

EFFECTS OF NASAL CONTINUOUS POSITIVE AIRWAY PRESSURE ON NUTRITIVE SWALLOWING IN LAMBS

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Short title: Nasal CPAP and nutritive swallowing

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ABSTRACT

Current knowledge suggests that, to be successful, oral feeding in preterm infants should be initiated as soon as possible, often at an age where immature respiration still requires ventilatory support in the form of nasal continuous positive airway pressure (nCPAP). While some neonatologist teams claim great success with initiation of oral feeding in immature infants with nCPAP, others strictly wait for this ventilatory support to be no longer necessary before any attempt at oral feeding, fearing laryngeal penetration and tracheal aspiration. Therefore, the aim of the present study was to provide a first assessment of the effect of various levels of nCPAP on bottle-feeding in a neonatal ovine model, including feeding safety, feeding efficiency and nutritive swallowing-breathing coordination. Eight lambs born at term were surgically instrumented 48 hours after birth, in order to collect recordings of electrical activity of laryngeal constrictor muscle, electrocardiography and arterial blood gases. Two days after surgery, lambs were bottle-fed under 5 randomized nCPAP conditions, including without any nCPAP or nasal mask and nCPAP of 0, 4, 7 and 10 cmH₂O. Results revealed that application of nCPAP in the full-term lamb had no deleterious effect on safety, feeding efficiency or on nutritive swallowing-breathing coordination. The present study provides a first and unique insight on the effect of nCPAP on oral feeding, demonstrating its safety in newborn lambs born at term. These results open the way for further research in preterm lambs to better mimic the problems encountered in neonatology.

KEYWORDS: Newborn, bottle-feeding, nutritive swallowing-breathing interaction, laryngeal chemoreflexes

INTRODUCTION

Feeding difficulties represent one of the most worrisome problems in preterm infants (1, 3). Preterm oral feeding is a complex developmental skill. Feeding readiness depends on neurological maturation, severity of illness and pre-feeding autonomic, motor, and behavioral state organization (36, 38). Overall neural immaturity, as well as various disorders frequently encountered in preterm infants such as neurological, digestive and respiratory problems, is often responsible for delaying feeding maturation and consequently extending hospital length of stay. In addition, the multiple aversive oral and/or nasal procedures such as suctioning of oronasal secretions, insertion and retrieval of nasogastric tube and endotracheal or nasal ventilatory support, which are used several times a day in preterm infants, often lead to hypersensitivity of the facial area and interfere with feeding maturation (1). Not infrequently, orofacial hypersensitivity leads to feeding problems for years (3, 37).

Various strategies have been developed in an attempt to enhance the acquisition of feeding skills and prevent the above difficulties in premature infants. Non-nutritive sucking has been shown to accelerate transition from tube to bottle-feeding and better bottle-feeding performance (23). Early introduction of oral feeding has also been reported to accelerate feeding maturation (22, 32). Hence, very early development of oral motor competence has been observed with initiation of breastfeeding between 29 and 33 weeks of postmenstrual age, full breastfeeding being reached at a postmenstrual age as low as 32 weeks (21).

However, preterm infants are often dependent on nasal continuous positive airway pressure (nCPAP) for several weeks after birth (16). A number of neonatology teams

refuse to initiate oral feeding in infants while on nCPAP (21), fearing laryngotracheal penetrations and pulmonary aspirations of milk with the consequent potentially deleterious cardiorespiratory events (25, 34, 35). On the other hand, some neonatologists defend the introduction of oral feeding in preterm infants while on nCPAP, as soon as cardiorespiratory stability is present (4, 6). To our knowledge, a detailed assessment of the effect of nCPAP on oral feeding in the neonatal period is not available. The aim of the present study was to provide a first assessment of the effect of various levels of nCPAP on bottle-feeding in a neonatal ovine model, including for safety (i.e. presence of cardiorespiratory events), feeding efficiency and nutritive swallowing-breathing coordination.

MATERIAL AND METHODS

Animals

A total of 8 mixed-breed lambs were included in the study. All lambs were born at term by spontaneous vaginal delivery at our local provider's farm and arrived in our animal quarters 1-2 days after birth. The study protocol was approved by the ethics committee for animal care and experimentation of our institution.

Surgical preparation

Aseptic surgery was performed in all lambs 2-3 days after birth under general anesthesia (2% isoflurane, 30% N₂O, 68% O₂). Anesthesia was preceded by an intramuscular injection of ketamine (10 mg/kg), atropine sulfate (0.1 mg/kg), morphine (0.016 ml/kg) and antibiotics (5 mg/kg gentamicin and 50 mg ampicillin) and an intravenous bolus of Ringer's lactate solution (10 ml/kg). One dose of ketoprofen (3 mg/kg) was also injected intramuscularly for analgesia and repeated if needed on the next day. Lambs were mechanically ventilated through an orotracheal tube (4.5 mm) during the surgical procedure. Heart rate, rectal temperature, pulse oximetry, end-tidal CO₂ and venous pH were continuously monitored throughout surgery.

Chronic instrumentation included placement of custom-made bipolar electrodes, built from right-angled gold connectors (Sullins Connector Solutions, Digi-Key Corporation, Thief River Falls, MN, USA), into both thyroarytenoid muscles (TA; a glottal constrictor) through the lateral aspect of the thyroid cartilage for electromyographic (EMG) recording. An electrocardiogram (ECG) was recorded with 2 needle-electrodes (F-E2M,

Grass Technologies, West Warwick, RI) inserted under the periosteum of the 5th rib, on both sides of the thorax, and directly glued on the rib (5). A third needle-electrode was inserted under the scalp as a ground. Finally, a catheter was placed into the left carotid artery to monitor arterial blood gases (PaO₂, PaCO₂) and pH and was left in place for the entire duration of the study. Leads from each electrode and catheter were subcutaneously tunneled to a common exit on the back of the animal. Post-operative care included intramuscular injection of gentamicin (5 mg/kg, daily) and ampicillin (50 mg, twice a day) until the end of the experimentation. The arterial catheter was flushed daily with heparin solution. Lambs were euthanized at the end of experiments by pentobarbital overdose (90mg/kg). Correct electrode and catheter positioning was systematically verified at necropsy.

