Cardiovascular Research (2009) **81**, 713–722
doi:10.1093/cvr/cvn341

Effects of hypoxia-induced intrauterine growth restriction on cardiopulmonary structure and function during adulthood

Christian F. Rueda-Clausen[1,2,4], Jude S. Morton[3,4], and Sandra T. Davidge[1,2,3,4]*

[1]*Department of Physiology, University of Alberta, Edmonton, Canada;* [2]*Cardiovascular Research Group, University of Alberta, Edmonton, Canada;* [3]*Department of Obstetrics and Gynecology, 220 HMRC, University of Alberta, Edmonton, AB, Canada T6G 2S2; and* [4]*Women and Children's Health Research Institute (WCHRI), Edmonton, Canada*

Received 8 September 2008; revised 11 November 2008; accepted 4 December 2008; online publish-ahead-of-print 16 December 2008

Time for primary review: 34 days

KEYWORDS
Fetal programming;
Hypoxia;
Cardiac function;
Ageing;
Sex;
Echocardiography;
Pulmonary hypertension;
Diastolic dysfunction

Aims Intrauterine growth restriction (IUGR), a condition affecting 7–15% of all pregnancies, is associated with an increased mortality rate during adulthood. Several animal models have been developed to study the effects of IUGR during adulthood. However, the *in vivo* characteristics of these models are still unknown. The main aim of this work was to evaluate, *in vivo*, the effects of IUGR on cardiopulmonary structure and function during adulthood.
Methods and results Pregnant Sprague Dawley rats were exposed to hypoxic (12% O_2) or normoxic (21% O_2) environments between day 15 and 21 of pregnancy. Offspring were raised to 4 or 12 months old when a complete *in vivo* echocardiographic study was performed. In addition, *ex vivo* morphometry and isolated working heart experiments were performed. At birth, pups exposed to hypoxia had a smaller body weight and larger heart/body weight than controls. At 4 months of age, there were no significant differences between the groups. At 12 months of age, male but not female offspring exposed to prenatal hypoxia had smaller body weights and signs of left ventricular hypertrophy. In addition, both male and females animals exposed to prenatal hypoxia showed *in vivo* and *ex vivo* signs of left ventricular diastolic dysfunction and pulmonary hypertension by 12 months of age.
Conclusion Our study demonstrated that hypoxia-induced IUGR is associated with the development of chronic cardiopulmonary dysfunction during ageing. The implication of these findings is the potential usefulness of neonatal diagnosis as a predictor of cardiopulmonary outcomes during adulthood.

1. Introduction

Up to 15% of all pregnancies worldwide exhibit some degree of intrauterine growth restriction (IUGR); meaning that the fetuses do not reach their growth potential for a given gestational age and are consequently at a higher risk of perinatal and childhood morbidity and mortality.[1] Moreover, the health problems associated with IUGR continue on into adulthood.[2,3] Several studies have shown that IUGR is not only associated with the development of chronic conditions such as hypertension,[4] dyslipidaemia,[5] and diabetes mellitus,[6] but also with an increased mortality rate in adulthood.[7] Taken together, these studies provide support for the 'fetal programming' theory, which states that exposure to particular environmental conditions during certain stages of fetal development can result in permanent alterations predisposing the individual to chronic diseases later in life.[8]

Since cardiovascular diseases are still the main cause of death and disability in modern society, the study of the long-term effects of certain prenatal insults on cardiac structure and function has become increasingly relevant. Previous studies have shown that prenatal exposure to stress, nutritional deprivation and other conditions leading to IUGR can affect the fetal cardiovascular development through mechanisms that include a decrease in the total number of cardiomyocytes and impaired endothelial function; conditions that could predispose offspring to the occurrence of cardiovascular events later in life.[9,10]

The aetiology of IUGR is variable, however, in most instances fetal growth is ultimately constrained by a limitation of oxygen and nutrient delivery.[11] Human fetuses have several mechanisms to compensate for an acute nutritional restriction[12] while they have limited reserves to compensate for oxygen insufficiencies. In fact, conditions associated with fetal hypoxia, such as living at high altitude, anaemia, pulmonary diseases, pre-eclampsia, and smoking, have also been associated with IUGR.[13,14] Using an animal

* Corresponding author. Tel: +1 780 492 1864; fax: +1 780 492 1308.
E-mail address: sandra.davidge@ualberta.ca

model of hypoxia-induced IUGR, our group has previously shown (*ex vivo*) that IUGR rat offspring have several cardiac structural and functional changes during adulthood. These include increased expression of collagen type I and III fibers, altered ß/α myosin heavy chains ratio (ß/αMHC), increased susceptibility to ischaemia reperfusion insult, and decreased expression of matrix metalloproteinase 2 (MMP-2).[15] However, no studies have been performed to determine whether these changes observed *ex vivo* have any effects on cardiac function *in vivo*. In order to address this question, the main objective of this study was to evaluate, *in vivo*, the cardiopulmonary structure and function of IUGR offspring. Additionally, it has been suggested that exposure to adverse environments during fetal stages may accelerate the development of chronic diseases during adulthood by affecting the response of different tissues to the normal ageing process (reviewed in Nilsson *et al.*[16]). Thus, one of the secondary objectives of this study was to evaluate the interaction of these two factors (exposure to hypoxia and ageing) by studying both young adult and aged animals previously exposed to prenatal hypoxia. Moreover, and despite the well-described differences between males and females in the pathophysiology of chronic cardiovascular diseases,[17,18] most studies of cardiac function in this field have been conducted in male animals and potential sex differences in this condition remain to be explored. Therefore, we conducted experiments in both male and female offspring.

2. Methods

2.1 Animals

Female Sprague Dawley rats were obtained at 3 months of age (Charles River, Quebec, Canada), acclimatized and then mated within the animal facility. A vaginal smear obtained the following morning was examined for the presence of sperm, which we signified as day 0 of pregnancy (term ≈21 days). Throughout pregnancy, rats were housed in standard rat cages with *ad libitum* access to water and food (standard lab rat chow) and were weighed daily. On day 15 of pregnancy, rats were randomized to control ($n = 16$) or maternal hypoxia ($n = 16$) and treated as previously described in detail.[15] Briefly, rats assigned to the maternal hypoxia group were placed inside a Plexiglas chamber continuously infused with nitrogen to maintain an oxygen concentration of 12% during the last 7 days of pregnancy. Just before birth, dams were returned to normal housing conditions (21% oxygen). At the time of birth, the litter was reduced to eight pups (four males and four females) in order to control the post-natal environment. Pups and dams were then weighed bi-weekly until weaning at week 3 and then on the experimental day at 4 or 12 months of age when they were considered to be young adults and aged adults, respectively. All animals were housed in standard rat cages with 60% humidity, a 12 h:12 h light:dark cycle and *ad libitum* access to water and food (standard lab rat chow) in the animal facilities of the University of Alberta. All procedures in this study were approved by the University of Alberta Animal Welfare Committee, and are in accordance with the guidelines of the Canadian Council on Animal Care and the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health.

