Elevated vascular resistance and afterload reduce the cardiac output response to dobutamine in early growth-restricted rats in adulthood

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Abstract

Epidemiological studies have linked intra-uterine growth restriction (IUGR) with an increased risk of CVD later in life. The aim of the present study was to examine the effect of maternal protein restriction on cardiac function in adulthood during dobutamine (DOB) stimulation. IUGR was induced in Wistar Kyoto dams through administration of a low-protein diet (LPD; 8·7 % casein) during pregnancy and lactation; the control group received a normal-protein diet (NPD; 20 % casein). At 14 weeks of age, cardiac function was assessed in male and female NPD (eight females and eight males) and LPD offspring (ten females and ten males) by pressure volumetry using an anaesthetised closed-chest approach. We determined mean arterial pressure (MAP), heart rate and left ventricular pressure–volume indices under baseline conditions and DOB stimulation (2 and 4 μg/kg per min). During β-adrenergic activation in LPD offspring, increases in cardiac output (CO, $P<0\cdot018$) and stroke volume (SV, $P<0\cdot005$) were attenuated in comparison with NPD offspring, while increases in ejection fraction and the maximal rate of ventricular pressure development were not affected. LPD females maintained a smaller end-diastolic volume ($P<0\cdot017$). MAP did not differ between the groups and did not change significantly during DOB infusion. Arterial elastance and total peripheral resistance decreased in all rats but remained significantly elevated in LPD offspring ($P<0\cdot015$ and $<0\cdot01$). Early growth restriction did not affect ventricular contractility but led to an increased afterload and impaired the ability to increase SV and CO during β-adrenergic stimulation.

Key words: β-Adrenergic stimulation: Fetal programming: Maternal protein restriction: Vascular resistance

Intra-uterine growth restriction (IUGR), which occurs in 4–10 % of all term pregnancies[1], is multifactorial in origin, with genetic and environmental influences adversely having an impact on fetal growth[2,3]. Epidemiological studies have shown a clear relationship between low birth weight, due to IUGR, and an increased incidence of CVD later in life[4–6]; hence, size at birth is an early indicator of disease risk.

It has been postulated that people born of low birth weight have fewer of the functional units in key organs, such as the heart and kidney, and this is likely to render these vital organs susceptible to disease later in life[7]. Recent studies from our laboratory have demonstrated that maternal protein restriction in rats leads to a reduction in heart weight and in the number of cardiomyocytes in the hearts of growth-restricted offspring at birth[8]. However, the total number of cardiomyocytes was found to be directly proportional to heart size at 4 weeks of age[9], approximately 2 weeks after

cardiomyocyte proliferation has ceased in the rat heart. It is not known whether the cardiomyocyte complement in IUGR hearts is capable of performing normal heart work at maturity; however, one study has shown that adult IUGR hearts have attenuated heart rate (HR) responses to β-adrenergic stimulation[10].

Importantly, we have shown in our IUGR rat model, where female breeder rats are fed a low-protein diet (LPD) during pregnancy and for 2 weeks during lactation, that there is a significant increase in the level of cardiac fibrosis in female offspring in early adulthood, and that this occurs in the absence of catch-up growth or elevation in blood pressure; here, male offspring were not examined[11]. In that study, it was not determined whether cardiac function in early adulthood was affected by the early growth restriction. Therefore, we address this in the present study. Interestingly, others have reported that when a maternal LPD is administered

during the gestation period, or any significant part thereof, there is accelerated postnatal growth associated with hypertension[12,13] and subsequent left ventricle (LV) dysfunction in male offspring[14], but not necessarily in females[15]. In this regard, Cheema et al.[14] reported a twofold elevation in LV end-diastolic pressure, increased wall thickness, reduced cardiac output (CO) and decreased maximum rates of contraction and relaxation in male offspring at 40 weeks of age. However, in the weaning period, the same offspring showed significant wall thinning, dilation and severely impaired ejection fraction (EF). Whether the systolic and diastolic impairment observed in the previous study[14] occurs entirely as a consequence of sympathetic overactivation in a compensatory phase of development during early adulthood or, in part, as a result of increased vulnerability of IUGR offspring to ageing is currently unknown.

