Inspired CO₂ and O₂ in sleeping infants rebreathing from bedding: relevance for sudden infant death syndrome

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Inspired CO₂ and O₂ in sleeping infants rebreathing from bedding: relevance for sudden infant death syndrome. J Appl Physiol 91: 2537–2545, 2001.—Some infants sleep facedown for long periods with no ill effects, whereas others become hypoxemic. Rebreathing of expired air has been determined by CO₂ measurement; however, O₂ levels under such conditions have not been determined. To evaluate this and other factors influencing inspired gas concentrations, we studied 21 healthy infants during natural sleep while facedown on soft bedding. We measured gas exchange with the environment and bedding, ventilatory response to rebreathing, and concentrations of inspired CO₂ and O₂. Two important factors influencing inspired gas concentrations were 1) a variable seal between bedding and infants’ faces and 2) gas gradients in the bedding beneath the infants, with O₂-poor and CO₂-rich air nearest to the face, fresher air distal to the face, and larger tidal volumes being associated with fresher inspired air. Minute ventilation increased significantly while rebreathing because of an increase in tidal volume, not frequency. The measured drop in inspired O₂ was significantly greater than the accompanying rise in inspired CO₂. This appears to be due to effects of the respiratory exchange ratio and differential tissue solubilities of CO₂ and O₂ during unsteady conditions.

METHODS

Subjects. Twenty-one healthy infants younger than 6 mo old from the St. Louis community were enrolled between September and November 1999. Subjects were 5–24 wk old. There were 8 males and 13 females. Nineteen of the 21 were born at term. At home, 4 (19%) slept prone, 4 (19%) slept...
supine or on the side, and 13 (62%) slept supine. Seventeen infants (81%) received routine periods of prone positioning while awake and supervised. The Washington University Human Studies Committee approved the study, and informed consent was obtained from the parent(s).

**Direct measurement of O2 and CO2 concentrations.** Infants were studied during natural sleep during a morning or afternoon nap after a feeding. They were placed in a plastic crib. Bedding used in this study consisted of a corrugated foam pad (5) covered by a polyester-filled comforter folded double (thickness of doubled comforter = 3 cm). A shallow depression (12.5 × 12.5 cm at surface, 4.5 cm deep) was cut into the foam mattress directly beneath the infant’s head. This created an environment with high rebreathing potential closely similar to the partially filled air mattress covered by a comforter described by Kemp and Thach (26). Breath-by-breath O2 and CO2 were measured via an 8-Fr silastic catheter with two small holes attached to the upper lip under the nares. The catheter was connected to a laser-diode O2 analyzer (VacuMed 17518C, Ventura, CA) at a low sampling rate (100–150 ml/min; response time < 200 ms) and an infrared photometer CO2 analyzer (Ohmeda 5200, Madison, WI) at a low sampling rate (150 ml/min; response time < 200 ms), with both analyzers arranged in parallel. Before each study, the O2 and CO2 analyzers were calibrated with standard gas mixtures. A separate nasal catheter also positioned in front of the nares was attached to a differential pressure transducer, comparing pressure at the narial opening to room pressure (28). During periods in which there was no change in head position, the resistance to flow produced by the bedding was constant; thus narial pressure was proportional to flow. This relationship has been previously demonstrated to be linear over a range of 0–8 l/min (28), allowing us to measure relative changes in nasal airflow. Flow signal was integrated to give tidal volume (VT). The infant’s heart rate, respiratory rate (Respirtrace, Noninvasive Monitoring Systems, Miami Beach, FL), and O2 saturation (Nellcor, Hayward, CA) were also monitored. All output data were continuously recorded on a polygraph (Beckman R611). Infant and polygraph tracings were recorded with an infrared video camera (Videonica, Campbell, CA) so as to allow correlation between infant behaviors and physiological recordings on analysis at a later date. After the infant fell asleep, baseline data were obtained. He or she was then turned prone, and the head was positioned facedown. Rebreathing was detected by an elevation in the inspired O2 level. To augment the degree of rebreathing, we often applied silk cloths around the face on the theory that these would decrease flow in occult air channels that might be present (28). However, we did not apply silk cloths in all infants, as those who slept facedown for relatively long periods achieved significant rebreathing on their own. In addition, not all infants underwent all protocols for evaluating their ventilatory responses or gas exchange with the environment and bedding due to either time constraints, nap duration, or the availability of the O2 analyzer, which arrived after five infants had been studied (Table 1).

