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ORIGINAL ARTICLE

# Transgenerational left ventricular hypertrophy and hypertension in offspring after uteroplacental insufficiency in male rats

**Jordanna S Master,* Monika A Zimanyi,† Kom V Yin,† Karen M Moritz,‡ Linda A Gallo,* Melanie Tran,* Mary E Wlodek*§ and Mary J Black†§**

**Department of Physiology, The University of Melbourne, Parkville, †Department of Anatomy and Developmental Biology, Monash University, Melbourne, Vic. and ‡School of Biomedical Sciences, University of Queensland, Brisbane, Qld, Australia*

## SUMMARY

**Epidemiological studies have shown an association between low birthweight and adult disease development with transmission to subsequent generations. The aim of the present study was to examine the effect of intrauterine growth restriction in rats, induced by uteroplacental insufficiency, on cardiac structure, number, size, nuclearity, and adult blood pressure in first (F1) and second (F2) generation male offspring. Uteroplacental insufficiency or sham surgery was induced in F0 Wistar-Kyoto pregnant rats in late gestation giving rise to F1 restricted and control offspring, respectively. F1 control and restricted females were mated with normal males, resulting in F2 control and restricted offspring, respectively. F1 restricted male offspring were significantly lighter at birth ($P < 0.05$), but there were no differences in birthweight of F2 offspring. Left ventricular weights and volumes were significantly increased ($P < 0.05$) in F1 and F2 restricted offspring at day 35. Left ventricular cardiomyocyte number was not different in F1 and F2 restricted offspring. At 6 months-of-age, F1 and F2 restricted offspring had elevated blood pressure (8–15 mmHg, $P < 0.05$). Our findings demonstrate the emergence of left ventricular hypertrophy and hypertension, with no change in cardiomyocyte number, in F1 restricted male offspring, and this was transmitted to the F2 offspring. The findings support transgenerational programming effects.**

**Key words: blood pressure, cardiomyocyte number, growth restriction, left ventricular hypertrophy, transgenerational transmission.**

## INTRODUCTION

Epidemiological studies, supported by a number of animal experimental models, have highlighted a strong correlation between low birthweight and the development of diseases in adult life.[1–3] The cardiovascular system has been identified as being particularly susceptible to prenatal programming, with intrauterine growth restriction (IUGR) linked to an increased incidence of hypertension and cardiovascular diseases in adulthood.[3,4] Remarkably, there is mounting epidemiological and experimental evidence to show that intrauterine programming effects are not limited to the first (directly exposed) generation, but can have transgenerational effects without recurring insults during pregnancy.[5–8] In this regard, important information has been derived from the study of women and their IUGR offspring who were exposed to a shortage of food throughout all, or part, of their pregnancy during the Dutch famine in 1944.[9,10] The IUGR children from these mothers showed an increased propensity for diabetes, obesity, cardiovascular disease and a range of other chronic illnesses in adulthood.[9,11] As adults, the IUGR first generation (F1) females, in a normal nutritional environment, subsequently gave birth to children with no alterations in birthweight, but their offspring showed increased neonatal adiposity and increased risk for disease in adulthood.[12] The cause of the increased vulnerability to cardiovascular disease as a result of IUGR in the first and subsequent generations is currently unknown.

To date, there have only been a few animal experimental studies that have examined transgenerational cardiovascular disease risk, with some studies supporting the concept.[5,8] In support of this concept, previous studies have highlighted that exposure to maternal protein restriction early in pregnancy, or late pregnancy, resulted in F1 and second generation (F2) male offspring with increased blood pressure and left ventricular thickness.[13] These effects were most pronounced when growth restriction was induced in late pregnancy compared with early pregnancy, and the effects in F2 offspring were exacerbated compared with F1 offspring, despite no interventions during their development *in utero*.[13] In other studies, maternal hypertension during pregnancy, caused by reduced uteroplacental perfusion on day 14 of gestation, resulted in F1 growth-restricted rat offspring with juvenile hypertension.[14] The severe reduction in birthweight (–20%) and hypertension were transmitted to the F2 generation, without any insults during the F1 pregnancy. In other studies of maternal undernutrition in rats, elevations in systolic blood pressure and endothelial dysfunction have been reported in F1 male rats,

Correspondence: Professor Mary E Wlodek, Department of Physiology, The University of Melbourne, Parkville, Vic. 3010, Australia. Email: m.wlodek@unimelb.edu.au

§Joint senior authors.