The aim of the present study was to compare cardiac function in situ at 14 weeks of age in male and female non-growth-restricted and early growth-restricted offspring (that had been exposed to maternal protein restriction during pregnancy and lactation). In particular, we have examined for the first time the haemodynamic and cardiac responses of the in situ growth-restricted heart during β-adrenergic activation with low doses of dobutamine (DOB) hydrochloride. To address these aims, we used a Millar pressure–volume (P–V) conductance catheter system, whereby LV P–V loops and arterial blood pressure were recorded in situ, in a closed-chest model, under steady-state conditions before, during and after β-adrenergic stimulation. Preload reductions in CO by vena caval occlusion provide load-insensitive indices of LV contractility, such as the end-systolic P–V relationship and the maximal rate of ventricular pressure development (dP/dt_{max}) relationship with the end-diastolic volume (EDV). However, such repeated manipulations might change the extent of cardiac sympathetic activation and loading conditions. In the present study, we were most interested in how loading conditions affect the regulation of CO by β-adrenergic activation, and thus load-sensitive indices of systolic and diastolic function were compared.

Experimental methods

Animals and diet treatment

Female and male Wistar Kyoto breeder rats (10 weeks old) were obtained from the Australian Resource Centre, Perth, Australia. Female breeders were placed on either a LPD (8·7 % casein and 15·6 MJ/kg) or a normal-protein diet (NPD; 20 % casein and 16·1 MJ/kg) 2 weeks before pregnancy (to allow them to adapt to the diet), during pregnancy and 2 weeks after birth (diets were commercially available from Specialty Feeds, Glen Forest, WA, Australia). Since cardiomyocyte proliferation is complete by the second week after birth, LPD rats in the present study received a normal diet after proliferation is assumed to have ceased. Female breeders were housed individually and maintained on a 12 h light–12 h dark cycle and at a constant temperature of 21 ± 1°C. Food and water were provided ad libitum. At birth, the litters

were reduced to eight pups per dam. Only one female and one male offspring from each litter were used for these studies (NPD, eight females/eight males and LPD, ten females/ten males); the remaining siblings were used in further studies and not included here. In order to avoid stress in the dams, the pups were first weighed at postnatal day 4. Body weight of the offspring was measured daily from day 4 until weaning (4 weeks of age) and then once a week until the experimental endpoint (14 weeks of age). Animal care and experimental and surgical procedures were carried out in accordance with the National Health and Medical Research Council of Australia 'Code of practice for the care and use of animals for scientific purposes', and were approved by the Monash University, School of Biomedical Sciences Animal Ethics Committee A (VIC, Australia).

Surgical preparation

Rat offspring at 14 weeks of age were anaesthetised with sodium pentobarbital (50 mg/kg, intraperitoneal) to induce general anaesthesia and maintained at supplemental doses thereafter (25 mg/kg per h, intravenous bolus) for the duration of the experiments. The trachea was intubated with a 14 gauge angiocath sheath, and the animals were artificially ventilated with a rodent ventilator (Ugo Basile Model 7025, Comerio, VA, Italy) at rates appropriate for their body size (frequency 50 breaths and tidal volume 1 ml/100 g body weight) by room air enriched with supplemental oxygen (30 % total). Body temperature was maintained at 37°C using a thermostatically controlled heating pad. The right jugular vein was catheterised with a polyethylene tube (PE-50) for drug delivery and to continuously deliver Hartmann's solution at 3–4 ml/h (KD Scientific Infusion Pump, Model 781210, Holliston, MA, USA) to maintain body fluid volume during the experiments. Another polyethylene catheter filled with heparinised saline (100 units/ml) was inserted into the left femoral artery for determination of arterial pressure. An intracardiac P–V catheter (1.4F Model-SPR 838, Millar Instruments, Houston, TX, USA) was inserted via the right carotid artery and advanced into the LV chamber, with the thorax intact, in order to obtain the P–V recordings continuously throughout the experiment[16]. Arterial blood pressure and cardiac P–V signals were recorded at a sampling rate of 1 kHz and processed using a Powerlab 16/30 Data Acquisition System (AD Instruments, Bella Vista, NSW, Australia).

Assessment of cardiac function

At 14 weeks of age, haemodynamic function derived from the left ventricular P–V relationship was assessed under baseline conditions and following stepwise increases in cardiac-selective β-adrenergic stimulation (DOB 2–4 µg/kg per min intravenously) in terminal anaesthetised experiments. DOB infusions were continued at each dose until HR and CO plateaued (about 3–5 min). After approximately 10 min, a post-DOB baseline was recorded. The following parameters were recorded or calculated offline on a beat-to-beat basis during brief periods (about 5 s) when the ventilation was

