Left ventricular output and aortic blood flow in response to changes in preload and afterload in the preterm piglet heart

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Eiby YA, Lumbers ER, Headrick JP, Lingwood BE. Left ventricular output and aortic blood flow in response to changes in preload and afterload in the preterm piglet heart. Am J Physiol Regul Integr Comp Physiol 303: R769–R777, 2012. First published August 15, 2012; doi:10.1152/ajpregu.00010.2012.—Low systemic blood flow occurs in up to 30% of infants born at less than 30 wk gestation. It is associated with increased morbidity and mortality, and current treatments are ineffective in 40% of cases. The aim of this study was to assess the ability of the preterm heart to respond to the acute shifts in preload and afterload that occur at the time of birth. Myocardial and coronary vascular function was assessed using an isolated working heart model in term (115 days) and preterm (92 days) piglets. Cardiac output/kg body wt in preterm hearts was ~50% lower than that of term hearts (P = 0.001). Pressure development was similar in term and preterm hearts. Elevations in preload increased cardiac output and aortic flow similarly in term and preterm hearts, demonstrating significant preload “reserve”. By contrast, elevations in afterload markedly depressed aortic flow, with a greater proportion of cardiac output being distributed to coronary flow in preterm hearts at high afterloads. The demands of increased workload were associated with greater increases in coronary flow in preterm hearts compared with term hearts. In preterm hearts, exposure to maternal glucocorticoids resulted in increased aortic flow when afterload was below 25 mmHg. These data suggest the preterm heart lacks the functional capacity to acutely adapt to postnatal afterload. To maximize systemic blood flow in preterm infants, treatments limiting afterload, while harnessing significant preload reserve, should be further explored.

preterm neonate; coronary blood flow; working heart model

AT BIRTH, THE CARDIOVASCULAR system undergoes profound structural and functional changes as it transitions from a fetal circulation (in which the ventricles work in parallel) to an adult circulation (in which the ventricles work in series). In addition, the low-resistance umbilical circulation is removed, and total peripheral resistance and left ventricular output are increased, leading to a substantial increase in left ventricular workload. Immature infants undergoing this transition are less able to sustain adequate systemic blood flow (SBF). Of infants born at less than 30 wk gestation, up to 30% have low SBF, which places them at risk of increased mortality and morbidity (30), including neurodevelopmental impairment (16). The causes of low SBF are poorly understood, although studies in fetal sheep (3), along with our own studies in the pig (Kim, MY, unpublished observations), indicate that the preterm heart is structurally immature, having smaller myocytes and fewer binucleated myocytes compared with the term heart. It is not known whether this structural immaturity is associated with functional immaturity, although a number of studies suggest functional intolerance of immature myocardium to afterload (13, 33).

Threatened preterm labor is often treated with maternal glucocorticoids to help develop the lungs and improve neonatal outcomes. There is evidence that maternal glucocorticoids advance the structural maturity of the neonatal heart in sheep (20, 21) and pigs (Kim, MY, unpublished observations). It is not known whether these structural changes result in improved myocardial function, although treatment does improve some cardiovascular parameters in human neonates (26), and infants exposed to maternal glucocorticoids have a reduced incidence of low SBF (27).

To understand the contribution of immature left ventricular function to the etiology of low SBF, it is necessary to study the heart in isolation, where loading conditions can be controlled, and free from the confounding effects of systemic neurohumoral control mechanisms. The fluid-ejecting working heart model permits physiologically relevant analysis of left ventricular mechanics under variable loading conditions (9, 43). Cardiac responses to changes in atrial filling pressure (preload) and systemic pressure (afterload) can be evaluated independently to assess the ability of the preterm heart to respond to the challenges that emerge on transition to adult circulation. The distribution of cardiac output between coronary and aortic blood flow can also be examined.

Thus, the primary aim of this study was to compare left ventricular function in preterm and term piglet hearts, to understand the possible contribution of poor left ventricular function to low systemic blood flow in preterm babies. A secondary aim was to assess the affect of maternal glucocorticoid treatment on preterm left ventricular function. We hypothesized that the preterm heart would have reduced cardiac output and aortic flow compared with the term heart, particularly in the face of increased afterload. We also hypothesized that this would be improved by maternal glucocorticoid treatment.

