dot{V}CO_2$ increased with increasing exercise level (table 6.1). The mean values for $\dot{V}O_2$ at rest and during exercise were: rest 0.18±0.04 $lmin^{-1}$ (range 0.09-0.24 $lmin^{-1}$); exercise level 1, 0.51±0.09 $lmin^{-1}$ (range 0.40-0.67 $lmin^{-1}$); level 2, 0.67±0.10$lmin^{-1}$ (range 0.53-0.87 $lmin^{-1}$); level 3, 0.91±0.15 $lmin^{-1}$ (range 0.71-1.04 $lmin^{-1}$). The respiratory quotient (RQ) was equal to or less than 1.0 at all exercise levels except for subject 9, in whom it was above 1.0 at the two higher exercise levels (1.15 at level 2 and 1.04 at level 3).

Baseline $P_{ET}CO_2$ was not significantly different for rest and the three levels of exercise (Appendix table 16), although in one subject (subject 5) the mean value during the highest exercise level was approximately 0.6kPa less than that at rest. There was no significant difference between mean $P_{ET}CO_2$ values during step change hypoxia and the corresponding exercise level baseline values for any subject, and the standard deviation of $P_{ET}CO_2$ about the mean during hypoxia ranged from 0.07 to 0.21 kPa in the nine subjects.

Inspiration of 12% O_2 at the different levels of exercise or 10% O_2 at rest produced similar maximal falls in SaO_2 (Appendix table 17), with the mean and range for rest being 83% (81-87%), exercise level 1, 85% (78-89%); level 2, 84% (81-87%); and level 3, 81% (77-85%).

Table 6.1: Baseline normoxic values for ventilation and gas exchange

Subject	Rest $\dot{V}_E$ $l min^{-1}$	Rest $\dot{V}O_2$ $l min^{-1}$	Rest $\dot{V}CO_2$ $l min^{-1}$	1 $\dot{V}_E$ $l min^{-1}$	1 $\dot{V}O_2$ $l min^{-1}$	1 $\dot{V}CO_2$ $l min^{-1}$	2 $\dot{V}_E$ $l min^{-1}$	2 $\dot{V}O_2$ $l min^{-1}$	2 $\dot{V}CO_2$ $l min^{-1}$	3 $\dot{V}_E$ $l min^{-1}$	3 $\dot{V}O_2$ $l min^{-1}$	3 $\dot{V}CO_2$ $l min^{-1}$	4 $\dot{V}_E$ $l min^{-1}$	4 $\dot{V}O_2$ $l min^{-1}$	4 $\dot{V}CO_2$ $l min^{-1}$
				EXERCISE LEVEL											
1	4.9	0.15	0.12	12.3	0.41	0.34	17.3	0.66	0.51	19.1	0.71	0.63	-	-	-
2	7.2	0.20	0.18	18.3	0.67	0.54	23.1	0.87	0.73	29.1	1.03	0.95	-	-	-
3	7.0	0.20	0.17	14.7	0.58	0.47	21.2	0.85	0.70	22.9	0.96	0.84	-	-	-
4	3.7	0.09	0.09	13.2	0.47	0.42	14.2	0.53	0.46	19.1	0.77	0.70	-	-	-
5	6.2	0.17	0.13	16.3	0.50	0.48	16.3	0.58	0.53	24.4	0.91	0.82	-	-	-
6	5.1	0.18	0.16	12.4	0.45	0.36	16.1	0.63	0.53	19.4	0.74	0.70	-	-	-
7	6.0	0.24	0.19	15.3	0.55	0.52	17.0	0.71	0.65	24.5	1.04	0.90	28.3	1.14	1.08
8	6.2	0.17	0.14	11.8	0.40	0.39	20.0	0.75	0.75	-	-	-	-	-	-
9	6.4	0.20	0.19	16.2	0.59	0.54	18.7	0.68	0.78	25.8	0.92	0.96	-	-	-

At rest, the $\dot{V}_E$inst/SaO_2 relationship ranged +0.02 to -1.85 $lmin^{-1}$ (table 6.2) in the 9 subjects. The $\dot{V}_E$inst/SaO_2 slope tended to increase (ie. to become more negative) with increasing exercise level (fig 6.1, table 6.2), and was significantly different from rest at levels 2 ($p<0.02$) and 3 ($p<0.01$) for the group as a whole. Also for the group there was a significant correlation between $\dot{V}_E$inst/SaO_2 and $\dot{V}O_2$ ($p<0.02$, fig 6.2), $\dot{V}O_2$/BSA ($p<0.05$, fig 6.2), $\dot{V}CO_2$ ($p<0.01$) and baseline normoxic $\dot{V}_E$inst ($p<0.01$). When the increase in $\dot{V}_E$inst measured as a result of step change hypoxia at a SaO_2 of 85% at each exercise level was expressed as a proportion of the baseline $\dot{V}_E$inst, the increase at rest ranged -2 to 219%, and during exercise the proportional increase ranged 14 to 168%. There was no correlation between the order in which the workloads were studied and the corresponding hypoxic ventilatory response measured. There was wide variation in $\dot{V}O_2$ between subjects at each exercise level. In order to investigate differences in the degree of potentiation of the ventilatory response to hypoxia between subjects, comparisons were made on the basis of $\dot{V}O_2$ at rest, and during light ($\dot{V}O_2$ 0.4-0.67 $lmin^{-1}$) and moderate ($\dot{V}O_2$ 0.7-0.92 $lmin^{-1}$) exercise. The increase in $\dot{V}_E$inst/SaO_2 from rest to light exercise ranged -0.15 to 0.70 $lmin^{-1}\%^{-1}$ (mean -0.21 $lmin^{-1}\%^{-1}$), and from light to moderate exercise ranged 0 to 1.93 $lmin^{-1}\%^{-1}$ (mean -0.46 $lmin^{-1}\%^{-1}$). However, there was no

significant difference between the percentage increase from rest to light exercise (range 0 to 119%) and light to moderate exercise (range 14 to 168%), for the group (Fig 6.3).

At rest, gain 1 ranged from 0.03 to 2.83 $lmin^{-1}\%^{-1}$, gain 2 from -0.56 to 5.33 $lmin^{-1}\%^{-1}$ and tau 2 from 36 to 313 sec (Fig 6.4, table 6.3). A positive value for the inhibition parameter was needed in 4 subjects, a negative value in one subject, and the parameter sigma was needed in 7 subjects (table 6.3). For the group as a whole, both gain 1 and gain 2 tended to increase with exercise (fig 6.4, table 6.3) and this increase was significantly different from rest for both gains at exercise levels 2 and 3 (gain 1, $p<0.02$; gain 2, $p<0.01$). A positive value for the inhibition parameter was consistently needed at rest and all exercise levels in four subjects, but was not always required in the remaining five subjects (table 6.3). A negative inhibition parameter was not needed in any subject during exercise . There appeared to be a trend with the inhibition parameter decreasing as the exercise level increased but this was not significant. Two subjects consistently did not require the parameter sigma at rest or on exercise, but in the remaining seven, sigma was required to obtain the best fit on some occasions (table 6.3). A wide variation in tau 2 was demonstrated both within and between subjects with resting values ranging 36 to 313 sec and exercise values ranging 1 to 329 sec

(table 6.3). However, tau 2 did not consistently change within an individual as the level of exercise increased. For the group there was a significant correlation between gain 1 and $\dot{V}O_2$ ($p<0.02$), $\dot{V}O_2$/BSA ($p<0.05$), $\dot{V}CO_2$ ($p<0.02$), $\dot{V}_E$inst ($p<0.01$) and $\dot{V}_E$inst/SaO_2 ($p<0.001$). Gain 2 correlated with $\dot{V}O_2$ ($p<0.01$), $\dot{V}O_2$/kg ($p<0.05$), $\dot{V}O_2$/BSA ($p<0.01$), $\dot{V}CO_2$ ($p<0.01$), $\dot{V}_E$inst ($p<0.01$) and $\dot{V}_E$inst/SaO_2 ($p<0.001$). Expression of $\dot{V}O_2$ for each subject per kg or m^2 body surface area did not improve the significance of the relationship with $\dot{V}_E$inst/SaO_2 (Fig 6.2), gain 1 or gain 2.

To compare differences between individuals in the effect of exercise on the gain parameters, the exercise levels were divided into light and moderate on the basis of $\dot{V}O_2$ as described above. The mean rise in gain 1 from rest to light exercise was 1.04 $lmin^{-1}\%^{-1}$ (range -0.45 to 2.57 $lmin^{-1}\%^{-1}$) and from light to moderate exercise 0.38 $lmin^{-1}\%^{-1}$ (range -2.26 1 to 1.78 $lmin^{-1}\%^{-1}$, Fig 6.5). The mean increase in gain 2 from rest to light exercise was 0.74 $lmin^{-1}\%^{-1}$ (range -2.59 to 1.98 $lmin^{-1}\%^{-1}$) and from light to moderate exercise was 0.15 $lmin^{-1}\%^{-1}$ (range -2.57 to 1.61 $lmin^{-1}\%^{-1}$, Fig 6.6).

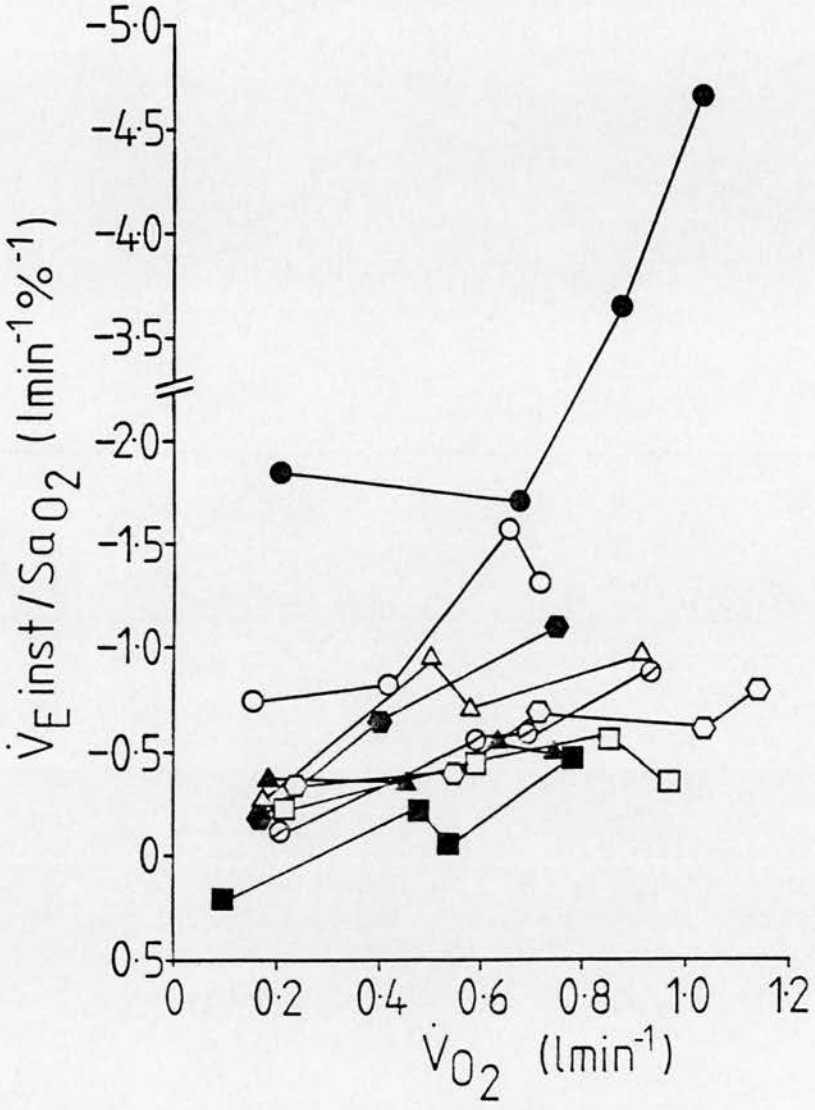
Table 6.2: $\dot{V}_E$ inst/SaO_2 slopes at rest and during steady state exercise.

$\dot{V}_E$ inst/$Sa0_2$ ($lmin^{-1}\%^{-1}$)

Subject	Rest	level 1	level 2	level 3	level 4
1	-0.75	-0.77	-1.57	-1.32	-
2	-1.85	-1.70	-3.63	-4.66	-
3	-0.23	-0.45	-0.56	-0.35	-
4	+0.02	-0.22	-0.06	-0.50	-
5	-0.26	-0.96	-0.73	-0.96	-
6	-0.32	-0.37	-0.53	-0.49	-
7	-0.33	-0.46	-0.70	-0.62	-0.79
8	-0.25	-0.65	-1.12	-	-
9	-0.19	-0.49	-0.57	-0.94	-

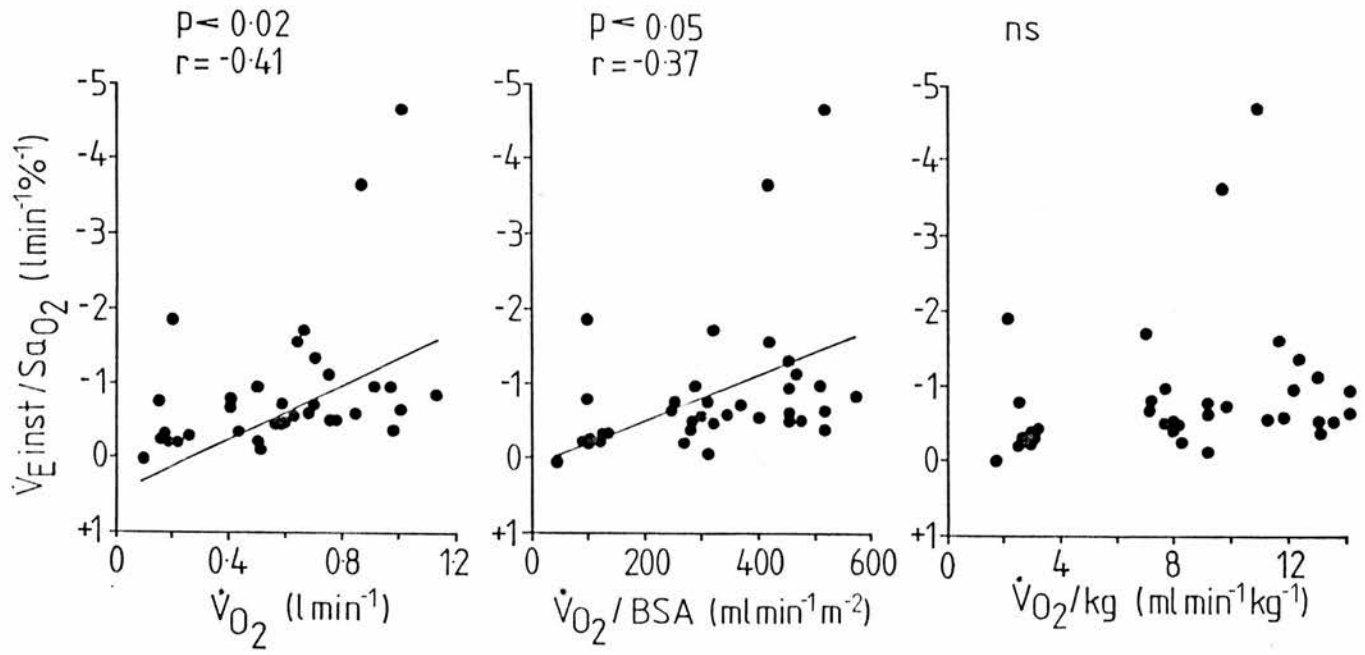
Hypoxic ventilatory drive expressed as the slopes of the $\dot{V}_E$inst/$Sa0_2$ relationship at rest and at various levels of steady state exercise arranged in order of increasing $\dot{V}0_2$.

Fig 6.1 : The effect of increasing exercise level on the $\dot{V}_E inst/SaO_2$ slope.



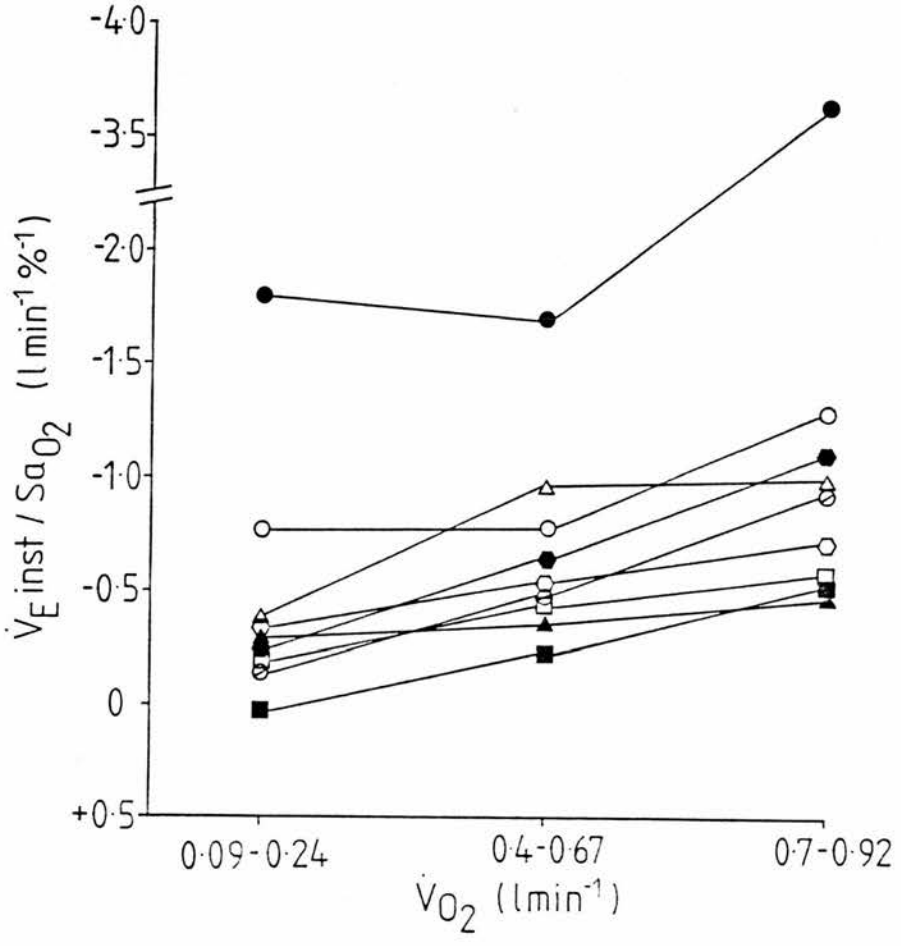
The $\dot{V}_E inst/SaO_2$ relationship is plotted against $\dot{V}O_2$ for all levels of exercise in the 9 subjects. Each point represents the value obtained in a subject at a given level of exercise and the symbols denote individual subjects (1 ○; 2 ●; 3 □; 4 ■; 5 △; 6 ▲; 7 ⬡; 8 ⬢; 9 ⊘). The hypoxic ventilatory response expressed as the $\dot{V}_E inst/SaO_2$ relationship increased with increasing exercise level.

Fig 6.2 : Relationship between the slope of $\dot{V}_E inst/SaO_2$ and $\dot{V}O_2$, $\dot{V}O_2$/BSA and $\dot{V}O_2$/kg.



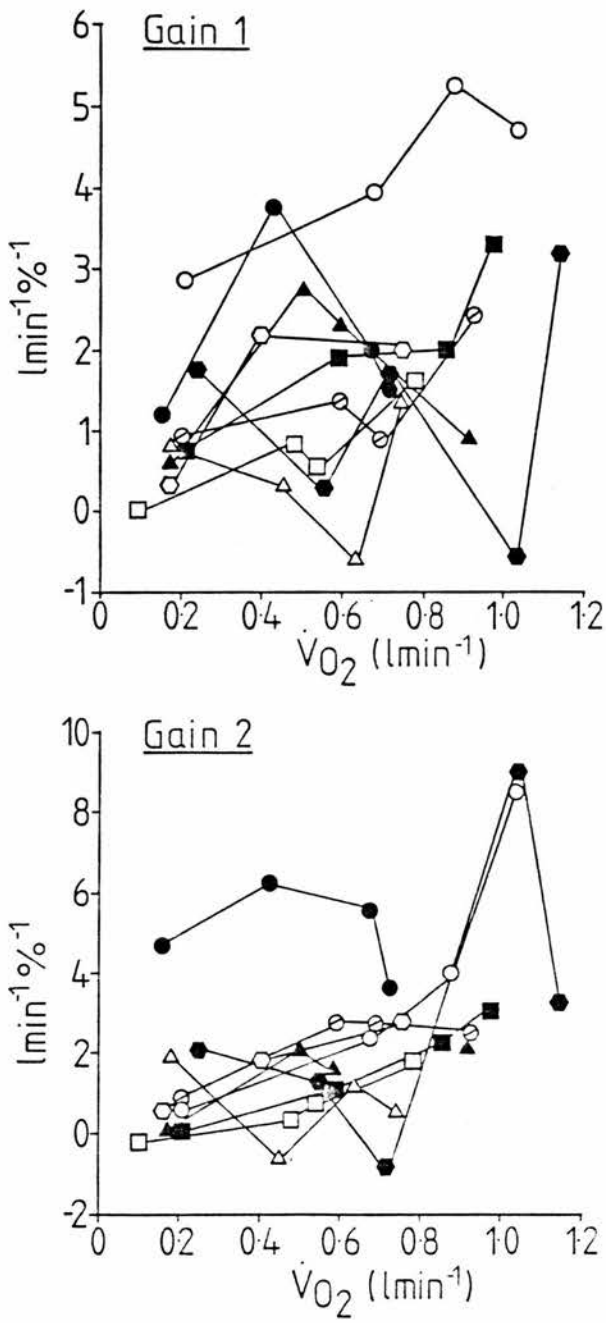
Each point represents the $\dot{V}_E inst/SaO_2$ slope in an individual either at rest or during exercise. Expression of $\dot{V}O_2$ either per BSA or kg did not improve the significance of the relationship with the $\dot{V}_E inst/SaO_2$ slope in this study of nine subjects.

Fig 6.3 : The $\dot{V}_E inst/Sa_{O_2}$ relationship at rest and during light and moderate levels of exercise.



The hypoxic ventilatory response expressed as the $\dot{V}_E inst/Sa_{O_2}$ relationship is plotted for each subject for rest, light ($\dot{V}O_2$ 0.4-0.67 l min^{-1}) and moderate exercise ($\dot{V}O_2$ 0.7-0.92 l min^{-1}). Each point represents the value obtained at that level in an individual. The symbols denoting individual subjects are the same as in Fig 6.1.

Fig 6.4 : The effect of increasing exercise level upon the model gain parameters.



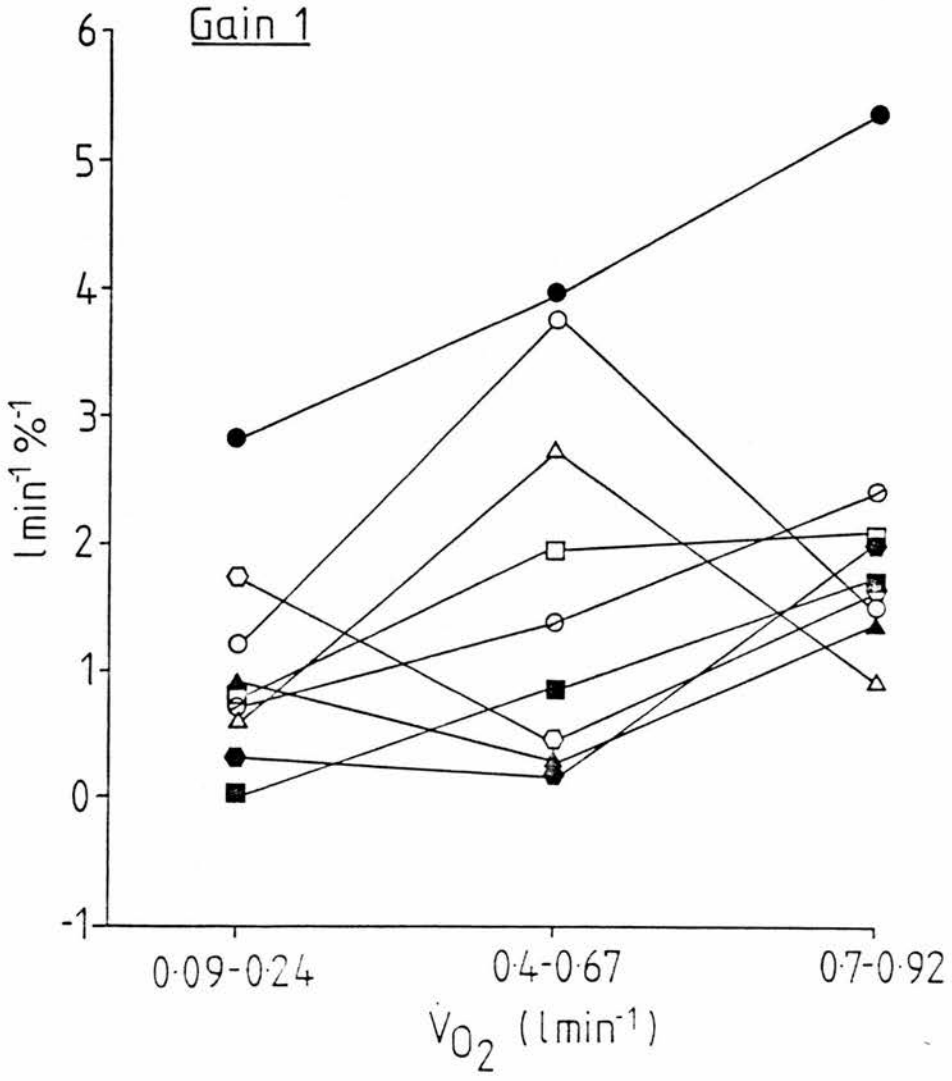
Each point represents the model gain 1 or gain 2 parameter for each subject (as illustrated in Fig 6.1) during rest and at the different levels of exercise. Both model gain parameters increased with increasing exercise level. Units for gain 2 are $lmin^{-1}$ if sigma also required, or $lmin^{-1}\%^{-1}$ if sigma not required to produce the model of best fit.

Table 6.3: Model parameter values for each subject at rest and increasing levels of exercise.

