Respiratory responses to changes in inspired oxygen levels in infancy: the effect of maternal smoking

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by

Kerry Ann Poole BSc, MSc

Department of Child Health

University of Leicester

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ABSTRACT

**Title:** Respiratory responses to changes in inspired oxygen levels in infancy: the effect of maternal smoking

**Author** Kerry Ann Poole

Deficits in respiratory control have been implicated in the aetiology of sudden infant death syndrome (SIDS). Victims of SIDS show signs consistent with chronic hypoxia prior to death and have abnormalities in both the brainstem and carotid body. Epidemiological studies have shown that maternal smoking is a major independent risk factor for SIDS. Pre and postnatal nicotine exposure may lead to changes in the brainstem and carotid body. This could result in respiratory control deficits which may be involved in SIDS.

The aim of the current work was to investigate the independent effect of maternal smoking on respiratory responses to changes in inspired oxygen in infants. Smoking is associated with other factors which may affect respiratory control, so a matched study design was implemented. Mother/infant pairs were matched for social class, maternal age and parity, gestational age, birthweight, infant gender and feeding intention.

Infants were seen overnight at approximately 10 weeks of age for tests of respiratory control using the alternating breath test. Ventilation was measured using respiratory inductance plethysmography and inspired and end-tidal oxygen levels by mass spectrometry. Fifty-nine infants were studied and data on 40 of these (17 smoking group) was included in the analysis. The respiratory responses were similar in both groups for all respiratory parameters, and there were no significant differences.

The mean end-tidal oxygen level when 40% $O_2$ was delivered was significantly higher in the smoking group even though the measured inspired oxygen levels were similar in the two groups. These unexpected findings seemed to be related to the ongoing nature of the response.

In conclusion, there does not appear to be an independent effect of maternal smoking on respiratory control in infants, and the differences in end-tidal oxygen levels may represent differences in alveolar ventilation during the alternating breath test.
ACKNOWLEDGEMENTS

I am grateful to all those friends, family and colleagues who have supported me over the past 4½ years. Special thanks should go to Dr Caroline Beardsmore who supervised my work throughout, assisted in practical work when required and always gave her time and guidance so willingly. I am indebted to Ms Hilda Hallinan for the role she played in this study in assisting with practical work and performing the tremendously difficult task of recruitment. Thanks also to Dr John Thompson who performed the complex statistical analysis required for this study and provided invaluable help and advice on statistical matters. I would also like to thank Mr Paul Whitacker for performing the urinary cotinine analysis and Mr Tony Bradstock for his support and help with some of the illustrations included in this thesis.

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SECTION I

BACKGROUND AND INTRODUCTION
Chapter 1

Introduction

Sudden infant death syndrome (SIDS) is a leading cause of postneonatal death in England and Wales. During 1997 25% (324) of deaths after 4 weeks of age were attributed to SIDS\(^1\). Numerous epidemiological studies have revealed that maternal smoking, particularly during pregnancy, increases the risk of SIDS by as much as 5 times\(^2\). This association remains when confounding factors of smoking, such as social class, are taken into account. Furthermore, the risk increases with increasing nicotine exposure.

In pathological studies SIDS victims have been shown to have abnormalities in areas important for respiratory control. The brainstems are immature\(^3\text{–}^5\) and show astrogliosis\(^6\), which may be secondary to hypoxic exposure. The carotid bodies, which are important for the regulation of breathing in response to hypoxia, contain elevated levels of dopamine\(^7\) which, when given by intravenous infusion, reduce ventilatory responses to hypoxia\(^8\text{,}^9\). These findings suggest that SIDS victims may have abnormalities in their respiratory control system, particularly in response to hypoxia. In support of this, SIDS victims often show signs consistent with chronic hypoxia before death\(^10\).

Nicotine exposure can result in changes in both the brainstem and carotid body. Prenatal exposure in rats leads to cell death in the medulla\(^11\). Postnatal exposure in newborn rats increases dopamine turnover and may interfere with postnatal resetting of the carotid chemoreceptors\(^12\). This may lead to the carotid chemoreceptors being less responsive to changes in arterial oxygen levels during development. Furthermore, the loss of functioning carotid chemoreceptors leads to respiratory instability and prolonged apnoea at a certain time in development\(^13\).

Studies investigating the effect of nicotine exposure on respiratory control have yielded contradictory results. Prenatal nicotine exposure in the rat did not affect the respiratory responses to hypoxia\(^14\), whereas postnatal exposure soon after birth led to reduced respiratory responses to hyperoxia, suggesting reduced tonic activity\(^12\). However, in the
lamb both pre\textsuperscript{15} and postnatal\textsuperscript{16} exposure have been shown to reduce ventilatory responses to hypoxia. Lewis and Bosque have investigated the effect of maternal smoking on postnatal respiratory responses to hypoxia in the human infant\textsuperscript{17}. They could not find any evidence of an effect of smoking on hypoxic responsiveness. However, this study did not control for confounding factors that may mask any independent effect of smoking, and their tests were performed during day-time sleep. The authors themselves acknowledge that results may have been different during night-time sleep. Since the occurrence of SIDS is primarily associated with a night-time period of sleep it is important to perform tests during this time period.

The main aim of the present work was to investigate the effect of maternal smoking on postnatal respiratory responses, and test the hypothesis that infants born to mothers who smoke have reduced ventilatory responses to changes in inspired oxygen levels. The study was designed so that confounding factors were controlled, and the independent effect of smoking could be assessed. Infants were studied during night-time sleep over the age range associated with the peak incidence of SIDS. It was not an aim of this study to further explore the relationship between smoking and SIDS.
Chapter 2

Sudden Infant Death Syndrome

2.1 Introduction

Sudden Infant Death Syndrome (SIDS) has been defined as "the sudden death of any infant or young child which is unexpected by history and in which a thorough post-mortem examination fails to demonstrate an adequate cause of death" 18.

The SIDS death rate was at its highest in England and Wales in 1988 with a rate of 2.01 per 1,000 live births during the postneonatal period 19. Since this time it has fallen, particularly following the introduction of the 'Back to Sleep' campaign, to a rate of 0.5 per 1,000 live births in 1997 1. However, although the death rate has fallen a large number of infants are still dying, such that in England and Wales a total of 324 infants died of SIDS during 1997 in the postneonatal period 1. This represents 25% of deaths in the postneonatal period 1.

This chapter will briefly summarise some of the main epidemiological factors related to the risk of SIDS and then focus on the relationship between smoking and SIDS in more detail.

2.2 Factors related to the risk of SIDS

2.2.1 General characteristics

Age distribution

Sudden infant death syndrome is relatively uncommon in the neonatal period but then rises sharply to reach a peak in the third and fourth months of life 20. The number of deaths then falls steadily so that at one year of age the occurrence is very rare. About 80% of deaths occur in the first six months and only 5% in the final quarter of the first year of life 20.

Seasonal incidence

A higher proportion of SIDS deaths occur in the autumn and winter months compared to spring and summer 21-23. Although there were clear seasonal differences in the SIDS
death rate until 1991, following the ‘Back to Sleep’ campaign seasonal differences apparently disappeared in 1992. However, this finding has been refuted in a more recent publication.  

2.2.2 Sociodemographic characteristics

Social class/deprivation
In a study containing 125 SIDS victims and 53,721 unmatched controls in the United States it was found that the mothers of SIDS victims were less likely to have graduated high school, and more likely to have a poorer socioeconomic index and live in more crowded conditions. Mason et al found that the death rate rose from 1.93 per 1,000 live births for infants in affluent families (social class I) to 5.24 for infants from more deprived families (social class V). These findings are supported by more recent studies in New Zealand and Scotland. Following the introduction of the ‘Back to Sleep’ campaign in Scotland the SIDS rate fell, but social deprivation was still an important risk factor. Furthermore although the SIDS death rate fell in New Zealand during the period between 1971 and 1991 it did not fall consistently across all social groups, with the low income group having the lowest fall. Consequently, in 1991 the death rate in the low income group was much higher (4.6 per 1,000 live births) than that in the middle (1.2 per 1,000) and high income groups (1.16 per 1,000).

Maternal age and parity
Reduced maternal age and increased parity increases the risk of SIDS. However, there is a very strong correlation between maternal age and parity, such that the increase in risk associated with increasing parity is much greater in younger mothers, particularly in those under 20 years of age. The risk of SIDS associated with the second born infant may be as much as seven times higher if the mother is 20 years of age or younger, compared to a mother aged over 30.

2.2.3 Infant characteristics

Gestational age
Infants born before 37 weeks gestation have been shown to have a 2.8 times greater risk of dying of SIDS than infants born over 37 weeks. The SIDS death rate increases with shorter gestational ages so that for infants born between 24 and 28 weeks the death
rate was 3.52 per 1,000, between 29 and 32 weeks it was 3.01, between 33 and 36 weeks it was 2.27, and 37 weeks or over it was 1.06.

**Birthweight**

Low birthweight has been shown to increase the risk of SIDS. Infants with birthweights between 1501 and 2000g have been shown to have an odds ratio of 5.3 for SIDS, when compared to infants with birthweights between 3001 and 4000g. The odds ratio falls to 2.8 when gestational age is taken into account. Similar results have been found in New Zealand and Scotland with odds ratios for birthweights of <2.5kg of 4.07 and 3.79 respectively.

**Gender**

Male infants are at greater risk of SIDS than females. The odds ratio for the risk of SIDS in male infants varies between 1.38 and 1.84 in different studies. This increase in risk cannot be explained by differences in gestational age, birthweight, social class or other potential confounding factors.

**2.2.4 Child care characteristics**

**Mode of feeding**

Breastfeeding has been shown to give a lower odds ratio of 0.5 compared to bottle feeding. Other studies have also shown that the incidence of SIDS increases with bottle feeding. Studies by Gilbert et al. and Brooke et al. have also shown that bottle feeding is associated with an increased odds ratio, but when confounding factors were taken into account there was no increase in risk. Similar results were found in the New Zealand Cot Death Study.

**Sleeping position**

The prone sleeping position is associated with an increased risk of SIDS. In Avon between November 1987 and April 1989, 93% of those infants who died were put down to sleep in the prone position compared to 57% of controls. The same group showed that a fall in mortality during the period between February 1990 and July 1991 could almost entirely be accounted for by reduced prevalence of the prone sleeping position.
In October 1991 the ‘Back to sleep’ campaign was launched by the Department of Health in the UK which advised parents to avoid placing their infants prone to sleep. This campaign, and others like it in other countries, appears to have been successful in reducing the number of SIDS deaths. The postneonatal SIDS death rate was 1.48 per 1,000 in 1990, whereas in 1997 it was 0.50. Following this intervention in the UK 16% of those infants who died of SIDS were placed prone, compared to 93% previously. The prone sleeping position was still associated with a high risk of SIDS with an odds ratio of 9.58, and a new worrying finding was that the side position also increased the risk. Other studies have also shown that although the prevalence of the prone position has fallen following intervention campaigns it is still associated with an increased risk.

**Thermal environment**

Overheating may be an important contributory factor in SIDS. Fleming *et al* have shown that infants who died of SIDS were more likely to have been more heavily wrapped, and to have had the heating left on for the whole night. Furthermore, following the ‘Back to Sleep’ campaign the same group also found that SIDS victims were more likely to have been more heavily wrapped, to have used a duvet or been found with covers over their head. When confounding factors were taken into account the infant being found with covers over their head gave a very high odds ratio of 21.58.

**2.3 Relationship between smoking and the risk of SIDS**

**Maternal smoking**

Over the past 30 years evidence has accumulated suggesting that maternal smoking increases the risk of SIDS. In 1967 it was reported that 68.2% of mothers of SIDS cases smoked during pregnancy compared to 39.5% of controls. Similar results were reported in 1976. Since then many studies have found that maternal smoking during pregnancy increases the risk of SIDS by between 1.5 and 6.05 times when potential confounding factors are not taken into account (table 2.3.1). However, smoking is related to social class, maternal age, feeding etc. which may themselves be related to the risk of SIDS. Therefore, potential confounding factors must be considered when investigating whether maternal smoking is an independent risk factor. Many studies have taken confounding factors into account, but the number and combination of factors...
varies, making comparisons difficult. Those studies that have taken confounding factors into account have found that maternal smoking during pregnancy is an independent risk factor for SIDS and is associated with an increased odds ratio between 1.3 and 6.9 (table 2.3.1). Furthermore, several studies have also shown that the risk of SIDS increases with increasing levels of smoking. In addition, studies conducted following advice about sleeping position have shown that the risk of maternal smoking remains.

The majority of women who smoke during pregnancy also smoke postnatally. Since the studies presented in table 2.3.1 did not take postnatal maternal smoking into account, these results may represent combined exposure. Schoendorf and Keily have investigated the separate effects of combined and passive exposure. Passive exposure in white infants was associated with an adjusted odds ratio of 1.75, whereas the odds ratio for combined exposure was 3.10. Therefore postnatal passive exposure from the mother was important, but combined exposure carried a much greater risk. It was suggested that maternal smoking during pregnancy was more important than postnatal passive smoking.
<table>
<thead>
<tr>
<th>Study</th>
<th>Date</th>
<th>Country</th>
<th>Unadjusted odds ratio (95% confidence interval)</th>
<th>Factors accounted for</th>
<th>Adjusted odds ratio (95% confidence interval)</th>
<th>Dose response relationship</th>
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<td>Alm et al 48</td>
<td>1998</td>
<td>Norway, Denmark, Sweden</td>
<td>4.0 (2.9-5.6)</td>
<td>age of infant, maternal age, education</td>
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<td>4.85 (2.76-8.53)</td>
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<td>Mitchell et al 34</td>
<td>1997</td>
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<td>Blair et al 49</td>
<td>1996</td>
<td>UK</td>
<td>4.84 (3.33-7.04)</td>
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<td>Klonoff-Cohen et al 55</td>
<td>1995</td>
<td>USA</td>
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<td>Poets et al 47</td>
<td>1995</td>
<td>Germany</td>
<td>3.2 (2.3-4.4)</td>
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<td>1-10 cigs per day: 2.6 (1.5-4.4)</td>
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<td>11-20 cigs per day: 2.8 (1.8-6.0)</td>
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<td>&gt; 20 cigs per day: 6.9 (1.9-25.5)</td>
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<td>Country</td>
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<td>Variables</td>
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<td>Taylor and Sanderson 32</td>
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<td>Mitchell et al 50</td>
<td>1993</td>
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<td>marital status, maternal age, maternal education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haglund and Cnattingius 52</td>
<td>1990</td>
<td>Sweden</td>
<td>1.8 (1.2-2.6)</td>
<td>maternal age, parity, partner, gender, type of birth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kraus et al 30</td>
<td>1989</td>
<td>USA</td>
<td>1.5 (0.9-2.4)</td>
<td>birthweight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malloy et al 56</td>
<td>1988</td>
<td>USA</td>
<td>2.92 (2.38-4.4)</td>
<td>Marital status, education level, age and parity</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Some studies have investigated the effect of postnatal maternal smoking on the risk of SIDS. Klonoff-Cohen et al have shown that the odds ratio associated with postnatal maternal smoking, adjusted for maternal smoking during pregnancy and other confounders, was 2.28. Other studies have also found a raised odds ratio but have not adjusted for maternal smoking during pregnancy.

Maternal smoking appears to be more important in those infants dying at a younger age. Nicholl and O'Cathain found that maternal smoking during pregnancy increased the risk of SIDS only in infants between 1 and 7 weeks of age. Haglund and Cnattingius have also found that maternal smoking is more important in early SIDS deaths. The relative risk associated with smoking ≥ 10 cigarettes per day was 3.6 in the early SIDS group (7-67 days) compared to 2.0 in the late SIDS group (68-145 days).

In summary, evidence has now accumulated that maternal smoking is associated with an increased risk of SIDS. There is some evidence that maternal smoking during pregnancy itself is an independent risk factor, and a more important factor than postnatal passive smoking.
Postnatal passive smoking by others in the household

In 1976 Bergman and Wiesner found that those infants who died of SIDS were more likely to have a father who smoked (59% in SIDS group, 43% controls) although this was not significant. Several studies have now investigated the impact of paternal smoking, and of others in the household on the risk of SIDS. Mitchell et al found that the odds ratio increased with increasing paternal smoking and increasing numbers of smokers in the household, so that when there were three or more smokers in the household the odds ratio was 5.72 in univariate analysis. However, paternal smoking only had an effect when the mother was also a smoker. Klonoff-Cohen et al found that the adjusted odds ratio associated with paternal smoking after birth was 3.46, increasing to 8.49 if the father smoked in the same room as the infant. Smoking from other live-in adults also increased the risk of SIDS. Similar results have been found in the UK. The risk of SIDS increased with greater numbers of smokers in the household, increasing number of cigarettes smoked in the same room as the infant and the number of hours the infant was exposed to cigarettes smoke per day. Whilst both paternal smoking and smoking from others in the household gave significant odds ratios in univariate analysis, only paternal smoking had an independent effect in multivariate analysis. However, these findings have not been confirmed by a more recent study which did not find a significant independent effect of paternal smoking or smoking from others in the household on risk of SIDS.

In summary, there is now some evidence that postnatal passive smoking from the father and other individuals in the household leads to an increased risk of SIDS. Furthermore, it has been suggested if all smoke exposure (antenatal and postnatal) was prevented the SIDS death rate may fall by almost two thirds.
Chapter 3
Control of ventilation in response to changes in arterial oxygen level

3.1 Introduction

The role of the respiratory control system is to adjust ventilation so that despite changing metabolic requirements or environments the arterial levels of oxygen stay fairly constant. Such a control system requires three elements: (i) sensors which gather information and send it to (ii) a central controller in the brain which integrates the information and sends signals to (iii) effectors (respiratory muscles) which change ventilation. The sensors involved in monitoring arterial oxygen levels are the peripheral chemoreceptors which are located in the carotid bodies at the bifurcation of the carotid artery, and in the aortic bodies in the aortic arch. The aortic chemoreceptors are thought to have little involvement in respiratory responses to hypoxia in the human. The carotid chemoreceptors respond very quickly to changes in arterial oxygen levels. The relationship between ventilation and arterial oxygen level is hyperbolic in nature, and large increases in ventilation are only seen when the arterial oxygen level falls to 7.98 kPa or below.

The central controller is positioned in the medulla of the brain stem and has a rhythmic ‘inspiratory ramp’ output. There are two main parts of the medulla responsible for this rhythmic output: (i) the nucleus tractus solitarius in the dorsal part of the medulla which has neurones that fire during inspiration, and (ii) the nucleus retroambigualis in the ventrolateral part of the medulla which contains neurones that fire during both inspiration and expiration. This ‘inspiratory ramp’ signal can be modified by areas in the pons, by information from the cortex of the brain, by information from the sensors already described, and from sensors not described here, such as stretch receptors. The ‘inspiratory ramp’ signal is output to respiratory muscles including the intercostal muscles, the diaphragm and upper airway muscles leading to a change in ventilation, and consequently changes in blood gases.
This chapter will discuss the development of this control system, focusing on the peripheral chemoreceptors and their response to changes in arterial oxygen level. The importance of the peripheral chemoreceptors will be discussed and evidence reviewed showing how nicotine exposure may affect the development of this system.

3.2 Development of control of breathing in infancy

3.2.1 In utero

Foetal lambs demonstrate periodic spontaneous breathing movements from around 40 days gestation (term 145-150 days), which are associated with periods of low voltage ECoG activity. In other words, foetal breathing activity is closely related to foetal sleep state with breathing movements occurring during REM sleep, but apnoea during non-REM sleep. During the last third of gestation in the foetal lamb breathing occurs for approximately 40% of the time, a similar proportion to the time spent in REM sleep. The human foetus spends a similar amount of time (31%) breathing in late gestation. Foetal breathing does not appear to be affected by normal fluctuations in foetal arterial oxygen or carbon dioxide levels. However, hypoxaemia abolishes breathing movements. The peripheral chemoreceptors are fully mature and capable of responding to chemical stimuli in the term foetus, but they are relatively insensitive to arterial oxygen because of the hypoxic environment they are exposed to during foetal life. In addition, information from the carotid bodies may be overruled by powerful inhibitory mechanisms.

The role of foetal breathing is not completely understood, but it is important for normal lung development.

3.2.2 Newborn infant

Two of the most important changes the foetus must make following delivery are (i) to breathe continuously rather than intermittently, and (ii) to adapt to an air breathing environment which is relatively rich in oxygen compared to the hypoxic intrauterine environment. The mechanism responsible for stimulating the first breath and maintaining continuous breathing is unknown. However, denervation of the carotid chemoreceptors does not affect initiation of continuous breathing at birth.
In the foetus, peripheral chemoreceptor activity is adapted to the hypoxic (approx. 3.33 kPa) environment. However, at birth the arterial oxygen level rises to between 7.98 and 11.97 kPa, and the peripheral chemoreceptors are silenced. Over the next few days of life the hypoxic sensitivity of the chemoreceptors is reset. If this did not occur the infant's arterial oxygen level would need to fall to below 3.33 kPa for a respiratory response to be initiated. Direct recordings of carotid sinus nerve activity in the lamb have revealed that the peripheral chemoreceptors are active during foetal life, become quiescent on the day of birth, and subsequently spontaneous activity returns at around 2 days of age. Hertzberg et al. have also shown a similar time course for resetting in the rat. A hyperoxic gas (100% O\textsubscript{2}) was given to rats to 'switch-off' the peripheral chemoreceptors and give a measure of tonic activity of the peripheral chemoreceptors. There was no ventilatory response on the day of birth, however by day 1 there was a significant fall in ventilation (-19.4%) suggesting that the peripheral chemoreceptors become active at around 24 hours after birth. This was accompanied by changes in the level of dopamine in the carotid body, which were much higher than foetal levels following birth, and then fell steadily. Dopamine reduces the sensitivity of the carotid body to hypoxia and reduces ventilatory responses. Consequently, the silencing of the chemoreceptors at birth may be a consequence of increased dopamine levels in the carotid body with resetting occurring when these levels fall postnatally. Resetting has also been studied in the human infant. Williams et al. found that the peripheral chemoreceptors were active 3-10 hours after birth, but the response was weak. Over the first 5-8 days postnatally the number of respiratory parameters involved and the size of the response increased. These results suggest that whilst resetting may start soon after birth in the human infant it is not complete until 5-8 days postnatally. However, these results have not been supported in a study conducted by the same group measuring the same infants longitudinally at two ages (43 hours and 47 days). There was no evidence of an increased response over time suggesting that resetting was essentially complete 24-48 hours after birth in the human infant.

In summary, it is clear that after birth the peripheral chemoreceptors are quiescent and must reset to their new relatively hyperoxic environment. This resetting appears to be achieved by reducing dopamine levels in the carotid body, however, the time period for this to occur is unclear. This process appears to start within the first 24 hours after birth and may continue until 8 days of age postnatally.
The neonates of several species (sheep, rabbit, human) have been shown to have a 'biphasic response' to hypoxia. This involves a rapid increase in ventilation over the first minute of exposure to hypoxia followed by a reduction in ventilation, often below baseline levels, over time. This response may be affected by environmental temperature. Ceruti found that when infants were studied in a cool environment (ambient temperature <28°C) the initial increase in ventilation was absent. This initial increase in ventilation is mediated by the peripheral chemoreceptors, but the mechanism by which ventilation is reduced is unknown. The peripheral chemoreceptors are active during the period of the 'biphasic response' in the kitten, and therefore this reduction is not due to failure of the chemoreceptors. This has led to the suggestion that the fall in ventilation seen with prolonged hypoxia may be the result of a central inhibitory mechanism, and it has been proposed that pathways involving the red nucleus may be involved.

The 'biphasic response' is peculiar to the neonate, and with maturity a sustained hyperventilatory response is seen with hypoxia. However, at what age does the neonate acquire the mature response? The term infant has been shown to demonstrate this mature pattern to hypoxia at around 1 week of age. However, a more recent study suggests that infants demonstrate the immature biphasic response up to 8 weeks postnatally. In the lamb Bureau et al have found differing results with two levels of hypoxia (7% and 12% O₂). At 2 days of age lambs demonstrated a biphasic response to both levels of hypoxia. At 7 days of age the lambs had a sustained hyperventilatory response to 12% O₂, but the immature biphasic pattern remained in response to 7% O₂. This may be a consequence of the balance between the drive from the peripheral chemoreceptors and the opposing central inhibitory effect. It is possible that the central inhibitory drive in response to 7% O₂ was greater than the peripheral chemoreceptor drive, and that a sustained hyperventilatory response is only seen when the drive from the chemoreceptors is great enough to overcome the central inhibitory effect.

Studies would suggest that after resetting of the peripheral chemoreceptors and development of the mature sustained hyperventilatory response to hypoxia no further development takes place. Wilkie et al found that the peripheral chemoreceptors had only a 10% contribution to tidal volume at 1-7 days of age, increasing to 26.6% between 8 and 42 days of age, with no further increase in older infants. Parks et al found that infants at 1, 2 and 3 months of age had similar responses and, thus, there was no
evidence of development over this time period 75. In addition, Calder et al measured responses to the alternating breath test in infants aged 43 hours and 47 days and found no evidence of maturation over this age range 67. However, these studies have not investigated respiratory responses over frequent intervals in the same individuals. A pilot study conducted within the University of Leicester measured respiratory responses using the alternating breath test in infants at weekly intervals between the ages of 6 and 18 weeks and found that respiratory responses increased 76.

In conclusion, it is clear that the foetus makes breathing movements in utero and that the peripheral chemoreceptors are functional. After birth the newborn must adjust to its new oxygen-rich environment and the peripheral chemoreceptors must be 'reset' and become responsive. The relatively weak chemoreceptor drive in response to hypoxia may, in part, result in the 'biphasic response' to hypoxia. However, during normal development this immature response is replaced by a mature sustained hyperventilatory response. Following this it is unclear whether any further development occurs, but a recent pilot study suggests that respiratory responses increase, at least until 18 weeks of age.

Clearly, if extrinsic factors, such as nicotine exposure, interfere with this complex pattern of development this may result in an infant who is vulnerable to hypoxia.

### 3.3 The possible effect of nicotine exposure on development of respiratory control

Maternal smoking appears to reduce foetal breathing movements in the human foetus 77,78. In addition, nicotine infusion in pregnant ewes leads to a reduction in foetal breathing movements 79. However, direct injection of nicotine into the sheep foetus stimulates foetal breathing 79. It has been suggested that the reduction in foetal breathing may be secondary to foetal hypoxia resulting from reduced placental gas exchange caused by nicotine exposure. If foetal breathing movements are reduced with nicotine exposure then this may have consequences for lung development, and perhaps development of respiratory control.

