



BASIC SCIENCE ARTICLE

Reducing lung liquid volume in fetal lambs decreases ventricular constraint

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BACKGROUND: This study evaluated whether an increased left ventricular (LV) pump function accompanying reduction of lung liquid volume in fetal lambs was related to increased LV preload, augmented LV contractility, or both.

METHODS: Eleven anesthetized preterm fetal lambs (gestation 128 ± 2 days) were instrumented with (1) an LV micromanometer-conductance catheter to obtain LV end-diastolic volume (EDV) and end-diastolic pressure (EDP), the maximal rate of rise of LV pressure (dP/dt_{max}), LV output, LV stroke work, and LV end-systolic elastance (E_{es}), a relatively load-independent measure of contractility; (2) an endotracheal tube to measure mean tracheal pressure and to reduce lung liquid volume. LV transmural pressure was calculated as LV EDP minus tracheal pressure.

RESULTS: Reducing lung liquid volume by 16 ± 4 ml kg^{-1} (1) augmented LV output (by 16%, $P = 0.001$) and stroke work (29%, $P < 0.001$), (2) increased LV EDV (12%, $P < 0.001$), (3) increased LV transmural pressure (2.2 mmHg, $P < 0.001$), (4) did not change LV dP/dt_{max} normalized for EDV ($P > 0.7$), and (5) decreased LV E_{es} (20%, $P < 0.01$).

CONCLUSION: These findings suggest a rise in LV pump function evident after reduction of lung liquid volume in fetal lambs was related to increased LV preload secondary to lessening of external LV constraint, without any associated rise in LV contractility.

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IMPACT:

- This study has shown that reducing the volume of liquid filling the fetal lungs lessens the degree of external constraint on the heart. This lesser constraint permits a rise in left ventricular dimensions and thus greater cardiac filling that leads to increased left ventricular pumping performance.
- This study has defined a mechanism whereby a reduction in lung liquid volume results in enhanced pumping performance of the fetal heart.
- These findings suggest that a reduction in lung liquid volume which occurs during the birth transition contributes to increases in left ventricular dimensions and pumping performance known to occur with birth.

INTRODUCTION

It is widely accepted that the volume of fluid which fills the future airways and alveoli of the lungs in the fetus is an important determinant of pulmonary perfusion, as a decrease in this “lung liquid” volume leads to an increase in pulmonary blood flow and a fall in pulmonary vascular resistance.^{1–5} More recently, however, findings from our laboratory have suggested that lung liquid volume also modulates ventricular pumping performance in the fetus, as reducing this volume increased not only right ventricular (RV) but also left ventricular (LV) output and hydraulic power, in spite of a decrease in ventricular filling pressures suggested by falls in mean right and left atrial (LA) blood pressures.^{3,4}

Although the specific mechanism(s) underlying an increase in fetal ventricular pump function after reduction of lung liquid volume have yet to be defined with direct experimental data, current information suggests that at least two factors may be involved. First, the fluid-filled lungs exert a physical constraint on the fetal heart which limits its pumping performance, as complete

retraction of the lungs away from the heart more than doubles not only RV stroke volume and work,^{6,7} but also LV stroke volume.^{7,8} Thus, in line with previous suggestions,^{3,4} a reduction in lung liquid volume may lessen external cardiac constraint and thus permit an increase in not only ventricular transmural pressure but also ventricular dimensions and filling, thereby augmenting cardiac output and work via a rise in ventricular preload. Second, an additional factor pertaining to the left ventricle is enhanced contractility, as the maximal rate of rise in blood pressure (dP/dt_{max}) within the aorta, a surrogate measure of LV dP/dt_{max} and LV contractility,^{9,10} increased after reduction of lung liquid volume.⁴ However, as changes in arterial dP/dt_{max} can be influenced by alterations in LV loading conditions,¹¹ this rise in dP/dt_{max} may have simply resulted from a larger LV chamber volume. Resolution of this issue therefore requires adjustment of dP/dt_{max} for LV end-diastolic volume and evaluation of LV contractility with a relatively load-independent index such as LV end-systolic elastance (E_{es}) derived from ventricular pressure–volume (P – V) loop analysis.^{12–14}

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Accordingly, the goal of the present study was to determine the extent to which an augmentation of LV pump function evident after a decrease in fetal lung liquid volume was related to increases in LV preload and/or LV contractility. Studies were performed in anesthetized fetal lambs instrumented with an LV micromanometer-conductance catheter to obtain high-fidelity P - V signals for (1) quantitation of changes in LV preload via measurement of LV EDV and estimation of LV transmural pressure, and (2) assessment of LV contractility via adjustment of dP/dt_{\max} for LV EDV and calculation of E_{es} .

