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Antenatal substance misuse and smoking and newborn hypoxic challenge response

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ABSTRACT

Objectives: Infants of smoking (S) and substance misusing (SM) mothers have an increased risk of sudden infant death syndrome. The aim of this study was to test the hypothesis that infants of SM or S mothers compared to infants of non substance misusing, non-smoking mothers (controls) would have a poorer ventilatory response to hypoxia, which was particularly marked in the SM infants.

Design: Physiological study.

Setting: Tertiary perinatal centre.

Patients: 21 SM; 21 S and 19 control infants. Infants were assessed before maternity/neonatal unit discharge.

Interventions: Maternal and infant urine samples were tested for cotinine, cannabinoids, opiates, amphetamines, methadone, cocaine and benzodiazepines.

Main out come measures: During quiet sleep, the infants were switched from breathing room air to 15% oxygen and changes in minute volume were assessed.

Results: The SM infants had a greater mean increase ($p=0.028$, $p= 0.034$ respectively) and a greater magnitude of decline ($p<0.001$, $p=0.018$ respectively) in minute volume than the S infants and the controls. The rate of decline in minute volume was greater in the SM infants ($p=0.008$) and the S infants ($p=0.011$) compared to the controls.

Conclusions: Both antenatal substance misuse and smoking affect the infant's ventilatory response to a hypoxic challenge.

INTRODUCTION

Substance misuse during pregnancy is common. A survey carried out by the Office for National Statistics showed that approximately one in a thousand women in Great Britain were dependent on opioids, the majority were of child bearing age. Anonymous screening of urine samples from women attending antenatal clinics in south London, demonstrated approximately 16% of the 807 mothers screened were taking at least one illicit substance.[1] In utero exposure to illicit drugs such as opioids, marijuana and cocaine is associated with an increased risk of sudden infant death syndrome (SIDS).[2] In a population based study, a seven fold increased risk of SIDS was reported amongst infants of substance using mothers.[2]

Globally, there has been a significant decline in maternal smoking during pregnancy. Across the UK, the percentage of mothers smoking before or during pregnancy fell from 33 percent to 26 percent between 2005 and 2010. A smaller percentage of all mothers smoked throughout pregnancy in 2010 (12 percent compared to 17 percent in 2005).[3] Nevertheless, maternal smoking in pregnancy remains an important problem, as evidenced in a population based study by the percentage of mothers of SIDS victims who smoked during pregnancy that increased from 57% in the 1980s to 86% in 2003.[4] Furthermore, since the 'back to sleep' campaign, cigarette smoke exposure in utero is the leading independent risk factor for the occurrence of SIDS.[5] The increased risk of SIDS of infants whose mother smoked during pregnancy has been reported to between two to four fold but, if combined with other risk factors, as high as six fold.[5-7] Over 60 studies have examined the relation

between maternal smoking during pregnancy and risk of SIDS. The pooled relative risk associated with maternal smoking was RR=2.86 (95% CI=2.77, 2.95).[8]

A possible explanation for the increased SIDS risk is that infants of SM or S mothers may have abnormalities of respiratory control. There is a body of evidence demonstrating nicotine exposure could and does influence respiratory control in animal models. For example, prenatal exposure to nicotine may alter the function of peripheral chemoreceptors, as in three-day old rabbit pups exposure to nicotine was associated with reduced dopamine levels and increased expression of tyrosine hydroxylase in the carotid bodies.[9] The ventilatory responses to hypoxia has been demonstrated to be attenuated by infusion of nicotine to 7, 17 and 27 day old lambs.[10] Furthermore, nicotine infusion to pregnant rats throughout gestation resulted in the pups having suppressed noradrenergic neuronal activity in the brainstem and forebrain and hyperresponsiveness to hypoxia.[11] In addition, intermittent hypoxia was demonstrated to result in transient delay in neuronal migration early in the postpartum period in Spragne-Dawley rats, amplified by concurrent nicotine administration.[12] Data from in vivo studies have highlighted that in utero exposure to substance misuse may also affect respiratory control. Infants prenatally exposed to cocaine were demonstrated to have a higher incidence of cardiorespiratory pattern abnormalities than infants with no prenatal drug exposure.[13] In another study, full term infants prenatally exposed to cocaine and opiates had less periodic breathing.[14] In addition, comparison of infants of substance misusing mothers to controls demonstrated the former group were