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independent of birthweight, which were transmitted to the F2 restricted male and female offspring.[15–17]

It has previously been reported in rodent and ovine animal models that early life growth restriction can lead to a significant reduction in the number of cardiomyocytes formed within the heart, with the complement of cardiomyocytes directly proportional to heart size.[18,19] This is likely to adversely impact on lifelong functional reserve, given the reduced proliferative capacity of cardiomyocytes in postnatal life. The heart grows by proliferation of cardiomyocytes early in gestation; however, during late gestation, cardiomyocytes undergo a process of maturation whereby they cease dividing and become terminally differentiated in preparation for the haemodynamic transition at the time of birth.[20–22] Growth of the heart muscle after this time is predominantly as a result of cardiomyocyte hypertrophy and deposition of extracellular matrix deposition. Proliferation of cardiomyocytes is not precluded in the adult heart; however, the proliferative and thus, regenerative capacity is markedly diminished. Hence, a reduced complement of cardiomyocytes in the heart of IUGR offspring at birth provides a plausible explanation for the increased vulnerability for heart disease in adult life; whether such deficits can persist into subsequent generations has not been explored and was the focus of the present study.

We have an established rat model of uteroplacental insufficiency, the leading cause of IUGR in developed countries.[23] In F1 male offspring, we have previously reported a reduction in birthweight and nephron number, the emergence of left ventricular hypertrophy, increased blood pressure and cardiac fibrosis at 6 months-of-age.[24] Growth-restricted female rats are born small and have nephron deficits, but do not develop hypertension, highlighting sex-specific disease programming associated with growth restriction.[8,25,26] Importantly, we have also shown in this model, that the growth-restricted male offspring present with a 28% reduction in cardiomyocyte number at postnatal day 7.[27] Recently, we reported fetal nephron deficits were restored at postnatal day 35 in F2 restricted male and female offspring, and only restricted male offspring developed hypertension in adult life.[8]

The aim of the present study was to examine the effect of uteroplacental insufficiency on cardiac structure, blood pressure, and cardiomyocyte number, size and nuclearity in the hearts of the F1 and F2 male offspring through the maternal line. We examined cardiomyocyte growth at day 35, a time-point when cardiomyocytes would have ceased proliferation. In general, in the rat heart the cardiomyocytes continue to proliferate in the first week or so after birth, and then they undergo a process of maturation and binucleation, which allows easy identification of mature cardiomyocytes.

## METHODS

### Experimental protocol and animal generation

The present study was approved by The University of Melbourne Animal Ethics Experimentation committee before all experimental procedures. Female Wistar-Kyoto (WKY) rats (F0 generation) were mated between 18 and 24 weeks-of-age. On day 18 of gestation, rats underwent either sham or bilateral uterine vessel ligation surgery to generate control or growth-restricted offspring, respectively.[28] On day 22 of gestation (term), rats gave birth naturally to F1 offspring. F1 control and growth-restricted female rats were mated between 18 and 24 weeks-of-age with normal males. The F1 pregnant rats gave rise to F2 control and restricted offspring, which were delivered naturally on day 22 of gestation without any interventions during pregnancy.[29] F1 and F2 control and restricted male offspring, one male from each litter at day 35 (weaning period), were studied for cardiac structure and cardiomyocyte number ($n$ = 6–10/group/generation). In separate sibling cohorts, systolic blood pressure was measured in F1 and F2 control and restricted male offspring at 2, 4 and 6 months-of-age ($n$ = 6/group/generation).