Experimental equipment

Ventilatory equipment

Nasal continuous positive airway pressure (nCPAP) was induced using the Infant Flow nCPAP system (Cardinal Health, Dublin, OH) with heated, humidified air. A custom-made nasal mask was installed on the lamb's muzzle to deliver nCPAP, in such a manner that the lamb was able to open its mouth at will and drink from a bottle (31).

Recording equipment

Lamb instrumentation was completed immediately before recordings. A pulse oximeter probe (Masimo Radical, Irvine, CA) was attached at the base of the tail for continuous monitoring of oxygen hemoglobin saturation by pulse oximetry (SpO₂). Arterial blood gases and pH were also measured (IL 1306; Instrumentation Laboratory, Lexington,

MA) and corrected for rectal temperature of the lamb (2). In addition, elastic bands for respiratory inductance plethysmography (Respirace, NIMS, Miami Beach, FL) were installed on the thorax and abdomen to monitor respiratory movements and assess lung volume variations qualitatively. Finally, nCPAP values were continuously monitored from the nasal mask (RX104A pressure transducer, Biopac System, Goleta, CA, USA). All recordings were performed in awake lambs, using our custom-designed radio telemetry system (29). All leads from each electrode were thus connected to this radio telemetry system, in order to obtain prolonged recordings in non-sedated lambs under the least possible restraining conditions. The raw EMG signals were rectified, integrated, and moving time averaged (100 ms). All parameters were continuously recorded on a PC using AcqKnowledge software (version 4.1; Biopac Systems, Goleta, CA, USA).

Design of the study

All lambs were cared for without their mother upon arrival in our animal quarters, due to specific needs of the study regarding bottle-feeding familiarization. They were placed in a Plexiglas chamber (1.2 m³; in agreement with recommendations by the Canadian Council for Animal Care for sheep housing) with holes to allow for air circulation. A bottle filled with reconstituted ewe's milk, from which lambs could drink freely, was placed permanently in the chamber.

The study was performed without sedation at least 45 hours after surgery and was designed to allow for simultaneous recording of nutritive swallowing (NS) activity, respiratory movements, ECG and SpO₂ while bottle-feeding under different nCPAP

conditions. The lambs were comfortably positioned in a sling with loose restraints. Two experimenters were present throughout the recordings to note lamb behavior.

Five different nCPAP conditions were randomly assessed, namely, no nasal mask, nasal mask without nCPAP (nCPAP0) and nCPAP 4, 7 and 10 cm H₂O. In each condition, following a basal recording of 5 minutes, the lambs were offered a bottle filled with reconstituted ewe's milk, heated to 39°C. The nipple of the bottle was the same as the one they had drank from in the Plexiglas chamber. The quantity of milk offered was the same in each condition and was determined from their feeding habits in the previous days. NCPAP conditions were separated from each other by two hours, during which the lambs were placed in the Plexiglas chamber without milk, to ensure that they were hungry enough for each condition. The first condition of the morning was also preceded by two hours without milk. In each condition, the bottle was offered to the lambs a maximum of three times, after which the feeding episode was considered finished. Reasons to pause feeding were mainly lamb discomfort/agitation or lamb refusing to drink. Recordings were continued for 10 minutes after feeding. Blood samples from the arterial catheter were taken before feeding and at 0, 5 and 10 minutes thereafter. Rectal temperature was also taken before and after feeding. Every effort was made to assess the five nCPAP conditions on the same day.

Data analysis

All signals were carefully observed and analyzed in relation with the time period (before, during or after feeding) and nCPAP condition, which could be verified via the mask pressure trace.

Cardiorespiratory variables

For each nCPAP condition, baseline values (i.e., pre-feeding values) for heart and respiratory rates (respectively, HR and RR) as well as SpO₂ were averaged on a period of 30 seconds. Every effort was made to choose periods close to the feeding episode and during which the lambs were calm. Heart rate, RR and SpO₂ during and after feeding were also calculated. Analyses of cardiorespiratory responses during and after feeding were mostly performed as previously described for laryngeal chemoreflex analysis (33). Briefly, the number of HR slowings (defined by a % decrease of HR \geq 33%) and bradycardias (HR slowing lasting $>$ 5s) were noted, and the % of time spent in bradycardia was tabulated. The number of apneas (defined as at least 2 missed breaths relative to baseline breathing) and the % of time spent in apnea were also noted. Finally, the number of desaturations $<$ 90 and 80% and the % of time spent with SpO₂ $<$ 90 and 80% were calculated.

Swallowing activity

Nutritive swallowing activity was recognized by a brief, high-amplitude TA EMG burst, as previously validated (27). To assess the relation between NS and respiration, NS were assigned to one of five types depending on the respiratory phase preceding and following NS: ee-type (preceded and followed by expiration), ei-type (at the transition from expiration to inspiration), ie-type (at the transition from inspiration to expiration), ii-type (preceded and followed by inspiration) (27) and ap-type (NS occurring during an apnea). Rhythmic stability of feeding was quantified using the coefficient of variation (COV: SD of the mean interval, divided by the mean interval) (11). Swallow-breath (NS-BR) and breath-breath (BR-BR) intervals were measured and their COVs calculated

(10). Only NS and breaths occurring during NS runs were used for analysis of rhythms. A NS run was defined as three or more NS with inter-swallow intervals of ≤ 2 seconds

(11). Feeding efficiency was also assessed and included volume of milk intake per unit time (mL/min) or per NS (mL/NS), NS frequency, % of total NS in runs and COV of NS-NS interval.

Statistical analysis

Results were first averaged in each lamb and then averaged for the 8 lambs as a whole. Values were expressed as means and SD. Statistical analyses were performed on raw data for all variables. Normality was tested using the Shapiro-Wilk test. Cardiorespiratory variables, arterial blood gases and NS-types were analyzed through a general linear model 2-way ANOVA for repeated measures. The independent variables for cardiorespiratory variables and blood gases were the nCPAP condition and the time period, while the independent variables for NS percentage were the nCPAP condition and the NS-type. Other data, if normally distributed (COV NS-NS and NS/min), were analyzed through a 1-way ANOVA for repeated measures with nCPAP condition as the independent variable. Both 1- and 2-way ANOVA for repeated measures were performed using PROC MIXED of SAS software, version 9.1. Results not normally distributed (mL/min, mL/NS, COV NS-BR, COV BR-BR and % of total NS in runs) were analyzed by Friedman's test followed by Wilcoxon signed-rank test, using GraphPad Prism software version 5.0. Differences were deemed significant if $P < 0.05$. In addition, given the relatively small number of studied lambs (related to both the complexity of the

ovine model and ethical constraints), it was decided to give full consideration to the presence of a significant trend, defined as $P \leq 0.1$.

