Retrograde lower body arterial reservoir discharge underlies rapid reversal of ductus arteriosus shunting after early cord clamping at birth in preterm lambs

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Abstract

Arterial reservoir (‘windkessel’) function, whereby a part of left ventricular (LV) output is stored in elastic arteries during systole and discharged in diastole, is a well-established physiological phenomenon. However, its role in rapid reversal (to left-to-right) and a systolic-to-diastolic shift of shunting across the ductus arteriosus after birth is unknown. To address this question, ductal and aortic isthmus flows were measured with high-fidelity transit-time probes in six anesthetized preterm fetal lambs before and after cord clamping and subsequent early mechanical ventilation, and for 30 min post-birth. Descending aortic flow was calculated as the sum of isthmus and ductal flows. Left-to-right ductal flow profiles were related to those of the isthmus and descending aorta, with upper body arterial reservoir discharge indicated by forward diastolic isthmus flow, and retrograde lower body arterial reservoir discharge by negative diastolic descending aortic flow. Left-to-right ductal shunting appeared immediately after cord clamping (P<0.001), due entirely to newly-emergent retrograde lower body reservoir discharge, and rose with ventilation via increased lower body reservoir discharge (P<0.005), supplemented by upper body reservoir discharge after 45 sec (P<0.025) and LV systolic flow after 3 min (P=0.025). The contribution of lower body reservoir discharge to left-to-right ductal shunting fell to 55±8% at ≥15 min (P<0.001), but remained higher (P<0.002) than LV systolic flow (33±8%) or upper body reservoir discharge (12±5%). These results suggest that retrograde lower body arterial reservoir discharge plays a key role in rapid reversal and a systolic-to-diastolic shift of ductal shunting after cord clamping and early ventilation at birth.

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The ductus arteriosus, a large vascular channel which connects the pulmonary trunk to the descending thoracic aorta just beyond the aortic isthmus, exhibits striking flow alterations during the birth transition. Thus, in the fetus, the ductus permits most of the right ventricular (RV) output to bypass the fluid-filled lungs, where vascular resistance is high, and instead undergo right-to-left shunting directly into the descending aorta, from where it perfuses tissues of the lower fetal body and passes to the placenta for re-oxygenation (11, 25, 37). Fetal ductal shunting occurs mainly in systole (8, 19, 21, 29, 33), with a minor degree of right-to-left shunting also evident in diastole (19, 21, 29, 34, 36). Within minutes after the onset of lung ventilation at birth, however, ductal shunting switches to a left-to-right direction, with this reversed shunting providing 30-50% of pulmonary blood flow (8, 32, 38) and occurring predominantly in diastole (8, 26).

The factors implicated in a postnatal emergence of left-to-right ductal shunting include a birth-related fall in pulmonary vascular resistance (26, 38) and a reversal of a positive pulmonary-to-aortic mean pressure difference present in utero (8). By contrast, the basis of an accompanying rapid systolic-to-diastolic shift in phasic ductal flow has not been established, although a proposed explanation is that left-to-right ductal flow arises directly from the left ventricular (LV) systolic outflow which, because the path-length of the ascending aorta and aortic isthmus exceeds that of the pulmonary trunk, does not reach the ductus until after RV systolic outflow has traversed this channel (15-17, 39). However, an additional but as yet unexamined possibility is that diastolic left-to-right ductal flow arises from systemic arterial reservoir discharge.

While a well-established feature of large systemic elastic arteries in the adult (5, 18, 28), reservoir (or ‘windkessel’) function has received scant attention in the fetal circulation, and none during the birth transition. Nonetheless, the hallmark of reservoir discharge, namely continuation of forward (i.e. positive) flow in distal arteries during diastole (5), when the heart is no longer contracting and the aortic valve is closed, is clearly evident in published blood flow/velocity profiles of many major fetal systemic arteries (1, 6, 7, 12, 32), and is particularly prominent in umbilical arteries (1, 2, 12). However, an added level of complexity is that, with the ‘in parallel’ and compartmentalized arrangement of the fetal arterial...
circulation (25, 31), two functionally discrete systemic arterial reservoirs are likely to exist in utero, interconnected through the aortic isthmus. The first is an upper body reservoir formed by the ascending aorta and major cephalic arteries, filled almost exclusively by LV outflow and discharging principally into cephalic organs. The second is a lower body arterial reservoir comprising the descending thoracic and abdominal aorta and its major branches, filled mainly by RV output and discharging into lower body tissues and the umbilico-placental circulation. Thus, any reservoir discharge passing left-to-right across the ductus during and after the birth transition could potentially arise via discharge from the upper body arterial reservoir crossing the aortic isthmus, retrograde discharge from the lower body arterial reservoir, or both.