The brainstem of the human foetus expresses nicotinic acetylcholine receptors which are more prevalent at midgestation, and can be found in areas important for cardiopulmonary
control 80. It has been suggested that the brainstem may be particularly susceptible to nicotine at this time, and that these receptors may play a critical role in development. Furthermore, chronic nicotine treatment during gestation and lactation leads to an increase in the number of nicotinic receptors in the brainstem in the neonatal rat 81. Prenatal nicotine exposure increases cell death in the medulla 11 and disrupts normal development of the midbrain and brainstem 82. In addition, prenatal nicotine exposure in rats may lead to inhibitory neurones in the midbrain and brainstem becoming hyperresponsive to hypoxia, contributing to respiratory arrest in response to severe hypoxia (5% O₂ for 60 minutes) 83. Therefore, evidence suggests that the development of the brainstem may be affected by prenatal nicotine exposure.

Postnatal nicotine exposure may interfere with resetting of the chemoreceptors 12. When 3 day old rat pups were injected with nicotine their reduction in ventilation following inspiration of 100% O₂ was lower (6.9%) than in control conditions (22.5%), suggesting reduced activity of the peripheral chemoreceptors during nicotine exposure. This effect of nicotine on the ventilatory response could be blocked by domperidone, a DA2 receptor antagonist, indicating that dopamine release may be involved. Although the dopamine content was lower in the carotid body with nicotine exposure, turnover was increased at a time in development when dopamine synthesis and release should be falling. It was concluded that nicotine may have two effects on the carotid body, (i) induction of dopamine release leading to attenuation of hypoxic sensitivity, and (ii) increase in dopamine synthesis leading to difficulties in postnatal resetting of the peripheral chemoreceptors.

If resetting of the chemoreceptors is affected by nicotine exposure this could mean that drive in response to hypoxia may be weaker than expected. Therefore, for a longer period during development the central inhibitory effect of hypoxia may be stronger that the excitatory peripheral chemoreceptor drive. Consequently, this could mean that (i) the initial increased ventilatory response to hypoxia would be reduced, and (ii) the biphasic response to hypoxia may continue past the first few weeks of life. In addition, lack of peripheral arterial chemoreceptor activity during development may lead to persistent impairment of hypoxic ventilatory responses 84. Even if resetting does occur, nicotine exposure may affect respiratory control by stimulating release of dopamine. Consequently, the possible effects of nicotine exposure on postnatal resetting, and
induction of release of dopamine, may mean that an infant is unable to respond appropriately to a hypoxic insult at a time in development when they should be able to do so, resulting in vulnerability.

It has been shown that postnatal administration of nicotine in the lamb attenuates the ventilatory response to hypoxia and increases the response to hyperoxia\(^\text{16}\). The authors concluded that postnatal nicotine exposure alters peripheral chemoreceptor oxygen sensitivity and also central processing of the chemoreceptor input. However, the augmentation of ventilatory responses to hyperoxia by nicotine may be species specific, since nicotine reduces the decrease in ventilation seen with hyperoxia in the rat\(^\text{12}\).

Prenatal nicotine exposure has been shown to reduce respiratory responses to hypoxia in the lamb\(^\text{15}\). However, these findings are not supported by investigations in the rat\(^\text{14}\). Lewis and Bosque have also failed to find a difference in respiratory responses to hypoxia in human infants born to women who smoke, compared to those born to mothers who do not\(^\text{17}\). Interestingly, it has been shown that normal healthy infants whose mothers smoked during pregnancy have an increase in the frequency and length of obstructive apnoeas, when compared to those born to nonsmoking women\(^\text{85}\).

In summary, nicotine exposure may cause changes in the brainstem and carotid body which may lead to a reduced ability to increase ventilation in response to hypoxia. Few studies have investigated the effects of nicotine exposure on respiratory responses to changes in oxygen, and those that have give contradictory findings.

### 3.4 Importance of functioning peripheral chemoreceptors during development

In the normal environment it is unlikely that an infant will encounter inspired oxygen levels which are low enough to lead to a reduction in arterial oxygen levels. However, if respiratory instability and apnoea were to occur then arterial oxygen levels may fall. The peripheral chemoreceptors play an important role in the termination of apnoea in the awake newborn lamb\(^\text{86}\). Animals with denervated carotid bodies were at increased risk of prolonged apnoea, but all survived.
The peripheral chemoreceptors are active during air breathing contributing to ventilation by as much as 38% in the human infant. Denervation of the carotid body in the lamb leads to a fall in ventilation, a reduction in arterial oxygen and an increase in arterial carbon dioxide levels. In addition, 3 of the 7 carotid body denervated lambs died suddenly and unexpectedly between 21 and 42 days of life. The authors suggested that the peripheral chemoreceptors are essential for postnatal maturation of breathing, and their absence rendered lambs vulnerable to unexpected death a few weeks after birth. There is evidence that the peripheral chemoreceptors may be important for maintaining a regular breathing pattern. In the neonatal rat carotid and aortic body denervation led to severe intermittent respiratory disturbance and cyanosis during sleep, which was more prevalent in the younger rats. More than half of the rats died when denervation was performed at 5 days of age, but was lower when denervation was performed at older ages. Cote et al have also found that the effect of carotid body denervation on respiratory pattern is age-dependent. Piglets had their carotid bodies denervated at either 4-5, 9-10, 12-15 or 21-22 days of age. It was found that carotid denervation between 12-15 days was associated with numerous, prolonged central apnoeas, which were associated with desaturation and tachycardia. These results show that there is a period during postnatal life when central rhythm generation may be dependent upon information from the peripheral chemoreceptors. In addition, the carotid chemoreceptors appear to play a role in establishment of oral breathing when the nasal airway is occluded. Carotid body denervation in lambs led to delayed onset of oral breathing and a greater degree of hypoxia during nasal occlusion.

In summary, evidence suggests that the peripheral chemoreceptors have two main roles, (i) to increase ventilation in response to a reduction in arterial oxygen level or an increase in arterial carbon dioxide level, and (ii) to stabilise respiration during normal air breathing. Therefore, poorly functioning peripheral chemoreceptors during development may lead to instability in the breathing pattern and vulnerability of the infant to prolonged apnoeic events. This may be more important at certain periods in development.
Chapter 4

Control of breathing, SIDS and smoking

4.1 Introduction

Previous chapters have presented evidence showing that the mechanisms involved in controlling ventilation in response to hypoxia undergo developmental changes over the first few months of life, and may be affected by pre and postnatal nicotine exposure. Functioning peripheral chemoreceptors are important for respiratory stability, particularly at critical points in development. Maternal smoking increases the risk of SIDS and, therefore, respiratory control deficits may be involved in the aetiology of SIDS. This chapter reviews the evidence that respiratory control deficits may be implicated in SIDS.

4.2 Pathological evidence suggesting that SIDS victims may have abnormalities in areas important for respiratory control

Approximately 80% of SIDS cases have intrathoracic petechiae on the surfaces of the thymus, heart and lungs, which are thought to be a consequence of continued respiratory effort against an upper airway obstruction. Therefore, the final common event in a proportion of SIDS deaths may be obstructive apnoea. Post-mortem findings of increased pulmonary arterial muscle, right ventricular hypertrophy, retention of periaxial brown fat and extramedullary haematopoiesis imply that SIDS victims may have experienced recurrent or chronic hypoxaemia before death. This could result from recurrent episodes of prolonged apnoea, chronic hypoventilation or both.

In 1976 Naeye showed that 14 out of the 28 SIDS victims had abnormal proliferation of astroglial fibres in the reticular formation of the brainstem. These findings have been confirmed by Kinney et al who measured the number of 'reactive' astrocytes in the medulla oblongata and found that SIDS victims have greater numbers of these in the nucleus tractus solitarius compared to controls. These abnormalities may be secondary to hypoxic/ischaemic insult, and it has been proposed that cerebral hypoperfusion during bradycardia accompanying apnoea may be important. This may set up a vicious cycle whereby hypoxaemia leads to brainstem gliosis resulting in respiratory dysfunction, and subsequent further hypoxaemia.
Other studies have shown that the brainstems of SIDS victims are immature. Takashima and Becker have found an increased number of dendritic spines in areas of the brainstem in SIDS victims. These findings are supported by a study by Quattochi et al who also found increased numbers of dendritic spines throughout the first year of postnatal life in areas of the brainstem important for respiratory control in SIDS victims. Since the number of dendritic spines fall after birth it is thought that this is a sign of immaturity. It has also been shown that the brainstems of SIDS victims contain more synapses that control infants, and exhibit delayed myelination.

Naeye et al have shown that 63% of SIDS victims have a subnormal volume of glomic cells and 23% an enlarged volume in their carotid bodies. Those infants with enlarged glomic cells showed evidence of chronic hypoxaemia suggesting that in a small proportion of SIDS deaths the carotid body was hyperplastic. Cole et al have shown that there is a reduction in cell number and size, and cytoplasmic granules in SIDS victims. These findings have not been supported by Perrin et al who found that the carotid bodies were morphologically similar in SIDS victims and control infants, and that the size, distribution and frequency of neurosecretory granules were also similar. However, increased levels of dopamine (10-fold) and noradrenaline (3-fold) have been found in the carotid bodies of SIDS victims. Dopamine is known to reduce the sensitivity of the carotid body to hypoxia and ventilatory responses. Therefore, if these changes were to occur in vivo, and result in increased release of dopamine from the carotid body, it is possible that this may reduce the infant's ability to increase ventilation appropriately to a hypoxic insult. These raised levels of dopamine may be secondary to chronic hypoxia, since exposure to hypoxia has been shown to increase the turnover and content of dopamine in the carotid body.

In summary, there is strong evidence that some infants who have died from SIDS have been chronically hypoxic before death. They have abnormalities in their brainstems, including areas important in respiratory control, consistent with hypoxic damage, and have also been shown to have delayed maturation. In addition, a proportion have raised dopamine levels in their carotid bodies which may affect their respiratory responses to hypoxia. Therefore, these observations have led to the suggestion that SIDS victims may have deficits in their ability to control breathing, particularly in response to hypoxia.
4.3 Studies investigating hypoxic ventilatory responses in infants at risk of SIDS

There are few studies investigating hypoxic ventilatory responses in infants thought to be at increased risk of SIDS. Hunt et al measured hypoxic ventilatory responses to a progressive reduction of inspired oxygen levels to 15% O$_2$ in two groups of infants. The first group had experienced a near-miss apnoeic attack with pallor or cyanosis (near-miss SIDS), and the second group (controls) apnoea while awake, often associated with gastroesophageal reflux. The near-miss SIDS group had a significantly reduced hypoxic ventilatory response slope compared to the control group. Ariagno et al were unable to find any differences in hypoxic ventilatory responses to 15% O$_2$ between near-miss SIDS and controls. However, the responses in this study were extremely variable and the number of infants involved was small (9 near-miss SIDS and 5 controls). Subsequent siblings of SIDS victims, apnoeic infants and controls have similar tonic activity of the peripheral chemoreceptors. When infants were given 100% O$_2$ to breathe the reduction in minute ventilation that was seen was 44.8% in SIDS siblings, 31.3% in apnoeic infants and 31.0% in controls during non-REM sleep.

Other studies have investigated the amount of periodic breathing and respiratory pauses in response to hypoxia. Brady et al found that SIDS siblings experienced more short apnoeic pauses than infants with a history of apnoea and control infants when exposed to 17% O$_2$. However, these findings have not been supported by other studies. Ariagno et al found that periodic breathing in response to 15% O$_2$ was slightly more prevalent in their control group compared to near-miss SIDS infants. Lindenberg et al found that periodic breathing in response to 17% O$_2$ was more prevalent in controls compared to near-miss SIDS infants and siblings of SIDS victims. There were no significant differences.

In summary, there is some evidence that infants at risk of SIDS may have reduced ventilatory responses to hypoxia.
4.4 Studies investigating breathing patterns during sleep in infants at risk of SIDS

Numerous studies have been conducted investigating breathing patterns, particularly apnoea, in infants who have subsequently died from SIDS, have had a near-miss episode, or are a subsequent sibling of a SIDS victim (table 4.4.1). In general, whilst some differences between high risk infants and controls have been found there is often overlap between groups, and therefore these respiratory parameters do not appear to be predictive. The overall findings are inconclusive with some studies finding differences and others not. Two of the studies have investigated the occurrence of obstructive apnoea in infants who have subsequently died of SIDS. One study showed that victims had more obstructive apnoea than matched controls, and the other study showed an increased frequency of apnoea, but not of the mixed/obstructive type. However, the latter study monitored infants in the first 7 days of life whereas the study by Kahn et al monitored infants at a median age of 9.2 weeks, an age closer to when SIDS is likely to occur. Other studies have investigated the incidence of central apnoea in infants who subsequently succumbed to SIDS. Southall et al found no difference in the number of central apnoeas between SIDS victims and age-matched controls. However, Schectman et al, using Southall's data, showed that in the second month of life SIDS victims had fewer central apnoeas.
Table 4.4.1 Summary of some of the studies investigating the occurrence of apnoea and periodic breathing in infants at risk of SIDS

<table>
<thead>
<tr>
<th>Author</th>
<th>Infants studied</th>
<th>Measurements</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hoppenbouwers et al (1993) 109</td>
<td>16 SIDS siblings 14 controls 19 born prematurely</td>
<td>Obstructive apnoeas ≥ 3 seconds</td>
<td>At 3 months of age similar number of apnoeas in controls and SIDS</td>
</tr>
<tr>
<td>Kahn et al (1992) 106</td>
<td>30 SIDS victims 60 matched controls</td>
<td>Central, obstructive and mixed apnoeas ≥ 3 seconds</td>
<td>Infants who died of SIDS had significantly more obstructive apnoea</td>
</tr>
<tr>
<td>Schectman et al (1991) 108</td>
<td>16 SIDS victims 66 controls</td>
<td>Central apnoea ≥ 4 seconds</td>
<td>In second month of life SIDS victims had significantly lower number of central apnoeas in both QS and AS</td>
</tr>
<tr>
<td>Southall et al (1986) 107</td>
<td>16 SIDS victims 127 controls</td>
<td>Central apnoea ≥ 3.6 seconds</td>
<td>No difference in central apnoeas</td>
</tr>
<tr>
<td>Kelly et al (1982) 110</td>
<td>10 siblings of SIDS 10 controls</td>
<td>Central apnoea ≥ 3 seconds</td>
<td>At two weeks of age SIDS siblings had greater number of apnoeas lasting 5-9.9 seconds and 10-14.9 seconds.</td>
</tr>
<tr>
<td>Steinschneider et al (1982) 105</td>
<td>10 SIDS victims 1301 controls</td>
<td>Central and mixed obstructive apnoea &gt; 2 seconds, in first 7 days of life</td>
<td>SIDS victims had greater frequency of apnoeic pauses during sleep but no difference in mixed/obstructive apnoea</td>
</tr>
<tr>
<td>Hoppenbrouwers et al (1980) 111</td>
<td>26 siblings of SIDS 25 controls</td>
<td>Central, mixed and obstructive apnoea ≥ 2 seconds</td>
<td>Mixed and obstructive apnoea was rare in either group. SIDS siblings had fewer central apnoeas at 3 months of age</td>
</tr>
<tr>
<td>Kelly et al (1979) 112</td>
<td>32 near-miss 32 controls</td>
<td>Periodic breathing</td>
<td>Periodic breathing occurred in 29/32 near-miss and 21/32 controls. Episodes in near-miss were more frequent and of longer duration</td>
</tr>
<tr>
<td>Guilleminault et al (1979) 113</td>
<td>29 near-miss 30 controls</td>
<td>Central, mixed and obstructive apnoea ≥ 3 seconds</td>
<td>Near-miss group had significantly increased obstructive and mixed apnoea</td>
</tr>
<tr>
<td>Hoppenbrouwers et al (1978) 114</td>
<td>9 siblings of SIDS 7 near-miss 9 controls</td>
<td>Central apnoea ≥ 6 seconds</td>
<td>Controls had significantly more central apnoea than other groups in AS</td>
</tr>
</tbody>
</table>
Subsequent siblings born into a family in which a cot death has occurred, and infants who have experienced an apnoeic attack requiring stimulation or resuscitation (near-miss), are at increased risk of SIDS compared to the general population. Therefore, these groups have been studied in an effort to learn more about SIDS. Two studies by the same group found that SIDS siblings had fewer central apnoeas than control infants\textsuperscript{111,114}. In contrast other studies have demonstrated increased central apnoea in SIDS siblings\textsuperscript{110}, increased obstructive and mixed apnoea in near-miss infants\textsuperscript{113} and increased periodic breathing in near-miss infants\textsuperscript{112}.

Therefore, some studies have shown that those infants who have subsequently died from SIDS or are at increased risk of SIDS exhibit increased apnoea, but the results remain contradictory. The results of the studies reviewed here should be viewed with the following caveats: (i) it is assumed that studying siblings of SIDS victims and near-miss infants will provide clues as to the aetiology of SIDS, which may not be the case, and (ii) it is assumed that any abnormalities are evident some time before the fatal event. Since SIDS seems to be related to development with a peak incidence at 8 to 12 weeks of age, it may be that any abnormalities may not be evident until this age period. It should be noted that in the studies by Southall \textit{et al}\textsuperscript{107} and Schectman \textit{et al}\textsuperscript{108}, for example, the time between studying the infant and death was sometimes considerable with a mean of 56 days and a range of 11 to 455 days. Consequently, any differences in SIDS victims compared to controls may have been missed.

The role of apnoea in SIDS is unknown. Two studies that were able to capture the moments before death in 12 SIDS victims showed that bradycardia was involved in nearly all the deaths, but that central apnoea was not\textsuperscript{115,116}. The authors proposed that the infants became severely hypoxaemic leading to bradycardia. The reasons for the hypoxaemia are unclear but since these studies did not monitor obstructive apnoea this cannot be ruled out. However, the fact that these infants may have been hypoxaemic would suggest that mechanisms normally involved in maintaining oxygen levels, for example the peripheral chemoreceptors, may not be functioning correctly. Indeed, it has been shown that near-miss SIDS infants hypoventilate during quiet sleep compared to control infants\textsuperscript{117}.
Other studies have investigated differences in respiratory rates and variability in infants at risk of SIDS and controls. Shannon et al. studied 10 SIDS victims and 34 age-matched controls and found that respiratory rate was more variable and higher in quiet breathing in those infants who subsequently died. Siblings of SIDS victims have been shown to have higher respiratory rates than controls at 3 months of age. Sudden infant death syndrome victims exhibit differences in dynamic breath-by-breath respiratory patterns. Schectman et al. found that SIDS victims exhibited restricted breath-to-breath variation in respiratory rates at slow respiratory frequencies, and this was present at, or soon after birth.

In summary, SIDS victims may have subtle changes in their breathing patterns, such as increased respiratory rates and reduced variability in breath-to-breath changes in respiratory rates. While some studies have shown an increased incidence of apnoea in infants at risk of SIDS the results remain unclear.

### 4.5 Smoking and vulnerability to SIDS: The triple-risk model

The evidence discussed above suggests that hypoxaemia and deficits in respiratory control may be implicated in SIDS. Furthermore, chapter 3.3 describes how nicotine exposure may affect development of respiratory control. Therefore, this may be one mechanism explaining the link between smoking and SIDS. Filiano and Kinney have proposed a triple risk model for SIDS that brings together findings from epidemiological and pathological studies. They suggest that for SIDS to occur three elements are required: (i) a vulnerable infant, (ii) a critical developmental period, and (iii) exogenous stressor(s) (figure 4.5.1). It is possible that nicotine exposure both pre and postnatally may result in a vulnerable infant by interfering with the normal development of respiratory control mechanisms, particularly in response to hypoxia (chapter 3.3). This could lead to respiratory instability, and also render the infant incapable of mounting an adequate ventilatory response to hypoxia. Consequently, the infant may be vulnerable to hypoxic insult. Certainly, SIDS victims show signs of recurrent or chronic hypoxaemia before death. Furthermore, evidence suggests that there may be a critical developmental period during which functioning peripheral chemoreceptors are important for the stability of the breathing pattern. This instability of the breathing pattern may lead to prolonged apnoea and hypoxaemia. Therefore, it is conceivable that nicotine exposure...
may lead to an infant who is vulnerable to hypoxic insult, particularly at a certain point in development. Exogenous stressor(s) such as respiratory tract infection, prone sleeping position and overheating may stress this already vulnerable system leading to respiratory instability. Therefore, for SIDS to occur these three factors must occur at the same time. This explains why all infants exposed to nicotine do not die of SIDS. However, it should be noted that whilst respiratory control deficits may be implicated in the pathogenesis of SIDS, other deficits such as deficient arousal responses and failure of autoresuscitation mechanisms would also be required for death to occur\textsuperscript{121}.

Figure 4.5.1 The triple-risk model for SIDS

![Venn diagram showing the triple-risk model for SIDS: critical developmental period, exogenous stressor(s), and vulnerable infant.]

Adapted from Filiano and Kinney\textsuperscript{120}.

### 4.6 Summary and aim of work

In summary, it has been shown that SIDS victims have been chronically hypoxaemic before death, and have abnormalities in areas of the brainstem important for respiratory control and the carotid bodies. These observations suggest that deficits in respiratory control mechanisms, particularly in response to hypoxia, may be implicated in SIDS. Some studies investigating respiratory responses to hypoxia and breathing patterns in infants who subsequently died of SIDS, or at high risk of SIDS, have shown differences compared to controls. However, the results are inconsistent.

Smoking is a major independent risk factor for SIDS and nicotine exposure may interfere with the normal development of respiratory control mechanisms both centrally and
peripherally. It is conceivable that nicotine exposure may lead to abnormalities in the brainstems and carotid bodies, similar to those seen in SIDS. This may render an infant vulnerable to hypoxic insult and may be involved in SIDS. So far, there are few studies investigating the relationship between nicotine exposure and respiratory control. Those that do exist give contradictory results. My aim was to investigate whether maternal smoking affects postnatal respiratory responses and test the hypothesis that infants born to mothers who smoke have reduced ventilatory responses to changes in inspired oxygen.
SECTION II

METHODOLOGY
Chapter 1

Measurement of peripheral chemoresponsiveness to oxygen in infants

1.1 Techniques that have been applied in infants

In animal studies it is possible to assess directly the sensitivity of the peripheral chemoreceptors to changes in arterial oxygen level by measuring the discharge of the carotid sinus nerve in response to a stimulus. Clearly this is not possible in the human infant and, consequently, peripheral chemoresponsiveness has been assessed by measuring the ventilatory response to different levels of inspired oxygen. One of the most widely used techniques involves delivery of a hypoxic gas for several minutes. For example, Cross and Warner delivered 15% O\textsubscript{2} for 5 minutes to newborn infants in a plethysmograph and found that whilst minute ventilation and tidal volume increased at first this was not sustained. However, there has been considerable variation in this technique between studies with the inspired levels of oxygen ranging between 12% and 17%, and time of exposure between 3 and 10 minutes. More recently, infants have been exposed to hypoxia (15% O\textsubscript{2}) for in excess of 6 hours. Furthermore, differences exist between studies in the way the respiratory response is assessed. Some studies have measured the change in ventilation over time, whereas others have used the number of apnoeic pauses and amount of periodic breathing. Although the initial response to hypoxia is determined by the peripheral chemoreceptors the latter response is affected by hypoxic central depression. The most extreme example of this is shown by the biphasic response of the newborn to hypoxia where after a few minutes exposure the infant is unable to maintain a hyperventilatory response and ventilation falls to below baseline levels (section I, 3.2.2). Furthermore, increased ventilation in response to hypoxia may lead to a fall in the arterial partial pressure of carbon dioxide ($P_a CO_2$) which may be avoided by addition of CO\textsubscript{2} to the inspirate. If uncontrolled these changes in $P_a CO_2$ may affect the ventilatory responses measured by reducing the drive from the peripheral chemoreceptors. Therefore, exposure to hypoxia for a prolonged period is not ideal for measuring peripheral chemoreceptor sensitivity.
Another technique that has been used in infants involves the delivery of a hyperoxic gas (100% O₂) for either a single breath ⁷⁴,⁷⁵, or for a longer period of time ranging between 15 seconds and 5 minutes ¹²⁵,¹³⁰-¹³². The increased arterial partial pressure of O₂ ($P_{aO_2}$) leads to a reduction in peripheral chemoreceptor drive and a consequent reduction in ventilation. If applied when the infant is breathing air this technique can be used to measure the tonic output of the peripheral chemoreceptors. In addition, investigators have also measured peripheral chemoresponses to hypoxia by applying this technique after infants have breathed hypoxic gases for a period of time ⁷⁵,¹³⁰. However, depending upon the length of time the hypoxic gas is supplied it is conceivable that $P_{aCO_2}$ may change and, therefore, the measured response may be the result of changes in both $P_{aO_2}$ and $P_{aCO_2}$.

An alternative approach involves delivering alternating levels of inspired oxygen for 1 or 2 breaths at a time over two minutes. This technique, the alternating breath test, can be used to measure the dynamic responses of the peripheral chemoreceptors to changes in oxygen without changing the average $P_{aO_2}$ and $P_{aCO_2}$. Furthermore, the same changes in inspired oxygen level can be delivered over many breaths and an average response calculated. Consequently, this is the method that has been used for measurement of peripheral chemoresponses in the work presented here and is described in more detail below.

