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

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Running head: Cardiac function in the preterm piglet

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Abstract

Aims: Low systemic blood flow occurs in up to 30% of infants born at less than 30 weeks 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.

Methods: Myocardial and coronary vascular function was assessed using an isolated working heart model in term (115d) and preterm (92d) piglets.

Results: Cardiac output /kg body weight in preterm hearts was approximately 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 to term hearts. In preterm hearts, exposure to maternal glucocorticoids resulted in increased aortic flow when afterload was below 25 mmHg.

Conclusion: These data suggest the preterm heart lacks the functional capacity to acutely adapt to post-natal afterload. To maximise systemic blood flow in preterm infants, treatments limiting afterload while harnessing significant preload reserve should be further explored.

Keywords

Preterm neonate; coronary blood flow, working heart model
Introduction

At birth, the cardiovascular system undergoes profound structural and functional changes as it transitions from a fetal circulation (where the ventricles work in parallel) to an adult circulation (where the ventricles work in series). In addition, the low resistance umbilicoplacental circulation is removed, 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 weeks 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 to the term heart. It is not known if 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 labour is often treated with maternal glucocorticoids to mature 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 if 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).
In order to understand the contribution of immature left ventricular function to the aetiology 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, in order 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 hypothesised that the preterm heart would have reduced cardiac output and aortic flow compared to the term heart, particularly in the face of increased afterload. We also hypothesised 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/999/08) and conforms to the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (7th edition 2004), and the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (NIH Publication No. 85-23, revised 1996).
**Animals**

Large White X Landrace piglets were delivered by caesarean 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 one day before the expected farrowing date. An additional group of preterm piglets (5 male, 6 female) was exposed to maternally administered glucocorticoids (betamethasone, 0.19mg/kg body wt, applied i.m.; Celestone Chronodose; Schering-Plough, USA) given 24h and 48h before delivery. The timing and dose/kg are equivalent to that given to women presenting with threatened preterm labour. 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 littermates 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.

**Caesarean delivery**

Pregnant sows (280-350kg) were premedicated with 400mg azaperone given i.m. (Stresnil; Janssen, Australia). Anaesthesia was induced with 200mg of alfaxalone given i.v. (Alfaxan-CD RTU; Jurox, Australia), 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. Anaesthesia was maintained with 2% isoflurane (Attane Isoflurane USP; Minrad, USA) in O₂ and sows breathed spontaneously. Throughout surgery, saline (2-3L of 0.15M NaCl) was administered via an ear vein and the following variables were monitored: arterial blood pressure by Doppler (Parks Medical Electronics Inc, Aloha, O, USA), O₂ saturation by pulse oximetry (Masimo, Irvine, CA,
USA), end tidal isoflurane and end tidal CO2 concentrations (Capnomac Anaesthesia Monitor, Datex-Ohmeda Inc, Madison, WI, USA).

Caesarean 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 approximately 10 min intervals. After all piglets were delivered the sow was euthanased by i.v. injection of pentobarbital sodium (60ml Lethabarb, Virbac, Australia).

Piglets were heparinised (~500i.u./kg DBL heparin sodium injection BP; Wasserburger Arzneimittelwerk GmbH, Germany) via the umbilical vein before anaesthesia with ~5mg/kg propofol (Provive 1%; AFT Pharmaceuticals, New Zealand). The umbilical cord was clamped and cut, and the piglet immediately weighed and sexed. The piglet’s chest was opened and the heart rapidly excised, along with approximately 20mm of the aorta and pulmonary artery, and placed into heparinised 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 (mmol/L): NaCl, 119; NaHCO3, 22; KCl, 4.7; KH2PO4, 1.2; MgCl2, 1.2; CaCl2, 2.5; glucose, 11; EDTA, 0.05. Following excision hearts were first placed in warm, heparinised (2 IU/mL) perfusate for 5-10s to remove excess blood then held in heparinised (2 IU/mL) perfusate at 5-10°C until suspended on the isolated working heart apparatus.

**Isolated working heart apparatus**
The working heart apparatus is depicted in Figure 1. Perfusate temperature was maintained at 37-38°C and bubbled with a mix of 5% CO₂ / 95% O₂ to maintain pH at 7.40 – 7.52. Hearts were suspended from an aortic cannula and underwent retrograde perfusion while a bevelled and flanged polyethylene catheter was passed through a pulmonary vein into the left ventricle and through the ventricular wall for connection to a transducer for ventricular pressure measurement. The left atrium was cannulated via the pulmonary vein and all other vessels occluded except the pulmonary artery. The heart was slowly switched from Langendorff (retrograde perfusion) to working heart mode by allowing flow into the left atrium via the atrial cannula. Pacing leads were attached to the right atrium with microaneurysm clips and the heart paced at 180 bpm to facilitate the comparison of rate-dependent contractile parameters between groups. This was the lowest rate at which the majority of hearts could be captured. Coronary flow emptied into the right atrium and was ejected through the pulmonary artery and discarded.

