Ductus arteriosus wave intensity analysis in fetal lambs: mid-systolic ductal flow augmentation is due to antegrade pulmonary arterial wave transmission

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**Running head:** Fetal ductus arteriosus wave intensity analysis

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Abstract

In mid-systole, fetal pulmonary trunk (PT) and arterial (PA) blood flows characteristically fall, despite pulmonary blood pressure increasing, while ductus arteriosus (DA) flow continues to rise to a delayed peak. Wave intensity (WI) analysis indicates that mid-systolic fetal PT and PA flow reductions are related to a very large mid-systolic PA backward-running compression wave (BCW_ms), which originates in the pulmonary microvasculature and is partially transmitted into the PT. This study tested the hypothesis that mid-systolic augmentation of DA blood flow was related to transmission of the PA BCW_ms into the DA. DA, PT and PA WI analysis was performed in eight anaesthetized late-gestation fetal sheep instrumented with DA, PT and left PA micromanometer catheters to measure pressure (P) and transit-time flow probes to obtain blood velocity (U). In a subgroup (n=5), the main PA was briefly occluded to abolish wave transmission from the lungs. WI was calculated as the product of P and U rates of change. PA and PT WI profiles both contained a prominent BCW_ms, ≈5-fold larger in the PA (P<0.005), which increased P but decreased U. By contrast, the DA WI profile demonstrated a large mid-systolic forward-running compression wave (FCW_ms), which increased DA P and U, and occurred 5 ms after PA BCW_ms. Furthermore, both DA FCW_ms and PT BCW_ms were abolished by main PA occlusion. These results suggest that the fetal PA BCW_ms undergoes retrograde transmission into the PT as a BCW_ms, but antegrade transmission into the DA as a FCW_ms that augments mid-systolic DA flow.

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The ductus arteriosus (DA) is a large fetal vascular shunt that is confluent at its proximal end with the pulmonary trunk (PT) and major pulmonary arteries (PA), and at its distal end with the aortic isthmus and descending thoracic aorta. The DA plays a key role in fetal circulatory physiology by permitting the fetal right ventricle (RV) to have a systemic output, with ~90% of RV output crossing the DA to form the major portion of the descending aortic blood stream that not only perfuses the lower fetal body, but also passes to the placenta for re-oxygenation (7, 28, 29, 38). Despite the close anatomical proximity, however, the blood flow profile of the DA is strikingly different from that of the PT or PA. Thus, both the PT and PA demonstrate a flow peak in early systole but, particularly in the main and large branch PA, flow then falls abruptly in mid-systole, even though pulmonary blood pressure continues to rise (19, 29, 36, 37). By contrast, DA flow also rises in early-systole but then increases further in mid-systole to a delayed peak (31). The specific mechanism responsible for the mid-systolic augmentation of DA flow is unknown, although it has been proposed that the high resistance and low compliance of the pulmonary circulation may play a role (31).

One potential means of gaining novel insights into the genesis of the fetal DA flow profile is the powerful and relatively new method of wave intensity ($WI$) analysis. This approach is founded on the premise that cardiovascular function is accompanied by the propagation of infinitesimal wavefronts defined by their pressure ($P$) and velocity ($U$) effects (2, 23), with the product of changes in $P$ and $U$ in the time domain (i.e. “$WI$”) related to the instantaneous energy carried by the wavefronts. Using $WI$ analysis (36, 37), we recently demonstrated that the mid-systolic fall in fetal PA blood flow was caused by an unusually large PA backward-running compression wave (BCW$_{ms}$) that originated in the pulmonary microvasculature and was of similar or greater magnitude than the forward-running compression wave (FCW$_{is}$) associated with impulsive RV ejection of blood. Furthermore, retrograde transmission of ~25% of PA BCW$_{ms}$ energy into the PT (36) produced the mid-systolic fall evident in the flow/velocity profile of this vessel (10, 29). Although the various fates of the PA BCW$_{ms}$ are yet to be fully elucidated, this relatively minor degree of PA-to-PT transmission raises the possibility that another portion of the PA BCW$_{ms}$ passed into the adjacent DA. This possibility is of particular relevance to the morphology of the DA flow profile as such transmission would be antegrade (i.e. in the direction of blood flow), rather
than retrograde, and should thus appear in the DA as a mid-systolic FCW acting to increase forward flow.

This study, in which simultaneous $WI$ analysis was performed in the DA, PT and PA of anesthetized near-term fetal lambs, therefore had three aims. The first was to define the features of the DA $WI$ profile. The second was to establish the relationship of this $WI$ profile to local changes in pressure and flow/velocity. The third was, via use of transient occlusion of the main PA to block wave transmission from the lungs (37), to specifically test the hypothesis that mid-systolic augmentation of DA flow was related to antegrade transmission of the fetal PA BCW$_{ms}$ into the DA as a FCW$_{ms}$.