Subject	Exercise level	G1 $lmin^{-1}\%^{-1}$	I $(lmin^{-1})^{-1}$	tau2 sec	G2 $lmin^{-1}\%^{-1}$	sigma %	Subject	Exercise level	G1 $lmin^{-1}\%^{-1}$	I $(lmin^{-1})^{-1}$	tau2 sec	G2 $lmin^{-1}\%^{-1}$	sigma %
1	Rest	1.20	0.07	114	4.71	0.44	6	Rest	0.76	0.20	47	1.95	0.76
	1	3.77	0.07	7	6.20	0.20		1	0.31	0.71	246	-0.64	*
	2	2.04	*	9	5.57	0.39		2	-0.55	0.58	38	1.15	*
	3	1.51	0.03	49	3.63	*		3	1.38	0.06	1	0.56	*
2	Rest	2.83	-0.13	313	0.64	*	7	Rest	1.73	0.37	39	2.03	0.14
	1	3.97	0.04	2	2.39	*		1	0.28	0.48	91	1.35	*
	2	5.33	*	24	4.00	*		2	1.58	*	284	-0.74	*
	3	4.72	*	8	8.53	*		3	-0.56	*	1	9.00	0.25
								4	3.21	0.10	2	3.28	0.15
3	Rest	0.76	0.42	250	0.10	*	8	Rest	0.33	0.41	124	0.62	0.77
	1	1.95	0.18	329	1.19	*		1	0.22	0.19	158	1.84	0.16
	2	2.01	0.11	146	2.36	0.14		2	2.00	0.05	1	2.89	0.27
	3	3.30	0.09	1	3.05	0.14							
4	Rest	0.03	0.78	249	-0.19	*	9	Rest	0.73	0.38	177	0.92	*
	1	0.83	0.32	221	0.35	*		1	1.38	0.09	9	2.74	0.36
	2	0.52	0.53	195	0.77	0.23		2	0.89	*	80	2.67	0.28
	3	1.64	0.34	128	1.88	0.35		3	2.42	0.09	12	2.08	*
5	Rest	0.60	0.68	36	0.03	*							
	1	2.71	0.15	8	2.01	*							
	2	2.30	0.19	16	1.51	*							
	3	0.93	*	184	2.12	*							

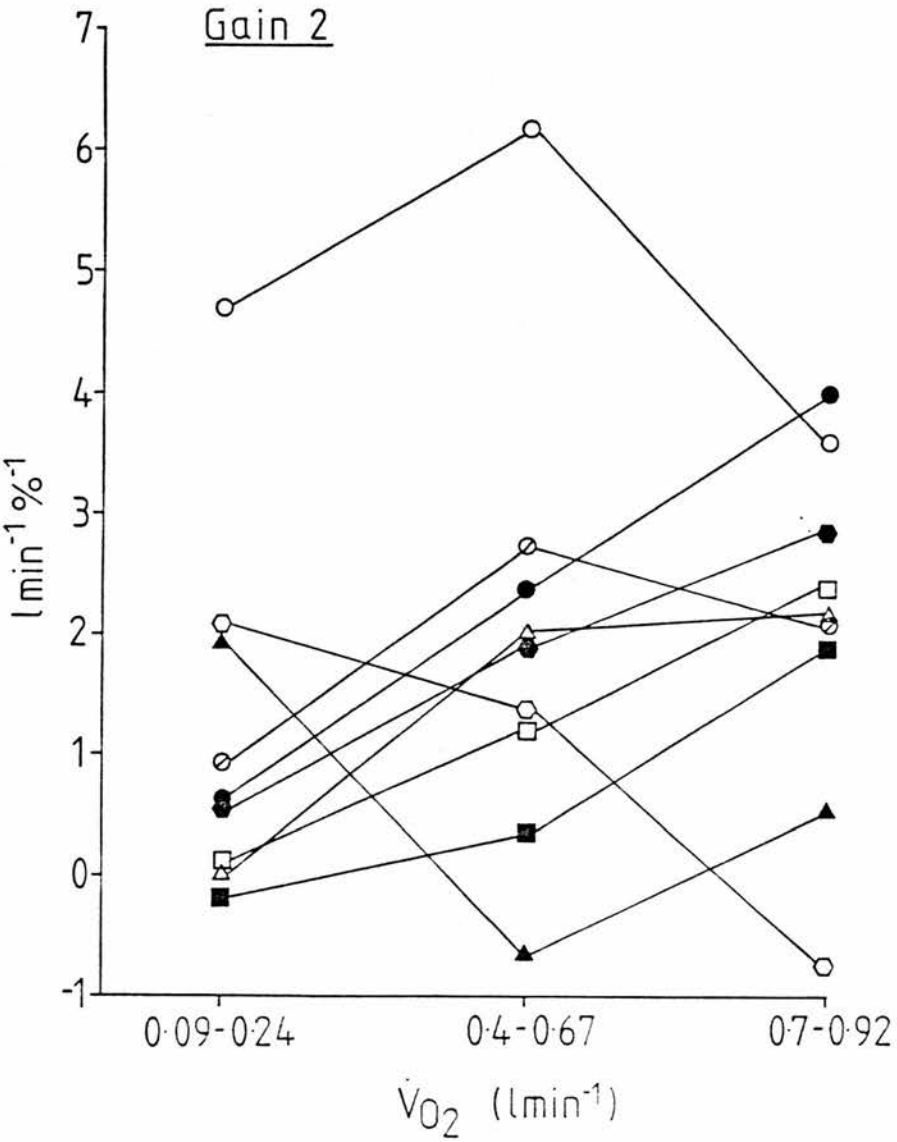
Units for gain 2 (G2) are $lmin^{-1}$ if sigma also required, or $lmin^{-1}\%^{-1}$ if sigma not required to produce the model of best fit.

Fig 6.5 : The effect of increasing exercise level on gain 1.



The hypoxic ventilatory response expressed as the model gain 1 parameter value is plotted for each subject for rest, light ($\dot{V}O_2$ 0.4 - 0.67 $l min^{-1}$) and moderate exercise ($\dot{V}O_2$ 0.7 - 0.92 $l min^{-1}$). Each point represents the value obtained at that level in an individual. The symbols denoting individual subjects are the same as in Fig 6.1.

Fig 6.6 : The effect of increasing exercise level on gain 2.



The hypoxic ventilatory response expressed as the model gain 2 parameter value is plotted for each subject for rest, light ($\dot{V}O_2$ 0.4 - 0.67 $l min^{-1}$), and moderate exercise ($\dot{V}O_2$ 0.7 - 0.92 $l min^{-1}$). Each point represents the value obtained at that level in an individual. The symbols denoting individual subjects are the same as in Fig 6.1.

IV DISCUSSION

The hypoxic ventilatory response expressed as the $\dot{V}_E inst/SaO_2$ relationship was greater during exercise than at rest and significantly correlated with baseline normoxic $\dot{V}O_2$, $\dot{V}CO_2$ and $\dot{V}_E inst$. This increase in the hypoxic ventilatory drive with exercise confirms the findings of Weil et al (1972) and Martin et al (1978). The hypoxic ventilatory response expressed as the model gain parameters was also greater during exercise than at rest and both gain 1 and gain 2 also correlated with the normoxic exercise $\dot{V}O_2$, $\dot{V}CO_2$ and $\dot{V}_E inst$. In this study, the proportional rise in $\dot{V}_E inst$ during hypoxia (expressed as a percentage of the normoxic baseline value) varied widely with increasing exercise level within an individual (range 74 to 159%). However, Martin et al (1978) found that the percentage rise in $\dot{V}_E inst$ to be constant at approximately 20 $\pm$ 2% above the baseline normoxic value over a wider range of exercise, although hyperoxia (ie. no chemoreceptor input) preceded progressive isocapnic hypoxia in that study.

There was not a consistent increase in $\dot{V}_E inst/SaO_2$ slope or the gain parameters as the intensity of exercise rose in each individual subject. Subject fatigue may have contributed to the variability of the increase in hypoxic ventilatory response with increasing exercise level although this was reduced by giving the workloads in random order and allowing the subjects to rest for 15 minutes between each exercise level.

Furthermore, there was no correlation between the order in which the workloads were studied and the hypoxic ventilatory response. Other factors must therefore account for the observed variability in the ventilatory response. Measurements of hypoxic ventilatory response are known to vary widely within an individual over a period similar to this study. Sahn et al (1977) have demonstrated a coefficient of variation for the hypoxic ventilatory response ranging from 8 to 64% over a period of 2 hours.

As O_2 and CO_2 interact at the peripheral chemoreceptors, any change in $PaCO_2$ may also affect the hypoxic ventilatory response (Nielson and Smith 1951, Eyzaguirre and Lewin 1961, Lahiri and Delaney 1975). Although the differences between baseline normoxic $P_{ET}CO_2$ at each exercise level were not statistically significant for the group, there was some individual variation which may partly account for the variability of the response. A further problem is that in this study, $P_{ET}CO_2$ is assumed to equal $PaCO_2$ and this assumption may not be correct. However, as discussed in Chapter 4, Jones et al (1966) and Whipp and Wasserman (1969) have shown that at mild to moderate exercise levels, as in this study, the differences between $P_{ET}CO_2$ and $PaCO_2$ probably contribute little to the variation in the hypoxic ventilatory response.

The increase in the $\dot{V}_E inst/SaO_2$ slope at a given level of exercise varied widely between subjects hence there

was individual variation in the potentiation of the hypoxic ventilatory response. If population studies are to be made at a particular level of exercise, eg. VO_2=1 $lmin^{-1}$, this variation in the degree of potentiation between individuals may influence the comparison of the hypoxic ventilatory response between individual subjects. In this study of only 9 subjects, the correction of $\dot{V}O_2$ for subject weight or body surface area did not improve the significance of the relationship with $\dot{V}_E inst/SaO_2$ slope or the gain parameters. However, in a population study with a wider range of subject age, body size and fitness, the level of exercise may contribute a varying level of exercise potentiation to the hypoxic ventilatory response between subjects, hence it may be more appropriate to correct $\dot{V}O_2$ for subject weight or body surface area.

The above factors probably also contribute to the high variability of the gain parameters with increasing exercise level. However, the model structure may not be applicable at different levels of exercise as the model was developed to describe the ventilatory response to both transient and step change hypoxic stimuli at an exercise level of approximately 1 $lmin^{-1}$. In this study, although transient hypoxic stimuli were not given, the step change hypoxic stimuli incorporated an abrupt fall in SaO_2 (one or two breaths 1% O_2 in N_2) to allow calculation of the short time constant component. Also, the lack of transient hypoxic stimuli was compensated by

the application of a double weighting to the start of each of the periods of step change hypoxia. Use of this double-weighting method to compensate for the lack of transient hypoxic stimuli appears to be valid as subjects 1, 2 and 3 also completed a repeatability study, involving both transient and step change hypoxic stimuli (Chapter 4, subjects 1, 2 and 4) and similar results were obtained during moderate exercise for $\dot{V}_E inst/SaO_2$ slope, gain 1 and gain 2. A quantitative analysis of the model goodness of fit is not possible, however similar F values were obtained at all levels of exercise for an individual, indicating that the fit was as good at all exercise levels.

The mechanism of the observed increase in hypoxic ventilatory response remains unclear but proposed mechanisms include increased sensitivity of the peripheral chemoreceptors, circulating chemical mediators, and increased muscle afferent activity interacting centrally with chemoreceptor input. Both gain 1 and gain 2 increased with increasing exercise level. If gain 1 is assumed to represent the peripheral chemoreceptor response (with a time constant of less than 3 sec), and gain 2, the central response, then the involvement of a combination of both mechanisms is implied. Enhancement of carotid chemosensitivity during mild exercise as shown by the increase in gain 1, is in agreement with previous suggestions of Briggs et al (1920), Hickham et al (1950), Asmussen and Nielson

(1957, 1958), Biscoe and Purves (1967), Bhattacharyya et al (1970), Lugliani et al (1971), Weil et al (1972), Masson and Lahiri (1974), Leitch et al (1976), Martin et al (1978) and Flenley et al (1979). Some form of central interaction from muscle afferent activity, as shown by an increase in gain 2, would be consistent with the proposal by Davies and Lahiri (1973) who were unable to demonstrate an increase in peripheral chemoreceptor activity in response to passive hindlimb exercise in either anaesthetised or decerebrate cats despite an increase in ventilation and Sergeant et al (1981). However, this hypothesis has been refuted by Duncan et al (1981) and Adams et al (1984a). Thus, both peripheral and central mechanisms are probably involved in the potentiation of the hypoxic ventilatory response with mild exercise.

This study has demonstrated an increase in hypoxic ventilatory response with increasing exercise level and suggests that the parameter analysis using the mathematical model can quantify physiological changes affecting both the peripheral and central mechanisms of the ventilatory response. Further studies are required to elucidate the precise mechanism of ventilatory stimulation such as the measurement or noradrenaline levels in the blood before, during and after hypoxia and investigation of the effect of lack of muscle afferent activity in patients with spinal cord lesions upon the ventilatory response to hypoxia.

CHAPTER 7: THE EFFECT OF ORAL THEOPHYLLINE UPON THE HYPOXIC VENTILATORY RESPONSE

I INTRODUCTION

The methylxanthines caffeine, theophylline and aminophylline can cause an increase in ventilation when given systemically. Aminophylline is a complex of two molecules of theophylline and the simple diamine, ethylenediamine which is used to increase solubility. It is assumed that only theophylline, which contributes 85% of the complex, causes the respiratory effect. This stimulation of ventilation has been shown to occur with either caffeine or aminophylline in normal adults (Lakshminarayan et al 1978), adults with pulmonary emphysema or Cheyne-Stokes respiration (Dowell et al 1965), human neonates (Gerhardt et al 1977) and in a variety of animal species including dogs, cats, sheep and rabbits (Eldridge et al 1983).

Methylxanthines have also been shown to increase the hypoxic ventilatory response in man. Lakshminarayan et al (1978) demonstrated an increase in hypoxic ventilatory response to progressive isocapnic hypoxia, resting $\dot{V}O_2$ and $\dot{V}CO_2$ in six normal subjects after intravenous administration of aminophylline (mean plasma level 9.41ugml^{-1}, range 7.7-11.3ugml^{-1}). Similarly, Sanders et al (1980) found an increase in the ventilatory response to progressive isocapnic hypoxia but only after P_ACO_2 was raised to the control level before the administration of either intravenous or oral

theophylline (oral aminophylline mean plasma level 8.8ugml^{-1}, standard deviation about the mean 1.7ugml^{-1}). Georgopoulos et al (1989) showed that the ventilatory decline due to prolonged hypoxia could be prevented by pretreatment with intravenous aminophylline in 10 normal subjects. However, an increase in the ventilatory response to hypoxia was not shown by Swaminathan et al (1989) with oral theophylline in 15 normal subjects (mean plasma level 11.3ugml^{-1}, range 5.3-22.1ugml^{-1}), although several subjects were below the therapeutic range of 10-20ugml^{-1}.

The bronchodilator effects of the methylxanthines have been attributed to the inhibition of phosphodiesterase (Butcher and Sutherland 1962), cellular uptake of calcium (Kolbek et al 1979), and adenosine antagonism (Ally and Nakatsu 1976). The central stimulation of ventilation by methylxanthines may be due to the alteration of dopamine (Lundberg et al 1981) and serotonin levels (Mueller et al 1981) or a change in the receptor activity in the brain of these compounds. However the stimulation of ventilation by theophylline is largely attributed to its action as an adenosine antagonist. This was demonstrated by Lagercrantz et al (1984), who showed that the respiratory depression due to administration of adenosine analogues to rabbit pups could be reversed by theophylline. Animal studies have indicated that the action of the methylxanthines is central (Eldridge et al 1985, 1983; Millhorn et al

1984). Eldridge et al (1985) showed that adenosine given systemically or injected directly into the third cerebral ventricle of 19 cats depressed respiration and systemically administered theophylline reversed this depression of ventilation. They therefore suggested that adenosine acts as a tonic modulator of respiration and that theophylline stimulates respiration by competitive antagonism of adenosine at neuronal receptor sites. Furthermore, cerebral adenosine has been shown to increase during hypoxia (Winn et al 1981, Daly et al 1981).

In humans, the evidence for the central stimulatory action of aminophylline upon the hypoxic ventilatory response comes mainly from the work of Easton et al (1988a). They found that the ventilatory decline during sustained hypoxia was not completely abolished by pretreatment with aminophylline but most of the ventilatory depression was due to changes in breathing pattern.

This aim of this study was to investigate the effects of oral theophylline, a proposed central stimulant of respiration, upon the hypoxic ventilatory response and to investigate whether the slow time constant equation of the mathematical model represents the central component of the ventilatory response to hypoxia.

II METHODS

i) Subjects

The subjects were 6 healthy volunteers (3 male, 3 female), aged 23 to 65 years. Their anthropomorphic and normal lung function details are given in Appendix table 18. All subjects were non-smokers apart from subject 1 who was an occasional cigarette smoker.

ii) Protocol

The subjects initially took oral theophylline (Astra, Theodur slow release preparation, Weinberger and Hendeles 1984), 12 hourly at 8am and 8pm in order to establish the required dose to produce a plasma theophylline level of 10-20 $ugml^{-1}$. This dosage was used in the subsequent study. The trial period was followed by a washout period of at least two weeks after which the subject took either theophylline or placebo for 15 days. The order was randomised and double-blind. All subjects abstained from taking coffee throughout the five week study period. The two limbs of the study were separated by at least one week, during which neither drug nor placebo were taken. Measurements were made on day 15 of each limb of the study and each individual was studied at the same time of day on both occasions. The subjects did not fast on day 15 but all took a light breakfast at least one hour before the measurement of hypoxic ventilatory response. The FEV_1 and specific airways conductance (SG_{aw}) were initially recorded. SG_{aw} was calculated from $(1/R_{aw})/VG$ measured using a Fenyves

body plethysmograph. A blood sample was then taken from the antecubital vein (2 to 4 hours after the last dose), and subsequently analysed for plasma theophylline concentration using a high performance liquid chromatographic method (Evenson and Warren 1976). Ventilation and gas exchange were then measured at rest, followed by the hypoxic ventilatory response during exercise using both transient and step change methods.

a) Measurement of resting ventilation and gas exchange

Resting ventilation and gas exchange were measured with the subject seated in an armchair and breathing room air through the apparatus described in Chapter 3. They rested initially for 10 minutes to establish steady state gas exchange. Mixed expired gas was then collected between 10-12 and 12.15-14.15 minutes after the start of the rest period for calculation of $\dot{V}_E$, $\dot{V}O_2$, $\dot{V}CO_2$ and RQ. Minute ventilation, tidal volume, respiratory frequency, inspired and expired gas concentrations and heart rate were also recorded breath-by-breath over the same 15 minute period.

b) Measurement of the hypoxic ventilatory response

The subjects walked on a level treadmill at speeds of 0.90-1.35 $msec^{-1}$ which had previously been selected to produce a $\dot{V}O_2$ of about 10 $mlmin^{-1}kg^{-1}$, and the same speed was used for each subject for both treadmill studies. They initially walked for 10 minutes breathing room air. Mixed expired gas was collected between 5-7

and 7.15-9.15 minutes after the start of exercise, and from 5-7 minutes after the last step change hypoxic stimulus for calculation of $\dot{V}_E$, $\dot{V}O_2$, $\dot{V}CO_2$ and RQ to confirm that steady state conditions existed during the measurement of the hypoxic ventilatory response.

The ventilatory response was measured first to transient hypoxia and then to step change hypoxia.

Transient hypoxia

Inspired gas was abruptly changed during an expiration from room air to 1% O_2 in N_2 for 1-3 breaths to cause a fall in SaO_2 to between 80-90%. Inspired gas was then returned to room air during an expiration. The transient hypoxic stimulus was repeated at 2 minute or at least 40 breath intervals until at least three acceptable stimuli were given (SaO_2 falling to between 80-90%). A maximum of 6 transient hypoxic stimuli were given during each study. Isocapnia was maintained during the transient stimuli by the addition of CO_2 close to the Hans-Rudolph valve in the inspiratory limb of the circuit to keep $P_{ET}CO_2$ constant.

Step change hypoxia

Following the last transient hypoxic stimulus the subjects breathed room air for at least 3 minutes.

The ventilatory responses to two levels of step change hypoxia (F_IO_2 0.15 and 0.12) were then measured. Inspired gas was abruptly changed during an expiration from room air to 1% O_2 in N_2 for one breath. In the

subsequent expiration, inspired gas was changed to 15% O_2 for the remainder of the 3 minute period. The subjects then breathed room air for at least 5 minutes. Inspired gas was then abruptly changed during an expiration to 1% O_2 in N_2 for two breaths followed by 12% O_2 for the remainder of the 3 minute period. Inspired gas was then returned to room air during an expiration. Isocapnia was maintained during the onset and duration of the step change hypoxic stimuli by the addition of CO_2 close to the Hans-Rudolph valve in the inspiratory limb of the circuit to keep $P_{ET}CO_2$ constant.

iii) Analysis

Resting respiratory frequency (f_R), tidal volume (V_t) and baseline $P_{ET}CO_2$ values were calculated as the mean of 20 breaths, eight minutes after the start of the rest period.

Baseline normoxic $P_{ET}CO_2$ during exercise was calculated as the mean of the 20 breaths before the first transient stimulus, 20 breaths before each of the step change hypoxic stimuli and 20 breaths five minutes after the end of the last hypoxic period. The mean $P_{ET}CO_2$ during transient hypoxia was calculated for all breaths used in the analysis of the ventilatory response. The mean $P_{ET}CO_2$ during step change hypoxia was calculated using 10 breaths before and all breaths during both 3 minute periods of hypoxia.

The results of the transient hypoxic stimuli were pooled and the response expressed as the slope of the

$\dot{V}_E$ inst/SaO_2 relationship as described in Chapter 3. The results for the step change hypoxic stimuli were also pooled and the response expressed as the slope of the linear regression relationship between $\dot{V}_E$inst and SaO_2 for 10 breaths before and all breaths during each 3 minute hypoxic period.

The ventilatory response to both transient and step change hypoxia was also analysed using the mathematical model and the parameters derived which best described the ventilatory response on each study day.

The Kruskal-Wallis one way analysis of variance test was used to compare normoxic baseline $P_{ET}CO_2$ with the corresponding values during transient and step change hypoxia for each subject. Other variables were compared using Wilcoxon's signed rank test.

III RESULTS

Theophylline was not detected in the plasma in any subject when taking placebo. When taking theophylline, plasma levels ranged from 4.4 to 11.4 $ugml^{-1}$, with two subjects (2 and 3) being outside the therapeutic range of 7-20 $ugml^{-1}$ (table 7.1). There were no significant differences in the resting baseline measurements of FEV_1, SGaw, $\dot{V}_E$, $\dot{V}O_2$, $\dot{V}O_2/kg$, $\dot{V}CO_2$, f_R, V_t and $P_{ET}CO_2$ when the subjects were taking placebo or theophylline (Appendix table 19). Due to technical problems, the SGaw could not be measured in subject 5 on the theophylline limb of the study. At rest, the RQ value was equal to, or less than 1.0 for all subjects taking either placebo or theophylline. There were no significant differences between the exercise baseline measurements of $\dot{V}_E$, $\dot{V}O_2$, $\dot{V}O_2/kg$ and $\dot{V}CO_2$ when the subjects were taking either placebo or theophylline (Appendix table 20). During exercise, the RQ value was less than or equal to 1.0 in all subjects taking either placebo or theophylline, except for subject 1 in whom it was 1.01 when taking placebo (Appendix table 20).

Baseline normoxic $P_{ET}CO_2$ values during exercise were not significantly different on theophylline or placebo, although the greatest difference between the two mean values was 0.99 kPa in subject 5 (Appendix table 21). There was no significant difference between the mean $P_{ET}CO_2$ during either transient or step change hypoxia and the corresponding baseline values for each subject

on either limb of the study (Appendix table 21).

The mean values for the fall in SaO_2 due to the transient hypoxic stimuli or the minimum value of SaO_2 during step change hypoxia, did not differ significantly when the subjects took either placebo or theophylline (table 7.2).

The ventilatory response to transient hypxia (expressed as the $\dot{V}_E inst/SaO_2$ relationship) ranged from -0.68 to -2.67 $lmin^{-1}\%^{-1}$ on placebo (although this measurement could not be made in subject 3 due to technical problems), and from -0.33 to -1.85 $lmin^{-1}\%^{-1}$ on theophylline (table 7.3). On theophylline the ventilatory response to transient hypoxia was increased in two subjects and was decreased in three subjects (Fig 7.1). The ventilatory response to step change hypoxia (expressed as the $\dot{V}_E inst/SaO_2$ relationship) ranged from -0.33 to -1.38 $lim^{-1}\%^{-1}$ on placebo, and -0.46 to -1.35 $lmin^{-1}\%^{-1}$ on theophylline (table 7.3). On theophylline the ventilatory response to step change hypoxia was increased in four subjects and decreased in two subjects (Fig 7.1). There was no significant difference between the $\dot{V}_E inst/SaO_2$ slopes in response to either transient or step change hypoxia when the subjects took either placebo or theophylline (Fig 7.1).

Table 7.1 Plasma theophylline levels for all subjects taking either placebo on one limb of the study and oral theophylline on the other, in a randomised order

Subject number	PLASMA THEOPHYLLINE LEVEL ($ugml^{-1}$) Study 1	Study 2
1	0	11.3
2	0	4.4
3	5.0	0
4	8.1	0
5	11.4	0
6	0	10.5

The plasma theophylline level considered to be therapeutic is 7 to 20 $ugml^{-1}$.

Table 7.2 Mean SaO_2 values for transient hypoxic stimuli and the minimum values during 12% O_2 step change hypoxia for each subject taking placebo and theophylline

Subject number	PLACEBO mean transient SaO_2 (%)	PLACEBO min. step change SaO_2 (%)	THEOPHYLLINE mean transient SaO_2 (%)	THEOPHYLLINE min. step change SaO_2 (%)
1	84	85	86	86
2	86	77	87	86
3	-	83	88	87
4	86	87	88	86
5	88	88	88	85
6	87	89	86	86

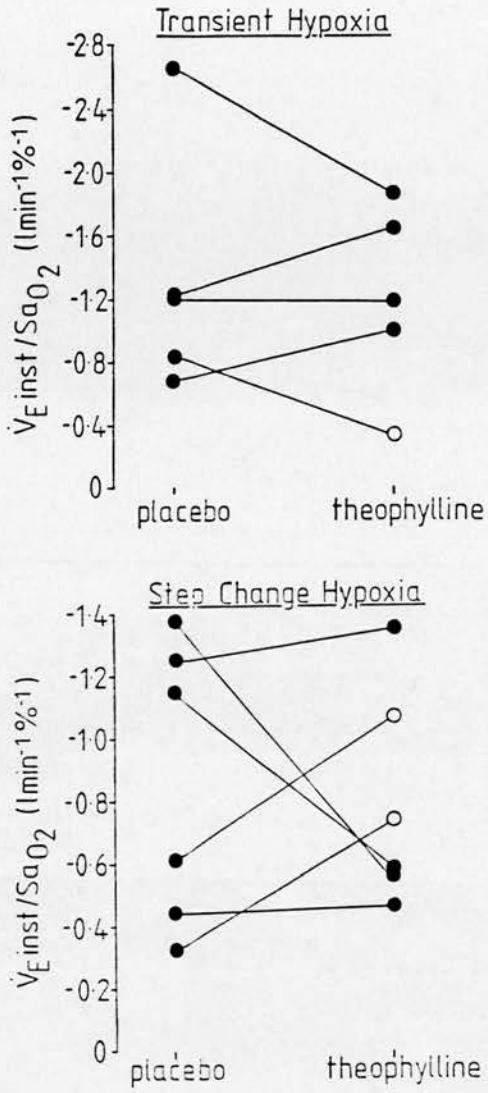
There was no statistical difference between the fall in SaO_2 due to either type of hypoxia for any subject taking either placebo or theophylline.