METHODS

Experiments were approved by the Murdoch Children's Research Institute Animal Ethics Committee and conformed to guidelines of the National Health and Medical Council of Australia.

Surgical preparation

The general features of the anesthetic and monitoring procedures were similar to those previously described.^{4,15} Briefly, 11 Border-Leicester cross ewes were anesthetized at a gestation of 128 ± 2 days (mean \pm SD, term = 147 days) with an intramuscular injection of ketamine (5 mg kg^{-1}) and xylazine (0.1 mg kg^{-1}), followed by 4% isoflurane delivered by mask. After insertion of a cuffed endotracheal tube, anesthesia was maintained with isoflurane (1–2%) and nitrous oxide (10–20%) delivered by the ventilator in O_2 -enriched air, and supplemented by an intravenous infusion of ketamine ($1\text{--}1.5 \text{ mg kg}^{-1} \text{ h}^{-1}$), midazolam ($0.1\text{--}0.15 \text{ mg kg}^{-1} \text{ h}^{-1}$), and fentanyl ($2\text{--}2.5 \mu\text{g kg}^{-1} \text{ h}^{-1}$). Transcutaneous O_2 saturation (S_{pO_2}) was monitored continuously with a pulse-oximetry sensor applied to the ear. The right common carotid artery was cannulated for monitoring of blood pressure and regular blood gas analyses (ABL800; Radiometer, Copenhagen, Denmark), with ventilation of the ewe adjusted to maintain arterial O_2 tension (P_{aO_2}) at 100–120 mmHg and CO_2 tension (P_{aCO_2}) at 35–40 mmHg.

Following a midline laparotomy, the fetal head was exteriorized via a hysterotomy and placed in a saline-filled glove to prevent loss of lung liquid. After delivery of the left forelimb and thorax, the neck was incised and a fluid-filled catheter passed into the superior vena cava via the left external jugular vein for fluid and drug administration. The left common carotid artery was cannulated with (1) a 6-Fr self-sealing vascular sheath for pressure measurement and blood sampling and (2) a 3.5-Fr micromanometer (model SPR-524; Millar Instruments, Houston, TX) that was passed into the aortic trunk (AoT) to obtain high-fidelity blood pressure. A thoracotomy was performed in the third interspace and major vessels carefully dissected for placement of non-constrictive flow probes (Transonic Systems, Ithaca, NY) around the brachiocephalic trunk (4 or 6 mm) and aortic isthmus (6 mm). A fluid-filled catheter was inserted into the LA appendage to measure pressure. An adjustable silk snare was placed around the descending thoracic aorta, 1–2 cm below its junction with the ductus arteriosus, for later production of transient rises in central arterial blood pressure, and thus LV afterload. A dual-field, multi-segment 3-Fr conductance-micromanometer catheter (model SPR-877; Millar Instruments) was subsequently inserted into the carotid artery sheath and advanced across the aortic valve to obtain high-fidelity and artifact-free LV pressure and volume signals. Correct placement of the conductance catheter was inferred from the presence of in-phase segmental P - V waveforms and square-like counterclockwise whole-chamber P - V loops. Finally, a clamped 4.5 mm endotracheal tube filled with normal saline and containing a proximal side-port for measurement of tracheal pressure was inserted to a depth of 4–5 cm via a proximal tracheostomy and tied into place. The saline-filled glove was then removed from the fetal head, with measurements before and after lung liquid removal made in the still partially exteriorized fetus.

