Nasal high-frequency oscillatory ventilation inhibits gastroesophageal reflux in the neonatal period

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Abstract
Nasal high-frequency oscillatory ventilation (nHFOV) in neonates is increasingly considered due to enhanced alveolar ventilation, absence of patient-ventilator asynchrony and lessened ventilator-induced lung injury. Although any type of non-invasive respiratory support can lead to gastric distension via esophageal air passage and thus promote gastroesophageal refluxes (GERs), we have shown that nasal continuous positive airway pressure (CPAP; 6 cmH₂O) and intermittent positive pressure ventilation (15/4 cmH₂O) conversely inhibit GERs in lambs. The current objective was to test the hypothesis that nHFOV also inhibits GERs compared to spontaneous ventilation without respiratory support. Eight lambs underwent five hours of polysomnographic and esophageal multichannel intraluminal impedance pHmetry recordings to assess GERs and air passage into the esophagus, with and without nHFOV (mean airway pressure = 8 cmH₂O, oscillation frequency = 8 Hz, amplitude = 20 cmH₂O and I:E = 1:2). Results revealed that GERs were decreased with nHFOV (p = .03), despite an increase in gas-containing swallows (p = .01). In conclusion, similarly to nasal CPAP and intermittent positive pressure ventilation, nHFOV inhibits GERs in newborn lambs.

1. Introduction
The use of non-invasive respiratory support has markedly increased in pediatrics over the last years. This is especially true in neonates, in an effort to counter the complications caused by endotracheal mechanical ventilation, such as bronchopulmonary dysplasia (BPD) (Davidson and Berkelhamer, 2017). Nasal continuous positive airway pressure (nCPAP), in particular, has become the standard initial care for preterm infants at birth (De Luca and Dell’Orto, 2016; Sweet et al., 2017). However, nCPAP often fails to prevent endotracheal ventilation in the most premature newborns at birth (Cummings and Polin, 2016) and is not always successful after endotracheal extubation in convalescing preterm infants (Lemyre et al., 2017).

Several non-invasive positive pressure ventilation modalities have been assessed in recent years in neonates, having as objective to improve the efficiency of alveolar ventilation without incurring deleterious effects on the lung. One significant addition to the respiratory support armamentarium consists in nasal high frequency oscillatory ventilation (nHFOV). Recent reviews have highlighted the advantages of nHFOV in human neonates and animals (De Luca and Dell’Orto, 2016; Mukerji and Dunn, 2016; Yoder et al., 2016), including increased alveolar ventilation and absence of patient-ventilator asynchrony. While mostly anecdotal, the use of nHFOV has also been reported in full-term newborns at birth (Dumas De La Roque et al., 2011; Yoder et al., 2016) and beyond (De Luca et al., 2016; Del Torre et al., 2014; Gregoretti et al., 2016), as well as in adult humans (Feltracco et al., 2012; Ogna et al., 2017).

Non-invasive respiratory support, regardless of the modality, can lead to abdominal distension via air passage into the esophagus (DiBlasi, 2011; Shepherd et al., 2013); this also includes under nHFOV (Fischer et al., 2015). The resulting adverse events in neonates are currently much less dramatic than initially reported (Garland et al., 1985) and mainly consist in abdominal distension with or without feeding intolerance (Jaile et al., 1992; Jeon, 2016). Notwithstanding, gastric distension is nonetheless an important trigger of transient relaxation of the lower esophageal sphincter (LES), which is the main mechanism responsible for gastroesophageal refluxes (GERs) in newborns (Davidson, 2003; Omari et al., 2002). Deleterious cardiovascular events can ensue in the immature newborn, especially when laryngopharyngeal refluxes trigger laryngeal chemoreflexes (Pickens et al., 1988; Praud, 2010; Thach, 1997). Of importance, we have recently shown that both nCPAP (6 cmH₂O) and nasal intermittent positive pressure ventilation at levels which do not induce significant gastric distension (15/4 cmH₂O), have a strong inhibitory effect on GERs in...
newborn lambs (Cantin et al., 2016; Djeddi et al., 2014). These unique results in the neonatal period are in agreement with previous results in adult humans with nCPAP, whether in healthy individuals (Kerr et al., 1993; Shepherd et al., 2007) or in the presence of obstructive sleep apnea syndrome (Tamanna et al., 2016; Tawk et al., 2006). In light of the above, the present physiological study pursues our exploration of the effect of non-invasive respiratory support on GERs, with the aim of providing the proof of concept that nHFOV can also inhibit GER.