### Growth profiles, blood pressure and tissue collection

Weights of individual male pups were recorded at postnatal day 1 and at post-mortem on day 35 or 6 months-of-age. Dimension measures including crown rump length, head length, head width and hindlimb length were also recorded before post-mortem in the day 35 cohort of animals using digital venier calipers (accurate to 0.01 mm). In a separate cohort of F1 and F2 male offspring, at 2, 4 and 6 months-of-age, systolic blood pressure was assessed by tail cuff after training to the restraint procedure as previously described.[24] Male offspring from the F1 and F2 cohorts were used for analysis of cardiac structure and cardiomyocyte morphology at day 35, and blood pressure at 6 months-of-age. At termination, all animals were killed with an overdose mixture of ketamine (Parnell laboratories, Alexandria, NSW, Australia; 50 mg/kg bodyweight) and Ilium Xylazil-20 (Troy Laboratories, Smithfield, NSW, Australia; 10 mg/kg bodyweight). In the cohort euthanized at day 35, the hearts were retrogradely perfusion fixed through the abdominal aorta with 4% paraformaldehyde.[18,27] The hearts were excised, trimmed of fat and connective tissue, and stored in 10% buffered formalin. The researcher was blinded to the experimental treatment groups.

### Heart weights and left ventricular weight and volumes

Heart weights and left ventricular plus septum weights were recorded, absolute and relative to bodyweight, in the fixed hearts at day 35 and fresh hearts at 6 months-of-age. The separated left ventricles plus septum (day 35) were cut into 1-mm slices using a razor blade slicing device, and ventricle wall and chamber volume were estimated using the Cavalieri principle.[18,27] Every second slice of the left ventricle plus septum was embedded in glycolmethacrylate (Technovit 7100 resin; Heraeus Kulzer, Wehrheim, Germany) for the stereological estimation of the number of cardiomyocytes, and the remaining slices were embedded in paraffin wax for assessment of cardiomyocyte nuclearity and cross-sectional area.[18,27]

### Cardiomyocyte number estimation

The glycolmethacrylate blocks were serially sectioned at 20 $\mu$m (Leica Microsystem GmbH, Nusstosh, Wetzlar, Germany) and every 20th section (commencing at a randomly selected number between 1 and 20) was stained with haematoxylin. An optical disector/fractionator approach as described in detail previously was used to estimate the number of cardiomyocyte nuclei in the left ventricle plus septum utilizing the stereological CASTGRID

program (Olympus, Albertslund, Copenhagen, Denmark).[18,27,30] The cardiomyocyte nuclei were easily distinguishable from other cells (e.g. endothelial cells and fibroblasts) by their elongated oval shape, light purple staining, visible chromatin and prominent nucleoli. The total number of cardiomyocyte nuclei was determined using the equation: $N_{\text{cardiomyocytes}} = Q^{-} \times 1/f_1 \times 1/f_2 \times 1/f_3 \times 1/f_4$, where $Q^{-}$ was the number of cardiomyocyte nuclei counted using the optical disector, and $f_1$, $f_2$, $f_3$ and $f_4$ were the sampling fractions.[18,27] The total number of cardiomyocytes within the left ventricle plus septum was then determined by adjusting for the relative proportions of mononucleated, binucleated and multinucleated cardiomyocytes.

### Cardiomyocyte nuclearity and cross-sectional area

The proportion of mononucleated and binucleated cardiomyocytes in the left ventricle plus adjoining septum was examined using confocal microscopy in 40-$\mu$m fluorescently labeled stained paraffin sections as previously described.[19,27] In brief, wheat germ agglutinin-Alexa Fluor 488 conjugate (Invitrogen, Mulgrave, Vic., Australia) was used to stain cell boundaries and 4′6-diamidino-2-phenylindole, dihydrochloride (DAPI) was used to stain cell nuclei (Invitrogen). Sections were systematically sampled, and utilizing 3-D software (Imaris Version 6.1/6.2; Bitplane, Zurich, Switzerland) the number of nuclei within at least 200 cardiomyocytes in each heart were examined. In 5-$\mu$m paraffin sections stained with wheat germ agglutinin-Alexa Fluor 488 (Invitrogen), the cell boundaries of cardiomyocytes in cross-section were traced, and the cross-sectional area determined using NIS-Elements software (Nikon, Kawasaki, Japan). Only cardiomyocytes in cross-section, where the nuclei could be seen in the center of the cell, were analyzed. The cross-sectional area of approximately 200 cardiomyocytes from each heart was measured.[19,27]