This study, which was performed in anesthetized and acutely-instrumented preterm lambs undergoing immediate clamping of the umbilical cord and early mechanical ventilation at delivery, therefore had two main aims. The first was to determine, via analysis of central high-fidelity blood flow profiles, the contributions of LV systolic flow, upper body arterial reservoir discharge and lower body arterial reservoir discharge to left-to-right ductal flow in the birth transition. The second aim was to establish if the relative proportion of contributing sources to reversed ductal flow changed as the degree of shunting increased, given that left-to-right ductal shunting rises rapidly to a peak by 10-15 min after birth (8, 32).

Methods

Studies conformed to guidelines of the National Health and Medical Council of Australia and were approved by the institutional Animal Ethics Committee.

Surgical preparation The general features of the surgical preparation were as previously described (32). Briefly, six Border-Leicester cross ewes were anesthetized at a gestation of 128 ± 1 days (mean ± SD, term = 147 days) with intramuscular ketamine 5 mg·kg⁻¹ and xylazine 0.1 mg·kg⁻¹, followed by 4% isoflurane given by mask. After intubation of the trachea, anesthesia was maintained with isoflurane (2-3%) and nitrous oxide (10-20%) delivered in O₂-enriched air using a ventilator, supplemented by intravenous infusion of ketamine (1-1.5 mg·kg⁻¹·hr⁻¹), midazolam (0.1-0.15 mg·kg⁻¹·hr⁻¹) and fentanyl (2-2.5 mg·kg⁻¹·hr⁻¹). Transcutaneous oxygen saturation (SpO₂) 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 blood gas analysis, with ventilation of the ewe adjusted to
maintain arterial Po$_2$ (P$_{aO_2}$) at 100-120 mmHg and arterial Pco$_2$ (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 adjacent thorax, a fluid-filled catheter was passed into the superior vena cava via the left
axillary vein for fluid and drug administration. In addition, the ascending aorta was
cannulated via the left axillary artery with a short fluid-filled catheter for pressure
measurement and blood sampling, and a 3.5-Fr micromanometer (SPR-524, Millar
Instruments, Houston, TX) to measure high-fidelity pressure. A thoracotomy was performed
in the 3rd interspace and vessels carefully dissected for placement of non-constrictive transit-
time flow probes around the aortic isthmus (6 mm), ductus arteriosus (8 or 10 mm) and left
pulmonary artery (4 or 6 mm). A fluid-filled catheter and another 3.5-Fr micromanometer
were inserted via purse-string sutures into the pulmonary trunk near the junction of the ductus
and common pulmonary artery to measure pressure (Fig. 1). After placement of a fluid-filled
catheter into the left atrial appendage for pressure measurement, a clamped 4.5 mm
endotracheal tube containing a proximal side-port for measurement of tracheal pressure was
inserted via a tracheostomy in a proximal intercartilaginous space and tied into place.

**Experimental protocol** After removal of the glove over the fetal head, the endotracheal
tube was unclamped to allow lung liquid to drain passively via gravity for ~20 sec, and then
re-clamped to prevent lung aeration prior to ventilation. While hemodynamics were recorded
continuously onto computer, the fetus was completely delivered from the uterus, placed on the
ewe’s abdomen without tension on the umbilical cord, and covered with warmed towels.
Following withdrawal of an aortic sample 30 sec after delivery for blood gas analysis, the
umbilical cord was occluded with a clamp 1-2 cm from its abdominal insertion site, and
another blood gas sample withdrawn 15 sec later. The endotracheal tube was then connected
to an infant ventilator (SLE 5000) and positive-pressure mechanical ventilation commenced
with a warmed and humidified O$_2$/air mixture 31 ± 9 sec after cord clamping, to avoid an
asphyxial state which occurs if the cord clamp-to-ventilation interval exceeds 45 sec (32).
Initial ventilator settings comprised a maximum peak inspiratory pressure of 50 cm H2O, a positive end-expiratory pressure of 8 cm H2O, a respiratory rate of 60 breaths per min, an inspiratory time of 0.4 sec, a tidal volume of 7 ml·kg⁻¹ estimated body weight and an inspired O2 concentration of 30%. Ventilator settings were adjusted as required to maintain a SpO2 of >90% measured with a pulse oximetry sensor applied to the cheek pouch. After delivery, anesthesia in newborn lambs was continued with an intravenous infusion of ketamine (4-8 mg·kg⁻¹·hr⁻¹) and midazolam (0.05-0.1 mg·kg⁻¹·hr⁻¹), with aortic blood gas samples obtained at 0.5, 1, 2, 3, 5 and 10 min after the start of ventilation.

The recording of physiological data onto computer that had been commenced just prior to delivery was continued for a further 10 min after the start of ventilation. After cutting of the umbilical cord and completion of this recording, the lamb was carefully transferred onto a heated neonatal resuscitation table. Hemodynamic data were subsequently recorded at 15 and 30 min after onset of ventilation, with each recording preceded by withdrawal of an aortic sample for blood gas analysis. Beyond the 10 min time-point, ventilator settings were adjusted on the basis of aortic blood gas results, with a target hemoglobin O2 saturation (SaO2) of 95-98% and PaCO2 of 45-55 mmHg. Ewes were euthanased with intravenous pentobarbitone (100 mg·kg⁻¹) after cord clamping, and lambs after completion of the study protocol, with zero-offset calibrations of all flow probes then confirmed in situ.

