Arterial hemodynamics and mechanical properties after circulatory intervention in the chick embryo

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Summary

Altered blood pressure and flow impact cardiac function during morphogenesis. How the arterial system supports cardiac morphogenesis after circulatory disruptions is not well characterized. We manipulated arterial flow *via* left atrial ligation (LAL) or arterial load *via* right vitelline artery ligation (VAL) in Hamburger-Hamilton (HH) stage 21 chick embryos. Embryos were re-incubated for 1 h (HH21), 14 h (HH24) or 30 h (HH27). At each stage we measured simultaneous dorsal aortic blood pressure and flow, and calculated arterial compliance, impedance and hydraulic power. LAL acutely reduced stroke volume (V_s), cardiac output ($\dot{Q}$) and hydraulic power. Arterial pressure was preserved by a compensatory increase in characteristic impedance and decrease in compliance. Impedance parameters and compliance normalized by HH24 and all parameters normalized by HH27. VAL acutely increased arterial resistance. Embryos maintained arterial pressure by decreasing V_s and $\dot{Q}$. These parameters remained altered through HH27. In summary, despite the intervention, compensatory alterations in V_s and arterial resistance maintained arterial pressure and fraction of oscillatory power within a narrow range. These results suggest that the maintenance of arterial pressure and circulatory energy efficiency, but not arterial flow, is critical to embryogenesis.

Key words: chick embryo, cardiovascular development, impedance, compliance, arterial load.

Introduction

The mechanical milieu is a critical epigenetic factor in cardiovascular development. Experimental models that alter blood pressure and/or flow through the developing cardiovascular system are known to cause structural and functional anomalies of the heart and outflow tract. For example, increasing ventricular afterload causes myocyte hyperplasia and changes in ventricular trabecular architecture (Gessner and Van Mierop, 1970; Keller et al., 1997; Clark et al., 1989; Miller et al., 2003). Reducing left ventricular preload leads to hypoplastic left heart syndrome (Harh et al., 1973; Sedmera et al., 1999), profound changes in right and left ventricular remodeling (Sedmera et al., 1999), increased left ventricular stiffness and decreased end-systolic pressure (Tobita et al., 2002), and reduced stroke volume (Harh et al., 1973). Manipulating venous return by vitelline vein ligation creates atrial and ventricular septal defects, outflow tract anomalies and altered ventricular filling characteristics (Hogers et al., 1997; Hogers et al., 1999; Ursem et al., 2004).

While the impact of hemodynamic alterations on cardiac functional development has been well studied, the compensatory responses of the vasculature are less well characterized. Chick embryos and *Xenopus* larvae quickly alter arterial resistance and stroke volume in response to acute changes in circulating blood volume (Yoshigi et al., 1996; Warburton and Fritsche, 2000). Within 5 h of right lateral vitelline vein ligation, embryos normalize the ligation-induced reductions in dorsal aortic blood flow (Stekelenburg-de Vos et al., 2003) even though ventricular dynamics may be altered. These studies suggest that the regulation or maintenance of certain hemodynamic parameters may be critical for surviving circulatory perturbations and continuing embryogenesis.

We hypothesize that the regulation of embryonic hemodynamics is a critical factor for embryo survival and that there are specific hemodynamic patterns associated with adaptation to altered circulation. Therefore, the objective of this study is to determine which hemodynamic parameters are maintained or altered as the chick embryo adapts to altered flow or altered resistance. In the present study we used microsurgical ligation techniques to acutely alter arterial flow or arterial resistance. At three successive time points, we measured dorsal aortic pressure and flow and calculated arterial impedance, compliance, and hydraulic power to assess a range of hemodynamic parameters. Knowledge of these patterns provides insight into the regulatory capacity of the embryonic cardiovascular system.

Materials and methods

Embryo preparation

Fertilized White Leghorn (*Gallus gallus* L.) eggs were obtained from Utah State University (Logan, Utah, USA) and incubated blunt-side up at 37–38°C and a relative humidity of approximately 70%. After approximately 90 h, Hamburger-Hamilton stage (HH) (Hamburger and Hamilton, 1951) 21, the egg was removed to a temperature controlled bench environment, and the embryo was exposed by creating a window in the shell above the air cell and resecting the membranes above the embryo. Dysmorphic or bleeding embryos were excluded. Each embryo underwent one of three treatments: left atrial ligation (LAL), vitelline artery ligation (VAL) or sham manipulation (CON). We chose to manipulate arterial flow and resistance *via* mechanical (ligation) methods to create sustained changes in these parameters. Ligation techniques are routinely used to investigate cardiovascular function in chick embryos.