Table 7.3 $\dot{V}_E inst/SaO_2$ slopes in response to transient (trans) and step change hypoxia (step) for each subject taking placebo or theophylline

Subject number	PLACEBO trans	PLACEBO step	THEOPHYLLINE trans	THEOPHYLLINE step
1	-1.22	-0.44	-1.64	-0.46
2	-0.83	-0.33	-0.33	-0.74
3	-	-0.61	-0.91	-1.07
4	-1.22	-1.15	-1.18	-0.57
5	-2.67	-1.38	-1.85	-0.56
6	-0.68	-1.25	-1.01	-1.35

$\dot{V}_E inst/SaO_2$ slopes expressed as $l min^{-1}\%^{-1}$

Fig 7.1 : Effect of theophylline upon the hypoxic ventilatory response.

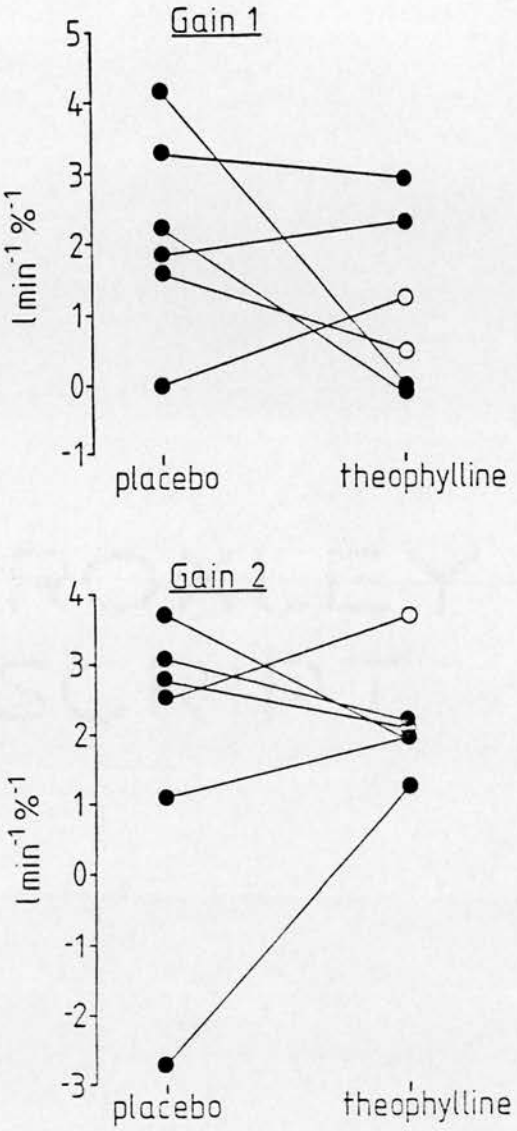


Each point represents the $\dot{V}_E inst/Sa_{O_2}$ relationship in response to transient (upper panel) and step change hypoxia (lower panel) for each of 6 subjects. For each subject, the ventilatory responses on placebo and theophylline are joined by a line. Closed circles on the theophylline limb of the study represent a plasma theophylline level within the therapeutic range and open circles, a level outside the range of 7-20ugml^{-1}.

The gain 1 parameter values ranged from 0.02 to 4.15 $lmin^{-1}\%^{-1}$ on placebo, and -0.02 to 2.94 $lmin^{-1}\%^{-1}$ on theophylline (table 7.4). On theophylline, the gain 1 parameter was increased in two subjects and decreased in four subjects (Fig 7.2). Gain 2 ranged from -2.70 to 3.71 $lmin^{-1}\%^{-1}$ on placebo, and 1.26 to 3.65 $lmin^{-1}\%^{-1}$ on theophylline (table 7.4). On theophylline, the gain 2 parameter increased in three subjects and decreased in three subjects (Fig 7.2). There was no significant difference between either the gain 1 or gain 2 values when the subjects took either placebo or theophylline (Fig 7.2). On placebo, the values for tau 2 ranged from 25 to 339 sec and on theophylline this range was 25 to 268 sec, with no consistent changes apparent (table 7.4). The inhibition parameter was required in three subjects (1, 2 and 4) on the placebo limb of the study (range 0.05 to 0.51 $(lmin^{-1})^{-1}$), and in two subjects (1 and 5) on the theophylline limb of the study (range 0.04 to 0.19 $(lmin^{-1})^{-1}$, table 7.4). The saturating effect parameter was required in three subjects on placebo (2, 4 and 6, range 0.05 to 0.24%), and in three subjects on theophylline (1,3 and 4,range 0.07 to 0.53%, table 7.4).

There was no obvious relationship between the plasma theophylline level of each subject during the theophylline limb of the study and the hypoxic ventilatory response expressed as either the $\dot{V}_E inst/SaO_2$ relationship, or as the model parameters gain 1 or gain 2 (Figs 7.1 and 7.2).

Fig 7.2 : Effect of theophylline upon the model gain parameters.



Each point represents the value derived for gain 1 (upper panel) or gain 2 (lower panel) for each of 6 subjects. For each subject, the gain values derived on placebo and theophylline are joined by a line. Closed circles on the theophylline limb of the study represent a plasma theophylline level within the therapeutic range and open circles, a level outside the range. Units for gain 2 are $l min^{-1}$ if sigma also required, or $l min^{-1} \%^{-1}$ if sigma not required to produce the model of best fit.

Table 7.4: Model parameter values for each subject taking placebo or theophylline.

Subject number	PLACEBO gain 1 $lmin^{-1}\%^{-1}$	PLACEBO I $(lmin^{-1})^{-1}$	PLACEBO tau 2 sec	PLACEBO gain 2 $lmin^{-1}\%^{-1}$	PLACEBO sigma %	THEOPHYLLINE gain 1 $lmin^{-1}\%^{-1}$	THEOPHYLLINE I $(lmin^{-1})^{-1}$	THEOPHYLLINE tau 2 sec	THEOPHYLLINE gain 2 $lmin^{-1}\%^{-1}$	THEOPHYLLINE sigma %
1	3.30	0.51	25	1.10	*	2.94	0.19	134	1.89	0.07
2	1.59	0.33	53	2.78	0.05	0.49	*	185	1.97	*
3	0.02	*	202	2.53	*	1.22	*	25	3.65	0.40
4	2.24	0.05	167	3.06	0.24	-0.02	*	133	2.12	0.53
5	4.15	*	339	-2.70	*	-0.01	*	268	1.26	*
6	1.80	*	46	3.71	0.08	2.29	0.04	134	1.93	*

Units for gain 2 are $lmin^{-1}\%^{-1}$ if sigma not required to produce the model of best fit, or $lmin^{-1}$ if sigma also required.
* represents parameter not required to produce the model of best fit.

IV DISCUSSION

This study has been unable to demonstrate any effect of oral theophylline upon the hypoxic ventilatory response expressed as either the $\dot{V}_E inst/SaO_2$ relationship or as the model gain parameters. However, only six subjects were studied, and in two of them the level of theophylline measured was outside the therapeutic range.

Previous evidence suggests that theophylline increases both resting normoxic ventilation and that during hypoxia by specific central adenosine antagonism (Eldridge et al 1983, 1985; Millhorn et al 1984; Lagercrantz et al 1984; Lakshminarayan et al 1978; Sanders et al 1980; Easton et al 1988b). Such an increase in the hypoxic ventilatory response due to intravenous aminophylline was demonstrated by Lakshminarayan et al (1978), and Sanders et al (1980) at similar plasma levels to those achieved in this study. However, an increase in the hypoxic ventilatory response could not be demonstrated by Swaminathan et al (1989), also with oral theophylline at plasma levels similar to those in this study.

Several reasons could account for this failure to demonstrate a consistent effect of theophylline upon the hypoxic ventilatory response. Previous hypoxic ventilatory response reproducibility studies by Sahn et al (1977) and Chapter 4 of this thesis have shown that the between-day variability of the response is at least 20% in normal subjects. Thus this variability may have

masked the effect of theophylline upon the hypoxic response. Furthermore, three of the subjects (3, 4 and 6) were female, and each were studied after a 15 day interval, hence potentiation of the hypoxic ventilatory response by progesterone (Sutton et al 1975, White et al 1983, Regensteiner et al 1989) may have contributed to the lack of any effect of theophylline upon the hypoxic ventilatory response in these subjects.

It is unlikely that the failure to demonstrate a consistent effect of theophylline was due to differing degrees of potentiation of the hypoxic ventilatory response by exercise on the two limbs of the study (Weil et al 1972, Martin et al 1978). In this study the subjects walked at the same speed on both limbs of the study and the $\dot{V}O_2$ did not vary significantly between placebo and theophylline days. Also, all subjects exercised below their anaerobic threshold (Buchfuhrer et al 1982). Also, although all subjects took a light breakfast approximately two hours before each study, the degree of potentiation of the hypoxic ventilatory response due to the ingestion of carbohydrates (Zwillich et al 1977) would have been similar on the two days. Each subject was studied at the same time of day (to within one hour), hence any diurnal variation in the response (as demonstrated for the hypercapnic ventilatory response, Koepchen et al 1954) was avoided. For all subjects, the fall in SaO_2 produced by either transient or step change hypoxic stimuli were consistent

between the two studies. Therefore the lack of a change in the hypoxic ventilatory response due to theophylline cannot be attributed to variation in the size of the stimuli. Although the differences between baseline normoxic $P_{ET}CO_2$ either at rest or during exercise were not statistically significant between the two limbs of the study, the variation may partly account for the lack of a response. An increase in the hypoxic ventilatory response could be demonstrated by Sanders et al (1980) only after P_ACO_2 was raised to the control level before the administration of intravenous or oral aminophylline. However they reported an increase in baseline resting ventilation and a consequent fall in P_ACO_2 following aminophylline. Since no such increase in resting ventilation was observed in this study, it was not necessary to increase $P_{ET}CO_2$ to the baseline placebo level and unlikely that the lack of a change in the response after theophylline was due to a change in P_ACO_2.

Adenosine has been shown to specifically stimulate the carotid body chemoreceptors in anaesthetised cats (McQueen and Ribeiro 1981, 1983). Maxwell et al (1986) demonstrated an increase in resting normoxic ventilation and hypoxic ventilatory response in six normal subjects after adenosine infusion. They attributed this increase in the hypoxic ventilatory response to peripheral chemoreceptor stimulation. As theophylline acts centrally as an adenosine antagonist, thus stimulating

ventilation, then any effect of theophylline is likely to be due to an imbalance in the opposing and interacting peripheral and central adenosine mechanisms and may therefore account for the disparity of published results.

This study has been unable to demonstrate any effect of oral theophylline upon the hypoxic ventilatory response expressed either as the $\dot{V}_E inst/Sa_{O_2}$ relationship or as the model gain parameters. As only four subjects demonstrated plasma theophylline levels within the therapeutic range, the lack of any consistent effect of theophylline upon the hypoxic ventilatory response may be due to the small number of subjects studied. Further studies are required with the plasma theophylline level well into the therapeutic range (ie. 15-20 $ugml^{-1}$) in order to confirm or refute previous work suggesting the central action of oral theophylline as a stimulant of the hypoxic ventilatory response.

CHAPTER 8: FAMILIAL ASPECTS OF THE HYPOXIC VENTILATORY RESPONSE

I INTRODUCTION

In man, the hypoxic and hypercapnic ventilatory drives to breathing vary widely between healthy individuals (Hirshman et al 1975) and the intensity of the drive may be important in the pathogenesis of chronic obstructive pulmonary disease (COPD). It was suggested by Dornhorst (1955) that patients suffering from COPD may be divided into two groups: the "pink puffers" who have relatively normal arterial blood gas tensions, and the "blue bloaters" who have low PaO_2, high $PaCO_2$, cor pulmonale and pulmonary hypertension. The two types of COPD cannot be distinguished by the degree of emphysema or the extent of airways obstruction (Thurlbeck 1976). Both Flenley et al (1970) and Bradley et al (1979) have shown that patients with the "blue and bloated" form of COPD had a low hypoxic ventilatory drive.

Various studies suggest that the intensity of the hypoxic ventilatory drive is partly determined genetically. Scoggin et al (1978) compared the ventilatory response to progressive isocapnic hypoxia in 16 non-athletic, healthy parents and siblings of 5 successful long-distance endurance athletes to those of 34 non-athletic controls. They found that the hypoxic ventilatory drive was decreased to a similar extent in the runners and their families when compared to the control subjects and concluded that familial factors

make a major contribution to the decreased hypoxic ventilatory drive observed in long-distance runners.

Several groups have investigated the familial aspects of hypoxic ventilatory drive by studying pairs of identical and nonidentical twins. Leitch et al (1975) measured the ventilatory responses to hypoxia and hypercapnia in one pair of identical twin athletes trained to a similar high degree and found the responses to be virtually identical. Collins et al (1978) measured the ventilatory response to progressive isocapnic hypoxia and hyperoxic hypercapnia at rest in 12 pairs of identical and 12 pairs of nonidentical twins. They found a significant correlation for hypoxic ventilatory drive for identical twin pairs but not for nonidentical twin pairs which were independent of body size, blood PCO_2 and pH. No such correlation was found for the ventilatory response to hyperoxic hypercapnia.

To investigate whether the genetically determined intensity of the ventilatory response to hypoxia determines the severity of hypoxaemia in COPD, Kawakami et al (1982a) compared the PaO_2 of 25 patients with stable COPD (air, 9.59 $\pm$ 1.52 kPa) with the hypoxic ventilatory response of their 34 sons measured using the progressive isocapnic hypoxia technique at rest. They showed that the hypoxic ventilatory drive of the sons expressed as the parameter A, as defined by Weil et al (1970), correlated significantly with the PaO_2 of the patients when in stable state, and with the $PaCO_2$ and

$PaCO_2$ during an acute exacerbation. They also made similar comparisons in an age-matched control group of 17 patients with silicosis and their 22 sons and found that in silicosis, no significant relationships were observed between patients and sons with respect to arterial blood gas determinations and the ventilatory responses, except for $PaCO_2$ of patients and hypercapnic ventilatory response of their sons. They concluded that familial factors are involved in determining the arterial blood gases and hypoxic ventilatory response in COPD, and blunted chemosensitivity to hypoxia may precede clinically manifest COPD.

The role of familial factors in determining arterial blood gas values in COPD was confirmed by Fleetham et al (1984) who showed that an abnormal chemical drive to breathing was present in patients with COPD which antedates the development of chronic CO_2 retention. They measured the ventilatory and $P_{0.1}$ responses to progressive hypoxia and hypercapnia in 14 patients with COPD (air, PaO_2 6.93 $\pm$ 0.81 kPa) and in 23 of their normal adult offspring and found that the hypoxic responses in the patients were positively correlated with the mean hypoxic responses of their offspring but neither the hypercapnic responses nor the resting breathing patterns of the patients correlated with those of their offspring. They also showed that the hypoxic ventilatory response was lower in offspring of hypercapnic patients than in offspring of normocapnic

patients. They concluded that blunt hypoxic responses in patients with COPD are influenced by familial factors and may represent a premorbid "risk factor" in the development of CO_2 retention in COPD.

It was proposed by Flenley (1978) that the observed interindividual differences in hypoxic ventilatory drive were due to differences in peripheral chemosensitivity to hypoxia. This hypothesis was recently supported by evidence of large interindividual differences in carotid sinus nerve responses to hypoxia observed in anaesthetised cat preparations (Vizek et al 1987). However, both Kawakami et al (1982a) and Fleetham et al (1984) used the progressive isocapnic hypoxia technique which may be susceptible to central depression of ventilation in certain individuals (Weiskopf and Gabel 1975, Easton et al 1986). The following study aims to investigate both the specific peripheral chemoreceptor and central hypoxic ventilatory responses by using both transient and short duration steady state isocapnic hypoxia techniques.

The aims of this study therefore were:

a) to determine the relationship between the PaO_2 of patients with COPD and the peripheral and/or central hypoxic ventilatory drive of their offspring.

b) to determine whether the spectrum of responses in the offspring differed from those of an age and sex matched population whose parents had no history of COPD.

II METHODS

i) Subjects

The 24 patients with chronic obstructive pulmonary disease (COPD) were recruited from those attending the Department's clinic. Arterial blood gas tensions were measured when they were all in a stable condition (no exacerbation of COPD for at least 3 weeks) and showed a wide range of arterial blood gas tensions (PaO_2 5.12-9.49 kPa, $PaCO_2$ 4.9-12.0 kPa, on air), and severity of airways obstruction (FEV_1 12-43 %pred, table 8a). Arterial blood samples for gas tensions and pH were obtained by direct puncture of the brachial artery. Samples were analysed in triplicate within 10 minutes of withdrawal using a Radiometer ABL II automatic gas analyser. The electrodes were calibrated automatically every two hours using high and low pH buffer solutions, and O_2 and CO_2 mixtures prepared by the analyser from primary gases. In addition, the analyser was calibrated manually twice weekly against blood tonometered with gas mixtures of known PO_2 and PCO_2. Thirty nine of their offspring were initially recruited. Four were subsequently withdrawn from the study as two were found to have abnormal lung function, one had mitral valve disease and one had rheumatoid arthritis. The 35 offspring of these patients were all first generation sons and daughters and their anthropomorphic and normal lung function details are given in Table 8b and Appendix table 22. The offspring group consisted of 20 males and

15 females (aged 18 to 47 years) and 19 of the group were cigarette smokers. Of the offspring group there were 16 single offspring, 5 pairs of siblings and 3 groups of three siblings.

Twenty six control subjects were studied who were sex and age-matched (to within 3 years) with the offspring group and whose parents had no history of COPD. They were recruited from friends or spouses of the offspring group and all had normal lung function. The control subjects anthropomorphic and lung function details are given in Table 8b and Appendix table 23. None of the control group were withdrawn from the study due to abnormal lung function or on medical grounds. The control group consisted of 15 males and 11 females (aged 19 to 50 years) and 5 of this group were cigarette smokers. All 26 control subjects came from different families.

Similar measurements were made in both groups. All subjects fasted for at least one hour before the hypoxic ventilatory response study and none were taking any medication (including the contraceptive pill) at the time of the study.

After the measurement of hypoxic ventilatory drive a 10ml venous blood sample was taken from 33 of the offspring and all 26 of the control group for determination of haemoglobin and carboxyhaemoglobin levels by co-oximetry using an Instrumentation Laboratory 282 co-oximeter.

Table 8a Summary of the lung function and arterial blood gas details of the patients

n	24
sex	10 M; 14 F
age (years)	68 (59-80)
FEV_1 (%pred)	23 (12-43)
FVC (%pred)	53 (22-85)
FEV_1/FVC (%)	32 (13-67)
PaO_2 (air, kPa)	7.01 (5.12-9.49)
$PaCO_2$ (air, kPa)	6.95 (4.90-8.70)

Values are given as mean and range

Table 8b Summary of anthropmorphic and lung function details of the offspring and control groups

	Offspring	Controls
n	35	26
sex	20 M; 15 F	15 M; 11 F
age (years)	35 (18-47)	33 (19-50)
height (m)	1.72 (1.58-1.85)	1.73 (1.59-1.84)
weight (kg)	71 (53-98)	70 (51-91)
FEV_1 (%pred)	94 (77-112)	97 (80-112)
FEV_1/FVC (%)	81 (61-93)	81 (69-92)
TLC (%pred)	96 (71-115)	97 (78-124)
RV/TLC (%)	28 (21-41)	26 (18-56)
TLCO (%pred)	80 (64-102)	92 (60-120)

Values are given as mean and range

ii) Protocol

The subjects walked on a level treadmill at speeds of 0.92 to 1.55ms^{-1} which had previously been selected to produce a $\dot{V}O_2$ of about 12mlmin^{-1}kg^{-1} for each individual. They initially walked for 10 minutes breathing room air to establish steady state gas exchange. Mixed expired gas was collected between 5-7 and 7.15-9.15 minutes after the start of exercise, and from 5-7 minutes after the last step change hypoxic stimulus for calculation of $\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}_E$ and R to confirm steady state conditions.

The ventilatory response was measured first to transient hypoxia and then to step change hypoxia.

iii) Transient hypoxia

Inspired gas was abruptly changed during an expiration from room air to 1% O_2 in N_2 for 1-3 breaths to cause a fall in SaO_2 to between 80-90%. Inspired gas was then returned to room air during an expiration. The transient hypoxic stimulus was repeated at 2 minute (or at least 40 breath) intervals until at least three acceptable stimuli were given (SaO_2 falling to between 80-90%). A maximum of 6 transient hypoxic stimuli were given during each study. Isocapnia was maintained by the addition of CO_2 close to the Hans-Rudolph valve in the inspiratory limb of the circuit to keep $P_{ET}CO_2$ constant.

iv) Step change hypoxia

Following the last transient hypoxic stimulus the subjects breathed room air for at least 3 minutes. The ventilatory response to two levels of step change hypoxia (F_IO_2 0.15 and 0.12) were then studied. Inspired gas was abruptly changed during an expiration from room air to 1% O_2 in N_2 for one breath. In the subsequent expiration, inspired gas was changed to 15% O_2 for the remainder of the 3 minute period. The subjects then breathed room air for at least 5 minutes. Inspired gas was then abruptly changed during an expiration to 1% O_2 in N_2 for two breaths followed by 12% O_2 for the remainder of the 3 minute period. Inspired gas was then returned to room air during an expiration. Isocapnia was maintained during the onset and duration of hypoxia by the addition of CO_2 close to the Hans-Rudolph valve in the inspiratory limb of the circuit to keep $P_{ET}CO_2$ constant.

v) Analysis

For each subject the results of the transient hypoxic stimuli were pooled and the response expressed as the slope of the $\dot{V}_E$inst/SaO_2 relationship as described in Chapter 3. The results for the step change hypoxic stimuli were also pooled and the response expressed as the slope of the linear regression relationship between $V_E$inst and SaO_2 for 10 breaths before and all breaths during each 3 minute period of hypoxia.

For each subject, unique parameters describing the

ventilatory response to both transient and step change hypoxia were derived using the mathematical model. For subjects in whom the ventilatory response to transient hypoxia could not be measured, a double weighting was applied to the start of each of the periods of step change hypoxia for parameter estimation by the mathematical model.

Baseline normoxic $P_{ET}CO_2$ was calculated as the mean of 20 breaths before the first transient stimulus, 20 breaths before each of the step change hypoxic stimuli and 20 breaths, five minutes after the end of the last hypoxic period. The mean $P_{ET}CO_2$ during transient hypoxia was calculated for all breaths used in the analysis of the ventilatory response. The mean $P_{ET}CO_2$ during step change hypoxia was calculated for 10 breaths before each and all breaths during both 3 minute periods of hypoxia.

Correlations between variables were assessed using least squares linear regression analysis. Baseline $P_{ET}CO_2$ was compared with that during transient and step change hypoxia for each subject using the Kruskal-Wallis one way analysis of variance method. The PaO_2 of the patients was related by stepwise multiple regression analysis in order to assess the combined effect of several variables, including body surface area, weight, $\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}_E$, slopes of $\dot{V}_E inst/SaO_2$ and model parameters. The results from the 26 age and sex matched pairs of control and offspring subjects were compared

using the Wilcoxon signed rank test.

III RESULTS

i) Offspring of patients with COPD

Values for baseline normoxic $\dot{V}_E$, $\dot{V}O_2$ and $\dot{V}CO_2$ for each subject are given in Appendix table 24. The mean $\dot{V}O_2$ was 0.85 $lmin^{-1}$ (s.d. 0.2 $lmin^{-1}$, range 0.52 to 1.43 $lmin^{-1}$), and when expressed per kg body weight was 11.9 $mlmin^{-1}kg^{-1}$ (s.d. 1.8$mlmin^{-1}kg^{-1}$, range 8.6 to 16.2 $mlmin^{-1}kg^{-1}$). The RQ value was below 1.0 in all but five subjects (subjects 1,11,19,26,27) in whom it ranged from 1.06 to 1.10. For each individual, there was no significant difference between baseline $P_{ET}CO_2$ and the mean values during either transient or step change hypoxia, and the standard deviation of $P_{ET}CO_2$ about the mean during hypoxia ranged from 0.08 to 0.36kPa (Appendix table 25). There was also no significant difference between the mean lowest SaO_2 in response to either transient or step change hypoxia in an individual (table 8.1).