**Hemodynamic data** Aortic, pulmonary, left atrial and tracheal catheter pressures were measured with transducers referenced to atmospheric pressure at left atrial level, and calibrated against a water manometer before each study. Signals from catheters, micromanometers and flow probes were digitized at a sampling rate of 1 kHz using programmable acquisition and analysis software (Spike2, Cambridge Electronic Design, Cambridge, UK).

As hemodynamics can change very rapidly during the birth transition, 5 sec data blocks were analyzed from the delivery data file 1) 15 sec after cord clamping, 2) immediately before the start of ventilation and 3) at 15 sec intervals in the first minute after the start of ventilation. In addition, 10 sec data blocks were analyzed 1) just before cord clamping and 2) at 2, 3, 4, 6, 8, 10, 15 and 30 min after ventilation onset. Apart from a 48 Hz low-pass filter to remove
electrical interference from signals, no filtering was employed during analysis, with analyses undertaken on ensemble-averaged signals typically generated from 12-25 beats.

During data analysis, 1) an instantaneous high-fidelity pulmonary-to-aortic pressure difference profile was derived after matching of mean aortic and pulmonary trunk micromanometer pressures to the corresponding catheter pressures; 2) combined left and right pulmonary arterial flow was calculated as the product of measured left pulmonary arterial flow and the total-to-left lung weight ratio (32), 3) pulmonary vascular resistance was computed as \( \frac{\text{mean pulmonary pressure} - \text{mean left atrial pressure}}{\text{mean pulmonary arterial flow}} \), and normalized to wet lung weight; 4) a descending aortic blood flow profile was generated as the instantaneous sum of the isthmus and ductal flows, with studies performed in a separate group of animals \( (n = 3) \) indicating that the morphology of this synthesized profile closely mirrored that of the waveform measured with a transit-time flow probe placed on the descending thoracic aorta just distal to its origin (data not shown).

**Phasic blood flow analyses**  Systole was defined as the interval between the onset of the systolic upstroke and the dicrotic notch in the aortic trunk micromanometer blood pressure profile, with diastole constituting the remainder of the cardiac cycle. To define the specific sources of left-to-right ductal shunting, the negative portion of the ductal flow profile was related to corresponding parts of the isthmus and descending aortic flow profiles as follows: 1) contributions arising directly from LV systolic ejection, which were usually separated by a mid-systolic area of RV-derived right-to-left flow, occurred in the early and late systolic segments of the isthmus flow profile; 2) discharge from the upper body arterial reservoir was evident as positive isthmus flow in diastole; 3) a contribution arising from retrograde discharge of the lower body arterial reservoir occurred where descending aortic flow was negative in diastole, and was calculated as total left-to-right ductal flow minus the summed LV systolic flow and upper body reservoir contributions. With these three flow sources, four main patterns were evident in their contribution to left-to-right ductal shunting, namely 1) combined lower body reservoir discharge and LV systolic flow in the systole-diastole borderzone, 2) only LV systolic flow in early and late systole, 3) only lower body reservoir discharge in early diastole, and 4) combined lower and upper body reservoir discharge in mid
and late diastole (Fig. 2). Raw measured flows of all segmental components were multiplied by the quotient of segment duration and heart period to yield reported flow values, which summed to total left-to-right ductal flow.

Statistical analysis Results were analyzed using GraphPad Prism (v6.02, La Jolla, CA). Longitudinal hemodynamic and blood gas data were analyzed with one way repeated measures analysis of variance and specific comparisons evaluated by partitioning the within-animal sums of squares into individual degrees of freedom, with a Bonferroni correction applied as required for multiple comparisons. Data are expressed as mean ± SD and significance was taken at $P < 0.05$.

Results

Blood gases and hemodynamics After umbilical cord clamping, pH, $\text{SaO}_2$ and $\text{PaO}_2$ fell rapidly, while $\text{PaCO}_2$ increased (all $P < 0.001$), with reversal of these changes after the start of ventilation (Table 1). PVR was unchanged after cord clamping, but fell by 55% with ventilation ($P < 0.001$, Fig. 3A). Mean aortic and pulmonary blood pressures increased by 8 mmHg after cord clamping ($P < 0.001$), but dropped 10-11 mmHg by 15 sec after ventilation ($P < 0.001$) before partially recovering over 3-4 min, with pulmonary blood pressure then falling progressively to the 15 min time-point ($P < 0.025$; Fig. 3B). The pulmonary-to-aortic mean pressure difference was positive in fetuses ($2.0 \pm 1.8$ mmHg, $P < 0.05$) and unaffected by cord clamping, but fell after ventilation to be negative at ≥8 min after birth ($P < 0.005$; Fig. 3C). Concurrently, the morphology of the pulmonary-to-aortic pressure difference profile was transformed from a positive and largely systolic deflection in the fetus, to an extensive negative waveform with a prominent early systolic spike in the newborn (Fig. 4).