2.2 Echocardiographic evaluation

At 4 or 12 months of age, rats were anaesthetized with sodium thiopental 50 mg/kg i.p. and placed in a supine position on a controlled heating pad. The chest and abdomen were shaved and the extremities were gently fixed to the electrodes in the pad surface using tape and a highly conductive electrode gel. A single channel electrocardiogram signal and respiratory rate were continuously recorded on the imaging system.

Body temperature was monitored by a rectal probe. Echocardiography evaluations were performed using a high-resolution *in vivo* micro-imaging system Vevo-770 (Visualsonics®) with a real-time micro visualization scan head of 17.5 MHz and following the guidelines of the American Society of Echocardiography.[19] Images were analysed using the Vevo 770 Protocol-Based Measurements and Calculations, Rev1.2, as previously described by other authors using rodents.[20] M-mode two-dimensional echocardiography images were obtained in the parasternal long- and short-axis. The dimensions of the left ventricle (LV) diameter and the thickness of its walls were made using a left parasternal short-axis view of the heart with the M-mode beam positioned just beyond the mitral leaflet tips, perpendicular to the long-axis of the ventricle and centered in the short-axis. The LV posterior wall endocardium was identified on the M-mode image as the most intense continuous line with the steepest systolic motion. The posterior wall epicardium was identified as the echo reflection immediately anterior to the posterior pericardium. The septal endocardium was identified as the steepest motion in systole with a continuous reflection through the cycle. The LV volumes were estimated using the 'cubed' formula, which assumes that the long-axis of the LV is equal to twice the short-axis diameter (D). The volume (V) of the LV at any given moment can then be approximated from a single short-axis measurement as $V = D$.[3] Since there are no formulae to estimate the ventricular mass in rodents based on echocardiographic images, the following formula for humans was used. $LV_{mass} = 1.04 \times [(LVIDd + PW + IVS)^3 - LVIDd^3] \times 0.8$, where LV =

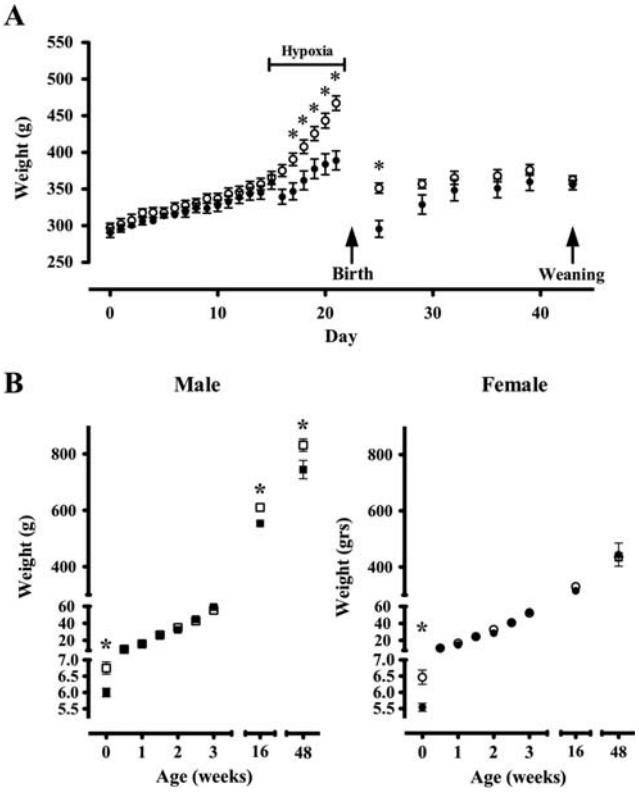


Figure 1 Effect of prenatal exposure to hypoxia on body weight change in the dams during pregnancy (*A*) and pups (*B*). Open signs represent controls and filled signs represent animals exposed to hypoxia. *$P < 0.01$ when compared with age-matched controls. (*A*) Includes the weights of 16 dams exposed to hypoxia and 16 controls, (*B*) includes data obtained from at least 37 animals (from at least 9 different litters) in each group.

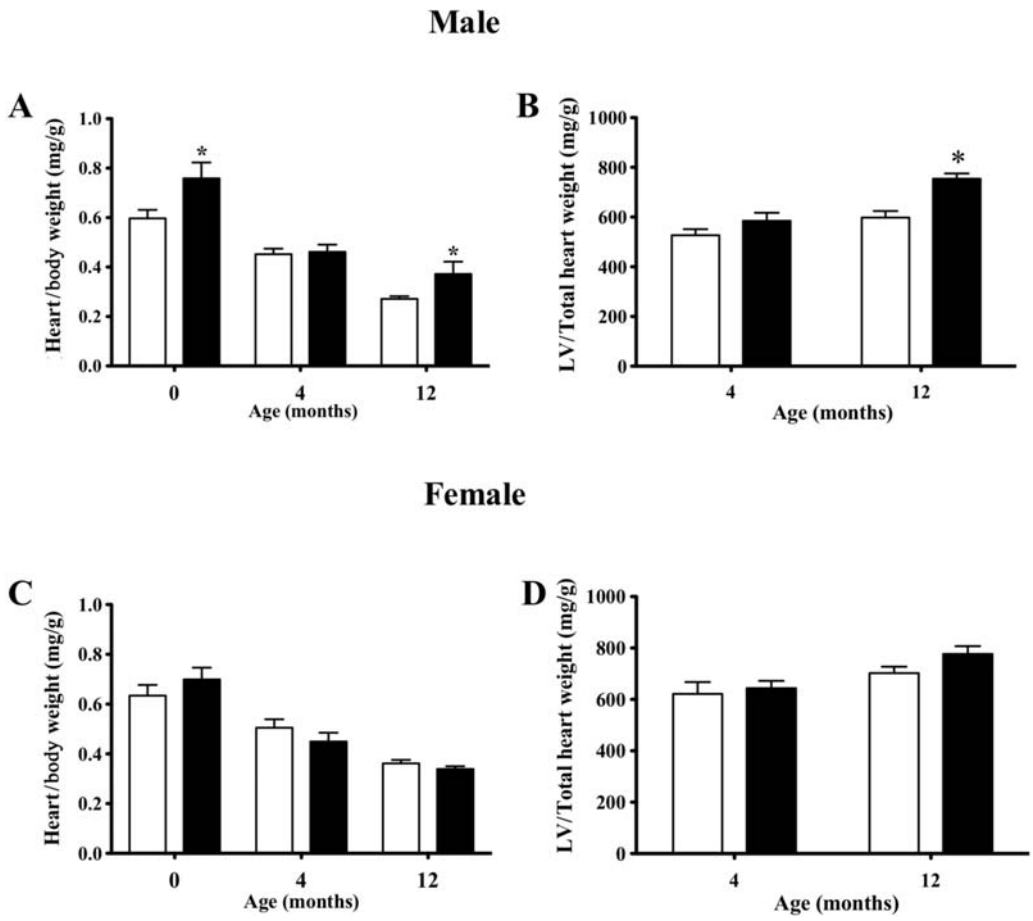


Figure 2 Effect of prenatal exposure to hypoxia on heart/body weight ratio (*A* and *C*) and left ventricle (LV)/total heart weight (*B* and *D*) in male (*A* and *B*) and female (*C* and *D*) offspring from dams exposed to hypoxia during pregnancy (solid bars) and controls (open bars). Heart/body weight at 0 months of age refers to data obtained from pups that were culled on the day of birth. $^*P < 0.01$ when compared with age- and sex-matched controls. Left ventricular mass used to estimate the LV/total heart weight ratio was calculated by echocardiography.

left ventricle, LVIDd = LV internal diameter in diastole, IVS = interventricular septum thickness.