### 1.2 Background to the use of the alternating breath test in infants

It has been shown that alternations in inspired oxygen levels lead to oscillations in respiratory parameters in newborn animals ¹³³-¹³⁵. Furthermore, in newborn lambs whose carotid sinus nerves were cut within 36 hours of birth the respiratory responses were abolished at 5-6 days postnatally ¹³⁵. Therefore, the ventilatory response to the alternating breath test is primarily mediated by the peripheral chemoreceptors. This test was first applied in the newborn infant by Blanco et al ¹³⁶. Infants aged 2-3 days were given two-breath alternations of normoxic (21% O₂) and hypoxic gas (16% O₂) to
breathe for 50-100 breaths. The infants were able to change tidal volume, frequency and minute ventilation in response to these changes in inspired oxygen level. The same group have used single-breath alternations of 21% and 16% O_2 to investigate maturation of peripheral chemoresponses. In addition, this technique has been used to demonstrate that infants with bronchopulmonary dysplasia fail to change ventilation in response to changes in inspired O_2. Recently, Bouferrache et al have applied single-breath alternations of 21% O_2 and 15%O_2 in infants and found that the test gives reproducible results under standardised conditions. When single-breath alternations of 21% O_2 and 16% O_2 were applied to infants in our laboratory it was found that the average change in end-tidal (alveolar) oxygen level was approximately 1%, and no consistent change in respiratory pattern was found. One possible reason for this may have been that in our laboratory gases were delivered via a facemask held close to the face, whereas in the previous studies gases had been delivered using nasal cannulae. Our practice may have led to a greater degree of entrainment of surrounding air and thus a smaller stimulus. Consequently, a range of hypoxic/hyperoxic inspired gas mixtures were tried in an attempt to increase ventilatory responses, but also to achieve an average end-tidal oxygen level which was close to that when breathing air. The inspired oxygen concentrations that were chosen to achieve these criteria were 42% O_2 and 0% O_2. It was estimated that single-breath alternations of these gases would yield end-tidal oxygen levels of 19 and 12%, whereas two-breath alternations would give values of 21% and 9%. Consequently, two-breath alternations were chosen to give the largest change in end-tidal oxygen level. This modified version of the original alternating breath test was investigated in infants of around 12 weeks of age. It was found that the test was safe with no value of oxygen saturation falling below 91%, and that the average end-tidal oxygen levels of 20.9% and 11% were similar to those estimated. However, the change in end-tidal oxygen level with the two gases when compared to baseline was greater with the hyperoxic gas (7.1%) than the hypoxic gas (-2.8%). Therefore, the average end-tidal oxygen level during the test increased by 2.3% above baseline. Significant ventilatory responses were seen in all variables, except frequency, when compared to a baseline air-breathing period. The average alternation in tidal volume was 26% (range 4-58%) which was considerably higher than those of around 5% seen in previous studies using the lower stimulus.
1.3 Application of the alternating breath test in the present study

In the present study two-breath alternations of 40% and 0% O\textsubscript{2} were used. The high inspired oxygen level was reduced from 42% to 40% O\textsubscript{2} in an attempt to achieve an average end-tidal oxygen level around that seen when breathing air. Further details of the collection and analysis of the alternating breath test are presented elsewhere (section III, 4.3.2).

1.4 Limitations of using changes in inspired oxygen levels to measure peripheral chemoreceptor sensitivity

The ventilatory response of an infant to a given inspired level of oxygen is dependent upon many factors (figure 1.4.1). If a hypoxic inspired gas is supplied for an infant to breathe the resulting reduced alveolar partial pressure of oxygen ($P_{A, O2}$) will depend upon the concentration of oxygen in the original inspired gas, but also upon the alveolar ventilation. The alveolar ventilation will itself be dependent upon the relationships between tidal volume, functional residual capacity and the anatomic deadspace. This resulting reduced $P_{A, O2}$ is then transmitted into the blood via diffusion and the peripheral chemoreceptors sense the reduced $P_{a, O2}$ sending information to the ‘respiratory centres’ in the medulla via the carotid sinus nerve. The medulla then integrates this information with other information from higher centres, central chemoreceptors and other sources, including stretch receptors in the lung, and signals to the diaphragm and intercostal muscles to increase ventilation. However, the change in ventilation achieved for a given output from the brainstem will be dependent upon the diaphragm, intercostal muscles and lung mechanics. The end result of this pathway is an increase in alveolar ventilation leading to an increase in $P_{a, O2}$. 
Reduced ventilatory responses to hypoxia could result from abnormalities in any of the components in this pathway even though the peripheral chemoreceptors may not be abnormal. For example, when a given reduced inspired oxygen level is delivered for an infant to breathe the resulting $P_{A\,O_2}$ will be higher in an infant who has a proportionately greater functional residual capacity (FRC) in relation to tidal volume ($V_T$), than in an infant with a smaller FRC in comparison to $V_T$. Therefore, even though these two infants would have received the same inspired level of oxygen the resulting $P_{A\,O_2}$ and $P_{a\,O_2}$ may be different, leading to different ventilatory responses. In addition, in infants with a similar $P_{a\,O_2}$ the resulting ventilatory response will be affected by lung mechanics, so that an infant with reduced lung compliance may have a lower ventilatory response than an
infant with normal lung compliance. Therefore, by delivering different levels of inspired oxygen for an infant to breathe and measuring the ventilatory response it is possible to measure the response of the complete pathway (the peripheral chemoreceptor). If an infant has a reduced response it is not necessarily the case that this is due to the peripheral chemoreceptors. Whilst it is possible to measure the $P_{A,02}$ and lung mechanics to investigate whether these factors are contributing to the reduced response it would not be possible to differentiate between the effects of the peripheral chemoreceptors and the brainstem.
2.1 Introduction

Respiratory Inductance Plethysmography (RIP) is a non-invasive technique that can be used to measure both the volume and timing components of the breathing pattern. Two inductance coils, positioned around the chest and abdomen, are used to monitor ventilation by measuring the movements of the rib cage (RC) and abdomen (AB) with each breath. Conventional methods of measuring respiration in the infant require the use of a facemask connected to a spirometer (SP) or a pneumotachograph (PNT). Application of a facemask to the face of a sleeping infant changes the breathing pattern, and may lead to awakening or a change in sleep state. Therefore, (depending upon the method of calibration chosen and the measurements required) the main advantage of RIP is that it does not require the use of a facemask. In addition, because RIP bands are comfortable to wear this technique is ideally suited to long-term monitoring in overnight studies. For these reasons RIP has been the method used for measuring changes in tidal volume ($V_T$) and other ventilatory parameters during the alternating breath test (a test of respiratory control) in unsedated sleeping infants.

2.2 Background

2.2.1 Theory of Respiratory Inductance Plethysmography

Each inductance coil consists of a Teflon insulated wire attached in a zigzag manner to a cotton elastic band. The inductance of each band changes with cross-sectional area. This means that RIP is able to respond to changes in both circumference and shape of the RC and AB. For example, as shown in figure 2.2.1.1 if coil (A) is stretched by increasing its circumference and the cross-sectional area enclosed (B) this would lead to an increase in volume from RIP. However, if the circumference were increased by the same extent but the cross-sectional area reduced (C) this would lead to a reduction in volume with RIP. It is this ability of RIP to respond to both changes in circumference
and shape that makes it such a powerful technique, and superior to others that only respond to displacement, such as magnetometry.

Figure 2.2.1.1 Principles of RIP operation

Adapted from Watson (1980) 143

In 1967 Konno and Mead 144 proposed that the respiratory system moves with two degrees of freedom of motion, the RC and AB. The movement of the RC reflects intercostal activity, whereas that of the AB reflects diaphragmatic activity. Therefore, changes in volume at the airway opening could be approximated by changes in the volume of the RC and AB compartments so that:

\[ V_T = \text{volume of RC} + \text{volume of AB} \]  \hspace{1cm} (1)

However, the RC and AB may reflect changes in volume with different sensitivities. The RC and AB can be assumed to be two separate cylinders with different heights and cross-sectional areas. If the height of each compartment stays constant with respiration and the same volume were to be displaced into both the RC and AB compartments the change in cross-sectional area would be related to the height of the cylinder (figure 2.2.1.2).

\[ V_T = \alpha \text{RC} + \beta \text{AB} \]  \hspace{1cm} (2)

where \( \alpha \) and \( \beta \) are the volume-motion coefficients of the RC and AB respectively. The equations for three parameters that can be obtained directly: (i) tidal volume (\( V_T \)), using a
Figure 2.2.1.2 Model demonstrating how the difference in height of a cylinder affects the change in cross-sectional area for a given volume

**RIB CAGE** radius = 10cm, height = 20cm

**ABDOMEN** radius = 30cm, height = 7.5cm

Volume of a cylinder = \( \pi r^2 h \)

Cross-sectional area = \( \pi r^2 \)

Volume of rib cage cylinder = \( \pi 10^2 \times 20 = 6283\text{cm}^3 \)

Volume of abdomen cylinder = \( \pi 30^2 \times 7.5 = 21205\text{cm}^3 \)

If 1000\text{cm}^3 added to each of these cylinders but the height stays constant:

Rib cage cylinder

\[
\frac{7283}{20} = 364\text{cm}^2
\]

Abdomen cylinder

\[
\frac{2205}{7.5} = 2958\text{cm}^2
\]

Therefore, the way in which the two compartments within an individual reflect changes in volume need to be taken into account and volume-motion coefficients for the RC and AB calculated, so that:

\[ V_T = \alpha \text{RC} + \beta \text{AB} \quad (2) \]

where \( \alpha \) and \( \beta \) are the volume-motion coefficients of the RC and AB respectively. This equation has three parameters that can be obtained directly: (i) tidal volume \( (V_T) \) using a

\[ V_T = \alpha \text{RC} + \beta \text{AB} \quad (2) \]
PNT, (ii) the RC signal, and (iii) the AB signal using RIP. The two unknowns, $\alpha$ and $\beta$, must be calculated using one of the following calibration procedures.

### 2.2.2 Calibration methods

Calibration may be performed in one or two stages. One stage calibration requires simultaneous use of a PNT and equation (2) is used. Two stage calibration uses a rearrangement of equation (2):

$$V_T = M [K (RC) + AB]$$  \hspace{1cm} (3)

where $K$ is equal to $\alpha/\beta$ and $M$ is a scaling factor to volume.

In the first stage $K$ is calculated without using a PNT. This is applied to the term in square brackets to give a value directly proportional to volume. The second stage requires the use of a PNT to calculate the scaling factor to volume, $M$. Therefore, all calibration methods require the use of a PNT for fully-quantitative volume measurements. Since this is not always required (for example, % changes in respiratory parameters are taken as a measure of response in the alternating breath test) only semi-quantitative measurements of volume may be necessary. An advantage of the two stage method of calibration is that semi-quantitative measurements can be obtained without using a PNT.

#### 2.2.2.1 One stage calibration methods

**Simultaneous equations and Least squares**

Two respiratory recordings are required where the volume-motion coefficients are the same but the relative contributions of RC and AB to volume are different, for example in two postures or sleep states. Simultaneous equations can then be solved to calculate $\alpha$ and $\beta$. This technique has been used in infants with an average error in minute ventilation of 0.2% and 0.8% in quiet and active sleep respectively.\(^{145}\)

The least squares method calculates the values of $\alpha$ and $\beta$ graphically. The values of RC/PNT and AB/PNT for each breath are plotted on the y and x axes respectively. The rib cage scaling factor ($\alpha$) is the reciprocal of the y intercept and the abdominal scaling factor ($\beta$) the reciprocal of the x intercept. Using this technique Dolfin et al found that it
was more accurate to use two sleep states for calibration rather than a single sleep state in infants. Warren and Alderson have suggested that it may be possible to use two breaths with differing RC/AB contributions within the same recording as an alternative to using different postures/sleep states. They found that in quietly awake infants 56% of tidal volumes measured using RIP were within 10% of the PNT measured tidal volume, and 92% were within 20%. However, these calibration factors were not appropriate when the infant fell asleep.

**Multiple Linear Regression (MLR)**

This technique assumes that there is enough variability in the RC and AB contributions to tidal volume between breaths during regular breathing to allow accurate determination of the volume-motion coefficients. Values of $\alpha$ and $\beta$ are determined by finding values which give the best regression relationship between tidal volume measured by RIP and PNT.

Banzett et al found that MLR occasionally gave physiologically improbable gain ratios which led to large errors when RC/AB changed from calibration conditions in adults. However, Loveridge et al found that when RIP was calibrated using MLR the average % difference between tidal volume measured by PNT ($V_{T_{PNT}}$) and RIP ($V_{T_{RIP}}$) was -1.3% 60 minutes after calibration.

This technique has been used in infants as a reference method by Gagliardi et al and compared to isovolume manoeuvres. They found that the average $\beta/\alpha$ ratio of 2.39 ($\alpha/\beta = 0.42$) was very different to that found using ISV (0.94). Unfortunately, % differences between $V_{T_{RIP}}$ and $V_{T_{PNT}}$ were not reported and it is difficult to conclude anything about the comparative accuracy of either of the two methods.

**2.2.2 Two stage calibration methods**

**Isovolume Manoeuvres (ISV)**

This technique was first described for use in adults by Konno and Mead. The manoeuvre requires the subject to stop breathing by closing the glottis and then gently displace the volume between the RC and AB. Consequently the subject must be well-
trained and co-operative. As the volume in the lungs does not change equation (2) can be rewritten:

\[ 0 = \alpha (RC) + \beta (AB) \]
\[ \alpha (RC) = - \beta (AB) \]
\[ RC = - \frac{\beta}{\alpha (AB)} \]

The RIP RC signal is plotted on the y axis and the AB signal on the x axis and K is equivalent to the reciprocal of the slope of the line.

This technique may not be valid because the infant has a compliant chest wall \(^{151}\), so distortion of the rib cage may occur such that it does not move as a single unit. Indeed, Gagliardi \textit{et al} found that only 51% of occlusions attempted in infants were analysable, and that the value of K was lower when compared to MLR \(^{150}\). In a pilot study I also found that the ISV was not feasible for use in infants as only 21% of the manoeuvres attempted were acceptable for analysis (unpublished).

**Qualitative Diagnostic Calibration (QDC)**

The Qualitative Diagnostic Calibration (QDC) was first proposed by Sackner \textit{et al} (1989) as a method of RIP calibration in adults \(^{152}\). It depends upon the natural variation in the relative contributions of the RC and AB between breaths during regular breathing to derive K. If a subject could breathe with a constant \(V_T\) then the variation in volume would be zero, and any changes in the relative contribution of RC to volume would be reciprocated in an equal and opposite way by the AB. This method is based upon the same equations as ISV such that when the change in \(V_T\) is zero:

\[ 0 = \alpha (RC) + \beta (AB) \]
\[ \alpha (RC) = - \beta (AB) \]
\[ \alpha/\beta \text{ or } K = - AB/RC \]

It was assumed that breath-to-breath variations in RC and AB followed a normal distribution, such that the standard deviations of the two signals could be calculated and were related to the gain of the two signals. The proportionality constant K could be calculated as the ratio of the standard deviations of the AB and RC, i.e. \(K = - SD(AB)/SD(RC)\).
The assumption of a constant $V_T$ is almost impossible to achieve even in a well-trained adult subject. Therefore, a large number of breaths are collected and are selected to approximate a constant $V_T$. The authors suggest that ideally 5 minutes of data should be collected and breaths outside $\pm 1.0$ SD of the mean sum uncalibrated [RC + AB] should be excluded.

In adults the QDC and ISV methods gave similar absolute % differences of 2.6% and 3.4% respectively $^{152}$. In full-term neonates aged 4 (±2) days the differences between $V_T$ measured using RIP calibrated using QDC and PNT were small and ranged between -2.38 and 1.64 mL in the supine position $^{153}$. The absolute % differences ranged between 0 and 10.7% with a mean of 3.0%. Recently, the QDC technique has been used in anaesthetised infants $^{154}$. During spontaneous ventilation agreement in $V_T$ between RIP and PNT was close (95% limits of agreement -2.3%, 3.0%), but this was reduced 10 minutes after calibration and when mechanical ventilation was used $^{154}$. On balance, studies suggest that the QDC method may be accurate in infants and it has been used for RIP calibration when the alternating breath test has been applied $^{67,137,139,142}$. However, it has not been assessed in postneonatal unanaesthetised infants or compared to other methods of calibration.

**Standard ratio of K**

Banzett *et al* have suggested that using a fixed value of K for all subjects might be just as accurate as using more complicated, sophisticated techniques $^{148}$. Using adult volunteers a range of fixed values of K between 8 and 1 were applied to RIP data and the average error calculated. During quiet breathing in both the standing and sitting positions the value of K used was not critical to the error and almost any value could be used with a similar error. However, when all types of breathing (quiet breathing, deep breathing, rib cage emphasis and abdominal emphasis) were included the value of K was more critical. A value of 2 was chosen as the standard ratio and during quiet breathing this was as accurate as using ISV. I examined this approach in infants (chapter 2.4).
2.2.2.3 Advantages and disadvantages of calibration methods available

All the methods currently available for calibration of RIP require the use of either a spirometer or pneumotachograph for fully-quantitative measurements. Calibration methods such as simultaneous equations, or least squares can be time consuming to perform because of the need for recordings in two sleep states or postures. Furthermore, changes in posture may lead to alterations in the volume-motion coefficients thereby invalidating these methods. Multiple linear regression is simpler to use as it only requires recordings in quiet breathing. However, this method has not been widely used in infant studies.

If only semi-quantitative measurements are required then ISV, QDC or a fixed K technique could be used. However, the ISV technique has been shown to be inaccurate and unfeasible for use in infants. The QDC technique has been used previously for RIP calibration when the alternating breath test was applied in infants. It has been shown to be accurate in neonates and anaesthetised infants, but has not been investigated in older infants or compared to other methods of calibration. One method of calibration that remains to be evaluated in infants and which seems particularly desirable because of its simplicity is the fixed K technique.

2.3 Aims

The primary aim of this study was to investigate whether the simple fixed K calibration method could be applied to infants and, if so, what value of K would be most appropriate. This method was compared to the MLR and QDC methods during regular breathing (quiet sleep) and under conditions where the relative contributions of the rib cage and abdomen to $V_T$ may change (active sleep).

2.4 Methods

2.4.1 Subjects

Twenty one infants were recruited. Twenty were recruited into a programme of research into respiratory control in healthy infants. The remaining infant was volunteered spontaneously by the mother after she had heard about the research. Two were born at 35 weeks gestation but were fit and well and did not require respiratory support. Although all infants were healthy at recruitment, a detailed questionnaire administered at
the time of study revealed that one infant was reported to have narrowed nasal passages and suspected gastroesophageal reflux. She was being treated with Sodium Cromoglycate nasal spray and Gaviscon thickened feeds. Another infant had been diagnosed as asthmatic and was being treated with Salbutamol (300μg twice daily, inhaled). All of the infants were studied in the hospital during an unsedated daytime nap and were well at the time of study.

All parents gave written informed consent and the study was approved by Leicestershire Health Authority Ethics Committee.

2.4.2 Data Collection

Figure 2.4.2.1 shows the equipment for data collection. Changes in cross-sectional area of the RC and AB were measured using Respiratory Inductance Plethysmography (Model 150, Studley Data Systems, Oxford, UK.) with both channels set to a gain of 1.0. Respiratory Inductance Plethysmography (RIP) bands 1.5cm wide were placed around the infant's abdomen at the level of the umbilicus and around the chest as close to the axillae as possible.

Respiratory flow was measured using an infant screen pneumotachograph with a linear range of over ± 1 L.S⁻¹ (Erich Jaeger, Market Harborough, UK.) attached to a facemask (Rusch size 3, Rendell Baker.). The flow was integrated by computer to provide a volume signal.

When the infant was asleep in the supine position a facemask and pneumotachograph were sealed around the nose and mouth using sterile putty, and data collection commenced. Simultaneous recordings of RC and AB movements and tidal flow and volume were made using an IBM compatible computer (Powerpaq pentium, City Business Systems, Leicester, UK.) and RASP software (PhysioLogic Ltd., Newbury, UK.) at a sampling rate of 50Hz. For each minute of recording the person holding the facemask classified the sleep state behaviourally as quiet (QS) or active (AS). A second investigator was responsible for data acquisition on computer. Data were collected for as long as the infant stayed asleep. The first 5 minutes of the recording in a sleep state were used for calculating calibration factors using MLR, QDC and a fixed K
method. Subsequent data in the same sleep state were used for validation and were split into one minute segments.

Figure 2.4.2.1 Equipment for data collection

2.4.3 Data Analysis

Changes in the PNT measured volume, RC and AB were calculated for each breath for all data collected. Inspiratory changes were calculated as the difference between the end-inspiratory value and previous end-expiratory value for each signal. Expiratory changes were calculated as the difference between the end-inspiratory value and the following end-expiratory value for each signal. The change in the three signals with each breath was computed as the average of the inspiratory and expiratory changes.
Previous studies have included a constant in equation (2) \(^{149,157}\). We have found that introducing a constant to the model increases the accuracy of RIP calibration in infants (data submitted for publication and not presented here). The equations used in this study included a constant \((\gamma)\) and were:

\[
V_T = \alpha RC + \beta AB + \gamma \quad (4)
\]
\[
V_T = M [K(RC) + AB] + \gamma \quad (5)
\]

2.4.3.1 Impact of range of values of \(K\) on accuracy of RIP: choosing a standard value

The impact of \(K\) on the accuracy of RIP calibration was investigated by applying a range of values of \(K\) to the changes in \(RC\) and \(AB\) with each breath in both quiet and active sleep. The values of \(K\) applied were 0.1-2. The effect of \(K=0\) \((\alpha=0)\) and \(K=\infty\) \((\beta=0)\) was also examined. The scaling factor to volume \(M\) and \(\gamma\) were calculated (see 2.4.3.2.1) and applied to the data. The absolute \% error using each value of \(K\) was calculated for the first 5 minutes of data, and for each minute of validation data. Based upon these data a standard value of \(K=0.5\) was chosen for the subsequent comparison of 3 calibration methods.

2.4.3.2 Comparison of the accuracy of the three calibration methods

2.4.3.2.1 Calculation of calibration factors

The first five minutes of data collected in either sleep state were used for the calculation of the calibration factors by the three methods. These factors were then applied to each subsequent minute of validation data in the same sleep state. Therefore, only recordings of a least six minutes duration were analysed.

Multiple Linear Regression (MLR)

Multiple linear regression was performed using PNT measured changes in volume \((V_T^{\text{PNT}})\) as the dependent variable and \(RC\) and \(AB\) changes with each breath to obtain the regression equation containing \(\alpha\), \(\beta\) and \(\gamma\) (equation 4), where \(\alpha/\beta\) is equivalent to \(K\) and \(\beta=M\) (equation 5).
**Qualitative Diagnostic Calibration (QDC)**

All breaths outside ± 1.0 SD of the mean sum [RC + AB] were excluded and the standard deviations of the RC and AB signals of the remaining breaths were calculated. The proportionality constant, K, was calculated as the ratio of the standard deviations of the AB and RC. This value of K was then reapplied to the first five minutes of RIP data to give a term proportional to volume [K(RC) + AB] in arbitrary units (au)(equation 5). A regression analysis was performed to predict $V_{TPNT}$ (mL) from the semi-calibrated RIP volume in arbitrary units ($V_{TRIP}$ (au)). The slope of this regression is the scaling factor to volume (M) and the intercept is equivalent to $\gamma$.

**Fixed K of 0.5 (K(0.5))**

A fixed value of K of 0.5 was applied to the RC and AB signals to give a term proportional to volume (equation 5). A regression analysis was then performed to calculate M and $\gamma$ in the same manner as described for QDC.

2.4.3.2.2. Validation of calibration factors: Accuracy of methods

Values of K, M and $\gamma$ calculated from the first five minutes of data for each calibration method were applied to each following minute of data in the same sleep period. For each breath the difference between $V_{TPNT}$ and $V_{TRIP}$ was calculated but the sign was ignored. The absolute errors (mL) were then expressed as a percentage of $V_{TPNT}$ for each breath.

$$\text{Absolute } \% \text{ error} = \left( \frac{|V_{TRIP} - V_{TPNT}|}{V_{TPNT}} \right) \times 100$$

The mean absolute % error was then calculated for each minute of validation data.

2.4.3.2.3 Statistical Analysis

The outcome variable, mean absolute % error, was not normally distributed and therefore these values were log transformed. A repeated measures ANOVA taking into account repeated measures over time in each subject was performed. The effects of calibration method and time were assessed using Wald tests.
2.5 Results

2.5.1 Data Collected

Eighteen continuous recordings (12 quiet sleep, 6 active sleep) of at least 6 minutes duration were collected from twelve infants whose characteristics are presented in table 2.5.1.1. The median (range) age, weight and length were 5.4 (3.0-12) months, 7.2 (5.6-9.5) kg and 63.6 (58.3-74.2) cms respectively. Recordings were obtained in more than one sleep period in 4 subjects; two had recordings in two sleep periods and two had recordings in three sleep periods. The reasons for not obtaining adequate data in the remaining infants were that the infant did not sleep or only slept for a short time (8 infants), or technical problems (1 infant).

Table 2.5.1.1. Characteristics of infants on whom adequate data was obtained

<table>
<thead>
<tr>
<th>Subject number</th>
<th>Age (months)</th>
<th>Weight (kg)</th>
<th>Length (cms)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.4</td>
<td>7.9</td>
<td>64.1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>9.7</td>
<td>8.3</td>
<td>70.2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>5.0</td>
<td>6.6</td>
<td>63.0</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>12.0</td>
<td>9.3</td>
<td>73.1</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>7.4</td>
<td>7.8</td>
<td>69.3</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>6.9</td>
<td>9.5</td>
<td>74.2</td>
<td>Asthmatic treated with Salbutamol</td>
</tr>
<tr>
<td>7</td>
<td>3.2</td>
<td>5.7</td>
<td>59.0</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>3.0</td>
<td>5.6</td>
<td>59.3</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>4.2</td>
<td>6.4</td>
<td>62.5</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>5.8</td>
<td>7.8</td>
<td>67.2</td>
<td>Treated with Sodium Chromoglycate and Gaviscon thickened feeds</td>
</tr>
<tr>
<td>11</td>
<td>7.2</td>
<td>6.1</td>
<td>61.1</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>4.6</td>
<td>6.0</td>
<td>58.3</td>
<td>Born prematurely at 35 weeks gestation, no respiratory problems</td>
</tr>
</tbody>
</table>

The average (SD) number of breaths used for the 5 minute calibration was 132 (33) in 16 of the 18 sleep periods recorded. The computer programme could analyse a maximum of 175 breaths in any recording, so that the two remaining calibration periods from a rapidly-breathing subject were reduced to 3.9 and 4.5 minutes. These included 173 and 174 breaths respectively. Therefore even though these recordings were of shorter duration they did not contain fewer breaths than other analysed recordings. Ninety-one minutes of validation data were collected, of which 18 were in active sleep. The average (SD) number of breaths in the one minute periods was 29 (8) in QS and 27 (8) in AS.
2.5.2 Impact of range of values of $K$ on accuracy of RIP: choosing a standard value

When a range of values of $K$ were applied to the data the absolute % error was not highly dependent upon the value of $K$ in either sleep state (figure 2.5.2.1). In all cases there were wide ranges of $K$ over which the absolute % error changed very little in either sleep state. For the first minute of validation data in quiet sleep the absolute % error was around 3% or below for a wide range of values of $K$ (0.5 to 2.0) (figure 2.5.2.1, A). In active sleep there was also a wide range of values of $K$ over which the error changed very little. Almost any value of $K$ gave an error of 11% or less.
Figure 2.5.2.1  Relationship between absolute % error and value of K applied in first minute after calibration

Each curve represents one recording in one subject, each subject has a different symbol. A is quiet sleep and B is active sleep. The dashed line shows the value of K = 0.5 chosen as the fixed ratio.
2.5.3 Comparison of the accuracy of the three methods of calibration

*Quiet sleep*

Figure 2.5.3.1 presents a box and whisker plot showing the median (range) absolute % errors for each of the calibration methods over the first six minutes following calibration. More than six minutes of data were collected in some individuals but there are too few for meaningful interpretation and, therefore, these have been excluded from the plot. The K(0.5) method of calibration generally gave a slightly higher median absolute % error than either of the other methods, with MLR giving the lowest. Although differences between methods were small the K(0.5) method gave significantly greater % error than either of the other two methods (p <0.0001). There was no significant difference between MLR and QDC. There was a trend for the median absolute % error to increase over time for all the methods but this was not statistically significant (p=0.08). The variability of the measurements increased over time with the range being particularly large in minute 6 using MLR and QDC. This was a consequence of one or two outlying individuals. The largest error of 16.08% was seen with the QDC technique in minute 6.
Figure 2.5.3.1 Box and Whisker plots of absolute % error for three methods of calibration over time in quiet sleep.

