Neonatal total liquid ventilation: is low-frequency forced oscillation technique suitable for respiratory mechanics assessment?
Dominick Bossé, Alexandre Beaulieu, Olivier Avoine, Philippe Micheau, Jean-Paul Praud and Hervé Walti


---

You might find this additional info useful...

This article cites 57 articles, 20 of which you can access for free at:
http://jap.physiology.org/content/109/2/501.full#ref-list-1

Updated information and services including high resolution figures, can be found at:
http://jap.physiology.org/content/109/2/501.full

Additional material and information about *Journal of Applied Physiology* can be found at:
http://www.the-aps.org/publications/jappl

This information is current as of August 28, 2012.
Neonatal total liquid ventilation: is low-frequency forced oscillation technique suitable for respiratory mechanics assessment?

Dominick Bossé,1 Alexandre Beaulieu,2 Olivier Avoine,1 Philippe Micheau,2 Jean-Paul Praud,1,3 and Hervé Walti1,3

1Faculté de Médecine et des Sciences de la Santé, Département de Pédiatrie, 2Faculté de Génie, Département de Génie Mécanique, and 3Faculté de Médecine et des Sciences de la Santé, Département de Physiologie et de Biophysique, Université de Sherbrooke, Sherbrooke, Québec, Canada

Submitted 15 September 2009; accepted in final form 8 June 2010

Bossé D, Beaulieu A, Avoine O, Micheau P, Praud J, Walti H. Neonatal total liquid ventilation: is low-frequency forced oscillation technique suitable for respiratory mechanics assessment? J Appl Physiol 109: 501–510, 2010. First published June 10, 2010; doi:10.1152/japplphysiol.01042.2009.—This study aimed to implement low-frequency forced oscillation technique (LFFOT) in neonatal total liquid ventilation (TLV) and to provide the first insight into respiratory impedance under this new modality of ventilation. Thirteen newborn lambs, weighing 2.5 ± 0.4 kg (mean ± SD), were premedicated, intubated, anesthetized, and then placed under TLV using a specially design liquid ventilator and a perfluorocarbon. The respiratory mechanics measurements protocol was started immediately after TLV initiation. Three blocks of measurements were first performed: one during initial respiratory system adaptation to TLV, followed by two other series during steady-state conditions. Lambs were then divided into two groups before undergoing another three blocks of measurements: the first group received a 10-min intravenous infusion of salbutamol (1.5 μg·kg⁻¹·min⁻¹) after continuous infusion of methacholine (9 μg·kg⁻¹·min⁻¹), while the second group of lambs was chest strapped. Respiratory impedance was measured using serial single-frequency tests at frequencies ranging between 0.05 and 2 Hz and then fitted with a constant-phase model. Harmonic test signals of 0.2 Hz were also launched every 10 min throughout the measurement protocol. Airway resistance and inercance were starkly increased in TLV compared with gas ventilation, with a resonant frequency ≤1.2 Hz. Resistance of 0.2 Hz and reactance were sensitive to bronchoconstriction and dilation, as well as during compliance reduction. We report successful implementation of LFFOT to neonatal TLV and present the first insight into respiratory impedance under this new modality of ventilation. We show that LFFOT is an effective tool to track respiratory mechanics under TLV.

l lung function test; perfluorocarbons; mechanical ventilation; sheep; methacholine chloride

THE ADVENT OF NEONATAL LIQUID-assisted ventilation (LAV) has concurred with global efforts to perfect strategies of ventilation, improving the morbidity and mortality of several clinical conditions, such as meconium aspiration and infant respiratory distress syndrome, while minimizing ventilator-induced injuries. To date, two type of LAV have been used: partial liquid ventilation (PLV) (12) and total liquid ventilation (TLV) (59, 60). The former consists in partly filling the lungs with a liquid, usually a perfluorocarbon (PFC), while a conventional gas ventilator allows gas exchanges. In contrast, during TLV, lungs are completely filled with PFC, and a dedicated liquid ventilator (39) ensures the oxygenation of the fluid and the renewal of a tidal volume of liquid. TLV appears to be superior to PLV (49, 60) and offers many advantages over conventional mechanical ventilation. Among others, TLV has anti-inflammatory properties (41, 48, 52, 53), recruits atelectatic zones of the lung (57), homogenizes ventilation (60) and pulmonary blood flow distribution (23), and reduces inflation pressure by increasing lung compliance (60). Thus TLV improves blood oxygenation and reduces occurrence of baro/volutrauma (15, 60). Moreover, tidal volumes of liquid allow meconium, exudate, and mucus removal and filtering (58), while ensuring adequate ventilation.

A total liquid ventilator must resemble other conventional ventilators, except that it must be able to conduct ventilation with a PFC fluid (8). Various types of liquid ventilators have been developed for conducting animal experiments (2, 21, 22, 28); however, our fourth prototype developed at the Université de Sherbrooke for experimental research on animal models of newborns (Inolivent-4) includes the latest up-to-date devices, findings, and control algorithms. In vivo experimental results with this prototype have demonstrated its efficiency in maintaining adequate gas exchange, normal acid-base equilibrium, and greater minute ventilation while nearing flow limits (38).

As during conventional mechanical ventilation, knowledge of the respiratory mechanics during TLV is of great interest, since it affords valuable insight into the lung state, as well as into underlying disease pathophysiology. Similarly, knowledge of the respiratory mechanics helps clinicians to optimize ventilation, follow treatment progression, plan timely weaning, and prevent iatrogenic injuries (16, 29, 36). The low-frequency forced oscillation technique (LFFOT) (46) appears particularly well suited for this purpose. Readers are referred to review articles for a complete understanding of the technique (13, 25, 27).