RESULTS

General characteristics

A sample tracing obtained in one lamb in nCPAP 10 cm H₂O is shown in Figure 1. Eight lambs weighing 3.8 kg (SD 0.3) (range 3.2 – 4.0) on the day of recordings were included in the study. Though all lambs were evaluated in every nCPAP condition, blood gases and saturation could not be analyzed in the case of one and two lambs, respectively (see below). Detailed results for RR, HR and SpO₂ are listed in Table 1. Neither nCPAP nor feeding had an effect on SpO₂, while RR was significantly decreased in the four conditions with nasal mask compared with the no mask condition, mostly after but also during feeding. In addition, HR was significantly increased by the four conditions with nasal mask both before and after feeding, while during feeding only nCPAP10 increased HR significantly. No significant interaction was found between nCPAP and feeding for RR ($P = 0.8$), HR and SpO₂ ($P = 1.0$ for both).

Cardiorespiratory events and arterial blood gases

The effect of nCPAP conditions and feeding on the % of time spent in apnea is shown in Figure 2. All other cardiorespiratory responses to nCPAP and feeding are detailed in Table 2. Blood gases and pH measurements are listed in Table 3. For all parameters, no significant interaction between nCPAP and feeding was found ($P > 0.1$ for all).

Apneas

While nCPAP had no significant effect on the frequency and the % of time spent in apnea ($P = 1.0$ for both), feeding significantly increased both parameters in almost all nCPAP levels when compared with before and after feeding ($P < 0.0001$ for both; see Table 2 and Figure 2 for details).

Bradycardias and heart rate slowings

Overall, only one bradycardia (i.e. lasting > 5 s) was found. It occurred during feeding in nCPAP7, lasted 10.7 s and was not associated with any apnea or desaturation. Therefore, statistical analyses were performed only for HR slowings (i.e. lasting < 5 s). There was no significant change in the frequency of HR slowings between all nCPAP levels ($P = 0.8$). However, the frequency of HR slowings was significantly increased during feeding compared with before and after, both in the no mask and nCPAP0 conditions (see Table 2 for details). Since not clinically relevant, the duration of HR slowings was not analyzed.

Arterial blood desaturations

Due to technical problems with the pulse oximeter probe in two lambs, saturation could only be analyzed in six of the eight lambs. SpO₂ fell below 80% only once, during 2 s in nCPAP7 before feeding, and was not associated with any apnea or bradycardia. Therefore, statistical analyses were performed only for SpO₂ $< 90\%$. There was no significant change in the frequency of desaturations between all nCPAP levels ($P = 0.5$). However, the % of time spent with SpO₂ $< 90\%$ was significantly increased in nCPAP7 compared to no mask and nCPAP4 during feeding ($P = 0.1$ for both) and compared to

nCPAP4 after feeding ($P = 0.1$). Feeding significantly increased the frequency of desaturations in nCPAP0 when compared to before and after feeding ($P = 0.01$ and 0.05 , respectively), but had no effect on the % of time spent with $SpO_2 < 90\%$ ($P = 0.5$).

Arterial blood gases and pH

Due to technical problems with the arterial catheter in one lamb, arterial blood gases and pH could only be measured in seven lambs. There was a significant decrease in pH in nCPAP7 and nCPAP10 when compared with nCPAP0 before feeding ($P = 0.04$ for both). Similarly, 10 minutes after feeding, pH was significantly decreased by nCPAP10 compared with nCPAP0 ($P = 0.1$). However, mean pH always remained within physiological limits (see Table 3). No significant effect of nCPAP was observed on either PaO_2 or $PaCO_2$ ($P = 0.5$ and 0.9 , respectively). Feeding had no effect on either pH, PaO_2 or $PaCO_2$ ($P = 0.6$, 1.0 and 0.2 , respectively).

In summary, nCPAP had no systematic effect on cardiorespiratory responses and blood gases. Similarly, feeding had no clear effect on all variables with the exception of apneas, for which feeding systematically increased frequency and % of time spent in apnea.

Feeding efficiency

The effects of nCPAP on feeding efficiency are listed in Table 4 and illustrated in Figure 3. There were no significant changes in total NS frequency and percentage of total NS in runs for all nCPAP levels ($P = 0.6$ and 0.2 , respectively). Similarly, the stability of NS

rhythm (COV of NS-NS interval) and the mL/min ratio were not disturbed in any nCPAP level ($P = 0.9$ and 0.3 , respectively). Nevertheless, when compared with every other condition, nCPAP10 significantly decreased the mL/NS ratio (see Figure 3 middle panel for details).

In summary, nCPAP had no effect on feeding efficiency except for a significant decrease of the mL/NS ratio by nCPAP10 when compared with all the other conditions.

Coordination between nutritive swallowing and phases of the respiratory cycle

The effects of nCPAP on NS-BR coordination are reported in Table 4 and Figure 4. Overall, NS-BR coordination was similar for all conditions. The COV of NS-BR interval did not differ between nCPAP levels ($P = 0.9$) and neither did the COV of BR-BR interval ($P = 0.4$). Similarly, no statistical differences were observed when the percentage of each NS type was compared among all nCPAP conditions ($P = 1.0$). Distribution of NS-types was similar between conditions, with ii-, ie- and ei-type NS significantly more frequent than both ee- and ap-type NS in almost all conditions (see Figure 4).

In summary, nCPAP had no significant effect on NS-BR coordination. In addition, although some NS-types were more frequent than others, NS could occur at any moment in the respiratory cycle.