The isolated working heart is a highly reproducible preparation provided appropriate exclusion criteria are applied. In this study hearts were excluded from the analysis if leaks were visualised from either the aortic or atrial cannula, total instrumentation time was prolonged, no cardiac output was produced at baseline loading conditions, the heart was arrhythmic beyond the first 5 min, or the intrinsic heart rate was >180bpm. In the current study, the exclusion rate was 50%. In term hearts this most commonly occurred due to atrial fibrillation and in preterm hearts 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 approximately 30mm 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 Inc., Ithaca, NY, USA). Pressure was measured using a Transpac® disposable pressure transducer (Hospira, USA). All data were acquired at 1 kHz on a Powerlab data acquisition system (ML880) using Labchart software version 7 (ADInstruments, Australia).

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- 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 2-16 mmHg range 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 step-wise 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/g heart wt and mL/min/kg body weight BW), relative aortic flow (mL/min/kg BW), developed pressure (mmHg) calculated as the difference between peak systolic and diastolic ventricular pressures, and cardiac power (mmHg.mL/min/g heart wt) calculated as the product of afterload and cardiac output per gram of heart. Coronary flow (mL/min/g heart wt) 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 percentage of the baseline values (17, 36). All values represent 10s averaged measures. Curves report cardiac output and aortic flow normalised to body weight as this best represents the ability of the heart to adequately supply the body.

Data are expressed as mean ± S.E.M. Pre- and post-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 of delay between removal and testing, perfusate pH and temperature on function were analysed using Pearson’s correlation. Differences in preload and afterload curves (between treatment groups and sexes and between
cardiac output and aortic flow) were analysed 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 (LSD) were used to analyse differences between groups. Linear regression analysis was used to determine if 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, USA) 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 6mmHg and afterload 35mmHg) 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 to term for females but not males (Table 1). In the preterm group absolute cardiac output and output per g heart was reduced in females compared to 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 obtained before measurements were made was similar in all groups (Table 1). Coronary flow was similar across groups (p=0.090) 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 minimised 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 hyperaemia), 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 to term hearts (p=0.013).
Mean cardiac output ($p=0.786$) and aortic flow ($p=0.495$) in glucocorticoid exposed preterm hearts was 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 to term hearts ($p<0.001$). The relationship between work (cardiac power) and coronary flow (Fig 3C) was steeper in preterm hearts compared to 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 25mmHg ($p>0.05$) (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 kg body weight 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 to term heart. 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 where 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/kg (18), 82 – 505 ml/min/kg (7), 159 – 500 ml/min/kg (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 five hours 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 hours after birth are those who are unable to produce this increase.

There were differences between males and females in cardiac output and cardiac output /g 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 based on 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. These sheep had limited preload reserve because the normal filling pressure is near the transition to the plateau phase. Both preterm and term piglet hearts possess significant preload 'reserve', such that they are able to produce substantially higher outputs under higher filling pressures. Thus, while left ventricular output may be developmentally limited, smaller preterm hearts can potentially accommodate increased perfusion requirements given appropriate preload.

However the full benefit of this preload reserve may not be available to the systemic circulation. Myocardial energetic demand increases with elevations in cardiac output or work, requiring increased coronary perfusion. This functional hyperaemia is reflected in linear relationships between cardiac power and coronary flow during elevations in preload (Fig 2C). Due to the necessity of maintaining adequate myocardial perfusion at higher workloads, aortic flow did not increase to the same extent as cardiac output (Fig 2D). This may contribute to the inconsistent outcomes observed following volume expansion in preterm neonates (5).

**Negative influences of afterload in preterm and term hearts**

The shift in left ventricular workload upon birth is substantial, and may well impact on the ability of immature myocardium to maintain cardiac output. As predicted for adult hearts, piglet left ventricular output fell slightly in the face of increased afterload. Interestingly,
aortic flow fell more dramatically owing to an increased distribution of cardiac output to the
coronary circuit during afterload-dependent work transitions. This observation is unlikely to
be the result of left-to-right shunting via the foramen ovale. Firstly, if the high measured
coronary flow was 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 which 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 Fig 2B and 3B). Secondly, coronary flow/g 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/g heart over a physiological afterload
range (9). In the adult guinea pig working heart model, coronary flow was 4-35mL/min/g
heart over a range of afterloads (1). These values are similar to coronary flow measured in
our model (3-25mL/min/g heart). 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 to 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, though 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 which 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 birthweight of male piglets only. This is consistent with a study in fetal sheep which found dose related effects in males only (23), however another study in sheep found reductions in birthweight 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, where glucocorticoid treatment resulted in maturational changes in female piglets, including increased terminal differentiation and larger myocytes – changes often associated with 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 arteriosis 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, in order to understand the possible contribution of poor left ventricular function to low systemic blood flow in preterm babies. It is possible that the presence of a patent ductus arteriosis would reduce left ventricular afterload and increase preload. The current results suggest this would result in improved cardiac function. Further research is required to determine if 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 Fig 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 preloading 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 BW in preterm hearts immediately after birth is approximately 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 focussed on increasing blood pressure in order 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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Conflict of interest: none declared
References


Figure Legends

Figure 1. Diagrammatic representation of the isolated working heart apparatus. Preload and afterload are adjusted by lowering or raising gravity fed reservoirs. RA right atrium, LA left atrium, RV right ventricle and LV left ventricle.