halted: mean arterial pressure (mmHg); HR (bpm); EDV (μl); end-systolic pressure (mmHg); stroke volume (SV; μl); EF (%); CO (ml/min); preload-adjusted maximal power (PAMP; mW/μl^2, determined as maximal power/(EDV)^2); total peripheral resistance (calculated as mean arterial pressure/CO; mmHg/ml); arterial elastance (Ea, mmHg/μl determined as end-systolic pressure/SV). Maximal and minimal rates of ventricular pressure changes during the cardiac cycle (dP/dt_{max}, mmHg/s; dP/dt_{min}, mmHg/s) and relaxation time constants calculated by the Glantz (Tau-G) method (regression of dP/dt *v.* pressure, ms) and the Weiss (Tau-W) method (regression of log pressure, ms) were computed using a cardiac $P–V$ analysis program (PVAN 3.5, Millar Instruments, Houston, TX, USA) as described previously[17]. At completion of the post-DOB baseline measurements, 2–3 ml of blood were collected from the femoral artery cannula. The blood sample from each animal was then used for a *post hoc* calibration of the volume conductance signal to absolute volume (μl) after a blood conductance correction (the conductance due to ventricular muscle using the hypertonic saline method) according to the recommended procedure[18]. The rats were then killed with a lethal overdose (150 mg/kg) of sodium pentobarbital, and the hearts were immediately excised. The hearts were trimmed of connective tissue and fat, and then weighed. In different male NPD rats, echocardiography was also used to validate the conductance catheter LV volume calibrations[19]. End-systolic and end-diastolic LV volumes were calculated using the Bullet formula[20] with long- and short-axis dimensions obtained by echocardiography in B-mode video.

Statistical analysis

All haemodynamic parameters were derived from 5 s averages during brief interruptions in ventilation to reduce respiratory rhythm artefacts during baseline conditions and DOB infusion. Parameters were analysed using SYSTAT version 12.0 (Systat Software, Inc., Chicago, IL, USA). The results are expressed as means with their standard errors of the mean. To compare treatment groups under baseline steady-state contractions, a two-way ANOVA was used with the factors maternal dietary treatment (P_T; NPD *v.* LPD), sex (P_S; females *v.* males) and their interactions ($P_{T\times S}$), followed by Bonferroni's *post hoc* test. To examine the dose response to DOB between males and females of both dietary treatment groups, a three-way ANOVA was employed with the factors dose of DOB (P_D), sex (P_S) and maternal dietary treatment (P_T) and their two-way and three-way interactions ($P_{S\times T}$, $P_{T\times D}$ and $P_{S\times T \times D}$). Statistical significance was accepted at $P < 0.05$. As only one female and one male from each litter were included in each treatment group, no adjustment for the litter of origin was necessary.

Results

Body weights and heart weights

Body weight in both female and male LPD offspring was significantly lower (21 and 11%, respectively) at postnatal day 4 when compared with NPD offspring ($P_T < 0.0001$; Table 1). At 14 weeks of age (early adulthood), female and male LPD offspring remained significantly smaller ($P_T < 0.0005$) in body mass compared with controls. Not surprisingly, absolute heart weights at 14 weeks of age were also significantly lower ($P_T < 0.025$) in female and male LPD offspring. Hence, the heart weight:body weight ratio was not significantly different between the dietary treatment groups but was greater in females than in males ($P_S < 0.0055$). It should be noted that while the heart weight:body weight ratio completely overlapped between the male and female NPD offspring, the same ratio overlapped little in the LPD group.

Pressure-volumetry assessment of cardiac function under baseline conditions

A comparison of LV function and haemodynamics during steady-state conditions (baseline) between the NPD and LPD offspring is shown in Table 1. At 14 weeks of age, we found little evidence of cardiac dysfunction in LPD offspring under steady-state basal contractions, except a significantly reduced EDV ($P_T < 0.02$). There was no evidence of elevation in arterial pressure and the slightly elevated Ea in LPD rats was not significant. Likewise, at baseline, HR, SV, CO, EF and LV pressure at end systole and end diastole did not differ between the treatment groups. Male rats differed significantly from female offspring only in basal maximal and minimal rates of LV pressure changes during the cardiac cycle and in the time relaxation constant (dP/dt_{max} ($P_S < 0.02$), dP/dt_{min} ($P_S < 0.02$) and Tau-G ($P_S < 0.009$), respectively). However, there was no significant difference between sexes in Tau-W.

Cardiac function and haemodynamics during and after dobutamine stimulation

HR increased significantly with increasing doses of DOB in both sexes of the NPD and LPD groups ($P_D < 0.001$; Fig. 1(a) and (b)) and almost completely returned to the baseline state (post-DOB *v.* base, $P < 0.073$), but the response did not differ between the treatment groups.

Female LPD offspring had a significantly lower EDV but not males, in comparison with NPD rats ($P_{S\times T} < 0.016$; Fig. 1(c) and (d)). The increasing doses of DOB led to a small but significant decrease in EDV in all offspring ($P_D < 0.002$; Fig. 1(c) and (d)), which was most pronounced in LPD females ($P_{S\times T} < 0.017$).