Briefly, LFFOT is a noninvasive technique to measure respiratory impedance (Zrs), from which mechanical properties of the overall respiratory system (resistance, elastance, and inercance) can be derived. Interestingly, this technique also enables the use of parametric models to discriminate the contribution of both tissues and airways to respiratory mechanics (14, 17, 34). Furthermore, it appears to be more sensitive to subtle mechanical changes than other lung function testing (45) and has already been used successfully in human infants (7, 30, 34, 46) and a preterm ovine model (32, 33), as well as during bronchoprovocation challenges (5, 18). Finally, since it does not require active collaboration of the patient, LFFOT can be easily performed during mechanical ventilation, especially under general anesthesia and curarization (10, 26).

Address for reprint requests and other correspondence: H. Walti, Faculté de Médecine et des Sciences de la Santé, Département de Pédiatrie, 3001, 12e Ave. Nord, Sherbrooke, Québec, Canada J1H 5N4 (e-mail: Herve.Walti@USherbrooke.ca).
However, unlike conventional mechanical ventilation, there is so far only scarce information relative to dynamic respiratory mechanics during TLV (1, 22, 57). In the present study, we implemented for the first time LFFOT to a total liquid ventilator and test whether this technique enables reliable respiratory mechanics characterization during neonatal TLV. For this purpose, we used healthy newborn lambs under steady-state ventilation conditions and assessed LFFOT responsiveness to changes in respiratory mechanics using methacholine (MCh) and salbutamol infusion, as well as chest bandage.

**Glossary**

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPM</td>
<td>Constant-phase model</td>
</tr>
<tr>
<td>(f)</td>
<td>Frequency (Hz)</td>
</tr>
<tr>
<td>(f_{res})</td>
<td>Resonant frequency (Hz)</td>
</tr>
<tr>
<td>FRF(_1)</td>
<td>Frequency-response complex function (cmH(_2)O/ml)</td>
</tr>
<tr>
<td>G</td>
<td>Tissue damping (cmH(_2)O·s·ml(^{-1}) at 1 rad/s)</td>
</tr>
<tr>
<td>GV</td>
<td>Gas ventilation</td>
</tr>
<tr>
<td>(G_{XY})</td>
<td>Cross-spectral density function estimate between X and Y</td>
</tr>
<tr>
<td>H</td>
<td>Tissue elastance (cmH(_2)O·s·ml(^{-1}))</td>
</tr>
<tr>
<td>Im(Z(_{rs}))</td>
<td>Imaginary part of respiratory system impedance (cmH(_2)O·s·ml(^{-1}))</td>
</tr>
<tr>
<td>I(_{rs})</td>
<td>Respiratory system inerntance (cmH(_2)O·s(^2)·ml(^{-1}))</td>
</tr>
<tr>
<td>j</td>
<td>Imaginary unit ((\sqrt{-1}))</td>
</tr>
<tr>
<td>LFFOT</td>
<td>Low-frequency forced oscillation technique</td>
</tr>
<tr>
<td>MCh</td>
<td>Methacholine</td>
</tr>
<tr>
<td>P</td>
<td>Indicates pressure signal (subscripts)</td>
</tr>
<tr>
<td>Paw</td>
<td>Airway pressure (cmH(_2)O)</td>
</tr>
<tr>
<td>PEEP</td>
<td>Positive end-expiratory pressure (cmH(_2)O)</td>
</tr>
<tr>
<td>PEEP(_{ref})</td>
<td>Reference positive end-expiratory pressure (cmH(_2)O)</td>
</tr>
<tr>
<td>PFC</td>
<td>Perfluorocarbon fluid</td>
</tr>
<tr>
<td>Raw</td>
<td>Airway resistance (cmH(_2)O·s·ml(^{-1}))</td>
</tr>
<tr>
<td>Re(Z(_{rs}))</td>
<td>Real part of respiratory system impedance (cmH(_2)O·s·ml(^{-1}))</td>
</tr>
<tr>
<td>RN</td>
<td>Random noise</td>
</tr>
<tr>
<td>R(_{rs})</td>
<td>Respiratory system resistance (cmH(_2)O·s·ml(^{-1}))</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>TLV</td>
<td>Total liquid ventilation</td>
</tr>
<tr>
<td>V</td>
<td>Indicates volume signal (subscripts)</td>
</tr>
<tr>
<td>(V_{pump})</td>
<td>Volume measured at the piston pump (ml)</td>
</tr>
<tr>
<td>X(_{rs})</td>
<td>Respiratory system reactance (cmH(_2)O·s·ml(^{-1}))</td>
</tr>
<tr>
<td>Z(_{rs})</td>
<td>Respiratory system impedance (cmH(_2)O·s·ml(^{-1}))</td>
</tr>
<tr>
<td>(Z_{total})</td>
<td>Total impedance (respiratory system + ventilator components) (cmH(_2)O·s·ml(^{-1}))</td>
</tr>
<tr>
<td>(\alpha)</td>
<td>Fractional exponent</td>
</tr>
<tr>
<td>(\gamma)</td>
<td>Coherence function</td>
</tr>
<tr>
<td>(\eta)</td>
<td>Tissue hysteresivity = G/H</td>
</tr>
<tr>
<td>(\omega)</td>
<td>Angular frequency (rad/s)</td>
</tr>
</tbody>
</table>

### MATERIALS AND METHODS

Experimentation was performed in accordance with the Canadian Council on Animal Care guidelines for the care and use of laboratory animals and was approved by our institutional Ethics Committee for Animal Care and Experimentation.