DISCUSSION

Statement of principal findings

The present study provides a first and unique insight on the effects of nasal continuous positive airway pressure on bottle-feeding in the newborn full-term lamb. Overall, our findings reveal that application of nCPAP in the full-term lamb has no deleterious effects on both the safety and efficiency of bottle-feeding, aside from a decreased efficiency with the highest nCPAP value (nCPAP10). In addition, our results show that nutritive-breathing coordination is not altered by nCPAP.

Safety of bottle-feeding during nCPAP

Nasal CPAP has been previously reported to induce dilatation of laryngeal opening in preterm infants (8). On this basis, several neonatology teams are reluctant to initiate oral feeding in infants receiving nCPAP (21), probably fearing the cardiorespiratory consequences of triggering laryngeal chemoreflexes due to increased laryngeal penetration of milk. On one hand, previous results from our laboratory showing severe cardiorespiratory events in preterm lambs during laryngeal chemoreflexes triggered by milk during spontaneous room air breathing may be seen as giving support to these fears (34). On the other hand however, very recent unpublished observations by our group in preterm lambs that nCPAP (6 cmH₂O) efficiently prevents these severe events justify further studies aiming at delineating the conditions where feeding can be attempted in newborns with nCPAP.

In the present study, both the frequency and the % of time spent in apnea were significantly increased during feeding in every experimental condition, including the no

mask condition. This result is not surprising given that deglutition apneas (NS occurring during apneas) are frequently observed both in term and preterm infants (12, 14, 15), owing to the well-known inhibition of the respiratory central pattern generator (CPG) induced by NS (18). Moreover, while the application of nCPAP at any studied level was associated with an increased HR, likely due to a sympathetic response to decreased venous return in an effort to maintain a normal cardiac flow (17), only nCPAP 10 cmH₂O further increased HR during feeding. No alterations in arterial blood gases / pH and no cardiorespiratory events such as apneas, bradycardias, desaturations were observed. Overall, the absence of clinically significant acute cardiorespiratory events under various nCPAP conditions, up to the maximal value reported to be used in newborns (20), is all the more reassuring for those teams pleading for feeding infants still on nCPAP.

Feeding efficiency during nCPAP

Various clinical conditions, such as post-menstrual age (19), intrauterine drug exposure (10), bronchopulmonary dysplasia (9) and acute viral bronchiolitis of infancy (24) have been shown to affect feeding efficiency, as measured by either mL/NS, % of total NS in runs, NS frequency or COV of NS-NS interval. However, results of the present study indicate that nCPAP has no effect on feeding efficiency. The only notable exception is the application of nCPAP 10 cmH₂O, which significantly decreased the mL/NS ratio and appeared to have a similar although non-significant effect on the mL/min ratio.

Previous studies from our group showed an inhibiting effect of nCPAP on non-nutritive swallowing (NNS) in newborn lambs during quiet sleep, mediated by stimulation of both

bronchopulmonary and upper airway receptors (30, 31). In addition, continuous lung inflation prompted by application of negative extrathoracic pressure in awake adult humans was also shown to inhibit water-triggered swallows (13). In the present study, NS frequency was not significantly altered under any nCPAP condition. We are unable to explain at this time the apparent discrepancy between the two above-quoted studies and the present study.

Overall, nCPAP 10 cmH₂O was the only condition to have a slight deleterious effect on feeding efficiency, a fact that has however little clinical impact since this level of nCPAP is rarely used in neonates (28).

Effect of nCPAP on nutritive swallowing-breathing coordination

Nutritive swallowing-breathing coordination is crucial for minimizing the risk of aspiration and the consequent deleterious cardiorespiratory events. Our previous studies (30, 31) revealed that nCPAP had no systematic effect on NNS-breathing coordination in full-term lambs. Similarly, the present results indicate that nCPAP has no effect on NS-breathing coordination in full-term lambs. Overall, swallowing-breathing coordination appears to be hardwired before birth and largely unaltered by various conditions such as preterm birth (26), hypoxia (7), nCPAP (30, 31) or nasal intermittent positive pressure ventilation (31).

Clinical implications and limitations of our study

Our observation in the present study that clinically relevant levels of nCPAP do not have deleterious consequences on the safety and efficacy of bottle-feeding, as well as on

swallowing-breathing coordination, is reassuring for those advocating for initiation of oral feeding despite the presence of nCPAP (4, 6). However, our results are obviously limited by the fact they were obtained in the healthy, full-term lamb. Such observations have to be considered as a first, albeit necessary, step towards the acquisition of a better knowledge of the relationships between nCPAP and oral feeding in the neonatal period. Further experiments will need to address the effect of nCPAP in preterm lambs, including with respiratory problems mimicking bronchopulmonary dysplasia, before contemplating a knowledge transfer to a clinical trial in the neonatal intensive care unit.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the expert technical assistance of Jean-Philippe Gagné and the statistical advice of Julie Hamon and Nathalie Carrier. The study was supported by grants from the Canadian Institutes for Health Research, the Canadian Foundation for Innovation and the Foundation of Stars (Quebec) allocated to J-P Praud. A Bernier is a MD-MSc scholar of the Fonds de la recherche en santé du Québec (FRSQ). Jean-Paul Praud is a member of the FRSQ-funded Clinical Research Center Étienne-Le Bel, Sherbrooke University Hospital, and the holder of the Canada Research Chair in Neonatal Respiratory Physiology.