For LAL, embryos were gently repositioned to their left side and the embryonic membranes above the developing left atrium were resected. A loop of 10-0 monofilament suture was positioned around the atrium and tightened, reducing effective chamber size by at least 50%. If the ligation visually appeared to reduce the chamber size by 50% or less or cause any blood loss, the embryo was immediately discarded. After successful ligation, the embryo was then repositioned, the egg was sealed with stretched parafilm and returned to the incubator. Overall survival rate was approximately 65% from ligation to time of measurement.

For VAL, membranes above the right lateral vitelline artery were gently resected and a length of 10-0 monofilament was passed below the artery and tied in an occlusive overhand knot with minimal disruption to the vitelline membrane. Venous return from this bed was not directly affected by arterial ligation. Eggs were sealed and reincubated. Overall survival rate was approximately 68%.

For CON, egg shells were windowed, the membranes were resected above the embryo, and eggs were sealed and reincubated. Survival was approximately 90%. After approximately 1 h of post-intervention recovery (HH21), 14 h (HH24) or 32 h (HH27), embryos were prepared for hemodynamic measurements. After measurements were taken, each embryo was discarded.

Hemodynamic measurements

We measured simultaneous dorsal aortic blood pressure with a servo-null system (900A, WPI, Sarasota, FL, USA) and dorsal aortic blood velocity with a pulsed-Doppler velocimeter (Triton, San Diego, CA, USA) and a 0.5 mm custom mounted probe (Iowa Doppler Products, Iowa City, IA, USA) as previously described (Tobita et al., 2002; Yoshigi et al., 1996) (Fig. 1A). Dorsal aortic diameter was imaged for individual HH21 and HH24 embryos using a video camera (model 70, Dage-MTI, Michigan City, IN, USA) mounted on a dissecting microscope. We calibrated the image analysis software (Scion Image, Scion Corp,
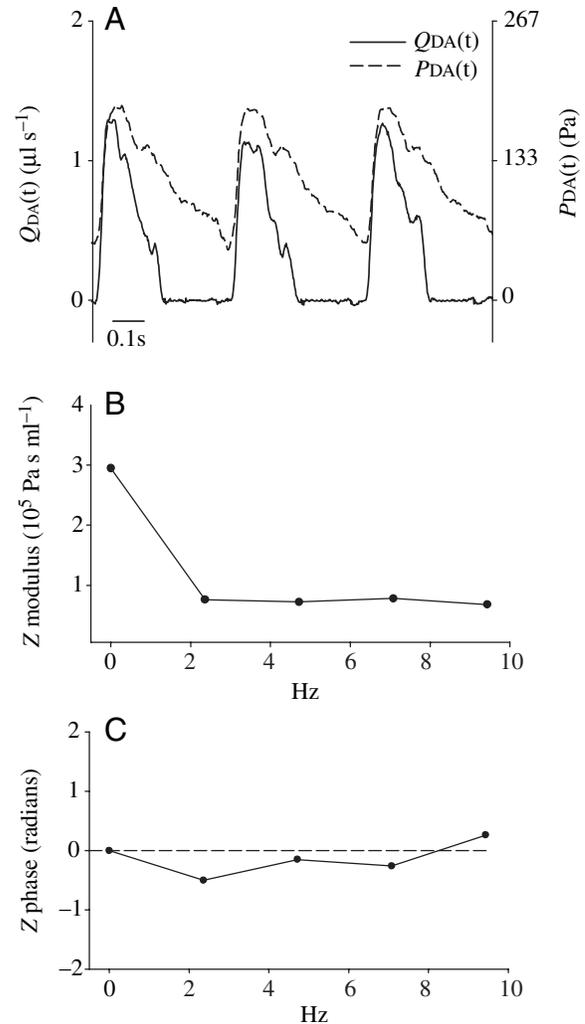

Fig. 1. Example of original data [$P_{DA}(t)$, dorsal aortic pressure; $Q_{DA}(t)$, dorsal aortic flow] obtained from a HH21 CON embryo (A). Data were recorded simultaneously in the dorsal aorta at the level of the sinus venosus. Waveforms were decomposed using Fourier transform to characterize the modulus (B) and phase (C) of arterial impedance.