The number of observations are 12 for minute 1, 11 for minute 2, 10 for minute 3, 11 for minute 4, 10 for minute 5 and 9 for minute 6. Horizontal lines represent the median, box represents data within 25th and 75th centile and whiskers represent the highest and lowest values.
Agreement between the three methods was also investigated within individual subjects using Bland-Altman plots (figure 2.5.3.2). The widest limits of agreement were seen when the MLR and K(0.5) methods were compared. There was good agreement between MLR and K(0.5) in the majority of subjects. However, MLR performed better than K(0.5) in two subjects (8 and 10). In the worst case (subject 10 minute 2) MLR gave an absolute % error of 1.67% and K(0.5) a value of 6.68%. The best agreement was seen between the MLR and QDC methods. There was good agreement in the majority of subjects, however the QDC method gave greater % errors in two subjects (subjects 1 and 10). The greatest difference between the two methods was in subject 1 minute 1 where MLR gave an error of 1.54% and QDC 5.23%. When the QDC and K(0.5) methods were compared agreement was good in most subjects. However, K(0.5) gave higher errors compared to QDC in subject 8, in the worst case K(0.5) gave an error of 4.25% compared to 1.19% with QDC. In addition, in subject 1 the QDC method performed worse than K(0.5), with the K(0.5) method giving an error of 1.58% and QDC 5.25% (minute 1).

**Active sleep**

The agreement between the three methods was also tested during AS where breathing pattern and the relative contribution of the RC and AB change. Figure 2.5.3.3 shows box and whisker plots comparing the three calibration methods for the first minute of validation data, for which there were 6 recordings. The median absolute % errors were greater than during quiet sleep and more variable. There was little difference in the median absolute % errors for the three calibration methods. However, when analysed statistically it was found that the QDC method gave significantly greater errors (p<0.0001) than either of the other two methods. There was no significant difference between MLR and K(0.5).

The agreement between methods of calibration was also investigated within individual subjects using Bland-Altman plots (figure 2.5.3.4). It can be seen that differences between methods were small. The best agreement was between MLR and K(0.5) with the latter being slightly more accurate. The worst was between the QDC and K(0.5) methods.
Figure 2.5.3.2 Bland-Altman plots comparing the three calibration methods in quiet sleep

Each data point is one minute of validation data. The solid line is the mean difference in absolute % error, the dotted lines represent the 95% limits of agreement.
Each data point is one minute of validation data. The solid line is the mean difference in absolute % error, the dotted lines represent the 95% limits of agreement.

Figure 2.5.3.3 Box and Whisker plots of absolute % error for three methods of calibration for first minute of validation data in active sleep

Horizontal line represents the median, box represents data within 25th and 75th centile and whiskers represent the highest and lowest values.
Figure 2.5.3.4 Bland-Altman plots comparing the three calibration methods in active sleep.

Each data point is one minute of validation data. The solid line is the mean difference in absolute % error, the dotted lines represent the 95% limits of agreement.
Each data point is one minute of validation data. The solid line is the mean difference in absolute % error, the dotted lines represent the 95% limits of agreement.

2.6 Discussion

2.6.1 Data Collected

These studies were performed on infants at a time during the day when they would normally be expected to have a nap. In spite of this, 8 infants either did not sleep or the duration of sleep was too short to obtain adequate recordings, even though they were often in the laboratory for several hours. Other studies of RIP calibration in infants have not highlighted the difficulty in obtaining recordings of adequate length. Some of these studies were performed on awake infants, and many have tended to concentrate on infants in the newborn period and those born preterm, whereas older infants tend to sleep less during the day and are more inquisitive than newborn infants. Two studies have included unsedated infants up to 4.5 months of age, only one of which included normal infants. Furthermore, the older infants in one study were studied overnight, when they would be expected to sleep for longer periods. The success rate of 60% in obtaining recordings is probably a consequence of the age range of the infants involved in this study (2.3-12 months), which is older than that in other studies. However, in spite of this good data were obtained from 12 infants, which is similar to the number reported in other published RIP calibration studies in adults and infants.
More recordings were obtained during quiet than active sleep, reflecting the difficulty in recording $V_T$ through a facemask during active sleep in infants of this age group. Infants would often rouse on the transition from quiet to active sleep and the facemask would have to be removed. Even though attempts were made to settle the infant back to sleep, these were often unsuccessful. Therefore, when planning to use RIP to measure $V_T$ in infants the difficulties associated with short sleep duration during the day and arousability when using a facemask should be borne in mind. Studies conducted overnight are more likely to be successful, however recruitment may be more difficult.

2.6.2 Derivation of a fixed value of $K$

In this study the ratio of 0.5 was chosen arbitrarily by studying the relationship between the absolute % error and value of $K$. In many of the infants studied the absolute % error was not highly dependent upon the value of $K$ in either sleep state. In other words, almost any value could be used with similar accuracy. In all infants almost any value of $K$ gave an absolute % error of 3% or below during quiet sleep. No value of $K$ gave a perfect calibration close to 0% error. In addition, in most infants using a combination of the RC and AB was more accurate than using either band alone ($K=0$ and $K=\infty$). Using the RC band alone ($K=\infty$) gave the least accurate results in the majority of infants.

In active sleep any combination of the RC and AB gave absolute % errors below 11%. Furthermore, as with quiet sleep the absolute % error was not highly dependent upon the value of $K$.

The ratio that was chosen in this study (0.5) was different to that used by Banzett and co-workers\(^{148}\) in adults. They used a ratio of 2.0 which suggested that the abdomen moves further than the rib cage. Our study suggests that in infants the opposite is true, i.e. the rib cage moves further than the abdomen for a given volume.
2.6.3 Comparison of the accuracy of the three calibration methods

**Quiet sleep**

The differences in error between the three calibration methods were small. Overall, the higher errors obtained using K(0.5) were slightly but significantly higher than either of the other two methods. However, these differences are unlikely to be of any practical significance. This is the first study to compare the QDC method of calibration to other methods in infants. Since it is also the first study in infants to attempt to use a fixed ratio, there are no other studies comparing it with other calibration methods. However, Banzett *et al.*[^148] found that the standard ratio technique compared favourably with ISV in adults.

It is difficult to compare the errors found in this study to those in other studies as mean absolute % errors have been calculated, whereas most of the other studies have presented their results either in terms of the percentage of breaths which lie within a given fraction of the PNT volume[^147,157,159], or as mean % errors[^147,154,159]. If the errors are scattered equally above and below zero, when the mean % error is calculated it will be close to zero. Consequently, it was felt that in the present study absolute % errors would give a better indication of error. In a study by Adams *et al.*[^153] involving neonates aged 4 ± 2 days the errors associated with using the QDC method could be derived from data presented in their paper. The calculated mean absolute % error for the 21 subjects in their report was 3.0% (range 0 to 10.7%), which was similar to that found in this study. In addition, Sackner *et al.*[^152] reported a similar error of 3.4% in a study using QDC calibration in adults. Therefore, the error associated with QDC calibration was similar in the present study to those published previously.

**Active sleep**

During active sleep the phase relationship between RC and AB changes and the RC/AB contribution to volume may change[^161]. Statistical analysis showed that the QDC method performed significantly worse than both the other methods in active sleep. However, as with quiet sleep the actual differences in % error between methods were so small as to be of little practical significance. For example, the median absolute % error immediately following calibration using MLR, QDC and K(0.5) were 7.35%, 7.40% and 7.23% respectively. However, it is clear that $V_{TRP}$ is less accurate during active sleep.
regardless of the method of calibration. The infant has a compliant chest wall\textsuperscript{151} which is prone to distortion\textsuperscript{162}, particularly during active sleep when the intercostal activity that usually helps to stabilise the chest wall is silenced and paradoxical movements occur\textsuperscript{163}. Therefore, the chest wall may move with additional degrees of freedom during active sleep such that assessment of ventilation using RIP is less accurate than during quiet sleep. However, another study has not shown any difference in the accuracy of RIP between the two sleep states in infants\textsuperscript{153}.

2.6.4 Limitations of the study

Since these studies were conducted during short periods of day-time sleep it was not possible to investigate the accuracy of RIP calibration over time, or the agreement between methods of calibration over time. This was not an aim of the study. However, we have found that at least over the first 6 minutes following calibration during quiet sleep that there is no significant increase in error for either method. Therefore, for the time period over which an alternating breath test is performed there is no significant increase in error. Brown \textit{et al} have shown that the accuracy in QDC calibration reduces 10 minutes after calibration in anaethetised infants\textsuperscript{154}. Future studies should aim to assess accuracy over longer periods, but this may need to be done overnight in infants of this age group.

A potential drawback of using a fixed ratio is that it may not give correct proportioning of the RC and AB signals and thus apnoeas may not be detected reliably\textsuperscript{164}. Although this study was not designed to investigate this, one infant had a spontaneous obstructive apnoea during data collection of 3 seconds duration. The use of RIP calibrated with K(0.5) technique showed a clear attenuation of breathing, but would not have enabled distinction between apnoea and hypopnoea with certainty. However, before advocating the use of this technique for calibration of RIP for this purpose further investigation is required. It should also be noted that accuracy of apnoea detection when the QDC method is used for calibration in infants has not been investigated either.

The majority of the infants studied were normal and healthy. Although the infant born prematurely and those receiving treatment did not yield results that were clearly different to the other infants future studies should investigate this more thoroughly before
advocating the use of a fixed ratio in infants who have been born prematurely or have respiratory disease.

2.6.5 Summary and Conclusions

Although some differences were seen in the accuracy of the three methods they were so small as to be of no practical importance. The fixed ratio $K(0.5)$ method of calibration can be used with similar accuracy to MLR or QDC in healthy unsedated sleeping infants in both quiet and active sleep. However, this technique has not been assessed as a method for apnoea detection, or for use in preterm infants, or those with respiratory disease. Further investigation is required if this technique is to be used in these groups.

This study has justified the use of $K = 0.5$ for the main work of this dissertation, namely using RIP to measure changes in ventilation associated with the alternating breath test in a study investigating the effect of maternal smoking on respiratory responses to alternations in inspired oxygen levels in infants.
SECTION III

RESPIRATORY RESPONSES TO CHANGES IN INSPIRED OXYGEN LEVELS IN INFANCY: THE EFFECT OF MATERNAL SMOKING
Studies in animals have shown that the brainstem and carotid bodies can be affected by nicotine exposure. It has been suggested that the human brainstem may be at its most vulnerable during midgestation when nicotinic acetylcholine receptors are prevalent. Indeed, rats exposed to nicotine in utero show increased cell death in the medulla. In addition, it has been found that inhibitory noradrenergic mechanisms become hyperresponsive to hypoxia in rat pups exposed to nicotine in utero. This was thought to contribute to respiratory arrest in response to hypoxia in 15% of the animals. Postnatal nicotine exposure has been shown to increase the synthesis and release of dopamine from the carotid body. Dopamine reduces the sensitivity of the carotid body to hypoxia and leads to reduced ventilatory responses. It has been proposed that the increased dopamine turnover seen with postnatal nicotine exposure may interfere with the usual postnatal resetting of peripheral chemoreceptor sensitivity to hypoxia. If this were to occur in the human infant this could result in the peripheral chemoreceptors being insensitive to changes in arterial oxygen at a time when they should be responsive. Therefore, these findings suggest that both antenatal and postnatal nicotine exposure may affect the brainstem and carotid body, such that ventilatory responses to changes in arterial oxygen level may be affected.

The peripheral chemoreceptors play an important role in the termination of apnoea. Studies in piglets have suggested that functioning chemoreceptors may be more critical for the termination of apnoea at certain times in development. Piglets who had their carotid bodies denervated at 12-15 days of age had more prolonged apnoea with desaturation than when denervation was carried out at any other age. The peripheral chemoreceptors also appear to be important for stabilising the breathing pattern during normoxia. Therefore, if nicotine were to reduce the activity of the chemoreceptors their lack of function may lead to respiratory instability. Indeed, it has been shown that infants born to women who smoke have longer and more frequent obstructive apnoea than those born to nonsmoking women.
The effect of prenatal nicotine exposure on ventilatory responses to hypoxia has been investigated in the lamb \cite{15} and rat \cite{14} yielding contradictory results. Hafstrom \textit{et al} treated pregnant ewes with 40mg/day of nicotine during the last trimester \cite{15}. At around 5 days of age the lambs that had been exposed to nicotine during pregnancy had significantly reduced ventilatory responses to hypoxia ($F_{1}O_{2} 0.1$ for 5 min). However, Bamford \textit{et al} found that rats exposed to nicotine (6mgKg$^{-1}$day$^{-1}$) throughout pregnancy did not have different ventilatory responses to hypoxia ($F_{1}O_{2} 0.1$ and 0.15 for 10 min) up to 34 days postnatally, compared to those who had not been exposed \cite{14}.

The effect of acute postnatal nicotine exposure has also been investigated in the lamb \cite{16} and the rat \cite{12}. Milerad \textit{et al} have investigated the effects of acute postnatal nicotine administration upon ventilatory responses to both hypoxia and hyperoxia in the developing lamb. Five lambs were studied at 7, 17 and 27 days of age, and ventilatory responses were measured to hyperoxia (100 % $O_{2}$) and hypoxia (10% $O_{2}$) with and without nicotine infusion \cite{16}. The hyperoxic test gives a measure of tonic discharge of the peripheral chemoreceptors and the hypoxic test a measure of chemoreceptor sensitivity. It was found that nicotine caused the hyperoxic ventilatory response to be augmented, but the hypoxic response to be attenuated. The authors suggested that these paradoxical findings could be explained if nicotine altered peripheral chemoreceptor oxygen sensitivity and the central processing of the chemoreceptor input \cite{16}. However, Holgert \textit{et al} found that ventilatory responses to hyperoxia were reduced following nicotine exposure in 3 day old rat pups \cite{12}. In control conditions ventilation fell by 22.5% during hyperoxia, compared to only 6.9% after nicotine exposure. These results suggest that the tonic activity of the peripheral chemoreceptors is reduced during nicotine exposure. Furthermore, this effect was blocked by the dopamine type 2 receptor antagonist domperidone suggesting that the effect was mediated by dopamine. Therefore, the small number of studies conducted in animals investigating antenatal and postnatal nicotine exposure give contradictory results.

The effect of maternal smoking on respiratory responses has been investigated in the human infant \cite{17}. Women were recruited during pregnancy and their smoking habit verified using urinary cotinine levels. Ventilatory and awakening responses to hypoxia and hypercapnia were investigated in 13 infants born to smoking and 34 infants born to nonsmoking women, at 8 to 12 weeks of age during a daytime nap. The authors found a
difference in the hypoxic awakening responses with more of the smoking group failing to
awaken in response to hypoxia (54% versus 15%), but ventilatory responses were not
different. However, in this study there were significant differences in maternal age,
postnatal age, birthweight, and the age of the infant at the time of study between the
smoking and nonsmoking groups. In addition, there were clear differences in the race
distribution between the two groups with only 23% of the smoking group being white,
compared to 65% of the nonsmoking group. It is possible that any or all of these factors
may influence respiratory control independently and, therefore, any differences between
the two groups due to smoking may be masked by these factors. Consequently, whilst
this study showed that there was no difference between the two groups which were
representative of the population residing in the San Francisco Bay area it did not
investigate the independent effect of smoking. One further study has attempted to
investigate the effect of maternal smoking on respiratory responses in the human infant
166. Fifteen infants born to smoking women and 16 born to nonsmoking women were
studied at four postnatal ages (1 day to 10 weeks). Fifteen second challenges with 100%
O₂ and 15% O₂ were performed during daytime sleep. The authors report that there
were 'no obvious effects of maternal smoking' but the results are not shown in their
abstract 166. Both of these studies were performed during daytime sleep, and it is
conceivable that results may be different during夜间time sleep. Since the majority of
cot deaths occur during night-time sleep it is imperative to conduct studies during the
overnight period. A previous study conducted within our department measuring
respiratory control in infants during night-time sleep found that infants with at least one
smoking parent had reduced ventilatory responses to alternations in inspired oxygen,
although this was not statistically significant 159. However, this study was not designed
to investigate the effect of smoking per se on respiratory control. These findings suggest
that smoking may affect ventilatory responses to inspired oxygen levels during night-time
sleep.

The aim of the present study was to investigate the independent effect of maternal
smoking on ventilatory responses to inspired oxygen levels, and test the hypothesis that
infants born to women who smoke have reduced responses during night-time sleep.
Since it has been shown that the human brainstem may be most vulnerable during
midgestation women were recruited during the first half of pregnancy and a urine sample
obtained to quantify their smoking habit. It was felt that this was preferable to relying on
retrospective information from the mother postnatally. Since most women who smoke during pregnancy also smoke postnatally it was not feasible to investigate the separate effects of prenatal and postnatal exposure. The main strength of this study was that potential confounding factors were controlled by matching each infant born to a smoking woman on an individual basis to an infant born to a nonsmoking woman. This enabled us to investigate, as far as possible, the independent effect of maternal smoking on respiratory responses.
Chapter 2
Study Design

2.1 Overview of Study

Eligible smoking and nonsmoking women were approached in the antenatal clinic at the
time of their first booking (usually around 13 weeks gestation) by the Research Nurse. They were given a short summary of the project and an information sheet to take home. Shortly afterwards the women were contacted at home to see if they might be interested in hearing about the project in more detail, those who agreed were visited in their own homes. Those women who were happy to take part were formally recruited and a questionnaire detailing information about the mother’s age, number of children, employment, smoking history etc. was completed. The home visit and recruitment lasted for about 1 hour. When the women were around 20 weeks pregnant a urine sample was obtained for measurement of cotinine, a metabolite of nicotine, to verify their smoking status. The women were then contacted at regular intervals throughout their pregnancy to check that it was proceeding satisfactorily and to ascertain their continued willingness to participate. The Research Nurse visited the maternity hospital on a daily basis to see if any of the women had delivered. A questionnaire concerning the delivery and the health of the infant was completed. When the infants were around 6 weeks of age the mothers were contacted to arrange an overnight visit to the hospital. Those infants who remained eligible and whose parents were happy for them to take part were studied between 8 and 12 weeks of age. This involved the infant staying in the laboratory for one whole night for respiratory control measurements and polysomnography. An overnight urine sample was collected from the infant and a questionnaire was completed concerning the infant’s health since delivery and child care practices. Figure 2.1.1 is a flow chart of the study.
2.2 Power Calculations

Previous work carried out in the department of Child Health investigating the effect of sleep deprivation on ventilatory responses to inspired oxygen levels in infants found that infants with at least one smoking parent had lower alternations in inspiratory drive (mean 8.3%) compared with those of nonsmoking parents (mean 21.3%), \( p=0.09 \)\(^{139}\). These findings were incidental and based on a small number of infants (5 and 13 respectively). Power calculations based upon these data suggested that 30 infants in each group would be required to have 91% power of detecting a difference in alternation of inspiratory drive of 10% in the group means.
2.3 Matching Criteria

Smoking is known to be associated with lifestyle and health factors such as social class, maternal age and parity, mode of feeding and prematurity. These factors have also been related to the risk of SIDS. It may be that respiratory control is also affected by these confounding factors, although this is not known. Therefore, to investigate the independent effect of smoking without interference from confounding factors a matched study design was used in which each infant born to a smoking mother was matched on an individual basis to an infant with a nonsmoking mother. A weighting system was developed for seven factors that were considered to be of importance in terms of their relationship with smoking, SIDS and respiratory control (table 2.3.1). These were social class, gender of the infant, maternal age, maternal parity, birthweight, gestational age and the way in which the mother intended to feed her baby after delivery.

Social class was assigned using OPCS criteria. If the mother was living with a partner this was based on the partner’s occupation. If he was currently unemployed it was based upon last employment within the previous 10 years. If the mother was living alone then it was based upon her employment. If she was currently unemployed it was based on past employment within the previous 10 years. The OPCS classifies the following social classes: I, II, IIIm(non manual), IIIm(manual), IV and V. One problem with these criteria is that it is not possible to classify people who have always been unemployed, look after the home, students, or servicemen/women. Therefore, in the present study, anyone who had always been unemployed or looked after the home was classified as unemployed, those currently a student were classified as student, and those in the services were classified as services. Individuals in any of these categories could only be matched with someone else from the same category and, therefore, matching these individuals was difficult.

The matching process was computerised using Microsoft Access and matches scoring 5 points or less were accepted. This would allow for small differences in factors, but would not tolerate larger differences in more than one factor. At the beginning of the study every infant was studied to build up a pool of studied babies. This meant that as the study progressed we were then able, with the aid of the computerised matching, to look for matches following delivery for infants who had already been studied or were
about to be studied and invite them to attend the laboratory. Thus infants were selected for testing on the basis of matching.

Table 2.3.1 Scoring system used for matching infants born to smoking and nonsmoking mothers

<table>
<thead>
<tr>
<th>Matching parameter</th>
<th>Difference</th>
<th>Points scored</th>
</tr>
</thead>
<tbody>
<tr>
<td>Social class</td>
<td>same</td>
<td>0</td>
</tr>
<tr>
<td>(I, II, IIInm, IIIm, IV, V)</td>
<td>one class apart (e.g. IIInm and IIIm)</td>
<td>2</td>
</tr>
<tr>
<td>(note unemployed/student/services only matched with infant from same category)</td>
<td>more than one class apart (e.g. II and IIIm)</td>
<td>unmatchable</td>
</tr>
<tr>
<td>Gender of infant</td>
<td>same</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>different</td>
<td>3</td>
</tr>
<tr>
<td>Maternal age (yrs)</td>
<td>&lt; 5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5-7</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>8-11</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>&gt;11</td>
<td>unmatchable</td>
</tr>
<tr>
<td>Parity</td>
<td>Each child different up to 3</td>
<td>1 point each</td>
</tr>
<tr>
<td></td>
<td>&gt;3 children</td>
<td>unmatchable</td>
</tr>
<tr>
<td>Birthweight (g)</td>
<td>Up to 499</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>500-749</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>750-999</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>&gt;999</td>
<td>unmatchable</td>
</tr>
<tr>
<td>Gestational age (wks)</td>
<td>Up to 2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>&gt;3</td>
<td>unmatchable</td>
</tr>
<tr>
<td>Feeding intention</td>
<td>Same</td>
<td>0</td>
</tr>
<tr>
<td>(Breast, mixed, bottle)</td>
<td>One step apart (e.g. breast and mixed)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Two steps apart (breast and bottle)</td>
<td>3</td>
</tr>
</tbody>
</table>
Chapter 3

Recruitment

3.1 Recruitment Target

From past experience using the alternating breath test overnight a 75% success rate in obtaining analysable data was anticipated. Therefore, we aimed to study a total of 80 infants to obtain data on 60 infants, the number suggested by the power calculations (section III, chapter 2.2). We planned to recruit an excess of women during pregnancy for three main reasons: i) a pool of women and infants was required so that we could match the tested infants closely, ii) a proportion of the women or infants would become ineligible, and iii) a proportion would withdraw from the study. It was felt that if 240 women (120 in each group) were recruited on to the study this would give enough reserve for withdrawals/ ineligibility and matching, and allow 80 infants to be studied.

3.2 Recruitment process

Eligible women were approached by the Research Nurse in the antenatal clinic at their first booking (usually around 13 weeks gestation) and given an introductory summary of the work. They were then contacted sometime later and a home visit was made to those who were agreeable. During the home visit the study was explained in detail and if the woman was happy to take part she was formally recruited and a questionnaire completed. Many women discussed the study with their partners before participating. No infant was included if the father was opposed to the study.

3.3 Eligibility

Initial Recruitment

The study required that the woman and her partner were both white Caucasian. Other eligibility criteria were that the woman should be: (i) eighteen or over, (ii) fit and well with no significant medical history, (iii) not taking prescribed medication (single course of antibiotics accepted), (iv) have a singleton pregnancy, (v) not using fertility treatment, and (vi) not using recreational drugs.
After recruitment

Women remained eligible if their pregnancy continued satisfactorily and were willing to remain on the study. Smokers reporting that they had given up smoking or had smoked inconsistently were excluded. Women whose urinary cotinine/creatinine levels did not verify their smoking/nonsmoking status were excluded.

After delivery

The infant remained eligible if they were born after at least 37 completed weeks gestation, birthweight was appropriate for gestation, no congenital abnormality, no respiratory support required and the parents remained willing to participate. During the period between delivery and testing if the infant became unwell, other than with minor illnesses such as coughs and colds, s/he became ineligible.

3.4 Recruitment and eligibility results

Over a 2½ year period a total of 199 women were recruited onto the study (97 smokers and 102 nonsmokers). During the time between recruitment and studying the infants a much greater proportion of the smoking group became ineligible or withdrew from the study (figure 3.4.1). The majority of women/infants became ineligible during the antenatal and postnatal periods, few immediately following delivery. The percentage excluded during any period was greater in the smoking group.

The main reasons for exclusion in the smoking group during the antenatal period were giving-up smoking, inconsistent smoking or admitting to smoking marijuana (table 3.4.1). The main reason for exclusion during this period in the nonsmoking group was the use of prescribed medication (9%). One nonsmoking woman was excluded for inconsistent smoking. This woman said that she was a nonsmoker at recruitment but later during a contact call by the Research Nurse admitted starting smoking. Very few women withdrew from the study during the antenatal period (4% and 3% in the smoking and nonsmoking groups).

The main reason for exclusion following delivery was premature delivery. In the smoking group 11% of those infants who remained eligible after the antenatal period
were born before 37 completed weeks gestation, compared to 4% in the nonsmoking group. In addition, there were 3 (5%) stillbirths in the smoking group.

In the postnatal period the main reason for exclusion in both groups was voluntary withdrawal from the study. In the smoking group 26 women chose to withdraw from the study, 51% of the women remaining after the delivery period. In the nonsmoking group 12 (16%) women withdrew. There were also 3 and 6 infants excluded from the study in the smoking and nonsmoking groups respectively because the infants were unwell. This was because of an upper respiratory tract infection or bronchiolitis. In addition, two women in the nonsmoking group were unable to bring their infants for testing, because one had broken her leg and the other had damaged her back.