Experimental protocol

After withdrawal of AoT samples from the fetus for measurement of blood resistivity (Rho calibration cuvette; Millar Instruments) and blood gas analysis (ABL800, Radiometer), a 20–30 s block of baseline steady-state physiological data was collected onto computer. Data were then recorded during progressive tightening of the snare around the descending thoracic aorta over 10–15 s. After release of this snare and return of hemodynamics to baseline levels, data were recorded during LA injection of $\sim 0.5 \text{ ml}$ of hypertonic (10%) saline for estimation of parallel conductance, i.e. the offset in the conductance catheter signal related to conductivity arising from structures surrounding the blood pool in the LV cavity.^{12,16}

Subsequently, the endotracheal tube was unclamped and lung liquid allowed to drain passively via gravity over $\sim 30 \text{ s}$ into a measuring cylinder until drainage largely stopped, with the volume of collected lung liquid recorded. The endotracheal tube was then re-clamped, taking care to ensure that the endotracheal tube remained entirely filled with lung liquid and that no air bubbles entered the lungs. After hemodynamics had stabilized, an AoT sample was again withdrawn for blood gas analysis, with recording of physiological variables repeated in the resting state, during transient tightening of the descending thoracic aortic snare and during LA injection of hypertonic saline. At the end of the study period, ewes and lambs were euthanized with an overdose of pentobarbital sodium (100 mg kg^{-1}).

Physiological data

AoT, LA, and tracheal fluid-filled catheter pressures were measured with transducers calibrated against a water manometer before each study and referenced to atmospheric pressure at LA level. Instantaneous LV pressure and volume were obtained from the micromanometer-conductance catheter via an interfacing P - V signal processing system (MV Ultra, Millar Instruments). Signals from fluid-filled micromanometer and conductance catheters, as well as flow probes, were digitized at a sampling rate of 1 kHz and displayed using programmable acquisition and analysis software (Spike2; Cambridge Electronic Design, Cambridge, UK). A 48 Hz low-pass filter was applied to digitized data at the time of analysis to remove any 50 Hz mains electrical interference from signals. Steady-state resting measurements before and after reduction of lung liquid volume were performed on ensemble-averaged signals generated from >40 beats.

Mean AoT micromanometer pressure was matched to the corresponding mean arterial fluid-filled catheter pressure. The rates of change of high-fidelity LV and AoT pressures were calculated using a running 3-point differentiation algorithm, and the amplitude of isovolumic phase LV dP/dt_{\max} and ejection phase AoT dP/dt_{\max} measured. LV pressure was subsequently matched to AoT micromanometer pressure over a period in mid-to-late systole where both LV and AoT differentials were near-zero. LV end-diastole was then defined at the foot of the upstroke in the LV pressure waveform using an automated curvature-based feature extraction algorithm.¹⁷ LV output (minus coronary blood flow) was obtained as the sum of the brachiocephalic trunk and aortic isthmus flows, noting that the brachiocephalic trunk is the only major cephalic branch of the ascending aorta in sheep. LV stroke volume was calculated as LV output divided by heart rate.

LV pressure and volume data

LV volume was calculated from the conductance catheter signal using standard methodology incorporating estimates of blood resistivity, parallel conductance, and the gain constant.^{12,13,16,18} Parallel conductance was similar before ($3.5 \pm 1.6 \text{ ml}$) and after reduction of lung liquid volume ($3.4 \pm 1.4 \text{ ml}$, $P = 0.4$). The gain constant was calculated as the ratio of conductance catheter and LV output-derived stroke volumes at steady state, both before and after reduction of lung liquid volume. The gain constant fell by

Table 1. Fetal pressures and heart rate before and after reduction of lung liquid volume.

	Baseline	LL reduction	<i>P</i>
AoT blood pressure, mmHg			
Systolic	50.9 ± 5.7	52.6 ± 5.6	<0.05
Mean	42.5 ± 5.7	43.0 ± 5.5	0.4
Diastolic	34.6 ± 4.9	33.8 ± 4.7	0.2
Mean LA blood pressure, mmHg	4.6 ± 1.7	2.3 ± 0.7	0.002
LV end-diastolic pressure, mmHg	8.5 ± 1.7	6.7 ± 1.2	<0.005
Mean tracheal pressure, mmHg	4.6 ± 1.7	0.6 ± 0.6	<0.001
LV transmural pressure, mmHg	3.9 ± 1.1	6.1 ± 1.4	<0.001
Heart rate, beats min ⁻¹	143 ± 10	148 ± 13	<0.025

Data are expressed as means ± SD; *n* = 11. LL lung liquid, AoT aortic trunk, LA left atrial, LV left ventricular.

