Measurement of the CO₂ apneic threshold in newborn infants: possible relevance for periodic breathing and apnea

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A BASELINE CONCENTRATION OF CO₂ is essential for breathing to occur (22, 34, 35). The level of CO₂ below which breathing ceases is known as the “CO₂ apneic threshold”. It is now well accepted that periodic breathing and apnea in human subjects is due to a decrease of CO₂ to levels below the apneic threshold (3, 4, 25, 28, 34, 42, 44, 51). The propensity for endogenous CO₂ to decrease below the apneic threshold would depend, to a large extent, on how close the baseline PCO₂ (eupneic PCO₂) is to the CO₂ apneic threshold. If the difference is small, minor behavioral changes would be enough to propel the PCO₂ below threshold. Apnea or periodic breathing would then ensue.

The underlying physiological mechanism responsible for this respiratory pattern in newborn infants has not been clearly defined. In 1972, our laboratory suggested that hypoxia was a crucial inducing factor, probably because of its central depressant effects, causing hypoventilation with a shift in the position and a decrease in the slope of the ventilatory response to CO₂ (40, 41). Our laboratory also postulated that sleep might be a contributing factor, because it also decreased the slope of the ventilatory response to CO₂ with a shift to the right, favoring the appearance of periodic breathing in a manner similar to that observed by Bulow (6) in adult subjects. This respiratory periodicity of neonates could easily be abolished by the administration of inhaled O₂, CO₂, or other respiratory stimulants, such as theophylline and doxapram (9–11, 18, 19, 29, 39, 40, 45, 46).

In normal adult subjects, the presence of apnea and periodic breathing is rare. It occurs primarily at advanced age during sleep, as central or obstructive apneas, affecting male individuals predominantly (5, 16, 23, 34). The physiological mechanism for this breathing instability in adult subjects remained largely unknown until the provocative work by Skatrud and Dempsey (44) in 1983. They suggested that the respiratory pauses characteristic of this breathing pattern likely occur because the baseline PCO₂ (eupneic PCO₂) decreases below the apneic threshold. This threshold is the minimal PCO₂ level essential to sustain breathing, and it is founded on the concept that breathing during non-rapid eye movement sleep has an intrinsic dependency on a baseline level of metabolic CO₂ (34, 35). The work of Skatrud and Dempsey (44) was confirmed and expanded in many of their subsequent publications and also in reports from other investigators (12, 13, 25, 28, 51).

More recent publications have suggested that the closer the eupneic PCO₂ is to the PCO₂ threshold, the more prone to instability breathing is (50). Hypoxia narrows this difference significantly, favoring the appearance of apnea. In adults, this narrowing is due to the decrease in baseline or eupneic PCO₂ as a result of hyperventilation mediated at the peripheral chemoreceptors. In newborn infants, the action of hypoxia differs from that in adults. With hypoxia, newborn infants decrease their ventilation, but PCO₂ also decreases because of a decrease in metabolism (31, 37). This lowered PCO₂ may decrease below the PCO₂ threshold, causing apnea.

In adult subjects the average PCO₂ apneic threshold is ~3.5 Torr below the eupneic PCO₂ level (12, 28, 32, 44, 49). This difference may be large enough to provide some stability of breathing pattern in adult subjects. Because tidal volume changes little under normal conditions, it would be unlikely for
P\textsubscript{CO2} oscillations to dive below the P\textsubscript{CO2} threshold. On the contrary, in newborn infants, there is a great variability in tidal volume, with major oscillations in P\textsubscript{CO2} under normal circumstances. If this is associated with a narrow difference between eupneic and threshold P\textsubscript{CO2}, triggered for example by these infants’ resting hypoxemia, breathing could become unstable, with increased respiratory periodicity and apneas. We reasoned that it would be important to measure the P\textsubscript{CO2} apneic threshold in neonates to know whether the difference from eupneic P\textsubscript{CO2} is indeed narrower than that reported for adult subjects.

We designed this study to measure the P\textsubscript{CO2} threshold in neonates. We hypothesized that this threshold would be much closer to the eupneic P\textsubscript{CO2} than that previously reported for adult subjects.

**MATERIALS AND METHODS**

*Subjects.* Thirty-two infants, previously studied for apnea of prematurity, were included in this study (27, 47). Sixteen were preterm (birth weight 1,540 ± 155 g, mean ± SE; gestational age 30 ± 1.0 wk; postnatal age 58 ± 7 days) and 16 were term (birth weight 3,380 ± 148 g, gestational age 39 ± 2 wk, postnatal age 27 ± 7 days).