Although basal SV did not differ between the treatments (Table 1), the increase in load-sensitive SV was significantly attenuated in LPD offspring of both sexes compared with NPD offspring ($P_T < 0.005$ and $P_{T\times D} < 0.064$; Fig. 1(e) and (f)). SV in NPD offspring was significantly larger than that in LPD offspring in the post-DOB period ($P_T < 0.018$). EF increased in all offspring of both the treatment groups in response to the DOB challenge, but not significantly so, due to within-animal variability in the response of males (Fig. 1(g) and (h)). Increasing doses of DOB led to a significant increase in CO in female and male NPD and LPD

Table 1. Morphometry and basal cardiac function in normal-protein diet (NPD, 20 %) and low-protein diet (LPD, 8·7 %) female and male offspring

(Mean values with their standard errors of the mean)

	Female NPD		Female LPD		Male NPD		Male LPD		
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	P
Morphometry									
Sample size (n)	8		10		8		10		
Neonatal weight at day 4 (g)	6·77	0·19	5·33	0·26	6·86	0·11	6·11	0·16	$P_S < 0.0336$ $P_T < 0.0001$
Body weight at 14 weeks (g)	231	4	213	3	340	6	316	7	$P_S < 0.0001$ $P_T < 0.0005$
Heart weight at 14 weeks (mg)	829	50	775	19	1126	34	1021	31	$P_S < 0.0001$ $P_T < 0.025$
Heart weight:body weight (mg/g)	3·59	0·21	3·65	0·11	3·31	0·06	3·23	0·05	$P_S < 0.0055$
Haemodynamics									
Heart rate (bpm)	332	20	327	15	347	19	343	15	NS
Cardiac output (ml/min)	28	3	24	2	34	4	27	3	NS
LV end-diastolic volume (μl)	171	8	140	7	166	8	149	13	$P_T < 0.02$
Stroke volume (μl)	84	7	73	5	95	8	79	7	NS
Arterial elastance (mmHg/μl)	1·37	0·19	1·67	0·13	1·36	0·15	1·68	0·16	NS
Systolic arterial pressure (mmHg)	97	11	108	8	110	8	116	6	NS
Diastolic arterial pressure (mmHg)	77	10	92	8	90	8	96	8	NS
Mean arterial pressure (mmHg)	88	10	101	8	100	8	105	7	NS
Ejection fraction (%)	47	4	49	3	54	4	52	3	NS
End-systolic pressure (mmHg)	108	11	121	9	123	9	115	8	NS
dP/dt_{max} (mmHg/s)	6221	553	7043	528	8313	685	7670	424	$P_S < 0.02$
dP/dt_{min} (mmHg/s)	−6166	753	−6584	507	−8262	737	−7805	518	$P_S < 0.02$
Preload-adjusted maximal power (mW/μl^2)	12·26	1·71	20·72	3·71	19·19	3·08	18·97	2·47	NS
Tau-W (ms)*	10·88	0·61	11·32	0·56	10·19	0·47	10·74	0·41	NS
Tau-G (ms)*	17·21	0·85	17·41	0·92	13·76	0·92	15·94	0·79	$P_S < 0.009$

S, sex; T, treatment; bpm, beats per minute; LV, left ventricle; dP/dt_{max}, maximal rate of ventricular pressure change; dP/dt_{min}, minimal rate of ventricular pressure change; Tau-W, Glantz method; Tau-G, Weiss method.
Mean values were significantly different with respect to sex and treatment ($P < 0.05$).
* Tau represents the relaxation time constant for ventricular pressure.

Heart function in growth-restricted rats

offspring ($P_D < 0.001$; Fig. 1(i) and (j)), but the rate of increase was significantly depressed in LPD rats ($P_{T \times D} < 0.024$). There was a strong trend for the depression of CO in LPD rats in the post-DOB period (post-DOB *v.* base, $P_T < 0.055$).

Increasing doses of DOB led to a significant decrease in Ea in all female and male offspring ($P_D < 0.001$; Fig. 1(k) and (l)). However, Ea was significantly elevated in the LPD treatment group ($P_T < 0.015$), indicating that ventricular afterload was higher in growth-restricted rats.

Adult offspring did not differ in mean arterial pressure (Fig. 2(a) and (b)), diastolic or systolic pressure at any dose of DOB or during the post-DOB period. End-systolic LV pressure was comparable between the groups, varying between

100 and 125 mmHg before, during and after DOB infusion (P_T, P_D and P_S, all not significant). Nonetheless, in agreement with the elevated Ea found in LPD offspring, total peripheral resistance (Fig. 2(c) and (d)) was significantly elevated in comparison with NPD offspring during DOB infusion ($P_T < 0.01$) and remained so in the post-DOB period (post-DOB *v.* base, $P_T < 0.028$).