**Animal Preparation**

Thirteen term and healthy newborn Romanov lambs (six females and seven males), weighing, on average, 2.5 ± 0.4 kg (SD) and <5 days of age (see Table 1 for lamb characteristics), were premedicated with intramuscular injections of ketamine (10 mg/kg), atropine (0.1 mg/kg), and midazolam (0.1 mg/kg), together with antibiotics (0.05 mg/kg duplocillin and 5 mg/kg gentamicin). Lambs were secured in supine position on an open cot equipped with a warming carpet and a radiant heater to maintain their central temperature at 39.5 ± 0.5°C throughout the experiment. Pressure-regulated volume-control ventilation was initiated (Servo 300 ventilator, Siemens-Elema, Solna, Sweden) following orotracheal intubation performed using a 5.5-G cuffed endotracheal tube (ET) (Mallinckrodt, St. Louis, MO). Ventilation settings were adjusted as follows: 55 breaths/min with an inspiratory-to-expiratory ratio of 1:3; end-inspiratory and end-expiratory pause of 0.5 s; tidal volume = 28 ml/kg; fraction of inspired oxygen = 1.0. Oxygen saturation was monitored using a pulse oximeter probe placed on the base of the tail (Radical, Masimo, Irvine, CA), and heart rate was recorded using a Hewlett-Packard cardiorespiratory monitor (model HP78342A, Palo Alto, CA). Anesthesia was induced with one intraperitoneal loading dose of thiopental (20 mg/kg) and followed by continuous infusion of 2 mg·kg\(^{-1}\)·h\(^{-1}\) via the left jugular vein. Complete paralysis was achieved with intravenous administration of vecuronium bromide (0.1 mg/kg) every 2 h, and continuous jugular infusion of 5% dextrose was given at 4 ml·kg\(^{-1}\)·h\(^{-1}\) via the right jugular vein. Complete paralysis was achieved with intravenous administration of vecuronium bromide (0.1 mg/kg) every 2 h, and continuous jugular infusion of 5% dextrose was given at 4 ml·kg\(^{-1}\)·h\(^{-1}\) via the right jugular vein. Complete paralysis was achieved with intravenous administration of vecuronium bromide (0.1 mg/kg) every 2 h, and continuous jugular infusion of 5% dextrose was given at 4 ml·kg\(^{-1}\)·h\(^{-1}\). The right jugular vein was cannulated in turn to provide eventual intravenous access for MCh and salbutamol delivery throughout the respiratory mechanics measurement protocol. A 3-Fr 7-cm catheter (PV2013L07, PiCCO catheter, Pulsion Medical System, Munich, Germany) was positioned into the femoral artery using a cut-down procedure for continuous monitoring of blood temperature and mean arterial, systolic, and diastolic pressures. The catheter also provided access for arterial blood gas sampling.

**Study Design**

TLV. Lambs were allowed 20 min for recovery and then gradually shifted from conventional mechanical ventilation to a volume-controlled, pressure-limited TLV using our specially designed ventilator (38). Transition was made as quickly as possible using 10-ml aliquots of warmed (39°C), preoxygenated perfluorodecalin (F2 Chemical, Lancashire, UK) and by increasing gas-ventilator PEEP from 4 to 7 cmH\(_2\)O (40). Total number of PFC aliquots was adjusted to achieve lamb calculated functional residual capacity (25 ml/kg). Gas ventilation (GV) was then interrupted, and TLV was initiated as followed: volume-controlled mode, 5.6 breaths/min with an inspiration-to-expiration ratio = 1:3; end-inspiratory and end-expiratory pause = 0.5 s; tidal volume = 28 ml/kg; PEEP\(_{ref}\) = 5 cmH\(_2\)O; and fraction of inspired oxygen at 0.95. Inspiration was volume controlled (38), while expiration was pressure regulated and volume targeted; both were pressure limited and time cycled (39).

**Table 1. Lamb characteristics**

<table>
<thead>
<tr>
<th>Lamb No.</th>
<th>Group</th>
<th>Sex</th>
<th>Weight, kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chest-strapped</td>
<td>M</td>
<td>2.85</td>
</tr>
<tr>
<td>2</td>
<td>Chest-strapped</td>
<td>M</td>
<td>2.50</td>
</tr>
<tr>
<td>3</td>
<td>Chest-strapped</td>
<td>F</td>
<td>2.06</td>
</tr>
<tr>
<td>4</td>
<td>Chest-strapped</td>
<td>M</td>
<td>1.90</td>
</tr>
<tr>
<td>5</td>
<td>Chest-strapped</td>
<td>F</td>
<td>2.34</td>
</tr>
<tr>
<td>11</td>
<td>Chest-strapped</td>
<td>F</td>
<td>3.09</td>
</tr>
<tr>
<td>13</td>
<td>Chest-strapped</td>
<td>F</td>
<td>2.45</td>
</tr>
<tr>
<td>6</td>
<td>MCh/salbutamol</td>
<td>M</td>
<td>3.04</td>
</tr>
<tr>
<td>7</td>
<td>MCh/salbutamol</td>
<td>F</td>
<td>2.54</td>
</tr>
<tr>
<td>8</td>
<td>MCh/salbutamol</td>
<td>F</td>
<td>2.00</td>
</tr>
<tr>
<td>9</td>
<td>MCh/salbutamol</td>
<td>M</td>
<td>2.10</td>
</tr>
<tr>
<td>10</td>
<td>MCh/salbutamol</td>
<td>M</td>
<td>2.06</td>
</tr>
<tr>
<td>12</td>
<td>MCh/salbutamol</td>
<td>M</td>
<td>3.01</td>
</tr>
</tbody>
</table>

Sex and weight distribution within both groups. *Lambs were not considereed in the results since lamb 2 had perfurothorax and lamb 6 had idopathic hemorhax (before total liquid ventilation initiation). **Impedance of chest-strapped lamb 11 was not counted due to a loosening of the bandage during the experiment. M, male; F, female; MCh, methacholine.
Additional interventions. Sodium bicarbonate or tromethamine was used to maintain pH > 7.25. Crystallloids (bolus of 10 ml/kg lactated Ringer solution) or vasopressor (dopamine 5–20 μg·kg\(^{-1}·\text{min}^{-1}\)) were used as needed to maintain a mean arterial pressure ≥ 50 mmHg. The rate of intravenous dextrose infusion was adjusted to maintain glucose blood level at 40–100 mg/dl. Once the protocol was completed, the lambs were euthanized with pentobarbital (60 mg/kg), and the lung and thoracic cavity were carefully inspected for evidence of perfluorothorax or gross abnormalities.