relatively insensitive to a carbon dioxide challenge.[15] Even apparently asymptomatic neonates with a maternal history of cocaine use may have degenerative changes or focal infarctions in their basal ganglia.[16] We, therefore, hypothesised that infants of mothers who smoked and/or substance misused during pregnancy would have a poor ventilatory response to hypoxia. The aim of this study was to test that hypothesis by comparing the ventilatory responses to hypoxia of infants of substance misusing mothers, infants of smoking mothers and infants of non-substance misusing, non-smoking mothers. In addition, we tested a further hypothesis that any change in the ventilatory response to hypoxia would be greater in newborns of mothers who substance misused, as such mothers usually also smoke, compared to those whose mothers had only smoked.

METHODS

Infants were eligible for entry into the study if they were born at 36 weeks of gestational age or greater at King's College Hospital NHS Foundation Trust and had no congenital abnormalities. Informed written parental consent was obtained and the study was approved by the Guy's and St Thomas's Hospitals NHS Foundation Trust Research Ethics Committee.

Three groups were recruited:

1. Infants of mothers who gave on antenatal screening a history of substance misuse during pregnancy (SM infants).
2. Infants of mothers who gave on antenatal screening a history of smoking during pregnancy (S infants).

3. Infants of mothers who neither smoked nor misused substances during pregnancy (controls).

Smoking was defined as any history of daily smoking regardless of the number of cigarettes smoked/day. Substance misuse was defined as use of any of the illicit drugs at any stage during pregnancy; mothers were enrolled from a methadone programme in a dedicated antenatal substance misuse clinic.

Hypoxic challenge

Physiological measurements were carried out when the infants were in quiet sleep. Sleep state was determined by observation of the behavioural state.[17] An infant was defined as being in quiet sleep when their eyes were closed, with no body or eye movement, no vocalization and their respiratory rate was regular. The hypoxic challenge was delivered via a facemask and custom-made open circuit system using 15% oxygen in balanced nitrogen from a cylinder (BOC Gases, UK). The facemask was placed over the infant's mouth and nose, therapeutic putty was put around the rim of the facemask to ensure an airtight seal around the face. Respiratory flow was measured using an appropriately sized pneumotachograph connected to the face mask (Mercury F10L, G M Instruments, Kilwinning, Scotland) with a dead space 0.8 ml and a resistance of 1.4 $cmH_2O/L/second$. The distal end of the pneumotachograph was connected to a two-way non-rebreathing valve contained within the open circuit system. A constant flow of medical air from a cylinder (BOC Gases, UK) was delivered to the inspiratory port of the two-way non-rebreathing valve via length of wide

bore (20mm), low resistance tubing. The pneumotachograph was attached to a differential pressure transducer/amplifier (13-4615-70, Gould, Cleveland OH, USA). Data were acquired and displayed in real time on a PC computer running Spectra software (Grove Medical, London, UK) with 100 Hz analog to digital sampling (PCI-MIO-16XE-50, National Instruments, Austin TX, USA). Respiratory rate, inspiratory time and the time to peak tidal expiratory flow were determined from the flow signal. Tidal volume was determined by digital integration of the flow signal by the acquisition software. Minute ventilation was calculated from the infant's respiratory rate and tidal volume and related to the infant's weight as maternal smoking and substance misuse are known to affect birth weight. Inspired and expired gases were sampled continuously using a small cannula inserted through the facemask and positioned close to the infant's mouth. The side stream method was used to measure carbon dioxide (CO_2) levels using a CO_2 sampling line and capnograph. The maximum $ETCO_2$ levels from the 60 second period immediately before the hypoxia challenge are reported. Oxygen saturation was measured using a pulse oximeter (Masimo rainbow SET Pulse Oximetry).