While the aforementioned findings indicate that LPD offspring of both sexes had attenuated SV responses to DOB stimulation, diet treatment did not affect dP/dt_{max} and dP/dt_{min} or PAMP. Overall, dP/dt_{max} was significantly higher in males compared with females irrespective of treatment ($P_S < 0.046$; Fig. 2(e) and (f)). DOB challenge led to a significant
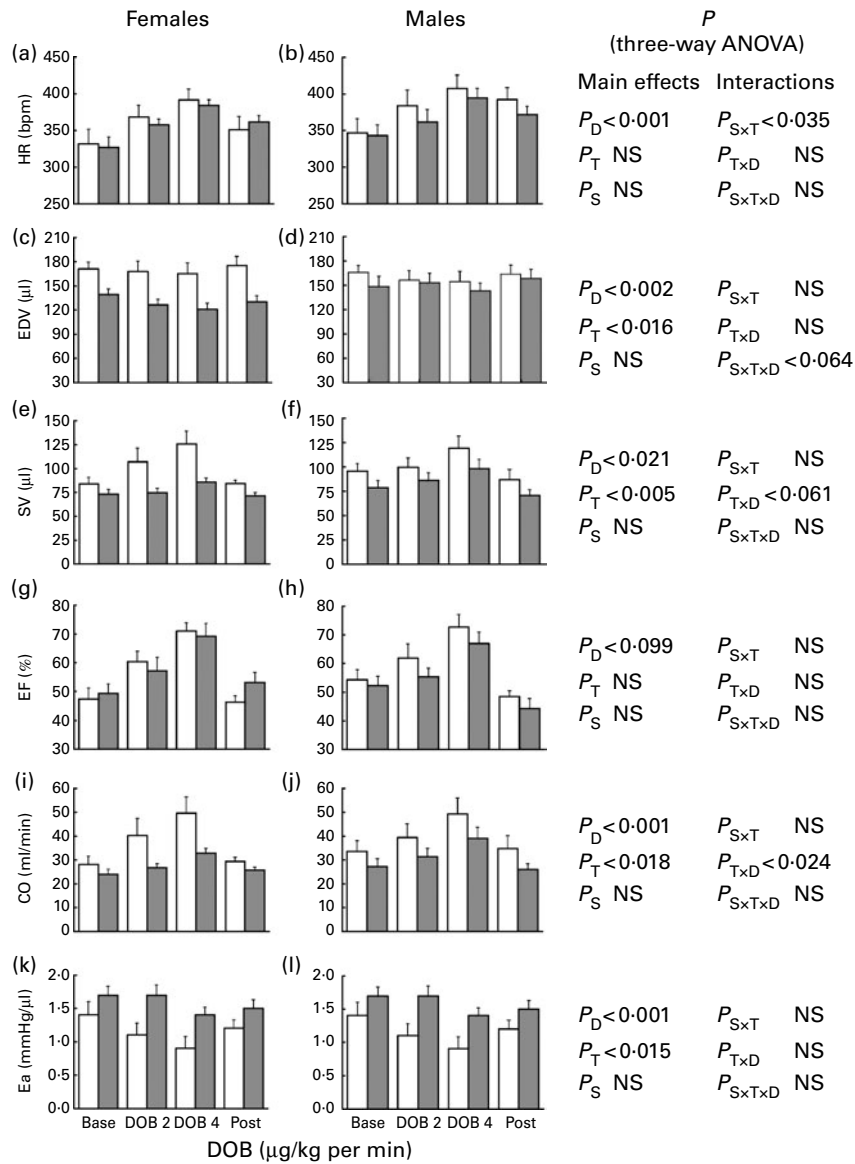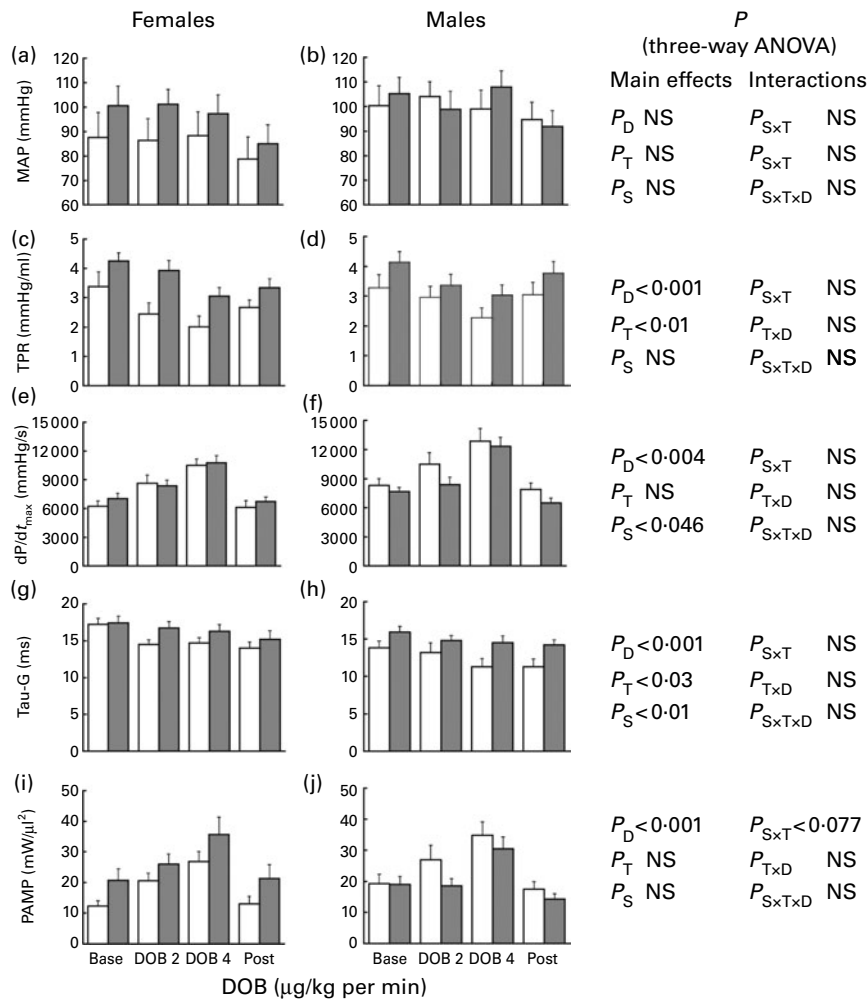
Fig. 1. Cardiac function parameters under steady-state baseline, during dobutamine (DOB) infusion (2–4 μg/kg per min) and the post-DOB (post) period in female and male normal-protein diet (NPD) and low-protein diet (LPD) offspring. (a, b) Heart rate (HR; beats per minute (bpm)), (c, d) end-diastolic volume (EDV), (e, f) stroke volume (SV), (g, h) ejection fraction (EF), (i, j) cardiac output (CO) and (k, l) arterial elastance (Ea) in female and male offspring are presented for the NPD (□) and LPD (■) treatment groups. Values are means, with standard errors of the mean represented by vertical bars. Mean values were significantly different with respect to maternal diet treatment for NPD or LPD (P_T), the dose of β-adrenergic stimulant dobutamine (P_D), and sex (P_S) and their interactions ($P_{T \times D}$, $P_{S \times T}$ and $P_{S \times D}$; $P < 0.05$).