Aortic root and left atrium diameters were obtained by M-mode with the scan head on the long-axis and the beam parallel to the axis of the aortic valve. LV systolic function was evaluated by estimating ejection fraction, shortening fraction and cardiac output from images obtained on M-mode of the LV short-axis. Ventricular diastolic function was assessed by describing the transmitral Doppler signal. *E* and *A* wave velocities and gradients were measured in a modified parasternal long-axis with the beam placed on the tip of the mitral leaflet. *E*/*A* index, mitral deceleration time, and myocardial performance index (Tei index)[21] were calculated. Pulmonary artery Doppler was measured in parasternal long-axis orientation after B-mode identification of the pulmonary artery lateral to the aortic root. Aortic Doppler was measured in a cephalic anterior long-axis view of the aorta immediately after the aortic valve.

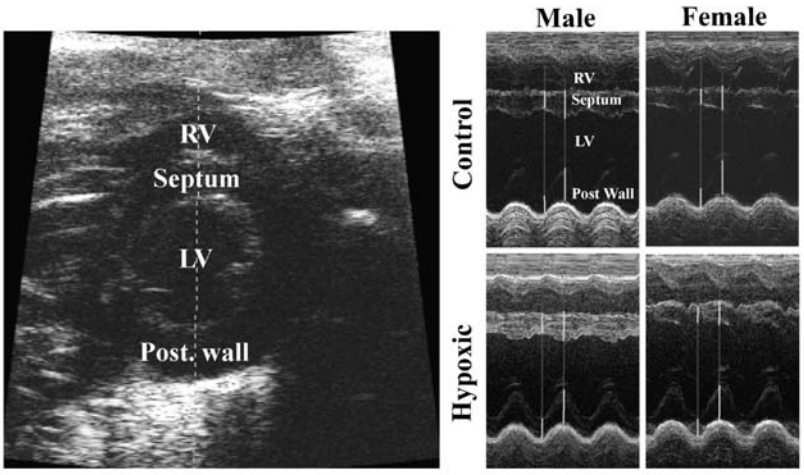
High-resolution echocardiography has proven to be an effective tool for the *in vivo* study of cardiovascular function and structure in small rodents.[22,23] However, being an operator-dependent technique it is inappropriate to make any firm conclusions before complementing the results with a non-echocardiographic method. Therefore, we confirmed our findings using non-echocardiographic approaches such as standard *ex vivo* morphometry, isolated working heart, and conventional histology.

2.3 Pulmonary histology and morphometry

A separate set of animals were euthanized with a lethal ip injection of sodium pentobarbital. Following the induction of a pneumothorax, lungs were perfused and fixed in 10% formalin. Histopathological preparations and haematoxylin and eosin staining were performed at the Alberta Diabetes Institute Histology Core (Edmonton, Canada). Small pulmonary arterioles (diameter 30–110 μm) were identified from five cross-sectional regions at the right lobe of the lung, per cent wall thickness of arteries was calculated using the following formula: Per cent wall thickness (%) = ((cross-sectional diameter − luminal diameter)/cross-sectional diameter) × 100 and a mean value was calculated for each animal.

2.4 Isolated working heart preparation

In a separate set of animals, hearts were excised from anaesthetized rats and perfused for 10 min in retrograde Langendorff mode; with Krebs-Henseleit prepared as reported previously,[15] gassed with 95% O_2 and 5% CO_2 against a constant perfusion pressure of 60 mmHg. Hearts were switched to anterograde working perfusion mode and perfused in a closed recirculating system at 37°C with 120 mL of modified Krebs-Henseleit solution prepared as previously described.[15] Buffer entered the cannulated left atrium at 11.5 mmHg, and passed to the LV from which it was spontaneously ejected through the aortic cannula against a pressure of 80 mmHg. LV pressure was continuously recorded using a 1.4 Fr micromanometer (Millar instruments, Houston, TX, USA) inserted through the left atrium. After 10 min of equilibration, hearts were paced at 300 beats/min (4-month-old) or 270 beats/min (12 month old) animals. Heart rate, preload pressure, aortic systolic and diastolic pressures, LV pressure, and the maximal first derivative of the pressure over time (dP/dt_{max}) were recorded using HSE Isoheart software for windows 2000 (Harvard Apparatus



	4 Months		12 Months		2 way ANOVA		
Male offspring	Control ($n = 14$)	Hypoxia ($n = 12$)	Control ($n = 13$)	Hypoxia ($n = 10$)	Int.	Hyp.	Age
RV diameter in diastole (mm)	2.3 (0.16)	2.6 (0.21)	2.2 (0.27)	3.2 (0.25)†		*	
End-diastolic septal thickness (mm)	1.8 (0.08)	1.7 (0.11)	2 (0.04)	2.2 (0.11)			
End-systolic septal thickness (mm)	2.42 (0.10)	2.36 (0.12)	2.53 (0.14)	3.13 (0.15)†		*	*
LV end-diastolic internal diameter (mm)	7.7 (0.29)	7.8 (0.25)	7.9 (0.5)	8.1 (0.48)			
LV end-systolic internal diameter (mm)	4.11 (0.17)	3.85 (0.18)	4.27 (0.17)	3.99 (0.16)		*	
LV end-diastolic posterior wall thickness (mm)	2.1 (0.07)	2.4 (0.18)	2.4 (0.08)	2.7 (0.05)			*
LV end-systolic posterior wall thickness (mm)	3.1 (0.07)	3.5 (0.15)	3.7 (0.11)	4.5 (0.2)†			*
M-mode trace LV end-diastolic vol. (μL)	316 (16)	355 (30)	352 (19)	368 (23)			*
Female offspring	($n = 10$)	($n = 11$)	($n = 9$)	($n = 9$)			
RV diameter in diastole (mm)	1.9 (0.14)	2.0 (0.21)	2.2 (0.24)	2.5 (0.3)			
End-diastolic septal thickness (mm)	1.6 (0.12)	1.5 (0.12)	1.7 (0.08)	1.7 (0.15)			
End-systolic septal thickness (mm)	2.12 (0.12)	2.31 (0.05)	2.45 (0.11)	2.49 (0.13)			
LV end-diastolic internal diameter (mm)	6.8 (0.16)	6.9 (0.27)	7.4 (0.28)	7.3 (0.26)			
LV end-systolic internal diameter (mm)	3.25 (0.16)	3.03 (0.2)	3.61 (0.3)	3.32 (0.18)			
LV end-diastolic posterior wall thickness (mm)	1.9 (0.21)	2.0 (0.07)	2.4 (0.09)	2.6 (0.08)			*
LV end-systolic posterior wall thickness (mm)	2.8 (0.11)	2.7 (0.12)	3.5 (0.12)	3.9 (0.2)			*
M-mode trace LV end-diastolic vol. (μL)	224 (14)	233 (23)	246 (30)	282 (27)			*