### Statistical analysis

Data were analyzed using two-way analysis of variance (ANOVA) with SPSS (SPSS, Chicago, IL, USA) to identify main effects of experimental groups (control and restricted) and generations (F1 and F2). If an interaction was present, data was split and analysed using an unpaired Student's *t*-test to determine where statistical significances existed. The level of significance was set at $P < 0.05$, and all data are presented as mean ± standard error of the mean.

## RESULTS

### Growth profiles

At postnatal day 1, average bodyweight was reduced by 12% in F1 male restricted offspring compared with the controls ($P < 0.05$; Fig. 1a). There were no differences in bodyweight at postnatal day 1 between groups in the F2 offspring, but F2 restricted offspring were heavier than F1 restricted offspring ($P < 0.05$; Fig. 1a). These findings were maintained at day 35 (Fig. 1b). Crown to rump length, leg length and head width measures were not different between groups or generations of male offspring on day 35; however, head length was reduced in the F2

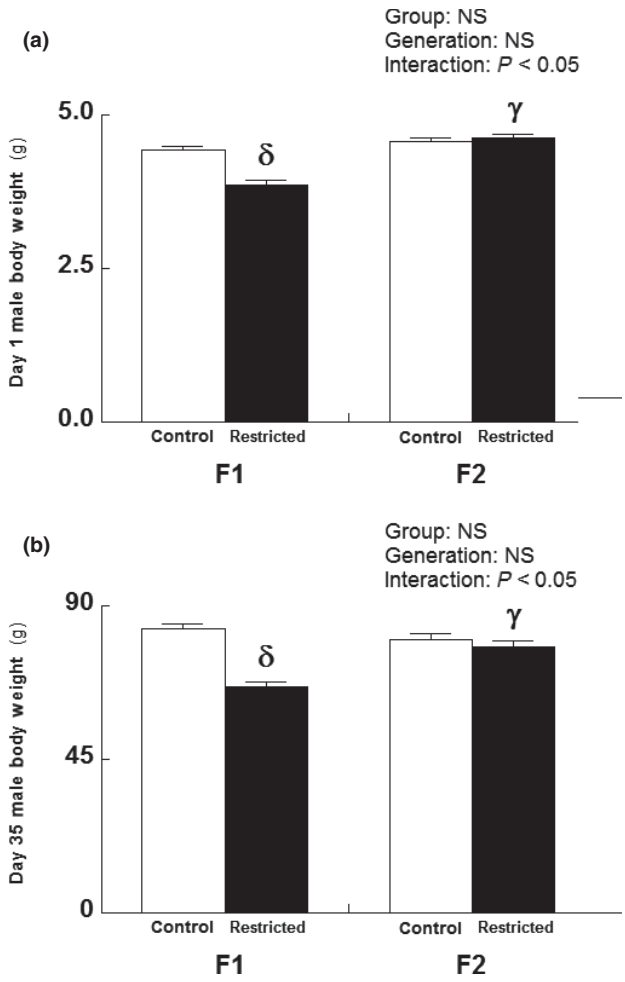


**Fig. 1** First generation (F1) and second generation (F2) male bodyweights. Bodyweights on (a) postnatal day 1 and on (b) day 35. All data are expressed as mean ± SEM; $n$ = 10/group/generation. $^{\delta}P < 0.05$ versus controls (after significant interaction) and $^{\gamma}P < 0.05$ versus generation (after significant interaction). NS, not significant.

male offspring compared with F1 males ($P < 0.05$; Table 1). By 6 months-of-age, there were no differences in bodyweight between control and restricted offspring in the F1 and F2 generations. However, the F2 offspring were heavier compared with the F1 offspring ($P < 0.05$; Table 1).