**Methods**

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

*Surgical preparation* The anesthetic and monitoring regimen was similar to that previously described (36, 37). Briefly, eight pregnant Border-Leicester cross ewes were anesthetised at a gestation of 139 ± 2 days (mean ± SD, term = 147 days) with intramuscular ketamine 5 mg/kg and xylazine 0.1 mg/kg, followed by 5% isoflurane delivered by mask. After tracheal intubation, anesthesia was maintained with 2-3% isoflurane and nitrous oxide (10-20%) in oxygen-enriched air delivered via a volume-controlled ventilator (900C Servo, Siemens-Elema, Solna, Sweden), supplemented by an intravenous infusion of ketamine (1-1.5 mg/kg/hr) and midazolam (0.1-0.15 mg/kg/hr). Oxygen saturation was monitored continuously with a pulse-oximetry sensor (Oximas Dura-Y, Tyco Healthcare, Pleasanton, CA) applied to the ear. The right common carotid artery was cannulated for monitoring of blood pressure (90308 Multiparameter Monitor, Spacelabs. Medical, Redmond, Wash) and arterial sampling for blood gas analysis (ABL 620, Radiometer, Copenhagen, Denmark), with ventilation of the ewe adjusted to maintain arterial O$_2$ tension at ≈120 mmHg and arterial CO$_2$ tension at ≈40 mmHg.

Following exposure of the uterus through a midline laparotomy, the head, left forelimb and upper thorax of the fetus were exteriorized and a saline-filled glove was placed over the fetal head. A cannula was inserted into the fetal left external jugular vein for fluid
administration, and a polyvinyl catheter was passed into the ascending aorta via the left common carotid artery for pressure measurement and blood sampling. A thoracotomy was performed in the 3\textsuperscript{rd} left interspace, with removal of the 3\textsuperscript{rd} and 4\textsuperscript{th} ribs to increase exposure of the heart and great vessels. After incision of the pericardium and careful dissection of major vessels, the DA was enclosed by an 8-10 mm ‘A series’ transit-time flow probe (Transonic Systems, Ithaca, NY), a 10-14 mm ‘A series’ flow probe was placed around the PT and a 4-6 mm ‘S series’ flow probe was positioned on the left PA. A cannula was inserted through a purse-string suture into the PT and connected to a polyvinyl catheter to measure pressure. Through separate purse string sutures, one 2.5F micromanometer catheter (Millar Instruments, Houston, TX) was inserted into the distal part of the PT and passed into the DA, a second was placed into the PT immediately distal to the PT flow probe, while a third was inserted into the PT close to the base of the main PA, and its tip advanced into the origin of the left PA (Fig. 1). The edges of the pericardial incision were then loosely re-approximated over the left PA flow probe.

\textit{Physiological data} Aortic and PT blood pressures were measured via the fluid-filled catheters with a transducer (Transpac IV, Abbott Critical Care Systems, Sligo, Ireland), referenced to atmospheric pressure at the level of the left atrium and calibrated against a water manometer before each study. Pressure signals from fluid-filled catheters were processed using a transducer amplifier (Transbridge TBM4M, World Precision Instruments, Sarasota, FL). High fidelity DA, PT and left PA pressures were obtained by interfacing micromanometers with transducer control units (TCB-500, Millar Instruments). DA, PT and left PA flows were measured with a flowmeter (model T206, Transonic Systems). All physiological signals were digitized at a sampling rate of 1000 Hz using an analogue-to-digital convertor (iNet-100B, GW Instruments, Somerville, MA) interfaced with programmable acquisition and analysis software (Spike2, Cambridge Electronic Design, Cambridge, UK). No data filtering was employed, apart from application of a 48 Hz low-pass filter at the time of analysis to remove electrical interference from signals. To calibrate the high-fidelity pressure signals, the mean PT micromanometer pressure was first matched to the mean pressure of the fluid-filled PT catheter. The diastolic portions of the DA and left PA
micromanometer waveforms were then matched to the corresponding segment of the PT
micromanometer pressure.

**Experimental protocol** Hemodynamics were allowed to stabilize for 10-15 min after
completion of surgery. After withdrawal of an aortic blood sample for gas analysis,
physiological data were recorded in all fetuses for baseline hemodynamic and $WI$ analysis.
Data were also recorded in a subgroup of five fetuses during a transient (~10 sec) occlusion of
the main PA with atraumatic tissue forceps to abolish wave transmission from the lungs (37).
At the end of the study, animals were killed with an overdose of pentobarbital sodium (100
mg/kg).

**Wave intensity analysis** As $WI$ analysis utilizes pressure and velocity data, DA, PT and
left PA blood flows were converted to velocity ($U$) using cross-sectional area derived from a
caliper measurement of vessel diameter. For $WI$ analysis, ensemble average P and $U$ signals
were generated from a mean of 38 ± 7 beats (mean ± SD), with subsequent calculation of the
rates of change of DA, PT and left PA blood pressure ($dP/dt$) and velocity ($dU/dt$), and the
product of these differentials (i.e. net $WI$). Note that $WI$ is a “time-corrected” variable that is
independent of the digitizing sample rate (4, 13, 24, 27, 36, 37).