Figure 3 Representative images of short-axis biventricular M-mode study at 12 months of age and a summary of the most relevant cardiac measurements obtained at 4 and 12 months of age using echocardiography. LV, left ventricle; Post wall, LV posterior wall; RV, right ventricle; vol, volume. †$P < 0.01$ when compared with age- and sex-matched controls using a Bonferroni *post*-test, *$P < 0.01$ when effect of hypoxia (Hyp.), ageing (Age) or their interaction (Int.) was evaluated by two-way ANOVA.

Canada, Saint-Laurent, Quebec, Canada). Cardiac output and aortic flow were measured using an in-line flow sensor (Transonic Systems, NY, USA). Cardiac function and coronary flows were calculated as previously described.[24,25] Measurements of cardiac function were carried out every 10 min during a 30 min period and averages of these measurements were calculated for each animal. Hearts that showed non-reversible cardiac arrhythmia were excluded. After perfusion, hearts were placed in dry gauze to remove excess water and weighed after all sutures and excess vascular tissues were removed.

2.5 Statistical analysis

Data are presented as means ± SEM, except where stated otherwise. A repeated measurements (mixed model) two-way analysis of variance (ANOVA) and Bonferroni *post*-test were performed to compare the impact of hypoxia on both dam weight during pregnancy and offspring weight change with time. Due to the marked phenotypical differences between sexes data obtained from male and female offspring were analysed separately. Differences in the measurements

performed among the groups were tested using two-way ANOVA with age and prenatal exposure to hypoxia as sources of variation. A Bonferroni *post*-test was then used to compare replicate means by groups. In order to decrease the multiple comparisons effect, $P < 0.01$ was considered statistically significant.

3. Results

3.1 Effect of hypoxia on maternal and fetal weight

There were no differences in the baseline characteristics of dams before the hypoxic insult. Hypoxia reduced maternal weight gain and the weight of pups at birth as shown in *Figure 1A* and *B*, respectively. The litter size (Controls 16 $\pm$ 2 vs. Hypoxic 15 $\pm$ 2 pups, $P = 0.54$), proportion of stillborn (Controls 0.6% vs. Hypoxic 1.7%, $P = 0.3$), and sex distribution (proportion of male offspring: Controls 53% vs. Hypoxic 45%, $P = 0.95$) from each litter were comparable between the groups. Newborns exposed to hypoxia *in utero* rapidly caught up with controls and a few days after birth there were no differences in body weight (*Figure 1B*). Male but not female offspring exposed to hypoxia during fetal development had a lower body weight than their respective controls at both 4 and 12 months of age (*Figure 1B*).

3.2 Effects of hypoxia-induced intrauterine growth restriction on left ventricle morphometry and function

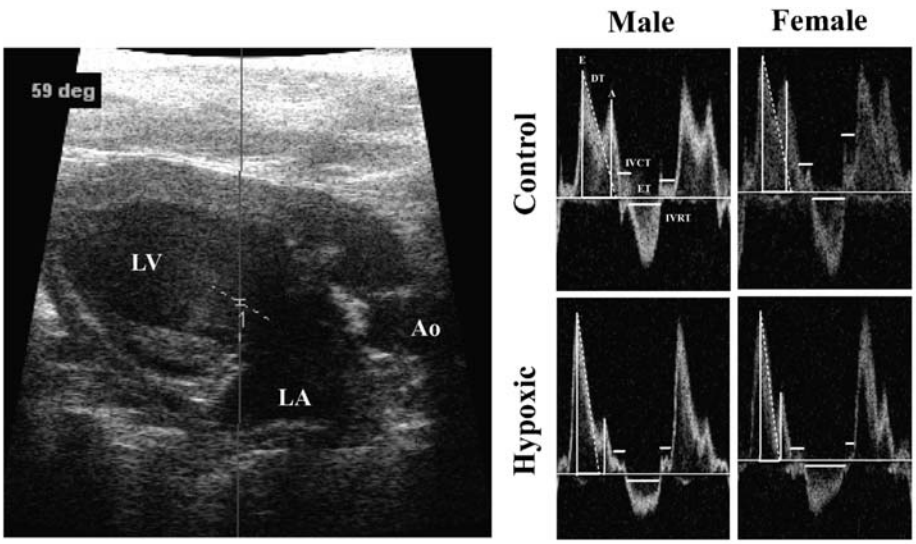
After adjusting for body weight, male but not female offspring had larger hearts than controls at birth (data obtained from culled pups are presented in *Figure 2A* and *C*). However, at 4 months of age, these differences were not observed and the heart/body weight ratio was comparable between the groups in both male and female offspring (*Figure 2A* and *C*, respectively). At 12 months of age, male (*Figure 2A*) but not female (*Figure 2C*) offspring exposed to prenatal hypoxia had a greater heart/body weight ratio than their respective controls. Since there were differences in the body weight of the male animals from the prenatal hypoxia group, the changes in LV mass are depicted after adjusting for the absolute heart weight (*Figure 2B* and *D*). Only aged (12-month-old) male animals exposed to hypoxia before birth had a higher relative ventricular weight when compared with their respective controls. Consistent with this finding, only aged male animals exposed to hypoxia had an augmented septal and posterior wall thickness *in vivo* when compared with controls (*Figure 3*).