increase in dP/dt_{max} ($P_D < 0.001$), but dP/dt_{min} was not significantly changed by DOB (P_D not significant; data not shown). There was a tendency for dP/dt_{min} to decrease with dose in female offspring ($P_S < 0.056$), while dP/dt_{min} was not different from baseline values in males. PAMP increased significantly with the DOB dose ($P_D < 0.001$; Fig. 2(i) and (j)); however, there was a trend for the response to differ between the treatments with respect to sex ($P_{S \times T} < 0.077$). These findings suggest that neither LV contractility nor relaxation was impaired by LPD treatment.

The relaxation time constants significantly decreased with DOB, Tau-W ($P_D < 0.001$) and Tau-G ($P_D < 0.001$) in all offspring (Fig. 2(g) and (h), Tau-G only presented). In both cases, the time constants were longest in LPD offspring ($P_T < 0.03$) and significantly shorter in male offspring than in females ($P_S < 0.01$).

Normalisation of stroke volume and cardiac output responses to dobutamine stimulation

Since the preceding analyses indicated that there were differences between the dietary treatment groups in the absolute SV and CO responses to DOB stimulation and in absolute and relative heart mass, we examined the regulation of CO after taking into account heart mass (Fig. 3). Group averages for relative CO and SV were determined following normalisation according to the heart mass of individual rats. The mean HR of male and female rats overlapped over the DOB dose range examined and did not differ between the treatment groups (Fig. 3(a)). The small difference maintained between the male LPD and NPD rats in absolute SV as the DOB dose increased (Fig. 1(e) and (f)) was entirely explained by the smaller heart mass of LPD rats (Fig. 3(b)). On the other hand, female NPD rats maintained at least a 25 % larger