They were all healthy at the time of the study and were not on /H11006. The cutaneous PCO2 (PtcPO2) was measured in a limited number of infants (model TCM3, Radiometer, Copenhagen, Denmark). The EEG was varying /H11021 and electrocardiogram has been described (17, 24, 30, 40 – 42). Briefly, a nosepiece and a screen flowmeter were used to measure respiratory flow. The valves were eliminated and dead space reduced by using a constant background flow (3 l/min), which was electronically balanced to an artificial zero. The screen flowmeter was linear response time of the system was 0.16 s. Hemoglobin O2 saturation (HbO2) was monitored by using a Nellcor pulse oximeter with a PE-20) attached to the left nostril adaptor and connected to a vacuum pump. The pump drew inspired gas through a Beckman analyzer (model LB2, Beckman Instruments, Fullerton, CA). The 95% response time of the system was 0.16 s. Hemoglobin O2 saturation (HbO2) was monitored by using a Nellcor pulse oximeter with a response time of 5 s (model N-100C, Nellcor, Hayward, CA). Transcutaneous PCO2 (PtcPCO2) was measured in a limited number of infants (model TCM3, Radiometer, Copenhagen, Denmark). The EEG was recorded with electrodes in the C4–A1 positions. The EOG was recorded from the upper outer canthus of the left eye and the lower outer canthus of the right eye and referred to the right earlobe. Heart rate was measured by using conventional leads, primarily as an indicator of the infants’ well-being and not as an end point for this study. All signals were recorded (at 5 mm/s) on a polygraph (model 4221, Nihon Kohden, Tokyo, Japan) and also were stored in a computer for further analysis.

Apneas were respiratory pauses longer than 5 s (28, 48). Presence or absence of the cardiac oscillation signal on the airflow and CO2 tracings was used to define central or obstructive apnea, respectively (26). Sleep states were monitored according to the criteria reported previously (14, 15, 17, 30). Briefly, quiet sleep was defined as the absence of rapid eye movements (REM) combined with, in preterm infants, discontinuous EEG, or, in term infants, trace alternant. REM sleep was defined by the presence of rapid eye movements on the EEG and continuous, irregular low voltage on the EEG. Transitional sleep with short epochs lasting 1–3 min was usually observed during the transition from quiet to REM sleep or vice versa. Indeterminate sleep was defined as that which could not be described by other definitions.

**Procedure.** The experimental procedure has been described previously (17, 30, 37). Briefly, the infant was brought to the laboratory and placed in an Ohio Intensive Care Unit. The skin temperature was servo-controlled and kept at 36.5 ± 0.03°C. After placement of the nosepiece and various leads, we waited for the infant to fall asleep and then all variables were recorded. Breathing was monitored continuously and as long as needed to contain at least one epoch of quiet sleep and one epoch of REM sleep. During the study, infants inhaled 21% O2.

Data collection and statistical analysis. Data were measured by hand and transferred to a computer for further analysis. We measured minute ventilation, respiratory frequency, tidal volume, alveolar P\textsubscript{CO2} (P\textsubscript{ACO2}), HbO2, PtcPCO2, fetal heart rate during epochs of quiet sleep. These measurements were made during 1) a baseline or eupneic period located 5 min or more preceding the switch from regular to periodic breathing, 2) the last three breaths preceding the first apnea of the periodic breathing epoch, and 3) the first three breaths after the last apnea of the same periodic breathing event. Averaging measurements over three breaths was done to reduce breath-to-breath variation, and to make sure that the value obtained reflected the P\textsubscript{ACO2} likely to be acting at the chemoreceptor level. All transitions were studied in quiet sleep only, and those associated with a change in state were not included in the analysis. During transitions, the average P\textsubscript{ACO2} of the three breaths immediately preceding periodic breathing was considered the P\textsubscript{CO2} apneic threshold.

We used one-way analysis of variance with repeated measures to test the significance of differences between respiratory variables. Tukey’s honestly significant difference test was used for post hoc comparisons. Values are expressed as means ± SE (and range when indicated). A probability value ≤0.05 was considered significant.

**RESULTS**

The duration of each study was 166 ± 10 min (mean ± SE). Infants spent 29% of their time in quiet sleep and 33% in REM sleep. Periodic breathing was present in 32% of the sleeping time in these infants. We analyzed all the transitions from regular to periodic breathing and vice versa (n = 64). The length of the periodic breathing cycle (breathing plus apneic intervals) was 14.4 ± 0.4 s, the length of the breathing interval was 8.1 ± 0.3 s, and the length of apnea was 6.3 ± 0.4 s (range 5–12 s). All apneas were central as they usually are in these infants with periodic breathing. The length of the periodic breathing epoch was 6 ± 0.2 min. Figure 1 is a representative tracing of pertinent variables during the transitions.

