Cardiovascular and pulmonary consequences of airway recruitment in preterm lambs

Graeme R. Polglase,1 Stuart B. Hooper, Andrew W. Gill,1 Beth J. Allison,2 Carryn J. McLean,1 Ilias Nitsos,1 J. Jane Pillow,1 and Martin Kluckow3

1School of Women’s and Infants’ Health, The University of Western Australia, Crawley, Western Australia; 2Department of Physiology, Monash University, Clayton, Victoria; and 3Department of Neonatal Medicine, Royal North Shore Hospital, and University of Sydney, Sydney, New South Wales, Australia

Submitted 3 November 2008; accepted in final form 12 February 2009

Polglase GR, Hooper SB, Gill AW, Allison BJ, McLean CJ, Nitsos I, Pillow JJ, Kluckow M. Cardiovascular and pulmonary consequences of airway recruitment in preterm lambs. J Appl Physiol 106: 1347–1355, 2009. First published February 12, 2009; doi:10.1152/japplphysiol.91445.2008.—Increases in positive end-expiratory pressure (PEEP) improve arterial oxygenation in preterm infants, but the effects on cardiopulmonary hemodynamics are understood poorly. We aimed to determine the effect of increased PEEP on cardiopulmonary hemodynamics and to compare measurements from indwelling flow probes with Doppler echocardiography. Preterm lambs (129 ± 1 days) were ventilated initially with a tidal volume of 7 ml/kg and 4 cmH2O of PEEP. In ramp lambs (n = 7), PEEP was increased by 2-cmH2O increments to 10 cmH2O and then in decrements back to 4 cmH2O. PEEP was unchanged in controls (n = 6). Doppler echocardiographic flow measurements in the left pulmonary artery (LPA) and ductus arteriosus (DA) were correlated with flow probe measurements. Compared with controls, high PEEP reduced LPA flow from baseline (10-cmH2O PEEP: 43 ± 8% vs. control: 83 ± 21%; P = 0.029). High PEEP increased the proportion of right-to-left (R-L) shunting through the DA, with a trend to an increased oxygenation index compared with controls (oxygenation index: 44.5 ± 13.5 at 10-cmH2O PEEP vs. 19.4 ± 4.5 in controls; P = 0.07). Increasing PEEP decreased heart rate (17 beats/min; P = 0.03) and tended to lower systolic arterial pressure (5.0 mmHg; P = 0.052) compared with controls. Doppler echocardiography measurement of LPA flows correlated strongly with indwelling flow probe (r2 = 0.73, P < 0.001), except during highly turbulent flows. Increases in PEEP have significant cardiopulmonary consequences in preterm lambs, including reduced LPA flow and increased R-L shunt through the DA. These changes are likely due to the concomitant increase in downstream pulmonary vascular resistance and increased cardiovascular constraint induced by PEEP.

pulmonary hemodynamics; Doppler echocardiography; waveform analysis; preterm; cardiovascular

POSITIVE PRESSURE ventilation, either with intubation and mechanical ventilation, or by application of continuous positive airway pressure, plays an important role in providing respiratory support for very preterm infants. However, the application of positive pressure to the lungs dramatically changes the interaction between the lung and the cardiovascular system (5). Normally, cardiac function is assisted by the subatmospheric intrathoracic pressure generated by spontaneous breathing, which enhances venous return to the heart and the reduction in pulmonary vascular resistance (PVR) immediately after birth, in turn augmenting pulmonary venous return (20). The application of positive airway pressure changes these interactions and potentially results in a decrease in cardiac output (6, 8, 14). The interactions between heart and lung are complex, as the improved lung inflation achieved using positive end-expiratory pressure (PEEP) may also impede pulmonary blood flow (PBF) and thereby reduce venous return and cardiac output (19). Previous studies in prematurely delivered lambs showed that increasing PEEP results in progressively reduced PBF, an effect that persists even after PEEP is reduced (17). Studies in humans have also shown that increased mean airway pressure is associated with a reduction in cardiac output (12, 15, 23). It is unclear whether the reduction in cardiac output and systemic blood flow is mediated via a direct pressure effect on the heart, a reduction in systemic or pulmonary venous return, or through impairment of myocardial function by increasing PVR.