MATERIALS AND METHODS

The project was approved by the University of Queensland Animal Ethics Committee (AEC approval number: UQCCR/9999/08) and conforms to the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (7th ed., 2004), and the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication No. 85–23, revised 1996).

Animals. Large White X Landrace piglets were delivered by cesarean section at two ages, preterm piglets (5 male, 4 female) delivered at 0.8 gestation (92/115 days) and term piglets (4 male, 4 female) delivered 1 day before the expected farrowing date. An additional
group of preterm piglets (5 male, 6 female) was exposed to maternally administered glucocorticoids (betamethasone: 0.19 mg/kg body wt im; Celestone Chronodose; Schering-Plough) given 24 h and 48 h before delivery. The timing and dose per kilogram are equivalent to that given to women presenting with threatened preterm labor. Each of the three treatment groups contained piglets from three litters. Between two and four piglets (similar sex ratios) from each litter were randomly assigned to this experiment, and four littersmates were randomly assigned to a sister experiment investigating cardiac structure (Kim MY, unpublished data). Piglets with a birth weight below the 10th centile were excluded from both studies.

**Cesarean delivery.** Pregnant sows (280–350 kg) were premedicated with 400 mg im atapeapone (Stresnil; Janssen). Anesthesia was induced with 200 mg iv of alfaxalone (Alfaxan-CD RTU; Jurox), followed by administration of additional alfaxalone, as required to allow intubation of the trachea with a 14–16-mm endotracheal tube. The total administered dose of alfaxalone was 300–700 mg. Anesthesia was maintained with 2% isoflurane (Attane isoflurane USP; Masimo), end-tidal isoflurane, and end-tidal CO₂ concentrations recorded at set preloads (mmHg, mean arterial pressure) and afterloads (mmHg, mean arterial pressure) included in the analysis. The total administered dose of alfaxalone was 300–700 mg. Anesthesia was maintained with 2% isoflurane (Attane isoflurane USP; Masimo), end-tidal isoflurane, and end-tidal CO₂ concentrations (Capnomac Anesthesia Monitor; Datex-Ommeda).

Cesarean delivery was performed via a ventral midline incision. Following incision into the linea alba, the uterus was exposed. Piglets were individually removed from the uterus at ~10-min intervals. After all piglets were delivered, the sow was euthanized by intravenous injection of pentobarbital sodium (60 ml Lethabarb; Virbac).

Piglets were heparinized (~500 IU/kg DBL heparin sodium injection Wasserburger Arzneimittelwerk) via the umbilical vein before anesthesia with ~5 mg/kg propofol (Provice 1%; AFT Pharmaceuticals). The umbilical cord was clamped and cut, and the piglet was immediately weighed, and the sex was determined. The piglet’s chest was opened and the heart was rapidly excised, along with ~20 mm of the aorta and pulmonary artery, and placed into heparinized perfusion solution. Total time from cord clamping to heart removal was <3 min.

**Composition of perfusate.** Perfusate was a modified Krebs-Henseleit solution composed of the following ingredients (in mmol/l): 119 NaCl, 22 NaHCO₃, 4.7 KCl, 1.2 KH₂PO₄, 1.2 MgCl₂, 2.5 CaCl₂, 11 glucose, and 0.05 EDTA. Following excision, hearts were first placed in warm, heparinized (2 IU/ml) perfusate at 5–10°C until the heart was arrhythmic beyond the first 5 min, or the intrinsic heart rate was >180 bpm. In the current study, the exclusion rate was 50%. In term hearts, this most commonly occurred due to atrial fibrillation, and in preterm hearts, it was usually the result of leaks from atrial tears.

**Data acquisition and recording.** Measures of left ventricular function recorded at set preloads (mmHg, mean venous or atrial filling pressure) and afterloads (mmHg, mean arterial pressure) included cardiac output (ml/min) measured as flow into the left atrium, aortic flow (ml/min) (as an indicator of systemic flow), and arterial pressure (mmHg), both measured ~30 mm above the aortic valve and distal to the coronary ostia, venous pressure (mmHg) measured immediately before the left atrium, and ventricular pressure (mmHg) measured inside the left ventricle. Flow was measured using in-line ultrasonic flow probes (Precision XN probes) connected to a T402 flowmeter (Transonic Systems, Ithaca, NY). Pressure was measured using a Transpac disposable pressure transducer (Hospira). All data were acquired at 1 kHz on a PowerLab data acquisition system (ML880) using LabChart software version 7 (ADInstruments).