Frederick, MD, USA) using an etched glass standard. Diameters were determined at two magnifications, compared with each other to verify accuracy, and then averaged to yield one diameter per embryo. After correction for the Doppler probe angle, flow was calculated as the product of the instantaneous velocity and the cross sectional area. Because the increasingly opaque body wall hampered dorsal aortic imaging at HH27, we imaged the dorsal aortas of separate groups of embryos using a high resolution ultrasound system equipped with a 40 MHz probe (Vevo 660, VisualSonics, Toronto, Ontario, Canada).

We used common hemodynamic measures as a gauge of arterial wall characteristics. Arterial impedance describes the opposition of the vasculature to both the steady and pulsatile aspects of blood flow and determines how much work the heart must perform to produce given pressure and flow pulse

contours. Total vascular resistance (*TVR*) represents the steady opposition to blood flow (Nichols and O'Rourke, 1998). Characteristic impedance (Z_C) quantifies impedance in the absence of reflected waves, a situation that is not physiological but can yield information regarding the site of measurement, such as relative stiffness (Nichols and O'Rourke, 1998). Impedance at the first harmonic (Z_1) refers to the impedance modulus that corresponds to the most prominent oscillatory frequency, the heart rate. Total arterial compliance (C_A) describes arterial wall distensibility. Total hydraulic power (W_T) is an index of the energy required by the heart to maintain pressure and flow pulsations while both expanding distensible vessels with blood (oscillatory component, W_O) and distributing blood to the periphery (steady component, W_S). Factors that influence these parameters include arterial diameter, the elastic properties of the arterial wall, wall thickness and vascular tone. Of note, the above measurements assess the arterial system at/peripheral to the dorsal aorta and do not include the cranial circulation or pharyngeal arch arteries.

Data analysis

We calculated basic hemodynamic parameters in a customized LabVIEW (National Instruments, Austin, TX, USA) environment. Cycle length and waveform features were determined manually. Input impedance spectra were generated as previously described (Yoshigi et al., 1996) (Fig. 1B,C). We defined *TVR* as the impedance modulus at 0 Hz, Z_1 as the impedance modulus at the first harmonic, and Z_C as the average of the impedance moduli from the third harmonic up to 10 Hz. Global C_A was calculated using the area under the pressure waveform as:

$$C_A = Vs / \{[(A_S + A_D)/A_D] \cdot (P_N - P_D)\}, \quad (1)$$

where Vs is stroke volume, P_N and P_D are pressure at the dicrotic notch and end diastole, respectively, and A_S and A_D are the areas under the aortic pressure waveform during systole and diastole, respectively. Steady power (W_S) was calculated as:

$$W_S = \bar{P} \cdot \bar{Q}, \quad (2)$$

where $\bar{P}$ and $\bar{Q}$ are mean pressure and flow, respectively. Oscillatory power (W_O) was calculated as:

$$W_O = (1/2)\Sigma(|Q_n|)^2 \cdot |Z_n| \cdot \cos\theta_n, \quad (3)$$

where $|Q_n|$, $|Z_n|$ and θ_n are the flow modulus, the impedance modulus, and the impedance phase angle at the 'nth' harmonic. Oscillatory fraction of hydraulic power is:

$$\%W_O = [W_O/(W_S + W_O)] \cdot 100. \quad (4)$$

Total power (W_T) was calculated as:

$$W_T = W_S + W_O. \quad (5)$$

Statistics

Data are reported as mean ± S.E.M. Heart rate, blood pressure, stroke volume, and changes in vascular impedance and hydraulic power were first analyzed by ANOVA followed by Tukey *post-hoc* analysis. Statistical significance was determined at a level of 5% alpha error between groups for a single measure ($P<0.05$).

Results
Left atrial ligation
HH21

One hour after ligation, systolic, diastolic, mean and pulse pressure were similar to control values although dicrotic notch pressure was decreased (Table 1). Stroke volume was reduced and may have been influenced by the increased heart rate observed in this group (Table 2). However, despite the increased f_H, $\dot{Q}$ remained depressed after ligation (Table 2). Total vascular resistance and Z_1 were unaffected by LAL although Z_C increased (Fig. 2A–C) and C_A decreased (Fig. 3). Total power, W_S, and W_O (Fig. 4A–C) were altered, although $\%W_O$ (Fig. 5) was not affected at any stage.

HH24

All pressure parameters were similar to controls. Stroke volume and $\dot{Q}$ remained depressed. Although Z_C normalized at this stage, *TVR* became elevated, reflecting a more global change in arterial resistance. Global compliance and W_S power normalized by HH24 while W_T and W_O remained lower than CON values ($P=0.08$ and $P=0.07$, respectively).