To accurately quantitate wave magnitude in the presence of large overlapping waves,
such as occur in the fetal PT and PA (36, 37), net $WI$ was separated into its forward and
backward components. To perform this separation, wave speed ($c$) was obtained with the
relation $\rho c = dP/dU$ (15), where $\rho$ is blood density (assumed as 1050 kg/m$^3$). Using the
ensemble-averaged beats, $dP/dU$ was calculated using least squares linear regression from the
P-$U$ slope during early systole, when the contribution of backward-running waves is minimal
(15, 17, 24, 36, 37). Time lags between P and $U$ data points related to hardware-related
delays (11) and any difference in the relative positions of the flow probe and micromanometer
measurement sites were corrected by aligning the peak second derivatives of these signals.
Despite the DA P-$U$ loop having a markedly different appearance to the PT and left PA loops,
all three sites displayed highly linear early-systolic P-$U$ relations ($R^2 \geq 0.99$, Fig. 2).

As per convention (2), waves propagating away from the ventricle were defined as
forward-running and those arising from the vasculature were defined as backward-running.
Using established methodology (4, 24, 36, 37), $W_I$ of forward-running waves was calculated as $(dP/dt + \rho c \cdot dU/dt)^2 / (4\rho c)$ and $W_I$ of backward-running waves as $-(dP/dt - \rho c \cdot dU/dt)^2 / (4\rho c)$. The pressure differential associated with a forward-running wavefront was given by $(dP/dt)_+ = \frac{1}{2} (dP/dt + \rho c \cdot dU/dt)$ and by $(dP/dt)_- = \frac{1}{2} (dP/dt - \rho c \cdot dU/dt)$ for a backward-running wavefront. The velocity differential associated with a forward-running wavefront was calculated as $(dU/dt)_+ = \frac{1}{2} [(dU/dt) + 1/\rho c \cdot (dP/dt)]$ and as $(dU/dt)_- = \frac{1}{2} [(dU/dt) - 1/\rho c \cdot (dP/dt)]$ for a backward-running wavefront. Waves were classified according to the pressure differential, such that a forward-running wave was a compression wave if $(dP/dt)_+ > 0$ and an expansion wave if $(dP/dt)_+ < 0$. Similarly, a backward-running wave was defined as a compression wave if $(dP/dt)_- > 0$ and an expansion wave if $(dP/dt)_- < 0$ (4, 24, 36, 37).

Forward and backward components of $P$ ($P_+$ and $P$, respectively) and $U$ ($U_+$ and $U$) were obtained by integration of pressure and velocity differentials (21, 36). Note that, in the remainder of the text, $\Delta P$ and $\Delta U$ refer respectively to changes in $P_+$ and $U_+$ associated with forward waves, and to changes in $P$ and $U$ accompanying backward waves.

The time interval between $W_I$ peaks was obtained from the separated profiles. The distance to the origin of reflected waves from the measurement site in the vessel of interest was estimated from the product of wave speed and one-half the time interval between wave peaks (11, 24, 36, 37).

To quantify wave size, the cumulative intensity ($CI$) of forward-running and backward-running waves, which is related to wave energy, was calculated by integrating the appropriate $W_I$ over the wave duration (4, 24, 36, 37). The effect of each wave on $P$ and $U$ was obtained by measuring $\Delta P$ and $\Delta U$ between the start and end of the wave (36, 37).

**Statistical Analysis** Data were analysed using Statistical Package for the Social Sciences Version 16.0 (SPSS Inc., Chicago). Baseline DA, PT and left PA $W_I$ data were analyzed using repeated measures analysis of variance. Specific comparisons were then evaluated by partitioning the within-animal sums of squares into individual degrees of freedom, with application of the Bonferroni adjustment, as appropriate, for multiple comparisons. Results are expressed as mean ± SD and significance was taken at $P < 0.05$. 
Results

Blood gases and hemodynamics  Ascending aortic pH was 7.29 ± 0.02, Hb 12.0 ± 1.3 g/dL, Hb O₂ saturation 69 ± 4%, Po₂ 25.4 ± 2.3 mmHg, Pco₂ 50.1 ± 2.9 mmHg and base excess -3.1 ± 0.8 mmol/L. Although DA, PT and left PA pressure profiles had a similar morphology, peak systolic blood pressure in the DA was 3-4 mmHg lower than in the PT and left PA (P < 0.001; Fig. 3A & Table 1). In addition, mean aortic pressure (57.3 ± 6.6 mmHg) was ∼1 mmHg less than mean PT pressure (P < 0.005). Whereas PT and left PA flow profiles displayed early-systolic peaks and mid-systolic reductions (36, 37), the DA profile had a distinctive stepped appearance with an initial early-systolic rise followed by a higher mid-systolic peak (Fig. 3B). Moreover, in accord with Doppler-echocardiographic findings (20, 31, 40), peak DA velocity exceeded maximal PT and left PA velocity (Fig. 3C & Table 1).