Morphology of blood flow profiles In the fetal state, ductal shunting was right-to-left throughout the cardiac cycle and descending aortic flow positive in diastole, with trivial negative ductal and descending aortic flow spikes occasionally evident in early diastole. However, clear-cut diastolic left-to-right ductal shunting appeared immediately after cord clamping, accompanied by diastolic backflow in both the isthmus and descending aorta (Fig. 5). This left-to-right ductal shunting rose further within 15 sec after the onset of ventilation.
and then increased progressively, in association with greater backflow from the descending
aorta and, after several minutes, the appearance of a transient early-systolic negative spike in
ductal flow that mirrored a positive spike in the isthmus profile. By contrast, however,
isthmus diastolic backflow initially fell with ventilation, with later emergence of a
progressively increasing degree of forward flow in diastole (Fig. 6A). As expected, rises in
left-to-right ductal shunting after cord clamping and ventilation were accompanied by a
progressively greater positive offset in the pulmonary arterial flow profile (Fig. 6B).

Blood flows Although falling abruptly \( (P < 0.001) \), mean ductal flow remained positive
with cord clamping but was negative at \( \geq 4 \) min after ventilation \( (P < 0.001) \), peaking at -415
ml/min by 15 min. However, significant left-to-right ductal shunting was first evident after
cord clamping \( (P < 0.001) \), and progressively increased following ventilation \( (P < 0.001) \) to a
nadir of -440 ml/min at 15 min. Correspondingly, right-to-left ductal flow fell with cord
clamping \( (P < 0.001) \), and then further with ventilation to be statistically zero at \( \geq 10 \) min (Fig.
7A). In association with a progressive rise in pulmonary arterial blood flow, mean isthmus
and descending aortic flows both fell after cord clamping \( (P < 0.001) \), but diverged after
ventilation, with isthmus flow increasing appreciably \( (P < 0.001) \) and descending aortic flow
falling further \( (P < 0.05; \) Fig. 7B).

Sources of left-to-right ductal flow Before and during cord clamping in the fetus, left-to-
right ductal shunting was entirely derived from lower body reservoir discharge. A significant
contribution from upper body reservoir discharge was evident by 45 sec after ventilation \( (8 \pm
6 \) ml, \( P < 0.025) \), and from LV systolic flow by 3 min \( (43 \pm 34 \) ml, \( P = 0.025) \) with the latter,
in particular, then increasing further \( (P < 0.002; \) Fig. 8A). However, while lower body
reservoir discharge also continued to rise after ventilation \( (P < 0.005) \), its relative contribution
to left-to-right ductal shunting diminished to \( 55 \pm 8\% \) at \( \geq 15 \) min \( (P < 0.001) \), which was still
higher than either LV systolic flow \( (33 \pm 8\%, P < 0.002) \) or upper body reservoir discharge
\( (12 \pm 5\%, P < 0.001; \) Fig. 8B).

Discussion
In the adult circulation, systemic arterial reservoir (or ‘windkessel’) function fulfills an
essential physiological role, as it enhances LV pumping efficiency and ventricular-vascular
coupling and, via a buffering effect, transforms a highly pulsatile LV systolic output into a relatively steady distal flow which spans both systole and diastole, thereby protecting the microvasculature from deleterious effects of excessive shear stress (5, 18, 28). However, until the present study, no direct assessment of systemic arterial reservoir function has been undertaken in the fetal and transitional circulations, even though forward blood flow/velocity in diastole, a distinctive feature of arterial reservoir discharge (5), is clearly evident in many major systemic arteries of the fetus and newborn in both experimental (1, 3, 7, 32) and clinical studies (6, 12).

In the adult, the systemic arterial reservoir is filled by LV outflow in systole and discharges into the microvasculature in diastole (5). However, as also observed in clinical Doppler-echocardiographic studies (12, 19, 21), both ductal and descending thoracic aortic flows were positive throughout systole and diastole in the fetus before cord clamping, while isthmus flow was positive in systole and much of diastole (Figs. 5&6). These flow patterns suggest that, in line with the biventricular origin of descending aortic blood flow (25, 31), systolic filling of the lower body portion of the systemic arterial reservoir in fetuses occurred from a combination of ductal (i.e. RV-derived) and isthmus (i.e. LV-derived) flow. Furthermore, diastolic flow from this reservoir was enhanced not only by continuous right-to-left ductal flow during diastole (19, 21), which is related to a combination of antegrade discharge from a central, pulmonary trunk reservoir and backflow from a conduit pulmonary arterial reservoir (34, 36), but also by discharge from the upper body arterial reservoir across the isthmus. Moreover, the increasing prominence of diastolic flow from the descending thoracic aorta to the umbilical arteries (1, 12) indicates that substantial additional reservoir function resides within the abdominal aorta and that antegrade discharge from the lower body arterial reservoir constitutes a major component of placental perfusion.