### Cardiac morphology at day 35

Relative heart weight on day 35 was increased by 9% in the F1 restricted group and by 16% in the F2 restricted group compared with their respective controls ($P < 0.05$; Fig. 2a). Relative left ventricular weight was increased by 11% in F1 restricted and by 22% in F2 restricted offspring compared with respective controls ($P < 0.05$; Fig. 2b). Relative left ventricular wall volume, determined using the Cavalieri principle, was increased by 17–21% in F1 restricted and F2 restricted offspring compared with controls ($P < 0.05$; Fig. 2c). There were no significant differences in left ventricular chamber volume between F1 and F2 offspring (data not shown). Absolute left ventricular cardiomyocyte number was not different between control and restricted offspring in the F1 or

**Table 1** First generation and second generation male bodyweights and dimensions

| Day 35 | F1 | | F2 | | 2-way ANOVA | | |
|---|---|---|---|---|---|---|---|
| | Control | Restricted | Control | Restricted | Group | Generation | Interaction |
| Crown rump length (mm) | 109 ± 0.79 | 100 ± 0.99 | 109 ± 1.06 | 107 ± 1.04 | NS | NS | NS |
| Head length (mm) | 39.81 ± 0.51 | 32.78 ± 0.34 | 33.06 ± 0.21 | 32.02 ± 0.17 | NS | $P < 0.05$ | NS |
| Head width (mm) | 19.84 ± 0.28 | 18.09 ± 0.15 | 18.64 ± 0.18 | 18.34 ± 0.14 | NS | NS | NS |
| Leg length (mm) | 35.57 ± 0.46 | 33.41 ± 0.36 | 35.43 ± 0.24 | 35.12 ± 0.24 | NS | NS | NS |
| Binucleated cardiomyocytes (%) | 90 ± 5.30 | 93 ± 4.99 | 89 ± 8.12 | 89 ± 3.40 | NS | NS | NS |
| 6 months | | | | | | | |
| Bodyweight (g) | 293 ± 30.85 | 340 ± 10.78 | 406 ± 5.37 | 396 ± 16.12 | NS | $P < 0.05$ | NS |
| Heart weight (%) | 0.36 ± 0.01 | 0.33 ± 0.01 | 0.33 ± 0.01 | 0.34 ± 0.01 | NS | NS | NS |
| Left ventricular weight (%) | 0.27 ± 0.01 | 0.24 ± 0.01 | 0.24 ± 0.004 | 0.24 ± 0.004 | NS | $P < 0.05$ | NS |

All data are expressed as mean ± SEM; $n = 10$/group/generation. $P < 0.05$ versus generation (main effect). F1, first generation; F2, second generation; NS, not significant.

F2 generations; however, F1 restricted offspring had a tendency to be reduced compared with controls, but this did not reach statistical significance (18%; Fig. 3a). The cross-sectional area of cardiomyocytes in F1 restricted offspring was reduced by 16% compared with controls ($P < 0.05$; Fig. 3b); however, no differences were apparent in the F2 offspring. In both the F1 and F2 control and restricted groups of offspring, ~ 90% of cardiomyocytes were binucleated (Table 1).

### Adult heart weight and blood pressure

There were no significant differences in relative heart weights between groups or generations at 6 months-of-age (Table 1). Relative left ventricular weight was not different between F1 and F2 control and restricted offspring; however, the F2 offspring were lighter than the F1 offspring at 6 months ($P < 0.05$; Table 1). There were no differences in blood pressure at 2 or 4 months-of-age in F1 and F2 restricted male offspring compared with controls (data not shown). At 6 months-of-age, there was a significant increase in blood pressure in F1 restricted offspring of 8 mmHg, and an increase of 15 mmHg in F2 restricted offspring compared with controls ($P < 0.05$; Fig. 4).