**LFFOT equipment.** Our total liquid ventilator prototype (38) was used to generate volumetric harmonic oscillations and record pressure and volume signals at specified expiratory or inspiratory pauses. Airway pressure (Paw) was measured using a stainless steel capillary tube inserted in the ET, with its capped end being located ~1 cm before ET distal end, thus reducing Venturi effect by measuring pressure in a constant section duct. Four equidistant radial holes ensured static pressure measurements by minimizing dynamic pressure effects. The capillary was connected to the pressure sensor (model 1620, Measurement Specialties, Hampton, VA) with 30-cm-long perfusion flexible tubing. The sensor was calibrated in vitro using a 1-m high water column, has a precision of ±1%, and 1.2-kHz bandwidth. Volumetric excitation signals were generated using the ventilator expiratory piston pump, while a linear position sensor (CS-250-AD, MTS Sensors, Cary, NC) measured piston displacement, and hence the volume of PFC in the pump, with a precision of ±0.15 ml and a bandwidth over 2 kHz. Flow spectra were obtained from Fourier analysis of the position sensor signals, as detailed below in Signal processing section. However, the flexible ventilator tubing adds a resonant behavior to the system, and the pressure sensor tubing adds a time delay, which must be measured and compensated for to retrieve the Zrs from the total impedance of the experimental system (Z\(_\text{total}\)).

**Respiratory mechanics measurement protocol.** Respiratory mechanics measurements were divided into six blocks (Fig. 1, B1–B6). While the first block was performed immediately after onset of TLV to monitor initial adaptation of the respiratory system to TLV, the next two blocks were performed to characterize TLV steady state. These first series of measurements were conducted similarly on every lamb, whereas the last three blocks where used to assess LFFOT sensibility to induced changes in respiratory mechanics (detailed below).

All experimental blocks were started and ended with one random noise (RN) signal test for respiratory mechanics assessment between 2 and 4 Hz. Single-frequency signals (9) were launched after the first RN (24) and over a narrow spectrum of 0.05–2 Hz inclusively, beginning with oscillations at 2 Hz and decreasing up to 0.05 Hz. Tests were interspaced every 0.2 Hz between 2 and 1 Hz and every 0.1 Hz between 1 and 0.1 Hz, with a final test at 0.05 Hz to closely characterize low-frequency Zrs. All oscillations were performed at fixed flow amplitude of 7.5 ml/s (i.e., a volume from 11.9 ml at 0.1 Hz down to 0.6 ml at 2 Hz), except for the 0.05 Hz sine wave, in which amplitude was set at 5 ml/s (15.9 ml volume) to avoid airway collapse and other nonlinearities associated with high volumetric oscillation amplitude.

Forced oscillations were performed during a 30-s apnea, except for the 0.05 Hz sinusoid, which necessitated a 45-s apnea to lower the influence of the transients on the spectra. All signals were launched in pairs and at two different lung volumes: the first at end expiration, the other at end inspiration. Lambs were allowed a minimum of 30 s between each pair of tests to maintain normal blood gases. Consequently, each block lasted between 60 and 90 min. Tests of 0.2 Hz were repeated every 10–12 min to assess LFFOT and short time variability of respiratory system mechanics, independently of frequency. Arterial blood-gas analyses were performed after each 10-min break between the first five blocks of measurements for ventilation recovery.

Lambs were then divided into two groups to assess LFFOT sensitivity to induced respiratory mechanics alterations. Sensitivity to compliance reduction was assessed in five lambs by gently wrapping the chest using a 10-cm-wide elastic bandage (Elastoplast, BSN Medical, Brierfield, UK). LFFOT performance in tracking resistance changes was assessed in five other lambs in two steps. Two blocks of measurement (B4 and B5) were first performed during MCh-induced rise of respiratory resistance. Continuous infusion of MCh was started at 6 μg·kg\(^{-1}·\text{min}^{-1}\), while preventing MCh-induced decline of mean systemic arterial pressure (54) below 50 mmHg. Dosage was then raised to 9 μg·kg\(^{-1}·\text{min}^{-1}\) after hemodynamic stabilization. Respiratory mechanics measurements began 10 min after ventilator peak pressure reached plateau. Then a last block of measurements (B6) was carried out after 10-min intravenous infusion of salbutamol (1.5 g·kg\(^{-1}·\text{min}^{-1}\)) to assess whether bronchodilation can be tracked by LFFOT.

**Signal processing.** The signal processing methodology followed a typical Welch’s overlapped segmented average frequency analysis procedure (6, 56). Pressure and volume signals were digitized [PCI-DAS1602/16, Measurement Computing, Norton, MA (16-bit resolution, 2-kHz sampling rate)], then digitally low-pass filtered (6th order Butterworth, −3-dB cutoff at 10 Hz) and downsampled at 50 Hz for recording. Each recorded test was then divided into overlapping segments (20 s for single-frequency signal, 10 s for noise, 67% overlap) and Hanning windowed. The frequency-response function estimate FRF\(_1\)(f), i.e., the transfer function between pump volume (V\(_\text{pump}\)) and Paw, was computed using

\[
\text{FRF}_1(f) = \frac{\hat{G}_{vp}(f)}{\hat{G}_{vp}(f)}
\]

where \(\hat{G}_{vp}(f)\) is the averaged cross-spectral density function between Paw and V\(_\text{pump}\), and \(G_{vv}(f)\) is the averaged autospectral density function of V\(_\text{pump}\). The Z\(_\text{total}(f)\) is then given by

\[
Z_{\text{total}}(f) = \frac{P_{aw}(f)}{V_{\text{pump}}(f)} = \frac{1}{j2\pi f} H(f)
\]

with \(j^2 = -1\).