**Experimental manipulations.** Hearts were stabilized in Langendorff mode for 10 min before switching to working heart mode and were stabilized a further 10 min before experimentation. Baseline measures at a fixed preload and afterload were recorded pre-test and post-test and were not significantly different, indicating that the preparation was stable over the study period. Preload (atrial filling pressure) and afterload (systemic resistance) were independently manipulated by lowering or raising gravity-fed reservoirs (Fig. 1). This overcomes difficulties associated with using mechanical resistors that may precipitate mechanical artefacts (9). Preload curves were recorded over a range of 2–16 mmHg at afterloads of 15, 25, and 35 mmHg for preterm hearts or 25, 35, and 45 mmHg for term hearts. Afterload curves were recorded over a range of 20–55 mmHg at preloads of both 6 and 12 mmHg. All curves were acquired in a stepwise fashion from low to high pressures. Preload curves were acquired prior to afterload curves. For simplicity, only preload curves acquired at an afterload of 35 mmHg, and afterload curves acquired at a preload of 6 mmHg, are reported, although observed trends were maintained in other curves.

**Data analysis.** Derived variables included relative cardiac output (ml·min⁻¹·kg⁻¹ body wt⁻¹), relative aortic flow (ml·min⁻¹·kg·body wt⁻¹), relative coronary flow (ml·min⁻¹·g·heart wt⁻¹). These parameters were calculated as the difference between peak systolic and diastolic ventricular pressures, and cardiac power (mmHg·ml·min⁻¹·g·heart wt⁻¹) calculated as the product of afterload and cardiac output per gram of heart. Coronary flow (ml·min⁻¹·g·heart wt⁻¹) was calculated as the difference between left atrial inflow and aortic outflow (9). Coronary flow above the physiological range is normal for the working heart model, as asanguinous perfusion fluids do not have the same oxygen carrying capacity as blood and can only provide sufficient oxygen if the coronary flow rates are several times the physiological norm (9, 10, 35). Consequently, coronary flow during changes in preload or afterload has been expressed as a percentage of the baseline values (17, 36). All values represent 10-s averaged measures. Curves report cardiac output and aortic flow normalized to body weight, as this represents the ability of the heart to adequately supply the body.

Data are expressed as means ± SE. Pre- and post hoc test baseline comparisons of cardiac function were made using paired t-tests. Effects of sex on piglet and heart weights and on cardiac function parameters under standardized conditions were detected by multivariate ANOVA (where sex and litter were fixed factors) separately for each group. Differences between groups within each sex were detected using multivariate ANOVA (where group was a fixed factor, and litter was nested in group). Significant differences are reported only where they exist independently of litter effects. Effects of length and weight on cardiac output were analyzed using Pearson’s correlation. Differences in preload and afterload curves (between treatment groups and sexes and conditions, the heart was arrhythmic beyond the first 5 min, or the intrinsic heart rate was >180 bpm. In the current study, the exclusion rate was 50%. In term hearts, this most commonly occurred due to atrial fibrillation, and in preterm hearts, it was usually the result of leaks from atrial tears.
between cardiac output and aortic flow) were analyzed using mixed between-within-subject ANOVA (treatment, sex, or flow as a fixed factor). Where differences were detected, post hoc analyses using least significant difference were used to analyze differences between groups. Linear regression analysis was used to determine whether the relationship between cardiac power and coronary flow differed between groups. The slopes of these relationships were compared using ANCOVA. Statistical significance was defined at $P < 0.05$. ANOVAs, and correlations were conducted using SPSS v. 17.0 (IBM), and the linear regression analysis was conducted with GraphPad Prism 5.

RESULTS

**Demographic parameters.** Piglet body and heart weights in preterm piglets were ~50% of values for term piglets, which also had larger hearts relative to body size (Table 1). Male and female piglets had similar body and heart weights in the untreated preterm and term groups; however, exposure to maternal glucocorticoids resulted in males that were significantly smaller than both treated preterm females and untreated preterm males (Table 1). Heart:body ratio differed consistently across all three groups, such that females had larger heart:body ratio compared with males.