At 4 months of age, both male and female offspring exhibited no changes in aortic diameter, aortic flow, or ventricular systolic function (*Table 1*). However, 12-month-old offspring from both sexes exposed to hypoxia during fetal stages presented with echocardiographic signs of LV diastolic dysfunction, including decreased mitral A-wave maximum velocity, isovolumetric relaxation time, and deceleration time of the mitral valve as well as increased E/A and Tei indexes (*Figure 4*). In addition, heart rate in aged males and females was significantly reduced compared with young animals ($P < 0.01$, *Table 1*). However, IUGR animals had no change in heart rate compared with controls. In order to

Table 1 Echocardiographic measurements obtained from male and female offspring

Male offspring	4 Months		12 Months		Two-way ANOVA		
	Control ($n = 14$)	Hypoxia ($n = 12$)	Control ($n = 13$)	Hypoxia ($n = 10$)	Int.	Hyp.	Age.
HR (bpm)	349 (10)	340 (11)	307 (7)	291 (9)		*	*
Parasternal short-axis							
CO/body weight (mL/min/kg)	140 (7)	150 (10)	160 (22)	150 (26)			
LV shortening fraction (%)	43 (1)	45 (1.4)	46 (1.3)	48 (1.7)			
LV ejection fraction (%)	71 (1.2)	74 (1.5)	73 (1.4)	78 (1.7)			
Parasternal long-axis apical							
Ao outflow vel./time integral (mmHg)	8.4 (0.9)	9.6 (1.1)	7.7 (0.7)	7.9 (1.6)			
Ao peak ejection vel. (cm/s)	1300 (110)	1277 (200)	1176 (116)	1077 (260)			
Ao ejection time (ms)	71 (2.5)	74 (3.7)	79 (4)	74 (5)			
Ao end-diastolic diameter (mm)	3.2 (0.1)	3.1 (0.1)	3.5 (0.1)	3.6 (0.1)			*
Ao end-systolic diameter (mm)	3.5 (0.08)	3.4 (0.14)	3.8 (0.2)	4 (0.1)			*
Female offspring	($n = 12$)	($n = 13$)	($n = 10$)	($n = 10$)			
HR (bpm)	338 (14)	325 (14)	290 (14)	280 (6)			*
Parasternal short-axis							
CO/body weight (mL/min/Kg)	200 (20)	211 (12)	172 (20)	186 (26)			*
LV shortening fraction (%)	46 (1.6)	43 (2)	47 (1.6)	47 (2.2)			
LV ejection fraction (%)	79 (2.6)	73 (2.2)	78 (2.1)	77 (2.3)			
Parasternal long-axis apical							
Ao outflow vel./time integral (mmHg)	8.3 (1)	9.1 (1.7)	7.5 (1.3)	8 (1.3)			*
Ao peak ejection vel. (cm/s)	1549 (162)	1593 (294)	1098 (117)	1197 (83)			*
Ao ejection time (ms)	77 (5)	78 (4)	81 (3)	82 (2.4)			
Ao end-diastolic diameter (mm)	3.1 (0.3)	2.8 (0.2)	3.3 (0.1)	3.4 (0.1)			
Ao end-systolic diameter (mm)	3.3 (0.33)	3 (0.08)	3.3 (0.1)	3.6 (0.2)			

Data presented as mean (SEM). HR, heart rate; CO, cardiac output; LV, left ventricle; Ao, aorta; vel, velocity. *$P < 0.01$ when effect of hypoxia (Hyp.), ageing (Age) or their interaction (Int.) was evaluated by two-way ANOVA.



	4 Months		12 Months		2 way ANOVA		
Male offspring	Control ($n=11$)	Hypoxia ($n=10$)	Control ($n=10$)	Hypoxia ($n=9$)	Int.	Hyp.	Age
Mitral E maximum vel. (mm/s)	1160 (257)	1275 (206)	1327 (130)	1309 (116)			
Mitral A maximum vel. (mm/s)	688 (94)	697 (98)	911 (73)	573 (58)†			
Mitral E/A index	1.8 (0.1)	1.8 (0.2)	1.4 (0.1)	2.2 (0.18)†	*	*	
Mitral deceleration time (ms)	49.3 (3.5)	45.6 (5.9)	41.2 (5.2)	32.1 (4.1)†		*	*
Myocardial performance (Tei) index	0.37 (0.04)	0.37 (0.03)	0.33 (0.03)	0.48 (0.04)†			
Mitral IVRT (ms)	17.53 (2.06)	14.35 (3.56)	16.32 (2.36)	19.56 (5.20)†		*	*
Mitral IVCT (ms)	13.56 (3.56)	12.25 (2.17)	9.98 (2.47)	10.11 (4.44)			*
Female offspring	($n=10$)	($n=11$)	($n=9$)	($n=9$)			
Mitral E maximum vel. (mm/s)	1319 (244)	1307 (148)	1481 (250)	1252 (198)			
Mitral A maximum vel. (mm/s)	696 (95)	796 (76)	945 (102)	670 (75)†			
Mitral E/A index	1.9 (0.2)	1.7 (0.2)	1.6 (0.1)	2.1 (0.1)†			
Mitral deceleration time (ms)	49.2 (3.5)	45 (5.2)	41.7 (4.1)	32.6 (3.9)†			*
Myocardial performance (Tei) index	0.33 (0.04)	0.37 (0.04)	0.31 (0.03)	0.43 (0.03)†		*	
Mitral IVRT (ms)	13.8 (2.35)	14.65 (3.25)	11.24 (2.36)	17.36 (3.15)†		*	
Mitral IVCT (ms)	11.26 (3.39)	13.25 (4.23)	10.02 (3.69)	12.4 (3.78)			

Figure 4 Representative images of transmitral Doppler signal at 12 months of age and a summary of the most relevant cardiac measurements obtained at 4 and 12 months of age using these images. LV, left ventricle; Ao, aorta; LA, left atrium; E, mitral E wave; A, mitral A wave; DT, mitral deceleration time; ET; LV ejection time; IVRT, isovolumetric relaxation time; IVCT, isovolumetric constriction time. $^{\dagger}P < 0.01$ when compared with age- and sex-matched controls using a Bonferroni *post*-test, $^{*}P < 0.01$ when effect of hypoxia (Hyp.), ageing (Age) or their interaction (Int.) was evaluated by two-way ANOVA.

complement these echocardiographic findings, cardiac hemodynamic parameters were evaluated during *ex vivo* heart perfusion experiments. The primary significant difference observed in the baseline function of hearts obtained from IUGR animals was an increased LV end-diastolic pressure (*Table 2*). This difference was significant in both male and female animals at 12 months of age.