WI analysis  After wave separation, six systolic waves were consistently present in the DA WI profile (Fig. 4). These comprised 1) a large FCW_is coinciding with an initial systolic increase in P and U, 2) a small BCW_ms within the tail region of FCW_is, 3) a mid-systolic FCW (FCW_ms) similar in size to FCW_is, and producing a further rise in P and U, 4) a small mid-systolic backward-running expansion wave (BEW_ms) in the latter part of the FCW_ms, that reduced P but increased U, 5) a large late-systolic forward-running expansion wave (FEW_is) that decreased P and U just prior to the incisura, and 6) a large late-systolic BCW (BCW_is) starting near the end of FEW_is, and increasing P but reducing U. The DA WI profile was more complex overall and strikingly different in mid-systole from the corresponding PT and left PA profiles (36, 37), which displayed a prominent BCW_ms that was temporally associated with a pronounced fall in U, as well as a small FCW_ms. In addition, no BCW_is was present in either the PT or left PA (Fig. 5).

WI data are presented in the Table 2. Wave speed in the DA was not different to that in the PT (P > 0.2), but was ∼80% higher than in the left PA (P < 0.005). FCW_is CI in the DA was ∼40% lower than in the PT or left PA (P < 0.005), with an accompanying higher PT ΔP (P = 0.005) and left PA ΔU (P < 0.005). However, DA FCW_ms CI was ∼5-fold larger than the PT and left PA FCW_ms CI (P < 0.001), with correspondingly greater effects on ΔP and ΔU (P < 0.001). Moreover, the magnitudes of CI, ΔP and ΔU related to DA FCW_ms and FCW_is were
not different ($P > 0.3$). By contrast, DA BCW\textsubscript{ms}, its $\Delta P$ and $\Delta U$ effects and the DA BCW\textsubscript{ms}/FCW\textsubscript{ls} CI, $\Delta P$ and $\Delta U$ ratios were much smaller than PT and left PA variables ($P \leq 0.01$). The DA BEW\textsubscript{ms} was much larger than the PT BEW\textsubscript{ms} ($P < 0.025$), with appropriately greater, albeit still minor, effects on $\Delta P$ and $\Delta U$ ($P < 0.01$). FEW\textsubscript{ls} CI was not different in the DA, PT and PA ($P > 0.8$), while the magnitudes of DA FEW\textsubscript{ls} CI, $\Delta P$ and $\Delta U$ were $\geq 2$ fold that of corresponding DA BCW\textsubscript{ls} variables ($P \leq 0.01$).

The DA FCW\textsubscript{ls} and FEW\textsubscript{ls} occurred $4 \pm 2$ ms ($P = 0.001$) and $4 \pm 1$ ms ($P < 0.001$) after their respective PT waves, while DA FCW\textsubscript{ms} occurred $5 \pm 4$ ms after left PA BCW\textsubscript{ms} ($P = 0.01$) but $9 \pm 4$ ms before PT FCW\textsubscript{ms} ($P < 0.001$). The DA FCW\textsubscript{ls}-FCW\textsubscript{ms} and FCW\textsubscript{ls}-FEW\textsubscript{ls} intervals were $47 \pm 9$ and $166 \pm 10$ ms ($P < 0.001$), while DA BCW\textsubscript{ms}, BEW\textsubscript{ms} and BCW\textsubscript{ls} occurred $17 \pm 4$, $8 \pm 8$ and $7 \pm 1$ ms after DA FCW\textsubscript{ls}, FCW\textsubscript{ms} and FEW\textsubscript{ls} respectively ($P \leq 0.025$). The latter intervals corresponded to reflection sites located $4.1 \pm 1.8$, $1.7 \pm 1.4$ and $1.7 \pm 0.6$ cm distal to the DA measurement site.

**Effect of main pulmonary artery occlusion on wave intensity profiles**

With occlusion of the main PA, the DA FCW\textsubscript{ms} and BEW\textsubscript{ms} disappeared, as did the stepped appearance of the upstroke of associated $P$ and $U$ profiles; these effects were completely and immediately reversed after removal of the main PA occlusion (Fig. 6). As reported previously (37), main PA occlusion also abolished the PT BCW\textsubscript{ms}, with a rounding of PT $P$ and $U$ profiles (see data supplement).