Contributions of authors

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Nathalie Samson: surgical instrumentation, recordings, analyses, manuscript writing

Pauline Bonneau: analyses of the results

Céline Catelin: design of the study, manuscript writing

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REFERENCES

1. **Amaizu N, Shulman RJ, Schanler RJ, Lau C.** Maturation of oral feeding skills in preterm infants. *Acta Paediatr* 97: 61–67, 2008.
2. **Andritsch RF, Muravchick S, Gold MI.** Temperature correction of arterial blood-gas parameters: A comparative review of methodology. *Anesthesiology* 55: 311–316, 1981.
3. **Barlow SM.** Oral and respiratory control for preterm feeding. *Curr Opin Otolaryngol Head Neck Surg* 17: 179-186, 2009.
4. **Bonner KM, Mainous RO.** The nursing care of the infant receiving bubble CPAP therapy. *Adv Neonat Care* 8: 78-95, 2008.
5. **Carreau AM, Patural H, Samson N, Doueik AA, Hamon J, Fortier PH, Praud J-P.** Effects of simulated reflux laryngitis on laryngeal chemoreflexes in newborn lambs. *J Appl Physiol* 111: 400–406, 2011.
6. **de Klerk R.** Nasal Prong Continuous Positive Airway Pressure: A Practical Guide. New York: Children’s Hospital of New York, 2004.
7. **Duvareille C, Lafrance M, Samson N, St-Hilaire M, Pladys P, Micheau P, Bournival V, Langlois C, Praud J-P.** Effects of hypoxia and hypercapnia on non-nutritive swallowing-breathing coordination in lambs. *J Appl Physiol* 103: 1180-1188, 2007.
8. **Gaon P, Lee S, Hannan S, Ingram D, Milner AD.** Assessment of effect of nasal continuous positive pressure on laryngeal opening using fibre optic laryngoscopy. *Arch Dis Child* 80: F230–F232, 1999.

9. **Gewolb IH, Bosma JF, Taciak VL, Vice FL.** Abnormal developmental patterns of suck and swallow rhythms during feeding in preterm infants with bronchopulmonary dysplasia. *Dev Med Child Neurol* 43: 454–459, 2001.
10. **Gewolb IH, Fishman D, Qureshi MA, Vice FL.** Coordination of suck-swallow-respiration in infants born to mothers with drug-abuse problems. *Dev Med Child Neurol* 46: 700–705, 2004.
11. **Gewolb IH, Vice FL, Schwietzer-Kenney EL, Taciak VL, Bosma JF.** Developmental patterns of rhythmic suck and swallow in preterm infants. *Dev Med Child Neurol* 43: 22–27, 2001.
12. **Hanlon MB, Tripp JH, Ellis RE, Flack FC, Selley WG, Shoemith HJ.** Deglutition apnoea as indicator of maturation of suckle feeding in bottle-fed preterm infants. *Dev Med Child Neurol* 39: 534–542, 1997.
13. **Kijima M, Isono S, Nishino T.** Modulation of swallowing reflex by lung volume changes. *Am J Respir Crit Care Med* 162: 1855–1858, 2000.
14. **Koenig JS, Davies AM, Thach BT.** Coordination of breathing, sucking, and swallowing during bottle feedings in human infants. *J Appl Physiol* 69: 1623-1629, 1990.
15. **Lau C, Smith EO, Schanler RJ.** Coordination of suck-swallow and swallow respiration in preterm infants. *Acta Paediatrica* 92: 721–727, 2003.
16. **Mahmoud RA, Roehr CC, Schmalisch G.** Current methods of non-invasive ventilatory support for neonates. *Paediatr Respir Rev* 12: 196-205, 2011.

17. **Marquez JM, Douglas ME, Downs JB, Wu WH, Mantini EL, Kuck EJ, Calderwood HW.** Renal function and cardiovascular responses during positive airway pressure. *Anesthesiology* 50: 393-398, 1979.
18. **Martin-Harris B.** Coordination of respiration and swallowing. In Shaker and Goyal GI Motility online-Part 1 Oral cavity, pharynx and esophagus (Goyal R, R. S eds.), vol. 2008. pp. web page. London: Nature Publishing Group; 2006: [<http://www.nature.com/gim o/contents/pt1 /full/gim o10.htm>]]
19. **Mizuno K, Ueda A.** The maturation and coordination of sucking, swallowing, and respiration in preterm infants. *J Pediatr* 142: 36–40, 2003.
20. **Morley CJ, Davis PG.** Continuous positive airway pressure: Scientific and clinical rationale. *Curr Opin Pediatr* 20:119–124, 2008.
21. **Nyqvist KH.** Early attainment of breastfeeding competence in very preterm infants. *Acta Paediatr* 97: 776–781, 2008.
22. **Pickler RH, Chiaranai C, Reyna BA.** Relationship of the first suck burst to feeding outcomes in preterm infants. *J Perinat Neonatal Nurs* 20: 157–162, 2006.
23. **Pinelli J, Symington A.** Non-nutritive sucking for promoting physiologic stability and nutrition in preterm infants. *Cochrane Database Syst Rev* 4: CD001071, 2005.
24. **Pinnington LL, Smith CM, Ellis RE, Morton RE.** Feeding efficiency and respiratory integration in infants with acute viral bronchiolitis. *J Pediatr* 137: 523–526, 2000.
25. **Praud J-P.** Upper airway reflexes in response to gastric reflux. *Paediatr Respir Rev* 11: 208-212, 2010.

26. **Reix P, Arsenault J, Langlois C, Niyonsenga T, Praud J-P.** Non-nutritive swallowing and respiration coordination in preterm lambs *J Appl Physiol* 97: 1283-1290, 2004.
27. **Reix P, Fortier PH, Niyonsenga T, Arsenault J, Letourneau P, Praud J-P.** Non-nutritive swallowing and respiration coordination in full-term newborn lambs. *Respir Physiol Neurobiol* 134: 209–218, 2003.
28. **Roehr CC, Schmalisch G, Khakban A, Proquitte H, Wauer RR.** Use of continuous positive airway pressure (CPAP) in neonatal units--a survey of current preferences and practice in Germany. *Eur J Med Res* 12: 139–144, 2007.
29. **Samson N, Dumont S, Specq ML, Praud J-P.** Radio telemetry devices to monitor breathing in non-sedated animals. *Respir Physiol Neurobiol* 179: 111-118, 2011.
30. **Samson N, Roy B, Ouimet A, Moreau-Bussiere F, Dorion D, Mayer S, Praud J-P.** Origins of the inhibiting effects of nasal CPAP on nonnutritive swallowing in newborn lambs. *J Appl Physiol* 105: 1083–1090, 2008.
31. **Samson N, St-Hilaire M, Nsegbe E, Reix P, Moreau-Bussiere F, Praud J-P.** Effect of nasal continuous or intermittent positive airway pressure on nonnutritive swallowing in the newborn lamb. *J Appl Physiol* 99: 1636–1642, 2005.
32. **Simpson C, Schanler RJ, Lau C.** Early Introduction of Oral Feeding in Preterm Infants. *Pediatrics* 110: 517–522, 2002.
33. **St-Hilaire M, Nsegbe E, Gagnon-Gervais K, Samson N, Moreau-Bussiere F, Fortier PH, Praud J-P.** Laryngeal chemoreflexes induced by acid, water, and saline