Finally, coherence function (\(\gamma^2\)) was computed from the spectral analysis of pressure and flow signals.

\[
\gamma^2(f) = \frac{|\hat{G}_{vp}(f)|^2}{\hat{G}_{vp}(f)\hat{G}_{vv}(f)}
\]

A value of \(\gamma^2 \geq 0.95\) was used as the criterion for test acceptance (47). This overall process is very similar to typical conventional mechanical ventilation forced oscillation technique analysis (35).

**Parametric identification.** Similar blocks of measurements (see Fig. 1) were ensemble averaged when possible, and a seven-parameter
inverse model was fitted to the average spectrum to improve fitting performance. Hence, prechallenge blocks were grouped (B2 and B3), as well as blocks performed during MCh infusion (B4 and B5) and on chest-strapped lambs (B4, B5, and B6). The complete system inverse model (Eq. 4) for which parameters needed to be identified consisted of the four-parameter, constant-phase model (CPM), $Z_{rs}(j\omega)$ (Eq. 5) (20), and a three-parameter linear transfer function representing ventilator flexible tubing dynamics, $Z_{v}
abla _{ing}(j\omega )$ (Eq. 6). 

$$Z_{\text{total}}(j\omega ) = Z_{rs}(j\omega ) \cdot Z_{v}
abla _{ing}(j\omega ) \tag{4}$$

where the $Z_{rs}$ is

$$Z_{rs}(j\omega ) = R_{aw} + j\omega I_{rs} + \frac{G - jH}{\omega ^{n}} \tag{5}$$

and the $Z_{v}
abla _{ing}$ is

$$Z_{v}
abla _{ing}(j\omega ) = \frac{\omega _{n}^{2}}{(j\omega )^{2} + 2\omega _{n}j\omega + \omega _{n}^{2}} e^{-d\omega /\omega _{n}} \tag{6}$$

In the $Z_{rs}(j\omega )$ model, $\omega$ is the angular frequency, $R_{aw}$ is the airway resistance, $I_{rs}$ is the respiratory system inertance, $G$ is the tissue damping, $H$ is the tissue elastance, and $\alpha = (2/\pi)\arctan(G/H)$ is a fractional exponent. The $Z_{v}
abla _{ing}$ was represented by an underdamped oscillation of natural pulsation $\omega _{n}$ and damping factor $\xi$ (37), and a time delay $d$ associated with pressure wave propagation in all flexible tubing (55). While these parameters gave no physiological information, their estimation was necessary for ventilator dynamics compensation and $Z_{v}
abla _{ing}$ estimation (Fig. 2).

The seven parameters (i.e., $R_{aw}, I_{rs}, G, H, \omega _{n}, \xi,$ and $d$) were identified by a nonlinear weighted least squares error minimization routine (44), the Matlab's lsqnonlin function (The MathWorks, Natick, MA), using experimental $Z_{\text{total}}$ from Eq. 2. After a first seven-parameter curve fit, the ventilator-associated parameters were set constant at the estimated value. The routine was launched a second time over a limited frequency range (0.05–1.75 Hz) to gain more precision on the four CPM parameters. This helped reduce computed parameter confidence intervals, yielding more precise physiological parameter values.

The real part of the $Z_{rs}$ [Re($Z_{rs}$)] is also called the respiratory system resistance ($R_{rs}$), and comprises $R_{aw}$ and $G$ parameters. $I_{rs}$ and $H$ form respiratory system reactance ($X_{rs}$), which is related to the imaginary part of the impedance [Im($Z_{rs}$)] by Im($Z_{rs}$) = $jX_{rs}$. Graphically, $G$ refers to the frequency-dependent pattern on $R_{rs}$ curve and $R_{aw}$ to its mainly frequency-independent part. $H$ mainly influences $X_{rs}$ at low frequencies, whereas $I_{rs}$ determines its shape for higher $f$. The discrete frequency that graphically discriminates $H$ and $I_{rs}$ contributions is where $X_{rs}$ is $0$ and is called the resonant frequency ($f_{r_0}$) (Fig. 2B). Hysteresivity was calculated as the ratio between $G$ and $H$: $\eta = G/H$ (11).

**RESULTS**

**LFFOT Implementation in TLV**

LFFOT implementation to our total liquid ventilator Inolvent-4 was straightforward and allowed easy measurement of $Z_{rs}$ in healthy newborn lambs. Ventilator piston pump forced oscillations enabled reliable assessment of total system mechanics using single-frequency signal as well as RN. $Z_{rs}$ was measured at very low frequency ($f \leq 2$ Hz) using single-frequency testing, while impedance of ventilator flexible tubing was dominant over 2 Hz and measured by RN. For the sake of simplicity, the reader is referred to Table 2 for results concerning 0.2-Hz single-frequency resistance and reactance ($R_{rs0.2}$ and $X_{rs0.2}$) and to Table 3 for CPM-derived parameter estimations ($R_{aw}, I_{rs}, G, H$) throughout the text.

Measurements, performed during several consecutive apneas, yielded moderate hypercarbia, but no significant hypoxia (see Fig. 3). Arterial oxygen saturation stayed close to 100%, but, during the MCh, saturation decreased down to $\sim 90\%$. Data are not available after salbutamol infusion since blood-gas analyses have been performed following each block of measurements, except after that last one since lambs were euthanized.