**Discussion**

The main finding of this study, which is the first to have evaluated hemodynamics in the fetal ductus arteriosus (DA) using wave intensity ($WI$) analysis, is that a characteristic mid-systolic augmentation of flow within this vascular shunt (31) has its genesis in a large mid-systolic backward-running compression wave (BCW\textsubscript{ms}) that originates from the pulmonary microvasculature and travels retrogradely into the major pulmonary arteries, where it increases blood pressure but reduces blood velocity. This BCW\textsubscript{ms} is then partially transmitted into the DA, but because this transmission is antegrade (i.e. in the direction of blood flow), it appears within the DA as a mid-systolic forward-running compression wave (FCW\textsubscript{ms}) that increases blood pressure and velocity/flow. Subsequent local reflection of this FCW\textsubscript{ms} results
in a smaller backward-running expansion wave (BEW\textsubscript{ms}) that further augments mid-systolic DA blood velocity/flow.

Our study suggested that forward (i.e. right-to-left) blood flow across the fetal DA consisted of three distinct temporal components. The first was a DA early-systolic forward-running forward compression wave (FCW\textsubscript{is}) that was responsible for the initial rise in DA velocity/flow. As it occurred 4 ms after the pulmonary trunk (PT) FCW\textsubscript{is} and was not abolished by occlusion of the main pulmonary artery (PA), this wave was transmitted from the PT, and thus arose from the right ventricle (RV). The DA FCW\textsubscript{is} was therefore a consequence of the ventricular impulse generated at the beginning of systole and which provides the forward momentum for blood movement from the ventricle into the vasculature (41).

The second contribution to DA forward flow was a mid-systolic forward-running compression wave (FCW\textsubscript{ms}), and it was this wave that was mainly responsible for the mid-systolic rise in the DA velocity/flow profile (Fig 4). Unlike the relatively small FCW\textsubscript{ms} present in the PT and PA, the magnitude of DA FCW\textsubscript{ms}, as well as its pressure (ΔP) and velocity (ΔU) effects, were similar to that of the preceding FCW\textsubscript{is} (Table 2). The observations that the DA FCW\textsubscript{ms} occurred 5 ms after the PA BCW\textsubscript{ms} and was abolished by occlusion of the main PA (Fig 6) demonstrated that the DA FCW\textsubscript{ms} arose from the pulmonary arterial circulation as an antegrade transmission of the PA BCW\textsubscript{ms}. Furthermore, such transmission was partial, as comparison of wave areas (Table 2) indicated that DA FCW\textsubscript{ms} energy was \(~30\%\) of the PA BCW\textsubscript{ms}.

Two other possible origins for the DA FCW\textsubscript{ms} can be discounted. Thus, the PT FCW\textsubscript{is} (and therefore the RV) could not have directly given rise to the DA FCW\textsubscript{ms} as the relatively long delay between these waves (~51 ms) and wave speed (~4.5 m/s) would then require that the distance between the PT and DA measurement sites be ~23 cm (i.e. 51 ms x 4.5 m/s), whereas it is typically 3-4 cm. It is also very unlikely that the DA FCW\textsubscript{ms} arose from the PT FCW\textsubscript{ms}, not only because the latter was much smaller (Table 2, Figs. 4&5), but also because its peak occurred ~9 ms after the DA FCW\textsubscript{ms}. 
The third component of forward flow across the DA was a BEW<sub>ms</sub> that enhanced mid-systolic DA flow via a “pulling” effect. This BEW<sub>ms</sub> was of relatively minor importance, however, as its energy and $\Delta U$ effect were only about a quarter that of the DA FCW<sub>ms</sub> (Table 2). Loss of the DA BEW<sub>ms</sub> after occlusion of the main PA (Fig. 6) was in accord with the proposition that this wave was a local reflection of the DA FCW<sub>ms</sub>, with the calculated origin of this reflection (1.7 cm distal to the DA measurement site) suggesting that the DA BEW<sub>ms</sub> arose within the confluence of the distal DA, terminal aortic isthmus and proximal descending aorta.

Although less striking than the variations in blood velocity/flow waveforms, differences in DA, PT and PA net blood pressure waveforms were also apparent in mid-systole (Fig. 3) and manifested as lower systolic and mean DA pressures (Table 1). WI analysis suggested that these mid-systolic regional pressure variations were related to differences in the make-up of component waves. Thus, DA mid-systolic pressure was related to the large FCW<sub>ms</sub> arising from the PA BCW<sub>ms</sub>, whose contribution to net pressure was partially offset by a pressure reduction accompanying the DA BEW<sub>ms</sub> (Fig. 4). On the other hand, the mid-systolic PT and PA net pressure was mainly due to the presence of a large BCW<sub>ms</sub>, supplemented by an additional pressure rise related to smaller FCW<sub>ms</sub> components (Fig. 5).