- in non-sedated newborn lambs during quiet sleep. *J Appl Physiol* 98: 2197–2203, 2005.
34. **St-Hilaire M, Samson N, Nsegbe E, Duvareille C, Moreau-Bussière F, Micheau P, Lebon J, Praud J-P.** Postnatal maturation of laryngeal chemoreflexes in the preterm lamb. *J Appl Physiol* 102: 1429-1438, 2007.
35. **Thach BT.** Some aspects of clinical relevance in the maturation of respiratory reflexes in infants. *J Appl Physiol* 104: 1828-1834, 2008.
36. **Thomas JA.** Guidelines for bottle feeding your premature baby. *Adv Neonatal Care* 7: 311–318, 2007.
37. **Thoyre SM.** Feeding outcomes of extremely premature infants after neonatal care. *J Obstet Gynecol Neonatal Nurs* 36: 366-375, 2007.
38. **White-Traut RC, Berbaum ML, Lessen B, McFarlin B, Cardenas L.** Feeding readiness in preterm infants: the relationship between preterm behavioral state and feeding readiness behaviors and efficiency during transition from gavage to oral feeding. *MCN Am J Matern Child Nurs* 30: 52–59, 2005.

FIGURE LEGENDS

Figure 1: Sample tracing obtained in one lamb during feeding with nCPAP 10 cmH₂O application

ECG, electrocardiogram; TA, electrical activity of the thyroarytenoid muscle (a glottal constrictor muscle); ∫TA, integrated TA; lung volume, sum signal of the respiratory inductance plethysmograph, allowing qualitative measurement of respiration (inspiration upward); mask pressure, nasal mask pressure, allowing verification of the pressure delivered to the lamb; SpO₂, oxygen hemoglobin saturation measured by pulse oximetry. Arrows indicate nutritive swallowing.

Figure 2: Percentage of time spent in apnea in every experimental condition

Before, before feeding; during, during feeding; after, after feeding; n, no mask condition; 0, nCPAP 0 cmH₂O condition; 4, nCPAP 4 cmH₂O condition; 7, nCPAP 7 cmH₂O condition; 10, nCPAP 10 cmH₂O condition. * vs. before; †: vs. after. Underlined symbols indicate $P < 0.05$, normal font symbols indicate $P \leq 0.1$. All other P values are greater than 0.1.

Figure 3: Feeding efficiency (NS frequency, mL/NS and mL/min) during each nCPAP condition

* : vs. nCPAP 10 cmH₂O condition. Underlined symbols indicate $P < 0.05$, normal font symbols indicate $P \leq 0.1$. All other P values are greater than 0.1. All other P values are greater than 0.1. See Figure 2 for abbreviations.

Figure 4: nCPAP does not modify nutritive swallowing-breathing coordination

ii, NS preceded and followed by inspiration; ie, NS at the transition from inspiration to expiration; ee, NS preceded and followed by expiration; ei, NS at the transition from expiration to inspiration; ap, NS occurring during an apnea. No statistical differences were observed between each NS type when compared among all nCPAP conditions ($P = 1.0$). Thus, nCPAP did not modify nutritive swallowing-breathing coordination. While NS could occur at any moment in the respiratory cycle, some NS-types were more frequent than others, with ii, ie and ei-types NS significantly more frequent than both ee and ap-type NS in almost all conditions. For reason of clarity, these significant interactions are not shown in figure 4, as these results are not part of the main objective of the present study. See Figure 2 for other abbreviations.

TABLES

Table 1: Effects of nCPAP conditions and feeding on cardiorespiratory variables

	No mask	nCPAP0	nCPAP4	nCPAP7	nCPAP10
RR, min⁻¹					
Before	35 (10)	37 (11)	34 (6)	32 (9)	36 (9)
During	41 (13)	39 (7)	33 (6)*	34 (8)*	38 (6)
After	45 (11) [§]	37 (9)*	37 (8)*	38 (8)*	36 (7)*
HR, min⁻¹					
Before	168 (25)	201 (31)*	199 (27)*,‡	211 (31)*	221 (38)*
During	164 (24)	183 (23) ^{‡,a}	180 (24) ^{‡,a}	184 (22) ^{‡,§,a}	205 (32)*
After	180 (25)	208 (30)*,‡	206 (32)*,‡	225 (31)*	240 (24)*
SpO₂, %					
Before	99 (1)	98 (2)	98 (2)	97 (2)	98 (2)
During	97 (2)	97 (3)	97 (2)	96 (3)	98 (1)
After	98 (2)	97 (2)	98 (1)	96 (3)	98 (2)

Values are expressed as mean (SD). RR, respiratory rate; HR, heart rate; SpO₂, hemoglobin O₂ saturation; before, before feeding; during, during feeding; after, after feeding. * : vs. no mask, § : vs. before, ‡ : vs. nCPAP10, ^a : vs. after, [‡] : vs. nCPAP7. Underlined symbols indicate $P < 0.05$, normal font symbols indicate $P \leq 0.1$. All other P values are greater than 0.1.