Assessment of the cardiovascular system in preterm infants is difficult, as the measurement of blood pressure invasively or noninvasively may not reflect cardiac output accurately (12). The use of invasive catheters for measurement of intravascular pressures and cardiac output is not feasible, due to the small catheter size required and the persistence of fetal shunts. Ultrasound machines with small high-frequency probes provide noninvasive relative measures of blood flow and cardiac output in the premature infant at the bedside (3). There is increasing use of this modality to assess cardiac output and function in the preterm infant (3). The importance of measuring cardiac output in vivo and relating these measures to blood flow and injury to other organs, including the brain, was recognized recently (11).

The premature sheep model provides an opportunity to study cardiorespiratory interactions and correlate measures obtained noninvasively using ultrasound with those obtained directly using flow probes implanted surgically. We aimed to assess the effects of a ramped increase of PEEP, within a neonatal therapeutic and physiological range, on PBF and shunting through the ductus arteriosus (DA). We hypothesized that stepwise increases in PEEP will improve oxygenation but have deleterious effects on PBF, and subsequently on cardiovascular indexes. A secondary aim was to assess the relationship between ultrasound derived measures of PBF and those obtained by invasive flow probes. The feasibility of using flow measure-
ment in the superior vena cava (SVC) as a proxy for cerebral blood flow was also explored.

**EXPERIMENTAL PROCEDURES**

The experimental protocol was approved by the animal ethics committees of the University of Western Australian, Monash University, and the University of Sydney.

Surgery was performed on anesthetized (Attane Isoflurane; Bovac, NSW, Australia) pregnant ewes (Merino), bearing single or twin fetuses (total 16 lambs), at 129 ± 1 days gestation (term is ~150 days). The fetal head and neck were exposed via hysterotomy for insertion of heparinized polyvinyl catheters (20 gauge) into a fetal carotid artery. A left thoracotomy was performed in the fourth intercostal space for placement of a 4-mm ultrasonic flow transducer (Transonic Systems, Ithaca, NY) around the left pulmonary artery (LPA) and insertion of a polyvinyl catheter into the main pulmonary artery, as described previously (17). A flow probe was placed on the SVC, just above the right atrium, via a right thoracotomy in the second intercostal space. Pulmonary and carotid arterial pressures (Transducers; DTX, Viggo-Spectramed) and blood flow through the LPA and SVC were measured and recorded digitally (1 kHz; Powerlab, ADI, Castle Hill, NSW, Australia). The fetal chest was closed, the fetal trachea intubated orally, and lung liquid was drained passively. Surfactant was not given as per previous publications using this model (17, 18).

Lambs were delivered, dried, weighed, and commenced on positive-pressure ventilation for 20 min [warmed humidified gas, initial peak inspiratory pressure (PIP) of 40 cmH2O, and a PEEP of 4 cmH2O] to allow birth-related changes in pulmonary hemodynamics to stabilize. Lambs were anesthetized with a continuous umbilical venous infusion of Remifentanil (Ultiva 0.05 mg.kg−1.min−1, GlaxoSmithKline, Victoria, Australia) and Propofol (Respose, 0.1 mg.kg−1.min−1, Norbrook Laboratories, Victoria, Australia). PIP was adjusted intermittently to maintain a tidal volume of ~7 ml/kg body wt (Florian Respiratory Monitor; Acutronic Medical Systems, Hirzel, Switzerland) and arterial partial pressure of carbon dioxide (Paco2) of 50–60 Torr.

At 20 min of age, lambs were commenced on one of two PEEP protocols, according to randomization before surgery. In control lambs, PEEP was maintained at 4 cmH2O throughout the ventilation protocol. In ramp lambs, PEEP was increased by 2 cmH2O every 10 min to 10 cmH2O and then decremented similarly back to 4-cmH2O (Fig. 1A). Peak insufflation pressure, cmH2O 30.6 ± 0.9 vs. 44.3 ± 2.7 PBF and arterial pressures.

LPA flow waveform analysis. Changes in the contour of the LPA waveform were measured by selecting five consecutive cardiac cycles, from six lambs from each group, at specific periods during the experiment: before birth; within the first 10 min after delivery, and at 10-min intervals during the study (corresponding with stepwise changes in PEEP in ramp lambs). The waveform parameters (mean systolic and diastolic flow, pulse amplitude, peak systolic and end-diastolic flow) measured from the LPA were analyzed, and pulsatility index calculated, as described previously (17).