In our study, immediate cord clamping followed by early ventilation produced highly reproducible changes in ductal shunting patterns, which arose from clearly discernible mechanisms. Thus, cord clamping resulted in the immediate appearance of a left-to-right ductal flow component in diastole, accompanied by similar negative components in both the isthmus and descending aortic flow profiles, as well as more positive diastolic flow in the
pulmonary arterial profile (Figs. 5&6). The most plausible explanation for these phasic flow changes is that, with abolition of antegrade discharge from the lower body arterial reservoir into the umbilico-placental circulation by umbilical cord clamping, discharge from this reservoir then abruptly switched direction, with the resultant retrograde flow mainly passing left-to-right across the ductus (thereby increasing diastolic pulmonary arterial blood flow), and also across the isthmus as backflow that enhanced cephalic discharge of the upper body arterial reservoir. Importantly, this initial change in direction of lower body arterial reservoir discharge (and thus the onset of significant diastolic left-to-right ductal shunting) was not dependent on falls in pulmonary vascular resistance or the pulmonary-to-aortic mean pressure difference, as both were unaffected by cord clamping (Fig. 3).

With the addition of ventilation, left-to-right ductal shunting, diastolic pulmonary arterial flow and the negative diastolic component of descending thoracic aortic flow increased further, whilst backflow across the isthmus fell (Fig. 6). These flow changes suggest that ventilation initially further increased retrograde discharge from the lower body arterial reservoir, presumably related to the ventilation-induced fall in pulmonary vascular resistance (Fig. 3), and also redistributed this discharge, so that even a greater portion passed left-to-right across the ductus, and thence to the lungs. With ongoing ventilation, isthmus flow became positive after 45 sec, first in late-diastole and later throughout most of diastole, indicating that discharge from the upper body arterial reservoir was then also supporting left-to-right ductal shunting. Subsequently, an increasing LV systolic flow contribution to left-right ductal shunting via isthmus flow in systole emerged by 3 min after ventilation onset, in parallel with a rise in LV output (32), and rapidly increased to exceed the contribution of upper body reservoir discharge. Thus, the systolic and diastolic proportions of left-to-right ductal shunting were quite dynamic in the birth transition, with an entirely diastolic flow during cord clamping in the fetus followed by a shift to combined diastolic and systolic shunting after several minutes’ ventilation, which stabilized at a proportion of two-thirds diastolic (i.e. the sum of lower and upper body reservoir discharge) and one-third systolic by 15 min (Fig. 8).
The results of the present study challenge two widely-held notions about left-to-right ductal shunting in the immediate period after birth. First, it is commonly presumed that blood undergoing such shunting is all derived from LV output (15-17, 39). However, our finding that retrograde discharge from a lower body arterial reservoir constitutes the main source of this shunting has an important corollary because, at least until systolic ductal flow falls to zero at ≥10 min after birth (Fig. 7), this reservoir will be filled by a combination of LV and RV systolic outflow. Moreover, comparison of ductal, isthmus and descending thoracic aortic flow patterns (Fig. 7) suggests that RV outflow was the main filling source of the lower body reservoir not only in the fetus, but also after cord clamping before ventilation and for the first minute after the onset of ventilation. A progressive shift to a predominant LV contribution then occurred over the next 10 or so minutes, as isthmus flow increased with a rise in LV output, and as systolic ductal flow fell to zero due to an increasingly greater distribution of a lowered RV output towards the lungs (32).

Second, it has been asserted that a predominant diastolic component of left-to-right ductal shunting is related to LV systolic outflow reaching the ductus in diastole, due to the path-length of the ascending aorta and aortic isthmus exceeding that of the pulmonary trunk (15-17, 39). However, the close temporal matching of positive isthmus and negative ductal flow components observed in systole after ventilation in the present study (Fig. 6) clearly demonstrate that LV systolic outflow crossed the ductus in systole, a conclusion also supported by two other lines of evidence. First, as wave speed in the perinatal aorta ranges from 3.6-5.8 m/s (13, 35, 40), it would take only 9-14 ms to travel the 51 ± 2 mm path-length (measured in 10 preterm lambs of similar gestation) from the aortic valve along ascending aorta and isthmus to the mid-length of the ductus, which is well within the duration of systole (183 ± 24 ms in the present study). Second, and perhaps more importantly, while LV and RV pre-ejection periods are similar in utero, the RV pre-ejection period increases but the LV pre-ejection period is unchanged in the initial 30 min after birth (14) i.e. the onset of LV ejection then precedes RV ejection. That LV ejection occurred before RV ejection in newborn lambs of our study was indicated by the appearance of a widening interval between the upstrokes of the high-fidelity aortic and pulmonary pressure profiles, which generated a very early and
progressively larger negative systolic spike in the pulmonary-to-aortic pressure difference profile after birth (Fig. 4). Importantly, this spike was accompanied by a corresponding early-systolic negative spike in the ductal flow profile (Fig. 6), a feature also evident at the 5 min newborn time-point in Fig. 5 of (8).