3.3 Hypoxia-induced intrauterine growth restriction and pulmonary hypertension

At 12 months of age, both male and female animals exposed to hypoxia exhibited echocardiographic signs of pulmonary hypertension, including decreased pulmonary artery acceleration time (*Figure 5*) and increased right ventricle

Table 2 Working heart baseline perfusion data

Male offspring	4 months		12 months		Two-way ANOVA		
	Control ($n = 4$)	Hypoxia ($n = 4$)	Control ($n = 5$)	Hypoxia ($n = 4$)	Int.	Hyp.	Age
Paced HR (bpm)	304 (6)	301 (5)	275 (6)	279 (4)			*
SBP (mmHg)	95.2 (1.7)	99.2 (3.3)	99.1 (2.2)	103.1 (3.1)			
DBP (mmHg)	77.8 (0.5)	79.2 (0.12)	71.8 (2.9)	72.9 (3.04)			*
Cardiac work (mmHg/mL/min/g)10^{-2}	1086 (157)	1193 (294)	950 (152)	1035 (77)			
LV dP/dt_{max} (mmHg/s)	4704 (676)	5240 (640)	4653 (707)	4283 (257)			
LVEDP (mmHg)	3.6 (0.98)	6.1 (1.2)	6.55 (1.78)	11.79 (1.14)†		*	*
Female offspring	($n = 4$)	($n = 4$)	($n = 4$)	($n = 6$)			
Paced HR (bpm)	303 (9)	300 (6)	271 (11)	276 (4)			*
SBP (mmHg)	90.1 (0.5)	85.3 (2.4)	85.9 (6.5)	92.1 (4.7)			
DBP (mmHg)	80.3 (0.6)	75.3 (2.4)	68.5 (4.7)	71.6 (3.1)			*
Cardiac work (mmHg/mL/min/g)10^{-2}	672.9 (102)	523 (66)	576 (53)	690 (124)			
LV dP/dt_{max} (mmHg/s)	3692 (579)	3141 (451)	4466 (279)	4140 (574)		*	*
LVEDP (mmHg)	4.3 (0.81)	5.58 (0.69)	7.58 (0.71)	13.24 (1.2)†		*	*

Data presented as mean (SEM). HR, heart rate; SBP, mean systolic blood pressure; DBP, mean diastolic blood pressure; LV dP/dt_{max}, the maximum first derivative of the LV pressure over time; LVEDP, LV end-diastolic pressure. †$P < 0.01$ when compared with age- and sex-matched controls using a Bonferroni *post*-test, ***P** < 0.01 when effect of hypoxia (Hyp.), ageing (Age) or its interaction (Int.) was evaluated by two-way ANOVA.

diameter in diastole (*Figure 3*) when compared with age- and sex-matched controls. In addition, histological studies of the lungs showed that the media thickness of small pulmonary arteries was increased in aged animals with a prenatal exposure to hypoxia when compared with controls (*Figure 6*).

4. Discussion

Our study provides the first *in vivo* description of the long-term effects of hypoxia-induced IUGR on cardiopulmonary function in young adult and aged offspring. These data further support that the previously observed microstructural and enzymatic changes in myocardial tissue evaluated *ex vivo* using this animal model also result in impaired cardiac function *in vivo*. Moreover, it is the first study to show that a prenatal hypoxic insult can induce the spontaneous development of LV diastolic dysfunction and pulmonary hypertension as the animals age.

Despite the changes in LV diastolic function observed in hypoxic animals, the LV systolic function (evaluated by ejection fraction, shortening fraction, and cardiac output) remained comparable to that of controls at 4 and 12 months of age. The presence of abnormalities in mechanical function during diastole that do not compromise the ejection fraction is also known as isolated LV diastolic dysfunction[26] and in this particular animal model could be explained by the changes in collagen deposition, impaired ß/αMHC, and decreased expression of MMP-2 previously described.[15]

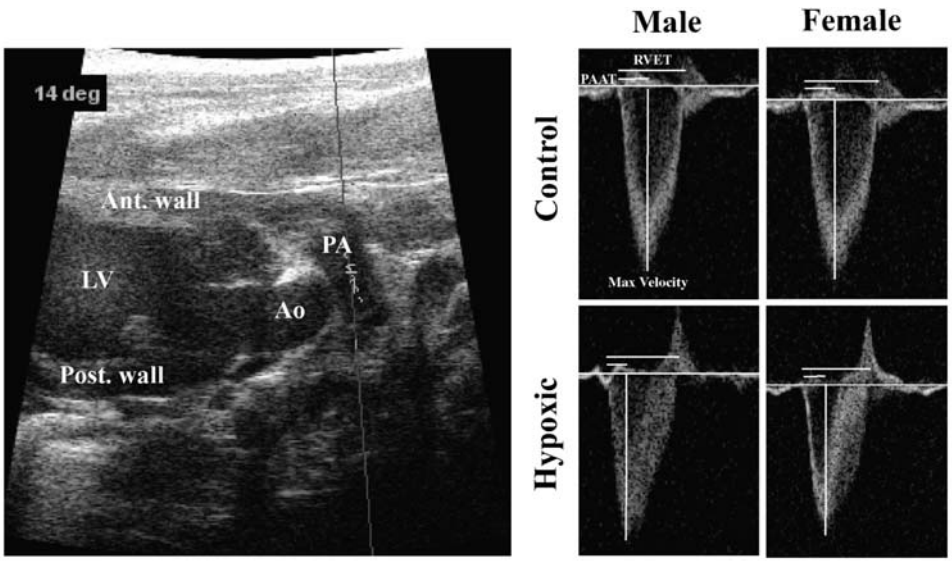
We also found that aged animals had a slight and consistent decrease in heart rate when compared with young adults, which is consistent with previous animal studies showing a reduction in heart rate associated with ageing in both males and females.[27] Interestingly, IUGR animals had no changes in heart rate when compared with their respective controls.

Another adverse finding in the aged offspring prenatally exposed to hypoxia was pulmonary hypertension. Low birth weight and conditions associated with low oxygen availability during fetal development have been extensively associated with acute respiratory distress, bronchopulmonary dysplasia, and persistent pulmonary hypertension of the newborn (reviewed in Tuder *et al.*[28]). However, to the best of our knowledge, this is the first study showing an association between hypoxia-induced IUGR and the development of primary pulmonary hypertension in aged rats. Moreover, in our model, pregnant dams were always placed in normal oxygen conditions before birth. Therefore, the chronic effects of this hypoxic insult during fetal development cannot be attributed to newborn exposure to a hypoxic environment during the early stages of life. Additionally, it is important to mention that our animal model of IUGR is not associated with prematurity; indeed, pregnant dams exposed to hypoxia experience a delay of around 6–12 h in the expected delivery time compared with controls.

In our study, signs of both LV diastolic dysfunction and pulmonary hypertension were evident at 12 but not 4 months of age, suggesting that in this particular model, the ageing process may act as a stressor that is less well tolerated by animals prenatally exposed to hypoxia. Additionally, none of the echocardiographic or direct *ex vivo* examinations of the hearts from adult animals prenatally exposed to hypoxia exhibited any kind of malformation. Therefore, the observed effects of prenatal hypoxia on cardiac function cannot be attributed to teratogenic effects.[29]

Since the animals were anaesthetized during the echocardiographic studies, the potential acute effects of anaesthetics on cardiovascular function need to be considered. It has been described that thiopental has a direct negative inotropic effect on rats,[30] and that at high dosage can cause a temporal drop in cardiac output.[31] However, these described acute effects of thiopental would not likely be related to the changes in ventricular morphometry, ventricular diastolic function, or pulmonary hypertension described in the IUGR animals.