Albeit a separate question from its physiological consequences for DA hemodynamics, the specific basis of PA BCW<sub>ms</sub> generation in the fetus still requires further investigation, as the mechanism suggested by WI analysis is not in accord with the conventional view of smooth muscle contraction. Thus, BCW<sub>ms</sub> is widely considered to be due to reflection of the preceding FCW<sub>is</sub> from “closed-end” reflection sites (11, 14, 15, 18, 23), with the magnitude of this reflection quantifiable as the BCW<sub>ms</sub>/FCW<sub>is</sub> ratio of $CI$, $\Delta P$ or $\Delta U$ (11, 16, 21, 24), that typically has values of <0.2. Thus, the small reflection coefficient of the DA BCW<sub>ms</sub> (0.04 using $CI$ and 0.17 using either $\Delta P$ or $\Delta U$), for example, is in accord with this wave being a reflection of the DA FCW<sub>is</sub>. However, using either steady-state WI analysis (37) or beat-by-beat WI analysis in the period surrounding a cardiac extrasystole (36), the magnitudes of the fetal PA BCW<sub>ms</sub>/FCW<sub>is</sub> $CI$, $\Delta P$ and $\Delta U$ ratios are commonly >1, indicating that more energy, $\Delta P$ and $\Delta U$ return from the lungs due to the BCW<sub>ms</sub> than enter this organ via the FCW<sub>is</sub>. This in turn implies that the fetal PA BCW<sub>ms</sub> arises not only via vascular reflection, but also via
generation of an additional pulmonary source of impulsive energy within each cardiac cycle, most likely in the form of a transitory vasoconstriction. The latter clearly runs counter to the notion that contraction in vascular smooth muscle is a relatively slow process (39), manifested as an alteration in vascular tone.

As in the PT and PA, a late-systolic forward-running expansion wave (FEW_{ls}) produced large falls in DA blood pressure and velocity/flow just prior to the incisura (Fig. 4). That the DA FEW_{ls} occurred 4 ms after PT FEW_{ls} and was not reduced by main PA occlusion (Fig. 6) was in accord with the proposition that this wave was a transmission of the PT FEW_{ls}, and thus arose from the RV as a vascular manifestation of a ventricular rarefaction (‘suction’) wave accompanying ventricular relaxation (41).

In addition to the FEW_{ls}, however, the fall in DA blood velocity/flow in late-systole was enhanced by a late-systolic backward-running compression wave (BCW_{ls}; Fig 4) that was not evident in either the PT or PA (Fig. 5). As this BCW_{ls} was smaller than and consistently occurred 7 ms after the DA FEW_{ls}, a likely origin was local reflection of the FEW_{ls} with the calculated origin of this reflection (1.7 cm distal to the DA measurement site) implying that the BCW_{ls} also originated from within the confluence of the DA, aortic isthmus and descending aorta.

The alternate possibility that the DA BCW_{ls} resulted from the ascending aortic FCW_{ls} traversing the aortic isthmus and then passing retrogradely into the DA was unlikely, given the temporal features of the DA WTI profile. Thus, assuming that the PT and ascending aortic FCW_{ls} occur at similar times in the cardiac cycle and that fetal aortic wave speed is 4.5 m/s (10), the very long interval between PT FCW_{ls} and DA BCW_{ls} (∼170 ms) would then require the distance between the ascending aorta and DA to be >76 cm, whereas it typically ranges from 6-8 cm. Our WTI analysis findings therefore suggest that the flow reversal evident across the fetal DA in late-systole (31) is related both directly (via the FEW_{ls}) and indirectly (via the BCW_{ls}) to RV relaxation, without any detectable contribution arising from an LV energy source.

A number of methodological issues require comment. Firstly, despite the presence of a very prominent FCW_{ms}, it is technically feasible and relatively straightforward to determine
DA wave speed using the slope of the P-U loop in early systole (Fig. 2). Somewhat surprisingly, however, given the recognized constrictive potential of the fetal DA (32), DA and PT wave speeds were not significantly different. This phenomenon may in part be related to structural alterations that occur within the DA in late-gestation, with formation of intimal cushions accompanied by transformation of smooth muscle cells into a synthetic phenotype and elaboration of dense connective tissue (9, 25).

Secondly, despite the peri-ductal dissection required prior to flow probe implantation, there was no evidence that any significant DA constriction occurred in our study. Although mean blood pressure in the PT was ~1 mmHg than in the ascending aorta, such a pressure difference is also observed in chronically instrumented near-term fetal sheep without any DA dissection (1, 3, 6, 8, 12, 34, 35). However, we cannot exclude the possibility that dissection around major vessels and subsequent placement of flow probes around the DA, PT and left PA may have altered wave transmission characteristics, although the magnitude of any such effect is likely to be quite minor.

Finally, because of the extent of surgical instrumentation required to obtain physiological measurements, it was necessary to perform the study under general anesthesia and open-chest conditions. However, blood gas and blood pressure data were within the normal range reported in unanesthetized, chronically-instrumented late-gestation fetal lambs (1, 5, 19, 22, 26, 30, 33, 35, 38). It is thus unlikely that the qualitative features of our findings were affected by our experimental approach.