Table 2: Effects of nCPAP conditions and feeding on cardiorespiratory events

	No mask	nCPAP0	nCPAP4	nCPAP7	nCPAP10
Apnea frequency, min⁻¹					
Before	0.1 (0.2) [†]	0.2 (0.2)	0.1 (0.2)	0.1 (0.1)	0.2 (0.3)
Feeding	0.7 (0.7) ^{‡,±}	0.5 (0.6) ^{*,±}	0.7 (0.7) ^{‡,±}	0.6 (0.5) ^{‡,±}	0.5 (0.5) ^{*,±}
After	0.03 (0.05)	0.04 (0.1)	0.1 (0.2)	0.05 (0.1)	0.05 (0.1)
HR slowing frequency, min⁻¹					
Before	0.1 (0.1)	0.2 (0.3)	0.05 (0.1)	0.1 (0.2)	0.4 (0.7)
Feeding	3 (5) ^{*,†}	2 (3) ^{*,†}	2 (3)	4 (7)	7 (15)
After	0.4 (0.9)	0.1 (0.1)	0.1 (0.2)	0.2 (0.3)	0.2 (0.3)
Desaturation frequency, min⁻¹					
Before	0	0	0	0.1 (0.2)	0.03 (0.08)
Feeding	0.04 (0.1)	0.4 (0.7) ^{‡,±}	0.1 (0.3)	0.2 (0.4)	0.1 (0.2)
After	0.03 (0.05)	0.1 (0.1)	0	0.1 (0.2)	0.02 (0.04)
% time spent in desaturation					
Before	0	0	0	4 (9)	2 (4)
Feeding	1 (3) [‡]	5 (9)	1 (3) [‡]	7 (17)	2 (4)
After	1 (3)	4 (6)	0 [‡]	6 (9)	0.2 (0.5)

Values are expressed as mean (SD). †: vs. after, *: vs. before, ‡: vs. nCPAP7. Underlined symbols indicate $P < 0.05$, normal font symbols indicate $P \leq 0.1$. All other P values are greater than 0.1. See Table 1 for abbreviations.

Table 3: Effects of nCPAP conditions and feeding on arterial blood gases and pH

	No mask	nCPAP0	nCPAP4	nCPAP7	nCPAP10
PaO₂, Torr					
Before	85 (12)	92 (11)	93 (12)	91 (4)	88 (9)
0 after	87 (17)	89 (10)	91 (10)	88 (19)	92 (8)
5 after	86 (18)	89 (12)	89 (15)	88 (19)	91 (8)
10 after	84 (15)	91 (17)	90 (13)	88 (13)	93 (11)
PaCO₂, Torr					
Before	43 (3)	43 (4)	42 (2)	43 (2)	43 (5)
0 after	40 (5)	40 (5)	41 (3)	42 (2)	42 (4)
5 after	42 (4)	41 (4)	42 (2)	41 (3)	41 (5)
10 after	42 (3)	42 (3)	40 (2)	40 (3)	42 (7)
pH					
Before	7.44 (0.05)	7.45 (0.04)	7.42 (0.04)	7.41 (0.02)*	7.41 (0.03)*
0 after	7.41 (0.02)	7.42 (0.02)	7.41 (0.04)	7.41 (0.02)	7.41 (0.03)
5 after	7.43 (0.03)	7.43 (0.03)	7.41 (0.04)	7.41 (0.02)	7.41 (0.03)
10 after	7.43 (0.04)	7.43 (0.02)	7.41 (0.04)	7.42 (0.02)	7.41 (0.03)*

Values are expressed as mean (SD). Before, before feeding; 0 after, immediately after feeding; 5 after, 5 minutes after feeding; 10 after, 10 minutes after feeding. * : vs. nCPAP0. Underlined symbols indicate $P < 0.05$, normal font symbols indicate $P \leq 0.1$. All other P values are greater than 0.1.

Table 4: Effects of nCPAP conditions on feeding efficiency and nutritive swallowing-breathing coordination

	No mask	nCPAP0	nCPAP4	nCPAP7	nCPAP10
Feeding efficiency					
% of NS in runs	96 (6)	86 (19)	95 (7)	93 (13)	97 (3)
COV NS-NS	0.5 (0.1)	0.5 (0.1)	0.4 (0.1)	0.5 (0.05)	0.5 (0.1)
Nutritive swallowing-breathing coordination					
COV NS-BR	0.7 (0.2)	0.7 (0.2)	0.7 (0.1)	0.7 (0.1)	0.7 (0.1)
COV BR-BR	0.5 (0.2)	0.4 (0.2)	0.5 (0.2)	0.5 (0.3)	0.4 (0.1)

Values are expressed as mean (SD). NS, nutritive swallowing; COV NS-NS, coefficient of variation of NS-NS intervals; COV NS-BR, COV of NS-breath intervals; COV BR-BR, COV of BR-BR intervals. All *P* values are greater than 0.1.