Findings in male and female animals were consistent in terms of the development of LV diastolic dysfunction and



	4 Months		12 Months		2 way ANOVA		
Male offspring	Control (n=14)	Hypoxia (n=12)	Control (n=13)	Hypoxia (n=10)	Int.	Hyp.	Age
PA vel./time integral (mmHg)	4.54 (0.18)	4.95 (0.3)	4.42 (0.17)	4.88 (0.17)			
PA maximum vel. (mm/s)	848 (19)	853 (24)	771 (28)	820 (38)			
RV ejection time (RVET) (ms)	84 (3)	85 (2.1)	89 (3)	94 (3.3)			*
PA acceleration time (PAAT) (ms)	37 (2.3)	36 (2.1)	38 (2.3)	28 (2.6)†		*	
PAAT/HR	0.111 (0.007)	0.128 (0.01)	0.12 (0.009)	0.089 (0.008)†			
(PAAT/RVET)	0.43 (0.01)	0.42 (0.02)	0.43 (0.02)	0.35 (0.02)†			
Female offspring	(n=12)	(n=13)	(n=10)	(n=10)			
PA vel./time integral (mmHg)	4.43 (0.24)	4.83 (0.26)	4.15 (0.2)	4.75 (0.2)		*	
PA maximum vel. (mm/s)	806 (29)	839 (40)	705 (43)	862 (37)			
RV ejection time (RVET) (ms)	81 (4)	86 (2.8)	85 (3.8)	88 (3)			
PA acceleration time (PAAT) (ms)	31 (3.1)	31 (2.9)	38 (2.1)	30 (1.9)		*	
PAAT/HR	0.10 (0.01)	0.11 (0.01)	0.13 (0.01)	0.11 (0.006)†	*		
(PAAT/RVET)	0.40 (0.03)	0.39 (0.02)	0.44 (0.02)	0.34 (0.02)			

Figure 5 Representative images of pulmonary artery echocardiographic findings at 12 months of age and a summary of the most relevant cardiac measurements obtained at 4 and 12 months of age using these images. LV, left ventricle; Ant wall, LV anterior wall; Ao, aorta; Pa, pulmonary artery; Post wall, LV posterior wall; RV, right ventricle; PAAT, pulmonary artery acceleration time; RVET, right ventricle ejection time. †$P < 0.01$ when compared with age- and sex-matched controls using a Bonferroni *post*-test, *$P < 0.01$ when effect of hypoxia (Hyp.), ageing (Age) or their interaction (Int.) was evaluated by two-way ANOVA.

pulmonary hypertension, however, male but not female offspring exposed to hypoxia had greater ventricular mass at 12 months of age when compared with their sex-matched controls. This suggests that the female sex may confer some form of protection against this particular long-term effect of IUGR. Consistent with this finding, *ex vivo* vascular studies previously conducted by our group described that male but not female offspring exposed to hypoxia during fetal development expressed changes in vascular myogenic tone during adulthood.[32] Given that antecedent, one potential mechanism that could be related to the development of LV hypertrophy in male aged animals exposed to hypoxia is higher blood pressure levels. Interestingly, previous results obtained using the tail-cuff technique in the same animal model suggest that the hypoxic insult has no effect on the blood pressure of adult male offspring.[15] Nonetheless, further studies specifically addressing effects on blood pressure regulation are necessary and are currently ongoing.

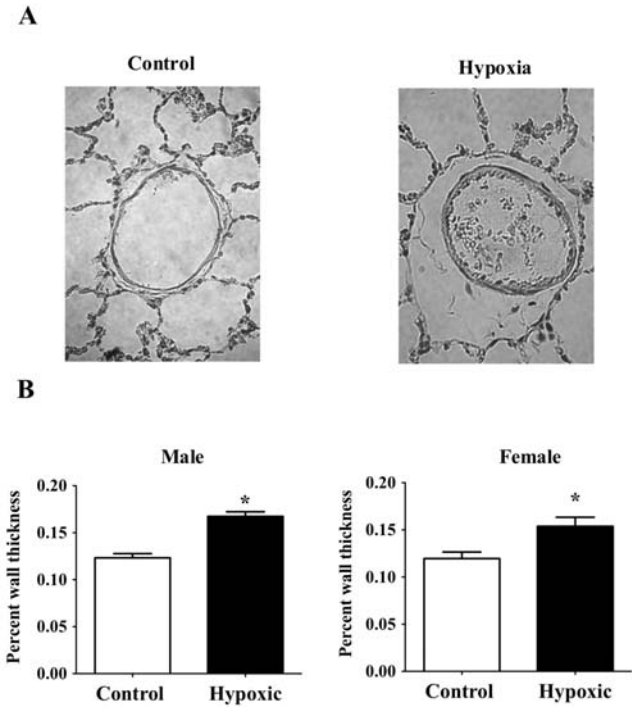


Figure 6 (*A*) Shows representative images of histological preparations of lungs from aged (12 month old) male offspring exposed to hypoxia and normal conditions during fetal development. (*B*) Summarizes information of arterial media thickness in the lung of 12 months old animals ($n \geq 3$ for each group). *$P < 0.01$ when compared with controls.

Before interpreting and extrapolating the results obtained in this animal model of IUGR to human situations there are species differences that need to be considered. For instance, the pulmonary development of the rat differs from humans. In fact, the degree of pulmonary development in a term rat is comparable to that observed in a 32-week-old human fetus. Therefore, it is plausible that maternal exposure to hypoxia during certain stages of fetal development would have a different effect in rats and humans. Another limitation of this model is in the fact that both dam and pups were exposed to hypoxia during pregnancy, which mimics conditions such as high altitude pregnancy or smoking during pregnancy but has some limitations emulating conditions like placental insufficiency or pre-eclampsia in which the fetus but not the mother has restricted oxygen availability. Additionally, it has to be mentioned that there are other anatomical and functional considerations regarding species differences in the cardiac structure and function that have to be made when interpreting these results. In healthy young rats and mice, for instance, the E/A index of the mitral valve is <1, while in healthy humans, this parameter is expected to be between 1.2 and 1.5 and values <1 are considered to be a diagnostic criteria for severe LV diastolic dysfunction. Despite these differences, the *ex vivo* confirmation of echocardiographic results supports the validity of the echocardiographic findings and their interpretation in the context of this animal model.

In conclusion, the results of the present study demonstrate that prenatal exposure to hypoxia has important deleterious consequences on the cardiopulmonary function of the offspring later in life, causing LV diastolic dysfunction, pulmonary hypertension, and an increased LV mass. Being female seems to confer some degree of protection against the development of LV hypertrophy but not against any of the other cardiopulmonary findings associated with hypoxia-induced IUGR. The mechanisms involved in this long-term programming phenomenon still need to be determined. However, the relevant implication of these findings in a clinical perspective is the potential usefulness of perinatal diagnosis as a predictor of cardiopulmonary outcomes during adulthood.

Acknowledgements

The authors would like to thank Ms Donna Baker and Dr Juliana Rey-Parra for their technical support.