During quiet breathing, the infants were switched from breathing room air to 15% oxygen in nitrogen (BOC Gases, UK). The final minute of tidal breathing in air was used as the baseline value. The hypoxic challenge was maintained for five minutes, but terminated if the oxygen saturation level fell below 85%. Infants respond to hypoxia with a biphasic response [18] . The responses to the hypoxic challenge were determined by

1) The magnitude of increase in minute ventilation from baseline to the peak ventilation
2) The magnitude of decline in minute volume from the peak to the lowest minute volume
3) The rate of decline in minute volume, calculated as the peak minute volume-lowest minute volume divided by the time from peak to the lowest minute volume.
4) The change in the oxygen saturation level from baseline to the lowest oxygen saturation level.

The time to peak minute ventilation was noted, as was the time to the lowest SaO_2 and the baseline end tidal CO_2.

Assessment of exposure to smoking and substance misuse

In the immediate postpartum period, urine samples were obtained from all mothers and infants to assess the profile of maternal substance misuse and for cotinine analysis. Urine samples were also obtained from the SM mothers during their antenatal out-patient attendance. The urine cotinine results for both maternal and infant urine samples were divided into categories: <10ng/ml, 10-500ng/ml and > 500ng/ml. The methadone doses used during pregnancy were obtained from the medical records for mothers prescribed methadone. Screening was carried out for cannabinoids, opiates, amphetamines, methadone, cocaine and benzodiazepines as previously described.[19]

Analysis

Differences between the three groups for continuous data were assessed for statistical significance using multiple regression where possible, with data transformation as needed to meet normality assumptions, if that was not possible the Kruskal-Wallis analysis of ranks test was used. Proportions were compared between groups using chi-squared test or Fisher's Exact test where the numbers in each group were small. Differences in baseline minute volume, the increase and decrease in minute volume were adjusted for gestational age using multiple regression on the transformed outcomes. Adjusted means and 95% confidence intervals are presented. The increase in minute volume data were highly skewed and so a square root transformation was required to satisfy Normality assumptions.[20] Since back-transformed confidence intervals are non-interpretable for square root-transformed data, p values are reported using the transformed data for rigour, but means and confidence intervals are presented on the natural scale. For data that could not be transformed, post hoc comparisons were conducted using the Mann Whitney-U test (continuous data) or Chi squared test (categorical data). Where regression analysis was able to be used, post hoc comparisons were made using likelihood ratio tests. Analysis was performed using Stata v11.

Sample size

Recruitment of 20 infants in each of the three groups allowed detection of a difference equivalent to one standard deviation in each outcome between the groups with 80% power at the 5% level. A similar magnitude of difference had

been detected in the ventilatory response to added dead space between newborns of smoking and non-smoking mothers.[21]

RESULTS

Twenty-one substance misuse (SM), 21 smoking (S) and 19 control infants were assessed (Table 1). The mean gestational age of the infants varied significantly between the three groups: SM infants were of significantly of lower mean gestational age than the S infants (37.3 vs 38.6; p=0.02) and the controls (37.3 vs 38.8; p=0.007). Similarly mean birth weight varied significantly between groups, the mean birth weight of the SM infants was lower than that of the S infants (2536 vs 3106; p=0.02) and the controls (2536 vs 3291; p=0.003). The urine analysis demonstrated that all the mothers of SM infants were also smoking, but that none of the mothers of the controls were smoking (Table 2). The urine analysis also demonstrated none of the mothers of S infants or the controls had been substance misusing. There were no significant differences in the percentage of mothers or infants with cotinine levels in predefined categories between the S and SM groups (Table 2). Analysis of urine samples during antenatal out-patient attendances confirmed that all SM women were substance misusing and misusing a wide range of substances (Table 3). In the SM group, urine analysis in the immediate post partum period demonstrated five of the 10 women who had been prescribed methadone were also taking it illicitly.