## DISCUSSION

The findings from the present study clearly demonstrate the transgenerational effects of fetal growth restriction caused by uteroplacental insufficiency, resulting in the developmental programming of left ventricular hypertrophy, and an elevation in blood pressure in both F1 and F2 restricted male progeny. Programming of left ventricular hypertrophy and hypertension in the F2 offspring occurred through the maternal line. These programming effects have long-term adverse implications for cardiovascular health in both generations, given that hypertension is a major risk factor for cardiovascular disease, and left ventricular hypertrophy is strongly associated with adverse cardiac health outcomes.

There are well-established links between low birthweight, as a result of IUGR, and an increased incidence of cardiovascular disease in adulthood.[1–3,31] However, insults during pregnancy do not always affect birthweight. Importantly in this regard, altered prenatal and postnatal growth trajectories increase relative long-term risk, with accelerated postnatal growth being independently linked to an increased propensity for adult disease.[32,33] In accordance with previous studies in our laboratory, as a result of bilateral uterine vessel ligation, there was a significant reduction in birthweight in the F1 restricted male offspring, but birthweights of the F2 restricted male offspring were not affected, even though the F2 offspring are likely to have experienced a compromised *in utero* environment.[8,34] We have previously reported that F1 growth restricted female rats are born small and have nephron deficits, but do not develop hypertension, highlighting sex-specific disease programming.[8,25,26] However, F1 restricted females have changes in the reactivity of the uterine artery,[35] and altered adaptations to pregnancy such that the dams develop glucose intolerance during late gestation,[29] consistent with other transgenerational studies.[32,36,37] Hence, it is likely that the *in utero* environment of the F2 restricted progeny was compromised and quite different to the F2 controls, and it is important to note that postnatal growth of the F1 and F2 restricted progeny was different to their respective controls. The F1 and F2 male restricted offspring were lighter than controls at birth, but caught up in weight at 6 months-of-age, which is indicative of accelerated growth supported by our recent publications.[8,34] Female offspring were not assessed in the present study, as they do not develop hypertension or cardiac hypertrophy, and future studies might inform mechanisms that underlie the reported cardiac differences.

In the present study, there was induction of left ventricular hypertrophy and hypertension in the male F1 Restricted offspring, which is in line with our previous finding, and importantly, these adverse effects in the heart and on blood pressure were observed in the F2 restricted generation.[24] Interestingly, the induction of left ventricular hypertrophy in both the F1 and F2 generations appears to be independent of enhanced cardiomyocyte growth. In the F1 offspring, there was no significant difference in the number of cardiomyocytes in the hypertrophied left ventricles of the restricted and control offspring, and cardiomyocyte cross-sectional area was significantly reduced, which is suggestive of a decrease in cardiomyocyte size. In the F2 generation, there was no significant difference in cardiomyocyte number or size in the hypertrophied left ventricles of the restricted offspring. In accordance with these findings, there was a significant