**Evidence for occult air channel formation.** Once the infant was asleep and rebreathing, additional studies were conducted to evaluate his or her air exchange with the bedding and the environment. Studies were done in three infants to detect air channels around the face. Once asleep and rebreathing, the O2 and CO2 analyzers were disconnected from the nose catheter and connected to a free catheter. This catheter was placed at various positions around the head just above the groove where the bedding contacts the infant’s face. Detection of an elevated CO2 level in currents of air in phase with respiration defined an air channel.

**Evidence for gas-gradient formation in bedding.** Before the start of the study, two 21-gauge lumbar puncture needles with blunted tips were inserted into the bedding into the pocket beneath the face. After a period of rebreathing when inspired gas had reached relatively steady-state concentrations, the O2 and CO2 analyzers were disconnected from the nose catheter and connected to the bedding needle. The needle was slowly withdrawn at 0.5-cm intervals until it was out of the bedding. This was repeated as tolerated by the infant. This procedure was done in the 7 of 21 infants who remained asleep and rebreathed for long enough periods for the sampling procedure to be completed.

**Infants’ ventilatory responses to rebreathing.** The change in end-inspiration CO2 that followed placement of cloths was evaluated in 31 episodes in 11 infants by comparing mean end-inspiration CO2 for the 10 breaths that preceded and followed cloth placement. In a different subset of 11 infants, subjects’ responses to rebreathing were evaluated for 31 episodes of rising end-inspiration CO2 and decreasing end-inspiration O2. Episodes of changing inspired gas concentrations were selected, and each breath, including at baseline, was evaluated as the index breath until the gas concentrations reached a relative steady state. In each subject, we measured respiratory frequency (f) and relative VT after the index breath at varying sequential CO2 and O2 levels. We studied the effect of tidal-volume size on inspired-gas (CO2 and O2) concentrations in five facedown infants who were chosen because they slept for relatively long periods with their faces straight down. The relative size of five sequential breaths, including a sigh or large breath, was compared with the inspired CO2 and O2 associated with each breath in each infant. Infants were studied for the duration of the nap, usually < 2 h.

**Effect of gas measurement technique on gas concentration in bedding.** In this study, it became clear that gas concentrations in bedding during rebreathing are influenced by a number of factors. Clearly, withdrawing gas from the bedding for determining concentration could have had some effect, even though we used the smallest feasible flow rates. We sought to estimate the effect of this sampling by using a simplified model in which concentration is primarily determined by two factors: 1) the relatively rapid rate of flow of CO2 (for example) into the bedding from the infant and 2) the relatively slow rate of flow of CO2 out of the bedding as a result of simple diffusion.

To estimate the rate of flow out of the bedding, we introduced a mixture of CO2, N2, and O2 (5% CO2, 13% O2, balance N2) into the bedding until it was saturated. We then placed a water-filled container with a shape and mass approximating that of an

### Table 1. Distribution of subjects among multiple analyses

<table>
<thead>
<tr>
<th>No. of Subjects</th>
<th>Gas evaluation</th>
<th>Protocols performed</th>
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</thead>
<tbody>
<tr>
<td>CO2</td>
<td>21</td>
<td>3</td>
</tr>
<tr>
<td>O2</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Air channel</td>
<td></td>
<td></td>
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<tr>
<td>Rise in CO2 after cloth</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Gas gradient</td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>Ventilatory response to rebreathing</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Effect of VT size on iCO2 and iO2</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Volume-averaged iCO2 and iO2</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td></td>
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</table>
Next, the rate of CO₂ flow into the bedding was estimated by determining the maximum rates of rise of CO₂ when infants first assumed the facedown position. Half-life was calculated for this rate of rise. Knowing the rates of flow into and out of the bedding allowed us to mathematically determine the differences in gas concentration in the bedding for continuous vs. intermittent sampling.