A potential limitation of our study was that it was performed under general anesthesia and open-chest conditions, an approach necessary because of the extent of instrumentation required to obtain required multi-site high-fidelity blood flow and pressure measurements. However, key features of the birth transition were similar to those previously reported in chronically-instrumental fetal lambs, including a rapid rise in pulmonary arterial flow and a marked fall in pulmonary vascular resistance, as well as a rapid switchover from fetal right-to-left to postnatal left-to-right ductal shunting (7-9, 11, 27). Nonetheless, we cannot exclude the possibility that dissection around central vessels with placement of flow probes altered changes in vessel compliance and flow patterns during the birth transition, although any effect is likely to be minor. In particular, while the ductus can constrict with mechanical manipulation (26), it is unlikely that any significant ductal constriction was present before birth in our study as the pulmonary-to-aortic mean pressure difference, a sensitive indicator of such constriction (36), was very similar to the value of 1-2 mmHg measured in chronically instrumented, normoxemic preterm fetal sheep without any ductal instrumentation (9, 10, 24).

In conclusion, the results of this study of the preterm birth transition strongly suggest that newly-emergent retrograde discharge from the lower body systemic arterial reservoir plays a key role in supporting rapid reversal and a systolic-to-diastolic shift of ductal shunting after immediate cord clamping followed by early ventilation, with this source of left-to-right ductal flow preceding and exceeding trans-isthmus contributions arising from LV systolic flow and upper body arterial reservoir discharge. Our findings also suggest that cord clamping and ventilation had specific but differing effects on left-to-right ductal flow patterns, in that cord clamping instigated an abrupt change in direction of the normal fetal antegrade discharge from the lower body arterial reservoir, with the resultant retrograde diastolic discharge passing both to the lungs via the ductus and to the fetal upper body across the aortic isthmus. On the other hand, presumably because of an associated fall in pulmonary vascular resistance,
ventilation not only enhanced retrograde lower body arterial reservoir discharge and directed it entirely across the ductus, but also promoted contributions to left-to-right ductal shunting via the isthmus from LV systolic flow and upper body arterial reservoir discharge. These divergent effects of cord clamping and ventilation thus imply that the temporal sequence of changes in flow patterns at the confluence of the isthmus, ductus and descending aorta, as well as the proportional make-up of left-to-right ductal flow sources, will differ from those described in the present study if ventilation precedes cord clamping at birth.

Finally, given that diminution, absence or even reversal of arterial diastolic blood flow/velocity accompanies many fetal and newborn cardiovascular disease states (20, 30), a wider implication of our study is that abnormalities of systemic arterial reservoir discharge may constitute a significant yet largely unrecognized contributory factor to perinatal hemodynamic disturbances. This is likely to be particularly relevant in preterm infants, where abnormal arterial diastolic blood flow/velocity profiles are a marker of systemic hypoperfusion (30), are more common in the presence of hypotension (23) and are a predictor of adverse neonatal outcomes (4, 22).
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References


Figure legends

Figure 1. Schematic diagram of lamb instrumentation. Abbreviations: AI FP, aortic isthmus flow probe; Ao F, ascending aortic fluid-filled catheter; Ao M, ascending aortic micromanometer catheter; DTA, descending thoracic aorta; Duct FP, ductus arteriosus flow probe; LPA FP, left pulmonary artery flow probe; LV, left ventricle; PT F, pulmonary trunk fluid-filled catheter; PT M, pulmonary trunk micromanometer catheter; RV, right ventricle.

Figure 2. Illustrative blood flow profiles in the ductus arteriosus (panel A), descending thoracic aorta (panel B) and aortic isthmus (panel C) after birth, showing negative left-to-right ductal flow arising from a combination of retrograde descending aortic and forward isthmus flows (region 1), forward isthmus flow alone (region 2), retrograde descending aortic flow alone (region 3) and combination of retrograde descending aortic and forward diastolic isthmus flows (region 4). Note that time zero is the beginning of systole, defined as the onset of the upstroke of the aortic micromanometer-derived pressure signal.

Figure 3. Pulmonary vascular resistance (panel A), ascending aortic (AoT) and pulmonary trunk (PT) blood pressures (panel B) and the pulmonary-to-aortic mean blood pressure difference (panel C) in the fetal state (F), 15 sec after umbilical cord clamping (CC), immediately before ventilation, and for 30 min after ventilation. Note that the time scale of the initial minute after ventilation (shaded area) has been magnified to aid visualization.