A response to transient hypoxia could not be measured in three subjects for technical reasons. The slope of the $\dot{V}_E inst/SaO_2$ slope ranged -0.24 to -2.36 $lmin^{-1}\%^{-1}$, and in response to step change hypoxia ranged +0.37 to -1.75 $lmin^{-1}\%^{-1}$ (table 8.2). In one subject (subject 11), minute ventilation fell during step change hypoxia, resulting in a positive $\dot{V}_E inst/SaO_2$ slope. For the group of patients with COPD, no correlation could be demonstrated between the PaO_2 on air, and either FEV_1 or the FEV_1/FVC ratio. The PaO_2 of the patients did not

correlate with the ventilatory response to transient hypoxia of their offspring expressed as the $\dot{V}_E inst/SaO_2$ relationship (Fig 8.1). Expression of the $\dot{V}_E inst/SaO_2$ slope in response to either transient or step change hypoxia per body surface area did not alter the significance of the relationships with the patients PaO_2 values. However, the PaO_2 of the patients did correlate ($p<0.01$) with the ventilatory response to step change hypoxia of their offspring expressed as the slope of the $\dot{V}_E inst/SaO_2$ relationship (Fig 8.1). The values assigned to the model parameters ranged from -1.45 to 4.17 $lmin^{-1}\%^{-1}$ for gain 1, from -1.83 to 3.85 $lmin^{-1}\%^{-1}$ for gain 2, and from 1 to 588 sec for tau 2. A negative value was required for the inhibition parameter, I in two subjects (range -0.05 to -0.26 $(lmin^{-1})^{-1}$) and a positive value in 15 subjects (range 0.08 to 0.62 $(lmin^{-1})^{-1}$). Sigma was required in 14 subjects and ranged from 0.11 to 3.92%. The individual values for all parameters are given in table 8.3. The PaO_2 of the patients did not correlate with the values for gain 1 derived in their offspring (Fig 8.2), but did correlate with the values for gain 2 ($p<0.05$, Fig 8.2). Stepwise multiple regression analysis showed that the PaO_2 of the patients was best predicted by a combination of both gain 1 and gain 2 of their offspring ($p<0.01$) as described by the equation:

$$PaO_2 \text{ (patient)} = (0.35\pm0.14) \text{ gain 2} + (0.30\pm0.15) \text{ gain 1} + (6.08\pm0.35)$$

Table 8.1 Mean SaO_2 of the transient hypoxic stimuli (trans) and lowest 12% O_2 step change SaO_2 (step) for each offspring and control subject

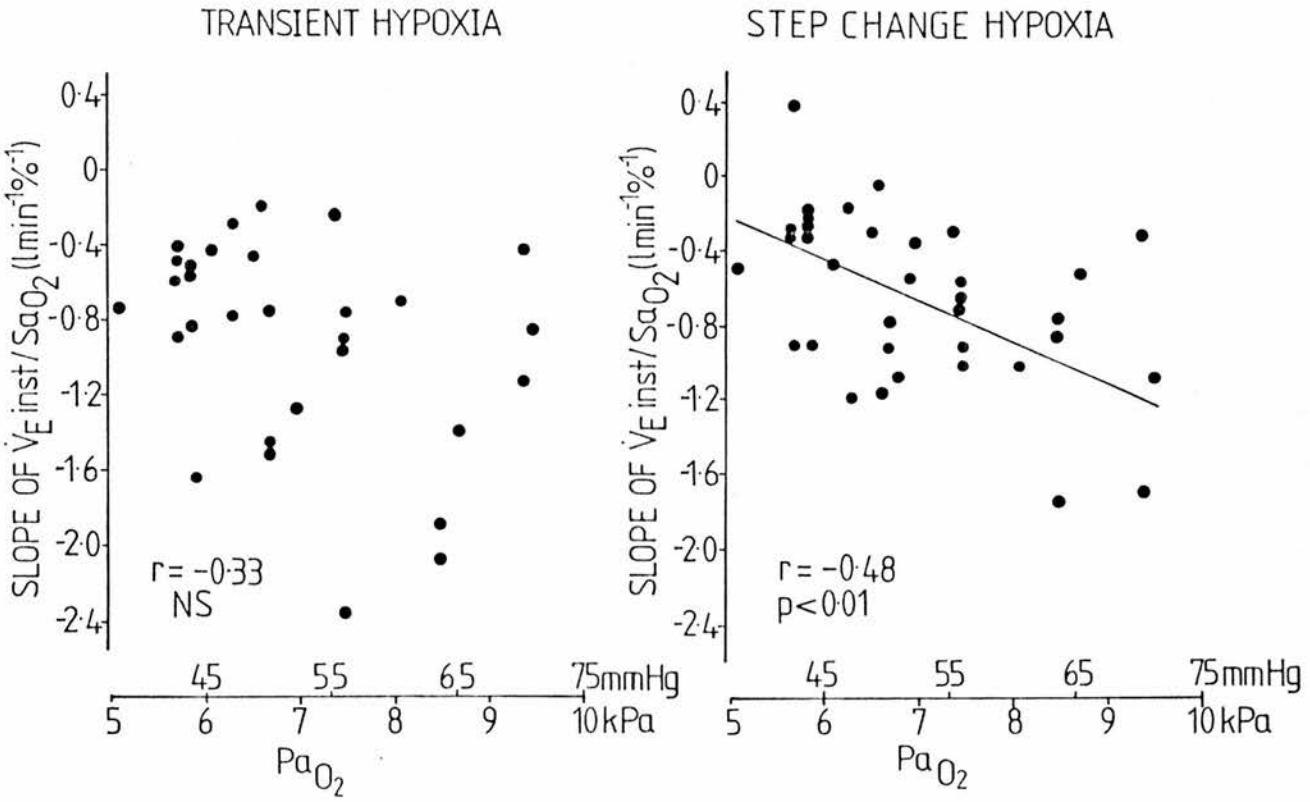
	OFFSPRING		CONTROLS	
Subject number	trans (%)	step (%)	trans (%)	step (%)
1	88	76	87	81
2	88	77	86	83
3	-	76	88	85
4	87	83	84	81
5	83	85	87	86
6	87	77	86	83
7	90	77	87	87
8	87	84	85	83
9	89	76	86	83
10	-	87	88	77
11	89	84	86	85
12	86	83	85	86
13	86	81	86	82
14	89	84	-	87
15	89	85	90	84
16	90	86	-	87
17	89	89	87	84
18	87	85	85	84
19	88	89	88	81
20	88	84	87	83
21	89	82	86	81
22	86	86	85	86
23	86	89	-	83
24	85	88	89	86
25	83	86	88	84
26	87	87	87	80
27	-	87		
28	89	87		
29	87	85		
30	83	82		
31	88	85		
32	90	87		
33	85	84		
34	89	79		
35	88	86		

Table 8.2 $\dot{V}_E inst/SaO_2$ slopes for transient and step change hypoxia for offspring and control subjects.

Subject number	OFFSPRING transient	OFFSPRING step	CONTROLS transient	CONTROLS step
1	-2.36	-0.73	-0.73	-0.66
2	-0.97	-0.68	-1.18	-0.52
3	-	-1.10	-1.98	-1.00
4	-1.89	-1.75	-1.12	-0.41
5	-0.87	-1.10	-1.24	-0.74
6	-0.24	-0.31	-0.87	-1.12
7	-1.28	-0.37	-1.04	-1.10
8	-0.75	-0.80	-1.14	-1.04
9	-0.43	-0.48	-0.14	-0.18
10	-	-0.79	-0.93	-0.44
11	-0.42	+0.37	-0.68	-0.62
12	-2.07	-0.89	-0.67	-0.81
13	-1.15	-1.71	-0.81	-0.44
14	-1.40	-0.54	-	-1.21
15	-0.53	-0.36	-0.93	-1.14
16	-0.83	-0.30	-	-1.59
17	-0.44	-0.33	-1.06	-0.88
18	-0.56	-0.20	-1.62	-1.25
19	-1.52	-1.18	-0.52	-0.08
20	-0.73	-0.51	-0.74	-0.81
21	-1.48	-0.96	-1.80	-0.49
22	-0.94	-1.21	-1.14	-1.23
23	-0.71	-1.06	-	-0.27
24	-0.76	-0.95	-0.84	-0.30
25	-0.85	-0.37	-0.94	-0.62
26	-1.64	-0.93	-0.69	-0.74
27	-	-0.58		
28	-0.92	-0.61		
29	-0.49	-0.37		
30	-0.78	-1.22		
31	-0.29	-0.21		
32	-0.60	-0.32		
33	-0.39	-0.08		
34	-0.46	-0.33		
35	-0.90	-0.92		

$\dot{V}_E inst/SaO_2$ slopes expressed as $l min^{-1}\%^{-1}$

Fig 8.1 : Relationship between the PaO_2 of patients with COPD and the $\dot{V}_E inst/SaO_2$ slopes measured in their offspring.



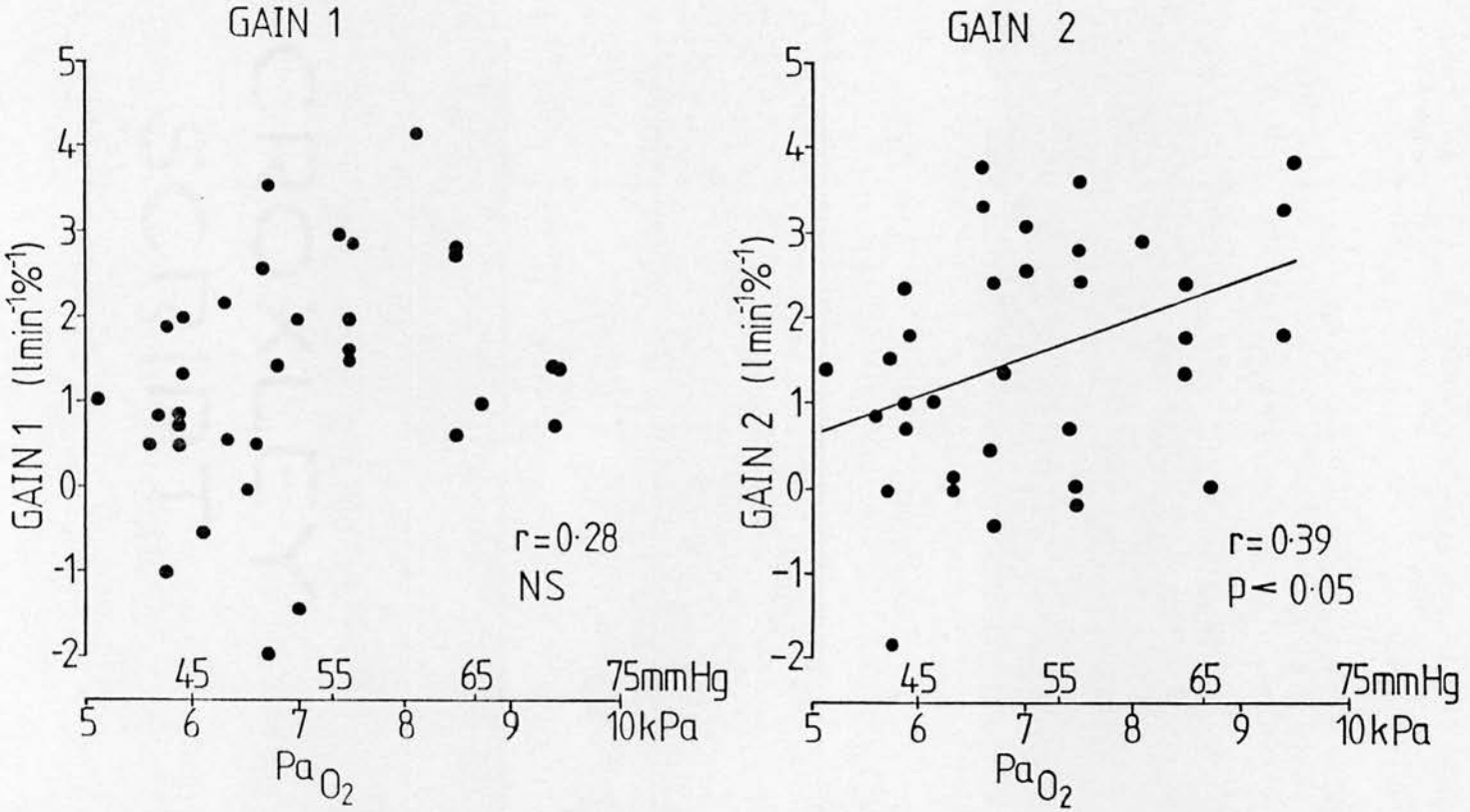
The PaO_2 of the patients is plotted against the hypoxic ventilatory response, expressed as the $\dot{V}_E inst/SaO_2$ relationship in response to transient (left hand panel) and step change hypoxia (right hand panel) of their offspring. Each point represents the response measured in an individual subject. The PaO_2 of the patients did not correlate with the response to transient hypoxia but did correlate with that to step change hypoxia ($p<0.01$).

Table 8.3 Model parameter values for each of the offspring group

Subject number	Gain 1 $l min^{-1}\%^{-1}$	Inhibition $(l min^{-1})^{-1}$	Tau 2 sec	Gain 2 $l min^{-1}\%^{-1}$	Sigma %
1	1.95	*	3	0.01	*
2	1.56	*	14	-0.16	*
3	1.41	*	209	1.38	*
4	2.70	*	31	1.34	*
5	1.48	*	36	3.85	0.17
6	2.95	0.09	1	0.71	*
7	-1.45	*	2	2.55	*
8	1.96	*	4	-0.44	*
9	-0.51	0.17	1	1.00	*
10	0.56	*	182	2.42	*
11	-1.02	*	588	1.52	*
12	2.75	0.23	50	1.79	*
13	1.43	*	46	3.26	*
14	0.96	*	50	0.04	0.20
15	0.80	*	159	0.71	0.20
16	0.70	0.08	75	0.98	0.72
17	0.71	*	256	1.82	*
18	0.45	0.41	28	2.31	3.92
19	2.54	*	180	0.45	0.50
20	1.01	*	32	1.38	0.23
21	3.55	0.19	16	2.45	*
22	2.89	0.18	4	2.48	*
23	4.17	0.21	37	2.97	*
24	1.41	0.22	58	2.85	*
25	1.31	-0.05	40	1.82	0.22
26	1.99	*	173	1.03	0.29
27	1.95	0.08	56	3.13	0.11
28	1.59	-0.26	93	3.59	0.53
29	0.83	0.62	240	0	*
30	2.13	0.45	27	0.18	*
31	0.51	0.52	204	0.04	*
32	0.49	0.10	28	0.88	0.34
33	0.49	*	43	3.78	0.25
34	-0.06	0.18	40	3.31	0.24
35	1.86	*	318	-1.83	*

* represents parameter not required to produce the model of best fit.

Fig 8.2 : Relationship between the PaO_2 of the patients with the model gain parameters derived in their offspring.



The PaO_2 of the patients is plotted against the hypoxic ventilatory response, expressed as the model parameters, gain 1 (left hand panel) and gain 2 (right hand panel) of their offspring. Each point represents the gain value derived for an individual subject. The PaO_2 of the patients did not correlate with gain 1 but did correlate with the gain 2 values of their offspring ($p<0.05$).

ii) Control subjects

Values for baseline normoxic $\dot{V}_E$, $\dot{V}O_2$ and $\dot{V}CO_2$ in individual subjects are given in Appendix table 24. The mean $\dot{V}O_2$ was 0.79 $lmin^{-1}$ (s.d. 0.17 $lmin^{-1}$, range 0.50 to 1.19 $lmin^{-1}$), and when expressed per kg body weight was 11.2 $mlmin^{-1}kg^{-1}$ (s.d. 1.6 $mlmin^{-1}kg^{-1}$, range 9.6 to 17.0 $mlmin^{-1}kg^{-1}$. The RQ value was less than 1.0 in all subjects. For each individual there was no significant difference between the mean baseline normoxic $P_{ET}CO_2$ and the mean values during either transient or step change hypoxia, and the standard deviation of $P_{ET}CO_2$ about the mean during hypoxia ranged from 0.09 to 0.32kPa (Appendix table 26). There was also no significant difference between the mean lowest SaO_2 in response to either transient or step change hypoxia in an individual (table 8.1).

A response to transient hypoxia could not be measured in three subjects for technical reasons. The slope of the $\dot{V}_E inst/SaO_2$ relationship in response to transient hypoxia ranged from -0.14 to -1.98 $lmin^{-1}\%^{-1}$, and in response to step change hypoxia ranged from -0.08 to -1.59 $lmin^{-1}\%^{-1}$ (table 8.2). The values assigned to the model parameters ranged from 0.24 to 3.94 $lmin^{-1}\%^{-1}$ for gain 1, -2.27 to 3.09 $lmin^{-1}\%^{-1}$ for gain 2, and 1 to 367 sec for tau 2. A negative value was required for the inhibition parameter I in 4 subjects (range -0.48 to -0.06 $(lmin^{-1})^{-1}$), and a positive value in 14 subjects (range 0.04 to 0.91 $(lmin^{-1})^{-1}$). Sigma was required in

15 subjects and ranged from 0.14 to 0.83 %. The individual values for all model parameters are given in table 8.4.

iii) Comparison of Offspring and Controls

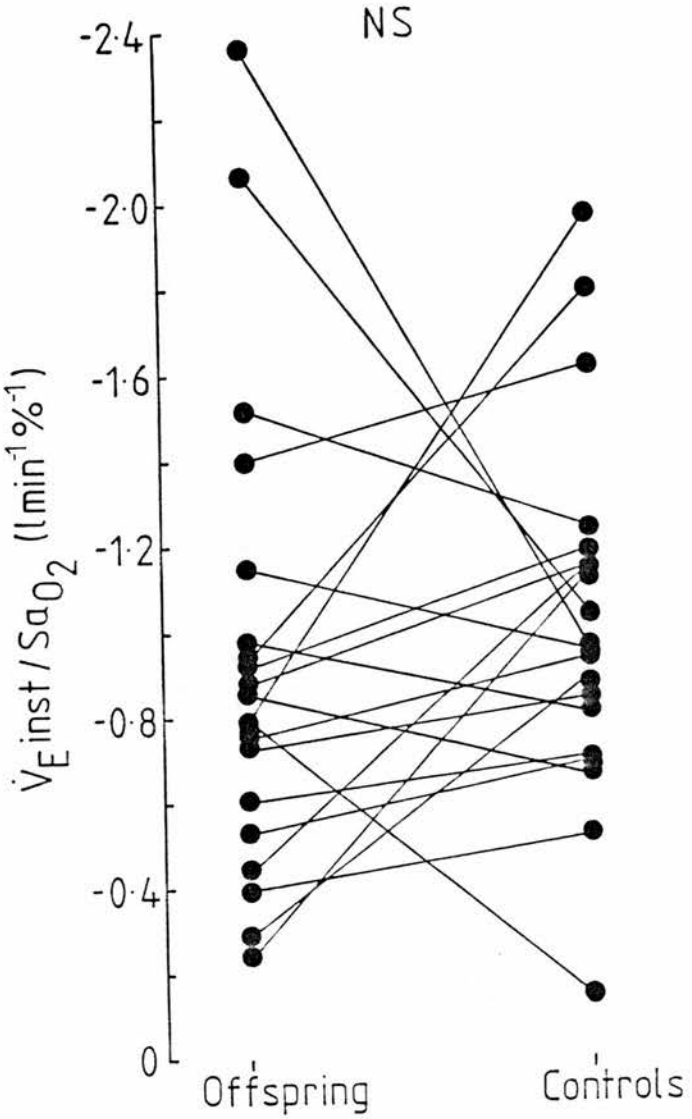
The nine offspring subjects who were not age and sex-matched with control subjects demonstrated hypoxic ventilatory response (expressed as either $\dot{V}_E inst/SaO_2$ slopes or model gain parameters) throughout the range of the 26 subjects for whom suitable control subjects were available. The two groups were correctly age-matched, as shown by no significant difference between the ages of the offspring and control groups. The two groups of subjects exercised at similar exercise levels as there was no significant difference between $\dot{V}O_2$ or $\dot{V}O_2/kg$. There was no significant difference between the hypoxic ventilatory response of offspring and control groups expressed as transient hypoxia $\dot{V}_E inst/SaO_2$ slope, step change hypoxia $\dot{V}_E inst/SaO_2$ slope, gain 1 or gain 2 (Figs 8.3 to 8.6).

Table 8.4 Model parameter values for each of the control group

Subject number	Gain 1 $l min^{-1}\%^{-1}$	Inhibition $(l min^{-1})^{-1}$	Tau 2 sec	Gain 2 $l min^{-1}\%^{-1}$	Sigma %
1	0.88	*	137	2.56	0.34
2	3.37	0.42	3	0.67	*
3	2.54	0.04	51	1.18	0.29
4	2.87	0.11	10	2.83	0.14
5	1.83	*	110	1.66	0.52
6	2.50	0.26	18	2.20	*
7	2.01	0.30	27	2.00	*
8	2.29	-0.11	155	2.54	0.83
9	0.24	*	38	3.09	0.42
10	1.42	0.06	5	2.83	0.30
11	2.40	0.50	11	1.16	*
12	1.25	-0.48	256	2.22	0.79
13	1.44	*	367	-2.27	*
14	1.59	0.17	38	2.85	*
15	2.06	*	80	2.17	0.22
16	2.77	*	46	1.29	*
17	3.10	0.64	23	1.99	*
18	2.59	-0.06	216	2.65	0.23
19	1.68	0.65	26	1.43	0.23
20	0.95	-0.17	32	2.94	0.76
21	3.94	0.09	1	2.21	0.17
22	1.66	*	81	2.99	*
23	0.30	0.65	183	0.62	0.55
24	0.45	*	245	2.11	0.63
25	3.26	0.91	39	1.68	*
26	2.22	0.17	17	1.76	*

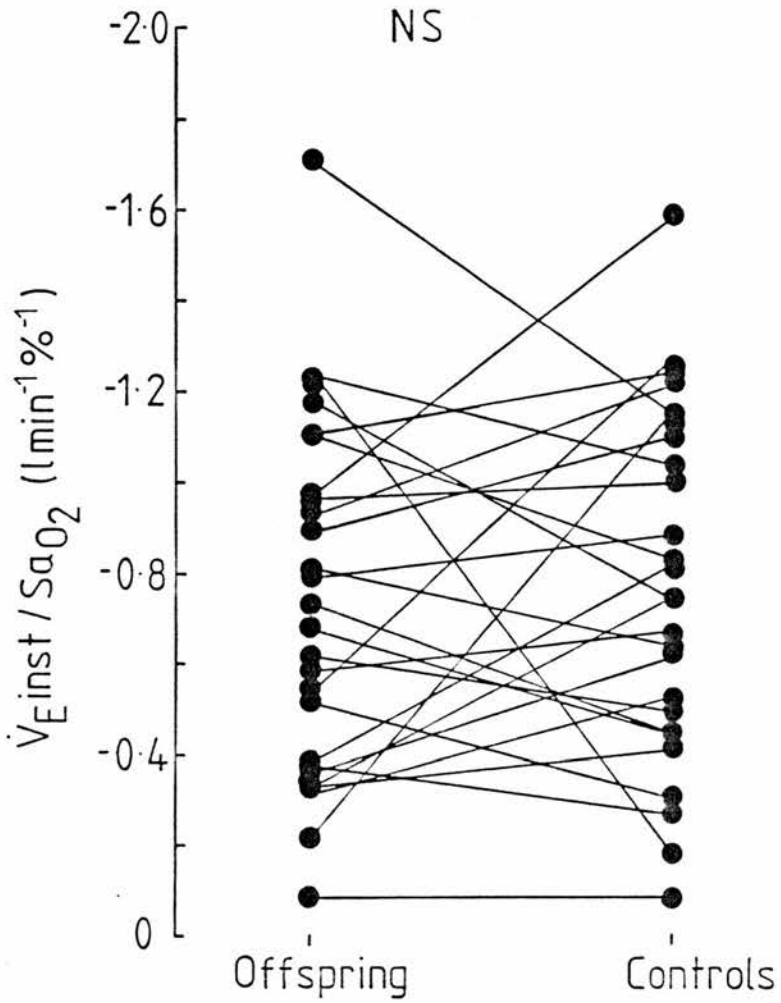
* represents parameter not required to produce the model of best fit.

Fig 8.3 : Comparison of $\dot{V}_E inst/Sa_{O_2}$ slopes in response to transient hypoxia in age and sex-matched pairs of offspring and control subjects.



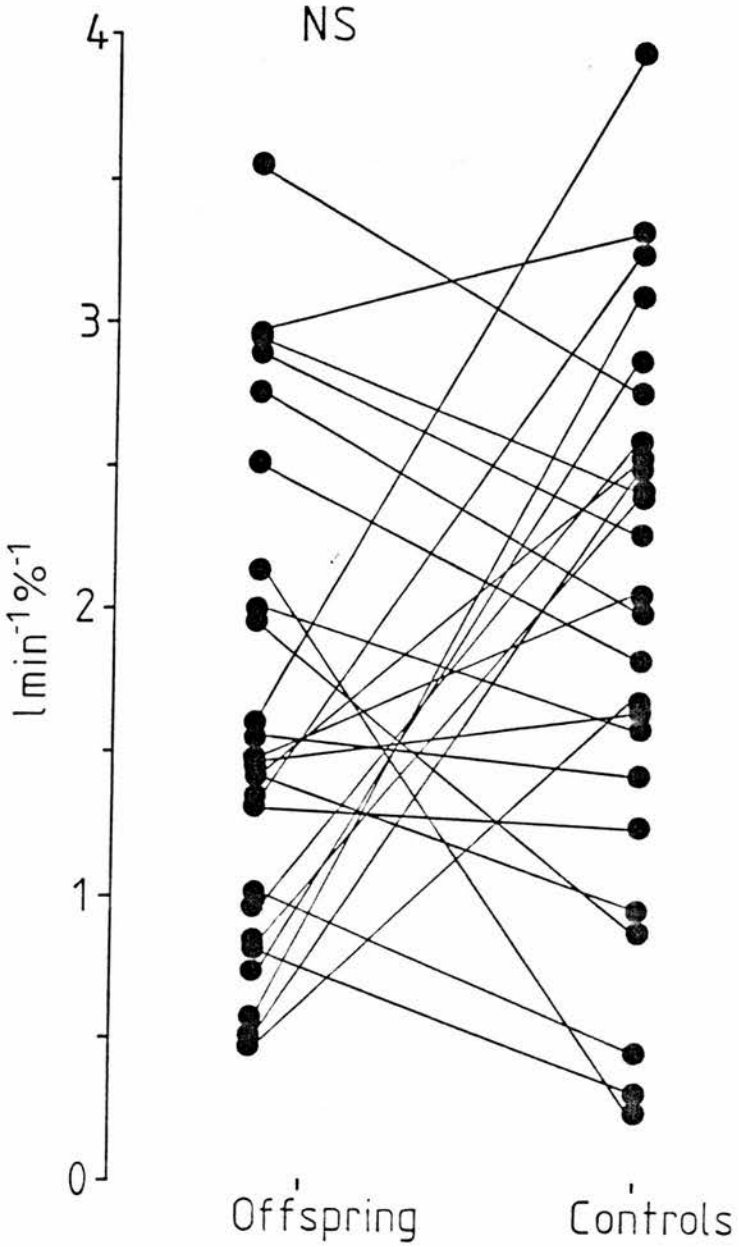
Each point represents the $\dot{V}_E inst/Sa_{O_2}$ slope for each offspring (left side) or control (right side) subject in response to transient hypoxia. Each age and sex-matched pair of offspring and control subjects are joined by a line. There was no statistical difference between the ventilatory response to transient hypoxia of the two groups of subjects.