Fig. 2. Cardiac function parameters under steady-state baseline, during dobutamine (DOB) infusion (2–4 μg/kg per min) and the post-DOB (post) period in female and male normal-protein diet (NPD) and low-protein diet (LPD) offspring. (a, b) Mean arterial pressure (MAP), (c, d) total peripheral resistance (TPR), (e, f) maximal rate of pressure development (dP/dt_{max}), (g, h) relaxation time constant of ventricular pressure (Tau-G) and (i, j) preload-adjusted maximal power (PAMP) in female and male offspring are presented for the NPD (□) and LPD (■) treatment groups. Values are means, with standard errors of the mean represented by vertical bars. Mean values were significantly different with respect to maternal diet treatment for NPD or LPD (P_T), the dose of β-adrenergic stimulant dobutamine (P_D), and sex (P_S) and their interactions ($P_{S \times T}$, $P_{T \times D}$ and $P_{S \times D}$; $P < 0.05$).
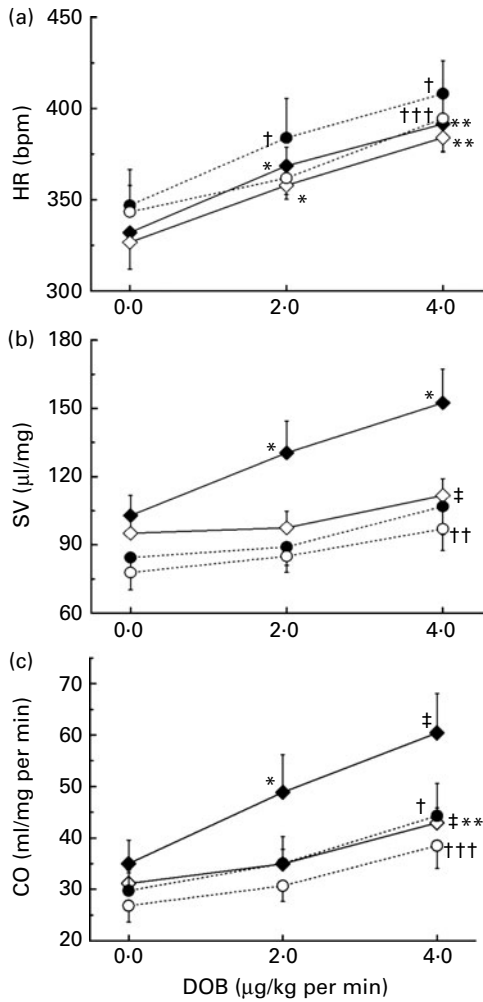
Fig. 3. Heart rate (HR; beats per min (bpm)), stroke volume (SV) and cardiac output (CO) responses to dobutamine (DOB) infusion (2–4 µg/kg per min) following normalisation for heart mass in female and male normal-protein diet (NPD) and low-protein diet (LPD) rats. Mean responses from Fig. 1 are presented for NPD females and males (◆, ●) and LPD females and males (◇, ○) following normalisation for heart mass in each individual rat. Values are means with their standard errors of the mean. Mean values were significantly different against female baseline: * $P<0.05$, ** $P<0.01$. Mean values were significantly different against male baseline: † $P<0.05$, †† $P<0.01$, ††† $P<0.001$. ‡ Mean values were significantly different for LPD females *v.* NPD females ($P<0.001$).

normalised SV in comparison with male rats and consequently a larger relative CO over male rats at the higher dose of DOB (Fig. 3(c)). In contrast, female LPD rats had mass-specific SV and CO responses to DOB that were intermediate between female NPD rats and male rats, and were significantly lower than female NPD rats at the higher DOB dose ($P<0.018$).

Discussion

The present findings demonstrate that in the absence of accelerated postnatal growth or elevated blood pressure, both male and female IUGR offspring in early adulthood exhibit a reduced capacity to increase CO during β-adrenergic stimulation. We attribute this impaired ability to increase CO during DOB infusion to an increased afterload in adult LPD

offspring acting to reduce the increase in SV. There was no evidence that systolic contractile function was impaired by the early growth restriction in either sex. Even though the increase in afterload was similar in male and female LPD offspring, we can conclude that β-adrenergic regulation of CO in female IUGR offspring was more adversely affected than male siblings, as female rats maintain higher CO relative to heart weight than males. Furthermore, the negative impact of an increased afterload on SV was compounded by a reduced preload in female LPD offspring.

Others have previously examined the effects of another β-adrenergic agonist, isoprenaline, on HR and LV-developed pressure in the offspring of protein-restricted dams[10,21]. Both studies have showed attenuated β-adrenergic responsivity. However, this is the first study, to our knowledge, that has examined the effects of ventricular loading conditions on the response of the IUGR heart to positive chronotropic and inotropic challenge in adulthood *in vivo*. While only load-sensitive indices of systolic and diastolic function were examined, the findings clearly demonstrate that growth restriction did not alter cardiac inotropic or chronotropic reserve, but the altered vascular resistance did have a large impact on SV and therefore CO.