Data analysis. O₂ and CO₂ concentrations were calculated by estimating to the nearest 0.25 mm on the output tracing and multiplying by the calibration constant (1 mm = 0.22% CO₂ or O₂). Relative inspired VT (in mm²) were calculated by integrating the flow curve during inspiration; f was calculated by counting the number of breaths in the preceding 10 s from the index breath and multiplying by six. Relative minute ventilation (VI) (units = 1 mm² box/min) was calculated by multiplying f and VT. The relationships between VI, VT, and f and inspired CO₂ and O₂ concentrations were evaluated by Student's two-tailed, unpaired t-test and multiple linear regression. The change in inspired CO₂ that followed cloth placement was analyzed by comparing mean inspired CO₂ before and after each cloth placement by Student's two-tailed, paired t-test. The significance of the relationship between VT size and inspired gas concentrations was evaluated by linear regression and Student's two-tailed, unpaired t-test (18).

Mean inspired gas concentration (volume-averaged) for each breath was calculated by weighting the gas concentration by the corresponding portion of VT for each millimeter subdivision (1 mm = 0.1 s) of inspiration divided by the VT. This method is depicted by the following formula, where i = each 0.1-s subdivision of inspiration and there are n total subdivisions: \[ \frac{\sum \text{(gas concentration)} \times \text{(volume)i}}{\text{VT}} \] (Fig. 1). Volume-averaged inspired CO₂ and O₂

![Fig. 1. Note that throughout inspiration (Insp), gas concentrations and flow pattern are changing. Volume-averaged gas concentration is calculated for each breath by multiplying volume (integrated flow) during 0.1-s segments of inspiration by the corresponding gas concentrations for each segment and dividing by tidal volume (VT), shown by the shaded area of flow.](image)

![Fig. 2. Air channels detected around one subject's head while facedown at positions D and G, where CO₂ is elevated over 1%.](image)
concentrations were compared with prior methods of estimating inspired gas concentrations (end-inspiration CO$_2$ and O$_2$ = room-air O$_2$ − end-inspiration CO$_2$) by Student’s two-tailed, paired $t$-test for each breath (30). Forty-five breaths during both rapidly changing and relatively steady-state conditions while rebreathing were analyzed in this manner in five infants who slept facedown for long periods. Results are expressed as means ± SE. Results for all statistical analyses were considered significant if $P < 0.05$.

RESULTS

Evidence for occult air channel formation. Gas exchange with the environment while facedown via air channels not apparent to visual inspection was demonstrated by a rise in CO$_2$ to 1.5% at certain positions around the infant’s face in the three infants tested, with gas changes in phase with respiration usually detected at one or more sites (Fig. 2, example of one infant). Location of channels and size of the CO$_2$ signal often changed with changes in the infant’s head position.

End-inspiration CO$_2$ rose by an average of 28.7% after placement of a silk cloth (1.34 ± 0.19 and 1.73 ± 0.17% before and after cloth placement, respectively) around the face in 11 prone infants ($P < 0.005$). Figure 3 shows a representative tracing from one infant.

Evidence for gas-gradient formation in bedding. As the needle was pulled from deep in the bedding into the pocket immediately beneath the face in seven infants, CO$_2$ levels gradually rose and O$_2$ levels fell. CO$_2$ levels rose to 3.96% and O$_2$ levels fell to 12.4% in the bedding beneath the face in a representative subject (Fig. 4). In addition, a rise and fall in gas concentration in phase with breathing was detected beneath the face in zones A and B, suggestive of bulk air movement.

Infants’ ventilatory responses to rebreathing. Larger VT was associated with a decrease in end-inspiration CO$_2$ (52.8 ± 8.93% lower) and an increase in end-inspiration O$_2$ (10.8 ± 0.53% higher) in the five facedown infants. These trends were significant with $P < 0.05$ for each of the five facedown infants studied (Fig. 5).