**Baseline hemodynamic parameters.** Cardiac output (at a fixed preload of 6 mmHg and afterload 35 mmHg) in untreated preterm piglets was significantly lower than at term when both absolute values and values relative to body weight were considered (Table 1). Cardiac output in preterm piglets was not altered by maternal glucocorticoid treatment. Cardiac output/g heart was lower in preterm hearts compared with term for
females but not males (Table 1). In the preterm group, absolute cardiac output and output per gram heart were reduced in females compared with males (Table 1). There were no sex differences in the absolute and relative cardiac outputs of glucocorticoid-exposed hearts or term hearts (Table 1).

The unpaced heart rate that was obtained before measurements were made was similar in all groups (Table 1). Coronary flow was similar across groups (P = 0.990) when assessed at standard conditions (preload 6 mmHg, afterload 35 mmHg). Developed pressure under standard conditions did not differ between groups or between male and female piglets (Table 1).

There was no significant difference in perfusate pH or temperature between groups, nor was there an influence of these on any cardiac function parameters. There was no significant effect of length of delay between removal of the heart and testing on cardiac output, aortic flow, or coronary flow in any of the groups, indicating that storage at low temperatures effectively minimized potentially detrimental effects of ischemia.

Cardiovascular response to preload. Cardiac output increased with preload over the preload range of 2–16 mmHg in all groups (P < 0.001) but was always lower in untreated preterm compared with term hearts (P = 0.024) (Fig. 2A). To support this preload-induced increase in cardiac output, coronary flow increased in all groups (Fig. 2B) (P < 0.001), so that there was a linear relationship between coronary perfusion and work (cardiac power) during changes in preload (Fig. 2C). This relationship, reflecting functional coupling of coronary perfusion to cardiac workload (i.e., functional hyperemia), was similar across groups (P = 0.819). As a result of this increase in coronary flow, aortic flow (SBF) increased to a lesser extent than cardiac output (interaction between preload and flow: P < 0.001 for all groups) (Fig. 2D). At all preloads measured, aortic flow was lower in untreated preterm hearts compared with term hearts (P = 0.013).

Mean cardiac output (P = 0.786) and aortic flow (P = 0.495) in glucocorticoid-exposed preterm hearts were not different from untreated preterm hearts, although variability in both of these parameters was reduced in glucocorticoid-exposed hearts due to a reduced number of hearts with extremely poor function. There was no significant difference in the response of male and female piglet hearts to preload (P > 0.05).

Cardiovascular response to afterload. Cardiac output was lower in untreated preterm vs. term hearts over the 20–55 mmHg afterload range (P = 0.008) and was similar in untreated and glucocorticoid-exposed preterm hearts (P = 0.914) (Fig. 3A). Output was generally stable up to an afterload of 45 mmHg in preterm hearts, beyond which it fell considerably (Fig. 3A). This transition point beyond which output declined was slightly higher in term hearts, at 50 mmHg. Coronary flow increased in all groups as afterload (and thus workload) was increased (Fig. 3B). This occurred, to a greater extent in preterm hearts compared with term hearts (P < 0.001). The relationship between work (cardiac power) and coronary flow (Fig. 3C) was steeper in preterm hearts compared with term hearts (P = 0.034). In glucocorticoid-exposed preterm hearts, the relationship was different to untreated preterm hearts (P < 0.001) and similar to term hearts (P = 0.155). In all groups, aortic flow was substantially reduced when afterload was increased (P < 0.001) (Fig. 3D) and was consistently lower in untreated preterm vs. term hearts (P = 0.014). When the afterload was low, aortic flow in glucocorticoid-exposed hearts was significantly higher than in untreated hearts (P < 0.05) and similar to term hearts (P > 0.05). This benefit was lost when the afterload exceeded 25 mmHg (P > 0.05) (Fig. 3D). There was no significant difference in the response of male and female piglet hearts to afterload (P > 0.05).

DISCUSSION

The present data reveal that the cardiac output and aortic blood flow per kilogram body weight that was able to be maintained by the preterm heart is significantly lower than that achieved at term under comparable conditions. This difference may involve changes both at the level of left ventricular contractile function and the coupling of coronary perfusion to cardiac workload. This reduced left ventricular function may contribute to low systemic blood flow, and this study suggests that appropriate modulation of determinants of preload and afterload may allow for improved systemic blood flow following premature birth.