HH27

Pressure parameters were similar to both CON. Stroke volume, $\dot{Q}$ and f_H also normalized to CON values. Compliance, all impedance and all hydraulic power parameters were similar to CON values.

Vitelline artery ligation
HH21

One hour after ligation, all pressure parameters were similar to CON values (Table 1). However, Vs and $\dot{Q}$ declined (Table 2). Total vascular resistance and Z_1 both significantly increased while characteristic impedance was unaffected (Fig. 2A–C). The C_A decreased (Fig. 3). The W_T and W_S were similar to controls although W_O was reduced (Fig. 4A–C). Percent oscillatory power was similar to controls (Fig. 5).

HH24

Dicrotic notch pressure and pulse pressure were elevated although other pressure parameters mirrored CON values. Total vascular resistance and Z_1 remained elevated and Z_C became elevated as well. Arterial compliance remained low. Total, steady and oscillatory power became low. Since W_O and W_T changed proportionally, $\%W_O$ remained similar to CON values.

HH27

Although pressure parameters normalized, Vs and $\dot{Q}$ remained low. Similarly, C_A remained low and *TVR*, Z_1 and Z_C

Table 1. *Pressure parameters in control and experimental embryos*

	Control	Left atrial ligation	Vitelline artery ligation	ANOVA
HH21				
N	10	8	13	
P_M, Pa	133±5	107±8‡	155±9	$P<0.01$
P_N	156±7	119±8*,‡	184±9	$P<0.01$
P_D	71±3	61±5‡	87±5	$P<0.01$
P_S	198±11	156±16‡	213±12	$P=0.01$
P_P	124±8	99±13	127±8	$P=0.11$
HH24				
N	14	10	10	
P_M	160±8	177±1	189±9	$P=0.10$
P_N	184±7	181±12‡	224±15*	$P=0.02$
P_D	87±4	85±7	92±11	$P=0.70$
P_S	256±12	235±17‡	305±20	$P=0.02$
P_P	169±8	148±12‡	209±13*	$P<0.01$
HH27				
N	12	9	10	
P_M	227±18	228±6	233±10	$P=0.84$
P_N	243±13	233±6	260±11	$P=0.23$
P_D	121±12	125±7	121±8	$P=0.95$
P_S	388±17	359±13	389±19	$P=0.34$
P_P	265±13	233±11	273±14	$P=0.11$

Values are mean ± S.E.M. Parameters at HH21, HH24 and HH27 for control (CON), left atrial-ligated (LAL), and right lateral vitelline artery-ligated (VAL) embryos. Data were compared using Oneway ANOVA and *post-hoc* analysis compared individual groups. *$P\leq0.05$ experimental group *versus* CON; $^\ddagger P\leq0.05$ LAL *versus* VAL.

Table 2. *Arterial flow-related parameters in control and experimental embryos*

	Control	Left atrial ligation	Vitelline artery ligation	ANOVA
HH21				
N	10	8	13	
V_S, 10^{-4} ml	1.49±0.17	0.67±0.07*	0.89±0.01*	$P<0.01$
$\dot{Q}$, 10^{-4} ml min^{-1}	21±2	11±1*	13±1*	$P<0.01$
f_H, beats min^{-1}	141±3	166±9*	150±3	$P=0.01$
HH24				
N	14	10	10	
V_S, 10^{-4} ml	2.26±0.21	1.52±0.13*	1.20±0.07*	$P<0.01$
$\dot{Q}$, 10^{-4} ml min^{-1}	34±3	24±2*	19±1*	$P<0.01$
f_H, beats min^{-1}	151±3	157±3	153±3	$P=0.41$
HH27				
N	12	9	10	
V_S, 10^{-4} ml	6.70±0.31	6.62±0.39‡	5.18±0.39*	$P<0.01$
$\dot{Q}$, 10^{-4} ml min^{-1}	120±7	118±8‡	85±8*	$P<0.01$
f_H, beats min^{-1}	180±6	179±9	163±4	$P=0.12$

Values are mean ± S.E.M. Parameters at HH21, HH24 and HH27 for control (CON), left atrial-ligated (LAL), and right lateral vitelline artery-ligated (VAL) embryos. Data were compared using Oneway ANOVA and *post-hoc* analysis compared individual groups. *$P\leq0.05$ experimental group *versus* CON; $^\ddagger P\leq0.05$ LAL *versus* VAL.