**Variability**

Mean short-term intraindividual variability that mirrored both the variation in the respiratory resistance over time and

![Figure 2](https://example.com/figure2.png)

**Fig. 2.** A: typical experimental total impedance ($Z_{\text{total}}$) distribution over frequency, with the line representing the seven-parameter inverse model fit on the data. Data are from lamb 11, baseline, end-inspiratory spectrum, fitting error $= 0.033$. B: respiratory system impedance ($Z_{rs}$) spectrum, given by the constant-phase model fit, after retrieval from the $Z_{\text{total}}$. Dashed lines and plus signs represent the imaginary part of impedance [Im($Z_{rs}$)]; solid lines and dots represent the real part of impedance [Re($Z_{rs}$)]. Constant-phase model parameters are graphically represented with $G$ (tissue damping). $R_{aw}$ (airway resistance) and $H$ (elastance) were separated from $I_{rs}$ (respiratory system inertance) by $f_{r_0}$ (resonant frequency). CIs, confidence intervals.
the variability of LFFOT was computed using Rrs0.2. Interestingly, the coefficient of variation was always lower at end inspiration than at end expiration, such that steady-state values obtained during baseline for the 11 lambs were 12.4 and 18.5%, respectively (Table 2). The variability was also higher immediately after TLV initiation (21%) and lower after salbutamol-induced bronchodilation (7%), when the resistive part of impedance was lower. Intraindividual variability was otherwise maintained within 10–15% at end inspiration and within 16–23% at end expiration.

**CPM**

During baseline conditions (Fig. 4), the frequency-independent part of the Rrs curve, associated with Raw, was within 0.3 and 1.2 cmH2O·s·ml\(^{-1}\) and preceded by a short frequency-

<table>
<thead>
<tr>
<th>Raw, cmH2O·s·ml(^{-1})</th>
<th>Irs, cmH2O·s·ml(^{-1})</th>
<th>G, cmH2O/ml</th>
<th>H, cmH2O/ml</th>
<th>η</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline (n = 11)</td>
<td>Mean IQR</td>
<td>Mean IQR</td>
<td>Mean IQR</td>
<td>Mean IQR</td>
</tr>
<tr>
<td>Adaptation (n = 11)</td>
<td>Mean IQR</td>
<td>Mean IQR</td>
<td>Mean IQR</td>
<td>Mean IQR</td>
</tr>
</tbody>
</table>

Values are presented as median with IQR = 25th to 75th for parameters airway resistance (Raw), respiratory system inertance (Irs), tissue damping (G), tissue elasticity (H), and hysteresivity (η), as provided by constant-phase model fitting on respiratory impedance, between 0.05 and 2 Hz, n. Of animals. Parameter values are given for end-inspiration and end-expiration volumes. *Statistically significant differences; †significant difference between parameters for each condition and its respective baseline; ‡comparison between MCh and salbutamol; P ≤ 0.05.

Table 2. Resistance and reactivity of the respiratory system as measured using a 0.2-Hz single-frequency signal

<table>
<thead>
<tr>
<th>Condition</th>
<th>Rrs0.2, cmH2O·s·ml(^{-1})</th>
<th>Xrs0.2, cmH2O·s·ml(^{-1})</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>IQR</td>
<td>Median</td>
<td>IQR</td>
</tr>
<tr>
<td>Adapt</td>
<td>0.75†</td>
<td>0.43–0.82</td>
<td>-0.21†</td>
</tr>
<tr>
<td>BL</td>
<td>0.60</td>
<td>0.41–0.81</td>
<td>-0.17</td>
</tr>
<tr>
<td>MCh</td>
<td>0.89†</td>
<td>0.78–0.99</td>
<td>-0.28</td>
</tr>
<tr>
<td>Salbut</td>
<td>0.43‡</td>
<td>0.35–0.52</td>
<td>-0.15‡</td>
</tr>
<tr>
<td>Bandage</td>
<td>0.41</td>
<td>0.36–0.50</td>
<td>-0.16</td>
</tr>
</tbody>
</table>

Group median respiratory system resistance (Rrs0.2) and reactance (Xrs0.2) for each experimental block. Values are presented as median with their respective interquartile range (IQR) = 25th to 75th. Coefficient of variation (CV) provides an insight into mean intraindividual variability within blocks and was computed using Rrs0.2. Measurements have been performed during initial respiratory system adaptation to total liquid ventilation (Adapt), during continuous MCh infusion (MCh), then after a 10-minutes salbutamol infusion (Salbut), and while lambs were chest-strapped (Bandage). Baseline steady-state condition (BL) values are provided for each of the three conditions. *Statistically difference from the respective baseline; †significant difference from the respective baseline; ‡comparison between MCh and salbutamol; P ≤ 0.05.

Table 3. Low-frequency forced oscillation technique respiratory mechanics during neonatal total liquid ventilation
dependent segment related to G. Note that the pattern of the Rrs curves was very similar among lambs. Conversely, the dispersion of the Xrs curve raised toward higher frequencies, suggesting greater variability of the inertance term. Furthermore, there was a significant inverse Pearson correlation between the lamb weight and $f_{\text{res}}$, with end-inspiratory $r = -0.634$ ($P = 0.018$) and end-expiratory $r = -0.541$ ($P = 0.043$). Irs also correlated with weight at both respiratory volumes ($r = 0.627$, $P = 0.019$ and $r = 0.619$, $P = 0.021$) at end inspiration and end expiration, respectively. Correlation between Rrs and weight did not otherwise reach statistical significance.