**DA analysis.** The DA was imaged from a left lateral view on the chest wall. The direction of flow in the DA was assessed using pulsed Doppler ultrasound. The proportion of time in the cardiac cycle of R-L shunting vs. left-to-right (L-R) shunting was calculated using the ratio L-R time/L-R time + R-L time; pure L-R shunting is represented by a ratio of 1, and progressive increases in R-L time will reduce the ratio.

Ultrasound derived LPA flow analysis. The ultrasound probe was directed toward the LPA to simultaneously measure flow in the LPA using both the transonic flow probe and Doppler echocardiography. The Doppler signal was obtained, and the velocity time signal recorded. The velocity time integral (VTI) was calculated as the area under the curve of the velocity time signal. The LPA diameter was measured from an optimized two-dimensional image and checked utilizing the color Doppler outline of the vessel. An average of three measurements was used. The flow in the LPA was calculated using the following equation:

\[
\text{Flow} = \text{VTI} \times \pi \times \text{LPA diameter}^2 / 4 \times \text{heart rate} \times 100
\]

LPA flow was calculated using the forward systolic component and also as a mean flow integrating the area under the curve of both systolic and diastolic velocity components to emulate the way that a transonic flow probe calculates flow. Measurements of both LPA flow and DA were calculated at a later stage from recordings, with the technician blind to the status of the animal.

Statistical analysis. Data are presented as means ± SE. Comparisons within groups were performed using two-way ANOVA with repeated measures (Sigmastat v3.0, SPSS). Post hoc comparisons were performed using the Holm-Sidak method. Depending on whether or not the data met normality, a Mann-Whitney rank sum test or a Student’s t-test was used to compare the two groups of ventilated animals at specific time points. Statistical significance was accepted as *P* < 0.05 for all analyses.

**RESULTS**

Thirteen out of 16 lambs survived instrumentation and were included in the study. Fetal body weights were not different between groups (**P** = 0.271, Table 1). Umbilical arterial blood-gas and acid/base values immediately after delivery were normal for all lambs and not different between groups (Table 1).

Tidal volume and minute ventilation, corrected for body weight, were not different between groups. PIP was not different between groups at any stage of the experiment. Mean airway pressure was significantly increased in ramp lambs at higher PEEP compared with control lambs, as expected (**P** < 0.001).

<table>
<thead>
<tr>
<th>Table 1. Subject characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Birth weights, kg</td>
</tr>
<tr>
<td>Male/female ratio</td>
</tr>
<tr>
<td>Single/twin ratio</td>
</tr>
<tr>
<td>Baseline pH</td>
</tr>
<tr>
<td>Baseline Paco2, Torr</td>
</tr>
<tr>
<td>Tidal volume, ml/kg</td>
</tr>
<tr>
<td>Minute ventilation, l·min−1·kg−1</td>
</tr>
<tr>
<td>Peak inspiratory pressure, cmH2O</td>
</tr>
</tbody>
</table>

Values are means ± SE. Paco2 arterial partial pressure of oxygen.
time point) (62.0 ± 6.5 vs. 24.2 ± 14.2%; P < 0.001). Calculated PVR was not different between groups throughout the ventilation protocol (Fig. 1B), although it tended to be elevated (P = 0.08) at high PEEP in ramp lambs.

Blood flow in the SVC, indicative of blood flow returning from the head and upper limbs, was lower in ramp than control lambs, but this difference did not reach significance at any point of the ventilation protocol (P = 0.329, Fig. 1C).

Pulmonary arterial pressure (PAP) was not different between control and ramp lambs (Fig. 1D). PAP appeared lower at 80 min in ramp lambs; however, this failed to reach statistical significance (P = 0.171). There was a trend toward lower systemic arterial pressure in ramp lambs than control lambs (P = 0.052). Heart rate was lower in ramp lambs compared with control lambs during the recruitment maneuver and remained lower at the end of the protocol (P = 0.03; Fig. 2B); heart rate progressively increased in control lambs throughout the ventilation protocol.