Figure 2. Effects of Preload. Panel A: Left ventricular output (LVO) (at afterload 35 mmHg) increased with preload in all groups (p<0.001) but was always lower in untreated and glucocorticoid exposed preterm hearts compared with term hearts (p=0.024). Panel B: To support this increase in left ventricular output, coronary flow (% baseline) increased but to a greater extent in untreated preterm and glucocorticoid hearts compared to term hearts (p<0.05). Panel C: This increase was linearly related to cardiac power. The slopes of the lines were not statistically different between groups (preterm: \( r^2=0.68, \) slope=0.017± 0.002, preterm + GC: \( r^2=0.43, \) slope=0.016± 0.002, term: \( r^2=0.62, \) slope=0.017± 0.002). Panel D: Aortic flow was also lower in untreated and glucocorticoid exposed preterm hearts than in term hearts (p=0.013). Aortic flow did not increase as much as cardiac output did with increased preload (p<0.001). Dotted line: untreated preterm (n=9). Dashed line: glucocorticoid exposed preterm (n=11). Solid line: term (n=8). Mean ± S.E.M. * indicates significant difference between term and preterm hearts.

Figure 3. Effects of Afterload. Panel A: Left ventricular output (LVO) was lower in all preterm vs. term hearts (p=0.008), and did not alter with increased afterload until afterload exceeded 45 mmHg. Panel B: Coronary flow (% baseline) increased as afterload was increased but to a greater extent in both preterm groups (p<0.05) compared to the term group. Panel C: The relationship between cardiac power and coronary flow was steeper (p=0.034) in untreated preterm hearts (\( r^2=0.69, \) slope=0.016± 0.001) compared to term hearts (\( r^2=0.52, \)
slope=0.012± 0.001). In glucocorticoid exposed preterm hearts ($r^2=0.27$, slope=0.009± 0.002) the relationship was different to untreated preterm hearts (p<0.001) and similar to term hearts (p=0.155). Panel D: In all groups aortic flow was substantially reduced when afterload was increased (p<0.001). Dotted line: untreated preterm (n=9). Dashed line: glucocorticoid exposed preterm (n=11). Solid line: term (n=8). Mean ± S.E.M. * indicates significant difference between term and preterm hearts. # indicates significant difference between untreated and glucocorticoid exposed preterm hearts.
Table 1. Weights and cardiac function at standardized conditions (preload 6mmHg and afterload 35mmHg) for female and male piglets in the three groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Preterm</th>
<th>Preterm + GC</th>
<th>Term</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female (n=3)</td>
<td>Male (n=4)</td>
<td>Female (n=6)</td>
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<tr>
<td>Body weight (g)</td>
<td>856 ± 72</td>
<td>878 ± 74</td>
<td>796 ± 46</td>
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<tr>
<td>Heart weight (g)</td>
<td>5.9 ± 0.5</td>
<td>5.4 ± 0.4</td>
<td>5.7 ± 0.3</td>
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<tr>
<td>Heart (% body weight)</td>
<td>0.69 ± 0.02</td>
<td>0.61 ± 0.02##</td>
<td>0.72 ± 0.10##</td>
</tr>
<tr>
<td>Cardiac output (mL/min)</td>
<td>103 ± 36</td>
<td>121 ± 15##</td>
<td>99 ± 9</td>
</tr>
<tr>
<td>Cardiac output (mL/min/g heart)</td>
<td>18.3 ± 6.8</td>
<td>23.5 ± 4.2##</td>
<td>17.4 ± 1.0</td>
</tr>
<tr>
<td>Cardiac output (mL/min/kg BW)</td>
<td>128 ± 48</td>
<td>144 ± 27</td>
<td>127 ± 13</td>
</tr>
<tr>
<td>Stroke volume (mL)</td>
<td>0.58 ± 0.20</td>
<td>0.68 ± 0.17##</td>
<td>0.56 ± 0.05</td>
</tr>
<tr>
<td>Developed pressure (mmHg)</td>
<td>59 ± 1</td>
<td>62 ± 5</td>
<td>58 ± 2</td>
</tr>
<tr>
<td>Unpaced heart rate (bpm)</td>
<td>142 ± 4</td>
<td>150 ± 8</td>
<td>151 ± 9</td>
</tr>
</tbody>
</table>
Values are mean ± S.E.M. Preterm + GC = preterm piglets exposed to maternal glucocorticoid treatment. Two preterm hearts that were unable to produce positive aortic flow at the standard conditions were not included in this analysis, but were included in all other analyses. * indicates a significant difference to untreated preterm animals of the same sex. # indicates a significant difference to females within the same group.