In preterm infants, the eupneic P\textsubscript{ACO2} was 38.6 ± 1.4 Torr, the preperiodic P\textsubscript{ACO2} apneic threshold was 37.3 ± 1.4 Torr, and the postperiodic P\textsubscript{ACO2} was 37.2 ± 1.4 Torr (P < 0.001; Table 1). In term infants, the eupneic P\textsubscript{ACO2} was 39.7 ± 1.1 Torr, the preperiodic P\textsubscript{ACO2} apneic threshold was 38.7 ± 1 Torr, and the postperiodic P\textsubscript{ACO2} was 37.9 ± 1.2 Torr (P < 0.01). Combining the differences between eupneic P\textsubscript{CO2} and P\textsubscript{CO2} threshold for preterm and term infants, the calculated P\textsubscript{CO2} apneic threshold was 1.15 ± 0.2 Torr (0.95–1.79, 95% confidence interval) below the eupneic value. Similarly, the eupneic-post periodic P\textsubscript{ACO2} difference was 1.6 Torr when three breaths were averaged and 1.15 Torr when only the first breath was considered. This was due to P\textsubscript{ACO2}, of the first postperiodic breathing being slightly higher and tidal volume slightly lower than the following two breaths (P\textsubscript{ACO2} 38.0 ±
1.4, 37.3 ± 1.2, and 37.4 ± 1.5 Torr, and minute ventilation 6.8 ± 0.4, 7.0 ± 0.5, and 7.1 ± 0.4 ml/kg for breaths 1, 2, and 3, respectively).

In parallel with these changes in $P_{\text{aco}}$, there was a minor and nonsignificant increase in minute ventilation from baseline to the preperiodic phase, primarily related to a slight increase in frequency. $HbO_2$ decreased slightly. Baseline $P_{\text{aco}}$ was available in only eight preterm infants (64 ± 3 Torr) and six term infants (87 ± 4 Torr). There was no change in sleep state during the resumption of breathing after the apneic pause.

**DISCUSSION**

We measured $P_{\text{aco}}$ and other ventilatory variables in preterm and term neonates during the transition from regular to periodic breathing and from periodic to regular breathing. We found that these infants breathe very close to their apneic threshold. The difference between eupneic $P_{\text{aco}}$ and $P_{\text{aco}}$ threshold was 1.15 ± 0.2 Torr (0.95–1.79, 95% confidence interval), a value significantly lower than that for adult subjects (3.5 ± 0.4 Torr) (28, 32, 49). The difference between eupneic and postperiodic $P_{\text{aco}}$ for all infants was similar to that preceding periodic breathing, whether values were considered for the first three breaths or only for the first breath postperiodic. The closeness of eupneic and threshold $P_{\text{aco}}$ likely confers a great vulnerability to respiratory stability in these infants. It is not surprising, therefore, that brief startles, movements, or change in sleep state could allow eupneic $P_{\text{aco}}$ to dive below the $P_{\text{aco}}$ apneic threshold, inducing periodic breathing and apnea in these infants.

There are no data in the literature regarding the $P_{\text{aco}}$ apneic threshold in newborn infants. Therefore, discussion regarding the mechanisms responsible for the narrow eupneic-apneic $P_{\text{aco}}$ threshold difference we observed in the present study must be largely speculative. Data from the fetal (22) and newborn (8) sheep, however, suggest that, like later in life, breathing is fundamentally dependent on the baseline level of $P_{\text{aco}}$. Kuipers et al. (22) have shown that fetal breathing decreases with hypocapnia induced by extracorporeal circulation. Canet et al. (8) used similar technique to control blood-gas levels and found that the average arterial $P_{\text{aco}}$ apneic threshold was 5.3 Torr below resting (eupneic) arterial $P_{\text{aco}}$ at 12 days of age. This value is close to values obtained in adult subjects and may explain the more regular, apnea-free, breathing pattern of the young lamb compared with human neonates.

One possible limitation of our methods relate to the variability of $P_{\text{aco}}$ and the implications to calculate the $P_{\text{aco}}$. The presence of the cardiac oscillation signal on the flow and $CO_2$ tracings indicates patency of the airways and defines the apneas as central (26). $HbO_2$, hemoglobin $O_2$ saturation; $Insp$, inspiration; $Exp$, expiration.