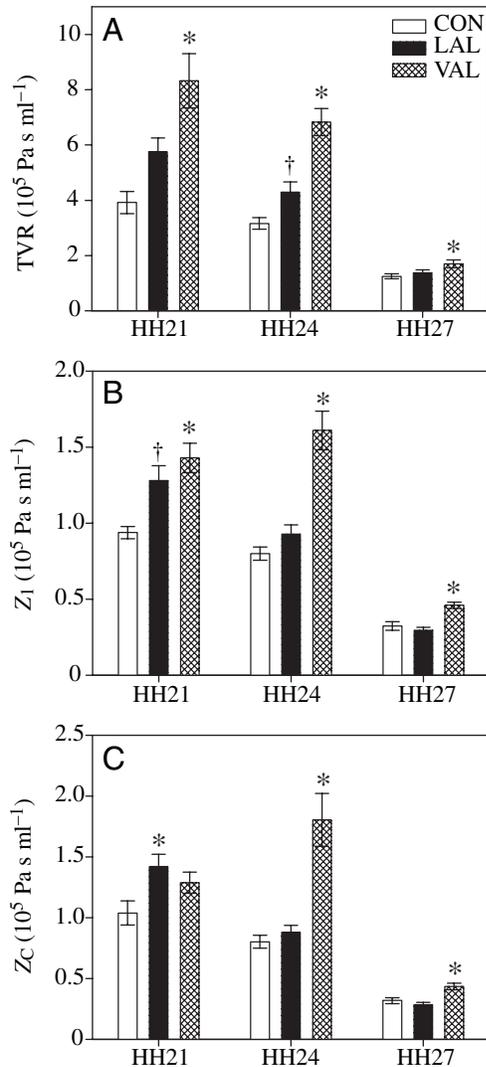Fig. 2. Total vascular resistance (*TVR*) in CON, left-atrial ligated (LAL) and right vitelline artery ligated (VAL) embryos at stages HH21 (*P*<0.01), HH24 (*P*<0.01) and HH27 (*P*=0.04) (A). VAL increased *TVR* at all stages. LAL caused an increase in *TVR* at HH24 that normalized by HH27. Fundamental impedance (Z_1) in CON, LAL and VAL embryos at HH21 (*P*<0.01), HH24 (*P*<0.01) and HH27 (*P*<0.01) (B). VAL increased Z_1 at all stages. LAL increased Z_1 at HH21 only. Characteristic impedance (Z_C) in CON, LAL and VAL embryos at HH21 (*P*=0.08), HH24 (*P*<0.01) and HH27 (*P*<0.01) (C). Z_C increased after LAL and then normalized by HH24. Z_C was unchanged in VAL embryos at HH21 but increased significantly at HH24 and HH27. Data are mean ± S.E.M. *$P \leqslant 0.05$, †$P \leqslant 0.1$ experimental group *versus* CON.
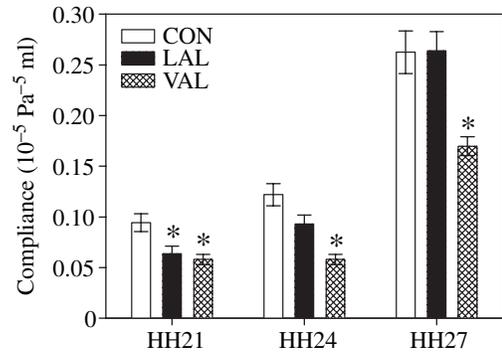
Fig. 3. Compliance (C_A) in CON, LAL and VAL embryos at HH21 (*P*<0.01), HH24 (*P*<0.01) and HH27 (*P*<0.01) embryos. C_A immediately decreased in both LAL and VAL embryos after ligation. C_A normalized by HH24 in LAL embryos but remained significantly low in VAL embryos through HH27. Symbols and abbreviations described in Fig. 2. Data are mean ± S.E.M.

some variation in the literature regarding hemodynamic values in chick embryos at specific Hamburger-Hamilton stages. For example, in the stage 24 embryo, f_H as low as 142 bpm (Broekhuizen et al., 1993) and as high as 183 bpm (Clark and Hu, 1982) has been reported. This variation is likely due to differences in incubator temperature, ambient environment during measurement recording, intra-stage variation, and possibly, genetic strain of chicken used. These factors may not affect within-study comparisons but may complicate between-study comparisons. However, the basic hemodynamic values reported here are similar to a number of previous reports (Clark and Hu, 1982; Hu and Clark, 1989; Broekhuizen et al., 1993; Hu and Keller, 1995). Impedance parameters at stage HH24 are higher and hydraulic power parameters are lower than those previously reported (Yoshigi et al., 1996) and is probably due to differences in V_S.