Baseline cardiac function in preterm and term hearts. At a set preload of 6 mmHg, an afterload of 35 mmHg and a heart rate of 180 bpm, preterm hearts exhibited lower cardiac output (both absolute and relative to body weight) compared with
term hearts. This may be partly due to their smaller heart size relative to body weight. Consistent with in vivo observations in neonates (29), these in vitro findings support depressed left ventricular function in premature hearts. Immature myocardial structure (smaller myocytes with fewer binucleated cells) as observed in littermates (Kim MY, unpublished data) may contribute to this immature function. Despite these differences in cardiac output, ventricular pressures were similar across all groups. This reflects the clinical situation in which blood pressure is not always a good indicator of circulatory status or systemic blood flow in preterm infants (5, 28).

The left ventricular output observed in our isolated piglet hearts at term is very similar to that reported in human studies (11, 18). In contrast, mean values of left ventricular output reported for preterm babies are often higher than those observed in preterm piglet hearts in this study; however, the range of measurements in preterm babies is very wide—100–400 ml·min⁻¹·kg⁻¹ (18), 82–505 ml·min⁻¹·kg⁻¹ (7), and 159–500 ml·min⁻¹·kg⁻¹ (12), and left ventricular output in some preterm babies may be increased as a result of high ductal flows (12), a factor absent in our model. In addition, in all reported studies in preterm babies, left ventricular output was measured at least 5 h after birth. Our measurements taken in hearts that have been exposed to ex utero circulation for only a few minutes would suggest that cardiac output may increase rapidly after birth. Perhaps those preterm babies who exhibit poor cardiovascular function in the 24 h after birth are those who are unable to produce this increase.

There were differences between males and females in cardiac output and cardiac output per gram heart in the untreated preterm group under standardized conditions. However, the functional significance of this observation is difficult to assess because the standardized conditions used to compare groups may represent less than ideal physiology for the preterm heart. It is difficult to define an appropriate set of baseline conditions that is relevant to both term and preterm groups and, therefore,
the function curves provide a better comparison. Heart weight as a proportion of body weight was lower in males than females across all groups. If this is the case in the human, this may contribute to the worse outcomes seen in male infants. The present study was unable to identify significant sexual dimorphism in responses to changes in preload and afterload that would explain the increased frequency of low SBF in male vs. female infants (6). If such a difference exists, it is much smaller than the variability within groups, and a power analysis indicates that more than 100 animals would be required to demonstrate a statistically significant difference. Alternatively, Stark et al. (34) reported differences in microvascular blood flow in male and female preterm infants, with dysregulation of vascular tone in males. This may potentially impact on loading of hearts in males vs. females, contributing to differing cardiac function in vivo.

**Functional responses to preload in preterm and term hearts.** Cardiac output in both preterm and term hearts increased progressively in response to elevated preload as expected on the basis of the Frank-Starling effect (31). This progressive increase is in contrast to that observed previously in fetal sheep (37), where output increases at low atrial pressures (0–4 mmHg) followed by a plateau phase at higher pressures. This observation is unlikely to be the result of left-to-right shunting via the foramen ovale. First, if the high measured coronary flow were the result of flow through the foramen ovale, this should be greatest during increases in preload, where changes in atrial pressure (and thus the driving pressure for left to right flow) were much greater than those that occurred during increased afterload (14 mmHg increase over preload curve vs. 1–2 mmHg over afterload curve and no
change in pulse pressure). This is not the case. Coronary flow increased by a similar amount in both experiments (compare Figs. 2B and 3B). Secondly, coronary flow per gram heart in our model is similar to that found in adult models, where the foramen ovale is not patent. In the mouse working heart model, coronary flow was 9–36 ml·min⁻¹·g heart⁻¹ over a physiological afterload range (9). In the adult guinea pig working heart model, coronary flow was 4–35 ml·min⁻¹·g heart⁻¹ over a range of afterloads (1). These values are similar to coronary flow measured in our model (3–25 ml·min⁻¹·g heart⁻¹). In addition, the pattern of change during alterations in afterload is similar to that seen in adult models. In the adult mouse isolated working-heart model, coronary flow increased 4-fold following a 2-fold increase in afterload (9). Likewise, in the guinea pig, coronary flow increased more than four-fold with increases in cardiac workload (1). This increase is, in fact, greater than the 100% increase in coronary flow in our preterm piglets, indicating that the large increase in coronary flow seen in our model is likely not due to a patent foramen ovale.