![Respiratory Measurements during Transitions from Regular to Periodic and Periodic to Regular Breathing in Preterm and Term Infants](https://jap.physiology.org/content/98/4/1173/F1)

**Table 1. Respiratory measurements during transitions from regular to periodic and periodic to regular breathing in preterm and term infants**

<table>
<thead>
<tr>
<th>Respiratory Variable</th>
<th>Preterm Infants ($n = 16$)</th>
<th>Term Infants ($n = 16$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline (eupnea)</td>
<td>Preperiodic</td>
</tr>
<tr>
<td>$V_e$, l/min $^{-1}$kg$^{-1}$</td>
<td>0.219±0.016</td>
<td>0.225±0.015</td>
</tr>
<tr>
<td>$f$, breaths/min</td>
<td>32.1±1.8</td>
<td>35.2±2.0</td>
</tr>
<tr>
<td>$V_t$, ml/kg</td>
<td>7.1±0.5</td>
<td>6.4±0.4</td>
</tr>
<tr>
<td>$P_{\text{aco}}$, Torr</td>
<td>38.6±1.4</td>
<td>37.3±1.4$^*$</td>
</tr>
<tr>
<td>$HbO_2$, %</td>
<td>97.4±0.7</td>
<td>97.0±0.7$^*$</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n$, no. of subjects. $V_e$, minute ventilation; $f$, respiratory frequency; $V_t$, tidal volume; $P_{\text{aco}}$, alveolar $P_{\text{aco}}$; $HbO_2$, hemoglobin $O_2$ saturation. $^*$ $P ≤ 0.05$ compared with baseline.

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apneic threshold. Breathing is irregular in neonates, with variability in tidal volume and frequency. As a consequence, end-tidal CO$_2$ also varies. To minimize the effect of this variability, we elected to calculate our ventilatory measurements over three breaths. In our experience, the values obtained for eupneic P$_{ACO_2}$ were similar to measurements we made previously over 1 min or more. We reasoned also that the three breaths should represent the P$_{CO_2}$ apneic threshold for the infant, because 1) they would comprise the first breath before apnea of a periodic breathing epoch, and 2) they would also comprise the second and third breaths before apnea, these being breaths with the lowest P$_{ACO_2}$, and thus most likely the stimulus for the central and peripheral chemoreceptors. Others have used one or more breaths preceding apnea to represent the P$_{CO_2}$ apneic threshold (28, 44, 50).

What is then the rationale for the P$_{CO_2}$ apneic threshold to be so close to the eupneic P$_{CO_2}$ in human newborn infants? We believe that the key element responsible for this narrow difference is the well-known hypoxemic status of these infants. Small preterm infants have a P$_{tCPO_2}$ of ~55–70 Torr and term infants of 85–90 Torr (38, 39). In the limited number of preterm and term infants in whom measurements were available in the present study, the P$_{tCPO_2}$ values were within these ranges. This low PO$_2$ tensions is a function of lung maturation. The lungs of neonates have a decreased alveolar-to-tissue ratio than older children and adult subjects (7). This low arterial PO$_2$ in newborn infants may keep the eupneic P$_{CO_2}$ relatively low, not by hyperventilation as in adult subjects but by a decrease in metabolism which parallels the decreased in arterial P$_{O_2}$ (32, 40). Indeed, in the present study, the baseline or eupneic P$_{ACO_2}$ was 38.6 ± 1.4 Torr in preterm infants and 39.7 ± 1.1 Torr in term infants, values lower than those in adult subjects during quiet sleep, 45.3 ± 2.3 Torr (28, 44, 49). The values for the P$_{ACO_2}$ apneic threshold, however, were much closer, being 37.3 ± 1.4 Torr in preterm infants, 38.7 ± 1 Torr in term infants, and 41.5 ± 0.2 Torr in adult subjects (28, 44, 49). This means that the difference in eupneic P$_{ACO_2}$ between the preterm infant and the adult subject is ~6 Torr, and the corresponding difference in the P$_{ACO_2}$ apneic threshold is ~4 Torr. This suggests that the narrower eupneic-apneic P$_{ACO_2}$ threshold difference in newborn infants is dependent more on their lower eupneic P$_{ACO_2}$ than on their higher P$_{ACO_2}$ apneic threshold. Other factors known to narrow the eupneic-apneic P$_{CO_2}$ threshold difference, such as metabolic alkalosis, are probably less relevant to neonates because, if anything, they more frequently exhibit a mild metabolic acidosis (32, 40).