Conflict of interest: none declared.

Funding

This work was supported by a research grant from Pfizer Canada, the Canadian Institutes of Health Research (CIHR), and a Personnel Award from the Heart and Stroke Foundation of Canada (H&S). C.F.R. is a fellow of the Strategic Training Program in Maternal; Fetal and Newborn Health (MFN) and the Tomorrow's Research Cardiovascular Health Professionals (TORCH). J.M. is supported by H&S, Alberta Heritage Foundation for Medical Research (AHFMR), and MFN. S.D. is an AHFMR Scientist and a Canada Research Chair in Women's Cardiovascular Health.

References

1. Manning FA. *Intrauterine Growth Retardation: Etiology, Pathophysiology, Diagnosis, and Treatment. Fetal Medicine: Principles and Practice*. Norwalk, CT: Appleton & Lange; 1995.
2. Louey S, Thornburg KL. The prenatal environment and later cardiovascular disease. *Early Hum Dev* 2005;**81**:745–751.
3. Schwartz J, Thornburg KL. The influence of various physiological challenges on permanent changes to the cardiovascular system. *Arch Physiol Biochem* 2003;**111**:3–7.
4. Ojeda NB, Grigore D, Alexander BT. Intrauterine growth restriction: fetal programming of hypertension and kidney disease. *Adv Chronic Kidney Dis* 2008;**15**:101–106.
5. Choi GY, Tosh DN, Garg A, Mansano R, Ross MG, Desai M. Gender-specific programmed hepatic lipid dysregulation in intrauterine growth-restricted offspring. *Am J Obstet Gynecol* 2007;**196**:477e471–477.
6. Stocker CJ, Arch JR, Cawthorne MA. Fetal origins of insulin resistance and obesity. *Proc Nutr Soc* 2005;**64**:143–151.
7. Barker DJ. Intrauterine programming of adult disease. *Mol Med Today* 1995;**1**:418–423.
8. Meyer K, Lubo Z. Fetal programming of cardiac function and disease. *Reprod Sci* 2007;**14**:209–216.
9. Thornburg KL, Louey S. Fetal roots of cardiac disease. *Heart* 2005;**91**:867–868.
10. Thornburg KL, Louey S, Giraud GD. The role of growth in heart development. *Nestle Nutr Workshop Ser Pediatr Program* 2008;**61**:39–51.
11. Marsal K. Intrauterine growth restriction. *Curr Opin Obstet Gynecol* 2002;**14**:127–135.
12. Jobgen WS, Ford SP, Jobgen SC, Feng CP, Hess BW, Nathanielsz PW *et al*. Baggs ewes adapt to maternal undernutrition and maintain conceptus growth by maintaining fetal plasma concentrations of amino acids. *J Anim Sci* 2008;**86**:820–826.
13. Hickey RJ, Clelland RC, Bowers EJ. Maternal smoking, birth weight, infant death, and the self-selection problem. *Am J Obstet Gynecol* 1978;**131**:805–811.
14. Cogswell ME, Yip R. The influence of fetal and maternal factors on the distribution of birthweight. *Semin Perinatol* 1995;**19**:222–240.
15. Xu Y, Williams SJ, O'Brien D, Davidge ST. Hypoxia or nutrient restriction during pregnancy in rats leads to progressive cardiac remodeling and impairs postischemic recovery in adult male offspring. *Faseb J* 2006;**20**:1251–1253.

16. Nilsson PM, Lurbe E, Laurent S. The early life origins of vascular ageing and cardiovascular risk: the EVA syndrome. *J Hypertens* 2008;**26**:1049–1057.
17. Miller AA, De Silva TM, Jackman KA, Sobey CG. Effect of gender and sex hormones on vascular oxidative stress. *Clin Exp Pharmacol Physiol* 2007; **34**:1037–1043.
18. Ordovas JM. Gender, a significant factor in the cross talk between genes, environment, and health. *Gend Med* 2007;**4**(Suppl. B):S111–S122.
19. Dittoe N, Stultz D, Schwartz BP, Hahn HS. Quantitative left ventricular systolic function: from chamber to myocardium. *Crit Care Med* 2007; **35**:S330–S339.
20. Watson LE, Sheth M, Denyer RF, Dostal DE. Baseline echocardiographic values for adult male rats. *J Am Soc Echocardiogr* 2004;**17**:161–167.
21. Pellett AA, Tolar WG, Merwin DG, Kerut EK. The Tei index: methodology and disease state values. *Echocardiography* 2004;**21**:669–672.
22. Coatney RW. Ultrasound imaging: principles and applications in rodent research. *Ilar J* 2001;**42**:233–247.
23. Williams R, Needles A, Cherin E, Zhou YQ, Henkelman RM, Adamson SL *et al*. Noninvasive ultrasonic measurement of regional and local pulse-wave velocity in mice. *Ultrasound Med Biol* 2007;**33**:1368–1375.
24. Xu Y, Clanachan AS, Jugdutt BI. Enhanced expression of angiotensin II type 2 receptor, inositol 1,4, 5-trisphosphate receptor, and protein kinase cepsilon during cardioprotection induced by angiotensin II type 2 receptor blockade. *Hypertension* 2000;**36**:506–510.
25. Xu Y, Kumar D, Dyck JR, Ford WR, Clanachan AS, Lopaschuk GD *et al*. (AT1) and (AT2) receptor expression and blockade after acute ischemia-reperfusion in isolated working rat hearts. *Am J Physiol Heart Circ Physiol* 2002;**282**:H1206–H1215.
26. Zile MR, Brutsaert DL. New concepts in diastolic dysfunction and diastolic heart failure: Part I: diagnosis, prognosis, and measurements of diastolic function. *Circulation* 2002;**105**:1387–1393.
27. Roberts J, Goldberg PB. Changes in basic cardiovascular activities during the lifetime of the rat. *Exp Aging Res* 1976;**2**:487–517.
28. Tuder RM, Yun JH, Bhunia A, Fijalkowska I. Hypoxia and chronic lung disease. *J Mol Med* 2007;**85**:1317–1324.
29. Webster WS, Abela D. The effect of hypoxia in development. *Birth Defects Res C Embryo Today* 2007;**81**:215–228.
30. Kanaya N, Zakhary DR, Murray PA, Damron DS. Thiopental alters contraction, intracellular Ca2+, and pH in rat ventricular myocytes. *Anesthesiology* 1998;**89**:202–214.
31. Wada DR, Harashima H, Ebling W, Osaki EW, Stanski DR. Effects of thiopental on regional blood flows in the rat. *Anesthesiology* 1996;**84**:596–604.
32. Hemmings DG, Williams SJ, Davidge ST. Increased myogenic tone in 7-month-old adult male but not female offspring from rat dams exposed to hypoxia during pregnancy. *Am J Physiol Heart Circ Physiol* 2005;**289**:H674–H682.