While it is difficult to relate coronary flow measurements directly to the human in vivo situation due to the higher coronary flow required by the in vitro model, the proportional changes associated with increased workload should be similar in vivo. Coronary flow in the fetal and newborn sheep is more than double that in the adult (38) and thus increases in coronary flow associated with increased cardiac work will be greater than in the adult and will have a greater impact on aortic flow than in the adult. In addition, in the absence of autoregulation, coronary blood flow could increase up to four-fold (38). Combining these two observations, coronary flow in vivo may increase from 10% to 40% of cardiac output, and so the fraction of cardiac output available for systemic circulation could fall by one-third from 90% to 60% of cardiac output. While our data do not suggest changes in coronary flow of this magnitude, they do indicate that the preterm heart requires considerably higher coronary perfusion compared with the term heart to meet the demands of additional cardiac work associated with increased afterload.

This increased coronary distribution could reflect exaggerated responsiveness of coronary vasoregulation to afterload, consistent with the steeper relationship between cardiac power and coronary flow in untreated preterm hearts. Such a shift could arise if efficiency of oxidative metabolism was lower in preterm hearts, necessitating greater increases in oxygen delivery to meet metabolic demands. However, coupling of coronary flow to cardiac work was the same in term and preterm piglets during shifts in preload, suggesting that afterload-dependent increases in coronary flow in preterm piglets are not due to lower metabolic efficiency. Alternatively, exaggerated coronary responses to afterload could reflect immaturity of coronary autoregulation (which can act in opposition to metabolic coronary control), such that myogenic contraction with coronary pressurization is impaired in untreated preterm hearts, allowing increases in coronary flow in excess of those required to maintain metabolic activity. We are unaware of prior studies of the ontogeny of coronary autoregulation in fetal vessels, although coronary vessels in newborn guinea pigs exhibit impaired myogenic responses compared with mature animals (41). This supports the possibility that, prior to term, the coronary vessels may, indeed, lack the ability to respond appropriately to the increases in coronary perfusion pressure that would occur with increased afterload.

Overall, the preterm heart appears to lack the structural or functional maturity to accommodate increased systemic pressures occurring during the transition to an adult circulation. Previous studies in both animals and human infants have also demonstrated that preterm neonates are unable to respond effectively to increased afterload (8, 13, 33, 44). Our study is the first to indicate that disproportionately high coronary flow may contribute to these observations. The results suggest that treatments that aim to increase mean arterial pressure in preterm infants toward that observed in term infants may not be beneficial. This would explain the observations of a small study in preterm babies, which reported an association between reduced aortic flow and increased mean blood pressure following dopamine treatment (46). These findings support the argument that lower mean arterial pressures in preterm infants, rather than being detrimental, may actually be beneficial, allowing for increased systemic flow and improved organ perfusion. This conclusion is further supported by recent studies that have found “little support for the concept that early postnatal hypotension is associated with developmental delay” in preterm infants (4, 19).

Effects of maternal glucocorticoid exposure on preterm hearts. Maternal glucocorticoid treatment resulted in a statistically significant reduction in birth weight of male piglets only. This is consistent with a study in fetal sheep that found dose-related effects in males only (23); however, another study in sheep found reductions in birth weight in both females and males (24). This area requires further investigation. Maternal glucocorticoid treatment also resulted in aortic flow equivalent to that in term hearts provided afterload remained low. The shift in the coronary flow-cardiac work relationship with glucocorticoid treatment suggests an improved oxidative efficiency and/or improved coronary vasoregulation in the face of increased loading pressures, possibly contributing to improved aortic flow.