Continuous Intravenous Infusion of MCh

An example of impedance spectra measured pre- and post-MCh challenge is presented in Fig. 5. Plots show vertical shift of Rrs at both respiratory volumes, consistent with an increase in Raw. Likewise, Rrs0.2 was statistically higher during MCh infusion (Table 2), although absolute values of CPM-derived resistive parameters (Raw and G) failed to show a significant increase (Table 3). Xrs also correlated with weight at both respiratory volumes ($r = 0.627$, $P = 0.019$ and $r = 0.619$, $P = 0.021$) at end inspiration and end expiration, respectively. Correlation between Rrs and weight did not otherwise reach statistical significance.

Intravenous Infusion of Salbutamol

Again, Fig. 5A shows a clear downward shift of the Xrs curve after salbutamol infusion compared with the resistance plateau during MCh infusion and before challenge. This difference was, however, substantially reduced at end expiration (Fig. 5B) compared with end inspiration. Similarly, both Rrs0.2 and Xrs0.2 decreased significantly at end-inspiration but not at end-expiration volume, a finding that concurs with the plots. CPM parameters failed to report a significant decrease in Raw and G compared with baseline.

Chest-Strapping

Zrs of lambs with a bandage strapped around their chest graphically revealed an increase in frequency dependence of Rrs at low frequencies (Fig. 6) and a right shift of the Xrs curve, consistent with a higher H (lower compliance). The later was more convincing at end expiration (Fig. 6B) than at end inspiration. Xrs0.2 was significantly lower at end expiration compared with baseline, but no change in Rrs0.2 was found at either volumes. CPM tissue parameters G and H were significantly higher than that observed during baseline at end-expiration volume, while only H was significantly increased at end inspiration. Rrs0.2 and Xrs0.2, as G and H, were, however, all statistically different at end inspiration compared with end expiration.

DISCUSSION

Clinical and Physiological Implications

We report herein the first implementation of LFFOT to TLV, as well as provide the first insight into Zrs of newborn lambs under this new modality of ventilation. We present spectral impedance within a very low range of frequencies (0.05–2 Hz inclusively) and show that a single-frequency signal of 0.2 Hz can be a simple and reliable tool to track pulmonary mechanics changes in TLV.

Likewise, since TLV prevents the lung from collapsing below functional residual capacity, several consecutive apneas can be induced without marked hypoxemia. Figure 3 shows no significant hypoxemia and moderate hypercarbia throughout the experiment. Blood-gas data deteriorated following MCh infusion, mainly because of bronchoconstriction. However, theoretical effects of these variations in arterial PCO2 and PO2 on respiratory mechanics were not investigated in the present study.

Methodological Considerations

Despite providing a good fit on our experimental data, the resistive parameters G and Raw were found dependent from each other during the parametric identification process (data not shown). This issue has also been reported by previous investigators (19, 51, 61) on conventional GV and is, therefore, unlikely to be attributable to PFC fluid in lungs, but to the mathematical structure of Zrs. Nevertheless, the dependence between both parameters decreases the sensitivity of the CPM...
and excludes reliable partitioning of tissue resistance and Raw. This phenomenon may also explain why no significant changes in Raw were found during MCh and salbutamol challenge, albeit Rrs modulations were graphically appreciable and noticeable by 0.2-Hz single-frequency signal tests.

Respiratory Mechanics in TLV

Respiratory mechanics of newborn lambs under TLV contrast with that found by other authors working with lambs under GV (31), mainly by Rrs predominance of the reactance spectrum under 2 Hz and a roughly 30- to 40-fold increase in Raw (40). Likewise, we found that \( f_{res} \) was predominantly below 1.2 Hz with PFC fluid into the lung (see Fig. 4), compared with \(~10\) Hz with air (31). This left shift of the reactance, as well as the increase in Rrs and Raw were to be expected, since 39°C perfluorodecalin density (1,880 kg/m³) and dynamic viscosity (3.4 mPa·s) are starkly greater than air at the same temperature. These observations also concur with results obtained at low pulmonary volume in PLV (when PFC-to-air ratio is maximal in the lungs) (43). However, compared with this latter type of LAV, respiratory mechanics is much less dependent on lung volumes in TLV, and the clinically relevant range of frequency is more confined to very low frequencies \((f \leq 2 \text{ Hz} \text{ instead of } f \leq 4 \text{ Hz in PLV}).

CPM spectra, as displayed in Fig. 4, showed some variability among lambs. Of note, part of this variability is inherent to the neonatal animal model itself, given that respiratory mechanics varies with height, lung maturity, and alveolarization of each newborn lamb (27, 34). On the other hand, the contribution of the method, especially the deconvolution process used for separating ventilator tubing dynamics from the respiratory system parameters, could not be overlooked as being a contributor to the observed variability.

Adequacy of the CPM to TLV and Interpretation

Adequacy of the CPM to explain TLV lung impedance spectra was one of the research hypotheses. While the model could be effectively fitted to TLV experimental data, questions arise regarding its interpretation. While in GV, Raw and airway impedance refer to the airways mechanics and G and H to the tissue, these assumptions could be misleading in TLV. For instance, a high-density PFC flow in the airways could yield nonnegligible fluid-structure interactions, such as elastic deformations and associated structural damping. Therefore, if the general behavior of the Zrs is similar, further evidence is needed to give the gas-CPM parameters the same meaning in TLV.