In summary, a total of 199 women were recruited on to the study, however, during the long time period (7 months) between recruitment and studying the infants a large proportion became ineligible or withdrew. This left 22 (22.7%) infants in the smoking group and 54 (52.9%) in the nonsmoking group who were eligible and available to be studied.
Figure 3.4.1 Percentage of the recruited smoking and nonsmoking groups remaining on the study after the antenatal, delivery and postnatal periods.
Table 3.4.1  Numbers and reasons for exclusion from the study in the smoking and nonsmoking groups

<table>
<thead>
<tr>
<th>Reasons</th>
<th>Smokers</th>
<th>Nonsmokers</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antenatal Exclusion</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gave up smoking during pregnancy</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Inconsistent smoking</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Smoke marijuana</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>High cotinine</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Alcohol abuse</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Use of medicines</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Mother unwell</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Miscarriage</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Ethnic partner</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Withdrew from study</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>62 (63.9%)</td>
<td>80 (78.4%)</td>
</tr>
<tr>
<td><strong>Delivery Exclusion</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stillbirth</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Infant born prematurely (&lt; 37 weeks)</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Infant admitted to neonatal unit</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Poor apgar</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>IUGR</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>51 (52.6%)</td>
<td>74 (72.5%)</td>
</tr>
<tr>
<td><strong>Postnatal Exclusion</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infant unwell</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Unable to come</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Withdrew from study</td>
<td>26</td>
<td>12</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>22 (22.7%)</td>
<td>54 (52.9%)</td>
</tr>
</tbody>
</table>
Chapter 4

Methods

4.1 Subjects

All 22 of the eligible infants in the smoking group were studied. In addition, two infants who were ineligible according to our strict criteria because they were born prematurely (36\textsuperscript{6} weeks) were also included to compensate for the high withdrawal rate in the smoking group. It was felt that this slight prematurity was unlikely to affect the outcome. Therefore, a total of 24 infants were studied in the smoking group.

At the onset of the study all infants born to nonsmoking women were studied, however later in the study we became more selective and studied infants on the basis of whether they matched with an infant in the smoking group. Consequently, 35 infants born to nonsmoking mothers were studied rather than all 54 available.

This study was passed by the Leicestershire Health Authority Ethics committee.

4.2 Measurement of urinary cotinine levels

Following recruitment, at around twenty weeks gestation women were asked to provide a urine sample. The women were not aware that the nicotine metabolite, cotinine, would be analysed in their urine unless they asked about this specifically. The main purpose for analysing cotinine levels in the women's urine was to verify the woman's reported smoking habit. Infant samples were also collected from those individuals who were studied in the sleep laboratory. These measurements gave information on the passive exposure of these infants to cigarette smoke postnatally.

Urine collection and storage

Maternal urine samples were collected in a urine pot. The infant samples were obtained by placing a cotton wool ball in the nappy. When the cotton wool ball was sodden it was removed and the urine squeezed out into eppendorf tubes. If the cotton wool ball was not damp enough to be squeezed out a small amount of distilled water was added. The urine samples were frozen and stored at -20°C until analysis.
Measurement of Urinary Cotinine

Cotinine was measured in the urine samples using an enzyme linked immunosorbent assay (ELISA). These measurements were performed in the Department of Chemical Pathology at Leicester Royal Infirmary. A commercial ELISA kit was used (Cozart Bioscience Ltd, Abingdon, Oxfordshire, UK.) and each urine sample was tested in duplicate. An enzyme-cotinine complex was added to a plate which contained an antibody specific for cotinine. The urine sample being tested was then added to the plate. The cotinine in the urine sample and the cotinine in the enzyme-cotinine complex compete for the sites on the antibody. The higher the concentration of cotinine in the urine sample the greater the amount that binds to the antibody and displaces the enzyme-cotinine complex. The plate was then washed to remove any displaced enzyme-cotinine complex. A substrate was then added which changes colour in the presence of the enzyme. The absorbance was then measured and is inversely related to the cotinine concentration. A standard curve was produced with a range of different cotinine concentrations and absorbances. The corresponding cotinine concentration for the absorbance was read off for the tested urine sample. The cotinine assay has a sensitivity of 1ng/mL and has been shown to discriminate between active smokers and nonsmokers with a sensitivity and specificity of 100% \(^{167}\). Creatinine was analysed using the Jaffé reaction \(^{168}\). All cotinine measurements were expressed per unit creatinine (\(\mu g/mMol\)).

4.3 Measurements made during overnight studies

Overview of Procedure

Parents were asked to bring their infant to the sleep laboratory for the last feed before they would normally put their infant down for the night. This time varied from 6pm to 10pm. The tests and procedures were explained in detail to the mother, or both parents, and written informed consent obtained. Parents were aware that there was a room for them to sleep if they wished and that they could come and go from the sleep laboratory as they pleased. Whilst the infant was still awake electrodes for monitoring the electrocardiogram (ECG), oxygen saturation, movement of the limb, rectal temperature and rib cage and abdomen movements were placed on the infant. The infant was then given their last feed and allowed to settle to sleep in their normal way. When the infant was asleep the remaining electrodes for monitoring the electroencephalograph (EEG) and electrooculogram (EOG) were placed in position. This was not done when the
infant was awake because the infants would usually pull them off! The polysomnography signals were then viewed and recorded continuously on the Jaeger Sleeplab for the whole night. When the infant was in polygraphically determined quiet sleep a fine tube from the mass spectrometer was placed just inside the nares. This allowed monitoring of inspired and end-tidal oxygen and carbon dioxide levels on a breath by breath basis. If the infant remained in quiet sleep the test of respiratory control, the alternating breath test, was performed and recorded on an IBM compatible pentium computer. We aimed to obtain at least 2 or 3 analysable tests in at least 2 separate periods of quiet sleep. Once this was achieved the infant was monitored polysomnographically for the remainder of the night. If the infant woke during the night the infant was cuddled or fed by either the parents or investigators depending upon the mode of feeding and the parents wishes. At the end of the study when the infants awoke the electrodes were removed and the infant was fed in the usual way.

4.3.1 Polysomnography recording for determination of sleep state

**Equipment**

Breathing movements were monitored using respiratory inductance plethysmography (RIP)(Model 150, Studley Data Systems, Oxford. UK.). The RIP, EEG, EOG, ECG and limb movement signals were fed into a headbox and then recorded on a commercial polysomnography computer (Sleeplab, Erich Jaeger, Market Harborough. UK.). Rectal temperature was monitored using an Edale thermometer (Edale Thermometer model C, Edale Instruments (Cambridge) Ltd., Cambridge. UK.) and oxygen saturation was monitored using a pulse oximeter (Biox 3700e, Ohmeda, Louisville. USA). The oximetry and rectal temperature signals were then passed through an isolating interface built within the department and recorded on the polysomnography computer. The EEG, EOG and ECG were recorded at a sampling rate of 128Hz, the limb movement was recorded at 64Hz, rectal temperature and breathing movements were recorded at 16Hz and oxygen saturation and pulse rate at 8Hz. A schematic diagram of the equipment set-up is shown in figure 4.3.1.1.
Figure 4.3.1.1 Equipment set-up for polysomnographic recording

- Headbox
- Edale
- Ohmeda Biox
- Isolating Interface
- Sleeplab Computer
- RIP
- ECG
- EOG
- EEG
- Movement detector
- Rectal Temperature
- Oxygen saturation
**Preparation and positioning of Electrodes**

Before positioning the EEG, EOG and ECG electrodes the skin was prepared by cleaning gently with an alcohol wipe.

**Electroencephalogram (EEG)**

The EEG was recorded using four silver-silver chloride cup electrodes (Henleys Medical Supplies Ltd., Welwyn Garden City. UK.). One electrode was placed behind each ear on the mastoid processes, A₁ and A₂. These were reference electrodes. The other two electrodes were placed on the scalp in the central and occipital regions (figure 4.3.1.2) on the opposite side of the head to that on which the infant preferred to sleep. If placed on the left hand side of the head they are referred to as C₃ and O₁, if on the right C₄ and O₂. Each cup electrode was filled with conductive paste (Ten20 conductive EEG paste, UniMed electrode supplies, Farnham. UK.) and placed in position. A piece of adhesive tape (Mefix, Molnlycke Health Care AB, Molnlycke, Sweden.) was placed over the reference electrodes to secure them into position. The central and occipital scalp electrodes were referred to the contralateral reference electrode during recording so that, for example, C₃ would be referenced to A₂.

**Electrooculogram (EOG)**

Two skin electrodes (Sensormedics Corporation, Yorba Linda. USA) were used to record the EOG. One electrode was placed on the outer canthus of each eye with one being placed higher than the other so that vertical and horizontal eye movements could be recorded (figure 4.3.1.2). They were secured to the skin using a double-sided sticky disc and conductive jelly was placed in the centre. The EOG electrodes were referred to the same reference as the EEG electrodes for recording.

**Electrocardiogram (ECG)**

Two skin electrodes were used and secured to the skin as described for the EOG. However, this time one electrode was placed below the middle of the right clavicle and the other just below the left nipple (figure 4.3.1.2).
Oxygen Saturation

A Flex II probe (Ohmeda, Louisville, USA.) was placed around the lateral surface of the left foot. Two small sticky discs on either end of the probe and a velcro strap were used to secure the probe in place. The signal was checked on the oxygen saturation monitor and, if satisfactory, a sock or baby grow were then placed over the top.

Limb movement

A movement detector (Henleys Medical Supplied Ltd., Welwyn Garden City. UK.) was placed on the lower part of the left leg so that artefact on the oxygen saturation resulting from limb movement could be excluded. The detector was taped onto the limb using adhesive tape.
Rectal temperature

A rectal probe (Grant Instruments (Cambridge) Ltd., Cambridge, UK.) was coated with Vaseline or KY jelly and inserted into the rectum 5cm from the anal margin. A small piece of Mefix tape was then used to secure this in place.

Respiratory Inductance Plethysmography (RIP)

Respiratory Inductance Plethysmography was used to monitor breathing movements of the rib cage and abdomen for determination of sleep state and measurement of ventilation during the test of respiratory control. Two 2cm wide bands were used and one was placed just below the axillae and the other at the level of the umbilicus.

Procedure

When the infant was asleep and all of the electrodes were in place polysomnography recording could commence. The EEG, EOG, ECG, oxygen saturation, limb movement, breathing movements and rectal temperature were recorded and could be viewed for the whole night. During the night if electrodes became detached one of the investigators would put them back in place. When the infant woke in the morning the electrodes were removed and recordings were saved onto an optical disk. Figure 4.3.1.3 shows a photograph of an infant wearing some of the equipment.

Figure 4.3.1.3 Photograph of an infant wearing some of the equipment
4.3.2 Measurement of Respiratory Control - The Alternating Breath Test

**Equipment**

Figure 4.3.2.1 shows a schematic diagram of the equipment used for the alternating breath test.

Respiratory Inductance Plethysmography (RIP)

Ventilation was measured using respiratory inductance plethysmography (RIP) with a band placed around the rib cage and the abdomen as described above. The RIP was set at a sensitivity high enough so that the resting breathing signal was taking up a large range of the span on the A/D board of the computer, but not so high that recordings of sighs and response to the alternating breath test were saturated. This was usually accomplished by using a sensitivity of 5 or 6 for both the rib cage and abdomen bands. The RIP was set for AC coupled output with a time constant of 10 seconds. The output of the RIP was passed through an A/D converter and collected on an IBM compatible computer utilising RASP software (PhysioLogic, Newbury, UK) and sampling at 100Hz.

Mass Spectrometer

The mass spectrometer (Airspec 2000, Airspec Ltd., Biggin Hill. UK.) was calibrated at the beginning of the night using a computerised calibration routine that involved using a certificated calibration gas containing known concentrations of oxygen and carbon dioxide, 100% N₂ and 100% O₂. After calibration with the three gases the mass spectrometer was set-up so that the 40% O₂ used for the alternating breath test would be within its optimal range. Where possible at the beginning and the end of each alternating breath test recordings of the calibration gas, 40% O₂ and 100% N₂ were collected so that correction curves could be generated if required. A fine tube was placed inside the infant’s nares to monitor inspired and expired (end-tidal) oxygen and carbon dioxide with each breath. The sampling flow rate was 14.7 mL/min and the sampling time was approximately 290ms. The mass spectrometer output was passed through the A/D converter of the computer and collected into the RASP software with the ventilatory signals.
Figure 4.3.2.1 Equipment set-up for the alternating breath test

- Computer
- A/D Converter
- Solenoid Valve Box
- Oxygen Saturation
- RIP
- Mass Spectrometer
- Air
- 100% \( \text{O}_2 \)
- 40% \( \text{N}_2 \)
Gas Delivery and switching of the solenoid valves

Three gas lines supplying 40% O₂, 100% N₂ and air were delivered to the facemask for the infant to breathe. Delivery of these gases was controlled by three solenoid valves and the flow rate was set at 7.0 L/min using rotameters attached to each valve. This flow rate was chosen to allow flushing of the system and to limit entrainment of air around the facemask. Operation of the valves was computer controlled and the infant’s respiratory pattern (RIP) was used to time the switching. When a new gas was delivered the appropriate valve was opened at the beginning of expiration so that the system would be completely flushed with the new gas before the infant took its next inspiration. The time taken for the computer to recognise expiration was 0.3 seconds, and for flushing the system with the new gas was 0.47 seconds. Therefore the total lag time from the start of expiration to delivery of the new gas was 0.77 seconds.

Oxygen saturation

Oxygen saturation was monitored, as described previously, for safety reasons. If the oxygen saturation fell to 94% or below during the test period of the alternating breath test the facemask was moved away from the infant’s face.

Procedure for the alternating breath test

During periods of polygraphically determined quiet sleep respiratory control was measured using the alternating breath test. Once the RIP signals and oxygen and carbon dioxide signals from the mass spectrometer were being recorded by the computer satisfactorily the measurements would start. The alternating breath test was approximately six minutes long. A facemask was held close to the infant’s face, but not touching. For the first two minutes air was delivered through the facemask (control period). This was followed by the test period which involved delivering alternately two breaths that were oxygen enriched (40% oxygen (balance N₂)), followed by two breaths that were oxygen depleted (100% N₂). This pattern would continue until at least 22 breaths were obtained, unless the infant sighed or oxygen saturation fell to 94% or below. If the infant sighed during the test period the facemask was removed and when the infant’s breathing pattern was steady again the facemask was put back in position and the test recommenced. Once a satisfactory test was obtained the infant would then be returned to air breathing. We aimed to obtain at least 2 or 3 satisfactory tests in separate
periods of quiet sleep during the night. Figure 4.3.2.2 shows a photograph of an alternating breath test being performed.

Figure 4.3.2.2. Photograph of an alternating breath test being performed

4.4 Analysis of Raw Data

4.4.1 Alternating breath test

Overview

The second breaths of 40% O\(_2\) were compared to the second breaths of 100% N\(_2\) for ten respiratory parameters. These were:

- \(V_{TI}\) Inspired tidal volume (arbitrary units, au)
- \(V_{TE}\) Expired tidal volume (au)
- \(V_{TT}\) Average of the inspired and expired tidal volume (au)
- \(t_i\) Inspired time (seconds)
- \(t_E\) Expired time (seconds)
- \(f_R\) Respiratory rate or number of breaths per minute
- \(V_{TI}/t_i\) Inspiratory drive (au/secs)
- \(V_{TE}/t_E\) Expiratory drive (au/secs)
- \(t_i/t_{tot}\) Inspiratory duty time where \(t_{tot} = t_i + t_E\)
- \(V_E\) Instantaneous ventilation (au/min)
Figure 4.4.1.1 shows a short excerpt of an alternating breath test. In this example, the second breath of 40% O\(_2\) (breath A) is compared to the second breath of 100% N\(_2\) (breath B), and breath B is then compared to the following second breath of 40% O\(_2\) (breath C). The % change in each variable for each alternation from 40% O\(_2\) to 100% N\(_2\) (high to low), and 100% N\(_2\) to 40% O\(_2\) (low to high) is calculated. In this example where \(V_t\) of breath A = 2.29 au, breath B = 1.64 au and breath C = 2.95 au:

\[
\text{% change in } V_t \text{ high to low} = \frac{(B-A)}{(B+A)/2} * 100
\]

\[
(1.64-2.29) *100 = -33.1\%
\]

\[
(2.29+1.64)/2
\]

\[
\text{% change } V_t \text{ low to high} = \frac{(C-B)}{(C+B)/2} * 100
\]

\[
(2.95-1.64) *100 = 57.1\%
\]

\[
(1.64+2.95)/2
\]

Figure 4.4.1.1 Excerpt of test period of alternating breath test

The alternating nature of the test means that every other % change will have a different sign. If the mean was taken of all the % changes within a test then it would be expected to be close to zero. Therefore, all of the % changes from high to low were multiplied by -1. The mean was then calculated for each variable and is referred to as the mean % alternation.
**Procedure**

Tests were selected for analysis on the basis that they should have at least one continuous period of six alternations (14 breaths) during the test and baseline periods. These periods were not required to be continuous. In addition, the end-tidal gas signals needed to be analysable. If the signals were too noisy or the end-tidal points were unclear the test was excluded from analysis. Sighs, defined as having a tidal volume at least twice the average for the test, were also excluded from the analysis. This was for two reasons: (i) volumes were often greater than twice normal tidal volume resulting in the RIP signals becoming saturated and (ii) the sighs were often followed by pauses in breathing which would cause mistriggering of the gases i.e. oscillations in an essentially flat RIP trace were interpreted as breaths and triggered the solenoid valve box. For this reason, if a sigh occurred the facemask was removed. The selected baseline and test periods were analysed in two stages. The first stage of the analysis was performed on specially commissioned software (RASP). The test or baseline period to be analysed was loaded into the analysis part of the software. A RIP sum signal was then created using 66.7% of the RIP abdomen signal and 33.3% of the RIP rib signal. This is equivalent to using a fixed value of $K$ of 0.5 (section II, chapter 2). This gave a RIP signal that was directly proportional to changes in volume. The programme was then used to calculate all of the respiratory parameters for each second breath of 40% $O_2$ and 100% $N_2$. These values were then exported from RASP and imported into Microsoft EXCEL. A macro in Microsoft EXCEL was then used to calculate the mean percentage alternations for all parameters.

4.4.2 Analysis of Inspired and End-tidal Oxygen and Carbon Dioxide

Figure 4.4.2.1 shows a typical oxygen and carbon dioxide trace at the end of a baseline period and the start of a test period. The levels of inspired and end-tidal oxygen and end-tidal carbon dioxide were calculated for the breaths used for the respiratory analysis i.e. second breaths of 40% $O_2$ and 100% $N_2$. The end-tidal levels of oxygen and carbon dioxide during the baseline period were also calculated for every second breath of the baseline period. In addition, the inspired and end-tidal oxygen levels were calculated for the very first breath of the test period.
Figure 4.4.2.1 Example of recording of oxygen and carbon dioxide at the nares during a baseline and test period showing points for analysis of inspired and end-tidal gases.

For some overnight studies the calibration of the mass spectrometer drifted during the night. Where possible recordings of calibration gas, 40% O$_2$ and 100% N$_2$ were made before and after alternating breath test recordings. The one closest in time to the beginning of the alternating breath test was analysed. The values of oxygen in the calibration gas, 40% O$_2$ and 100% N$_2$ were calculated. If these recorded values were within 2% of the actual known values then a correction was not required i.e. the mass spectrometer calibration had not drifted sufficiently to require correction of the alternating breath test gases. However, if the % difference for any of the gases was greater than 2% a correction was calculated. The known values of oxygen in the gases were plotted against the measured values of oxygen by the mass spectrometer. A regression analysis was performed and the regression equation was used for correction of the alternating breath test inspired and end-tidal oxygen values to give absolute levels. In those tests that were corrected, or those for which correction was not required, the following values were calculated:
Inspired \( \text{O}_2 \) levels for second breaths of 40\% \( \text{O}_2 \) and 100\% \( \text{N}_2 \)

End-tidal \( \text{O}_2 \) levels for second breaths of 40\% \( \text{O}_2 \) and 100\% \( \text{N}_2 \)

Change in end-tidal \( \text{O}_2 \) (difference between end-tidal \( \text{O}_2 \) for second breath 40\% \( \text{O}_2 \) and end-tidal \( \text{O}_2 \) for second breath of 100\% \( \text{N}_2 \)).

Those tests that did not have recordings of calibration gas, 40\% \( \text{O}_2 \) and 100\% \( \text{N}_2 \) before or after the test were excluded from the analysis.

It was not possible to correct the end-tidal carbon dioxide levels and, therefore, absolute values were not calculated. However, changes in end-tidal carbon dioxide during the test period of the alternating breath test compared to the baseline were calculated. The difference between the end-tidal carbon dioxide level of each analysed breath in the test period and the mean baseline end-tidal carbon dioxide was calculated. The mean of all these differences is referred to as the mean change in end-tidal carbon dioxide during the test period.

### 4.5 Statistical Analysis of Data

The analysis involved fitting a mixed effects model with normal error structure. A random effect was used to allow for the correlation between repeat runs on the same infant and fixed effects were used for the matching strata, smoking and confounding factors such as inspired oxygen levels and baseline breathing parameters. Significance was assessed using Wald tests.
Chapter 5
Data obtained and characteristics of population

5.1 Subjects on whom data was obtained and those included in the analysis

The temperature in the laboratory during the overnight studies ranged between 21°C and 25°C. A total of 59 infants (24 smoking and 35 nonsmoking) were seen for overnight study and data on 40 (17 smoking, 23 nonsmoking) of these were included in the analysis (figure 5.1.1). We were unable to obtain at least one test of a minimum length of 14 breaths in five infants (1 smoking, 4 nonsmoking). This was equivalent to a success rate of 96% and 90% in the smoking and nonsmoking groups respectively. In a further six studies (1 smoking) technical problems were experienced with the mass spectrometer and consequently inspired and end-tidal gases were not available. A further 7 infants (4 smoking) were excluded because we were either unable to find a match for them, or obtain data on their match when they attended the laboratory. One infant in the smoking group became unwell during the period of the overnight study and was subsequently treated with antibiotics for a chest infection. Therefore, data obtained on this infant was also excluded. In six cases infants were studied in either the prone (2 smoking) or side positions (3 nonsmoking, 1 smoking), all other infants were studied in the supine position. The characteristics at the time of testing of the 40 infants included in the analysis are shown in table 5.1.2.

Table 5.1.2 Characteristics at time of overnight testing of the two groups of infants included in the analysis

<table>
<thead>
<tr>
<th></th>
<th>Nonsmoking Mean (SD)</th>
<th>Smoking Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postnatal age (weeks)</td>
<td>11.5 (1.6)</td>
<td>11.2 (2.2)</td>
</tr>
<tr>
<td>Postconceptional age (weeks)</td>
<td>51.6 (2.1)</td>
<td>51.0 (2.9)</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>5.7 (0.7)</td>
<td>5.4 (0.7)</td>
</tr>
<tr>
<td>Length (cms)</td>
<td>59.1 (1.9)</td>
<td>57.2 (2.1)</td>
</tr>
</tbody>
</table>
5.2 Tests included in the Analysis

A total of 261 tests (122 smokers, 139 nonsmokers) were attempted in the 40 infants included in the analysis. This was equivalent to an average of 7 attempts per infant in the smoking group and 6 attempts in the nonsmoking group. A similar proportion of these attempts in the two groups (52.5% smokers and 55.4% nonsmokers) resulted in tests that met the minimum criteria for analysis (continuous period of at least 14 breaths). The main reasons for tests not reaching the required length were termination of the test because of the occurrence of a sigh (70.8%) (figure 5.2.1) and mistriggering of the inspired gases by the computer (25.8%) (figure 5.2.2).

Termination of the test because of a sigh was more common in the nonsmoking group (82.3%) than the smoking group (58.6%). In contrast, mistriggering of the inspired gases was more prevalent in the smoking (37.9%) rather than the nonsmoking group.
(14.5%). There was a total of 64 tests in the smoking group and 77 in the nonsmoking group that met the minimum length. However, 4 of these tests were excluded because they did not have acceptable end-tidal traces, and 2 were excluded because the abdominal RIP signal was technically unacceptable. When performing the test our aim was to collect a continuous period of 22 breaths, although we accepted a period of 14 breaths for analysis. If the first attempt during a data collection run did not achieve this then the facemask would be removed for at least 10 breaths and then returned and the gases delivered once more. Therefore, within a data collection run several attempts may have been made (figure 5.2.3). If there were several acceptable attempts only one period from one data collection run was included in the analysis, and this was the longest continuous period.
Figure 5.2.1 Example of a test terminated by a sigh

SIGH on breath 6 of test

- Rib RIP signal
- Saturated AB signal
- Pause after sigh
- Abdomen RIP signal
- Sum RIP signal
- O₂ (%) at nares
- CO₂ (%) at nares

Figure 5.2.2 Example of a test terminated by a mistrigger of the inspired gases

Short pause/swallow leading to mistrigger of the inspired gases

- Rib RIP signal
- Abdomen RIP signal
- Sum RIP signal
- O₂ (%) at nostril
Figure 5.2.3 Example of a test with two attempts

Sigh followed by a pause

FIRST ATTEMPT

SECOND ATTEMPT

Rib RIP signal
Abdomen RIP signal
Sum RIP signal
$O_2$ (%) at nostril
A total of 127 tests of acceptable length were included in the final analysis; 56 from the smoking and 71 from the nonsmoking groups respectively. The median number of tests obtained in each infant was 3 in both groups with a range of 1 to 6 in the nonsmoking and 1 to 7 in the smoking group. Table 5.2.1 presents the distribution of tests in the two groups.

**Table 5.2.1 Distribution of the number of tests obtained in each infant for the two groups**

<table>
<thead>
<tr>
<th>Number of tests obtained</th>
<th>Nonsmoking group</th>
<th>Smoking group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of infants (%)</td>
<td>Number of infants (%)</td>
</tr>
<tr>
<td>1</td>
<td>1 (4)</td>
<td>2 (12)</td>
</tr>
<tr>
<td>2</td>
<td>8 (35)</td>
<td>3 (18)</td>
</tr>
<tr>
<td>3</td>
<td>6 (26)</td>
<td>6 (35)</td>
</tr>
<tr>
<td>4</td>
<td>5 (22)</td>
<td>3 (18)</td>
</tr>
<tr>
<td>5</td>
<td>2 (9)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>6</td>
<td>1 (4)</td>
<td>1 (6)</td>
</tr>
<tr>
<td>7</td>
<td>0 (0)</td>
<td>1 (6)</td>
</tr>
</tbody>
</table>

The tests included in the analysis had an average (SD) length of 33 (15) breaths in the smoking group and 32 (12) breaths in the nonsmoking group.