There was significant variability between the groups in mean baseline minute volume, which remained significant after adjustment for gestational age (Table 4). The SM infants had a significantly higher adjusted mean baseline minute

volume than controls (395 vs 348; p=0.005) (Table 4). The initial increase in minute volume during the hypoxic challenge differed significantly between the groups before and after adjustment for gestational age, there was a significantly higher mean increase in the SM group compared to the S group (117 vs 57; p=0.028) and the controls (p=0.034) (Table 4). The magnitude of the subsequent decline in minute volume varied significantly between the groups with a greater magnitude of decline occurring in the SM compared to the controls (220 vs 106; p<0.001) and the S infants (220 vs 151; p=0.018). The mean rate of decline in minute volume also varied significantly between the groups overall and was greater in the SM infants compared to the controls (4.29 vs 160; p=0.008) and the S infants compared to the controls (4.29 vs 3.88; p=0.011) (Figure 1). There were no significant differences in either the mean baseline oxygen saturation or mean lowest saturation level in response to the hypoxic challenge between the groups (Table 5). The mean time to the lowest oxygen saturation, however, was greater in the controls compared to both the infants of S or SM mothers (115, 100, 100 seconds respectively; p=0.017) (Table 5). There were no significant differences in the mean time of peak minute ventilation or the maximum baseline end tidal CO_2 levels between the three groups (Table 5). It was noted, however, that the capnogram results often did not return to zero baseline on inspiration and this may have influenced some of our results.

DISCUSSION

We have demonstrated that infants of SM mothers had a greater magnitude of decline in ventilation than the controls and S infants and both the SM and S

infants had a greater rate of decline in minute ventilation compared to controls. The three groups' gestational ages differed significantly and, as maturity at birth affects respiratory control, we adjusted for gestational age. Similar significant differences were found after that adjustment. Similarly, differences in the initial increase in minute ventilation between the three groups remarked significantly after adjustment for gestational age. To our knowledge, this is the first study that has compared the responses to hypoxia in neonates of substance-misuse/smoking mothers and smoking mothers. Our results suggest in utero exposure to substance abuse and smoking have an additive effect on the response to a hypoxic challenge and may explain their increase risk of SIDS.

The early excitatory phase of the biphasic ventilatory response to hypoxia is the result of peripheral chemoreceptor stimulation. It is mediated through N-methyl-D-aspartate (NMDA) glutamate receptors in the caudal brainstem. Prematurely born infants with neonatal apnoea were shown to have an increased ventilatory response to hypoxia.[22] Reduction in arterial oxygen tension strongly stimulates the carotid bodies which mediate the rapid increase in ventilation. The authors [22], therefore, speculated that previous episodes of apnoea with resultant intermittent hypoxia led to the enhanced peripheral chemoreceptor response to hypoxia. The peripheral chemoreptors are active from at least 28 weeks of gestation.[23] Fetuses exposed to nicotine and other misused substances are exposed to intermittent/chronic hypoxia in utero which may explain the initial increased ventilatory response to hypoxia we document.[24] Our results demonstrating infants of misusing/smoking mothers had a greater initial increase in ventilation are supported by the finding that infants who were chronically exposed in utero to opiates had a significantly greater initial ventilatory increase to hypoxia compared to non-opiate exposed controls.[25]

Exposure to intermittent chronic hypoxia may explain our findings that, compared to controls, there was a greater rate of decline in the ventilatory response to the SM and S infants and a greater magnitude of decline in the SM infants. In a piglet model, the decline was more marked following exposure to chronic intermittent hypoxia until day ten after birth [26] as evidenced by a decline in phrenic electroneurograms (ENGphr). Several neurotransmitters including ∝-aminobutyric acid (GABA) [27], adenosine [28, 29], serotoma [30] and opiods [31] are involved in the late component of the hypoxic ventilator response. Intracisternal injection of bicuculline, a $GABA_A$ antagonist, in piglets aged two to ten days old reversed the effects of recurrent hypoxia on the ENGphr hypoxic response and eliminated apnoea during hypoxia.[26] The authors, therefore, speculated that in the newborn period, GABA is released within the brainstem in direct proportion to the number of episodes of recurrent hypoxia, resulting in activation of $GABA_A$ receptors and worsening of hypoxic respiratory depression.[26] The late phase is mediated in part through platelet derived growth factor (PDGF) foreceptors maturation of the neuromodulators, particularly NMDA and PDGF-β Receptors mediated pathways occurs during the postnatal period.[30]