2. Materials and methods

Eight full-term lambs aged 4–5 days and weighing 3.8 ± 0.4 kg (mean ± SD) were involved in the study. The Ethics Committee for Animal Care and Experimentation of the Université de Sherbrooke approved the study (protocol # 283-11). The care and handling of the animals were in accord with the Canadian Council on Animal Care.

2.1. Chronic instrumentation and experimental equipment

Chronic surgical instrumentation was performed under general anesthesia and included insertion of i) custom-built bipolar electrodes into both thyroarytenoid (a laryngeal constrictor) muscles for recording swallowing activity, ii) a catheter into the left carotid artery for blood gas analysis and iii) a transcutaneous catheter between the fourth and fifth tracheal ring to monitor tracheal pressure (Carriere et al., 2015).

Lamb instrumentation was completed immediately before the recordings. Needle electrodes were inserted subcutaneously for electroencephalogram (EEG), electrooculogram (EOG) and electrocardiogram (ECG) recordings. Elastic bands were installed on the chest and abdomen to record lung volume variations via respiratory inductance plethysmography (Ambulatory Monitoring, Ardsley, NY). Continuous monitoring of oxygen hemoglobin saturation (SpO2) was performed using a pulse oximeter probe (LNOP YI reflectance sensor, Masimo Radical, Irvine, CA) at the tail base (Carriere et al., 2015).

Nasal HFOV was delivered using a high-frequency oscillatory ventilator (Sensormedics 3100a, Cardinal Health, Canada) with heated and humidified air through a plastic nasal mask custom-tailored for newborn lambs (Hadj-Ahmed et al., 2015). The mask was filled with dental paste to decrease the dead space and prevent leaks. The nasal mask was not installed for the control condition (= spontaneous breathing). Finally, both GERs and swallows were continuously assessed via a MII-pH catheter (Unisensor, Portsmouth, USA) inserted transnasally, its position being confirmed by radiography and secured with sutures (Djeddi et al., 2012).

Physiological signals were transmitted wirelessly and recorded on a PC (Samson et al., 2011). The entire recording period was also filmed with a webcam, and an experimenter was present throughout the recordings.

Blood gases (GEM Premier 3000 PAK, Instrumentation Laboratory, Lexington, MA) and temperature were measured before, in the midportion, and at the end of the recording. The abdominal perimeter was measured before and at the end of the recording to grossly assess gastric distension.

2.2. Design of the study

Chronic instrumentation was performed on the day of arrival of the lamb in our animal quarters. Forty-eight hours later, five-hour polysomnographic recordings were performed without sedation during nHFOV and control conditions, on two successive days in each lamb, according to a cross-over design. The order of the recordings was randomized such that nHFOV was applied on the first day in half of the lambs. Lambs were bottle-fed one hour prior to recordings with 15 ml/kg of reconstituted ewe milk (corresponding to the usual amount ingested from a bottle by a full-term newborn lamb). They were not fed during the recordings. For nHFOV, the same settings as in our previous study in lambs were used, namely an oscillation frequency of 8 Hz, a mean airway pressure (MAP) of 8 cmH2O, an I:E ratio of 1:2 and an oscillation amplitude (delta P) level when visible abdominal wall oscillations were present, i.e. 17–20 cmH2O in the present study (Hadj-Ahmed et al., 2015). At the time of the study, these settings were chosen so as to correspond to the settings most often reported in previous clinical studies in preterm infants (Fischer et al., 2015; Mukerji and Dunn, 2016; Yoder et al., 2016). Lambs were placed in a sling and able to sleep ad libitum throughout the five-hour sessions.

3. Data analysis

3.1. States of alertness

Standard electrophysiological and behavioral criteria were used to define quiet and active wakefulness, as well as non REM (NREM) and REM sleep (REM) (Renolleau et al., 1999).