**5.3 Comparison of urinary cotinine/creatinine levels in the two groups included in the analysis**

We were unable to obtain maternal urine samples at 20 weeks gestation in two smoking women; one refused to provide a sample and the other was never at home when the Research Nurse called. There was a clear separation in maternal urinary cotinine/creatinine levels between the two groups (figure 5.3.1). The median values were 1.1 µg/mMol in the nonsmoking and 106.9 µg/mMol in the smoking group. This was significantly different (p<0.001) when analysed using the Mann-U-Whitney test for independent non-parametric data.
We were unable to obtain urine samples during the overnight studies in 3 of the 20 infants in the nonsmoking group. There was some overlap in infant urinary cotinine/creatinine between the two groups and there was a wide range of values in the smoking group (figure 5.3.2). The median levels were 3.33 and 18.0 μg/mMol in the nonsmoking and smoking groups respectively and these were significantly different (p=0.0005). All women in the smoking group smoked throughout pregnancy, however, two women reported giving-up smoking following delivery. The urinary cotinine/creatinine values in their infants at testing were 3.33 and 6.67 μg/mMol, and were similar to those in the nonsmoking group.
Figure 5.3.2. Infant urinary cotinine/creatinine levels at the time of overnight testing in the two groups

Box and whisker plots where box represents middle 25-75%, horizontal line represents the median and whiskers represent the range of the data.

5.4 Comparison of the recruited and analysed populations

The group recruited on to the study and that included in the analysis were similar in respect of social class distribution, maternal age, parity, gestational age, mode of feeding, gender of infant, birthweight, maternal cotinine/creatinine ratio and number of cigarettes per day smoked (table 5.4.1). However, there were a few individuals on whom we were unable to obtain this information, mainly in the group who were recruited but not tested.

There were significant differences in social class distribution, maternal age, mode of feeding and birthweight between the nonsmoking and smoking groups recruited. Following matching some of these differences were minimised, but significant differences in birthweight remained.
Table 5.4.1  Characteristics of recruited and analysed groups

<table>
<thead>
<tr>
<th></th>
<th>no. with social class</th>
<th>I and II (%)</th>
<th>IIInm and IIIm (%)</th>
<th>IV and V (%)</th>
<th>Unemployed and other (%)</th>
<th>Maternal age (yrs)</th>
<th>Parity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Recruited</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonsmoking</td>
<td>101</td>
<td>34.7*</td>
<td>49.5</td>
<td>12.9*</td>
<td>2.9*</td>
<td>29.1* (19.5,43.1)</td>
<td>0.4</td>
</tr>
<tr>
<td>Smoking</td>
<td>94</td>
<td>6.5*</td>
<td>51.6</td>
<td>25.8*</td>
<td>16.1*</td>
<td>26.3* (18.5,43.1)</td>
<td>0.6</td>
</tr>
<tr>
<td><strong>Analysed</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonsmoking</td>
<td>23</td>
<td>21.7</td>
<td>60.9</td>
<td>13.0</td>
<td>4.4</td>
<td>27.3 (20.0,36.5)</td>
<td>0.3</td>
</tr>
<tr>
<td>Smoking</td>
<td>17</td>
<td>11.8</td>
<td>58.8</td>
<td>23.5</td>
<td>5.9</td>
<td>27.9 (18.5,35.1)</td>
<td>0.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Breast feeding (%)</th>
<th>Male infants (%)</th>
<th>Birthweight (kg)</th>
<th>Gestational age (wks)</th>
<th>Maternal cotinine/creatinine (ug/mMol)</th>
<th>Number of cigarettes per day reported</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Recruited</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonsmoking</td>
<td>66.3*</td>
<td>50</td>
<td>3.55* (2.48,4.78)</td>
<td>40.0 (34.0,42.4)</td>
<td>1.5* (0.1,196.3)</td>
<td>10 (0,30)</td>
</tr>
<tr>
<td>Smoking</td>
<td>42.9*</td>
<td>38</td>
<td>3.28* (0.99,4.40)</td>
<td>40.0 (32.0,42.4)</td>
<td>103.5* (0.3,1000)</td>
<td>10 (0,30)</td>
</tr>
<tr>
<td><strong>Analysed</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonsmoking</td>
<td>69.6</td>
<td>44</td>
<td>3.40* (2.98,3.91)</td>
<td>40.0 (38.1,41.6)</td>
<td>1.1* (0.1,26.4)</td>
<td>10 (3,30)</td>
</tr>
<tr>
<td>Smoking</td>
<td>47.1</td>
<td>41</td>
<td>3.18* (2.72,3.91)</td>
<td>39.9 (37.6,41.3)</td>
<td>106.9* (50.9,348.8)</td>
<td>10 (3,30)</td>
</tr>
</tbody>
</table>

All values are median (range), except (%), analysed statistically using Mann-U-Whitney test for independent non-parametric data. n refers to total number in groups, * denotes a significant difference between the two recruited groups, * denotes a significant difference between the two groups studied.
5.5 Details of matched design used for statistical analysis

The 40 eligible, matchable infants with acceptable data were divided into 17 closely matched sets. Each set contained 1 infant born to a smoking mother and between 1 and 3 nonsmokers (Appendix 1). Within each matched set the nonsmoking mother/infant pair had to match with the smoking mother/infant pair, and also each nonsmoking pair had to match with the other nonsmoking pairs in the set. Whilst our aim was to accept matches if they scored 5 points or less on our weighted scoring system we did accept one match of 6 points (matched set 17). Each set of data on an infant was only used once in the analysis.
Chapter 6

Summary of main findings

One hundred and ninety nine women were originally recruited onto the study and 59 infants (24 smoking group) were studied. The final analysis includes data from 40 infants (17 smoking group). Nineteen remaining infants were excluded from the analysis because (i) there was no acceptable data, (ii) a match could not be found or, (iii) they were ineligible.

The respiratory responses measured using the alternating breath test were similar in both groups for all ten respiratory parameters measured, and there were no significant differences. The lag in the respiratory response affected the results of individuals, but was not systematically different between the two groups. Consequently, the comparison of inspiratory drive in the two groups was unaffected when the response was recalculated taking the lag into account, and remained non-significant. However, whilst smoking did not appear to affect the respiratory response other factors including the baseline breathing parameters, and postnatal age and length at testing were related to the response.

The mean end-tidal oxygen level when 40\% \text{O}_2 was inspired during the alternating breath test was an average of 1.13\% higher in the smoking group and this difference was highly significant (p=0.007). In addition, the mean change in end-tidal oxygen when breathing 40\% \text{O}_2 and 100\% \text{N}_2 was an average of 1.24\% higher in the smoking group (p=0.02). There were no differences between the groups in the end-tidal oxygen level when 100\% \text{N}_2 was inspired. These differences in end-tidal oxygen level were unexpected and could not be accounted for by differences in inspired \text{O}_2 level, weight at testing or birthweight between the groups. When length of the infant at testing was taken into account the change in end-tidal oxygen level during the test became non-significant (p=0.12), but the end-tidal oxygen level when 40\% \text{O}_2 was inspired remained significantly higher in the smoking group (p=0.032). However, the end-tidal oxygen level for the first breath of each test (40\% \text{O}_2) was not different between the two groups (p=0.202) suggesting that the raised mean end-tidal \text{O}_2 concentration seen over the whole test may be related to the development of the response.
Chapter 7

Results: Inspired and end-tidal oxygen and carbon dioxide levels

7.1 Comparison of the inspired oxygen levels in the two groups

When performing the alternating breath test a facemask is held close to the face but does not touch the skin. The inspired levels of oxygen that the infant receives will be dependent upon the flow rate of the gas delivered, how close the facemask is to the face and the direction of gas flow across the face. If the flow rate of the gas delivered is lower than the infant’s peak inspiratory flow rate this will lead to entrainment of room air. In this study the flow rate of the gas supplied was set to exceed the expected peak inspiratory flow rate, and thus is unlikely to have been an important contributory factor. Clearly, when the facemask is held further away from the face, or the delivered gas blows across the top of the face, a reduced amount of the gas supplied will be inspired. Since the investigators were not blind to the smoking status of the infant’s mother at the time of the measurements it is theoretically possible that the level of the inspired gases may have varied by the way in which the facemask was held. Therefore, it is essential to demonstrate that there was no systematic difference in the actual inspired oxygen levels that the infants in the two groups received.

Figure 7.1.1 shows the relationship between the average inspired oxygen level when 40% $O_2$ was delivered and the average inspired oxygen level when 100% $N_2$ was delivered for each test in the smoking and nonsmoking groups. There was considerable variability in the levels of inspired gases between tests with the levels ranging between 32.3% and 40.9% when 40% $O_2$ was delivered, and 0.1% and 8.8% when 100% $N_2$ was delivered. The mean values for each subject are presented in appendix 1. As expected there was an inverse relationship between the high and low inspired oxygen levels (figure 7.1.1). When 40% $O_2$ was delivered entrainment of air reduced the actual mean inspired level by approximately 2% in both groups (table 7.1.1). The mean inspired oxygen level when 0% $O_2$ (100% $N_2$) was delivered was increased by a similar amount (table 7.1.1).
Figure 7.1.1 Relationship between the actual inspired oxygen level when 40% O₂ and 100% N₂ delivered for both groups

Each data point is the result for one test.

Table 7.1.1 Mean (SD) inspired levels of oxygen measured in the two groups

<table>
<thead>
<tr>
<th>Gas delivered</th>
<th>Nonsmoking</th>
<th>Smoking</th>
</tr>
</thead>
<tbody>
<tr>
<td>40% O₂</td>
<td>38.1 (1.3)</td>
<td>38.5 (1.0)</td>
</tr>
<tr>
<td>100% N₂</td>
<td>2.3 (1.5)</td>
<td>2.2 (1.6)</td>
</tr>
</tbody>
</table>

The mean of all tests in each subject was calculated. The mean (SD) of the subject means was calculated and presented in table.

The mean inspired oxygen levels presented in table 7.1.1 were similar in the two groups. However, these are not weighted according to the number of tests obtained for each subject, and do not take account of the matching. If the difference in the inspired oxygen levels between the infants of smoking and nonsmoking women is calculated for each
matched set it is possible to investigate the differences in inspired gases taking account of
the matching. If the infants in the smoking group had a tendency for higher inspired
levels when 40% O₂ was delivered then the differences would be above zero. For
matched sets with more than one infant in the nonsmoking group the average differences
between the infant in the smoking group and those in the nonsmoking group was
calculated. There was no clear pattern in the differences within matched sets, and they
were equally scattered around zero for both the high and low inspired gases (figure
7.1.2). Statistical analysis of these data taking account of the number of tests within
individuals and the matching found that these differences were not statistically
significant. The average difference between the groups when 40% O₂ was delivered was
0.17% (p=0.63), and when 100% N₂ was delivered was 0.03% (p=0.949) (figure 7.2.2).

These data show that the same levels of inspired oxygen were delivered to both the
smoking and nonsmoking groups.
Figure 7.1.2 Differences in inspired gases within each matched set

A presents the differences in the high inspired gas, B presents the differences in the low inspired gas. Each data point is the average difference between the infant in the smoking group and the infant(s) in the nonsmoking group. Dotted line is zero.
7.2 Comparison of the end-tidal oxygen and carbon dioxide levels in the two groups

End-tidal oxygen levels during the baseline air-breathing period

The baseline end-tidal oxygen levels were similar in the two groups. The mean (SD) in the smoking group was 14.81 (0.48)% and in the nonsmoking group 14.91 (0.62)%.

End-tidal oxygen levels during the test period of the alternating breath test

The end-tidal or alveolar oxygen level is thought to reflect the arterial levels of oxygen, which drive the respiratory response. Consequently, even though the inspired levels of oxygen were the same in both groups it is important to measure the end-tidal oxygen levels. The mean end-tidal oxygen levels for each subject are presented in appendix 1. The mean end-tidal oxygen level for the second breaths of 40% O\textsubscript{2} was higher in the smoking group compared to the nonsmoking group (table 7.2.1). The mean change in end-tidal oxygen from the second breaths of 40% O\textsubscript{2} to 100% N\textsubscript{2} was also higher in the smoking group (table 7.2.1). However, there was little difference in the mean end-tidal oxygen level between the groups when 100% N\textsubscript{2} was delivered (table 7.2.1).

Table 7.2.1 Mean (SD) end-tidal oxygen levels in the nonsmoking and smoking groups

<table>
<thead>
<tr>
<th></th>
<th>Nonsmoking</th>
<th>Smoking</th>
</tr>
</thead>
<tbody>
<tr>
<td>End-tidal O\textsubscript{2} when 40% O\textsubscript{2} delivered (%)</td>
<td>20.86 (1.31)</td>
<td>21.87 (1.46)</td>
</tr>
<tr>
<td>End-tidal O\textsubscript{2} when 100% N\textsubscript{2} delivered (%)</td>
<td>10.22 (1.30)</td>
<td>10.01 (1.47)</td>
</tr>
<tr>
<td>Change in end-tidal O\textsubscript{2} (%)</td>
<td>10.61 (1.93)</td>
<td>11.86 (1.28)</td>
</tr>
</tbody>
</table>

The mean of all tests in each subject was calculated. The mean (SD) of the subject means was calculated and presented in table.
The differences in end-tidal oxygen levels between the infant in the smoking group and infant(s) in the nonsmoking group for each matched set were calculated as described previously. When 40% $O_2$ was delivered more of the differences within matched sets were above zero (figure 7.2.1, A). This was also true of the change in end-tidal oxygen level (figure 7.2.1, B). When these differences were analysed statistically taking into account the number of tests within individuals and the matching they were significantly different. The end-tidal oxygen level when 40% $O_2$ was delivered was an average of 1.13% ($p=0.007$) higher in the smoking group, and the change in oxygen level was an average of 1.24% ($p=0.02$) higher in smoking group (figure 7.2.2). When inspired levels of oxygen were taken account of in the analysis the differences remained significant ($p=0.012$ and 0.025 respectively), and therefore could not be explained by differences in inspired oxygen levels. When birthweight and weight at time of testing were taken into account these differences remained statistically significant. However, when length at time of testing was accounted for the end-tidal oxygen when 40% $O_2$ was delivered remained significantly higher in the smoking group ($p=0.032$), but the change in end-tidal oxygen level became non-significant ($p=0.12$).

Although there seemed to be a trend for more of the differences within matched sets to be below zero for the end-tidal oxygen level following delivery of 100% $N_2$ (figure 7.2.1, C), there was no significant difference between the groups ($p=0.81$) (figure 7.2.2).

Figure 7.2.1 Differences in end-tidal oxygen levels within matched sets
A presents the differences in end-tidal oxygen level when 40% O\textsubscript{2} delivered, B presents the differences in the change in end-tidal oxygen level and C presents the difference in end-tidal oxygen level when 100% N\textsubscript{2} delivered.

Each data point is the average difference between the infant in the smoking group and the infant(s) in the nonsmoking group.

Dotted line is zero.
As expected, the end-tidal oxygen level was related to the inspired oxygen level, however there was considerable scatter suggesting that the inspired oxygen level was not the only determinant of the end-tidal level (figure 7.2.3). The end-tidal oxygen level could vary during the test period even when the inspired oxygen level was fairly constant (figure 7.2.4). Although there was a slight difference in the inspired oxygen levels for breaths A and B (40.3% and 39.5% respectively), a much greater difference was seen between the end-tidal oxygen levels (21.3% and 25.3% respectively)(figure 7.2.4). This seems to be related to tidal volume since breaths A and C have similar tidal volumes and end-tidal oxygen levels. The tidal volume for breath B was considerably larger resulting in a higher end-tidal oxygen level. Therefore, when tidal volume is altered in response to alternation in inspired gases the nature of the response may affect the end-tidal oxygen levels. Consequently, it is possible that the higher end-tidal oxygen levels seen in the smoking group when 40% $O_2$ was delivered may be related to the response. To investigate whether this was the case the end-tidal oxygen level for the first breath of
Figure 7.2.3 Relationship between the inspired and end-tidal oxygen level

A presents relationship when 40% O₂ delivered and B presents relationship when 100% N₂ delivered. Each data point is the result for one test.
Figure 7.2.4 Example of an alternating breath test where the response contributes to the variability in end-tidal oxygen level

For explanation of figure see text.

the test (40% O₂) was compared for the two groups. This breath was chosen because it occurs before the onset of the ventilatory response (section III, chapter 9.1). Four infants did not have acceptable data for the first breath of the test and therefore values were calculated for 36 infants (14 smoking group). When the differences in end-tidal oxygen within matched sets were calculated, as described previously, there was a trend for more of the differences to be greater than zero (figure 7.2.5). However, when these were analysed statistically taking into account the number of tests within individuals and matching the average difference between the groups was 0.71% (standard error 0.55%), and was non-significant (p=0.202). These results suggest that the mean end-tidal oxygen level for all the second breaths of 40% O₂ during the test was higher in the smoking group because of differences between the groups when the ventilatory response occurs.

Changes in end-tidal carbon dioxide level during the alternating breath test

The absolute end-tidal carbon dioxide levels during the test were not calculated as it was not possible to correct the mass spectrometer readings. However, changes in the end-tidal carbon dioxide level occurring during the test period compared to baseline levels were calculated. The mean change in the end-tidal carbon dioxide level was small and
similar in both groups, with a mean (SD) of -0.10 (0.17)% in the nonsmoking and -0.14 (0.15)% in the smoking groups respectively.

**Figure 7.2.5** Differences in the end-tidal oxygen level for the first breath of the test in each matched set

Each data point is the difference between the infant in the smoking group and infant(s) in the nonsmoking group in each matched set. Four matched sets are excluded because of unacceptable data for first breath.

Dotted line represents zero.

### 7.3 Errors in the measurement of the inspired and end-tidal oxygen levels

Even though the highest level of O<sub>2</sub> delivered to the infant was only 40%, in some tests the calculated inspired oxygen level was greater than this (figure 7.1.1), with 40.9% being the highest value measured. Clearly this is not possible and the most likely explanation for these findings was that the gases measured during the tests were not corrected appropriately. Four hypothetical reasons that might explain these findings were investigated.
(i) Time between run used for correction and test run
The six tests with the highest inspired oxygen levels were used to investigate whether the time between the test run and the calibration check could account for the high levels. For 4 of these tests the difference in time ranged between 124 and 383 minutes. The test with the largest time difference also had the highest inspired oxygen level. However, for two of these tests the time difference was only 2 and 11 minutes. Therefore, whilst it is possible that the time between the test run and the calibration check may affect the accuracy of the correction it cannot account for all of the elevated values.

(ii) Sampling error when reading-off the values for the 40% O₂, 0% O₂ and calibration gas used for correction
When obtaining values for the three gases from the recording used for correction one point was chosen from the stable part of the trace for each run of the three gases. Therefore, if the traces were noisy this may have led to sampling error leading to inaccurate correction. An alternative approach would have been to measure more points for each gas and take the mean. The impact of this was assessed by analysing 2 tests with high inspired oxygen levels taking an average of 10 points for one gas run, and comparing this to taking one point. The corrections were applied to the inspired gases and in both tests the high inspired oxygen level actually increased when the 10 point calibration method was used. In one subject the high inspired oxygen level rose from 40.4% to 40.6%, and in the other it rose from 40.8% to 41.1%. Therefore, taking more points for correction would be unlikely to have prevented the high inspired oxygen levels seen.

(iii) Sampling error when measuring the inspired and end-tidal O₂ levels
When the inspired gases were measured from the traces the high oxygen measurement was always taken at the highest point on the trace and the low oxygen measurement at the lowest point on the trace. However, if the signals were noisy it is conceivable that this may have led to errors in measurement.
(iv) **Sampling lines of the mass spectrometer did not give identical values**

The mass spectrometer has two sampling lines. For all of the studies the two sampling lines were identical in length, bore of the tubing and had a nasal prong inserted in them. At the beginning of the night the mass spectrometer was fully calibrated using one of the lines. This line was then used for measuring the inspired and end-tidal oxygen levels from the infant. It was not practical or desirable to remove the nasal probe from the infant in order to collect gases to check the accuracy of the calibration throughout the night so the second line was used for this purpose. In a study conducted within the sleep laboratory I found that even when identical sampling lines were used that there were slight differences in the measured gas concentrations. The mass spectrometer was calibrated using one of the sampling lines (A) and then four gases, 0% O\(_2\) (100%N\(_2\)), 14.7% O\(_2\), air and 40% O\(_2\), were sampled through both lines. The percentages of oxygen measured by the mass spectrometer are shown in table 7.3.1.

<table>
<thead>
<tr>
<th></th>
<th>0% Oxygen</th>
<th>14.7% Oxygen</th>
<th>Air</th>
<th>40% Oxygen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Line (A)</td>
<td>0.0</td>
<td>14.4</td>
<td>20.7</td>
<td>39.7</td>
</tr>
<tr>
<td>Line (B)</td>
<td>0.6</td>
<td>14.7</td>
<td>20.8</td>
<td>39.2</td>
</tr>
</tbody>
</table>

Each value is an average of 10 measurements.

The sampling line used for calibration (Line (A)) gave slightly more accurate results than line (B). During the night studies the line used for calibration was used for the measurement of the inspired and end-tidal O\(_2\) levels from the infant. The other line (line (B)) was used for the correction. If the results from line (B) are used to correct line (A), in this example, then instead of the 40% O\(_2\) reading 39.7% it would actually be corrected to 40.5%. Imbalances between the mass spectrometer lines may lead to discrepancies of a similar magnitude to those seen in the inspired gases following delivery of 40% O\(_2\).
Chapter 8

Results: Comparison of ventilatory responses to changes in inspired oxygen in infants born to smoking and nonsmoking mothers

8.1 Breathing parameters during baseline air breathing period

Baseline frequency of breathing, inspiratory time, expiratory time and duty time were similar in the two groups during the air breathing period (table 8.1.1). When these were analysed statistically taking into account the matching the average differences between the groups was small and there were no statistically significant differences (table 8.1.1). Absolute measurements of volume-related parameters were not available as RIP was used in a semi-quantitative manner.

Table 8.1.1 Comparison of respiratory parameters during baseline air breathing period in the two groups

<table>
<thead>
<tr>
<th></th>
<th>Nonsmoking Mean (SD)</th>
<th>Smoking Mean (SD)</th>
<th>Average difference Mean (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inspiratory time (secs)</td>
<td>0.81 (0.14)</td>
<td>0.78 (0.18)</td>
<td>-0.021 (0.04), p=0.57</td>
</tr>
<tr>
<td>Expiratory time (secs)</td>
<td>1.50 (0.32)</td>
<td>1.40 (0.31)</td>
<td>-0.002 (0.10), p=0.98</td>
</tr>
<tr>
<td>Duty time</td>
<td>0.35 (0.05)</td>
<td>0.35 (0.05)</td>
<td>-0.005 (0.02), p=0.77</td>
</tr>
<tr>
<td>Frequency (breaths/min)</td>
<td>27.3 (4.7)</td>
<td>28.3 (5.3)</td>
<td>0.435 (1.45), p=0.76</td>
</tr>
</tbody>
</table>

The mean of all tests in each subject was calculated. The mean (SD) of the subject means in each group was calculated and presented in table. Average difference (SEM) is from statistical analysis taking into account the matching.
8.2 Comparison of alternations in respiratory parameters during the test period in the two groups

There was considerable variation in the respiratory response between subjects. Two extremes of response in two subjects are shown in figures 8.2.1 and 8.2.2. Figure 8.2.1 presents a recording resulting in one of the largest responses measured in the study. Changes in tidal volume can be seen clearly in response to alternations in the inspired and end-tidal gases and the % alternation in $V_{TT}$ was 72%. Figure 8.2.2 shows a trace from an infant with one of the smallest responses. Here changes in tidal volume in response to the alternations in inspired oxygen cannot be seen clearly and the % alternation in $V_{TT}$ was only 3%.

Figure 8.2.1 Excerpt of trace for test with one of the largest responses (subject 157 test 4)
Figure 8.2.2  Excerpt of trace for test with one of the smallest responses (subject 138 test 1)

There was also considerable within subject variability in response. Figures 8.2.3 and 8.2.4 present the results for the alternation in mean tidal volume for every test in the nonsmoking and smoking groups respectively. It can be seen that in some subjects repeated measurements are quite reproducible (e.g. subject 54) whereas in others reproducibility was poor (e.g. subject 128). Alternation in inspiratory drive showed a similar pattern of variability (figures 8.2.5 and 8.2.6). The mean percentage alternation for all ten respiratory parameters for each subject are presented in appendix 3.
Figure 8.2.3 Alternation in mean tidal volume (%) for every test in each infant in the nonsmoking group

Each data point is the result for one test. Each subject has a different symbol.
Figure 8.2.4 Alternation in mean tidal volume (%) for every test in each infant in the smoking group.
Figure 8.2.5 Alternation in inspiratory drive (%) for every test in each infant in the nonsmoking group

Each data point is the result for a test. Each subject has a different symbol.
Figure 8.2.6 Alternation in inspiratory drive (%) for every test in each infant in the smoking group

Each data point is the result for a test. Each subject has a different symbol.
The smoking group had a tendency for slightly lower respiratory responses compared to the nonsmoking group (figure 8.2.7). For example, the mean alternation in inspiratory drive in the smoking and nonsmoking groups was 23.72% and 28.78%. However, there was considerable overlap between the responses of the two groups as shown by the error bars. In both groups the volume-related variables ($V_{TI}$, $V_{TE}$, $V_{TT}$, $V_{TI}/t_i$, $V_{TE}/t_E$, $V_E$) showed the greatest response, whereas the response in timing variables ($t_i$, $t_E$, $f_R$, $t_i/t_{tot}$) was small.

Figure 8.2.7 Comparison of respiratory responses in the two groups

Each bar represents the mean ($\pm 2$SEM) adjusted for the number of tests within individuals.

However, these data do not take account of the individual matching. The differences between the infant in the smoking group and infant(s) in the nonsmoking group were calculated as described previously. There was no clear pattern in the differences within each matched set for either of the ten respiratory parameters measured, suggesting that there was no systematic effect of smoking on response (figures 8.2.8 to 8.2.17).
Each data point is the difference between infant in the smoking and infant(s) in nonsmoking group in each matched set.
Figure 8.2.10 Difference within each matched set for alternation in $V_{TT}$ (%)

Each data point is the difference between infant in the smoking and infant(s) in nonsmoking group in each matched set.

Figure 8.2.11 Difference within each matched set for alternation in $t_{i}$ (%)
Figure 8.2.12 Difference within each matched set for alternation in $t_E$ (%)

Each data point is the difference between infant in the smoking and infant(s) in nonsmoking group in each matched set.

Figure 8.2.13 Difference within each matched set for alternation in $f_R$ (%)
Figure 8.2.14 Difference within each matched set for alternation in $V_{T_{I}/t_{I}}$ (%) 

Each data point is the difference between infant in the smoking and infant(s) in nonsmoking group in each matched set.