Fig 8.4 : Comparison of $\dot{V}_E inst/SaO_2$ slopes in response to step change hypoxia in age and sex-matched pairs of offspring and control subjects.



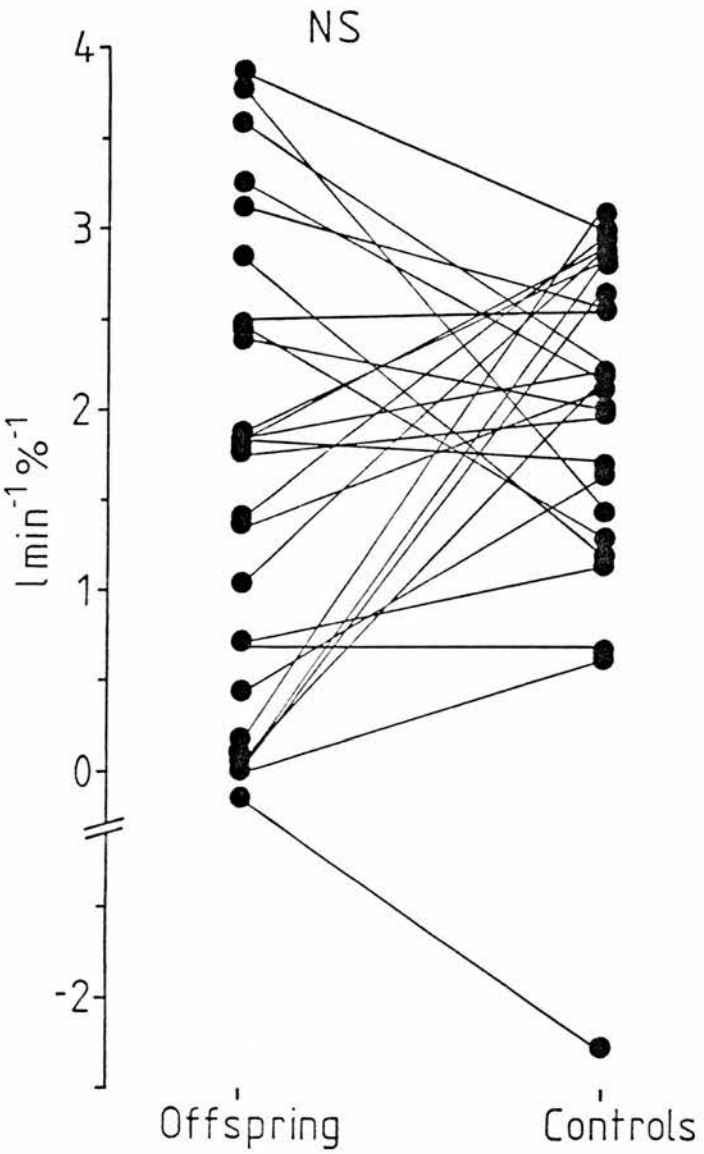
Each point represents the $\dot{V}_E inst/SaO_2$ slope for each offspring (left side) or control subject (right side) in response to step change hypoxia. Each age and sex-matched pair of offspring and control subjects are joined by a line. There was no statistical difference between the ventilatory response to step change hypoxia of the two groups of subjects.

Fig 8.5 : Comparison of gain 1 values in age and sex-matched pairs of offspring and control subjects.



Each point represents the gain 1 value for each offspring (left hand side) or control subject (right hand side). Each age and sex-matched pair of offspring and control subjects are joined by a line. There was no statistical difference between the gain 1 values for the two groups of subjects.

Fig 8.6 : Comparison of gain 2 values in age and sex-matched pairs of offspring and control subjects.



Each point represents the gain 2 value for each offspring (left side) or control subject (right side). Each age and sex-matched pair of offspring and control subjects are joined by a line. There was no statistical difference between the gain 2 values for the two groups of subjects.

IV DISCUSSION

This study has shown that the PaO_2 of patients with COPD, when in a stable state and breathing air, correlated with estimates of the hypoxic ventilatory response measured in their first generation offspring. These results confirm previous findings (Kawakami et al 1982a, Fleetham et al 1984). Furthermore, there was no significant difference between the intensity of the hypoxic ventilatory response measured in the offspring subjects and that recorded in a group of age and sex-matched control subjects whose parents did not have COPD.

Patients with the "blue and bloated" form of COPD have been shown to have blunted hypoxic ventilatory responses whereas an intact response has been reported in patients with the "pink and puffing" form of the disease (Flenley et al 1970, Bradley et al 1979). This led to the hypothesis that a genetically determined (Scoggin et al 1978) low premorbid ventilatory response may predispose an individual with COPD to develop severe hypoxaemia (Flenley 1978). The correlation between the PaO_2 of patients with COPD and estimates of the hypoxic ventilatory response in their offspring found in both this and previous studies (Kawakami et al 1982a) supports this hypothesis.

No previous attempts have been made to determine whether the spectrum of hypoxaemia in COPD can be explained by the wide normal variation in the hypoxic

ventilatory response. Kawakami et al (1982a) found a significant relationship between the PaO_2 of 25 patients with COPD and the hypoxic ventilatory response of their 34 sons whereas no correlation could be detected between the PaO_2 of 17 patients with silicosis and the response of their 22 sons. They did not, however compare the hypoxic ventilatory rsponses of the two offspring groups. In this study, the hypoxic ventilatory response values obtained for both the offspring and control subjects fell within the normal range measured using a rebreathing technique by Rebuck and Campbell (1974). This, coupled with the fact that there was no significant difference between the responses recorded in the offspring and the age and sex-matched control subjects, implies that the offspring reflect the normal variability and are not a sub-group of the population with abnormally low responses. Thus the degree of hypoxaemia developed in COPD appears to be related to the genetically determined premorbid intensity of the hypoxic ventilatory response.

In spite of the significant correlation between the PaO_2 of the patients and the hypoxic ventilatory response of their offspring, this relationship only accounted for approximately 25% of the variance in the degree of hypoxaemia of the patients. Several experimental factors may have contributed to the unexplained variance. The subjects were not requested to fast for a specified time before each study. Thus in

some individuals the response may have been affected by eating (Zwillich et al 1977) or drinking coffee (Lakshminarayan et al 1970). However, these effects were probably minimal as the subjects would have abstained from food or beverages for at least one hour prior to the study. A more significant effect may have resulted from the fact that the hypoxic ventilatory response was measured on only one occasion in each subject, and not all subjects were studied at the same time of day. The hypoxic ventilatory response varies both within and between days (Sahn et al 1977) and the single measurement may not represent the true value. However, as discussed in Chapter 4, even though the extent of the between-day variability was 19 to 45 %, between-subject differences were apparent. Furthermore, 15 of the 35 offspring subjects were females aged between 18 and 47 years. In women, the hypoxic ventilatory response has been shown to be 43% greater in the luteal phase compared with the follicular phase of the cycle (White et al 1983) and significantly less than in men. Hormonal differences will therefore have influenced the correlation between patients' PaO_2 and offspring hypoxic ventilatory response to an unknown extent. However, there was no difference between the male and female hypoxic ventilatory responses in this study, and as discussed in Chapter 4, the ventilatory responses of the female subjects studied were no less reproducible than those of the male subjects.

The hypoxic ventilatory response was measured during exercise so that an adequate transient hypoxic stimulus could be obtained with only 2-3 breaths of 1% O_2 in N_2. However, use of exercise may have contributed to the variance for two reasons. Firstly, $P_{ET}CO_2$ was assumed to equal $PaCO_2$ and this may not be correct. However, as discussed in Chapter 4, Jones et al (1966) and Whipp and Wasserman (1969) have shown that at mild to moderate exercise levels, as in this study, the differences between $P_{ET}CO_2$ and $PaCO_2$ are small and probably contribute little to the variation in hypoxic ventilatory response. Secondly, the hypoxic ventilatory response is potentiated by exercise (Weil et al 1972, Martin et al 1978) and the degree of potentiation may vary between subjects. However, in this study the level of exercise was similar in all subjects expressed as the $\dot{V}O_2$/kg and expression of the hypoxic ventilatory response per m^2 body surface area did not change the significance of the relationship with the patients' PaO_2. Also, as shown in Chapter 7, mild exercise at this level caused similar degrees of potentiation in all subjects. Therefore, whilst small differences in potentiation between subjects may have contributed to the unexplained variance, the observed correlation between the PaO_2 of the patients and the hypoxic ventilatory response of their offspring is unlikely to be due to the degree of exercise potentiation in each individual.

Another source of variability was that only one parent with COPD contributed to the genetic make-up of each offspring subject. In some cases the hypoxic ventilatory responses of siblings were almost identical whereas in other families, the responses were diverse. However, Collins et al (1978) showed that the hypoxic ventilatory response was almost identical between pairs of homozygous twins but not between pairs of heterozygous twins.

The major difficulty in interpreting these results and those of similar studies (Kawakami et al 1982a) arises from the selection of the PaO_2 of the patients. In this study, a single PaO_2 value was taken for each patient when they were in a stable state breathing air. However, little is documented on the natural history of the development of hypoxaemia. Middleton et al (1979) reported a mean annual decline in PaO_2 of only 0.02 kPa in 85 patients with stable COPD. This would account for a fall of only 0.4 kPa on average in an individual patient assuming a twenty year history of the disease which would have little effect on the observed correlation. The steady age-related decline in PaO_2 of 0.4-0.5 kPa per decade (Mellemgaard et al 1966, Sorbini et al 1968) would also have had little effect between subjects on the single PaO_2 values used for each patient as 21 of the 24 patients were aged between 60 and 75 years. Middleton et al (1979) also found that hypoxaemic patients with COPD suffer a terminal rapid decline in

PaO_2, possibly due to pathological changes in the lungs. It is unlikely that the values selected for any of the patients in this study were affected by this terminal fall, since the value selected was within 0.5 kPa of the previous measurement made within the preceding twelve months. However, the interpretation assumes that patients become, and remain, either "pink puffers" or "blue bloaters". Factors other than the hypoxic ventilatory response may also reduce the PaO_2 in COPD. The degree of airways obstruction may affect the PaO_2 although no correlation could be demonstrated in this study between the degree of airways obstruction (FEV_1 or FEV_1/FVC) and the PaO_2 breathing air, therefore the extent of airways obstruction was not the major factor governing the PaO_2. Wagner et al (1977) demonstrated different $\dot{V}_A/Q$ patterns in patients with the "pink and puffing" and "blue and bloated" forms of COPD but showed that the measurement was not definative. Thus some of the variability of the PaO_2 measurements may be due to ventilation-perfusion inequalities of the COPD patients. Also, the reduced hypoxic ventilatory response in patients with COPD may have developed as a result of the chronic hypoxaemia due to peripheral chemoreceptor adaptation. Such peripheral chemoreceptor adaptation is thought to occur in high-altitude natives during the first two years of life probably due to an irreversible desensitisation of the peripheral chemoreceptor response to hypoxia (Sorensen and Severinghaus 1968, Weil et al

1981). Further information is therefore required regarding the natural history of the disease before the degree of hypoxaemia due to a blunted hypoxic ventilatory response or other factors can be established.

Finally, the initial hypothesis proposed that, since the increase in ventilation in response to hypoxia is mediated by the peripheral carotid bodies (Lugliani 1971), individual differences in the premorbid peripheral chemoreceptor sensitivity were responsible for determining the severity of hypoxaemia in COPD (Flenley et al 1978). Such individual variation in the carotid chemoreceptor sensitivity has been demonstrated in cats (Vizek et al 1987), but is difficult to quantitate in humans. Previous studies comparing the PaO_2 of patients with COPD to the hypoxic ventilatory response of their offspring have used the progressive isocapnic hypoxia method (Scoggin et al 1978, Collins et al 1978, Kawakami et al 1982a, Fleetham et al 1984) which may cause central depression of ventilation in some subjects (Weil and Zwillich 1976, Easton et al 1986). This study has shown that the PaO_2 of the patients was best predicted by a combination of the gains of the two equations of the mathematical model. If the equation with the fast time constant quantifies the peripheral chemoreceptor response whilst the slow time constant equation reflects a combination of peripheral and central activity, then the results suggest that some

central regulation, possibly central depression of ventilation, may also be involved in determining the PaO_2 in COPD. Although central depression of ventilation has been demonstrated in conscious humans (Easton et al 1986, 1988), the variability in the normal population has not been assessed. Investigation of the effect of prolonged hypoxia, similar to that described by Anthonisen's group (Easton and Anthonisen 1988b; Easton et al 1986, 1988) both in the offspring of patients with COPD and in age and sex-matched control subjects, as used in this study, would help to elucidate the role of central hypoxic depression of ventilation in the pathogenesis of the "blue and bloated" form of COPD.

SUMMARY AND CONCLUSIONS

A computer based two-compartment mathematical model has been used to analyse the dynamic ventilatory response to hypoxia and to quantitate both peripheral and central components of the response. The model consists of two linear differential equations (I and II) in parallel; both using the fall in SaO_2 as the input, with the summed output (I + II) giving the rise in $\dot{V}_E$ during hypoxia above that in normoxic exercise. Equation I has a fast time constant (< 3 sec) and equation II has a slower time constant (> 3 sec). Each equation incorporates a time constant and a gain. Non-linearities also included are a "saturating effect" which reduces the gain of equation II as SaO_2 falls, and "inhibition" or "potentiation" of the gain of equation I as the output of equation II increases. The model was derived empirically to fit the hypoxic ventilatory responses of ten subjects to transient and step change hypoxia at one particular exercise level.

The aims of this project were to assess the repeatability of the mathematical model parameter values obtained in an individual, to investigate the physiological interpretation of the model structure and to use the model to study the role of the hypoxic ventilatory response in determining the severity of hypoxaemia developed by patients with chronic obstructive pulmonary disease.

In order to assess the effects of physiological

changes upon the model parameter estimation, reproducibility of the model parameter estimation, the effect of the rate of onset of an hypoxic stimulus, the effect of increasing exercise level and the effect of the adenosine antagonist theophylline upon the hypoxic ventilatory response were studied.

The hypoxic ventilatory response expressed either as the $\dot{V}_E inst/SaO_2$ relationship or as the mathematical model parameters was measured ten times in four normal subjects over a period of at least ten weeks and was reproducible with no significant within-subject differences, but with significant between-subject differences present. Also, the model gain 1 and gain 2 parameters significantly correlated with the $\dot{V}_E inst/SaO_2$ relationship in response to step change hypoxia for each subject, suggesting that these parameters reflect the intensity of the hypoxic ventilatory response. Therefore, the model parameters are reproducible within a subject and allow identification of differences in the hypoxic ventilatory response between individuals.

No effect could be demonstrated of the rate of onset of an hypoxic stimulus (rate of onset range 17-50 sec) upon the hypoxic ventilatory response measured as the $\dot{V}_E inst/SaO_2$ relationship or as the model parameters. An increase in the $\dot{V}_E inst/SaO_2$ relationship in response to step change hypoxia as the level of exercise rose and also a concomitant increase in the model gain parameters were demonstrated. This increase in the model gain

parameters indicates that they are capable of reflecting physiological changes. The increase in the hypoxic ventilatory response may be due to increased peripheral chemosensitivity, increased muscle afferent activity or circulating chemical mediators such as noradrenaline acting centrally. Further studies are necessary to investigate the mechanism of ventilatory stimulation during exercise, such as measurement of noradrenaline levels in the blood before, during and after hypoxic exercise, and investigation of the effect of a lack of muscle afferent activity in patients with spinal cord lesions upon the hypoxic ventilatory response.

The ventilatory stimulant theophylline which acts centrally as an adenosine antagonist had no consistent effect upon the hypoxic ventilatory response whether expressed as the $\dot{V}_E inst/SaO_2$ relationship or as the model gain parameters. However, only six subjects were studied, and in two of them the plasma theophylline level was outside the therapeutic range. Further studies are therefore required with greater numbers of subjects and therapeutic levels of theophylline to investigate the effect of theophylline on the model parameters.

Previous work had suggested that the hypoxaemia seen in patients with the "blue and bloated" form of COPD develops as a result of diminished peripheral chemoreceptor mediated ventilatory drive and this may be an inherited characteristic, which exists premorbidly. However, the PaO_2 of patients with COPD studied

correlated with the hypoxic ventilatory response of their offspring measured as the $\dot{V}_E$inst/SaO_2 relationship to step change hypoxia, or as a combination of both gain 1 and gain 2 parameters. If gain 1 represents the peripheral response, and gain 2 the combined peripheral and central response, then both peripheral and central mechanisms of the hypoxic ventilatory response may be inherited and a low premorbid ventilatory response may make an individual susceptible to the "blue and bloated" form should COPD develop. The hypoxic ventilatory responses of the group of offspring subjects (expressed as either the $\dot{V}_E$inst/SaO_2 relationship or as the model gain parameters) fell within the normal wide range of a group of age and sex-matched control subjects whose parents did not have COPD. Thus the responses of the offspring group demonstrated the variability of the normal population and did not form a distinct group with abnormally low responses. These results support the hypothesis that a genetically determined pre-morbid intensity of the hypoxic ventilatory response partly determines the PaO_2 in COPD.

Further work is necessary to conclusively determine the physiological mechanisms reresented by each of the model components, such as using the specific peripheral chemoreceptor stimulant almitrine or inducing central depression of ventilation with prolonged hypoxia. It is also necessary to determine to what extent the severity of hypoxaemia developed by patients with COPD is due to

blunted peripheral chemoreceptor sensitivity or central depression of ventilation in response to hypoxia by investigating the variability of the intensity of central hypoxic depression within a normal population and comparing it with that of offspring of patients with COPD.

APPENDIX A

MODELLING THE DYNAMIC VENTILATORY RESPONSE TO HYPOXIA IN NORMAL SUBJECTS

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Running Head: Modelling the dynamic hypoxic ventilatory response.

ABSTRACT

We have developed a mathematical model to describe the dynamic ventilatory response to hypoxia. The ventilatory response to both transient and step change hypoxic stimuli were measured in 11 normal subjects during moderate exercise ($\dot{V}_{O2}$ 0.96 ± 0.08 l min^{-1}). The simplest model which adequately described the responses in all subjects consisted of two linear differential equations (1 and 2) in parallel; both using the fall in arterial oxygen saturation as input, and with the outputs summed (1+2) to give the rise in ventilation. Equation 1 had a fast time constant (<3 sec), and equation 2 a slow time constant. Non-linear terms included were a) a "saturating" effect, similar to that described by the Michaelis-Menten equation, reducing gain 2 as oxygen saturation falls, and b) "inhibition" or "potentiation" of gain 1 as the output of equation 2 increased. Repeated measurements in four subjects showed that parameter estimation was reproducible in an individual. The model gives a more precise description of the hypoxic ventilatory response than the linear ventilation/oxygen saturation relationship.

Index Terms: hypoxic ventilatory control, mathematical model, transient hypoxic stimuli, step change hypoxic stimuli.

INTRODUCTION

Wide individual variation in the carotid body mediated hypoxic ventilatory drive (18) has been shown in normal subjects (22). Studies in humans (3,15) and animals (26) suggest that this may arise from genetic differences in the carotid chemoreceptor sensitivity to hypoxia. It has been proposed that this inherited sensitivity to hypoxia may relate to the pathogenesis of the two forms of chronic obstructive pulmonary disease; those individuals with genetically low drives developing the "blue and bloated" syndrome characterised by hypoxemia, CO2 retention, pulmonary hypertension, and right heart failure, and those with brisk drives tending to become "pink and puffing" with relatively well preserved arterial blood gas tensions (10), despite similar severity of airflow obstruction. Patients with the "blue and bloated" syndrome have a poor prognosis (28), so that identification of individuals at risk, either premorbidly or at an early stage of their disease, may aid in their clinical management.

Conventional methods of assessing hypoxic ventilatory drive using either steady state (17) or progressive isocapnic hypoxia (29) may underestimate the peripheral chemoreceptor response as central depression of ventilation may result from the relatively prolonged hypoxia (7). To overcome this, transient hypoxic (8,12,16,25) or hyperoxic (5,11) methods have been used. A good correlation has been shown between the responses

to transient and to progressive isocapnic stimuli (25). However we have found that, during moderate exercise, the response to transient hypoxic stimuli expressed as the linear relationship between minute ventilation and ear (arterial) oxygen saturation (21) did not accurately reflect chemoreceptor activity alone (1). Furthermore, the relationship between minute ventilation and oxygen saturation in response to rapid onset, short duration transient stimulus was less than to slower onset, more prolonged hypoxia, and the two responses could not be described adequately by a single linear system (27).

Mathematical models have been used to describe the ventilatory response to O2 (23) and CO2 (2,13,24). However, modelling techniques have not previously been used to investigate the dynamics of the hypoxic ventilatory controller. We have therefore developed a mathematical model to relate minute ventilation to oxygen saturation. This was systematically developed to fit the observed responses of normal subjects to both transient and more prolonged hypoxia. The model parameters give a more precise description of the individual's response to hypoxia than the simple linear relationship between ventilation and oxygen saturation allowing investigation of the physiological mechanisms involved in the ventilatory response to hypoxia.

EXPERIMENTAL METHODS

Subjects

The 11 subjects (7M,4F; age 21-40 years) were drawn from laboratory staff who had no previous history of cardiovascular or respiratory disease, and who were taking no current medication. All had a normal FEV1, lung volumes, and DLCO. The model was developed from results in subjects 1-10 and repeated measurements were subsequently made in subjects 1,6,10 and 11. All were non-smokers except for subject 1, who occasionally smoked cigarettes. The subjects fasted for at least four hours prior to a study. They each gave informed consent to the study after the nature and purpose had been explained. The study was approved by the Ethics of Medical Research Sub-Committee of the Lothian Health Board.

Measurements of Ventilatory Variables

The subjects breathed through a mouthpiece and custom built two-way respiratory valve with a total deadspace of 100 ml. The valve had two inspiratory ports operated by a remote control switch which allowed for rapid change of inspired gas without the subject's knowledge. Inspiratory and expiratory flows were recorded with separate pneumotachographs (Fleisch no. 2), and expiratory flow integrated to give expiratory tidal volume. Expired gas passed via the pneumotachograph through a 3.2 litre mixing chamber to a Parkinson Cowan CD4 gas meter modified to give a digital electrical

output of expired volume. Inspiratory and end-tidal PO2 (PET_{O2}) and PCO2 (PET_{CO2}) were measured at the lips by a mass spectrometer (VG Medical Spectralab-M, Middlewich, England, U.K.) previously calibrated using five gas mixtures of known O2, CO2, N2 and Argon concentrations. The inspiratory and end-tidal gas concentrations were calculated from the concentrations of the four gases summed to 100% and adjusted for barometric pressure, after allowing for the delay in the mass spectrometer response which was measured prior to each study. Ear oxygen saturation (Sa_{O2}; Hewlett Packard 47201A ear oximeter), and heart rate (Hewlett Packard 78351A ECG monitor) were monitored throughout the study. Signals from the pneumotachographs, CD4 gas meter, thermistor in the expiratory line, mouth pressure micromanometer (Furness Controls Ltd) to determine end-expiration, mass spectrometer, and ECG monitor were sampled on-line by a PDP 11/23 computer (Digital Equipment Corporation). The integrated expiratory flow signal was calibrated against the output of the CD4 gas meter every 10 litres. Custom written programs provided breath-by-breath outputs of respiratory frequency (fR), tidal volume (Vt; l/min BTPS), instantaneous minute ventilation ($\dot{V}_E inst$ = Vt x fR; l/min BTPS), and inspiratory and end-tidal gas concentrations.

Oxygen consumption ($\dot{V}_{O2}$) and CO2 output ($\dot{V}_{CO2}$), both expressed as l min^{-1} STPD, were calculated over two minute periods using minute ventilation measured with

the dry gas meter, and O2 and CO2 concentrations of mixed expired gas sampled simultaneously from the mixing chamber. Oxygen concentration was measured with a Servomex O2 analyzer (model 570A), and CO2 with a Gould capnograph (Mark III), both previously calibrated with four gas mixtures of known O2 and CO2 concentrations.

Ventilatory responses to hypoxia were measured during moderate steady state exercise on a level treadmill (Woodway ELG2). The subjects walked at a speed (approximately 3 mph) which raised $\dot{V}_{O2}$ to about 1 l/min.

Ventilatory Responses to Transient and Step Change Hypoxia

Ten subjects were each studied on one occasion. The subjects exercised for two 45 minute periods separated by 30 minutes rest. The ventilatory response to transient hypoxia was measured during the first period of exercise and to step change hypoxia during the second period. For each exercise period, the subjects initially walked for 11 minutes breathing room air to establish steady state conditions. Duplicate measurements of $\dot{V}_{O2}$ and $\dot{V}_{CO2}$ were made after seven minutes of exercise, and repeated two minutes after both the third and the final transient hypoxic stimulus, and five minutes after the final step change hypoxic stimulus.

A transient hypoxic stimulus was produced by changing the inspired gas from room air to 100% nitrogen during an expiration. The subjects took between one and three breaths of 100% N2 in order to reduce Sa_{O2} to

approximately 85%. The inspired gas was then returned to room air during expiration. The transient hypoxic stimulus was repeated six times, separated by at least 60 breaths of room air (Fig 1).

A step change hypoxic stimulus was produced by changing the inspired gas from room air to 15% O2 during expiration. Hypoxia was maintained for three minutes. The inspired gas was then changed back to room air for six minutes. The step change hypoxic stimulus was then repeated using an inspired concentration of 12% O2, which reduced Sa_{O2} to approximately 85%. After a further six minutes breathing room air this sequence of step change stimuli was repeated (Fig 1). Isocapnia was maintained initially by priming the hypoxic gas mixture with an appropriate concentration of CO2, and thereafter by adjusting the inspired CO2 concentration so as to keep PET_{CO2} constant at the normoxic level during the hypoxia. However, no attempt was made to maintain isocapnia after the return to room air.

Variables were compared using Wilcoxon's test for paired data.

Repeatability Studies

Four subjects were studied on ten occasions at the same time of day at least one week apart. Subjects 1,10 and 11 were studied on ten consecutive weeks and subject 6 over a period of 34 weeks. A modified protocol was used for these studies. Each individual walked on a level

treadmill at the same speed (approximately 3 mph) for all ten studies. They initially walked for 9 minutes breathing room air. Duplicate measurements of $\dot{V}_{O2}$ and $\dot{V}_{CO2}$ were made after five minutes of exercise, and five minutes after the second step change hypoxic stimulus. Transient hypoxic stimuli were repeated four times, each separated by 40 breaths of room air. After the fourth transient stimulus, the subjects continued to walk breathing room air for three minutes. The responses to 15% and 12% O2 step changes in inspired gas were then measured once only at each level of hypoxia. The onset of the step change hypoxic stimuli was also modified to produce a fall in Sa_{O2} which approximated more closely to a square wave. The inspired gas was initially changed from room air to 1% O2 in N2 for one breath before changing to 15% O2 mixture, and from room air to 1% O2 in N2 for two breaths before changing to 12% O2. Isocapnia was maintained during transient hypoxia, and during the onset and duration of the step change hypoxic stimuli by addition of CO2 to the inspired air so that PET_{CO2} was kept constant.