Figure 4. Illustrative example of changes in high-fidelity aortic (black line) and pulmonary trunk blood pressures (thick gray line) and the instantaneous pulmonary-to-aortic blood pressure difference (thin gray line) from the fetus to 15 sec after cord clamping (15sCC), followed by selected intervals after the onset of ventilation. Note 1) the shaded gray area in each time segment represents duration of systole; 2) the clear time delay between the upstrokes of the aortic and pulmonary profiles at 4 and 10 min (clear arrows), accompanied by a prominent negative spike in the pulmonary-to-aortic blood pressure difference (black arrows).
Figure 5. Representative example of phasic changes in pulmonary trunk blood pressure profile (panel A) and blood flow waveforms in the ductus arteriosus (panel B), descending thoracic aorta (panel C) and aortic isthmus (panel D) occurring with cord clamping (CC).

Figure 6. Illustrative example of changes in blood flows of ductus arteriosus (thick black line), descending thoracic aorta (gray line) and aortic isthmus (thin black line; panel A) and pulmonary artery (panel B) in the birth transition from the same study as in Fig. 4. Note 1) shaded gray area in each time segment represents duration of systole; 2) the prominent early-systolic negative spike in the ductal flow profile at 4 and 10 min (black arrows).

Figure 7. Mean, right-to-left (R→L) and left-to-right (L→R) ductal blood flow (panel A), and mean descending thoracic aortic (DTA), pulmonary arterial (PA) and aortic isthmus (AI) blood flows (panel B) in the fetal state (F), 15 sec after umbilical cord clamping (CC), immediately before ventilation, and for 30 min after ventilation. Note that the time scale of the initial minute after ventilation (shaded area) has been magnified to aid visualization.

Figure 8. Absolute (panel A) and percentage (panel B) contribution to left-to-right ductal shunting arising from lower body arterial reservoir discharge (LB reservoir), upper body arterial reservoir discharge (UB reservoir) and directly from left ventricular systolic flow (LV systolic) in the fetal state (F), 15 sec after umbilical cord clamping (CC), immediately before ventilation, and for 30 min after ventilation. Note that 1) the solid thick line in panel A is the magnitude of total left-to-right ductal flow depicted in Fig. 7A; 2) the time scale of the initial minute after ventilation (shaded area) has been magnified to aid visualization.
Figure 1. Schematic diagram of lamb instrumentation. Abbreviations: AI FP, aortic isthmus flow probe; Ao F, ascending aortic fluid-filled catheter; Ao M, ascending aortic micromanometer catheter; DTA, descending thoracic aorta; Duct FP, ductus arteriosus flow probe; LPA FP, left pulmonary artery flow probe; LV, left ventricle; PT F, pulmonary trunk fluid-filled catheter; PT M, pulmonary trunk micromanometer catheter; RV, right ventricle.
Figure 2. Illustrative blood flow profiles in the ductus arteriosus (panel A), descending thoracic aorta (panel B) and aortic isthmus (panel C) after birth, showing negative left-to-right ductal flow arising from a combination of retrograde descending aortic and forward isthmus flows (region 1), forward isthmus flow alone (region 2), retrograde descending aortic flow alone (region 3) and combination of retrograde descending aortic and forward diastolic isthmus flows (region 4). Note that time zero is the beginning of systole, defined as the onset of the upstroke of the aortic micromanometer-derived pressure signal.
Figure 3. Pulmonary vascular resistance (panel A), ascending aortic (AoT) and pulmonary trunk (PT) blood pressures (panel B) and the pulmonary-to-aortic mean blood pressure difference (panel C) in the fetal state (F), 15 sec after umbilical cord clamping (CC), immediately before ventilation, and for 30 min after ventilation. Note that the time scale of the initial minute after ventilation (shaded area) has been magnified to aid visualization.
Figure 4. Illustrative example of changes in high-fidelity aortic (black line) and pulmonary trunk blood pressures (thick gray line) and the instantaneous pulmonary-to-aortic blood pressure difference (thin gray line) from the fetus to 15 sec after cord clamping (15sCC), followed by selected intervals after the onset of ventilation. Note 1) the shaded gray area in each time segment represents duration of systole; 2) the clear time delay between the upstrokes of the aortic and pulmonary profiles at 4 and 10 min (clear arrows), accompanied by a prominent negative spike in the pulmonary-to-aortic blood pressure difference (black arrows).
Figure 5. Representative example of phasic changes in pulmonary trunk blood pressure profile (panel A) and blood flow waveforms in the ductus arteriosus (panel B), descending thoracic aorta (panel C) and aortic isthmus (panel D) occurring with cord clamping (CC).
Figure 6. Illustrative example of changes in blood flows of ductus arteriosus (thick black line), descending thoracic aorta (gray line) and aortic isthmus (thin black line; panel A) and pulmonary artery (panel B) in the birth transition from the same study as in Fig. 4. Note 1) shaded gray area in each time segment represents duration of systole; 2) the prominent early-systolic negative spike in the ductal flow profile at 4 and 10 min (black arrows).
Figure 7. Mean, right-to-left (R→L) and left-to-right (L→R) ductal blood flow (panel A), and mean descending thoracic aortic (DTA), pulmonary arterial (PA) and aortic isthmus (AI) blood flows (panel B) in the fetal state (F), 15 sec after umbilical cord clamping (CC), immediately before ventilation, and for 30 min after ventilation. Note that the time scale of the initial minute after ventilation (shaded area) has been magnified to aid visualization.
Figure 8. Absolute (panel A) and percentage (panel B) contribution to left-to-right ductal shunting arising from lower body arterial reservoir discharge (LB reservoir), upper body arterial reservoir discharge (UB reservoir) and directly from left ventricular systolic flow (LV systolic) in the fetal state (F), 15 sec after umbilical cord clamping (CC), immediately before ventilation, and for 30 min after ventilation. Note that 1) the solid thick line in panel A is the magnitude of total left-to-right ductal flow depicted in Fig. 7A; 2) the time scale of the initial minute after ventilation (shaded area) has been magnified to aid visualization.
### TABLE 1. Ascending aortic blood gas variables before and after clamping of the umbilical cord, and following ventilation.