The narrow difference between eupneic and apneic P$_{CO_2}$ threshold observed in neonates favors the appearance of apnea, but for breathing to become frequently periodic or apneic, instability of baseline breathing is also required. It could be argued that if the baseline breathing drive is absolutely regular, even with a narrow eupneic-threshold P$_{CO_2}$ difference, breathing may not become unstable as it normally is in neonates. The basic reason for background instability appears to be the major contribution of the peripheral chemoreceptor drive to normal breathing at this age. Indeed, the arterial P$_{O_2}$ tension of these infants sits on the steep portion of the minute ventilation-arterial P$_{O_2}$ regression curve for human adults (2, 43). This means that small changes in baseline arterial P$_{O_2}$ produce large changes in baseline ventilation. Administration of high O$_2$ (≥40%) to these infants invariably induces complete apnea, suggesting very active peripheral chemoreceptors (9, 40). Because their baseline P$_{O_2}$ varies greatly with their daily activity and behavior, breathing becomes highly erratic and unstable. This instability can drive the eupneic P$_{CO_2}$ below threshold levels, inducing periodic breathing and more prolonged apneas.

Other factors may predispose these infants to respiratory instability, such as sleep state, low functional residual capacity (FRC), and neuronal immaturity. Newborn infants sleep most of the time and sleep favors the instability of breathing (6, 34, 39). Also, depth of sleep alters baseline arterial O$_2$ tension which may predispose the system to periodic breathing (39). Lack of chest wall recoil due to a very compliant chest wall is one of the major handicaps of the newborn respiratory apparatus, particularly in the preterm infant (1). These infants breathe with their FRC at 10% of their vital capacity, very near the closing volume (38). Low FRC leads to hypoxemia, and the cascade toward periodic breathing and apnea is set in motion. Finally, neonates have some degree of neuronal immaturity, which may enhance their predisposition for respiratory instability. They lack dendritic arborization and axodendritic synaptic connections (36). This may alter neuronal traffic and impair respiratory drive. All of these factors are contributory to respiratory instability in neonates, potentially favoring the dive of eupneic P$_{CO_2}$ below threshold levels.

The changes in ventilation associated with the changes in P$_{CO_2}$ were minor and in the same direction as changes our laboratory observed previously between baseline regular breathing and oscillatory breathing pattern without apnea, a cyclic waxing and waning of tidal volume and frequency of breathing, which may precede periodic breathing with respiratory pauses (20). In that previous study, our laboratory also observed that minute ventilation did not change significantly in the period preceding periodic breathing compared with baseline values, but there was an increase in frequency with a decrease in tidal volume. There was also a slight decrease in HbO$_2$ preceding periodic breathing, similar to our observations in the present study. Although the mechanism for this discrete change in pattern and oxygenation is unknown, a slight change in FRC with consequent hypoxemia may be a predisposing factor. According to the theoretical analysis of Khoo et al. (21), a decrease in FRC would alter lung O$_2$ and CO$_2$ storage volume and thereby the plant gain. This may explain why changes in ventilation associated with changes in P$_{CO_2}$ were minor.

The present observations support previous findings that inhalation of CO$_2$ abolishes periodic breathing (9, 29, 41). Indeed, we observed that even low concentrations of CO$_2$ (0.5–1%) are effective in reducing periodic breathing and apnea in these infants (3). Mechanistically, inhaled CO$_2$ would tend to increase CO$_2$ stores and baseline eupneic arterial CO$_2$ tension, likely increasing ventilatory output and the eupneic-apneic P$_{CO_2}$ threshold difference. This would provide more stability to breathing. This effect of CO$_2$ has been used to treat adult subjects with idiopathic central sleep apnea, in which 2–3% inhaled CO$_2$ was effective, decreasing their respiratory pauses (48). In a previous study in preterm infants, our laboratory found that 0.8% inhaled CO$_2$ was as effective as theophylline in treating apnea of prematurity, with none of the adverse side effects of methylxanthines (4). In these infants, breathing increased with baseline P$_{CO_2}$ rising by ~1–2 Torr,
without causing any respiratory discomfort. This major ability of CO₂ to stabilize breathing is being tested now in a clinical trial in infants with apnea (Al Saif S., Alvaro R., Manfreda J., Kwiatkowski K., Qurashi, M. and Rigatto H., unpublished observations).

In summary, we found that the PCO₂ apneic threshold in newborn infants is only slightly below the eupneic PCO₂. The average difference of 1.15 Torr is very small, compared with that in adult subjects, which is ~3.5 Torr. This small difference is likely to make these infants more prone to respiratory oscillations, with frequent decreases in baseline PCO₂ to levels below the apneic threshold, favoring the appearance of periodic breathing and apnea. We suggest that measures such as increasing the baseline eupneic CO₂ by inhalation of small concentrations of CO₂ may minimize apnea by increasing the distance between the eupneic and the CO₂ apneic threshold.

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GRANTS

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