Basic hemodynamics following LAL and VAL

Increasing arterial resistance with VAL caused a drastic decrease in V_S and $\dot{Q}$. Conversely, reducing V_S and $\dot{Q}$ *via* LAL caused an increase in resistance parameters. Regardless of intervention, systolic, diastolic and mean pressures were maintained 1 h after both LAL and VAL and at subsequent stages. These observations indicate that maintaining mean and pulsatile pressure, as opposed to arterial flow or resistance, is essential for embryo survival. Forces associated with intraluminal pressures, such as cyclic stretch and strain, are known to modulate vascular smooth muscle cell proliferation, phenotype, and a variety of signaling cascades in both vascular smooth muscle and endothelial cells (Birukov et al., 1995; Sackin, 1995; Reusch et al., 1996; Xu et al., 1996; Birukov et al., 1997; Hishikawa and Luscher, 1997; Cheng et al., 1998; Lehoux and Tedgui, 1998; Iwasaki et al., 2000). Since the arterial vasculature undergoes rapid growth and differentiation at these stages (i.e. only a thin layer of vascular smooth muscle cells surround the proximal arteries and many ECM proteins, such as elastin, are not yet present (Hughes, 1943; Bergwerff

remained elevated. All hydraulic power parameters were similar to CON values.

Discussion

This study describes the acute and chronic patterns of arterial hemodynamic resulting from altered arterial blood flow (LAL) and resistance (VAL) in HH21 chick embryos. There is
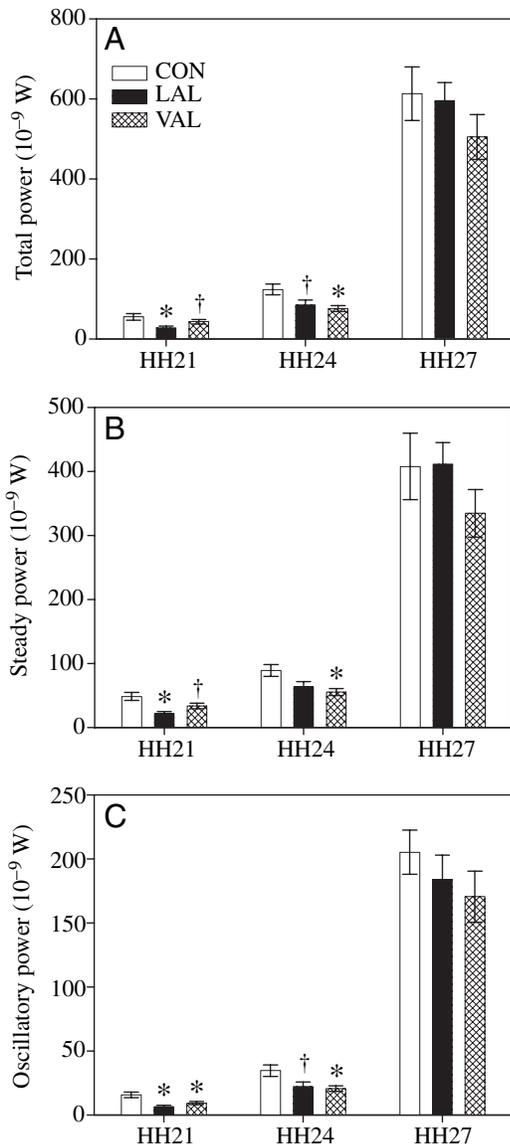
Fig. 4. Total power (W_T) (A), steady power (W_S) (B) and oscillatory power (W_O) (C) in HH21 (W_T: $P<0.01$; W_S: $P<0.01$; W_O: $P<0.01$) HH24 (W_T: $P=0.02$; W_S: $P=0.02$; W_O: $P=0.03$) and HH27 (W_T: $P=0.40$; W_S: $P=0.41$; W_O: $P=0.42$) control and experimental groups. All power parameters significantly declined post-ligation in LAL groups, reflecting a decrease in preload. However, by HH24, power parameters became more similar to CON values and normalized by HH27. Power parameters also decreased in VAL embryos after ligation and normalized by HH27. Symbols and abbreviations described in Fig. 2. Data are mean ± S.E.M.