Table 2. Fetal left ventricular systolic indices before and after reduction of lung liquid volume.

	Baseline	LL reduction	<i>P</i>
LV output, ml min ⁻¹	306 ± 96	356 ± 119	0.001
Stroke volume, ml	2.13 ± 0.67	2.40 ± 0.77	0.002
LV dP/dt _{max} , mmHg s ⁻¹	1,177 ± 229	1,330 ± 282	<0.001
AoT dP/dt _{max} , mmHg s ⁻¹	670 ± 165	749 ± 155	<0.001
Normalized LV dP/dt _{max} , mmHg s ⁻¹ ml ⁻¹	403 ± 147	400 ± 151	>0.7
Normalized AoT dP/dt _{max} , mmHg s ⁻¹ ml ⁻¹	233 ± 102	230 ± 103	0.6

Data are expressed as means ± SD; *n* = 11. AoT aortic trunk, dP/dt_{max} maximal rate of pressure rise, LL lung liquid, LV left ventricle. Note that LV and AoT dP/dt_{max} have been normalized to LV end-diastolic volume.

Table 3. LV pressure–volume variables before and after reduction of lung liquid volume.

	Baseline	LL reduction	<i>P</i>
LV end-diastolic volume, ml	3.29 ± 1.29	3.69 ± 1.27	<0.001
LV stroke work, J × 10 ⁻³	10.4 ± 4.5	13.4 ± 5.4	<0.001
LV <i>E</i> _{es} , mmHg ml ⁻¹	56.5 ± 23.5	45.2 ± 14.4	<0.01
LV <i>V</i> ₀ , ml	0.4 ± 0.8	0.4 ± 1.1	0.8

Data are expressed as means ± SD; *n* = 11. LL lung liquid, LV left ventricle, *E*_{es} end-systolic elastance, *V*₀ volume intercept of end-systolic pressure–volume relation.

~8% after a decrease in lung liquid volume (from 1.01 ± 0.51 to 0.93 ± 0.46, *P* < 0.05), but in accord with the assumption made in prior fetal studies,^{19,20} neither value differed from unity (both *P* > 0.6).

LV end-diastolic pressure and volume were measured, and LV transmural pressure estimated as LV end-diastolic pressure minus mean tracheal pressure. LV stroke work in the resting state before and after reduction of liquid volume was computed as the area of the *P*–*V* loop. LV *E*_{es} was obtained from the series of *P*–*V* loops generated during a transient increase in LV afterload produced by progressive tightening of the snare around the descending thoracic aorta. The end-systolic point located at the top left hand corner of each *P*–*V* loop was defined as the maximal *P*-to-*V* ratio. The end-systolic points for all the loops formed a highly linear relationship during the increase in LV afterload, both before (*R*² = 0.98 ± 0.02, fitting over 19 ± 4 points) and after reduction of lung liquid volume (*R*² = 0.98 ± 0.02, fitting over 24 ± 7 points). The slope of this end-systolic *P*–*V* relation (i.e. *E*_{es}), and the volume intercept of this relation (*V*₀), were obtained using a standard iterative method.^{12,13,18}

Statistical analysis

Results were analyzed using GraphPad Prism 7 (GraphPad Software Inc., La Jolla, CA). The effects of a reduction in lung liquid volume on hemodynamic, blood gas, and LV *P*–*V* data were analyzed with one-way repeated measures ANOVA. Data are expressed as mean ± SD and significance was accepted at *P* < 0.05.