These results are consistent with clinical observations that maternal glucocorticoid treatment reduces the need for circulatory support in preterm infants (26). Glucocorticoid exposure increases the ATP and creatine kinase levels in fetal heart (25, 42), potentially contributing to improved performance in glucocorticoid-exposed hearts. The observations are also consistent with results from structural studies in littermates, in which glucocorticoid treatment resulted in maturational changes in female piglets, including increased terminal differentiation and larger myocytes, changes often associated with an increased amount and organization of contractile proteins. In addition, proliferation and apoptosis profiles in female glucocorticoid-treated preterm hearts were more like those of term hearts (Kim MY, unpublished data). Once again, the current study was not able to demonstrate a statistically significant difference in response to preload and afterload between glucocorticoid-exposed male and female hearts with power analysis, indicating that more than 100 animals would be required.

Limitations of the working heart model. The working heart model does not allow the assessment of flow through the ductus arteriosus and its contribution to cardiac function. However, that is beyond the stated aims of the study, which was to compare left ventricular function in preterm and term piglet hearts, to understand the possible contribution of poor left {\textsuperscript{AJP-Regul Integr Comp Physiol} • doi:10.1152/ajpregu.00010.2012 • www.ajpregu.org}
ventricular function to low systemic blood flow in preterm babies. It is possible that the presence of a patent ductus arteriosus would reduce left ventricular afterload and increase preload. The current results suggest that this would result in improved cardiac function. Further research is required to determine whether this is the case, and whether any improvements in cardiac function outweigh the disadvantage of reduced systemic flow due to left-right shunting.

The high coronary flow required by the perfused working heart model means that relevant values of absolute coronary flow cannot be obtained. However, the patterns of change in coronary flow with alterations in preload or afterload are similar to those seen in the adult mouse and dog working heart models (9, 17), and the relationship between coronary flow and workload is linear (see Figs. 2 and 3), suggesting that the increase in flow is due to increased workload as observed in vivo and not an artefact of the model. It is, therefore, likely that although absolute coronary flow is high, proportional changes are consistent with in vivo physiology.

Although this model excludes extrinsic factors such as neural and hormonal controls of coronary vascular tone, the buffer-perfused heart exhibits normal functional hyperemia during increased workloads (1, 9) or adrenergic activation (1, 14, 15, 40). Similarly, profound reactive hyperemia arises after coronary occlusion (1, 45), together with hypoxic hyperemia (1, 22), and coronary responses to major regulatory substances are preserved (2, 15, 32, 39). In short, functional coupling of coronary and cardiac function, and responsiveness of coronary vessels to local and extrinsic stimuli, is well preserved in these models, hence, their utility in assessing mechanisms of coronary vasoregulation (1, 2, 32). Data in the current study evidence cardiac-coronary coupling, with coronary perfusion increasing predictably in response to increased preload of the heart.

Conclusions. This study of left ventricular function in the isolated working heart from term and preterm piglets has shown that left ventricular output/kg body wt in preterm hearts immediately after birth is ∼50% of that in term hearts under comparable conditions of preload and afterload. Preterm hearts, nonetheless, possess substantial preload “reserve” and are able to significantly increase output at higher filling pressures. In addition, preterm hearts have reduced aortic flow during elevations in afterload. This is due to their requirement for higher coronary perfusion in the face of the additional cardiac work associated with increased afterload. Exposure to maternal glucocorticoids significantly improves aortic flow when afterload is low, although this benefit is lost at high afterloads. Our results suggest that reduced left ventricular function may be a significant contributor to low systemic blood flow in preterm infants and that treatments that limit afterload while maintaining or augmenting preload should be further explored.

Perspectives and Significance

Left ventricular load increases significantly at birth, and premature infants may be unable to produce sufficient cardiac output to meet circulatory requirements. Clinical management of preterm infants with poor cardiovascular function remains controversial as many treatments are focused on increasing blood pressure to maintain perfusion, rather than improving blood flow. However, these treatments often fail to improve outcomes, and the effect of raising systemic pressure on the function of the immature heart are not widely studied. This study provides evidence, that while elevations in preload may enhance the performance of the preterm heart, elevations in afterload may compromise systemic blood flow. Maternal glucocorticoid exposure appears to increase systemic flow, but only when afterload remains low. These findings suggest that treatments designed to increase blood pressure in preterm infants may be detrimental rather than beneficial for the infant. Further study is required to determine whether the alternative strategy of maintaining, or even reducing afterload, will result in improved cardiovascular function in preterm infants. It may also be informative to examine the mechanistic basis of immature cardiac function, to identify cellular processes that are immature and could be targeted by novel treatments.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