3.2. Effect of nasal high-frequency oscillatory ventilation on gastroesophageal reflexes

MII-pH recordings were automatically analyzed with the MMS software (Medical Measurement Systems USA, Dover, NH) and visually verified. The total number of GERs during five hours, as well as the number of GERs per hour of recording and per hour of each state of alertness were calculated. Characterization of GERs as liquid, purely gaseous or mixed, as well as their pH, was performed according to published criteria (Djeddi et al., 2012).

3.3. Effect of nasal high-frequency oscillatory ventilation on swallowing

3.3.1. Gas-containing swallows

Calculation of gas-containing swallows was performed to assess whether nHFOV increased air passage into the esophagus/stomach. MII-pH recordings were used to count the total number of gas-containing swallows and to calculate the gas-containing swallows index (number of gas-containing swallows per hour). The effect of the state of alertness on gas-containing swallows was also assessed. A gas-containing swallow was defined as a short and high-amplitude burst of laryngeal constrictor muscle activity (= swallow) together with an increased esophageal impedance of at least 1000 ohm on at least four of the six impedance channels. Gas-containing swallows were then sorted as complete (the increased impedance reached the lowest channel) or partial (the increased impedance did not reach the lowest channel). A complete gas swallow was deemed particularly relevant since it could cause gastric distension. Of note, the swallowing assessment did not allow quantifying the amount of air swallowed.

3.3.2. Non-nutritive swallows in NREM

Non-nutritive swallows, i.e. swallows unrelated to feeding, corresponding to the sum of gas-containing swallows and purely liquid swallows were computed in NREM only. Non-nutritive swallows were defined in the present study as a burst of laryngeal constrictor muscle activity together with a change of at least 1000 ohm on at least four of the six impedance channels. Comparison of the non-nutritive swallow index during nHFOV and control condition allowed assessing whether nHFOV has a similar inhibiting effect on non-nutritive swallows as nCPAP in NREM (Samson et al., 2005).

3.4. Statistical analysis

All statistical tests were performed under the close supervision of the biostatistician of our research center. Normality was systematically tested using the Shapiro-Wilk test, which revealed that distributions were not normal. All data are expressed as median and interquartile range (Q1, Q3). Statistical analyses were performed on raw data.
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The total number of gastroesophageal refluxes (top portion) and the gastroesophageal reflux index (bottom portion) are shown in control and nasal high-frequency oscillatory ventilation conditions for each state of alertness. REM sleep periods were not analyzed due to minimal time spent in this state. AW: active wakefulness, QW: quiet wakefulness, NREM: non REM sleep.

Comparisons between control and nHFOV conditions were carried out using the Wilcoxon signed-rank test for all variables. The Friedman test completed by the Wilcoxon signed-rank test was used to assess the effects of the states of alertness. A p value < 0.05 was deemed significant. Statistical analyses were performed using SPSS software (version 22, Chicago, IL, USA).

4. Results

4.1. Effect of nasal high-frequency oscillatory ventilation on gastroesophageal refluxes

GERs were strongly inhibited by nHFOV [0 (0, 1)] compared to control condition [7 (3, 20)] (p = .03). Moreover, GER frequency was lower during nHFOV compared to control condition in active wakefulness, quiet wakefulness and NREM (p < 0.05) (Table 1). REM sleep could not be considered due to the very limited time spent in this sleep state. Of note, the total number of GERs was higher during the first two hours in control condition (p = .03 for the first hour) (Fig. 1).

4.2. Effect of nasal high-frequency oscillatory ventilation on swallowing

The total number of gas-containing swallows was higher in nHFOV [292 (214, 425)] compared to control condition [121 (64, 183)] (p = .01). In addition, complete gas-containing swallows were more frequent during nHFOV [273 (163, 423)] compared to control condition [101 (44, 164)] (p = .01). Conversely, no difference was observed for partial gas-containing swallows. Note that for both conditions, 90% of the gas-containing swallows were complete (change in impedance down to the lower esophageal channel).

The total number and index of non-nutritive swallows in nHFOV did not differ relative to control condition during NREM (Fig. 2). However, the number and index of liquid swallows was decreased during nHFOV (p = .01 for both) while the number and index of gas-containing swallows was higher in nHFOV (p = .01 for both) (Fig. 2).

4.3. States of alertness

Compared to control condition, a higher percentage of time was spent in quiet wakefulness in nHFOV [62 (61, 67) % vs. 56 (52, 59) %, p = .02], while there was no difference in time spent in active wakefulness [5 (3, 8) % vs. 12 (10, 13) %] and NREM [26 (22, 34) % vs. 20 (25, 33) %]. The time spent in REM was insufficient to enable comparison.