RESULTS

Blood gas analysis

Lung liquid volume decreased by 16 ± 4 ml kg⁻¹ body weight, and was associated with minor falls in fetal pH (7.320 ± 0.02 vs. 7.311 ± 0.02, *P* < 0.01), hemoglobin O₂ saturation (63 ± 8 vs. 60 ± 8%, *P* < 0.05), and *P*_aO₂ (24.1 ± 2.3 vs. 23.2 ± 2.1 mmHg, *P* < 0.05), but no change in *P*_aCO₂ (45.1 ± 2.0 vs. 45.6 ± 1.8 mmHg, *P* = 0.4).

Pressures and heart rate

After reduction of lung liquid volume, AoT systolic blood pressure, LV transmural pressure, and heart rate increased, while mean LA, LV end-diastolic, and mean tracheal pressures fell (Table 1). Moreover, the decline in tracheal pressure (4.0 ± 1.8 mmHg) exceeded (*P* < 0.001) falls in mean LA (2.3 ± 1.9 mmHg) and LV end-diastolic pressure (1.8 ± 1.6 mmHg). However, linear relationships were evident between decreases in mean tracheal pressure (*X*) and both mean LA pressure (*Y* = 0.97*X* – 1.59, *R*² = 0.90, *P* < 0.001) and LV end-diastolic pressure (*Y* = 0.58*X* – 0.53, *R*² = 0.43, *P* < 0.05).

Ventricular indices

With reduction of lung liquid volume, LV output rose by 16% (*P* = 0.001), LV stroke volume by 13% (*P* = 0.002), LV dP/dt_{max} by 13% (*P* < 0.001), and AoT dP/dt_{max} by 12% (*P* < 0.001). However, LV and AoT dP/dt_{max} normalized for LV end-diastolic volume were unaltered (Table 2).

Following reduction of lung liquid volume, LV stroke work rose by 29% (*P* < 0.001) and LV end-diastolic volume by 12% (*P* < 0.001; Table 3), with the resting LV *P*–*V* loop increased in area and noticeably shifted to the right (Fig. 1). Transient constriction of the descending thoracic aorta resulted in large and highly linear rises in LV end-systolic *P*–*V* points, both before and after a decrease in lung liquid volume, but only minor fluctuations in end-diastolic pressure and volume (Fig. 2). After reduction of lung liquid volume, *E*_{es} declined by 20% (*P* < 0.01) but *V*₀ was unchanged (Table 3).

DISCUSSION

This is the first experimental study that has utilized gold-standard *P*–*V* analysis to examine the basis of an increased LV pumping performance evident after reduction of lung liquid volume in the fetus. Our findings indicated that rises in LV output, stroke volume and stroke work occurring after a reduction in lung liquid volume were accompanied by increases in LV end-diastolic volume and LV transmural pressure, no change in LV dP/dt_{max} normalized for LV end-diastolic volume and a decrease in LV *E*_{es}, a relatively load-independent measure of cardiac contractility. Taken together, these findings suggest that an augmented LV pump function evident after a reduction of lung liquid volume in fetal lambs arose primarily from an increase in LV preload related to a lessening of LV external constraint by the lungs, without any associated rise in LV contractility.

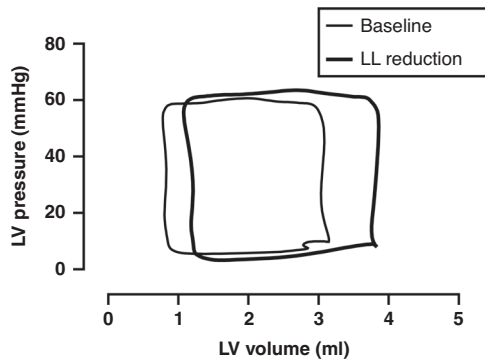


Fig. 1 Left ventricular (LV) pressure–volume loops under resting conditions. Illustrative loops relate to the baseline state (fine line) and after reduction of lung liquid (LL) volume (bold line).