Respiratory response to rebreathing was evaluated in 11 infants (represented by subject IA in Fig. 6). All demonstrated an increase in $\dot{V}t$ (average of 332%) and $\dot{V}t$ (average of 319%) as infants were exposed to an increasingly asphyxial environment. $f$ varied irregularly with regard to O$_2$ and CO$_2$. Slopes of $\dot{V}t$ and $\dot{V}t$ vs. end-inspiration O$_2$ and end-inspiration CO$_2$ were significantly different from zero by $t$-test for all patients ($P = 0.01$ and $P < 0.01$, respectively). In contrast, the slope of $f$ vs. end-inspiration O$_2$ and end-inspiration CO$_2$ was not statistically significantly different from zero ($P = 0.57$). The high level of correlation between CO$_2$ and O$_2$ ($r = -0.9$) precluded use of multiple-regression analysis.

Direct measurement of O$_2$ and CO$_2$ concentrations. Inspired-gas concentrations were analyzed from five infants who rebreathed while sleeping in a facedown position without requiring additional cloths around the face. These infants were similar to the entire study group with regard to age and sex. Of these five, two (40%) slept prone at home. Volume-averaged inspired CO$_2$ concentration was 3.43 ± 0.19%, range 5.78–5.75%. Volume-averaged inspired O$_2$ concentration was 15.94 ± 0.23, range = 13.32–19.65%. Linear regression of the change in CO$_2$ on the change in O$_2$ concentration from room air did not reveal an equal relationship, as the slope was <1 for each subject. The
decrease in $O_2$ was significantly greater in magnitude than the increase in $CO_2$ ($P < 0.01$ for each infant; Fig. 7).

Analysis of the 45 breaths showed the volume-averaged inspired $CO_2$ concentration was significantly higher than the end-inspiration $CO_2$ concentration for each subject (18.2–122.2% higher; $P < 0.005$). In each subject, volume-averaged inspired $O_2$ concentration was significantly lower than prior estimates (inspired $O_2 = room~air~O_2 - end-inspiration~CO_2$) of $O_2$ levels (6.7–17.2% lower, $P < 0.005$; Fig. 8). Two of our subjects who showed the most pronounced differences in $O_2$ concentrations (16.1 and 17.2% lower) also experienced rapid desaturations to 84–85%. Concurrently, volume-averaged $O_2$ concentration decreased to 13% over 20–30 s with a much slower accompanying rise in $CO_2$ (Fig. 9). Figure 10 shows the relationship between change in $CO_2$ and change in $O_2$ in both expired and inspired air during this rapid desaturation, which is best fit by a curve. One of these infants failed to arouse on his own and required our intervention when his $O_2$ saturation reached 85%.

**Effect of gas measurement technique on gas concentration in bedding.** The intermittent method of gas sampling approximated the natural time course of diffusion of gas out of the bedding, as minimal flow was directed to the gas analyzers. Average half-life of flow of $CO_2$ out of the bedding for intermittent sampling was 125 vs. 88 s for continuous sampling, which had an effect of increasing flow of gas out of the bedding. Similar results were obtained for $O_2$. Half-life of flow of gas into the bedding was 10.4 s for $CO_2$ and 10.5 s for $O_2$. Thus, although the continuous method changed gas concentration faster than the intermittent method by a factor of 1.4, the rate of change in inflow was still roughly 10 times faster than the rate of outflow, irrespective of the method of sampling. The ratio of gas-concentration equations showed that the effect of continuous sampling was to lower measured values of $CO_2$ and raise measured values of $O_2$ by 5%, compared with our estimates of gas concentrations if no sampling had occurred.

**DISCUSSION**

We verified preliminary data that there are often air channels around the infant’s face, while sleeping face-down, which allow gas exchange with the environment and that they are occult, in that direct inspection does not suggest their presence. In addition, we noted that slight movements of the infant’s head could increase or decrease flow through these channels. This principle was also demonstrated by the significant rise in $CO_2$ after the placement of cloths near infants’ faces, thus increasing the bedding’s effect on rebreathing. When the infant has the ability to exchange air via air channels, the degree of rebreathing may be limited (5). However, this also highlights the subtleties of infant and bedding positioning, which are not immediately visible and may allow some infants to sleep facedown, whereas others who do so may experience dangerous asphyxia. Although we investigated only one type of bedding, it is conceivable that certain characteristics of the composition of the bedding influence the occurrence and size of channels and thereby would be relevant to its safety for infants (26).