4.4. Abdominal circumference

The increase in abdominal circumference was significantly greater during nHFOV compared to control condition [median = + 1.8 (0.5, 3.0) cm vs. −0.8 (-1.0, −0.5) cm, p = .02].

5. Discussion

This novel physiological study demonstrates that nasal high-frequency oscillatory ventilation, with an oscillation frequency of 8 Hz, an I:E ratio of 1:2, an oscillation amplitude nearing 20 cmH2O and a mean airway pressure of 8 cmH2O, inhibits gastroesophageal reflux in newborn lambs.

5.1. The newborn lamb model for studying nasal respiratory support and gastroesophageal refluxes

Over the past ten years, we have gained a unique and extensive expertise in non-invasive ventilatory support in lambs (Cantin et al., 2016; Djeddi et al., 2014; Hadj-Ahmed et al., 2012; Hadj-Ahmed et al., 2015; Moreau-Bussiere et al., 2007) as well as headlined studies using GER recordings in the newborn full-term lamb in which GERs can be readily characterized using MII-pH in non-sedated conditions (Djeddi et al., 2012). The preruminant, monogastric newborn lamb has a well-defined lower esophageal sphincter and presents spontaneous GERs, which bear several similar features to those of the human infant.

![Time course of the number of gastroesophageal refluxes in control and nasal high-frequency oscillatory ventilation conditions during the five-hour recording. In control condition, the number of gastroesophageal refluxes was higher during the first two hours of the recording period (p = .03 during the first hour and p = .1 during the second hour) while no differences were observed during the last three hours. Solid line = control condition, dotted line = nasal high-frequency oscillatory ventilation condition. **: p < 0.05](image)
Moreover, the lamb offers a unique means to perform a prospective study where nasal respiratory support conditions can be strictly controlled and randomized in the same subject. This allows gaining unique physiological knowledge, paving the way for the design of clinical studies in neonates.

Due to its different size and maturation, the full-term lamb however cannot pretend to mimic the very low birth weight infant, in whom nHFOV has been mostly used clinically. Forthcoming studies using our unique preterm lamb model (Samson et al., 2018), despite not being as immature as a very low birth weight infant, will nonetheless help determine whether prematurity affects the results obtained in the present study. Aside from the current high interest of using nHFOV in premature infants, the potential usefulness of nHFOV in full-term newborns (Dumas De La Roque et al., 2011) as well as beyond the neonatal period (De Luca et al., 2016; Del Torre et al., 2014; Gregoretti et al., 2016) further highlights the relevance of our present study for human infants.

5.2. Decrease in gastroesophageal refluxes during nasal high-frequency oscillatory ventilation

With nHFOV, as with any other nasal respiratory support, the gas applied at positive pressure into the upper aerodigestive tract can be deviated into the esophagus. This in turn can cause gastric distension, whose consequences include GERs (DiBlasi, 2011; Shepherd et al., 2013). The high prevalence of the latter, as well as GER disease in the neonatal period (Vandenplas et al., 2009), underlines the importance of our results showing that nHFOV, on the contrary, can decrease GERs. Interestingly, a decrease in refluxes during nHFOV occurred despite a significantly greater abdominal distension compared to the control condition.

The present findings complement our previous observations in newborn lambs that GERs are inhibited by nCPAP at 6 cmH2O (Djeddi et al., 2014) as well as by two different modes of nasal intermittent positive pressure ventilation at 15/4 cmH2O (Cantin et al., 2016). As a whole, our results in lambs thus extend similar reports of GER inhibition with nCPAP in adult humans (Kerr et al., 1993; Shepherd et al., 2007; Tamanna et al., 2016; Tawk et al., 2006) to the neonatal period and to all types of non-invasive positive airway pressure support tested. The mechanisms by which nHFOV inhibits GER still remains to be fully elucidated. Beyond decreasing the thoraco-abdominal pressure gradient, which drives GER through the LES, it is suggested that nCPAP primarily acts by decreasing the duration and depth of LES relaxations (Shepherd et al., 2007), the latter of which are the principal mechanism responsible for GER. Our previous results also supported this hypothesis, by showing that nCPAP decreased the duration and depth of swallow-induced LES relaxation in lambs (Djeddi et al., 2014). Another proposed mechanism stems from the fact that transient LES relaxations are initiated by a longitudinal contraction of the esophagus, which displaces the LES orally. By displacing the diaphragm and the mediastinum caudally, hence increasing esophagus length, the nasally applied positive pressure thereby putatively increase the preload of the longitudinal esophageal muscle. This would decrease its ability to displace the LES and in turn impede LES relaxation (Shepherd et al., 2007).
Regardless of the mechanism(s) inhibiting GER during nCPAP, the similar findings previously obtained with nasal intermittent positive pressure (Cantin et al., 2016) and now with nHFOV substantiate the universal effect nasal positive airway pressure support can have on GER.