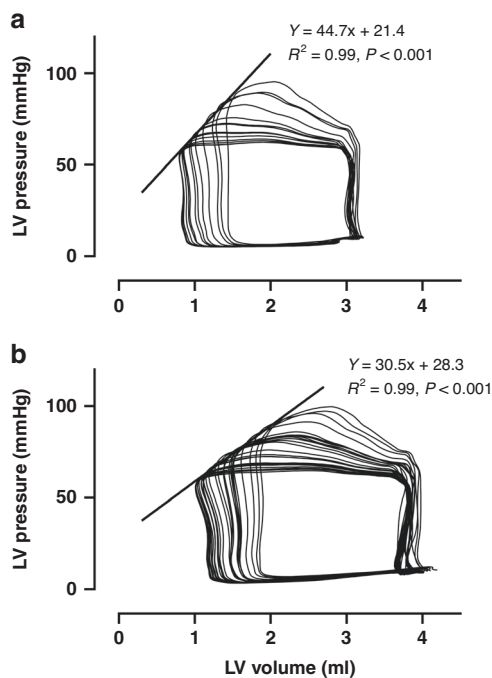


Fig. 2 Left ventricular (LV) pressure–volume loops during transient constriction of the descending thoracic aorta. Highly linear end-systolic pressure–volume relations are depicted before (a) and after (b) reduction of lung liquid volume.

In the fetus, the lungs are filled with fluid which is secreted by the epithelial lining of future airways and airspaces.⁵ This lung liquid hyper-expands the lungs⁵ and exerts extravascular compressive effects on pulmonary microvessels¹ that contribute to a characteristically low level of pulmonary blood flow evident in the fetus.^{5,21} As the fluid-filled lungs are in close apposition to the fetal heart, this hyper-expansion also imposes a physical constraint on the heart which limits its pumping performance. Thus, removal of this external constraint by complete retraction of the lungs away from the fetal heart more than doubles not only RV stroke volume and work,^{6,7} but also LV stroke volume.^{7,8} As evident in the present study (Tables 2 and 3), qualitatively similar (albeit quantitatively smaller) effects on LV stroke volume and stroke work occurred if lung-to-heart contact was maintained, but the degree of lung distension lessened by reducing lung liquid volume.

As lung liquid volume in fetal lambs at the gestation used in the present study is $\sim 40 \text{ ml kg}^{-1}$,²² the amount of liquid drained in

our study (16 ml kg^{-1}) represented approximately a 40% decrease in this volume. Following this reduction of lung liquid volume, increases in LV output, stroke volume, and stroke work were accompanied by a rise in LV end-diastolic chamber volume (and therefore LV filling) as well as LV transmural pressure. These rises in LV end-diastolic volume and transmural pressure were indicative of an increase in LV preload, and thus represent the first direct experimental evidence to support our previous suggestion that reducing lung liquid volume in the fetus diminished LV external constraint.⁴ Note that an additional factor contributing to a rise in LV preload was an increase in pulmonary venous return accompanying a rise in pulmonary blood flow which occurs with a decrease in lung liquid volume.^{1–4} Importantly, the functional consequence of an increased LV preload was greater LV myocardial stretch, which facilitates rises in LV output, stroke volume, and stroke work mediated via the Frank-Starling mechanism.^{23,24}

In our study, linear relationships were evident between a fall in tracheal fluid pressure and declines in LV end-diastolic or mean LA blood pressures. A plausible explanation for these relationships was that the contribution of transmitted pressure from fetal lungs to the left heart chambers decreased following a decline in lung liquid volume. However, the finding that LV end-diastolic volume and LV transmural pressure increased at the same time (Tables 1 and 3) provide further support for the proposition that both LV end-diastolic and mean LA blood pressure are inaccurate measures of LV preload in the fetus, if changes in these pressures are accompanied by alterations in the degree of cardiac constraint.^{7,8} Note that falls observed in LV end-diastolic and mean LA blood pressure were only about half the decrease in mean tracheal pressure, as a decrement in transmitted pressure from the lungs following reduction of lung liquid volume was partially offset by a pressure-increasing effect of increased pulmonary venous return to the left heart chambers that occurred secondary to a rise in pulmonary arterial blood flow.⁴