**Blood-gas status.** FiO₂ was not different between groups at any stage of the protocol; however, FiO₂ significantly increased in both groups throughout the protocol (from 0.50 ± 0.07 at 10 min to 0.71 ± 0.10 at 80 min; P = 0.016). PaO₂ (mean controls: 39.5 ± 1.0 Torr; ramp: 45.3 ± 4.4 Torr) and SaO₂ (mean controls: 87.8 ± 1.3 mmHg; ramp: 85.8 ± 2.1 mmHg) were not different between groups, nor did they significantly change during the ventilation protocols. OI tended to be higher in ramp lambs compared with control lambs at 10-cmH₂O PEEP (Fig. 2C, P = 0.07), but not at
any other point during the protocol. There was a significant increase in OI from baseline in controls (by 11.9 ± 3.6; \( P = 0.044 \)), suggesting worsening respiratory disease. In contrast, OI was not different in ramp lambs between the start and the end of the protocol. \( \text{PaCO}_2 \) was not different between groups (Fig. 3D), and, although it tended to increase in both groups over the ventilation period, this did not reach significance.

**LPA blood flow waveform analyses.** Mean systolic, mean diastolic, peak systolic, and end-diastolic LPA flow were all significantly reduced at high PEEP in ramp lambs compared with control lambs (Fig. 3). The pulse amplitude (peak flow – postsystolic minimum flow) was reduced significantly in ramp lambs at maximum PEEP compared with controls, suggestive of cardiovascular constraint. The pulse amplitude returned to control values in ramp lambs when PEEP was reduced back to 4 cmH\(_2\)O. Pulsatility index, a measure of downstream resistance to blood flow, increased significantly in both groups throughout the ventilation protocol (\( P = 0.009 \)), but no difference was determined between groups (Fig. 3D).

Negative values of postsystolic minimum LPA flow, reflecting retrograde flow, were observed in seven of seven ramp lambs and four of six control lambs. In all ramp lambs, the onset of retrograde flow occurred at maximum PEEP and persisted despite subsequent stepwise reductions in PEEP, whereas, in control lambs, retrograde flow occurred after 70 min of ventilation. The contour of the LPA flow waveform
in ramp lambs at maximum PEEP resembled that of the fetus (Fig. 4), suggesting raised pulmonary pressures.

**Doppler echocardiography measurements and correlation.**

The ratio of L-R-to-R-L ductal flow showed resumption of R-L shunting in seven of seven ramp lambs, and four of six control lambs. Overall, ramp lambs had significantly reduced L-R-to-R-L ratio compared with control lambs ($P = 0.004$; Fig. 5), indicating increased R-L shunting through the DA. The resumption of R-L shunting as measured by ultrasound closely correlated with retrograde (negative) PBF during postsystole.

Mean LPA flow values (systolic + diastolic PBF) extrapolated from velocity and vessel diameter measurements (over 3–5 cardiac cycles), obtained using Doppler ultrasound, were closely correlated with in-dwelling flow probe measurements ($r^2 = 0.73$, $P < 0.001$; Fig. 6A). Systolic LPA flow calculated using echocardiography also correlated with in-dwelling flow probe measurements, although this relationship was weaker ($r^2 = 0.55$, $P = 0.001$). Bland-Altman analysis of mean LPA flow showed good agreement for flow $< 100$ ml·min$^{-1}$·kg$^{-1}$ with a tendency for Doppler ultrasound to overestimate LPA flow. At higher flows ($>100$ ml·min$^{-1}$·kg$^{-1}$), Doppler ultrasound markedly overestimated LPA flow (Fig. 6B). Bland-Altman analysis of systolic LPA flow showed similar results (data not shown).

Blood flow through the DA before birth was predominantly R-L. At birth, a large increase in DA flow and rapid reversal of direction (to L-R) was observed coinciding with a fall in PVR. Flow in the DA was very turbulent soon after birth and correlated poorly with echocardiography LPA flow measurements. Within 15 min, flow became more laminar, the propor-

---

Fig. 3. LPA waveform analysis. Mean systolic (A), mean diastolic (B), and pulse amplitude (peak systolic — end systolic; C) blood flow through the LPA and pulsatility index (D) are shown in control (●) and ramp (○) groups. Shaded region indicates time of ramp recruitment. *Significant difference ($P < 0.05$) ramp vs. control groups.
tion of L-R shunting was reduced, and the correlation with echocardiography measurements improved (Fig. 7).