5.3. Swallows

Our present observations further show that the number of gas-containing swallows was significantly increased with nHFOV. In addition, the vast majority of gas-containing swallows traveled along the length of the esophagus. These results were expected as both nCPAP and nIPPV are known to increase air passage into the esophagus (Cantin et al., 2016; Mahmoud et al., 2011). Our findings are however the first, to our knowledge, to report gas-containing swallows in nHFOV.

We have previously shown that nCPAP decreases the number of non-nutritive swallows (purely liquid swallows + gas-containing swallows) in NREM (Samson et al., 2005). Moreover, we have shown that this inhibition is mediated by stimulation of bronchopulmonary receptors (most likely the slowly adapting stretch receptors) and can also be mediated by stimulation of upper airway reflexes in certain situations (Samson et al., 2008). The present results show that, conversely to nCPAP, nHFOV did not decrease non-nutritive swallows. However, nHFOV decreased the purely liquid swallows whereas the gas-containing swallows were increased. These differences may be related to the presence of oscillations in nHFOV compared to nCPAP.

5.4. Study limitations

Given that lambs did not wear the nasal mask during the control condition, we cannot exclude that some of the effects on GER and swallowing ascribed to nHFOV may be partly related to the mask itself. Moreover, the effects of nHFOV on GER occurrence and swallowing were tested using fixed settings adapted to non-sedated, healthy full-term lambs without respiratory difficulties. Review of the literature shows that different settings, especially higher oscillation frequency, mean airway pressure, oscillation amplitude and I:E ratio are necessary in infants in need of high-level respiratory support (see reviews in De Luca and Dell’Orto, 2016; MUKer et al., 2017; Yoder et al., 2016). We acknowledge that the effects of nHFOV may be different with such higher settings. The settings used herein remain however within or close to the lower end of the range of values used or suggested in recent publications on the clinical use of nHFOV (De Luca and Dell’Orto, 2016; Mükjeri et al., 2017; Yoder et al., 2016; Zhu et al., 2017). In addition, application of nHFOV with higher settings for several hours could lead to lamb discomfort and thus important alterations in breathing (Hadj-Ahmed et al., 2015), which in themselves could add unwanted effects on the results. Moreover, from a clinical standpoint, the study was conducted in healthy lambs for only 5 h, while patients requiring respiratory support may need such support for several days or more, in a setting marked by increased inspiratory efforts. Lastly, as already alluded to, our full-term lamb model cannot pretend to mimic very low birth weight infants with respiratory distress, who currently represent the main clinical indication of nHFOV. Preterm infants may be more prone to air passage into the esophagus and consequently to more frequent complications related to gastric distension (Fischer et al., 2015; Garland et al., 1985). Our forthcoming studies on non-invasive positive pressure respiratory support in general, and on nHFOV in particular, will involve preterm lambs. We will investigate, among others, the effect of prematurity on air passage into the esophagus, on lower esophageal sphincter physiology and on GER occurrence; results from the present study will thus serve as comparison to infer the effect of prematurity. Overall, despite the above limitations, our results constitute a unique proof of concept that nHFOV can inhibit GER in the neonatal period.

6. Conclusion

Results from the present physiological study and previous knowledge show that all types of non-invasive positive pressure respiratory support tested, including nHFOV, efficiently inhibit GERs in full-term newborn lambs, at least in the absence of important gastric distension. Pending similar observations in humans, we propose that the present observations may be of significant clinical importance in infants under nHFOV.

Conflicts of interest and source of funding

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