Fig. 5. Percent oscillatory power in control and experimental groups at HH21 ($P=0.16$), HH24 ($P=0.38$) and HH27 ($P=0.40$). Despite ligation technique, percent oscillatory power was similar at all stages. Symbols and abbreviations described in Figs 1 and 2. Data are mean ± S.E.M.

et al., 1996), precise regulation of these processes may be critical to embryo survival. Because vascular tone is not centrally regulated during these early stages of avian embryogenesis (Altimiras and Crossley, 2000; Crossley and Altimiras, 2000) arterial responses must be exerted *via* local control of tone and/or passive tissue properties.

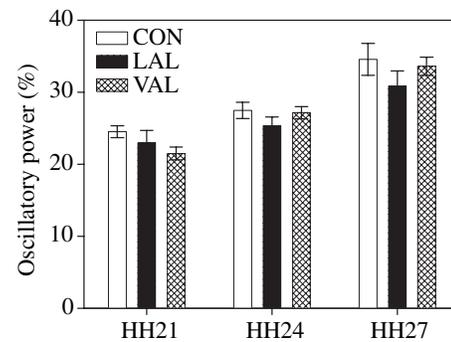Although all pressure values were similar to CON values 1 h after ligation, most were different between VAL and LAL groups. In HH24 chick embryos, infusion and withdrawal of vascular volume causes immediate dose-dependent increases and decreases, respectively, in mean and peak arterial pressure that were countered by changes arterial impedance within the 30–100 s observation period (Yoshigi et al., 1996). Similarly, bolus injections of saline and a volume expander increase arterial pressure by 6–15% in the relatively older Nieuwkoop-Faber (Nieuwkoop and Faber, 1994) stage 49–51 *Xenpous laevis* larvae (Warburton and Fritsche, 2000). Larvae quickly attenuated arterial pressure changes *via* changes in stroke volume and arterial resistance and most restored arterial pressure to pre-injection levels within 15 min. In the present study, it is possible that arterial pressure decreased immediately after LAL and increased immediately after VAL but normalized within the one hour period between ligation and measurement. Regardless, it is evident that the rapid restoration of arterial pressure is a primary directive of the embryonic vascular system.

An essential role of blood flow is the delivery of oxygen to tissue and the removal of metabolic waste products. Burggren et al. (2000) completely occluded the outflow tract of approximately HH18–HH25 chick embryos and observed that ligated embryos grew at the same rate as control embryos for the 4–8 h observation period. The authors speculated that embryonic oxygen requirements were likely met through diffusion and that blood flow was not immediately critical for survival at these stages. This same group partially occluded conotruncal flow in the HH18 chick embryo. This treatment did not affect body, eye or chorioallantoic membrane growth in the subsequent 36 h (to HH24) (Burggren et al., 2004). The authors propose that, at these stages, arterial flow may occur in advance of the need for convective flow to tissues to ensure adequate vascular performance at the time when convective flow becomes critically important to survival. It is well established that blood flow stimulates cellular activities *via* shear stress responsive elements associated with endothelial cells and the extracellular matrix and that arterial flow patterns may be important for normal vascular expansion and

wall maturation. Thus, the relatively simple embryonic cardiovascular system may tolerate certain functional deficits that could become problematic in fetal and post-natal life. The long-term consequences of developing under conditions of low arterial flow are not known. In light of the present data, we propose that, during these stages of embryogenesis, reducing arterial flow, rather than arterial pressure, is the preferred mechanism employed to balance circulation deficiencies.

Impedance and compliance following LAL and VAL

Infusion and withdrawal of peripheral blood volume in HH24 chick embryos causes immediate compensatory changes in peripheral impedance and Z_1 but not Z_C (Yoshigi et al., 1996). Although we did not remove blood volume, we reduced aortic flow by reducing V_S *via* LAL. Acutely reduced aortic flow may have triggered local vasoconstriction within the large arteries and account for the observed change in Z_C, Z_1 and C_A but not *TVR* at HH21. Continually low $\dot{Q}$ may cause volume to shift from the embryonic arterial system to the compliant venous system. Reduced arterial volume, potentially coupled with associated changes in vascular structure, may then be responsible for inducing peripheral vasoconstriction, as noted by the increase in *TVR* by HH24. However, adaptive cardiac remodeling is known to occur after LAL (Sedmera et al., 1999) and presumably, once V_S and $\dot{Q}$ recovered, Z_C, Z_1 and *TVR* and C_A normalized.