**Perspectives and Significance** The results of this study suggest that the very large PA BCW<sub>ms</sub> present in the fetus has major hemodynamic consequences which extend beyond local PT and PA effects. In particular, the augmentation of mid-systolic forward flow across the DA which accompanies partial antegrade transmission of the PA BCW<sub>ms</sub> into the DA as a FCW<sub>ms</sub> appears to be a unique mechanism that facilitates maintenance of fetal right-to-left transductal shunting at a point in the cardiac cycle where PT flow (and therefore RV output) is decreasing. Furthermore, as blood crossing the DA constitutes the major portion of the descending aortic stream, it is likely that subsequent transmission of the DA FCW<sub>ms</sub> into the descending aorta will augment both mid-systolic pressure and velocity/flow in this vessel.
Together with previous observations (36, 37), our findings thus point to a pivotal role for the PA BCW_{mu} in the regulation of central hemodynamics within the fetus.
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References


**Figure legends**

**Figure 1.** Schematic diagram of fetal instrumentation. **Abbreviations:** AA, ascending aorta; Ao F, aortic fluid-filled catheter; DA FP, ductus arteriosus flow probe; DA M, ductus arteriosus micromanometer catheter; LA, left atrium; LPA FP, left pulmonary artery flow probe; LPA M, left pulmonary artery micromanometer catheter; LV, left ventricle; PT F, pulmonary trunk fluid-filled catheter; PT FP, pulmonary trunk flow probe; PT M, pulmonary trunk micromanometer catheter; RV, right ventricle.

**Figure 2.** Blood pressure-velocity loops in the ductus arteriosus (upper panel), pulmonary trunk (middle panel) and left pulmonary artery (lower panel). Note the highly linear portion of each loop in early systole (between arrows), the slope of which was used to calculate wave speed.

**Figure 3.** Blood pressure (A), flow (B) and velocity waveforms (C) in the ductus arteriosus (thick line), left pulmonary artery (thin black line) and pulmonary trunk (thin gray line).

**Figure 4.** Net blood pressure (A) and blood velocity (B), shown in thick line with forward and backward components shown in thin lines, as well as net wave intensity (C) and separated forward and backward wave intensities (D) in the fetal ductus arteriosus. Abbreviations in panel D as in Table 1.

**Figure 5.** Net blood pressure (A) and blood velocity (B), shown in thick line with forward and backward components shown in thin lines, as well as net wave intensity (C) and separated forward and backward wave intensities (D) in the fetal left pulmonary artery (left panel) and pulmonary trunk (right panel). Abbreviations in panel D as in Table 1.

**Figure 6.** Net blood pressure (A) and blood velocity (B), shown in thick line with forward and backward components shown in thin lines, as well as net wave intensity (C) and separated forward and backward wave intensities (D) in the fetal ductus arteriosus before (left panel), during (middle panel) and after (right panel) transient occlusion of the main pulmonary artery (MPA). Abbreviations in panel D as in Table 1.
## TABLE 1. Blood pressures, flows and velocities in fetal ductus arteriosus, pulmonary trunk and left pulmonary artery.

<table>
<thead>
<tr>
<th></th>
<th>DA</th>
<th>PT</th>
<th>LPA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blood pressure (mmHg)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak systolic</td>
<td>70.7 ± 9.1c</td>
<td>74.6 ± 8.5</td>
<td>73.8 ± 8.3</td>
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<tr>
<td>Mean</td>
<td>57.8 ± 6.9b</td>
<td>58.6 ± 6.9</td>
<td>58.4 ± 6.8</td>
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<tr>
<td>Minimum diastolic</td>
<td>47.8 ± 5.7</td>
<td>47.7 ± 5.7</td>
<td>47.9 ± 5.9</td>
</tr>
<tr>
<td><strong>Blood flow (mL/min)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak positive</td>
<td>2644 ± 838</td>
<td>3011 ± 605</td>
<td>657 ± 191c</td>
</tr>
<tr>
<td>Mean</td>
<td>622 ± 226d</td>
<td>752 ± 192</td>
<td>66 ± 35c</td>
</tr>
<tr>
<td><strong>Blood velocity (m/s)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak positive</td>
<td>0.66 ± 0.17a</td>
<td>0.44 ± 0.12</td>
<td>0.54 ± 0.21</td>
</tr>
<tr>
<td>Mean</td>
<td>0.15 ± 0.04c</td>
<td>0.11 ± 0.04</td>
<td>0.05 ± 0.03e</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD; n=8. Abbreviations: DA, ductus arteriosus; PT, pulmonary trunk; LPA, left pulmonary artery. a $P < 0.025$, b $P < 0.005$, c $P < 0.001$ compared to other vascular sites; d $P < 0.05$, e $P < 0.001$ compared to PT.
TABLE 2. Wave intensity analysis in fetal ductus arteriosus, pulmonary trunk and left pulmonary artery.