Figure 8.2.15 Difference within each matched set for alternation in $V_{T_{E}/t_{E}}$ (%)
Figure 8.2.16 Difference within each matched set for alternation in $t_{t/40k}$ (%)

Each data point is the difference between infant in the smoking and infant(s) in nonsmoking group in each matched set.

Figure 8.2.17 Difference within each matched set for alternation in $V_E$ (%)
Statistical comparison of the two groups taking into account the matching found small differences between the groups which were not statistically significant (figure 8.2.18). For example, the average difference in alternation of inspiratory drive was only -2.83% (SEM 5.39%) (p=0.59). Taking into account confounding factors such as the inspired levels of oxygen, baseline breathing parameters and weight and length at time of testing did not affect the outcome. Therefore, there was no evidence of an effect of smoking on respiratory responses in the group as a whole.

**Figure 8.2.18** Differences between the smoking and nonsmoking groups for the ten respiratory parameters from the matched analysis

Each bar represents the mean ± 1 SEM.

In addition, there was no evidence of a dose-dependent effect of antenatal or postnatal nicotine exposure on respiratory responses. There was no relationship between the difference in inspiratory drive within matched sets and the urinary cotinine/creatinine levels in the mother at 20 weeks gestation (figure 8.2.19), or in the infant at the time of testing (figure 8.2.20).
Figure 8.2.19 Relationship between the difference in alternation in inspiratory drive within matched sets and the difference in maternal urinary cotinine/creatinine level

Figure 8.2.20 Relationship between the difference in alternation in inspiratory drive within matched sets and the difference in infant urinary cotinine/creatinine level at testing
Chapter 9

Further investigation of factors that affect respiratory responses measured using the alternating breath test

The previous chapters in this section have investigated the independent effect of smoking on the respiratory responses measured by the alternating breath test. The aim of this chapter is to investigate other factors which may influence the response measured.

9.1 Lag in the respiratory response

Ventilation does not change instantaneously in response to changes in alveolar oxygen level. This lag in response may be considered to be the result of (i) the time taken for the change in alveolar oxygen level to be transmitted to the carotid body (lung-carotid transit time), and (ii) the time taken for the carotid body to elicit a change in ventilation (reflex latency). It has been shown by Ward et al.\textsuperscript{169} that once the information arrives at the carotid body the ventilatory response is almost instantaneous, so that the principal contributor to the overall lag is the lung-carotid transit time. This was estimated in adults by using ear oximetry during an alternating oxygen stimulus, and was found to be an average of 4.7 seconds\textsuperscript{169}. Similar results were found in two infants on whom I was able to collect beat-to-beat oxygen saturation on the ear lobe during an alternating breath test. Figure 9.1.1 shows a recording of RIP tidal volume, oxygen saturation and end-tidal oxygen level during an alternating breath test in subject 197. It can clearly be seen that each series of two breaths of 100% N\textsubscript{2} is followed by a dip in oxygen saturation a short while later. If the time between the beginning of inspiration of the second breath of 100% N\textsubscript{2} and the nadir in oxygen saturation following this is taken as the estimated
Figure 9.1.1 Example of recording of alternating breath test with beat-to-beat oximetry on ear lobe (subject 197, test 5)
lung-carotid transit time then in this example the average time was 5.1 seconds. In the other subject (189) on whom beat-to-beat oxygen saturation was collected the average time of 4.1 seconds was slightly shorter. Although these data are limited they do suggest that the lung-carotid transit time is short and likely to be similar to that found in adults. In addition, in the example shown (figure 9.1.1) it can be seen that breaths with larger tidal volumes occur at the time the maximal dips in oxygen saturation occur. These breaths are likely to be a response to the fall in end-tidal oxygen level immediately beforehand, and suggest that the reflex latency is very short, which supports the findings of Ward et al in adults. Alvaro et al have shown that preterm infants hypoventilate within 3 breaths following inhalation of 100% O2. They first saw hypoventilation at an average of 3.6 seconds after the beginning of inhalation of 100% O2; a maximal response was seen at 6.8 seconds. Therefore, it would appear that ventilatory changes in response to changes in alveolar oxygen levels are likely to occur within around 5 seconds in the 3 month old infant. However, if the lag between a change in alveolar oxygen level and its corresponding change in ventilation were around 5 seconds the number of breaths this would correspond to would depend upon respiratory frequency. In an infant breathing at a respiratory rate of 20-25 breaths per minute the maximal response would be likely to be seen 2 breaths later, whereas an infant breathing at a frequency of 30-35 breaths per minute would be likely to have a maximal ventilatory response 3 breaths later.

When performing the alternating breath test a four breath pattern is delivered (2 breaths 40% O2 and 2 breaths 100% N2), and the ventilatory responses on the second breaths of the 40% O2 and 100% N2 gases (breaths 2 and 4) are compared. It is assumed that these are the response breaths related to maximal stimulation (second breaths of 40% O2 and 100% N2). However, if an infant had a lag in response such that maximal response occurred on breaths 1 and 3 the response could be underestimated. Figures 9.1.2 and 9.1.3 show examples of recordings from subjects who appear to have different lags in response that affect the measured response. If one assumes that breaths with the largest tidal volume correspond to the lowest end-tidal oxygen level a few breaths before, and similarly breaths with the smallest tidal volumes correspond to the highest end-tidal oxygen levels then in the first example (figure 9.1.2) it can be seen that breath A is most likely to be the consequence of the low end-tidal oxygen level 2 breaths before (1). In addition, the high end-tidal oxygen level (2) results in a breath with a reduced tidal volume 2 breaths later (B). In this example there appears to be a 2 breath lag between
the change in alveolar oxygen level and the ventilatory response. Therefore, although there is a time lag between stimulus and response this does not cause any problems with the analysis because these response breaths occur on the second breaths of the high and low gas (A1 and A2), which are the analysed breaths.

Figure 9.1.2. Extract of a recording from subject 194 test number 5

![Graph](image)

For explanation of figure see text.

However, if the lag between the stimulus and response were three breaths this could lead to an underestimation in the response. Figure 9.1.3 presents an example where the response appears to occur 3 breaths after the stimulus. In this example the low end-tidal oxygen of breath 1 is most likely to correspond to the increased tidal volume of breath A which is 3 breaths along. Likewise the reduced tidal volume of breath B is likely to be the consequence of the high end-tidal of breath 2. However, these response breaths do not occur on the second breaths of the high and low gases which are analysed (A1 and A2), but on the subsequent (unanalysed) breaths. This means that the alternation between breaths A1 and A2 would be calculated, rather than the correct alternation between breaths A and B. This would lead to an underestimation of the response.
Therefore, conceivably differences in the lag of the response both within and between subjects could lead to increased variability in response. In addition, although unlikely, if there were a systematic difference in lag between the smoking and nonsmoking groups, such that the response of the nonsmoking group was underestimated, this would have affected the ability of the study to find a difference in response between the two groups. Since inspiratory drive was the primary outcome measure of the study the lag in response of inspiratory drive was estimated and the respiratory response recalculated taking this into account.

9.1.1 Estimation of the lag in inspiratory drive

Inspiratory drive and end-tidal oxygen levels were measured for every breath in each alternating breath test. For each test the end-tidal oxygen level for a given breath was correlated to the inspiratory drive for the same breath, and to inspiratory drive values of each of the following 6 breaths. Inspiratory drive and oxygen level should be inversely related to each other, such that when oxygen level falls inspiratory drive should increase. Therefore, the breath number at which the best negative correlation coefficient occurred was taken as the estimated lag, so that if the peak correlation occurred 2 breaths along a lag of 2 breaths was used. A similar analysis has been used by Ward et al to estimate the lag in adults. Figure 9.1.1.1 shows an example of the cross-correlation analysis in one subject (125) for the recording shown in figure 9.1.3. It can be seen that the best
negative correlation occurred when a 3 breath lag was used, and therefore this was taken as the estimated lag for this subject and test.

**Figure 9.1.1.1 Example of cross-correlation analysis in subject 125 test number 4**

![Correlation Coefficient vs. Number of Breaths](image)

**9.1.2 Recalculation of the alternation in inspiratory drive**

The end-tidal oxygen levels for each breath were lined up with the appropriate inspiratory drive values according to the lag calculated. Once this was achieved the mean percentage alternation in inspiratory drive comparing the second breaths of the 40% $\text{O}_2$ and 100% $\text{N}_2$ were calculated as described previously (Section III, chapter 4.4.1).

**9.1.3 Impact of the lag in response on results of individual infants**

All of the tests were found to have a lag of either 2 or 3 breaths using the cross-correlation analysis. However, a greater proportion of the 127 tests were found to have a 3 breath (78), rather than a 2 breath (49) lag. When the % alternations in inspiratory drive were recalculated adjusting for the estimated lag in response this made a considerable difference to the response in some individuals, for example the % alternation for the data shown in figure 9.1.3 increased from 10.3% to 50.8%. Figures
9.1.3.1 and 9.1.3.2 present the difference between the alternation in inspiratory drive adjusting for the lag and the unadjusted value for all tests in the nonsmoking and smoking groups respectively. It can be seen that in the majority of subjects and tests adjusting for the lag increased the alternation in inspiratory drive by as much as 56.5% (subject 119, smoking group). Adjusting for the lag reduced the variability in response in some subjects. For example in subject 128 (smoking group), who had the most variable response, before adjustment the range of values measured was -1.22% to 47.04% (figures 8.2.5 and 8.3.6, chapter 8), and after adjustment this was reduced slightly (19.3% to 47.3%) (figure 9.1.3.4). Therefore, even after adjustment there was still considerable intrasubject variation (figures 9.1.3.3 and 9.1.3.4).

9.1.4 Influence of lag on comparison between smoking and nonsmoking groups
Since adjusting for the lag in individual subjects did appear to affect the measured ventilatory response it was possible that this may also have affected the comparison of the smoking and nonsmoking groups. However, the relative proportion of tests with a 3-breath lag was similar in the two groups with 61% and 62% in the smoking and nonsmoking groups respectively. The remaining tests had a 2-breath lag. The overall mean alternation in inspiratory drive was higher in both groups when adjusted for the lag, but was increased by a similar amount in both the smoking and nonsmoking groups (figure 9.1.4.1). The average difference in adjusted inspiratory drive in the matched analysis was -1.35% (p=0.77), compared to -2.83% when unadjusted. Therefore, the lag did not affect the outcome of the comparison between the two groups.
Figure 9.1.3.1 Difference between alternation in inspiratory drive adjusted and unadjusted for the lag in response for the nonsmoking group

Each data point is the result for a test, each subject has a different symbol
Figure 9.1.3.2 Difference between alternation in inspiratory drive adjusted and unadjusted for the lag in response for the smoking group.
Figure 9.1.3.3 Adjusted alternation in inspiratory drive for the nonsmoking group

- Each data point is the result for one test, each subject has a different symbol.
Figure 9.1.3.4 Adjusted alternation in inspiratory drive for the smoking group

Each data point is the result for one test, each subject has a different symbol.
Each bar represents the mean ±SEM weighted for amount of data in each subject, and adjusted for matching.

### 9.1.5 Relationship between lag in response and other factors

#### Baseline frequency of breathing

The tests with a 3-breath lag tended to have a higher frequency of breathing during the air breathing baseline period when compared to tests with a 2-breath lag, although there was some overlap (figure 9.1.5.1). The mean (SEM) baseline frequency of breathing for tests with a 2-breath lag was 24.3 (0.6) breaths per minute compared to 30.4 (0.6) breaths per minute for tests with a 3-breath lag. This difference was highly significant (p<0.001) when tested using an unpaired t-test.
Figure 9.1.5.1  Relationship between the lag in response and baseline frequency of breathing during air breathing

End-tidal oxygen levels

The mean end-tidal oxygen level when 40% O₂ was delivered was significantly higher in the tests with a 2-breath lag compared to those with a 3-breath lag (table 9.1.5.1). The mean change in end-tidal oxygen level throughout the test was also significantly higher in the tests with a 2-breath lag. Although the end-tidal oxygen levels when 100% N₂ was delivered were slightly higher in the tests with a 2-breath lag this was not significant.

Table 9.1.5.1  Mean (SEM) end-tidal oxygen levels in tests with 2 or 3-breath lags in response

<table>
<thead>
<tr>
<th></th>
<th>2-breath lag</th>
<th>3-breath lag</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>End-tidal oxygen level when 40% O₂ delivered (%)</td>
<td>22.0 (0.2)</td>
<td>20.9 (0.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>End-tidal oxygen level when 100% N₂ delivered (%)</td>
<td>10.3 (0.2)</td>
<td>9.9 (0.2)</td>
<td>0.21</td>
</tr>
<tr>
<td>Change in end-tidal oxygen (%)</td>
<td>11.8 (0.3)</td>
<td>11.0 (0.2)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

* difference between means tested using unpaired t-test. Factors such as number of tests per subject, smoking etc. were not taken into account.
9.2 Relationship between alternation in inspiratory drive and end-tidal oxygen levels

Alternations in inspiratory drive were significantly correlated with the end-tidal oxygen levels following delivery of 40% O₂ in both groups, although there was considerable scatter leading to poor correlation coefficients (figure 9.2.1). There was no significant difference in the slopes of the regression lines for the two groups, but the intercept was significantly higher in the smoking group (p=0.002). However, there was no relationship between the % alternation in inspiratory drive and the end-tidal oxygen level when 100% N₂ was delivered in either group (figure 9.2.2). In addition, whilst there was a significant relationship between alternation in inspiratory drive and change in end-tidal oxygen level in the smoking group, no such relationship was seen in the nonsmoking group (figure 9.2.3).

Figure 9.2.1 Relationship between alternation in inspiratory drive and end-tidal oxygen level when 40% O₂ delivered

Each data point is the result for one test.
Figure 9.2.2 Relationship between alternation in inspiratory drive and end-tidal oxygen level when 100% N₂ delivered

- nonsmoking
- smoking

Figure 9.2.3 Relationship between alternation in inspiratory drive and change in end-tidal oxygen level

- nonsmoking  \( r=0.19, p=0.10 \)
- smoking    \( r=0.39, p=0.003 \)

Each data point is the result for one test.
9.3 Relationship between alternation in inspiratory drive and baseline breathing parameters

The ventilatory response was significantly related to the frequency of breathing during the air breathing baseline period, such that the higher the frequency the lower the response (figure 9.3.1, A). However, breathing frequency is related to the lag, such that at higher frequencies the lag is more likely to be three breaths (figure 9.1.5.1). This may mean that responses at the higher frequencies are underestimated leading to a relationship between response and frequency. Indeed, the relationship between alternation in inspiratory drive and frequency becomes non-significant in the smoking group when the response is adjusted for lag, but remains significant in the nonsmoking group (figure 9.3.1, B).
Figure 9.3.1 Relationship between baseline breathing frequency and unadjusted and adjusted alternation in inspiratory drive

Each point is the result for one test. A presents alternation in unadjusted inspiratory drive. B presents alternation in adjusted inspiratory drive.
Other aspects of the baseline breathing pattern, inspiratory and expiratory time, were also related to the measured ventilatory response. As inspiratory and expiratory times increased so did the alternation in inspiratory drive (figures 9.3.2 and 9.3.3). However, this is not surprising since both inspiratory and expiratory times are related to respiratory frequency. When the alternation in inspiratory drive was adjusted for the lag the relationships between inspiratory and expiratory times and response were weaker, suggesting that these relationships were mainly a consequence of underestimation of responses at higher breathing frequencies.

9.4 Relationship between age and length of infant at the time of testing and alternation in inspiratory drive

Increasing postnatal age at the time of testing was associated with an increase in alternation in inspiratory drive in both groups (figure 9.4.1, A). This relationship was significant in the nonsmoking group ($r=0.42$, $p=0.04$), but was non-significant in the smoking group ($r=0.35$, $p=0.16$). However, one of the infants in the smoking group was a clear outlier and when these data were omitted from the regression analysis the relationship was significant ($r=0.58$, $p=0.02$) (figure 9.4.1, B). There was also a trend for the alternation in inspiratory drive to increase with postconceptional age but this did not reach statistical significance in either group.

There was a tendency for the alternation in inspiratory drive to increase as the length of the infant at the time of testing increased (figure 9.4.2). However, this relationship did not reach statistical significance in the smoking group. There was no such relationship between response and weight at the time of testing (data not shown).
Figure 9.3.2. Relationship between baseline inspiratory time and unadjusted and adjusted alternation in inspiratory drive

A

Baseline inspiratory time (secs)

- nonsmoking  $r=0.64$, $p<0.001$
- smoking  $r=0.44$, $p=0.001$

B

Baseline inspiratory time (secs)

- nonsmoking  $r=0.39$, $p=0.001$
- smoking  $r=0.20$, $p=0.14$

Each point is the result for one test. A presents alternation in unadjusted inspiratory drive. B presents alternation in adjusted inspiratory drive.
Figure 9.3.3  Relationship between baseline expiratory time and unadjusted and adjusted alternation in inspiratory drive

Each point is the result for one test. A presents alternation in unadjusted inspiratory drive. B presents alternation in adjusted inspiratory drive.
Figure 9.4.1 Relationship between alternation in inspiratory drive and postnatal age at the time of testing.

A

B

Each data point is the mean of the tests in one subject. A shows all data including the outlier, B shows the regression analysis excluding the outlier.
Figure 9.4.2 Relationship between length of the infants at time of testing and alternation in inspiratory drive

Each data point is the mean of all tests in one subject.
SECTION IV

DISCUSSION AND FUTURE DIRECTIONS
1.1 Study Design

Women who smoke are more likely to come from the lower social classes, are younger and more likely to bottle feed their infants than nonsmoking women. Additionally, infants born to women who smoke during pregnancy are more likely to be born earlier in gestation and be of lighter birthweights \(^{171,172}\). It is conceivable that any of these confounding factors of smoking may affect respiratory control independently. Consequently, if a study were to find a difference in ventilatory response between groups this could be attributable to a confounding factor of smoking, or smoking per se. Alternatively, these confounding factors may mask any effect of smoking. For example, one study that has investigated respiratory responses to hypoxia in infants born to smoking and nonsmoking women did not make any attempt to control confounding factors \(^{17}\). As a result the two groups were significantly different in terms of maternal age, gestational age, birthweight, race and postnatal age at testing. The differences in these factors between the groups may have masked any effect of maternal smoking on respiratory responses to hypoxia. In particular, we have found that increasing postnatal age results in greater respiratory responses \(^{76}\) (section III, figure 9.4.1). Since the smoking group in the study by Lewis and Bosque were studied at an older age (10.4 weeks) compared to the nonsmoking group (8.7 weeks) this may have minimised any difference in respiratory control between the groups \(^{17}\). The aim of the present study was to investigate the independent effect of maternal smoking on respiratory control in infants. To do this potential confounding factors must be controlled and this may be achieved in two ways: (i) perform a large study, perhaps involving many hundreds of infants, and control for confounding factors retrospectively in the statistical analysis, or (ii) perform a smaller study matching the two groups for the confounding factors. The former approach has been used in studies investigating the effect of maternal smoking on infant lung function \(^{173-175}\). However, it would have been impractical to study large numbers of infants if each required an overnight study. Therefore, the latter approach was chosen to enable investigation of the independent effect of maternal smoking while restricting the number of infants to a more manageable size. The disadvantage of this approach is that it is not possible to investigate the effect of each of the confounding factors on respiratory control. Each infant born to a smoking woman was matched on an individual basis to an infant born to a nonsmoking woman for seven factors; social class, gestational age, birthweight, gender of the infant, maternal age, maternal parity and
feeding. This matched design is a major strength of the study. An arbitrarily weighted matching system was implemented so that small differences in two or three of the matching factors would be accepted but that large differences in any would not be tolerated. Close matching on seven factors was not always possible to achieve, and consequently data from seven infants were excluded because an acceptable match with data could not be found. Matches were deemed acceptable if they scored 5 points or less, except on one occasion when a match scoring 6 points (match 17, appendix 1) was accepted as it was felt that it was better to accept a slightly weaker match than exclude valuable infant data. This matched pair was not different from the other matches in terms of the effect of smoking upon respiratory control or the end-tidal oxygen levels.

1.2 Recruitment and subjects studied

1.2.1 Recruitment and eligibility

Power calculations based upon previous work within the Department of Child Health suggested that data would be required on a total of 60 infants to have 91% power of detecting a 10% difference between the groups. Previous experience using the alternating breath test suggested that a success rate of 75% could be expected for obtaining the technically acceptable data required. Consequently, we aimed to recruit 240 women so that 80 infants could be studied. It was envisaged that this over-recruitment would allow for matching, withdrawal and women/infants becoming ineligible.

A total of 199 women (97 smokers) were successfully recruited onto the study. However, during the period between recruitment and studying the infants the drop-out rate in the smoking group (77%) was almost twice that in the nonsmoking group (47%). The greatest number of women became ineligible during the antenatal period with 36% and 22% of the smoking and nonsmoking groups respectively being excluded from the study. The single most important factor for exclusion from the study was postnatal voluntary withdrawal (27% and 12% in the smoking and nonsmoking groups respectively). Therefore, from the 199 women originally recruited 22 from the smoking and 54 from the nonsmoking groups remained eligible and willing to take part. Recruitment and retainment of individuals was difficult and time consuming, particularly in the smoking group. This is reflected by the fact that in order to study 10 infants from
the smoking group in the laboratory it was calculated that the research nurse needed to approach 429 women in the antenatal clinic, whereas only 55 needed to be approached in the nonsmoking group.

Other studies investigating the effect of maternal smoking on respiratory control and lung function have also found that a large proportion of women withdraw/ become ineligible after recruitment. Lewis and Bosque recruited women antenatally for their study investigating the effect of maternal smoking on respiratory control in infants by sending advertisements to pregnant women in San Francisco. A total of 126 women (56 smokers) responded to the advertisement and 73 (29 smokers) were recruited onto the study. During the period between recruitment and studying the infants a considerably greater proportion of the smoking group (54%) became ineligible or withdrew from the study when compared to the nonsmoking group (15%). Although the withdrawal/exclusion rates are lower than in the present study this is not surprising since these women responded positively to an advertisement, and therefore are probably less likely to withdraw from the study. It should also be appreciated that the measurements on infants were performed during a daytime nap, whereas in the present study the measurements required an overnight stay in hospital. This may have contributed to the greater rate of exclusion/withdrawal in the present study. However, these problems of retaining recruited women/infants following antenatal recruitment are not confined to studies investigating respiratory control in infants. Hanrahan et al have investigated the effect of maternal smoking on infant lung function. Following recruitment in the antenatal clinic during the early part of pregnancy, 77% of the group became ineligible or withdrew before testing of the infants could take place (62% in present study). It is not possible to say whether there was a greater withdrawal/exclusion of those in the smoking group as the individual values for each group are not reported.

It could be argued that if women had been recruited postnatally then the time spent recruiting women who would subsequently become ineligible or withdraw from the study could have been minimised. However, it was imperative in this study that reliable information concerning smoking and nicotine exposure of the infant during and after pregnancy was obtained. Maternal self-reports of smoking habits during pregnancy may be unreliable with one study showing that as many as 22% of women who had cotinine levels equivalent to smokers denied active smoking and the amount of smoking was
often under reported 177. Furthermore, maternal cotinine levels during pregnancy are better related to birthweight and birth length than reported smoking habits 178. Therefore, it was decided that in order to get the most accurate information about smoking habits during pregnancy antenatal recruitment would be undertaken and an objective measurement of nicotine exposure (urinary cotinine/creatinine) obtained rather than rely upon retrospective information. Only two women were excluded from the current study on the basis of their maternal urinary/cotinine levels. Both denied smoking despite having cotinine/creatinine levels well within the range of the smoking group. Antenatal recruitment also allowed the health and progress of the woman’s pregnancy to be monitored and the research nurse was able to develop a relationship with the women. This relationship was undoubtedly important, and resulted in our being able to study more infants than we would otherwise have been able to do.

1.2.2 Characteristics of the population recruited and population included in the final analysis

The fact that such a large proportion of the recruited groups became ineligible or withdrew, particularly in the smoking group, was a potential concern since this may introduce bias into the studied groups. For example, if women from poorer social classes were more likely to withdraw then this could have resulted in a more affluent population being studied and any effects of smoking being minimised. However, when the social class distribution, maternal age, parity, gestational age, gender of the infant, birthweight and mode of feeding for the recruited and studied smoking and nonsmoking groups were compared there were no significant differences (Section III, table 5.4.1). Furthermore, the reported number of cigarettes smoked during pregnancy and the maternal urinary cotinine/creatinine levels were similar in the recruited and studied groups. Therefore, the populations studied were representative of the populations recruited for these factors.

However, there were some differences between the smoking and nonsmoking groups recruited. There was a clear difference in the social class distribution in the two groups with the nonsmoking group containing a significantly greater proportion of social class I and II women, and the smoking group having a preponderance of lower social classes (IV and V). The women in the recruited smoking group were also significantly younger
than those in the nonsmoking group and significantly more likely to bottle feed their infants. The infants born to smoking women were significantly lighter than those born to nonsmoking women. The difference between the birthweights (250 g) was similar to that found in other studies\(^\text{172}\). The fact that these factors were significantly different between the two recruited groups supports the use of a matched design. The matched design was successful with differences in social class, maternal age and feeding being reduced so that these factors were no longer statistically significant between the groups. However, within the scoring system for matching a difference in birthweight of 499 g was allowed before any points were scored. Therefore, matching did not diminish the difference in birthweight between the infants in the two studied groups.

1.3 **Inspired and end-tidal oxygen levels**

1.3.1 **Errors in the measurement of the inspired and end-tidal oxygen levels**

The mass spectrometer was calibrated at the beginning of the night, but was not usually recalibrated during the period of the study as this was not practical. Consequently, the calibration sometimes drifted during the night. Therefore, so that the validity of the calibration could be checked and any drift corrected, recordings of 40% O\(_2\), 0% O\(_2\) (100% N\(_2\) ) and a calibration gas (14.7% O\(_2\) ) were generally collected before and after each alternating breath test. Recordings that were closest in time to an alternating breath test were used for correction of the inspired and end-tidal gases. However, following correction eight tests gave inspired oxygen levels greater than 40% when 40% O\(_2\) was delivered; the highest level seen was 40.9%. Clearly, this is not possible and reasons why this might have occurred were investigated. These included the time between the correction recording and alternating breath test, sampling error when measuring the gas concentrations and disagreement between the readings of the two lines of the mass spectrometer. The most likely reason for the apparently raised inspired gases after correction was small imbalances between the two sample lines available on the mass spectrometer. The mass spectrometer was calibrated at the beginning of the study using the line that would be used for the measurements on the infant (line A). During the period of the study the other line (line B) was used for recording the three gases used for correction. When the measurements of these two lines were compared following delivery of three known gases there were slight differences, particularly with 40% O\(_2\).
When the results from line B were used to correct the measurements of line A this had the effect of increasing the level of oxygen measured by a similar magnitude to the discrepancies seen in the high inspired gases in the infants (Section III, chapter 7.3).