The mean PET_{CO2} during transient hypoxia was compared to the mean normoxic baseline level using Wilcoxon's test for paired data. Other comparisons were made using Friedman's Analysis of Variance.

Development of the Model

The simplest model structure relating $\dot{V}_E$inst to Sa_{O2} was sought which would use the minimum number of parameters

and which would adequately describe the experimental data from all subjects. The Sa_{O2} recorded by the ear oximeter was assumed to be an accurate measure of the hypoxic stimulus (with all other ventilatory stimuli assumed constant), and $\dot{V}_E$inst was assumed to be a measure of the response. The model structure also had to be compatible with a proportional relationship between $\dot{V}_E$inst and Sa_{O2} during steady state isocapnic hypoxia as implied by previous observations during rebreathing (21). For all models, the fall in Sa_{O2} during hypoxia (S), where S = Sa_{O2}(normoxia) - Sa_{O2}(t), was taken as the input. The output of the model ($\dot{V}$calc), was calculated and compared to the experimentally recorded rise in $\dot{V}_E$inst. The details of the computer program are given in the Appendix.

The complexity of model was increased progressively in the order 1) a linear differential equation, 2) a non-linear differential equation, 3) various arrangements of two linear differential equations, and 4) two linear differential equations with non-linear terms, until a structure was found which adequately fitted all experimental data. At each stage, the best fit to the data was found, and the process stopped at the level when the addition of further terms did not yield a statistically significant decrease in the residual deviations of $\dot{V}_E$inst from the values for ventilation calculated by the model. A decrease on addition of one new parameter was considered to be significant if the

quantity:

$$F = \frac{(RSS_{before} - RSS_{after})}{(RSS_{after}/(n-(p+1))}$$

was greater than the 5% level of Snedecor's F distributed with 1 and (n-(p+1)) degrees of freedom, where RSS_{before} and RSS_{after} were the residual sum of squares before and after inclusion of the additional parameter, p was the original number of parameters, and n was the number of data points being fitted. This is a form of Analysis of Variance.

RESULTS

Physiological Responses

There was no significant difference in the normoxic steady state levels of $\dot{V}_E$, $\dot{V}_{O2}$, $\dot{V}_{CO2}$, or PET_{CO2} between the two periods of exercise (Table 1). During transient hypoxia PET_{CO2} fell significantly ($P<0.01$) from a mean baseline of 42 $\pm$ 3 mmHg to a mean lowest value of 37 $\pm$4 mmHg. The largest standard deviation about the mean for PET_{CO2} during step change hypoxia was 1.4 mmHg. The mean lowest Sa_{O2} in response to the repeated transient hypoxic stimuli (range 76 - 87 mmHg) was not significantly different from the mean lowest Sa_{O2} in response to the 12% O2 step change stimuli (range 75 - 89 mmHg).

Model Development Results

The simplest model used was a single linear differential equation as illustrated in Fig 2. In the steady state (i.e. dV/dt = 0) the equation reduces to:

$$V = GS$$

thus fulfilling the criterion that in steady state isocapnic conditions $\dot{V}_E$inst is proportional to Sa_{O2}, while the differential equation includes time as a variable to allow dynamic analysis. Applying this equation, parameters giving a good fit between $\dot{V}$calc and $\dot{V}_E$inst in response to transient hypoxia underestimated the increase in response to step change hypoxia (Fig 2), whereas those giving a good fit for step change hypoxia overestimated the response to transient hypoxia. This

confirms that the ventilatory response to the two types of hypoxic stimuli cannot be described adequately by a single linear system (27).

Two non-linear terms were then added into the single linear differential equation. The first allowed the gain to change at a threshold of S, dS/dt, $\dot{V}$calc, or dVcalc/dt. The second allowed the gain of the equation to "saturate" in a way analogous to the Michaelis-Menten equation by replacing the gain G with the term G/(S + r). In no case was a significantly better fit obtained.

Various forms of interaction of two linear differential equations were then investigated. These included a) arrangement in series with the output of one being the input of the other, and with the output of the second equation forming the overall output, and b) arrangement in parallel with both differential equations receiving the same input with the output of both being either summed or multiplied to give the overall output. The best fit was consistently obtained using two equations in parallel with both outputs summed (Fig 3) with Equation 1 having a fast time constant (1 <3 sec) and Equation 2 having a slow time constant (2). The slow time constant resulted in a slow return of Vcalc to baseline after the return to normoxia. Since isocapnia was not maintained during the recovery from hypoxia good agreement between $\dot{V}$calc and $\dot{V}_E$inst could not be expected here. The output (Y2) of the equation with the slow time constant was therefore set to zero when S < 2%, which

eliminated the slow recovery in Vcalc. This was necessary to limit the weighting given to the recovery period during the fitting process. Even after elimination of the slow recovery, this model did not adequately describe all data.

Finally, simple non-linear terms were added to the two differential equations. These included the "saturating" effect (described above) in either equation, changes in the gain of one equation dependent on the output of the other (simulating inhibition or potentiation), and the introduction of time delays in different parts of the equations.

The Final Model

The simplest model which adequately described the experimental data in all subjects is shown in Fig 4. The time constant of equation 1 ($\tau 1$; Fig 4) was found to be fast (< 3 sec) and was therefore arbitrarily set to 0.1 sec since the time constant of the ear oximeter (1.6 seconds; 6) prevented this parameter from being measureable. Equation 2 had a long time constant ($\tau 2$) and its gain was allowed to saturate as described above. Finally, the output of equation 1 was "inhibited" (positive parameter I) or "potentiated" (negative parameter I) by the output of equation 2.

Parameter Estimation in 10 Normal Subjects

The parameters giving the best fit between the model output and the experimental data are summarized in Table 2. In each case the addition of further parameters did not result in a significantly improved fit. In three of the 10 subjects only the 3 parameters G1, G2, and $\tau 2$ were needed (Fig 5, subject 4). In the remaining subjects, the parameter r was also needed in three (Fig 5, subjects 5,10), a positive I parameter ("inhibiting" effect) was needed in two, and a negative I parameter ("potentiating" effect) in a further three subjects (Fig 5, subject 3). In one subject both the parameters r and a negative I were also required.

Repeatability in Four Subjects

Normoxic baseline values for $\dot{V}_E$, $\dot{V}_{O2}$, $\dot{V}_{CO2}$, and PET_{CO2} (Table 3) were not significantly different on different days in an individual. The PET_{CO2} did not change significantly from normoxic baseline during transient hypoxia, and the largest standard deviation about the mean during step change hypoxia was 1.7 mmHg.

Gain 1, gain 2 and $\tau 2$ varied between days within an individual (Fig 6) but these differences were not significant. The coefficients of variation ranged from 21-34% for gain 1, from 20-45% for gain 2, and from 75-121% for $\tau 2$. There was a significant difference ($P<0.001$) between subjects for gains 1 and 2 but not for $\tau 2$. The absolute values of the non-linear terms I and r varied between days in an individual (Fig 6). However

the parameter I tended to be consistently either positive (inhibition) or negative (potentiation) within an individual (Fig 6), with a positive value needed for all 10 measurements in subjects 1 and 11, and with a negative value needed on eight occasions in subject 6 and nine occasions in subject 10. The parameter r was consistently needed in subject 10 and on only one occasion in subject 1. In subjects 6 and 10, r was needed on four and six occasions respectively (Fig 6).

DISCUSSION

To explore the apparent differences in the hypoxic drive to ventilation as measured using transient or step change hypoxic stimuli, we have developed a mathematical model based on two linear differential equations and incorporating non-linear terms to describe the dynamic relationship between $\dot{V}_E$ and Sa_{O2} during both types of stimulus. This model provided a consistently better fit to the data than models based on a single differential equation, with or without non-linear terms, and of other combinations of two differential equations with and without non-linear terms. The model depends upon several assumptions. Arterial oxygen saturation was taken as a measure of the stimulus and $V_E$inst as the output. The P_{O2} within the carotid glomus is considered to be the stimulus to the peripheral arterial chemoreceptors (9), but end-tidal P_{O2} cannot be used as index of the stimulus to the chemoreceptors since it will not accurately reflect arterial P_{O2} during the rapid changes of the inspired gas from room air to 100% nitrogen. While Sa_{O2} is not the actual chemoreceptor stimulus, it is related to arterial P_{O2} (and thus to carotid glomus P_{O2}), and provides the only available non-invasive measure of arterial oxygenation since the response time of transcutaneous P_{O2} electrodes would be inadequate to follow the rapid changes.

Although ventilation is a linear function of Sa_{O2} during rebreathing (21), we are concerned with

describing the relationships during rapid changes in Sa_{O2}. Use of two linear differential equations in parallel with different time constants for each equation yields an output which is intrinsically non-linear, but which approximates to a linear model in steady state conditions (ie. Sa_{O2} remaining constant for a period of at least twice the slow time constant). The non-linear terms needed to describe the response in some subjects distort this long-term appearance of linearity, although the output with "saturating" effect appears to be approximately linear at high levels of stimulus. Our model has been developed to describe our observations in studies using stimuli lasting up to 3 minutes. It may therefore be less adequate during more prolonged hypoxia.

A further assumption was that arterial P_{CO2} was constant and equal to end-tidal P_{CO2}. End-tidal P_{CO2} has been directly compared with simultaneously measured Pa_{CO2} in normal subjects at rest and on exercise (4,14) showing a small positive PET_{CO2}-Pa_{CO2} difference as tidal volume increases during exercise. However there are insufficient data to allow the application of a reliable empirical correlation to calculate Pa_{CO2} (the presumed other stimulus to ventilation) in our studies. During the onset and plateau of the step change hypoxic stimuli we adjusted the inspired CO2 concentration so as to keep end-tidal P_{CO2} constant. Possible inadequate control of Pa_{CO2} was disregarded in the model. The

PET_{CO2} was allowed to fall during the return to breathing room air with a resultant reduction in $\dot{V}_E$inst at this time. This phase of the response is reflected in the model by setting the output of the slow time constant equation to zero as the Sa_{O2} returns to its baseline.

Assessing the goodness of fit of the model to the data poses problems. A perfect fit is clearly impossible due to the uncertainty in measuring both stimulus (Sa_{O2}) and response ($\dot{V}_E$inst). The uncertainty in the response can be estimated as measurement errors are small compared with the easily measured random physiological variation in $\dot{V}_E$inst. However, the uncertainty in the stimulus cannot be assessed due to problems in measuring Sa_{O2}. The time constant of the HP4710A ear oximeter in the fast mode is 1.61 seconds (6) and the oximeter only provides integer output values. In some subjects Sa_{O2} fell by only 2 or 3% when breathing 15% oxygen, so that this restriction to integer values could conceal an error of up to 50%. Furthermore, the time course of the measured Sa_{O2} is distorted by averaging the readings within each breath, and this distortion is worse during transient stimuli when the true nadir values may be lost. As the size of these experimental inaccuracies cannot be assessed, the standard chi-squared "goodness of fit" test cannot be used quantitatively. The residual sum of squares method which was used to assess goodness of fit does not give an absolute measure as it depends

upon the variability in $\dot{V}_E$inst at any given Sa_{O2}, which may differ between normoxia and hypoxia. However it allows the goodness of fit between one model or set of parameters and another to be compared objectively. We have therefore shown that the final model fitted the data statistically better than all the simpler models tested, and at least as well as the more complex ones studied.

Many different structures were studied during the development of the model. We consider that the final model is the simplest using the approach adopted. Although there may be other combinations of terms which would give an equally good or better fit it is likely that this would be at the expense of greater complexity and hence greater parameter uncertainty. Even with the simplest model the complexity precludes unique estimation of all parameters, and the parameters found may not necessarily be the best possible. Ideally, the parameters derived for an individual from one set of data should be confirmed by data from a different set of stimuli. This was done implicitly when we used a reduced data set to derive the model initially (see Appendix) with subsequent application of the model to the full data sets. Furthermore, the repeated studies confirm the value of the model and suggest that it gives a quantitative and reproducible measure of hypoxic ventilatory control in normal subjects. The between-day differences in the estimated parameters are compatible

with the previous reported day-to-day variablity in the hypoxic ventilatory response in an individual (22). The differences may therefore reflect physiological changes such as variations in blood pH which has been shown to account partly for the between-day variability in the ventilatory response to hypoxia (22). The greater between-subject compared to within-subject variability in the gains suggests that the model is capable of quantifying genetically-determined individual differences in hypoxic ventilatory control (3).

Finally, however, it must be emphasised that the form of the model was developed empirically on a purely mathematical basis without consideration of possible underlying physiological mechanisms. Physiological interpretation of the model must be treated with caution. It is tempting to ascribe the components of the response described by the differential equation with the fast time constant to the activity of the carotid chemoreceptor, and that with the longer time constant to a central "controller", but further studies are needed to explore these interpretations.

APPENDIX

The Computer Program

The program (written in Fortran IV and assembler on a DEC PDP 11/73) allowed for any arrangement of one or two differential equations, with or without the non-linear terms described above. An input file contained a sequence of values for an individual of Sa_{O2}, time and $\dot{V}_E$inst, including both step change and transient events. To reduce processing time, only 15 normoxic breaths before and after each period of hypoxia were included in the file. A standard Runge-Kutta integration routine (20) was used to calculate values of ventilation for a given set of equation parameters, and the parameters were adjusted to minimize the sum of weighted squared differences between the calculated and observed ventilation using the "Simplex" method (20). The weighting prevented the fitting process being biased towards the step changes (due to their greater number of experimental points contributing to the sum of squares) by multiplying the squared differences during step changes by the factor W, where

$$W = \frac{\text{sum of durations of transients}}{\text{sum of durations of steps}}$$

To allow the use of delays within the model and compensation for the delay in response of the ear oximeter (about 2 sec), the Sa_{O2} was interpolated to one second intervals within the program using the method of Lagrange (19).

During development of the model, a reduced data set consisting of the response to three transient and two step change hypoxic stimuli (one 15% O2 and one 12% O2 stimulus) for a subject was used to reduce computing time. The best fit was then confirmed on the full data set and adjusted if necessary.

When estimating model parameters using the final model, a fit was made initially without either of the non-linear terms, and was repeated using first the "saturating" effect, and then the inhibition term. At each stage a significant improvement in fit was sought. If this did not occur then the parameter values from the previous computation were taken as representative of the subject.

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TABLE 1

Ventilation and gas exchange during normoxic steady state exercise in 10 normal subjects.

Subject No.	Period 1 (Transient Hypoxia)				Period 2 (Step Change Hypoxia)			
	$\dot{V}_E$ $l min^{-1}$	$\dot{V}_{O2}$ $l min^{-1}$	$\dot{V}_{CO2}$ $l min^{-1}$	PET_{CO2} mmHg	$\dot{V}_E$ $l min^{-1}$	$\dot{V}_{O2}$ $l min^{-1}$	$\dot{V}_{CO2}$ $l min^{-1}$	PET_{CO2} mmHg
1	22.9	0.98	0.84	44	23.1	0.93	0.80	44
2	26.3	0.89	0.84	38	27.1	0.92	0.88	38
3	22.5	1.04	0.84	44	25.2	1.14	0.97	42
4	19.4	1.01	0.84	46	19.2	0.99	0.83	45
5	22.2	0.95	0.80	41	22.6	0.95	0.78	41
6	27.9	1.04	0.90	39	28.1	1.07	0.92	38
7	29.5	0.96	0.94	41	28.4	0.99	1.01	43
8	19.7	0.90	0.77	43	22.0	0.96	0.83	40
9	24.0	0.93	0.84	42	21.6	0.95	0.81	42
10	20.7	0.80	0.69	38	21.1	0.78	0.68	38
Mean	23.6	0.95	0.83	42	23.8	0.97	0.85	41
SD	3.5	0.07	0.07	3	3.2	0.09	0.10	3

Footnote : $\dot{V}_E$, minute ventilation (l min^{-1} BTPS); $\dot{V}_{O2}$ oxygen consumption (l min^{-1} STPD); $\dot{V}_{CO2}$ carbon dioxide output (l min^{-1} STPD); PET_{CO2}, end-tidal P_{CO2} (mmHg).

TABLE 2

Parameters giving the best fit between $\dot{V}calc$ and $\dot{V}_E inst$ in the 10 normal subjects.

Subject No.	Equation 1		Equation 2		
	G1	I	τ_2	G2	r
1	3.0	- 0.001	15	3.0	0.1
2	0.6	*	70	3.2	*
3	1.3	- 0.015	8	4.8	*
4	0.1	*	20	1.8	*
5	1.0	*	70	11.0	0.26
6	9.0	- 0.014	15	10.0	*
7	3.0	0.05	15	0.8	*
8	0.7	*	16	1.3	*
9	0.9	0.009	8	3.4	*
10	1.7	*	30	12.0	0.3

Footnote: G1: gain of equation 1 (1 $min^{-1}\%^{-1}$)

I: parameter describing "inhibition" (positive value) or "potentiation" (negative value) of gain 1 $(1\ min^{-1})^{-1}$

τ_2: time constant of equation 2 (sec)

G2: gain of equation 2 (without r, 1 $min^{-1}\%^{-1}$; with r, 1 min^{-1})

r: parameter describing "saturating" effect acting on G2 (%)

* indicates parameter not needed for best fit between Vcalc and $V_E inst$.

TABLE 3

Mean ventilation and gas exchange during normoxic steady state exercise for 10 repeated studies in four subjects.

Subject No.	$\dot{V}_E$ l min^{-1}	$\dot{V}_{O2}$ l min^{-1}	$\dot{V}_{CO2}$ l min^{-1}	PET_{CO2} mmHg
1	19.3 ± 0.8	0.84 ± 0.05	0.72 ± 0.06	43 ± 1.3
6	23.9 ± 0.9	0.92 ± 0.02	0.89 ± 0.04	39 ± 2.8
10	19.6 ± 1.3	0.66 ± 0.03	0.61 ± 0.05	38 ± 1.6
11	26.5 ± 2.6	1.07 ± 0.08	1.03 ± 0.10	42 ± 2.1

Footnote: $\dot{V}_E$, minute ventilation (l min^{-1} BTPS); $\dot{V}_{O2}$, oxygen consumption (l min^{-1} STPD); $\dot{V}_{CO2}$, carbon dioxide output (l min^{-1} STPD); PET_{CO2}, end-tidal P_{CO2} (mmHg).

FIGURE LEGENDS

Figure 1: Breath-by-breath measurements of $\dot{V}_E$inst (upper trace), Sa_{O2} (middle trace), and end-tidal P_{CO2} (lower trace) in one subject showing the responses to transient and step change hypoxic stimuli. Hypoxic stimuli consisting of 2 breaths of 100% N2, given at times indicated by the arrows, caused a transient fall in Sa_{O2} and brief rise in $\dot{V}_E$inst. Step change stimuli, produced by changing the inspired gas abruptly to either 15 or 12% O2, caused a slower onset, sustained fall in SaO2 accompanied by a gradual and maintained rise in $\dot{V}_E$inst. End-tidal PET_{CO2} fell slightly during the response to the transient stimulus, but was maintained constant at the normoxic baseline level during the onset and duration of the step change hypoxic stimuli.

Figure 2: A. Schematic representation of the model consisting of a single linear differential equation.

B. Comparison of the model output, $\dot{V}$calc (upper trace, and dotted line in the lower trace), and the experimentally recorded $\dot{V}_E$inst (solid line, lower trace) in response to three transient and two step change (FI_{O2} 0.15 and 0.12) hypoxic stimuli in one subject. The model output tended to overestimate the response to transient hypoxia and underestimate the response to step change hypoxia.

Figure 3: A. Schematic representation of the model consisting of two linear differential equations in

parallel with both outputs summed (Y1 + Y2).

B. Comparison of the model output, $\dot{V}$calc, and the experimentally recorded $\dot{V}_E$inst, illustrated as in Fig 2. The model did not adequately describe the response to all hypoxic stimuli, tending to underestimate the response to the 15% O2 step change stimulus, and with the slow time constant of equation 2 resulting in a slow return in $\dot{V}$calc to baseline after the return to normoxia.

Figure 4: A. Schematic representation of the final model consisting of two linear differential equations in parallel, with both outputs summed and incorporating two non-linear terms describing i) "inhibition" (positive I) or "potentiation" (negative I) of gain of equation 1 (G1) by the output of equation 2 (Y2), and ii) allowing the gain of equation 2 to "saturate" by replacing G2 with G2/(S + r). The slow recovery in Vcalc on return to normoxia was eliminated by setting the output of equation 2 (Y2) to zero when S < 2%.

B. Comparison of the model output, $\dot{V}$calc, and the experimentally recorded $\dot{V}_E$inst, illustrated as in Fig 2, showing a close fit between the model output and the experimentally recorded response to transient and step change hypoxic stimuli with elimination of the slow recovery in Vcalc on removal of the hypoxic stimulus.

Figure 5: Illustration of the model output giving the best fit to the experimentally recorded $\dot{V}_E$inst in four

subjects. For each subject the model output ($\dot{V}$calc, upper trace and dotted line in middle trace), and breath-by-breath measurements of $\dot{V}_E$inst (middle trace) and Sa_{O2} (lower trace) are shown. In subject 4 only the parameters G1, G2 and γ_2 were needed to give the best fit, whereas a negative I parameter ("potentiation") was also needed in subject 3, and r needed in subjects 5 and 10.

Figure 6: Histograms of the estimated parameters G1, G2, I, r and γ_2 measured on 10 occasions in each of four subjects. The units given for G2 of 1 min^{-1} $\%^{-1}$ apply to those cases where r was not required; for those cases where r was needed the units for G2 are 1 min^{-1}.

Fig 1

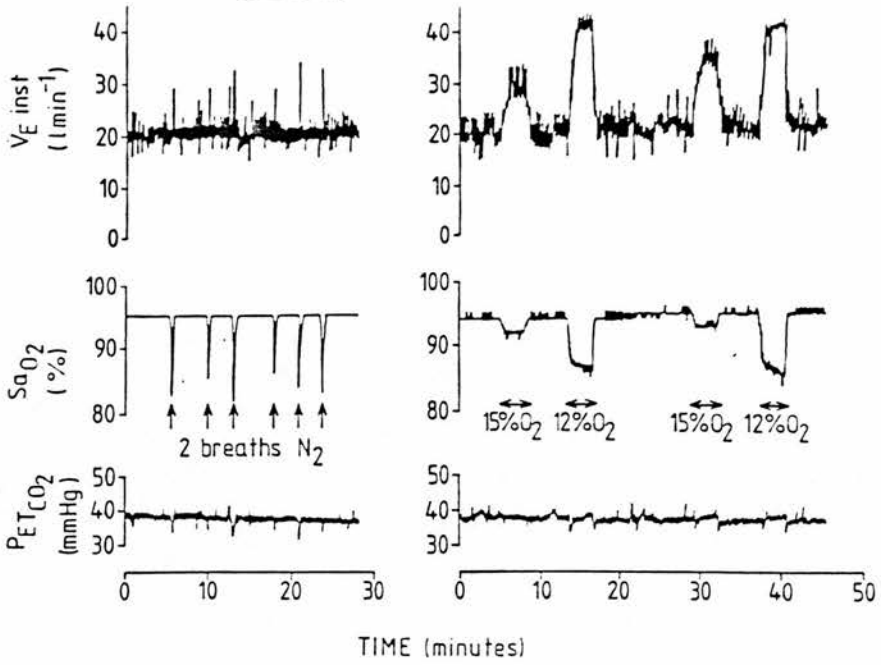


Fig 2

A.

Fall in Saturation → S → $\tau \frac{d\dot{V}}{dt} + \dot{V} = G.S$ → $\dot{V}$ → Rise in Ventilation

τ = time constant; G = gain

$$S = Sa_{O_{2\,(normoxia)}} - Sa_{O_2}(t)$$

$$\dot{V}calc = \dot{V} + \dot{V}_{E\,(normoxia)}$$

B.