<table>
<thead>
<tr>
<th></th>
<th>DA</th>
<th>PT</th>
<th>Left PA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wave speed (m/s)</td>
<td>5.0 ± 1.7</td>
<td>4.3 ± 0.9</td>
<td>2.8 ± 0.9&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>CI (Wm&lt;sup&gt;-2&lt;/sup&gt;s&lt;sup&gt;-1&lt;/sup&gt;x10&lt;sup&gt;4&lt;/sup&gt;)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FCW&lt;sub&gt;is&lt;/sub&gt;</td>
<td>1.53 ± 0.63&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.42 ± 0.88</td>
<td>2.63 ± 1.25</td>
</tr>
<tr>
<td>FCW&lt;sub&gt;ms&lt;/sub&gt;</td>
<td>1.24 ± 0.71&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.21 ± 0.15</td>
<td>0.17 ± 0.16</td>
</tr>
<tr>
<td>BCW&lt;sub&gt;ms&lt;/sub&gt;</td>
<td>0.06 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.87 ± 0.41</td>
<td>4.01 ± 3.35&lt;sup&gt;f&lt;/sup&gt;</td>
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<tr>
<td>BEW&lt;sub&gt;ms&lt;/sub&gt;</td>
<td>0.14 ± 0.11&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.01 ± 0.01</td>
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<tr>
<td>FEW&lt;sub&gt;ls&lt;/sub&gt;</td>
<td>1.63 ± 1.04</td>
<td>1.77 ± 0.85</td>
<td>1.58 ± 1.00</td>
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<tr>
<td>BCW&lt;sub&gt;ls&lt;/sub&gt;</td>
<td>0.51 ± 0.35</td>
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<tr>
<td>CI ratio</td>
<td></td>
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<tr>
<td>BCW&lt;sub&gt;ms&lt;/sub&gt;/FCW&lt;sub&gt;is&lt;/sub&gt;</td>
<td>0.04 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.35 ± 0.07</td>
<td>1.36 ± 0.49&lt;sup&gt;g&lt;/sup&gt;</td>
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<tr>
<td>ΔP (mmHg)</td>
<td></td>
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<tr>
<td>FCW&lt;sub&gt;is&lt;/sub&gt;</td>
<td>10.5 ± 1.3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>12.7 ± 2.4</td>
<td>10.2 ± 1.1&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>FCW&lt;sub&gt;ms&lt;/sub&gt;</td>
<td>10.6 ± 3.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.7± 1.3</td>
<td>2.1± 1.1</td>
</tr>
<tr>
<td>BCW&lt;sub&gt;ms&lt;/sub&gt;</td>
<td>1.7 ± 0.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.0± 1.2</td>
<td>11.7± 2.2&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>BEW&lt;sub&gt;ms&lt;/sub&gt;</td>
<td>-2.9 ± 1.7&lt;sup&gt;e&lt;/sup&gt;</td>
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<td></td>
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<tr>
<td>FEW&lt;sub&gt;ls&lt;/sub&gt;</td>
<td>-9.6 ± 2.7</td>
<td>-8.8± 1.9</td>
<td>-6.5± 1.4&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>BCW&lt;sub&gt;ls&lt;/sub&gt;</td>
<td>4.9 ± 1.7</td>
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<tr>
<td>ΔP ratio</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>BCW&lt;sub&gt;ms&lt;/sub&gt;/FCW&lt;sub&gt;is&lt;/sub&gt;</td>
<td>0.17 ± 0.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.64± 0.07</td>
<td>1.13 ± 0.19&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>ΔU (m/s)</td>
<td></td>
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<tr>
<td>FCW&lt;sub&gt;is&lt;/sub&gt;</td>
<td>0.29 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.39± 0.11</td>
<td>0.52± 0.21</td>
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<tr>
<td>FCW&lt;sub&gt;ms&lt;/sub&gt;</td>
<td>0.28 ± 0.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.11± 0.05</td>
<td>0.11± 0.08</td>
</tr>
<tr>
<td>BCW&lt;sub&gt;ms&lt;/sub&gt;</td>
<td>-0.05 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-0.25± 0.07</td>
<td>-0.61± 0.30&lt;sup&gt;g&lt;/sup&gt;</td>
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<tr>
<td>BEW&lt;sub&gt;ms&lt;/sub&gt;</td>
<td>0.07 ± 0.04&lt;sup&gt;e&lt;/sup&gt;</td>
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<tr>
<td>FEW&lt;sub&gt;ls&lt;/sub&gt;</td>
<td>-0.26 ± 0.10</td>
<td>-0.27± 0.09</td>
<td>-0.33± 0.15</td>
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<td>BCW&lt;sub&gt;ls&lt;/sub&gt;</td>
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<td>-1.13 ± 0.19&lt;sup&gt;g&lt;/sup&gt;</td>
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