Whilst there may be some small errors in the inspired and end-tidal oxygen levels measured these do not invalidate the comparison of the smoking and nonsmoking groups. Both groups were treated in exactly the same way and the corrections were done in an identical manner.

1.3.2 Comparison of the inspired and end-tidal oxygen levels in the smoking and nonsmoking groups

When comparing the respiratory responses of two groups of infants to changes in inspired oxygen levels it is important to demonstrate that the two groups were treated in the same way. This was particularly so in this study as the investigators were not blinded to the smoking status of the mother when the infant was studied. Therefore, it is conceivable that the smoking group may have subconsciously been treated differently to the nonsmoking group. For example, the facemask may have been held further away from the face for infants in the smoking group reducing the levels of oxygen inspired. The end-tidal oxygen levels reflect the arterial oxygen concentration which drives the change in respiratory response and can be thought of as the stimulus. It is therefore important to show that these levels are the same in both groups.

The mean inspired oxygen levels when either 40% \( \text{O}_2 \) or 100% \( \text{N}_2 \) was delivered were not significantly different between the two groups. This shows that the two groups were treated in an identical way. However, surprisingly the end-tidal oxygen level when 40% \( \text{O}_2 \) was inspired was significantly higher in the smoking group. In addition, the change in end-tidal oxygen from the second breath of the 40% \( \text{O}_2 \) to the second breath of 100% \( \text{N}_2 \) was also significantly higher in the smoking group. There were no differences in end-tidal oxygen between the groups when 100% \( \text{N}_2 \) was inspired. In other words, even though the inspired oxygen levels were similar, differences in the end-tidal oxygen levels occurred between the groups with the smoking group receiving a larger stimulus.

Other studies that have investigated the effect of maturation\(^{66,67}\), bronchopulmonary dysplasia\(^{137}\) or chronic lung disease\(^ {132}\) on respiratory responses to changes in inspired
oxygen in infants have not measured the inspired and end-tidal oxygen levels. Therefore, it is difficult to conclude that differences seen between the groups in respiratory responses are not the result of the groups receiving different stimuli. The measurement of end-tidal and inspired oxygen levels is an advantage of this study.

1.3.3 Factors that may contribute to the difference in end-tidal oxygen level between the two groups

Approximately 50% of the alternating breath tests attempted did not continue for a minimum of 14 breaths and were thus excluded from the analysis. The main reason for this was that the infant sighed and the test was terminated. A greater proportion of tests were excluded from the analysis for this reason in the nonsmoking group (36%) than the smoking group (28%). It could be argued that the reason we found that infants in the smoking group had higher end-tidal oxygen levels when 40% O\textsubscript{2} was delivered was that the tests with the highest end-tidal oxygen levels in the nonsmoking group were not long enough and were excluded. This would imply that (i) there is a relationship between high end-tidal oxygen levels and increasing tidal volume, and (ii) the two groups respond to these high end-tidal oxygen levels in different ways. This seems unlikely since (i) the increased tidal volume seen with a sigh is more likely to be stimulated by a low end-tidal oxygen level and these were not different between the two groups, and (ii) this study has not found any difference in the ventilatory responses of the two groups. Therefore, exclusion of tests because of a sigh in the nonsmoking group does not explain the difference in end-tidal oxygen levels between the groups.

The end-tidal oxygen levels are not purely determined by the levels of inspired oxygen there are several factors involved, including alveolar ventilation. This represents the amount of fresh inspired air that reaches the alveoli and is available for gas exchange. It is determined by the relative relationship between the tidal volume (\(V_T\)) and anatomic deadspace. The resulting end-tidal (alveolar) oxygen level will depend upon the concentration of oxygen in the inspired gas, the relationship between the alveolar ventilation and resting lung volume (FRC), and the concentration of oxygen in the gas already in the lung. Clearly, if there were systematic differences in \(V_T\), FRC or anatomic deadspace between the two groups this could affect the end-tidal oxygen levels. Hanrahan \textit{et al} have reported that infants born to mothers who smoke have a similar
FRC but reduced $V_T$ in comparison to those born to nonsmoking women\textsuperscript{173}. If this were the case in the groups in the present study one would expect that if anatomic deadspace were the same in the two groups the end-tidal oxygen levels would not change as much in the smoking group. However, this was not the case. In addition, although the mean end-tidal oxygen level following inspiration of 40\% $O_2$ was higher during the alternating breath test in the smoking group, it was not significantly higher for the first breath of the test. These findings suggest that this higher end-tidal oxygen in the smoking group is related to the nature of the ongoing response rather than any systematic difference between the groups before the onset of a ventilatory response. During the test period when $V_T$ changes the relative relationships will change and the end-tidal oxygen levels will be affected. Consequently, the end-tidal oxygen levels may also be viewed as a measure of response (Section III, figure 7.2.4). The alternating nature of the test means that the lag in response may affect the end-tidal oxygen levels. For example, when 100\% $N_2$ is inspired for two breaths this will result in two breaths with an increased $V_T$. If an infant has a 2-breath lag the second response breath will occur on the second breath when 40\% $O_2$ is being delivered (Section III, figure 9.1.2). Alternatively, if an infant has a 3-breath lag the second response breath would occur on the first breath when 100\% $N_2$ is delivered, and a smaller breath would occur on the second breath of 40\% $O_2$ (Section III, figure 9.1.3). Therefore, if the end-tidal oxygen level for the second breath of 40\% $O_2$ was measured it would be higher for the test with the 2-breath lag. Tests with a 2-breath lag had a significantly higher end-tidal oxygen level when 40\% $O_2$ was inspired when compared to tests with a 3-breath lag (Section III, table 9.1.5.1). However, there was no systematic difference in the estimated lag between the two groups, and therefore it is unlikely that this explains the differences observed in end-tidal oxygen in the two groups.

The fact that the end-tidal $O_2$ level was higher in the smoking group when 40\% $O_2$ was inspired suggests that alveolar ventilation when the hyperoxic gas was being delivered was greater in the smoking group, this may be in response to previous delivery of the hypoxic gas. If alveolar ventilation were the same in both groups for the subsequent delivery of hypoxic gas (100\% $N_2$) one would expect that the end-tidal $O_2$ when 100\% $N_2$ was inspired would also be higher in the smoking group. However, this was not the case, the end-tidal $O_2$ level when 100\% $N_2$ was inspired was similar in the two groups suggesting that alveolar ventilation was also greater in the smoking group when the
hypoxic gas was delivered. If it is assumed that FRC and anatomic deadspace remain constant throughout the test then this could be achieved by changes in $V_T$. If the smoking group had a greater $V_T$ on the hyperoxic and hypoxic breaths compared to the nonsmoking group this could explain these findings.

Other factors that may affect the end-tidal oxygen levels include gas transfer across the lung, ventilation/perfusion relationships, gas mixing in the lung, lung mechanics and metabolic rate. It was not possible to investigate the effect of these factors on the end-tidal oxygen levels in this study.

### 1.4 Measurement of ventilatory responses to oxygen

#### 1.4.1 Confounding factors of the ventilatory responses measured

When investigating the effect of smoking on ventilatory responses it is necessary to establish whether other factors may affect this comparison. If there were a systematic difference in a confounding factor between the two groups ventilatory responses may be different between the groups simply because of this, rather than as a result of smoking.

#### 1.4.1.1 Lag in response

When the alternating breath test is performed a four breath pattern of inspired levels of oxygen is delivered (2 breaths of 40% $O_2$ followed by 2 breaths of 100% $N_2$), and breaths 2 and 4 are analysed. It is assumed that the responses related to the highest end-tidal level (second breath 40% $O_2$) and the lowest end-tidal level (second breath 100% $N_2$) occur on these breaths. However, if the maximal response breaths were to fall on breaths 1 and 3, rather than on breaths 2 and 4, this could lead to the response being underestimated. Where the response breaths fall will be dependent upon the lag in response. If an infant had a 2-breath lag then the maximal response breaths would occur on breaths 2 and 4, however a 3-breath lag would result in maximal response breaths occurring on breaths 1 and 3 (Section III, figures 9.1.2 and 9.1.3). Consequently, the lag in response may be an important confounding factor.

The impact of the lag on measurements of alternation in inspiratory drive was investigated by using a cross-correlation technique to calculate the lag, and applying this delay to recalculate the response. Ward et al used a similar technique to estimate the lag.
in adults. In their study a lag was accepted if there was a clear peak in the correlation coefficient associated with this breath delay, and it was significantly different to the values when using a lag of one fewer, or one greater breath. If this had been applied in the present study then tests on infants with small responses would have been excluded from the analysis leading to a bias in the results. Consequently, following on from the basic analysis which assumes a 2-breath lag, in the present study the breath delay with the highest correlation coefficient was taken as an estimate of the lag, regardless of whether there was a clear peak, or whether it was significantly different to those on either side. It was felt that this was justifiable as all breaths would be of similar size and duration in an infant who did not respond well, and therefore the lag would be of little consequence. Using this technique I have found that all tests had either a 2 or 3-breath lag. However, there was a much greater proportion of tests with a 3-breath lag (overall 61.4%, 61% in smoking and 62% in nonsmoking groups respectively), suggesting that the response in a large proportion of the tests may have been underestimated. The mean alternation in inspiratory drive when adjusted for the lag was considerably higher in some individuals. For example, the largest difference seen was in subject 119 where adjusting for the lag in response increased the alternation in inspiratory drive from 25.2% to 81.7%. This suggests that the measured response was underestimated in some individuals as a result of maximal response breaths falling on unanalysed breaths.

The impact of the lag was only investigated for inspiratory drive as this was the main outcome variable. It was not appropriate to apply the lag calculated for inspiratory drive to other variables, as it has been shown that different variables may have different lags.

Other studies that have applied the alternating breath test to infants have not attempted to measure the lag in response. However, these studies have used single-breath alternations in gases rather than the two-breath alternations used in the present study. The single-breath alternation technique is probably less susceptible to the difficulties in analysis described above as every breath is included in the analysis. However, if the lag were to change throughout the test this may lead to errors in the analysis.
The lag consists of the time taken for the stimulus to be transported from the lung to the carotid body (lung-carotid transit time), and the time taken for the carotid body to elicit a ventilatory response (reflex latency). The lag in terms of time rather than number of breaths can be estimated from the mean frequency of breathing for the tests with a 2-breath (24.3 breaths/min) and 3-breath (30.4 breaths/min) lag. The estimated time lag for the tests with the 2-breath lag was 4.9 seconds and for the 3-breath lag 5.9 seconds. This time lag is similar to that found in studies on adult subjects \(^{169,179}\) and preterm infants \(^{170}\). Similar results were found when the lag was estimated using beat-to-beat oxygen saturation on two infants in the present study (Section III, chapter 9.1). The major constituent of the delay is the lung-carotid body transit time \(^{169}\), and the timing of its arrival can affect the ventilatory response. Arrival of an excitatory stimulus at the carotid body during early inspiration has little or no effect upon the inspired volume of the breath, but does reduce the duration of inspiration in cats \(^{180}\). However, arrival during late inspiration increases the inspired volume and duration of inspiration \(^{180,181}\). In contrast, expiratory arrival prolongs the duration of expiration \(^{180,181}\). Differences in timing of arrival of stimuli at the carotid body also affects ventilatory responses in man \(^{169}\). Arrival during late inspiration increases the inspiratory activity (inspiratory flow and volume) of that breath, and increases the expiratory activity (shortened expiratory time) of the following expiration. This pattern of response is described as 'in-phase' since increased inspiratory and expiratory activity occur in the same breath. In contrast, expiratory arrival is associated with an 'out-of-phase' response where increased expiratory activity occurs on one breath followed by increased inspiratory activity on the following breath. Therefore, the lag may affect the timing of arrival of the stimulus at the carotid body leading to different patterns of response.

1.4.1.2 Baseline breathing parameters

The size of the response in volume-related parameters was inversely related to the baseline frequency of breathing (Section III, figure 9.3.1) suggesting that infants with a higher frequency of breathing have lower ventilatory responses. In anaesthetised cats it has been shown that the output of the peripheral chemoreceptors is related to the frequency of the stimulus \(^{182}\). Alternations in inspired oxygen with different cycle durations (2, 4 and 8 seconds) were given, and the output of the carotid sinus nerve measured. The largest output was seen with the 8 second cycle, and the smallest with
the 2 second cycle. This may contribute to the apparent reduction in response with increasing frequency of breathing.

Alternatively, the baseline frequency of breathing was related to the lag, such that infants with a 3-breath lag tended to have a higher respiratory rate when compared to those with a 2-breath lag. Therefore, it may be that the relationship seen between baseline breathing parameters and response may be a consequence of underestimation of responses in infants with a higher frequency of breathing. Indeed, following adjustment of inspiratory drive for the lag the relationship between response and frequency became non-significant in the smoking group, but remained significant in the nonsmoking group (Section III, figure 9.3.1).

1.4.1.3 Postnatal age

The alternation in volume-related parameters increased with postnatal age in both groups and was unrelated to changes in end-tidal oxygen levels with age. Although younger infants tended to have a higher frequency of breathing, when inspiratory drive was adjusted for the lag the relationship between response and postnatal age remained. One infant in the smoking group clearly had a lower response than expected for their age (Section III, figure 9.4.1). Although this infant’s mother reported smoking more cigarettes (30 per day) than the other women in the group, neither the maternal nor the infant urinary cotinine/creatinine levels were higher than those of the rest of the group.

These findings of an increase in respiratory response with age are supported by recent observations. Respiratory responses were measured in the infant’s homes using the alternating breath test when they were aged between 37 and 128 days. There was a clear increase in alternation of volume-related parameters with age.
1.4.2 Comparison of the ventilatory response of the smoking and nonsmoking groups

Clearly, it was not possible to control the lag in response. The adjustment of inspiratory drive for the lag made a considerable difference to the measured response in some individuals. However, the proportion of tests with a 3-breath lag was similar in the two groups (61% and 62% in the smoking and nonsmoking groups respectively). Furthermore, adjustment of inspiratory drive for the lag led to a similar increase in response in the two groups (Section III, figure 9.1.4.1). Therefore, differences in lag did not affect the comparison of alternation in inspiratory drive in the two groups. The lag was not calculated for the other variables, therefore a systematic difference in the lag of other variables between groups cannot be ruled out. Since there was no difference in the lag of inspiratory drive between the groups it seems unlikely that there would be differences in other variables, therefore the comparison of the two groups would be unaffected.

Baseline breathing parameters clearly could not be controlled in the design of the study. Therefore, these were adjusted for in the statistical analysis of the matched data.

As far as possible postnatal age was controlled in the design of the study, since every effort was made to study matched infants in the two groups at similar postnatal ages. However, this was not always possible as tests were sometimes postponed because the parents were unable to attend on any particular evening. The postnatal ages of the two groups studied were similar and the average difference in postnatal ages within each match was -0.3 weeks (smoker-nonsmoker).

There were no differences in the respiratory responses of the ten parameters between the two groups. The average difference due to smoking was small in all variables with a difference of only -2.83% in inspiratory drive. After adjustment of inspiratory drive for the lag in response the average adjusted difference due to smoking was slightly lower (-1.35%). Therefore, this carefully controlled matched study has failed to find any differences in respiratory responses between the two groups and, at least for inspiratory drive, the comparison was unaffected by the lag in response.
Lewis and Bosque also found that infants born to smoking and nonsmoking mothers had similar respiratory responses to hypoxia\textsuperscript{17}. In this study infants were supplied 17\%, 15\% and 13\% O\textsubscript{2} for five minutes and the change in alveolar ventilation was measured. However, although the results of the present study agree with those found by Lewis and Bosque fundamental differences exist between the two studies. The main difference is that Lewis and Bosque did not attempt to match the two groups, and consequently the two groups were significantly different in terms of maternal age, gestational age, birthweight and possibly socioeconomic status (information not provided). In addition, infants of different racial groups were studied leading to a much greater representation of black infants (46\%) in the smoking group than in the nonsmoking group (3\%). At the time of the study the infants in the smoking group were significantly older than those in the nonsmoking group. Therefore, any effect of smoking in this study may have been masked by one or all of these confounding factors.

Animal studies have yielded contradictory results. Bamford et al have shown that rat pups exposed to nicotine \textit{in utero} do not have different ventilatory responses to hypoxia ($F_{1}O_{2} = 0.1$ and 0.15) when studied at four postnatal ages (3, 8, 18 and 34 days)\textsuperscript{14}. However, in the newborn rat exposure to nicotine reduces ventilatory responses to hyperoxia\textsuperscript{12}. In the newborn lamb both prenatal\textsuperscript{15} and postnatal\textsuperscript{16} nicotine exposure leads to an attenuation in the respiratory response to hypoxia.

Although it was not an aim of this study to investigate the relationship between maternal smoking and SIDS, the results would suggest that maternal smoking itself is not related to deficits in respiratory responses to changes in inspired oxygen. If respiratory control in response to changes in oxygen were important in SIDS it may be that this is mediated by other factors related to smoking such as maternal nutrition, birthweight, social class etc. Alternatively it may be that the link between maternal smoking and SIDS cannot be explained by respiratory responses to changes in oxygen, and therefore deficits in this mechanism may not be involved in the pathway leading to SIDS. However, it should be appreciated that infants at particularly high risk of SIDS (e.g. those born prematurely, those with intrauterine growth retardation etc.) were not involved in this study, and an effect of smoking on respiratory responses in these infants cannot be ruled out.
1.5 Possible limitations of the study

One potential concern was that the study may not have had enough power to detect a difference in respiratory response between the two groups. The total number of infants included in this study was 40 (17 smokers, 23 nonsmokers). Power calculations based upon previous work using the alternating breath test\textsuperscript{139} suggested that 30 infants in each group would be required to have 91% power of detecting a difference of 10% in alternation of inspiratory drive. As a result of difficulties in recruitment and obtaining acceptable data the number of infants included in the final analysis was fewer than desired. However, in the present study an average difference in inspiratory drive of -2.83% (standard error 5.39) was found, which is smaller than the 10% in the original calculations. In order to show that such a difference is statistically significant, data on approximately 247 pairs of infants would be required. Therefore, the size of any difference between the groups would be small and unlikely to be of physiological significance.

In addition, the ventilatory responses were variable both within and between subjects using this test. This lack of reproducibility may have restricted the ability of the study to find any differences. Bouferrache \textit{et al} using single-breath alternations of air and 15% O\textsubscript{2} found that when two tests were performed during daytime quiet sleep in infants the respiratory responses were reproducible\textsuperscript{138}. It is possible that the lag in response contributes to the variability in the present study. However, even when inspiratory drive was adjusted for the lag considerable intra and intersubject variability remained (Section III, figures 9.1.3.3 and 9.1.3.4). Differences in inspired oxygen levels, baseline breathing parameters, and size of the infant at testing may contribute to this variability, and were taken into account in the statistical analysis. During the period of the night there are considerable changes in rectal temperature, falling soon after the onset of sleep, reaching a nadir within around two hours after the onset of sleep, and then rising steadily until waking\textsuperscript{183-186}. The extent to which body temperature falls overnight is dependent upon the age of the infant\textsuperscript{184}, and it has been shown that temperature can change from 37.8 to 36.3°C during the night\textsuperscript{186}. Furthermore, there are more frequent smaller oscillations in temperature of about 0.2°C in amplitude that are likely to be related to sleep state, such that temperature rises during active sleep and falls during quiet sleep\textsuperscript{186}. Changes in temperature may affect respiratory control\textsuperscript{187} and, therefore, it is possible that variability
in temperature may be a contributory factor to the variability in respiratory response. This was not investigated in the present study as 50% of parents declined measurement of rectal temperature in their infants. Future studies should attempt to investigate which factors contribute to the variability in response.

The group of smokers studied reported light to medium smoking habits with 47.1% (n=8) smoking between 1 and 9 cigarettes per day, 47.1% (n=8) smoking between 11 and 19 cigarettes per day, and one woman smoked 30 per day. The median was 10 per day. Similar reported maternal smoking habits have been seen in smoking groups involved in studies investigating the effect of smoking on respiratory control or infant lung function. It could be argued that the reason a difference in respiratory control between the groups was not found was because a large proportion of the smoking group were light smokers (1-9 cigs per day), and that any affect may only become apparent at higher levels of exposure. However, there was no evidence of an increased effect on respiratory responses with (i) increasing numbers of cigarettes the mother smoked pre or postnatally, (ii) the number of cigarettes smoked in the home, or (iii) maternal and infant cotinine/creatinine levels. An effect of greater nicotine exposures than encountered in this study cannot be ruled out. Future studies should endeavour to recruit women who smoke more heavily so that this can be investigated. This would not be an easy task since such women often live in more deprived circumstances, and are less likely to volunteer their children to take part in research. Furthermore, even if it were possible to recruit these women they may be more likely to become ineligible for medical reasons or withdraw. Therefore, studies such as these are limited by the fact that the most exposed group are not amenable to study.

This study failed to find an independent effect of smoking on respiratory control in infants. However, this does not mean that no differences exist between unselected infants born to smokers and nonsmokers. In an unmatched uncontrolled study factors such as social class would have been different between the two groups which could have led to differences in respiratory control. One limitation of this study is that infants who were born preterm, or who were otherwise at increased risk of SIDS, were excluded. Our aim was to investigate the effect of smoking in those infants born at appropriate gestation and studied at a defined age. An effect of smoking in other populations of infants cannot be ruled out.
Although the matched design is the main strength of this study one potential criticism may be that the smoking effect may be underestimated by matching birthweight. If the pathway by which respiratory control and birthweight were affected by smoking was the same then the effect of smoking may be underestimated by controlling for birthweight. Whilst this may be true it was felt necessary to control for differences in birthweight which might otherwise exaggerate any effect of smoking. The matching system therefore allowed small differences in birthweight (up to 499g without a score) but would not allow differences of more than 999g.

The finding that the high end-tidal oxygen level was significantly higher in the smoking group may, as discussed earlier, suggest that differences in alveolar ventilation in response to alternation in inspired oxygen may have occurred between the two groups. It may be that alveolar ventilation is greater in the smoking group when either the hyperoxic and hypoxic gases are delivered. If it is assumed that FRC and deadspace are constant then this must be achieved by increasing $V_T$. Therefore, if this were the case why wasn’t a difference in respiratory response between the two groups seen with the alternating breath test? When the alternating breath test is analysed % changes in respiratory parameters from the second breath of 40% to the second breath of 100% N$_2$ are calculated. This is necessary because respiratory inductive plethysmography is calibrated semi-quantitatively, and consequently absolute values of volume-related parameters cannot be measured. Whilst this is a good technique for measuring breath-to-breath changes in response with alternation in inspired gases it is possible to obtain the same % alternation in two different infants when the tidal volumes are very different. For example, if in one infant the tidal volumes measured when the second breath of the high and low gases were delivered were 50mL and 30mL, and in a second infant they were 60mL and 36mL respectively, the % alternation in both examples would be 50%. However, the tidal volumes in the second infant are 20% higher than in the first infant. Therefore, if something similar were to occur in the two groups of infants this could explain the differences in end-tidal oxygen levels occurring without differences in respiratory response.

Alternatively, it may be that alveolar ventilation is a more accurate measure of response than measuring changes in ventilation by body surface measurements. However, Lewis and Bosque did not find any differences in alveolar ventilation in response to hypoxia in
infants of smoking and nonsmoking mothers, but the limitations of their study mean that this question remains open.

Finally, in the present study, infants were studied on one occasion over the narrow age range at which SIDS is most prevalent. The two groups appeared to have similar relationships between age and responsiveness over the age range of this study. Whilst it may be that differences exist between the two groups outside the age range studied it was not an aim of the present study to investigate this.

1.6 Conclusions
This matched study has failed to find any evidence of an independent effect of maternal smoking on ventilatory responses, and does not support the hypothesis that infants born to mothers who smoke have reduced respiratory responses to changes in inspired oxygen in infants. However, differences in the end-tidal oxygen levels were found between the two groups. The smoking group had a significantly higher end-tidal oxygen level when the second breath of 40% O$_2$ was inspired compared to the nonsmoking group. This finding was unrelated to the actual inspired levels of oxygen, and appeared to be associated with the ongoing response.

1.7 Future Directions
The findings that the smoking group had a significantly greater end-tidal oxygen level when 40% O$_2$ was inspired warrant further investigation. In the present study this appeared to be related to the ongoing nature of the response, and may suggest differences in response between the groups. Interpretation of the end-tidal oxygen levels and respiratory responses are complicated by the lag in response during the alternating breath test. Therefore, my future work will apply single-breaths of inspired levels of oxygen to infants born to smoking and nonsmoking women. The inspired levels of oxygen will range between 0% O$_2$ (100% N$_2$) and 100% O$_2$. The response breath will be identified as the first breath to have a $V_T$ more than 2 standard deviations away from the baseline level. Inspired, end-tidal O$_2$ and CO$_2$ levels and beat-to-beat oxygen saturation on the ear-lobe will also be measured simultaneously. Respiratory inductance plethysmography will be used to monitor ventilation and a pneumotachograph will be used to measure absolute volumes. This study will enable the following:
1) Dose-response relationships between ventilation and end-tidal oxygen level for the single-breaths will be obtained for the two groups. This is not possible with the alternating breath test, and will provide important information.

2) It will also be possible to measure $V_T$ from the PNT recordings and ascertain whether the smoking group have greater tidal volumes during the response compared to the nonsmoking group which may explain the findings of the present study.

3) Measurements of ventilatory response using RIP, PNT or alveolar ventilation (calculated from end-tidal CO$_2$ levels) will be compared.

4) The lung-carotid transit time and reflex latency for the hypoxic breaths will be estimated using the beat-to-beat oxygen saturation measurements. This will give greater understanding of the nature of this lag in infants and its effect upon respiratory responses.

5) The simultaneous measurement of end-tidal oxygen and oxygen saturation with a range of inspired oxygen levels will allow oxygen dissociation curves to be produced for the two groups. If they are the same this will suggest that gas transfer and foetal haemoglobin levels are similar in the two groups. The usefulness of beat-to-beat oxygen saturation as a measure of stimulus will also be determined, which may be important if studies are to be performed in a location other than where a mass spectrometer is available.
APPENDICES
### APPENDIX 1 - Details of the 17 matched sets

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APPENDIX 2 Mean value for each subject for inspired and end-tidal oxygen levels

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Each value is the mean of all the tests in a particular subject.
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Each value is the mean of all the tests in a particular subject, $V_{TI}/t_1$ adj is the inspiratory drive adjusted for the lag in response.
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