We evaluated the effect of our method of continuously sampling gas for analysis by comparing it to intermittent sampling. We estimated that the continuous withdrawal of gas in the bedding altered gas values by ~5% compared with actual values that would have been present during rebreathing in the absence of sampling. Thus we slightly underestimated the asphyxial challenge to the infant during rebreathing. Because the overall effect of continuous sampling was to cause an apparent increase in the background diffusion of gas out of the bedding and because $CO_2$ and $O_2$ were affected equally, our findings regarding proportional differences in gas concentrations and gradients in bedding were unaffected.

Presence of $CO_2$ and $O_2$ gradients in the bedding, with high $CO_2$ and low $O_2$ just beneath the nose and low $CO_2$ and high $O_2$ furthest from the nose, demonstrates that the infant exchanges gas with bedding during rebreathing as well. These findings confirm preliminary observations made earlier by this laboratory (5). Reasons for formation of gradients are not clearly identified. We theorize that the microstructure of the bedding (23, 24, 26) allows airflow with minimal turbulence and consequently little air mixing. Also, exchange of gases at the interface between the bedding and the environment must be occurring by diffusion. As air is exhaled, end-expired air, which is of highest $CO_2$ and lowest $O_2$ content, is trapped closest to the face. Although convection does occur, as demonstrated...
by the pulsatile flow detected in the bedding nearest the face, it diminishes further out from the infant's face and thus limits mixing. In effect, the bedding expands the dead space in series with the infant's dead space. Due to the relatively large nature of this space, the infant inspires end-expiratory gas before accessing fresher air that is further away in the bedding (Fig. 4). This is confirmed by the effect of VT on inspired-gas concentrations, with larger VT allowing access to fresher air, in the facedown infants seen in this study.

Fig. 6. Example in one representative subject of change in minute ventilation (Vt), VT, and respiratory frequency (f) with simultaneously increasing end-i CO2 and decreasing end-i O2 while rebreathing. Vt, VT, and f are displayed on the y axis. End-i O2 is displayed on x axis. End-i CO2 value is displayed both visually and numerically by the “bubble” diameter and adjacent number. This three-dimensional graph is to aid in visual interpretation of changes in Vt, VT, and f with an increasingly asphyxial environment (Excel, 1997). Note positive correlation between Vt and VT with an increasing hypercapnic-hypoxic environment (P = 0.01 and P < 0.01, respectively). There is no significant change in f with an increasing hypercapnic-hypoxic environment (P = 0.57).

Fig. 7. Mean change in CO2 from room air (RA) vs. mean change in O2 from room air in 5 facedown rebreathing infants (modified conventional PCO2 and PO2 diagram) (37). Note that each infant’s regression line has a slope of < 1, which is shown by the line of identity. Therefore, for each infant, the absolute decrease in O2 is larger in magnitude than the absolute increase in CO2 (P < 0.01 for each regression line). •, TA; x, BM; ○, MD; ●, IA; ◦, RM.

Fig. 8. Measured volume-averaged inspired O2 (black bars) vs. estimated O2 (gray bars) from previous method (inspired O2 = room air O2 − end-i CO2)(30) in 5 facedown, rebreathing infants (mean ± SE). Note that, for each infant, measured O2 is significantly lower than previously theorized. *P < 0.005.
The relationship between VT size and inspired-air composition highlights the importance of volume averaging inspired-gas concentrations. Nunn (34) reviews a method for determining the composition of gas rebreathed from apparatus dead space. Because inspired rebreathed gas is of changing composition, as shown by Fig. 1, the true effective mean concentration is volume based (2). When facedown and breathing from a relatively large reservoir of exhaled air in the bedding, true inspired-gas concentration depends on the volume and corresponding concentration of air breathed early as well as late in inspiration. For example, at the beginning of inspiration, the infant breathes in end-expiratory gas near the nose. As the inspiratory volume increases, more air is drawn from distant, fresher areas of the bedding. Thus the volume-averaged concentration reflects the various sources of gas inhaled during the entire inspiration.