DISCUSSION

We have confirmed the reduction in LPA blood flow with increasing PEEP seen in previous studies, (17, 18) and demonstrated the cardiorespiratory hemodynamic consequences of altered pulmonary pressures. PBF also progressively reduced in control lambs throughout the ventilation procedure, which is likely due to a reduction in the contribution from L-R shunting through the DA. A gradual reduction in PBF may be due to ventilating premature lambs without surfactant, with progressive increases in PVR, and worsening respiratory distress syndrome with time (as evidenced in this study by worsening OI and increasing FIO2). However, of particular note was the reduction in PBF in response to increasing airway pressure achieved by increasing PEEP, especially at maximum PEEP settings. Importantly, as in previous studies (16–18), the fall in LPA flow with incremental PEEP in the ramp lambs did not return to baseline with subsequent PEEP reduction, suggesting a physiological change occurred in the lung, referred to as a lung “history” effect (17).

There are several possible explanations for the decrease in PBF in response to increasing PEEP. We measured flow in the LPA, where the flow receives contributions from both the right ventricular output and the L-R flow through the DA (a proportion of left ventricular output). Changes in either of these contributions would alter LPA flow. The determinants of right ventricular output include preload, contractility, and afterload. Both the preload and afterload can be affected by changes in the lung, particularly those induced by positive pressure ventilation. The effect of increasing afterload in the absence of corticosteroids on right ventricular contractility during the transition to air breathing after premature birth has not been studied extensively. Indeed, increasing afterload on the right ventricle, either by increasing airway pressure or through increased PVR as respiratory distress syndrome develops, may lead to cardiovascular dysfunction (2). Studies of fetal animals have consistently shown a marked inability of the heart to contract with the imposition of afterload, with the rapid onset of ventricular failure as afterload is increased (22). This is in contrast to studies in more mature newborn lambs, which found that stepwise increases in afterload increase contractility considerably, as reflected by the maintenance of stroke volume at higher afterloads (10).

High PEEP in ramp lambs causes a reduction in LPA PBF pulse amplitude (Fig. 3). We speculate that this is due to the concurrent increase in PVR, which the right ventricle has to pump against, and to a physical constraint (increasing intrathoracic pressure) placed on the heart by the overexpanded lung.
Pulse amplitude in these lambs returned to normal once PEEP was reduced, suggesting that the PBF history effect is not a function of continued reduced cardiovascular function. The increase in heart rate, critical in maintaining cardiac output, was also suppressed in ramp lambs compared with controls, further supporting the suggestion of depressed cardiac function in these lambs. The depressed cardiac function as a result of PEEP may incur a critical adverse effect in the preterm infant, particularly in the presence of an immature α-adrenergic response, which would hinder an increase in mean systolic filling pressure required to counteract the effects of PEEP; this area is poorly understood in the preterm model and warrants further attention.

The DA demonstrates large L-R blood flow in the prematurely delivered lamb, and this provides a significant proportion of the blood flow to the lungs immediately after delivery; similar large increases in systemic-to-pulmonary shunting were shown recently in preterm infants shortly after delivery (7, 21). We demonstrated high-velocity blood flow and turbulence in the LPA in the first 30–40 min of postnatal life due to increased DA flow into the lungs. The immediate L-R shunt through the DA at birth occurs due to the reduction in PVR and thus the pressure gradient from the systemic to pulmonary circulations. However, with progressive ductal constriction, the flow through the DA is limited, and this may account for some of the reduction in LPA flow over time.

More importantly, we found evidence in this study using Doppler assessment of ductal blood flow direction that the PEEP increments in the ramp lambs were associated with a relative increase in the proportion of R-L flow through the DA. This correlated with the observation of negative flow in the LPA waveforms. R-L flow in the DA also reduces LPA flow and may explain some of the reduced PBF seen in these lambs compared with controls. The increased amount of ductal R-L shunting in lambs exposed to higher PEEP settings may be explained by an increase in PVR secondary to increased airway pressure. Reduced blood flow to the lungs may also result in reduced left atrial filling and ultimately, a reduced left ventricular output. The observation that R-L shunting does not return to baseline as PEEP is decreased, and the increased severity in ramp animals suggests some longer lasting effect within the lung. We have suggested previously that this may be a function of interstitial edema from lung damage (18). Compression of pulmonary vessels by interstitial fluid accumulation, resulting in an increase in interstitial pressure and a reduction in the interstitial capillary wall transmural pressure, could result in an increase in PVR and a direct reduction in PBF.