Our study has strengths and some limitations. We studied infants prior to maternity/neonatal unit discharge and thus were able to assess the effect of only the antenatal exposure to smoking and illicit substances. We assessed substance misuse by analysing samples from the mothers and their infants and cotinine levels were also measured, hence we had objective evidence of substance misuse and antenatal smoking. We were unable to assess the effect

of substance misuse alone as all the mothers from the substance-misuse group smoked. In addition, we were unable to assess the effect of specific drug misuse as the majority of mothers were misusing multiple drugs. We did not find any significant differences between the groups in the lowest saturation level in response to the hypoxic challenge, but this is likely because we terminated the challenge if the oxygen saturation level fell below 85%. The oxygen saturation level did not fall below 85% in all infants, hence we compared the time to the lowest oxygen saturation level in the three groups. The time taken to reach the lowest SaO_2 was significantly longer in the controls compared to either infants in the S or SM groups, likely reflecting the latter two groups had a greater magnitude of decline in ventilation than the controls. We used a sidestream method to measure $ETCO_2$ levels using a CO_2 sampling line and capnograph. The sidestream method does not adequately monitor both nasal and oral airflow. When we examined the capnographic waves, it is often noted that capnogram trace did not return to zero baseline on inspiration which may have influenced some of our results. Given the method we used to measure $ETCO_2$, it was not possible to evaluate the changes in $ETCO_2$ at different periods during the hypoxic challenge. The differences in the maximum baseline $ETCO_2$ levels were not statistically significant but there was a trend for the S infants to have lower levels than the controls and the SM infants lower levels than the S infants. This trend likely reflects the significantly higher baseline minute volumes in the SM infants compared to the controls and the trend for the SM infants to have a higher baseline minute ventilation than the S infants and the S infants to have higher levels compared to the controls. The infants of substance misusing mothers had higher baseline minute ventilation than the other two groups, reflecting they were withdrawing from the substances their mothers had

misused. Nevertheless, we were able to show a significant difference in the response of the SM infants to the hypoxic challenge compared to both the controls and the S infants.

In conclusion, both SM and S infants compared to controls had a greater rate of decline in ventilation as well as a greater increase in ventilation in response to a hypoxic challenge in the newborn period. Our results also highlight that SM infants had a greater magnitude of decline in ventilation than the S infants or the controls suggesting that substance misuse and smoking may have an additive effect.

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Competing interests: None.

Contributor statement: AG and RB designed the study, KA collected the data, KW was responsible for the drug screening, KA, TR and JLP undertook the analysis, all authors were responsible for production of the manuscript.

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What is already known on this topic:

- Infants of substance misusing and/or smoking mothers are at increased risk of SIDS.

What this study adds:

- The ventilatory response to a hypoxic challenge was compared in infants of substance misusing (SM) and smoking (S) mothers and controls.
- The initial increase and magnitude of decline were greater in the SM Infants.
- The rate of decline was greater in the SM and S infants.

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Table 1: Demographics by maternal smoking and substance misuse status

Data are presented as mean (SD) [median; range]

	Controls (n=19)	**Smoking (S) (n=21)**	**Substance misuse (SM) (n=21)**	**Overall p-value**	**Post-hoc tests* p- values where applicable**
Gestational age (wks)	38.8 (1.7) [39; 36-42]	38.6 (1.7) [39; 36-42]	37.3 (1.6) [37; 36-40]	0.01	S versus control: 0.77 SM versus S: 0.02 SM versus control: 0.007
Birth weight (gms)	3291 (657) [3282; 2500-4972]	3106 (759) [2988; 1860-4384]	2536 (487) [2465; 1730-3192]	0.002	S versus control: 0.41 SM versus S: 0.02 SM versus control: 0.003
Head circumference (cms)	34.2 (1.5) [35; 32-37]	34.3 (1.4) [35; 32-36]	33.6 (0.75) [34; 32-35]	0.15	
Maternal age (yrs.)	30.9 (5.1) [31; 23-38]	27.9 (7.8) [29; 18-43]	30.5 (6.2) [30; 20-40]	0.41	
Gender (male)	58% (11)	71% (15)	43% (9)	0.17	
Mode of delivery (SVD)	23% (11)	40% (19)	36% (17)	0.06	
Age at study (days)	2 (1-5)	2(1-9)	2(1-8)	0.35	