Unfortunately, the diastolic P – V relation was unable to be assessed in the present study because, as shown in Fig. 2, transient constriction of the descending thoracic aorta produced large changes in the magnitude of end-systolic P – V points, but only minor fluctuations in corresponding end-diastolic points. However, the combination of an increase in LV end-diastolic volume and decrease in LV end-diastolic pressure evident under steady-state conditions (Fig. 1 and Tables 1 and 3) is consistent with a downward and rightward shift of the diastolic P – V relation which occurs after lessening of external cardiac constraint.⁷

In contrast to an augmented LV preload, two lines of evidence suggested that LV contractility was not increased following reduction of lung liquid volume. First, consistent with an increase in AoT dP/dt_{max} reported previously,⁴ AoT and LV dP/dt_{max} both rose after lung liquid volume was reduced in the present study. However, in accord with the known dependency of AoT and LV dP/dt_{max} on loading conditions,¹¹ both of these variables were unchanged after normalization to LV end-diastolic volume (Table 2). Second, in conjunction with a shift of LV P – V loops to the right in the resting state, E_{es} declined by $\sim 20\%$ following a reduction in lung liquid volume, with no change in V_0 . Interestingly, a proportionally similar decrease in LV E_{es} , accompanied by rises in LV stroke volume, stroke work, and end-diastolic volume, was seen with the combination of lung liquid drainage followed by positive pressure mechanical ventilation during the birth transition²⁵ implying that removal of lung liquid per se makes a substantial contribution to LV functional changes occurring at birth.

Several methodological issues require comment. First, a potential limitation of our study was that experiments were performed using an anaesthetized and acutely instrumented fetal

preparation. While we cannot exclude the possibility that use of such a preparation may have altered fetal hemodynamics, any effects were likely to have been quite minor and unlikely to have confounded evaluation of changes arising from a reduction in lung liquid volume, given that (1) variables such as arterial blood gases, heart rate, arterial blood pressure, major central blood flows, and circulating norepinephrine and epinephrine concentrations in our type of preparation at baseline^{3,4,15,26} are similar to those reported in chronically instrumented fetal preparations,^{27–30} and (2) hemodynamic and pulmonary blood flow responses to a decrease in lung liquid volume in our preparation^{3,4} resemble those in chronically instrumented fetal lambs.²

Second, E_{es} in our study was obtained via a transient increase in LV afterload induced by a brief and gradual constriction of the descending thoracic aorta, whereas in the juvenile and adult circulations, E_{es} is usually obtained via a decrease in preload produced by brief occlusion of the inferior vena cava.^{12–14} However, the latter has at least two potential disadvantages in the fetal setting. First, it may result in inconsistent findings, including paradoxical increases in LV stroke volume that presumably result from altered flow patterns across the foramen ovale.³¹ Second, while LV P – V relations were highly linear with an afterload increase (Fig. 2), this relation becomes curvilinear at low pressures and LV chamber volumes that may be encountered during preload reduction,³² which is particularly relevant given that resting arterial blood pressure is already low in preterm fetal lambs (Table 1).^{27–29} Although E_{es} is steeper if generated by aortic occlusion, compared to inferior vena caval occlusion,^{32,33} this is unlikely to have confounded evaluation of changes related to reduction of lung liquid volume.

The results of our study have significant implications for cardiovascular changes occurring in the birth transition. Thus, a fall in lung liquid volume has been reported to occur both before and during labor at birth.^{34,35} Our findings suggest that this fall in lung liquid volume, via a decrease in LV constraint, is likely to contribute to perinatal rises observed in not only LV output and systemic arterial blood flows,^{7,25,36,37} but also LV end-diastolic dimensions.^{8,25,38–40}

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AUTHOR CONTRIBUTIONS

J.J.S.: (1) study conception and design, (2) substantial contributions to data acquisition, analysis, and interpretation, (3) drafted and critically revised article for important intellectual content, and (4) gave final approval of version to be published. M.M.H.C.: (1) substantial contribution to data interpretation, (2) revised article critically for important intellectual content, and (3) gave final approval of version to be published. J.P.M.: (1) substantial contribution to data interpretation, (2) revised article critically for important intellectual content, and (3) gave final approval of version to be published.

ADDITIONAL INFORMATION

Competing interests: J.P.M. is a consultant to the Brain Protection Company and Masimo Corporation. These companies provided no input into this study.

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