In this study, we demonstrated the feasibility of obtaining Doppler measurement of both LPA flow and the direction of shunting in the DA. The correlation between Doppler-derived LPA flow and the flow derived from an indwelling ultrasonic flow probe was good, particularly when the mean calculated flow (both systolic and diastolic components) was used. However, when there was high-velocity turbulent flow entering the LPA due to L-R shunting through the DA, particularly in the first 30 min of life, the correlation was worse. These results support the validity of noninvasive Doppler ultrasound to estimate PBF in clinical practice. We also used Doppler to assess the direction of blood flow in the DA and demonstrated that this is a simple technique to show changes in the proportion of R-L flow in real time. The accurate ductal velocity and flow direction measurements may be used clinically as a proxy for lung injury or an overexpanded lung during ventilation, giving the clinician a useful tool to assess cardiopulmonary hemodynamic homeostasis in the first hours after preterm delivery.

Assessment of cardiac output and PBF provides important information about cardiorespiratory interactions. The relevance of reduced PBF, however, may be more important in relation to blood flow to other areas of the body, particularly the brain. Measurement of true systemic blood flow in the fetal or newborn at birth is complicated by the presence of
persisting fetal shunts through the DA and foramen ovale (4). Consequently, other methods of assessing true systemic blood flow, and in particular adequacy of blood flow to essential organs such as the brain, become important. We placed a flow probe on the SVC to document changes in the flow in this vessel as lambs transitioned from fetal to neonatal life, which were comparable with those obtained in human infants (13). SVC flow was not different between control and ramp lambs throughout the ventilation period and was extremely variable between lambs. There was, however, a significant reduction to systolic arterial pressure (measured in the carotid artery) in ramp lambs compared with controls. In human preterm infants, low SVC flow is strongly associated with subsequent periventricular/intraventricular hemorrhage (11) and impaired neurodevelopmental outcomes at 3 yr of age (9). Our observation of altered systemic pressure confirms the intricate and complex interaction between the pulmonary, cardiovascular, and cerebral circulations in the first hours after birth and how each needs to be considered to prevent possible short- and long-term complications.

Our study has several limitations. First, this is a complex instrumented model, and there may have been some variation in animals due to the anesthetic agent used and placement of flow probes. The model is of moderately immature animals that most likely have some degree of surfactant deficiency. We did not treat with surfactant as these lambs have only moderate surfactant deficiency. PEEP is an alternative management strategy for stabilizing alveoli in the presence of surfactant deficiency and was used in this protocol. We have shown previously that the application of surfactant in lambs of this gestation improves OI, which may explain, in part, the respiratory deterioration seen over time in control lambs. However, surfactant administration fails to prevent the deleterious effects of PEEP on PBF (1). The myocardial contractility was not measured, so there is no direct measure of afterload, and calculated vascular resistance is used as a measure of afterload.

In conclusion, this study highlights the complex but critically important interaction between the lungs and heart in the first hours of preterm life. The recruitment maneuver (ramp increase and decrease of PEEP) protected against the progressive deterioration in OI between the start and end of the study. However, at maximum PEEP of 10 cmH₂O, there was a trend toward a higher OI in the ramp group (indicative of worse arterial oxygenation) that was accompanied by a potentially deleterious fall in PBF. Further effects of maximum PEEP include increased R-L shunting through the DA, reduced heart rate, and increased right ventricular afterload that persisted for up until 20 min after maximum PEEP was reached. These findings suggest that, while ramp recruitment maneuvers with PEEP may have beneficial pulmonary effects, there is potential for acute pulmonary overdistention accompanied by impaired cardiovascular function. Doppler echocardiographic measures of LPA flow correlated strongly with measurements achieved from indwelling flow probes during laminar but not during high turbulent flow.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the wonderful assistance of JRL Hall & Co. for the supply and care of the sheep, in particular Ross Wales, Sarah Ritchie, and Fiona Hall, and the expert technical assistance of Gabby Musk.

GRANTS

This research was supported by the National Heart Foundation of Australia (NHFA), Women and Infants Research Foundation, North Shore Heart Research Foundation, a NHFA and National Health and Medical Research Council of Australia (NH&MRC) Fellowship (G. R. Polglase), a Sylvia and Charles Viertel Senior Medical Research Fellowship (J. J. Pillow), and NH&MRC Research Fellowships (S. B. Hooper and C. J. McLean).
REFERENCES