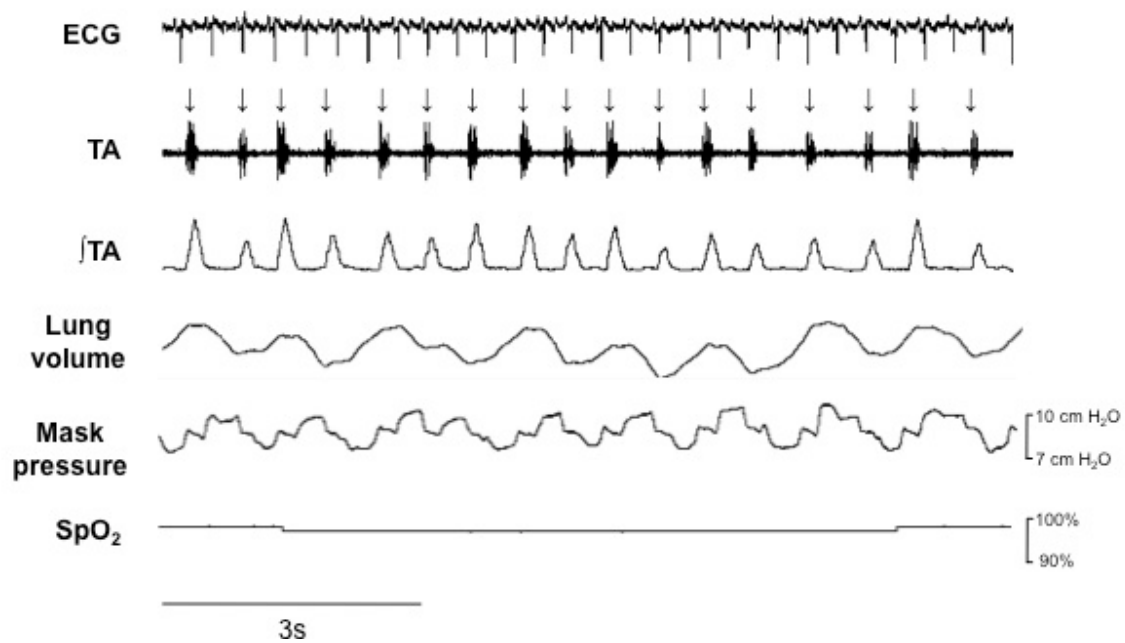
Figure 1

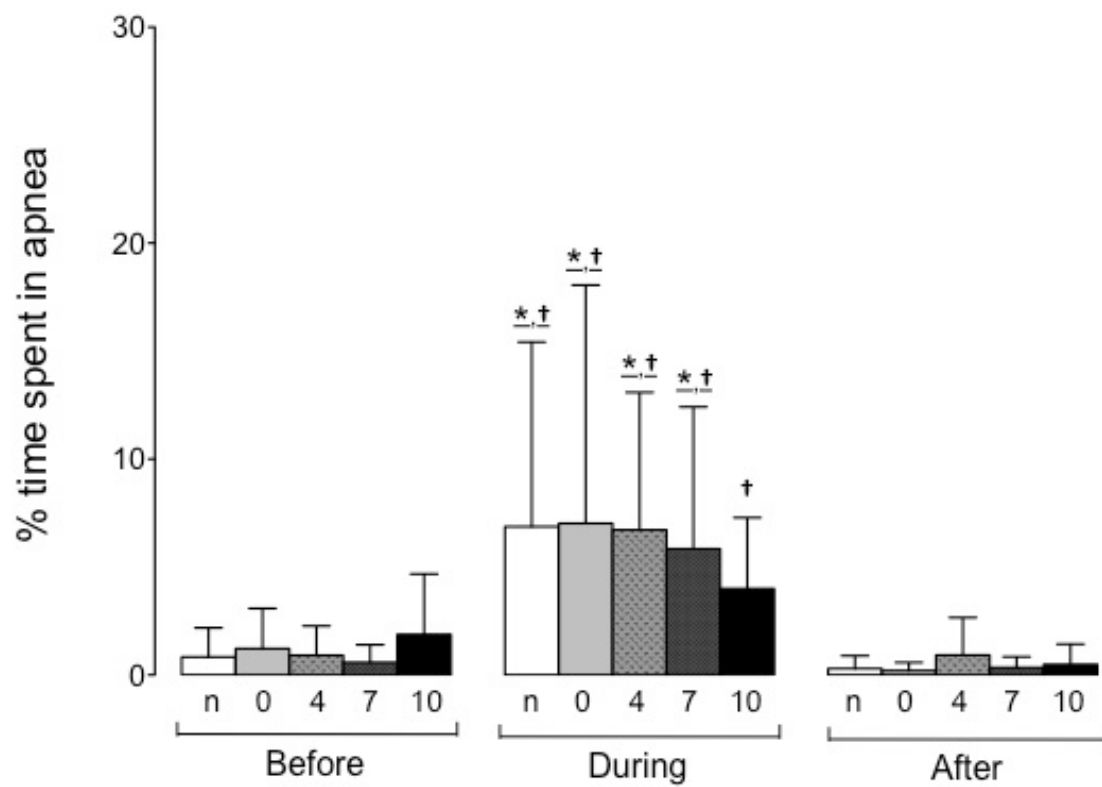
Figure 2

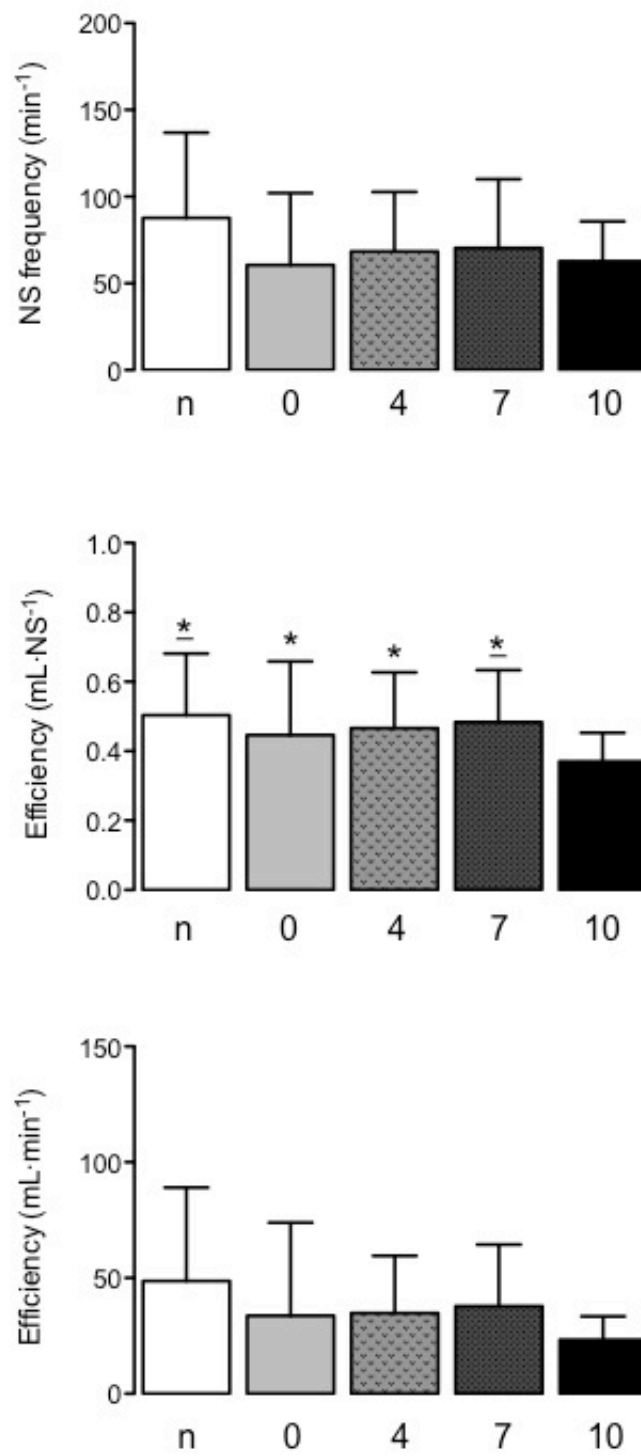
Figure 3

Figure 4