<table>
<thead>
<tr>
<th></th>
<th>Hb (g/dl)</th>
<th>pH</th>
<th>SaO2 (%)</th>
<th>PaO2 (mmHg)</th>
<th>PaCO2 (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fetal</strong></td>
<td>10.9 ± 0.8</td>
<td>7.324 ± 0.015&lt;sup&gt;a&lt;/sup&gt;</td>
<td>60.5 ± 4.3</td>
<td>21.5 ± 2.1</td>
<td>47.2 ± 3.4</td>
</tr>
<tr>
<td><strong>Post-cord clamp, 15 sec</strong></td>
<td>11.1 ± 0.9</td>
<td>7.300 ± 0.014&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31.2 ± 8.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.4 ± 2.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>52.6 ± 3.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Post-ventilation, 0.5 min</strong></td>
<td>11.1 ± 0.7</td>
<td>7.295 ± 0.013&lt;sup&gt;b&lt;/sup&gt;</td>
<td>63.8 ± 21.6</td>
<td>25.1 ± 9.6</td>
<td>48.7 ± 2.7</td>
</tr>
<tr>
<td><strong>Post-ventilation, 1 min</strong></td>
<td>11.1 ± 0.8</td>
<td>7.299 ± 0.013&lt;sup&gt;b&lt;/sup&gt;</td>
<td>72.6 ± 22.2</td>
<td>29.1 ± 9.5</td>
<td>47.6 ± 1.9</td>
</tr>
<tr>
<td><strong>Post-ventilation, 2 min</strong></td>
<td>11.0 ± 0.9</td>
<td>7.317 ± 0.024&lt;sup&gt;b&lt;/sup&gt;</td>
<td>79.5 ± 27.0</td>
<td>35.8 ± 14.2</td>
<td>43.9 ± 3.0</td>
</tr>
<tr>
<td><strong>Post-ventilation, 3 min</strong></td>
<td>10.8 ± 0.9</td>
<td>7.321 ± 0.049&lt;sup&gt;b&lt;/sup&gt;</td>
<td>86.2 ± 24.8</td>
<td>57.7 ± 44.0</td>
<td>42.9 ± 5.7</td>
</tr>
<tr>
<td><strong>Post-ventilation, 5 min</strong></td>
<td>10.7 ± 0.8</td>
<td>7.317 ± 0.071&lt;sup&gt;b&lt;/sup&gt;</td>
<td>94.9 ± 6.7</td>
<td>65.8 ± 33.6</td>
<td>43.3 ± 7.4</td>
</tr>
<tr>
<td><strong>Post-ventilation, 10 min</strong></td>
<td>10.9 ± 0.6</td>
<td>7.342 ± 0.093&lt;sup&gt;b&lt;/sup&gt;</td>
<td>97.3 ± 4.0</td>
<td>72.6 ± 39.3</td>
<td>41.6 ± 9.0</td>
</tr>
<tr>
<td><strong>Post-ventilation, 15 min</strong></td>
<td>10.8 ± 0.7</td>
<td>7.338 ± 0.093&lt;sup&gt;b&lt;/sup&gt;</td>
<td>97.7 ± 3.0</td>
<td>58.4 ± 19.0</td>
<td>42.2 ± 9.9</td>
</tr>
<tr>
<td><strong>Post-ventilation, 30 min</strong></td>
<td>10.7 ± 0.6</td>
<td>7.303 ± 0.080&lt;sup&gt;b&lt;/sup&gt;</td>
<td>95.3 ± 4.4</td>
<td>46.6 ± 11.3</td>
<td>45.9 ± 8.7</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD; n = 6. Abbreviations: Hb, hemoglobin concentration; SaO2, hemoglobin oxygen saturation; PaO2, ascending aortic O2 tension; PaCO2, ascending aortic CO2 tension.

<sup>a</sup>P < 0.001, compared to post-cord-clamp value, <sup>b</sup>P < 0.001, compared to fetal and post-ventilation values.