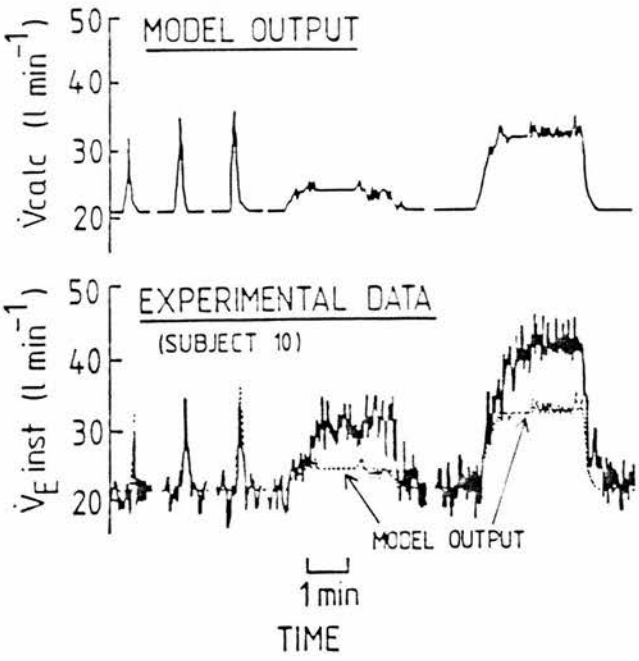


Fig 3

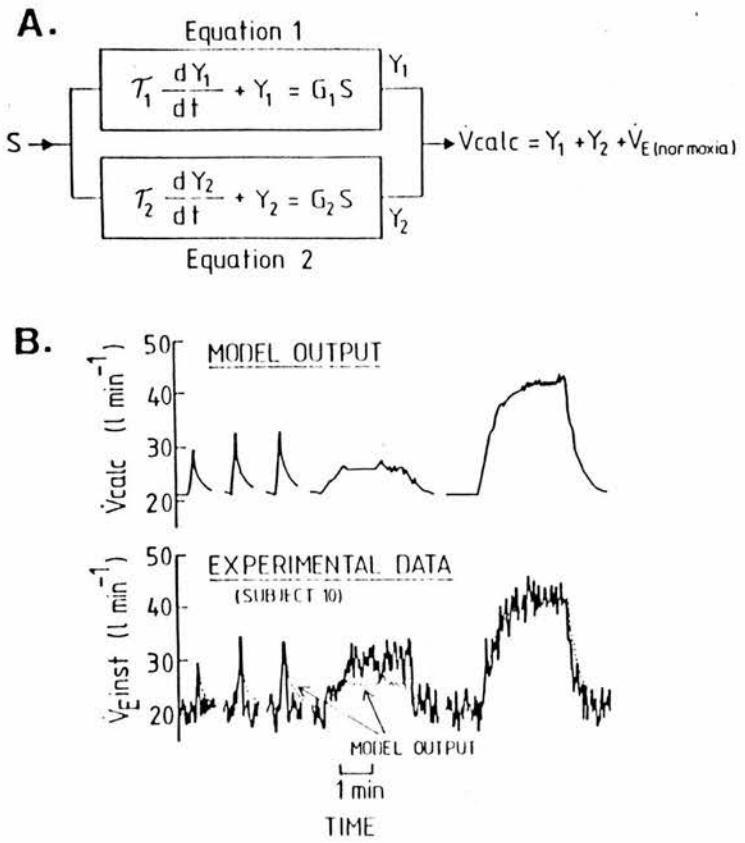

A.
Equation 1
$\tau_1 \frac{dY_1}{dt} + Y_1 = G_1 S$
Y_1
S
$\dot{V}calc = Y_1 + Y_2 + \dot{V}_{E\,(normoxia)}$
$\tau_2 \frac{dY_2}{dt} + Y_2 = G_2 S$
Y_2
Equation 2
B.
MODEL OUTPUT
$\dot{V}calc$ (l min^{-1})
50
40
30
20
EXPERIMENTAL DATA
(SUBJECT 10)
$\dot{V}_E inst$ (l min^{-1})
MODEL OUTPUT
1 min
TIME

Fig 4

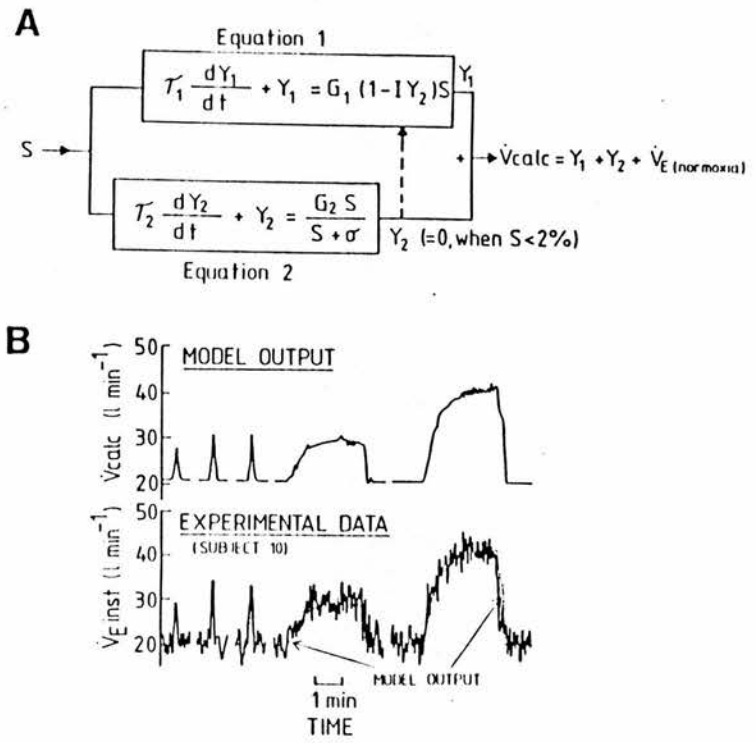

A
Equation 1
$\tau_1 \frac{dY_1}{dt} + Y_1 = G_1 (1 - I Y_2) S$
Y_1
S
$\dot{V}calc = Y_1 + Y_2 + \dot{V}_{E\,(normoxia)}$
$\tau_2 \frac{dY_2}{dt} + Y_2 = \frac{G_2 S}{S + \sigma}$
Y_2 (=0, when S<2%)
Equation 2
B
MODEL OUTPUT
$\dot{V}calc$ (l min^{-1})
50
40
30
20
EXPERIMENTAL DATA
(SUBJECT 10)
$\dot{V}_E inst$ (l min^{-1})
MODEL OUTPUT
1 min
TIME

Fig 5

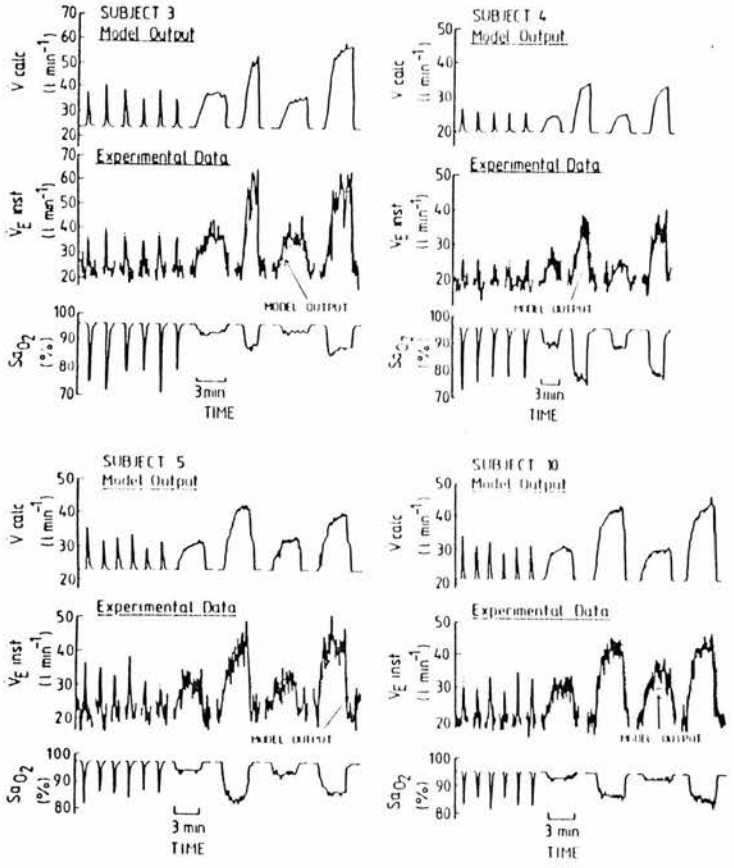
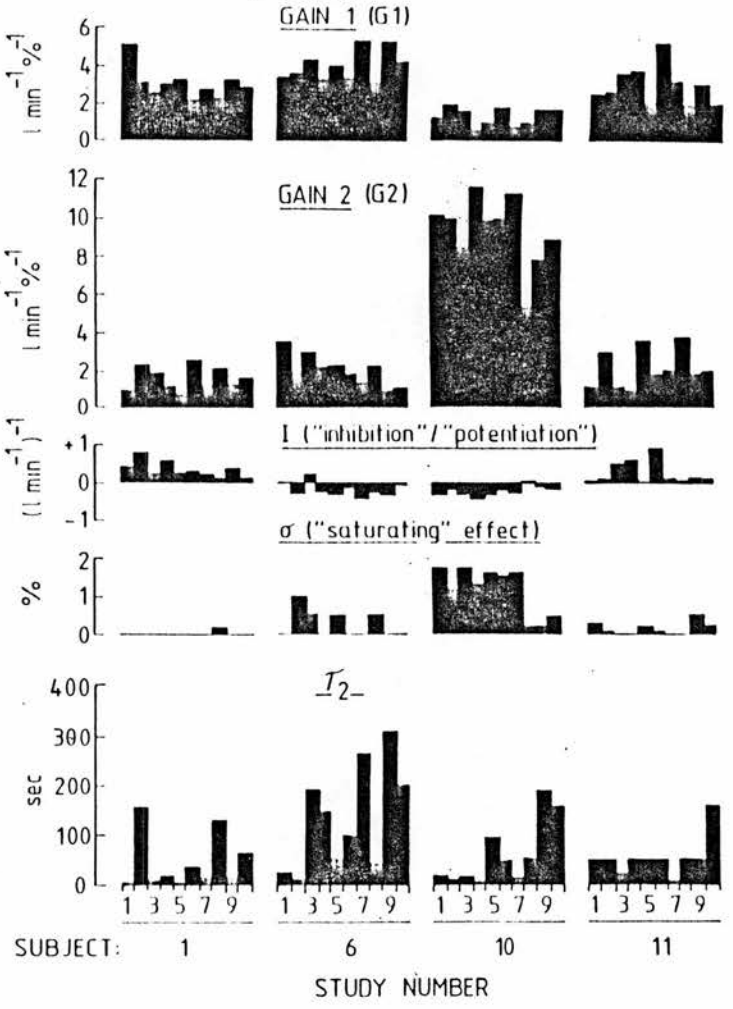

SUBJECT 3
Model Output
V calc (l min⁻¹)
Experimental Data
V̇E inst (l min⁻¹)
MODEL OUTPUT
SaO2 (%)
3min
TIME
SUBJECT 4
Model Output
Experimental Data
SUBJECT 5
Model Output
Experimental Data
3 min
SUBJECT 10
Model Output
Experimental Data

Fig 6


GAIN 1 (G1)
l min⁻¹ %⁻¹
GAIN 2 (G2)
I ("inhibition"/"potentiation")
(l min⁻¹)⁻¹
σ ("saturating" effect)
%
T2-
sec
1 3 5 7 9
SUBJECT: 1 6 10 11
STUDY NUMBER

Appendix B: Table 1 : Anthropomorphic and lung function measurements

Subject Number	Sex	Age (years)	Height (m)	Weight (kg)	FEV_1 (l)	FEV_1 (% pred)	VC (l)	VC (% pred)	FEV_1/VC (%)	FEV_1/VC (% pred)	TLC (l)	TLC (% pred)	RV (l)	RV (% pred)	RV/TLC (%)	TL(CO) (mmol/kPa.min)	TLCO (% pred
1	F	23	1.52	57	2.59	89	3.16	90	82	105	3.81	77	0.65	60	17	8.14	95
2	M	30	1.75	70	4.42	103	5.04	97	88	106	6.74	101	1.70	106	25	11.70	105
3	F	40	1.63	93	3.0	107	3.75	104	80	106	5.27	101	1.39	82	27	9.16	108
4	M	33	1.71	72	3.66	89	4.59	94	80	96	6.45	99	1.72	108	27	12.31	116

Table 2 : Baseline normoxic values for ventilation and gas exchange

Study day	Subject 1			Subject 2			Subject 3			Subject 4		
	$\dot{V}_E$ $l min^{-1}$	$\dot{V}O_2$ $l min^{-1}$	$\dot{V}CO_2$ $l min^{-1}$	$\dot{V}_E$ $l min^{-1}$	$\dot{V}O_2$ $l min^{-1}$	$\dot{V}CO_2$ $l min^{-1}$	$\dot{V}_E$ $l min^{-1}$	$\dot{V}O_2$ $l min^{-1}$	$\dot{V}CO_2$ $l min^{-1}$	$\dot{V}_E$ $l min^{-1}$	$\dot{V}O_2$ $l min^{-1}$	$\dot{V}CO_2$ $l min^{-1}$
1	20.7	0.66	0.66	30.0	1.19	1.19	24.3	0.95	0.88	20.3	0.84	0.73
2	18.3	0.64	0.51	26.6	1.09	1.02	23.7	0.92	0.87	20.2	0.84	0.76
3	18.6	0.67	0.57	26.2	1.18	1.11	24.2	0.91	0.89	19.2	0.83	0.74
4	17.7	0.64	0.54	21.5	0.94	0.92	25.5	0.91	0.94	19.1	0.83	0.76
5	20.7	0.72	0.67	29.0	1.10	1.14	23.5	0.91	0.86	20.4	0.96	0.82
6	21.1	0.72	0.63	23.9	0.96	0.91	23.1	0.89	0.90	17.9	0.77	0.72
7	18.5	0.65	0.62	29.6	1.13	1.09	25.3	0.93	0.93	19.1	0.83	0.68
8	19.9	0.67	0.64	25.9	1.03	0.94	23.1	0.92	0.82	19.1	0.88	0.66
9	21.0	0.72	0.65	27.0	1.04	1.00	23.5	0.93	0.84	18.6	0.80	0.72
10	10.9	0.65	0.62	25.6	1.04	0.94	24.1	0.94	0.90	19.2	0.84	0.62
mean	19.6	0.66	0.61	26.5	1.07	1.03	24.0	0.92	0.88	19.3	0.84	0.72
SD	1.3	0.03	0.05	2.6	0.08	0.10	0.8	0.02	0.04	0.8	0.05	0.06

$\dot{V}_E$ expressed as $l min^{-1}$ BTPS, $\dot{V}O_2$ and $\dot{V}CO_2$ expressed as $l min^{-1}$ STPD.

Table 3 Mean $P_{ET}CO_2$ values (kPa) for each subject on each study day

Study day	SUBJECT 1			2			3			4		
	Baseline	Transient	Step	Baseline	Transient	Step	Baseline	Transient	Step	Baseline	Transient	Step
1	4.62±0.11	4.57±0.24	4.60±0.10	5.14±0.25	5.12±0.21	5.15±0.23	4.25±0.07	4.17±0.20	4.21±0.09	5.58±0.10	5.40±0.25	5.45±0.10
2	5.19±0.12	5.24±0.17	5.16±0.12	5.85±0.20	5.98±0.26	5.88±0.17	5.30±0.13	5.13±0.15	5.26±0.16	5.83±0.10	5.64±0.24	5.68±0.19
3	5.39±0.16	5.12±0.25	5.30±0.13	6.15±0.21	6.13±0.20	6.00±0.15	4.99±0.24	4.67±0.22	4.97±0.22	5.93±0.12	5.91±0.15	5.89±0.15
4	5.19±0.14	5.17±0.18	5.07±0.18	5.62±0.26	5.50±0.22	5.56±0.20	5.06±0.16	5.00±0.19	5.09±0.14	5.84±0.15	5.70±0.23	5.77±0.14
5	5.13±0.14	5.25±0.18	5.07±0.11	5.81±0.32	5.74±0.29	5.84±0.19	5.39±0.13	5.23±0.27	5.37±0.16	5.69±0.19	5.79±0.20	5.61±0.20
6	5.09±0.15	5.10±0.21	5.05±0.13	5.54±0.26	5.62±0.34	5.44±0.15	5.39±0.12	5.31±0.25	5.40±0.15	5.78±0.22	5.70±0.18	5.78±0.20
7	5.26±0.14	5.33±0.21	5.23±0.13	5.58±0.27	5.53±0.19	5.53±0.18	5.05±0.12	4.93±0.20	5.04±0.13	5.37±0.26	5.38±0.37	5.37±0.20
8	5.08±0.14	5.21±0.22	5.06±0.10	5.50±0.17	5.38±0.17	5.48±0.20	5.24±0.13	5.16±0.31	5.26±0.15	5.80±0.19	5.70±0.21	5.76±0.14
9	5.17±0.15	5.23±0.19	5.09±0.10	5.53±0.18	5.48±0.20	5.63±0.21	5.47±0.13	5.31±0.23	5.48±0.17	5.89±0.12	5.87±0.20	5.82±0.11
10	4.92±0.17	5.02±0.20	4.92±0.17	5.55±0.18	5.53±0.24	5.52±0.17	5.41±0.17	5.22±0.22	5.41±0.17	5.75±0.17	5.73±0.22	5.77±0.10

Table 4 Mean SaO_2 of the transient hypoxic stimuli (trans) and lowest 12% O_2 step change SaO_2 (step) values for each subject on each study day (%)

Study day	Subject 1		2		3		4	
	trans	step	trans	step	trans	step	trans	step
1	80	85	88	85	82	83	89	80
2	82	85	88	86	85	83	86	82
3	82	86	88	88	85	82	87	82
4	81	87	87	86	88	87	87	84
5	84	86	81	83	86	89	84	76
6	86	84	88	89	85	85	88	86
7	82	83	81	80	89	90	85	81
8	82	86	83	84	83	79	88	79
9	86	84	82	85	86	87	88	81
10	86	86	85	82	85	84	84	78
mean	83	85	85	85	85	85	87	81
s.d.	2	1	3	3	2	3	2	3

Table 5 Transient hypoxic stimuli slopes of response ($l min^{-1} \%^{-1}$) for each subject on each study day

Study day	Subject 1	2	3	4
1	-1.26	-1.98	-2.27	-2.54
2	-1.48	-2.38	-1.43	-1.17
3	-1.19	-1.38	-2.74	-1.33
4	-0.99	-1.74	-2.31	-1.35
5	-1.51	-1.50	-2.36	-1.33
6	-1.17	-1.32	-2.41	-1.13
7	-0.95	-1.26	-3.83	-0.88
8	-1.21	-1.60	-1.95	-1.50
9	-1.58	-1.94	-2.80	-1.42
10	-1.93	-1.36	-2.76	-1.26
mean	-1.33	-1.65	-2.49	-1.39
s.d.	0.30	0.36	0.63	0.44

Table 6 Step change hypoxic stimui slopes of response ($l min^{-1} \%^{-1}$) for each subject for each study day

	Subject			
Study day	1	2	3	4
1	-1.85	-1.00	-4.33	-0.42
2	-1.78	-0.45	-2.96	-0.41
3	-1.60	-0.98	-1.26	-0.76
4	-1.35	-0.52	-4.15	-0.66
5	-1.15	-1.05	-3.35	-0.35
6	-1.69	-0.58	-3.03	-1.06
7	-1.11	-1.19	-4.06	-0.45
8	-1.06	-1.55	-2.49	-0.51
9	-1.44	-1.07	-4.32	-0.68
10	-1.52	-0.76	-2.97	-0.79
mean	-1.46	-0.92	-3.29	-0.61
s.d.	0.28	0.34	0.98	0.22

Table 7 Model parameter gain 1 values for each subject given for each study day ($l min^{-1} \%^{-1}$)

Study day	Subject 1	2	3	4
1	1.55	2.54	3.54	5.31
2	1.94	2.64	3.67	3.31
3	1.74	3.48	4.33	2.58
4	0.77	3.57	3.37	3.03
5	1.18	2.00	3.82	3.53
6	1.87	5.19	3.37	2.17
7	0.96	3.27	5.44	2.69
8	1.09	1.93	3.25	2.21
9	1.73	3.11	5.24	3.49
10	1.75	1.99	4.17	2.78
mean	1.46	2.97	4.02	3.11
s.d.	0.42	0.99	0.78	0.91

Table 8 Model parameter inhibition values for each subject given for each study day ($(1min^{-1})^{-1}$)

Study day	Subject 1	2	3	4
1	-0.19	0.04	*	0.44
2	-0.09	0.12	-0.18	0.82
3	-0.20	0.56	0.20	0.21
4	-0.26	0.63	-0.22	0.59
5	-0.21	0.03	-0.31	0.19
6	-0.14	0.96	-0.06	0.30
7	-0.17	0.13	-0.42	0.26
8	0.02	0.07	-0.21	0.11
9	-0.01	0.09	-0.24	0.43
10	-0.08	0.11	-0.05	0.15

where * represents parameter not required by model of best fit

Table 9 Model parameter time constant of equation 2, tau 2 (sec) values for each subject for each study day

Study day	Subject 1	2	3	4
1	34	51	24	4
2	24	51	15	158
3	28	26	180	11
4	14	50	145	20
5	90	50	48	3
6	45	50	95	37
7	29	12	272	14
8	56	50	40	129
9	180	50	307	15
10	167	170	197	67
mean	67	56	132	46
s.d.	60	42	105	55
co.of var.	90%	75%	79%	121%

where co. of var. represents the coefficient of variation

Table 10 Model parameter gain 2 values for each subject for each study day

Study day	Subject 1	2	3	4
1	10.28	1.18	3.64	0.96
2	10.08	3.01	2.01	2.37
3	8.34	1.18	2.97	1.74
4	11.63	1.14	2.20	1.31
5	9.99	3.64	2.32	0.76
6	10.08	1.89	1.82	2.84
7	11.39	2.12	1.42	1.25
8	5.43	3.76	2.26	2.27
9	7.89	1.87	0.99	1.43
10	8.82	2.03	1.04	1.67
mean	9.39	2.18	2.07	1.66
s.d.	1.84	0.98	0.82	0.66

Units for gain 2 if sigma also included are $lmin^{-1}\%^{-1}$, or if sigma not required to produce the model of best fit then $lmin^{-1}$.

Table 11 Model parameter saturating effect, sigma values for each subject for each study day (%)

Study day	Subject 1	2	3	4
1	1.70	0.29	*	*
2	1.23	0.05	1.02	*
3	1.73	*	0.51	*
4	1.35	*	*	*
5	1.63	0.21	0.47	*
6	1.59	0.05	*	*
7	1.68	*	*	*
8	0.24	*	0.46	0.18
9	0.25	0.49	*	*
10	0.49	0.19	*	*

where * represents parameter not required by model of best fit

Table 12 Anthropomorphic and lung function details of the subjects

Subject Number	Sex	Age (years)	Height (m)	Weight (kg)	FEV_1 (l)	FEV % pred	VC (l)	VC (% pred)	FEV/VC %	FEV_1/VC (% pred)	TLC (l)	TLC (% pred)	RV (l)	RV (% pred)	RV/TLC (%)	TLCO mmol/kPa.min	TLCO % pred
1	F	22	1.52	57	2.59	89	3.16	90	82	100	3.81	83	0.65	59	17	8.14	95
2	F	39	1.63	93	3.00	107	3.75	104	80	103	5.27	101	1.39	82	26	9.16	108
3	M	33	1.71	72	3.66	89	4.59	94	80	96	6.45	101	1.72	108	27	12.31	116
4	F	25	1.75	68	4.10	114	4.65	101	88	109	6.41	102	1.66	92	26	10.32	101
5	M	24	1.88	73	4.31	86	6.10	100	71	87	8.01	104	1.91	112	24	13.16	101
6	M	37	1.80	75	4.99	113	5.75	107	87	107	7.45	105	1.70	94	23	11.00	97

Table 13 Baseline normoxic values for ventilation and gas exchange

Subject Number	Transient Hypoxia			Step change hypoxia 1% + 12% O_2			6% + 12% O_2			12% O_2		
	$\dot{V}_E$ $lmin^{-1}$	$\dot{V}O_2$ $lmin^{-1}$	$\dot{V}CO_2$ $lmin^{-1}$	$\dot{V}_E$ $lmin^{-1}$	$\dot{V}O_2$ $lmin^{-1}$	$\dot{V}CO_2$ $lmin^{-1}$	$\dot{V}_E$ $lmin^{-1}$	$\dot{V}O_2$ $lmin^{-1}$	$\dot{V}CO_2$ $lmin^{-1}$	$\dot{V}_E$ $lmin^{-1}$	$\dot{V}O_2$ $lmin^{-1}$	$\dot{V}CO_2$ $lmin^{-1}$
1	17.2	0.65	0.56	17.4	0.65	0.54	18.4	0.68	0.59	17.9	0.69	0.54
2	23.0	0.78	0.77	23.9	0.85	0.78	22.9	0.80	0.76	22.9	0.86	0.71
3	21.0	0.86	0.72	21.9	0.83	0.71	21.1	0.83	0.73	21.9	0.83	0.75
4	22.1	0.76	0.64	21.7	0.76	0.70	23.1	0.80	0.67	21.0	0.80	0.65
5	22.9	0.89	0.75	23.4	0.91	0.75	22.9	0.90	0.77	22.3	0.88	0.76
6	24.6	0.95	0.84	21.6	0.87	0.78	22.4	0.91	0.80	22.1	0.86	0.87

$\dot{V}_E$ expressed as $lmin^{-1}$ BTPS, $\dot{V}O_2$ and $\dot{V}CO_2$ expressed as $lmin^{-1}$ STPD

Table 14 Baseline and hypoxic $P_{ET}CO_2$ values for each section of the study

Subject Number	Baseline $P_{ET}CO_2$ (kPa)				Hypoxic $P_{ET}CO_2$ (kPa)			
	Transient	1% + 12% O_2	6% + 12% O_2	12% O_2	Transient	1% + 12% O_2	6% + 12% O_2	12% O_2
1	5.60 ± 0.12	5.51 ± 0.14	5.46 ± 0.19	5.55 ± 0.11	5.66 ± 0.13	5.50 ± 0.10	5.47 ± 0.12	5.57 ± 0.13
2	5.39 ± 0.10	5.37 ± 0.11	5.41 ± 0.09	5.36 ± 0.11	5.45 ± 0.13	5.35 ± 0.12	5.49 ± 0.11	5.35 ± 0.12
3	5.70 ± 0.21	5.79 ± 0.19	5.71 ± 0.22	5.69 ± 0.18	5.73 ± 0.21	5.76 ± 0.19	5.77 ± 0.20	5.77 ± 0.19
4	5.36 ± 0.18	5.27 ± 0.20	5.31 ± 0.21	5.39 ± 0.17	5.30 ± 0.10	5.47 ± 0.09	5.16 ± 0.10	5.42 ± 0.09
5	5.45 ± 0.18	5.40 ± 0.21	5.32 ± 0.17	5.37 ± 0.19	5.45 ± 0.16	5.41 ± 0.18	5.26 ± 0.18	5.43 ± 0.20
6	5.49 ± 0.20	5.53 ± 0.16	5.52 ± 0.15	5.65 ± 0.20	5.67 ± 0.14	5.57 ± 0.16	5.45 ± 0.14	5.69 ± 0.16

Table 15: Anthropomorphic and lung function measurements

Subj No	Sex	Age (yrs)	Ht (m)	Wt (kg)	BSA (m^2)	FEV_1 (l)	FEV_1 (% pred)	VC (l)	VC (% pred)	FEV_1/VC(%)	FEV_1/VC (% pred)	TLC (l)	TLC (% pred)	RV (l)	RV (% pred)	RV/TLC (%)	TLCO (mmol/kPa.min)	TLCO (% pred)
1	F	23	1.52	57	1.52	2.59	89	3.16	90	82	105	3.81	77	0.65	60	17	8.14	95
2	F	40	1.63	93	1.98	3.00	107	3.75	104	80	106	5.27	101	1.39	82	27	9.16	108
3	M	33	1.71	72	1.83	3.66	89	4.59	96	80	96	6.45	90	1.77	108	27	12.31	116
4	F	25	1.76	57	1.69	3.59	100	4.21	92	86	106	5.39	86	1.18	69	21	8.24	81
5	M	32	1.76	64	1.78	3.86	89	4.70	81	81	111	7.16	106	3.30	195	42	9.04	82
6	F	25	1.61	56	1.57	3.16	105	3.98	105	79	97	5.51	111	1.36	94	25	7.26	80
7	M	24	1.88	73	1.97	4.32	86	5.76	94	75	91	7.87	102	1.89	111	24	13.00	105
8	F	28	1.67	56	1.61	3.23	98	3.69	90	87	109	4.73	83	1.18	73	25	7.20	76
9	M	29	1.84	75	1.96	3.83	81	5.42	93	79	96	7.91	105	1.95	110	25	12.39	84

Table 16: Baseline and step change $P_{ET}CO_2$ values at each exercise level.