The present findings reaffirm that there are sex-specific differences in β-adrenergic modulation of LV force development and relaxation times in the rat heart at early adulthood, as first reported by Elmes *et al.*[21]. However, we did not find any evidence of sexual dimorphism in the programming of cardiac and haemodynamic dysfunction as a result of IUGR and/or maternal protein restriction, other than a reduced ability to increase LV filling during β-adrenergic activation in growth-restricted female offspring. Ventricular afterload (Ea) and total peripheral resistance were similarly elevated in both sexes of LPD offspring, suggesting that early growth restriction increased vascular resistance in young adult rats, but not to the extent of developing systemic hypertension. Although many groups have reported hypertension following IUGR[12,13,22], Rodford *et al.*[23] also reported that neither male nor female early growth-restricted offspring developed hypertension as adults. In contrast to this study, there have been a number of experimental studies reporting differential effects between male and female offspring in response to insults *in utero*[15,23–31]. In contrast to the present findings, it is generally reported that male offspring are more adversely affected.

In the present study, both female and male growth-restricted offspring attained lower body weights at 14 weeks of age compared with NPD controls, indicating that IUGR offspring did not fully catch up in growth when placed on an *ad libitum* standard laboratory chow 2 weeks after birth; heart weight was in proportion with adult body weight, albeit significantly larger in female rats. Even though IUGR offspring were smaller, basal SV and CO when normalised for heart weight were similar compared with control animals. In support of our findings, Cheema *et al.*[14] found no differences in EF (assessed by echocardiography) under basal conditions in male (female data reported but not shown) LPD offspring in early adulthood when compared with NPD controls. Interestingly, in that long-term study, there was

overt cardiac dysfunction at 40 weeks of age as assessed using the $P-V$ method. In this regard, it is likely that more pronounced cardiac dysfunction may also ensue with ageing in our offspring (especially the IUGR females), given that at 14 weeks of age we observed an impaired ability to increase CO and that systemic vascular resistance was elevated.

In the present study, the hearts of female IUGR offspring were unable to match the SV increase of the hearts of female control offspring during positive inotropic stimulation. All rats demonstrated dose-dependent increases in HR and SV during β-adrenergic stimulation (Fig. 1(a), (b) and (e), (f), respectively), but notably, in female IUGR offspring, the increase in SV and CO was significantly depressed compared with controls (CO increase of 78% in NPD $v.$ 64% in LPD). LV preload decreased during DOB infusion in female IUGR hearts as EDV showed a trend towards larger decreases with the DOB dose, and remained significantly smaller than that of control offspring. The present findings thus demonstrate that in early adulthood (14 weeks of age), only female LPD rats have significantly diminished capacity to increase CO that cannot be accounted for by the smaller heart mass. Indeed, normalising CO and SV to heart mass revealed marked differences in LPD females $v.$ NPD females but not in males; HR was on average only 10–20 bpm lower in LPD males $v.$ controls at the doses examined and females were approximately 2–10 bpm lower (Fig. 3(a)). Hence, in male growth-restricted offspring, the diminished CO response to DOB, at this age at least, appears to be largely attributable to the smaller heart mass rather than the increase in afterload per se.

The reduced ability to increase SV during β-adrenergic stimulation in female IUGR offspring is unlikely to be attributed to a reduction in β-adrenergic sensitivity as LPD offspring were equally able to increase HR. Likewise, the reduced blood ejection in female IUGR offspring does not appear to be attributed to a reduced ability to maximally increase force development, as their hearts developed pressure normally. Previously, we have shown that in the rat heart, cross-bridge cycling is essentially completed in the isovolumetric contraction phase[16], and in this study, female LPD hearts did not have impaired rates of force development in this first phase of the $P-V$ relationship. Therefore, cross-bridge cycling and myofilament Ca^{2+} sensitivity are presumed to be normal in the IUGR heart. The fact that the hearts of LPD female offspring required higher PAMP to attain the same end-systolic pressure and dP/dt_{max} as NPD offspring also argues against abnormal calcium handling in the cardiomyocytes of IUGR females. Rather, the diminished capacity to increase SV appears to be due to diminished preload and impaired fibre shortening in the face of elevated afterload. Further studies are currently determining whether the LV extracellular matrix composition is altered by an early growth restriction.

In conclusion, it is now well established that the antecedents of CVD can be programmed in early life, with IUGR directly linked to an increased risk of adult CVD. The present findings strongly suggest that both the smaller absolute heart mass and the increased afterload reduce the growth-restricted capacity of the heart to increase SV and CO during β-adrenergic activation in early adulthood.

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