![Fig. 5. Plots exemplifying typical changes (in one lamb) in Zrs during continuous infusion of MCh (dark solid lines) and after a 10-min infusion of salbutamol (dashed shaded lines) compared with baseline Zrs conditions (thin solid lines) at both end-inspiration (A) and end-expiration (B) volumes.](image)

![Fig. 6. Plots exemplifying typical changes in Zrs following chest-strapping of one lamb at both end-inspiration (A) and end-expiration (B) volumes. Thin line, Zrs at baseline conditions; thick line: Zrs on chest-strapped lamb.](image)
According to the gas constant-phase theory, G and H should be elementary coupled, and, therefore, changes in G value should always be followed by H, such that hysteresivity $\eta = G/H$ remains constant (11). However, we observed relatively high variability in parameter $\eta$ among lambs within the same experimental block, as well as from one block to another (see Table 3). This indicates that G could possibly account for other phenomena than pure tissue viscoance, and, therefore, its value should be interpreted with precaution. In GV, the artifactual rise in G has been extensively ascribed to airway inhomogeneities, a phenomenon well described by Bates and Lutchen (4) and particularly apparent during inhaled MCh challenge, when inhomogeneities are probably greater (42, 50, 53). On the other hand, explanation is unlikely to be true in TLV, since airway recruitment and uniform distribution of PFC into the respiratory system enhance ventilation homogeneity and uphold alveolar patency even at end-expiration volume (15, 59).

Herein, Fig. 4 shows that the real part of the impedance response, which refers to Rrs, is only frequency dependent at very low frequencies (typically 0.05 and 0.1 Hz) and constant at upper frequencies. Such distribution of the measured impedance real part implies two possible biases on the value of G. 1) Parameter G in the CPM equation (Eq. 4) is the gain of the frequency dependence on the real part (inverse of the frequency). However, a good fit is not a guarantee for identification of parameter G of the CPM: if an increase in Raw values for these low frequency experiments is indeed generated, it will be identified by parameter G. Indeed, it was observed that a too large volume amplitude at low frequencies could generate a nonlinear response of the airways (and eventually collapses, which is equivalent to an increase in Raw). Actually, few collapses were observed for the high-volume 0.05-Hz tests, but the results were always rejected based on the resulting low coherence values. As suggested in Ref. 3, airway collapses in TLV induce neither structural changes in the respiratory system, nor any noticeable airway injury, so we can expect that subsequent measurements were unaffected by these events. Despite the fact that heavily nonlinear responses were discarded from our analysis, an undetected nonlinear behavior of the airways could explain the increase in resistance and, consequently, create a bias for the value of G. A recommendation could be to perform measurements at the lowest amplitudes to ensure a linear response of the airways. 2) The identification of the parameter G, which characterizes the frequency dependence, is strongly sensitive of the few very-low-frequency measurements: a potential noisy measurement on one recording would strongly affect the identified parameter G (and indirectly the estimated parameter Raw). This could explain the large dispersion on the value of G from one experimental block to the other. A recommendation could be to perform more measurements at very low frequencies to obtain additional data showing the frequency dependence of the real part in TLV.

**Single Frequency vs. Spectral Analysis**

Authors are aware that spectral analysis would have required multiple-frequency signal tests (24), which afford a more straightforward approach to track respiratory mechanics and reduce temporal bias. However, as a first step in TLV, we reasoned that it was better to use single-frequency signals at $f \leq 2$ Hz, to closely characterize respiratory mechanics at very low frequencies with high signal-to-noise ratio ($\geq 20$ dB) by avoiding flexible tubing resonance excitation. On the other hand, fixed-frequency tests are easy to implement and fairly understandable by clinicians. Rs0.2 and Xs0.2 seem particularly suitable in newborn lambs under TLV, since they provide a quick overview of respiratory mechanics and show good responsiveness to bronchoconstriction and bronchodilation challenges, as well as to compliance reduction. In fact, the reduction in compliance using bandage wrapping around the lamb’s chest induced a significant lowering of Xs0.2, whereas MCh bronchoconstriction induced a significant increase in Rs0.2 that is at both pulmonary volumes. A higher frequency (e.g., 1.0 or 1.5 Hz) reflecting only the frequency-independent resistance (Raw) could also be used, for instance, to track airway or ET obstruction.

**Conclusion**

In summary, we have successfully implemented the LFFOT to neonatal TLV and present the first report of Zrs under this modality of ventilation. The process revealed considerable differences between conventional mechanical ventilation and TLV lung mechanics. Namely, the high viscosity and density of PFC shift frequencies of interest toward lower values and yield considerably higher inertance and resistance values. In addition, we show that a 0.2-Hz single-frequency signal represents an optimal tool to track pulmonary mechanics modulations under diverse physiological circumstances, such as bronchoconstriction and dilation, as well as compliance reduction. This experiment, the first of a series, is a key element toward enhancing our knowledge of lung dynamics in TLV. Further studies will be needed to adapt multiple-frequency signal to TLV to improve and enable a more effective assessment of spectral respiratory mechanics at $f \leq 2$ Hz. Moreover, the physiological explanation of the model parameter meaning, mostly unusual G and $\eta$ values, is yet to be determined. The main long-term objective is to achieve the implementation of LFFOT in future total liquid ventilators ready to be introduced in neonatal intensive care units. Hence, as in GV, LFFOT is expected to provide insight into treatment progression and help plan optimal weaning procedures in TLV.

**ACKNOWLEDGMENTS**

The authors gratefully acknowledge Nathalie Carrier for statistical consultations, together with Raymond Robert and Nathalie Samson for technical assistance. We also acknowledge Pulsion Medical Systems for providing the PiCCO device used for hemodynamic monitoring.

**GRANTS**

This work was supported in part by the Fondation des Étoiles and the Faculté de Médecine et des Sciences de la Santé of the Université de Sherbrooke (Perinatal Research Team on Ovines), the Natural Sciences and Engineering Research Council of Canada, and the Fonds Québécois de la Recherche sur la Nature et les Technologies. P. Micheau, J.-P. Praud, and H. Walti are members of the Fonds de la Recherche en Santé du Québec funded Centre de Recherche Clinique Étienne-Le Bel of the Centre Hospitalier Universitaire de Sherbrooke.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).
REFERENCES