	Baseline $P_{ET}CO_2$ Exercise					Hypoxic $P_{ET}CO_2$ Exercise				
Subject	rest	1	2	3	4	rest	1	2	3	4
1	5.31±0.13	5.19±0.13	5.15±0.08	5.19±0.14	-	5.32±0.11	5.23±0.14	5.23±0.13	5.24±0.16	-
2	4.99±0.07	5.01±0.12	5.09±0.11	5.07±0.10	-	4.93±0.09	5.07±0.15	5.10±0.13	5.16±0.19	-
3	5.46±0.15	5.58±0.13	5.64±0.12	5.75±0.15	-	5.37±0.12	5.51±0.10	5.62±0.14	5.75±0.14	-
4	5.38±0.17	5.33±0.18	5.36±0.11	5.63±0.14	-	5.37±0.13	5.29±0.13	5.30±0.12	5.56±0.18	-
5	6.11±0.11	5.44±0.12	5.57±0.19	5.49±0.12	-	6.03±0.13	5.48±0.13	5.65±0.13	5.52±0.15	-
6	5.52±0.09	5.45±0.15	5.40±0.13	5.59±0.13	-	5.45±0.19	5.39±0.19	5.43±0.14	5.54±0.21	-
7	5.72±0.13	5.60±0.13	5.71±0.12	5.64±0.17	5.36±0.24	5.59±0.11	5.63±0.13	5.52±0.09	5.60±0.15	5.41±0.17
8	5.18±0.21	5.46±0.09	5.50±0.15	-	-	5.22±0.17	5.52±0.20	5.42±0.18	-	-
9	5.48±0.10	5.43±0.28	5.60±0.19	5.55±0.22	-	5.53±0.07	5.49±0.17	5.64±0.15	5.66±0.15	-

Table 17 Lowest SaO_2 values during hypoxia at rest and at each exercise level during step change hypoxia for each subject

		Exercise level			
Subject	Rest	1	2	3	4
number	SaO_2 (%)	SaO_2 (%)	SaO_2 (%)	SaO_2 (%)	SaO_2 (%)
1	87	89	87	85	-
2	84	89	87	85	-
3	83	84	84	81	-
4	82	78	82	77	-
5	84	84	83	80	-
6	81	83	84	77	-
7	82	89	85	81	87
8	82	87	81	-	-
9	83	82	82	83	-
mean	83	85	84	81	
s.d.	2	4	2	3	

Table 18: Anthropomorphic and lung function details of the subjects.

Subject Number	Sex	Age years	Ht m	Wt kg	FEV_1 l	FEV_1 % pred	VC l	VC % pred	FEV_1/VC %	FEV_1/VC % pred	TLC l	TLC % pred	RV l	RV % pred	RV/TLC %	TLCO mmol/kPa.min	TLCO % pred
1	M	34	1.71	70	3.66	89	4.59	94	80	96	6.45	99	1.72	108	27	12.31	116
2	M	65	1.72	88	2.05	108	2.80	112	73	96	4.12	70	1.47	65	36	7.10	84
3	F	52	1.59	60	2.35	102	2.90	97	81	108	4.80	100	2.16	127	45	5.60	73
4	F	27	1.71	73	3.45	102	4.50	105	77	96	5.33	89	1.00	59	19	6.45	67
5	M	38	1.81	89	4.80	110	5.70	106	85	105	7.52	104	1.54	81	21	13.33	118
6	F	23	1.66	58	3.90	118	4.35	104	90	111	6.08	107	1.73	115	29	8.85	92

Table 19: FEV_1, airways conductance, respiratory frequency, tidal volume, $P_{ET}CO_2$, ventilation and gas exchange for all subjects at rest, taking placebo or theophylline.

	PLACEBO								THEOPHYLLINE							
Subject number	FEV_1 l	SGaw sec.kPa	$\dot{V}E$ $l min^{-1}$	$\dot{V}O_2$ $l min^{-1}$	$\dot{V}CO_2$ $l min^{-1}$	F_R breaths min^{-1}	Vt l	$P_{ET}CO_2$ kPa	FEV_1 l	SGaw sec.kPa	$\dot{V}E$ $l min^{-1}$	$\dot{V}O_2$ $l min^{-1}$	$\dot{V}CO_2$ $l min^{-1}$	F_R breaths min^{-1}	Vt l	$P_{ET}CO_2$ kPa
1	3.75	1.56	6.1	0.18	0.15	17.7	0.38	5.57	3.60	0.51	8.6	0.23	0.23	18.2	0.96	5.54
2	1.95	2.07	9.0	0.20	0.15	22.0	0.45	4.82	1.70	0.20	7.0	0.17	0.14	16.0	0.39	4.93
3	1.95	1.36	4.2	0.10	0.10	14.5	0.33	5.74	2.35	0.29	5.5	0.13	0.12	14.7	0.35	5.35
4	3.60	1.00	7.4	0.22	0.19	15.7	0.51	4.85	3.60	0.27	8.0	0.20	0.19	15.2	0.54	4.48
5	4.40	1.19	7.7	0.22	0.20	12.7	0.64	5.77	4.60	-	9.1	0.24	0.22	14.9	0.67	4.76
6	3.75	1.29	6.6	0.19	0.17	13.7	0.53	5.06	3.80	0.19	7.6	0.22	0.18	14.8	0.58	5.35

Table 20 Steady state exercise ventilation and gas exchange values for each subject taking either placebo or theophylline

Subject number	PLACEBO $\dot{V}_E$	PLACEBO $\dot{V}O_2$	PLACEBO $\dot{V}CO_2$	THEOPHYLLINE $\dot{V}_E$	THEOPHYLLINE $\dot{V}O_2$	THEOPHYLLINE $\dot{V}CO_2$
1	20.4	0.83	0.84	20.9	0.79	0.79
2	21.7	0.79	0.66	20.7	0.70	0.58
3	17.0	0.47	0.46	15.2	0.53	0.48
4	15.8	0.62	0.53	19.0	0.65	0.57
5	20.2	0.77	0.72	22.5	0.88	0.76
6	16.2	0.63	0.54	17.6	0.67	0.56

$\dot{V}_E$ expressed as $lmin^{-1}$ BTPS, $\dot{V}O_2$ and $\dot{V}CO_2$ as $lmin^{-1}$ STPD.

Table 21 Baseline normoxic exercise $P_{ET}CO_2$ values (base) and those during transient (trans) and step change hypoxia (step) for each subject taking placebo or theophylline

Subject number	PLACEBO base	PLACEBO trans	PLACEBO step	THEOPHYLLINE base	THEOPHYLLINE trans	THEOPHYLLINE step
1	5.71 ±0.49	5.85 ±0.33	5.92 ±0.25	5.50 ±0.17	5.47 ±0.23	5.50 ±0.16
2	5.33 ±0.15	5.31 ±0.16	5.22 ±0.11	5.17 ±0.12	5.11 ±0.17	5.13 ±0.12
3	5.20 ±0.37	- -	5.18 ±0.18	5.14 ±0.17	5.03 ±0.18	5.21 ±0.10
4	5.02 ±0.14	5.05 ±0.22	4.97 ±0.14	4.49 ±0.15	4.56 ±0.10	4.50 ±0.12
5	5.95 ±0.21	5.79 ±0.31	5.89 ±0.16	4.96 ±0.14	4.89 ±0.22	4.99 ±0.17
6	5.18 ±0.15	5.06 ±0.21	5.19 ±0.13	5.31 ±0.17	5.11 ±0.23	5.20 ±0.12

Table 22 Anthropomorphic and lung function measurements for the offspring group

Subject Number	Sex	Age yrs	Ht m	Wt kg	BSA m^2	FEV_1 l	FEV_1 % pred	VC l	VC % pred	FEV_1/VC %	FEV_1/VC % pred	TLC l	TLC % pred	RV l	RV % pred	RV/TLC %	TLCO mmol/ kPa.min	TLCO % pred	THb gdl^{-1}	COHb %
1	M	28	1.84	82	2.04	3.71	78	4.99	86	75	92	6.65	90	2.27	134	34	9.92	81	-	-
2	M	28	1.74	62	1.73	3.71	86	5.03	97	90	107	6.84	104	1.81	121	27	9.00	80	14.2	6.8
3	M	46	1.83	80	2.00	3.29	77	4.64	86	72	90	7.24	98	2.61	124	36	8.22	75	15.8	1.8
4	M	44	1.76	75	1.90	3.85	96	4.75	95	83	103	6.93	102	2.03	107	29	8.27	80	14.4	7.9
5	M	39	1.75	68	1.81	3.53	86	5.03	101	69	84	7.27	109	2.25	125	31	6.03	64	12.5	3.4
6	F	41	1.59	57	1.57	2.70	104	3.33	101	81	105	4.64	95	1.31	82	28	5.48	68	11.7	0.6
7	M	45	1.64	69	1.74	3.37	96	4.08	97	83	101	5.30	91	1.62	95	31	6.85	77	13.4	1.9
8	F	35	1.65	60	1.65	2.92	97	3.40	90	86	109	4.69	87	0.97	57	21	7.64	86	11.3	1.1
9	M	41	1.75	69	1.82	3.60	90	5.25	105	69	85	7.14	107	2.40	133	34	7.03	68	13.3	4.7
10	M	26	1.78	69	1.85	4.34	94	4.79	87	91	110	6.51	94	1.73	108	27	11.22	95	14.0	2.7
11	M	40	1.75	78	1.92	3.79	92	4.65	93	82	100	6.09	91	1.45	81	24	8.89	85	14.6	1.7
12	F	47	1.73	70	1.82	3.25	112	4.15	109	78	103	5.69	96	1.74	83	31	7.80	89	12.5	0.4
13	M	24	1.84	98	2.20	4.98	102	5.45	92	91	110	7.12	96	1.77	111	25	9.51	76	14.9	1.2
14	M	23	1.84	81	2.02	5.21	106	5.65	96	92	111	7.35	99	1.70	106	23	10.11	80	13.0	1.1
15	F	25	1.66	54	1.58	3.46	105	4.01	98	86	106	5.44	97	1.59	106	29	5.99	65	-	-
16	F	35	1.68	53	1.59	2.77	89	3.31	85	84	106	4.49	79	1.22	72	27	7.35	81	12.9	1.1
17	M	30	1.82	67	1.85	4.85	105	5.60	100	87	106	6.79	94	1.74	102	26	7.81	71	13.8	8.0
18	F	37	1.74	60	1.71.	3.32	104	4.34	106	77	99	6.00	98	1.66	87	28	8.29	88	13.5	0.6

Table 22 Anthropomorphic and lung function measurements for the offspring group

Subject Number	Sex	Age yrs	Ht m	Wt kg	BSA m^2	FEV_1 l	FEV_1 % pred	VC l	VC % pred	FEV_1/VC %	FEV_1/VC % pred	TLC l	TLC % pred	RV l	RV % pred	RV/TLC %	TLCO mmol/ kPa.min	TLCO % pred	THb gdl^{-1}	COHb %
19	F	18	1.67	66	1.73	3.40	94	4.10	93	83	100	5.22	90	1.20	86	23	6.71	68	11.9	3.1
20	F	44	1.60	66	1.68	2.32	89	3.35	102	69	90	5.73	115	1.91	119	33	5.78	72	14.1	4.3
21	M	46	1.75	74	1.88	3.40	85	3.65	75	93	115	6.65	99	1.96	103	30	10.28	102	13.9	1.2
22	M	38	1.73	84	1.97	3.75	92	5.00	102	75	92	6.34	98	1.80	106	28	6.20	66	15.1	6.7
23	M	42	1.70	67	1.76	3.23	85	4.45	95	73	89	6.04	96	1.59	94	26	9.50	97	13.7	1.2
24	M	30	1.75	71	1.85	3.75	89	4.20	84	89	107	5.75	86	1.18	74	21	8.32	75	13.6	5.3
25	F	33	1.61	76	1.79	3.05	105	3.85	107	79	100	4.87	94	1.16	77	24	7.62	88	12.5	4.2
26	F	28	1.59	61	1.62	2.70	90	3.40	92	79	99	4.45	87	1.01	72	23	7.07	80	13.5	4.3
27	M	29	1.69	69	1.78	3.75	92	4.65	95	81	96	5.91	95	1.22	87	21	7.10	68	12.7	1.9
28	M	27	1.76	75	1.90	4.56	104	5.45	103	84	101	7.28	109	1.86	124	26	11.20	98	13.4	6.3
29	F	29	1.58	65	1.65	3.01	104	3.81	106	79	99	4.91	98	1.03	74	21	8.22	95	11.8	0.8
30	F	40	1.72	95	2.11	2.60	84	3.20	80	81	104	4.21	71	1.34	71	32	6.08	66	11.9	4.8
31	F	41	1.62	58	1.60	2.80	104	3.40	97	82	107	4.55	89	1.33	78	29	5.43	64	12.3	1.6
32	M	38	1.83	91	2.12	3.45	78	4.65	85	74	91	7.37	101	3.03	159	41	10.18	89	15.2	5.2
33	M	24	1.85	70	1.91	4.95	103	6.10	105	81	98	7.61	103	1.60	94	21	11.32	92	14.7	4.4
34	F	37	1.68	71	1.79	2.57	83	4.20	108	61	78	5.82	104	1.62	90	28	6.87	76	14.1	5.0
35	F	40	1.67	63	1.70	2.75	95	3.50	95	79	101	4.99	91	1.51	84	30	7.00	83	13.8	1.4

Table 23 Anthropomorphic and lung function measurements for the control group

Subject Number	Sex	Age yrs	Ht m	Wt kg	BSA m^2	FEV_1 l	FEV_1 % pred	VC l	VC % pred	FEV_1/VC %	FEV_1/VC % pred	TLC l	TLC % pred	RV l	RV % pred	RV/TLC %	TLCO mmol/ kPa.min	TLCO % pred	THB gdl^{-1}	COHB %
1	M	29	1.79	73	1.90	4.74	105	5.99	109	79	95	7.62	109	1.40	82	18	12.04	104	14.1	0.7
2	F	41	1.65	61	1.66	2.80	100	3.85	104	73	95	4.86	90	1.44	85	30	6.58	77	12.5	4.2
3	M	30	1.75	70	1.84	4.42	103	5.04	97	88	106	6.74	101	1.70	106	25	11.70	105	13.4	0.9
4	M	31	1.78	70	1.86	3.95	88	4.55	84	87	106	7.53	109	2.96	174	39	10.00	88	14.2	2.2
5	F	19	1.65	60	1.65	3.25	96	3.55	85	92	112	5.03	90	1.29	92	26	8.14	84	12.2	0.4
6	F	42	1.59	53	1.53	2.35	90	3.00	91	78	101	4.42	90	1.54	96	35	6.32	78	13.4	3.2
7	F	50	1.63	65	1.69	2.80	112	3.50	106	80	107	5.11	100	1.43	79	28	7.07	90	10.8	1.5
8	M	38	1.79	80	1.98	3.80	88	4.95	93	77	95	6.37	91	1.33	74	21	11.89	107	14.4	1.4
9	F	39	1.64	51	1.53	2.50	89	3.15	88	79	101	4.61	87	1.34	79	29	7.61	89	12.3	0.6
10	M	29	1.77	91	2.07	4.50	102	5.13	97	88	106	6.67	98	1.30	81	19	10.39	91	14.5	1.0
11	F	23	1.75	67	1.80	3.15	88	3.43	76	92	114	4.89	78	1.16	68	24	9.63	94	11.9	1.5
12	F	31	1.64	59	1.62	3.00	97	3.65	95	82	104	4.91	91	1.14	71	23	9.40	103	13.2	0.8
13	M	29	1.84	70	1.91	4.25	90	5.60	97	76	93	7.46	101	1.82	101	24	10.03	82	13.5	0.9
14	F	28	1.60	52	1.52	2.83	94	3.35	88	85	106	5.07	98	2.83	202	56	7.95	88	9.6	0.8
15	M	23	1.65	66	1.72	3.70	90	4.20	89	88	104	5.77	98	1.48	123	26	10.38	99	13.2	1.6

Table 23 Anthropomorphic and lung function measurements for the control group

Subject Number	Sex	Age yrs	Ht m	Wt kg	BSA m^2	FEV_1 l	FEV_1 % pred	VC l	VC % pred	FEV_1/VC %	FEV_1/VC % pred	TLC l	TLC % pred	RV l	RV % pred	RV/TLC %	TLCO mmol/ kPa.min	TLCO % pred	THB gdl^{-1}	COHB %
16	M	46	1.84	81	2.02	4.60	107	6.65	123	69	86	9.17	124	2.54	121	28	10.87	98	12.6	1.8
17	M	28	1.73	74	1.87	4.65	108	5.85	115	80	95	6.97	107	1.29	86	19	13.23	119	14.7	0.7
18	M	22	1.77	66	1.80	3.25	80	4.25	79	77	92	5.68	84	1.29	92	23	10.33	87	13.3	1.6
19	M	24	1.79	73	1.90	4.65	101	5.55	99	84	100	7.25	104	1.59	106	22	11.86	99	13.1	1.9
20	M	49	1.76	80	1.95	3.70	95	4.75	97	78	98	6.72	99	2.06	103	31	8.14	83	12.8	6.5
21	M	27	1.79	75	1.92	4.55	101	5.50	100	83	100	7.38	105	1.77	111	24	12.60	109	13.0	0.9
22	M	39	1.72	86	1.97	4.05	101	5.05	105	80	98	6.75	104	1.73	102	26	12.83	120	16.9	0.7
23	F	27	1.71	69	1.80	3.20	94	4.40	102	73	91	5.63	94	1.35	79	24	5.75	60	13.0	3.5
24	F	42	1.75	65	1.77	3.25	105	4.25	104	77	100	5.61	92	1.33	67	24	6.85	74	12.6	0.1
25	F	33	1.69	72	1.81	3.24	101	3.65	91	89	113	4.67	81	0.97	68	21	6.92	77	11.8	0.7
26	M	39	1.80	88	2.06	4.65	108	5.65	107	82	101	7.57	107	1.67	88	22	9.85	90	15.8	3.0

Table 24 Baseline normoxic values for ventilation and gas exchange for offspring and control groups

Subject number	OFFSPRING $\dot{V}_E$	$\dot{V}O_2$	$\dot{V}CO_2$	CONTROLS $\dot{V}_E$	$\dot{V}O_2$	$\dot{V}CO_2$
1	28.9	0.99	1.09	16.8	0.76	0.70
2	22.4	0.94	0.90	19.8	0.68	0.58
3	27.2	1.14	1.10	30.0	1.19	1.19
4	25.8	0.92	0.75	20.3	0.75	0.75
5	23.1	0.92	0.74	18.2	0.66	0.63
6	19.0	0.67	0.63	19.7	0.66	0.53
7	25.4	0.90	0.81	19.5	0.71	0.60
8	18.0	0.63	0.63	26.0	1.07	1.06
9	24.3	0.93	0.93	13.8	0.50	0.40
10	21.1	0.97	0.95	23.5	0.99	0.86
11	29.1	0.99	1.05	18.6	0.75	0.70
12	25.1	0.92	0.86	18.2	0.61	0.52
13	32.2	1.43	1.22	20.4	0.79	0.74
14	20.5	0.92	0.83	17.7	0.50	0.45
15	17.0	0.56	0.47	24.1	0.85	0.80
16	16.0	0.52	0.49	24.2	0.96	0.81
17	21.0	0.76	0.65	19.3	0.76	0.71
18	18.8	0.60	0.54	18.5	0.82	0.69
19	21.4	0.63	0.67	20.3	0.71	0.69
20	16.1	0.57	0.50	23.1	0.81	0.69
21	30.8	1.20	1.03	22.3	0.88	0.88
22	24.7	0.89	0.79	26.1	1.09	0.84
23	20.0	0.81	0.74	16.4	0.69	0.52
24	21.3	0.93	0.89	16.9	0.68	0.61
25	29.0	0.93	0.97	19.3	0.69	0.57
26	20.8	0.68	0.74	21.0	0.84	0.79
27	21.4	0.88	0.96			
28	17.4	0.71	0.63			
29	19.5	0.79	0.77			
30	26.0	0.89	0.90			
31	15.7	0.55	0.46			
32	32.5	1.09	1.07			
33	26.9	0.93	0.87			
34	21.3	0.86	0.74			
35	18.4	0.67	0.60			

$\dot{V}_E$ expressed as $l min^{-1}$ BTPS, $\dot{V}O_2$ and $\dot{V}CO_2$ as $l min^{-1}$ STPD.

Table 25 Mean $P_{ET}CO_2$ values for each of the offspring group

Subject number	$P_{ET}CO_2$ (kPa) Baseline	Transient hypoxia	Step change hypoxia
1	5.65±0.19	5.37±0.34	5.52±0.22
2	5.96±0.12	5.99±0.18	5.93±0.11
3	5.67±0.30	-	5.67±0.22
4	4.75±0.16	4.70±0.17	4.80±0.10
5	5.10±0.18	4.91±0.31	5.06±0.15
6	5.09±0.09	5.08±0.15	5.08±0.14
7	5.32±0.13	5.23±0.25	5.28±0.17
8	5.19±0.20	5.07±0.23	5.21±0.20
9	5.46±0.17	5.44±0.20	5.41±0.18
10	6.47±0.29	-	6.57±0.27
11	4.64±0.44	4.90±0.31	4.81±0.36
12	5.43±0.21	5.33±0.30	5.33±0.18
13	5.68±0.19	5.64±0.19	5.71±0.22
14	6.07±0.15	6.13±0.23	6.07±0.17
15	4.85±0.19	4.84±0.14	4.80±0.16
16	5.06±0.13	5.12±0.13	5.00±0.12
17	5.73±0.13	5.81±0.12	5.80±0.17
18	4.75±0.18	4.82±0.17	4.85±0.12
19	4.51±0.20	4.47±0.20	4.35±0.17
20	4.92±0.20	5.07±0.16	4.82±0.13
21	4.88±0.12	4.88±0.14	4.82±0.10
22	5.07±0.13	5.03±0.21	5.05±0.12
23	5.52±0.21	5.47±0.21	5.58±0.20
24	5.88±0.16	5.88±0.25	5.87±0.11
25	4.81±0.25	4.90±0.34	4.87±0.14
26	4.98±0.14	4.82±0.22	5.06±0.09
27	4.98±0.20	-	4.91±0.18
28	6.14±0.18	6.18±0.25	6.02±0.22
29	4.92±0.19	4.76±0.22	4.95±0.20
30	5.38±0.33	5.26±0.27	5.29±0.26
31	5.05±0.10	5.10±0.08	5.04±0.08
32	5.00±0.18	4.92±0.13	4.90±0.14
33	4.98±0.43	5.32±0.22	4.96±0.31
34	5.42±0.16	5.58±0.15	5.46±0.17
35	5.06±0.22	4.98±0.10	4.96±0.11

Table 26 Mean $P_{ET}CO_2$ values for each of the control group

Subject number	$P_{ET}CO_2$ (kPa) Baseline	Transient hypoxia	Step change hypoxia
1	4.82±0.20	4.66±0.22	4.79±0.19
2	4.42±0.13	4.45±0.23	4.39±0.12
3	5.14±0.25	5.12±0.21	5.15±0.23
4	5.29±0.23	5.20±0.18	5.22±0.13
5	5.72±0.19	5.77±0.32	5.72±0.24
6	4.61±0.19	4.60±0.20	4.59±0.17
7	5.25±0.11	5.17±0.17	5.18±0.09
8	5.81±0.17	5.78±0.26	5.73±0.20
9	5.29±0.29	5.63±0.25	5.49±0.18
10	5.79±0.22	5.74±0.21	5.70±0.19
11	6.05±0.20	5.80±0.19	5.92±0.18
12	4.77±0.11	4.85±0.17	4.77±0.12
13	5.57±0.17	5.55±0.19	5.48±0.12
14	4.90±0.14	-	4.87±0.10
15	5.35±0.32	5.25±0.15	5.46±0.17
16	5.16±0.11	-	5.14±0.12
17	5.74±0.13	5.66±0.27	5.69±0.10
18	5.49±0.18	5.24±0.27	5.44±0.15
19	5.65±0.18	5.49±0.09	5.64±0.13
20	4.99±0.09	4.94±0.18	4.97±0.10
21	6.17±0.19	6.01±0.30	6.07±0.19
22	5.32±0.16	5.21±0.29	5.30±0.13
23	5.19±0.21	-	5.14±0.14
24	5.32±0.24	5.29±0.21	5.24±0.15
25	5.19±0.14	5.09±0.18	5.16±0.10
26	5.71±0.15	5.53±0.18	5.65±0.10

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GLOSSARY

FEV_1	Forced expiratory volume in 1 second (l)
VC	Vital capacity (l)
RV	Residual volume (l)
FRC	Functional residual capacity (l)
TLC	Total lung capacity (l)
ERV	Expiratory reserve volume (l)
TLCO	Single-breath carbon monoxide diffusion factor (mmol/kPa.min)
R_{AW}	Airways resistance (kPa.sec)/l
SG_{aw}	Airways specific conductance $(kPa.sec)^{-1}$
VTG	Thoracic gas volume (l)
BTPS	Body temperature and pressure, saturated
STPD	Standard temperature and pressure, dry
$\dot{V}_E$	Expired ventilation ($lmin^{-1}$)
$\dot{V}_E inst$	Instantaneous expired minute ventilation ($lmin^{-1}$)
V_t	Tidal volume (l)
f_R	Respiratory frequency (breaths min^{-1})
$\dot{V}O_2$	Oxygen consumption ($lmin^{-1}$)
$\dot{V}CO_2$	Carbon dioxide elimination ($lmin^{-1}$)
RQ	Respiratory exchange ratio ($\dot{V}CO_2/\dot{V}O_2$)
SaO_2	Arterial oxygen saturation (%)
P_I	Partial pressure of inspired gas (kPa or mmHg)
P_E	Partial pressure of end-tidal gas (kPa or mmHg)
P_A	Partial pressure of alveolar gas (kPa or mmHg)
P_a	Partial pressure of arterial gas (kPa or mmHg)
F_I	Fractional concentration of inspired gas (%)
F_E	Fractional concentration of expired gas (%)