***post-hoc tests only undertaken when the overall comparison of the three groups was statistically significant.**

Table 2: Urinary cotinine data by maternal smoking and substance misuse status

Data are expressed as the percentage in each category

	S	SM
Maternal urine cotinine <10ng/ml	0%	0%
10-500ng/ml	40%	23.5%
> 500ng/ml	60%	76.5%
Infant urine cotinine		
<10ng/ml	0%	0%
10-500ng/ml	73%	56%
> 500ng/ml	27%	44%

Table 3: Results of the urine analysis of the substance misuse mothers

	Number of subjects	Mean (SD)	Range
Visits to antenatal clinic and urine collected	20	9.33 (6.8)	1-26
Number of occasions a particular substance was detected			
Methadone	13	6.86 (7.14)	0-26
Illicit drug	20	6.95 (4.97)	1-20
>1 illicit drug	17	3.95 (2.99)	1-19
Ecstasy	6	0.90 (1.29)	0-5
Cocaine	13	2.90 (2.84)	0-19
Cannabis	11	4.24 (5.01)	0-18
Morphine	11	2.19 (2.48)	0-20
Any opioid metabolite	11	3.19 (3.68)	0-25
Dihydrocodeine	5	0.52 (0.80)	0-3
Benzodiazepines	6	0.90 (1.29)	0-6

Table 4: Changes in minute ventilation by maternal smoking and substance misuse status

Data are presented as mean; median (range)

	Unadjusted values mean; median (range)				**Adjusted for gestational age[1] Mean (95% CI)**			
	Control n=19	**Smoking n= 21**	**Substance misuse n=21**	**Over-all P**	**Control n=19**	**Smoking (S) n= 21**	**Substance misuse (SM) n=21**	**Overall P P for pairs of means**
Baseline minute volume (ml/kg/min)	317; 317 (203-405)	354; 344 (253-524)	416; 408 (266-614)	0.003	315 (283, 350)	348 (315, 385)	395 (356, 438)	0.014 S versus control: 0.16 SM versus control: 0.005 SM versus S: 0.10
Increase in minute volume (ml/kg/min)	44; 37 (0-126)	55; 45 (0-163)	121; 89 (0-374)	0.017	47 (13, 81)	57 (25, 89)	117 (83, 150)	0.04 S versus control: >0.99 SM versus control: 0.034 SM versus S: 0.028
Magnitude of decline in minute volume (ml/kg/min)	100; 102 (15-233)	147; 129 (54-243)	230; 225 (56-444)	<0.001	106 (70, 142)	151 (117, 185)	220 (185, 255)	0.0002 S versus control: 0.038 SM versus control: <0.001 SM versus S: 0.018

Rate of decline in minute volume (ml/kg/min)	1.90; 1.70 (0.6-5.0)	7.51;2.58 (0.59-32.17)	7.21 ;4.64 (0.10-31.32)	0.006	1.60 (0.98, 2.60)	3.88 (2.45, 6.12)	4.29 (2.66, 6.92)	0.009 S versus control: 0.011 SM versus control: 0.008 SM versus S: 0.77

[1] Note: adjusted estimates for Increase in minute volume and magnitude of decline in minute volume are calculated from untransformed data as confidence intervals based on square root transformed data cannot be back-transformed. Significance tests are from transformed data

Table 5: Oxygen saturation by maternal smoking and substance-misuse status.

Data are presented as median [range]

	Controls (n=19)	**Smoking (n=21)**	**Substance (n=21)**	**p**
Baseline SpO_2 (%)	99 (96-100)	99 (97-100)	100 (96-100)	0.81
Lowest SpO_2 (%) with hypoxic challenge	88 (85-94)	88 (85-94)	87 (85-96)	0.65
Time to lowest SpO_2 (seconds)	115 (60-300)	100 (50-160)	100 (30-200)	0.017
Change in SpO_2 (%)	10 (5-15)	10 (6-15)	11 (4-15)	0.66
Time to peak minute ventilation (seconds)	94 (10-282)	115 (35-256)	135 (35-286)	0.177
Maximum baseline end tidal CO_2	35 (27-41)	33 (26-42)	31 (27-38)	0.21

FIGURE LEGENDS

Figure 1: Box plot of the magnitude of decline in minute volume by maternal smoking and substance misuse status