Subjects demonstrated appropriate ventilatory responses to an asphyxial environment. Of note, our subjects were exposed to a hypercarbic and hypoxic stimulus, depending on the degree of rebreathing achieved by each infant. Although the usual ventilatory response to hypoxia is hyperbolic in shape, the responses of these infants fit a linear model best (11). Hypercarbia normally results in a linear increase in Vt (29). The observed response likely reflects the dual stimuli as the ventilatory response to asphyxia is greater than that to the individual stimuli of the rise in CO2 and the fall in O2 (9, 29). Our subjects increased Vt primarily by increasing VT, with inconsistent changes in f. It has been shown that humans, unlike other animals, primarily change VT to affect ventilation (1, 5, 7, 11, 15). In a facedown rebreathing environment, the increase in VT is beneficial as it allows access to fresher air in the bedding. If the primary response consisted of shallow, rapid breathing, then the infant would be exposed to a more threatening environment. This may explain the greater increased risk of SIDS associated with prone positioning in infants with infectious respiratory illness as opposed to well infants (36). Such illnesses tend to be associated with stimuli that increase f.

High resistance to airflow associated with covering the nose and mouth has been associated with respiratory failure and/or death (40, 41). Although the facedown infants in our study did have their mouths and noses covered, the resistance of our bedding has been previously shown to be well below the level of resistive load associated with development of hypercapnia in infants (24, 41). High resistive loads in infants have been associated with hypoventilation, effected primarily through changes in f instead of VT (41). Thus the ventilatory changes seen in our study reflect a response to an asphyxial environment due to rebreathing, not to a high resistive load.

Previously, estimates of the CO2 concentrations in infants’ environments have used the end-inspiration point (6, 30). Actual inspired-O2 concentration while rebreathing has not been measured, but it has been assumed to reciprocate elevated CO2 levels (30). We attempted to find a more accurate estimate of the inspired gases by continuously measuring both CO2 and O2 and calculating the volume-averaged mean concentrations. Our data showed that the decrease in actual O2 concentration does not reciprocate the elevation in CO2 but is of significantly greater magnitude. During quasi-steady-state conditions, the inequality

![Figure 9: Desaturation to 87% in one subject while sleeping facedown. Note rapid decrease in O2 from 15.4 to 12.6% with a much slower accompanying rise in CO2 from 3 to 3.7%. Sat, saturation.](http://jap.physiology.org/)

![Figure 10: Scatter diagram of simultaneous changes in CO2 and O2 concentrations from room air during a period of rapid desaturation in one subject. Note nonlinear shape of best-fit lines for both expired (●) and inspired (○) gas concentrations, which is suggestive of an unsteady state.](http://jap.physiology.org/)
between changes in O₂ and CO₂ is partially due to the respiratory quotient, reflecting the relatively larger consumption of O₂ than CO₂ production. The respiratory quotient remains relatively constant as the data best fit a line for each subject (Fig. 7).

Additionally, the measured O₂ inspired by rebreathing infants was significantly lower than predicted when rapid change in inspired gas composition occurred soon after assuming the facedown position. This was most evident in the two infants who experienced rapid desaturations to 84–85%. The time course of these episodes was notable in that, although the O₂ concentration fell rapidly, the CO₂ concentration changed minimally. The relationship of change in CO₂ and change in O₂ is best fit by a curve during these rapidly changing episodes (Fig. 10), as opposed to a linear relationship during quasisteady-state conditions. This likely reflects the body’s ability to store CO₂ 100-fold more than O₂ (34). Before reaching steady-state conditions, the respiratory exchange ratio temporarily changes, making the difference between O₂ and CO₂ even larger (34). The larger decrease in O₂ vs. increase in CO₂ while sleeping and rebreathing may have a deleterious effect on an infant’s ability to respond to an asphyxiating environment. The inspired O₂ reached a nadir of 13.3%, which is below the level of an asphyxiating environment. The inspired O₂ concentration changed minimally. The relationship of change in CO₂ and change in O₂ is best fit by a line for each subject (Fig. 7).

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