Total vascular resistance and impedance parameters generally decrease as the embryo develops due to the rapid growth and expansion of the arterial circulation (Zahka et al., 1989; Yoshigi et al., 1996). Resistance parameters followed this trend in both CON and LAL embryos. By contrast, these parameters, as well as compliance, did not change between HH21 and HH24 in VAL embryos. Considering that dicrotic notch and pulse pressure, which are indices of arterial stiffness, also increased at this stage, arterial stiffness increased appreciably by HH24 without observable detrimental effects to morphogenesis. It is worth noting that although aortic valve leaflets have not yet developed, outflow tract cushions appear to function as valves (VanMierop and Bertuck, 1971; Keller et al., 1990), though they may not be fully competent (Ursem et al., 2001). As ventricular pressure exceeds aortic pressure, blood is ejected into the aortic sac until sac pressure exceeds ventricular pressure. We assume that wave reflection occurs in the viscoelastic, branching embryonic arterial system and that, similar to mature cardiovascular systems, summation of reflected pressure waves terminates ventricular ejection earlier in the cardiac cycle (thus, at a higher pressure) in stiffer conduit arteries due to a faster transit time. Although resistance and impedance were still elevated at HH27, they became much closer to CON values. We speculate that between HH24 and HH27, adaptive vascular remodeling within the embryo and/or accelerated expansion of the extra-embryonic vascular systems occurred to reduce opposition to blood flow. These embryos showed a remarkable ability to maintain a normal growth trajectory while experiencing a marked increase in arterial stiffness and arterial flow opposition.

The observation of an increase in f_H after LAL was unexpected because, in general, the early embryo does not adjust f_H to compensate for moderate changes in cardiac function (Keller et al., 1997). At HH21, pacemaker depolarization occurs in the region of the sinus venosus and the depolarization front travels through the ventricular area to the outflow tract at varying velocities, depending on the cardiac segment (de Jong et al., 1992). Ligating a large percentage of the left atrium may significantly deform the atrium and while bringing the sinus venosus and atrioventricular canal closer together at the inner curvature of the heart. This deformation of the atrium may have increased the resting stretch on pacemaking cells within the atrium with a subsequent alteration in stretch-sensitive ion channels and decay in membrane potential, resulting in more rapid triggering of myocyte depolarization. By HH24, f_H in LAL embryos was similar to CON and VAL embryos, consistent with a return to normal depolarization rates.

Hydraulic power following LAL and VAL

Left atrial ligation caused an acute decrease in hydraulic power parameters and increase in HR. Theoretically, increased HR favors steady power at the expense of oscillatory power. However, percent oscillatory power was unaffected by LAL. In fact, $\%W_O$ did not change in any experimental group at any stage. Hydraulic power parameters were altered after acute alteration of blood volume in chick embryos (Yoshigi et al., 1996). However, $\%W_O$ remained stable except at the highest level of volume alterations. Often referred to as 'wasted' energy, oscillatory energy produces cyclic stretch. Cyclic stretch impacts cellular activities *via* mechanotransducers and affects many functions that are essential to arterial growth (Birukov et al., 1995; Wilson et al., 1995). In the adult circulation, $\%W_O$ is approximately 10% (O'Rourke, 1967) and is much lower than the approximately 23–33% observed over three developmental stages in this study and the approximately 25% observed by Yoshigi et al. (1996) in HH24 chick embryos. A precise balance between oscillatory and steady hydraulic power may be required for normal embryonic growth, despite changes in absolute values.

In conclusion, we found that arterial pressure is maintained at the expense of stroke volume and blood flow at all stages observed. Additionally, the $\%W_O$ was conserved in all groups at all stages regardless of changes in other hydraulic power parameters. Together, these data suggest that the maintenance of arterial pressure and oscillatory vascular stretch is necessary for short-term embryogenesis while arterial flow is not, although the long-term consequences of this compensatory mechanism are not known.

Abbreviations

LAL	left atrial ligation
VAL	vitelline artery ligation
CON	sham treatment
TVR	total vascular resistance

Z_C	characteristic impedance
Z_1	fundamental impedance
C_A	arterial compliance
W_T	total hydraulic power
W_O	oscillatory power
W_S	steady power
$\%W_O$	fraction of oscillatory power
f_H	heart rate
V_S	stroke volume
$\dot{Q}$	cardiac output
$\bar{P}$	mean pressure
P_N	dicrotic notch pressure
P_S	peak systolic pressure
P_D	end diastolic pressure
P_P	pulse pressure
P_{DA}	dorsal aortic pressure

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