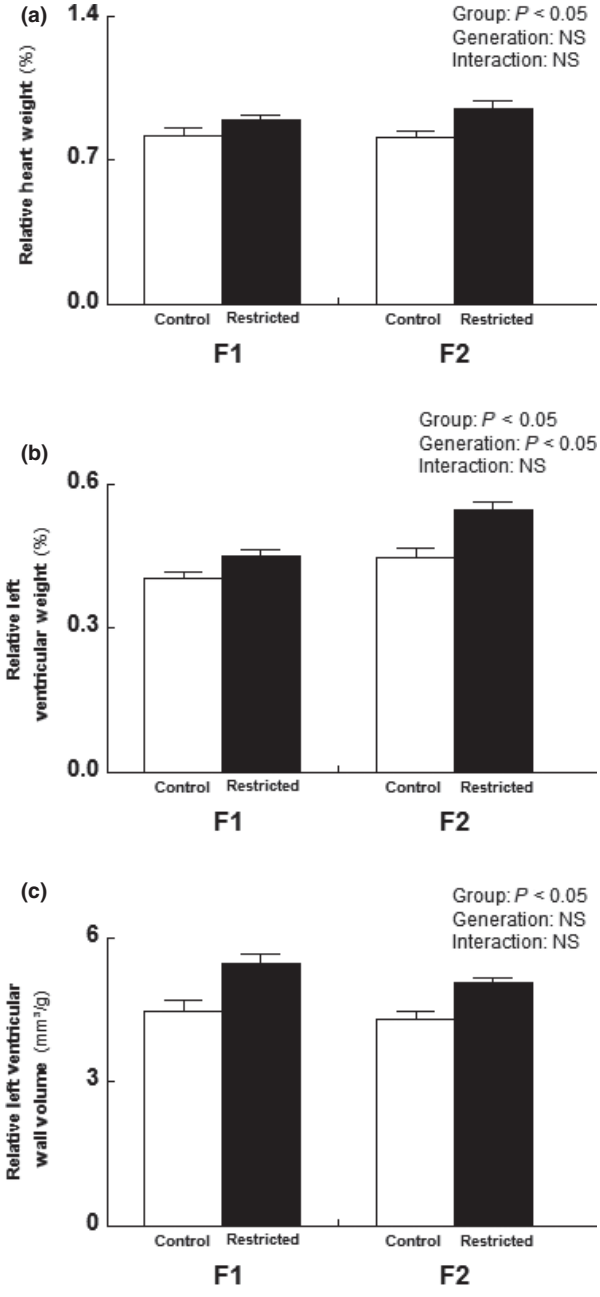


**Fig. 2** First generation (F1) and second generation (F2) male day 35 cardiac structure. (a) Relative heart weight, (b) relative left ventricular weight and (c) relative left ventricular wall volume in male offspring on day 35. All data are expressed as mean ± SEM; $n$ = 10/group/generation. $P < 0.05$ versus controls (main group effect) and $P < 0.05$ versus generation (main generation effect). NS, not significant.

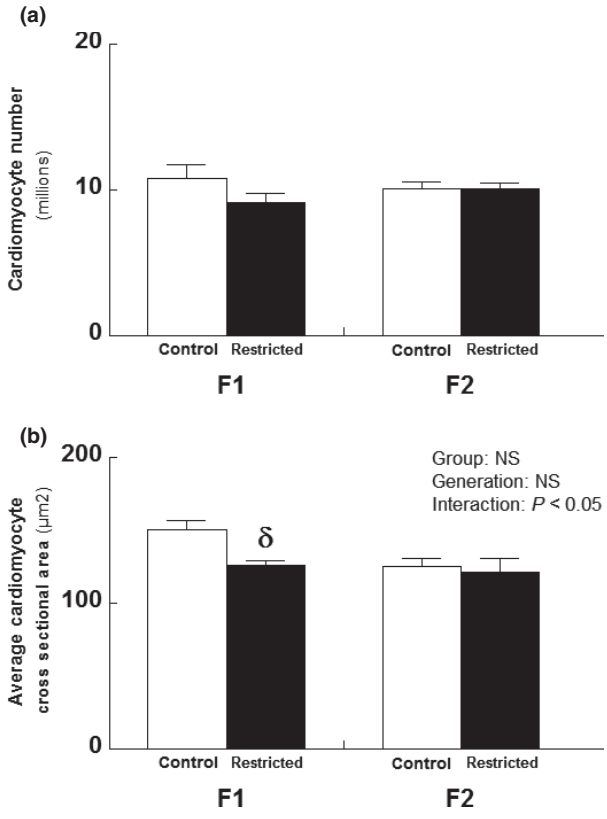


**Fig. 3** First generation (F1) and second generation (F2) male day 35 cardiomyocyte number and area. (a) Left ventricular cardiomyocyte number and (b) cardiomyocyte cross-sectional area in male offspring on day 35. All data are expressed as mean ± SEM; $n$ = 6/group/generation. $P < 0.05$ versus controls (main group effect) and $^{\delta}P < 0.05$ versus controls (after significant interaction).

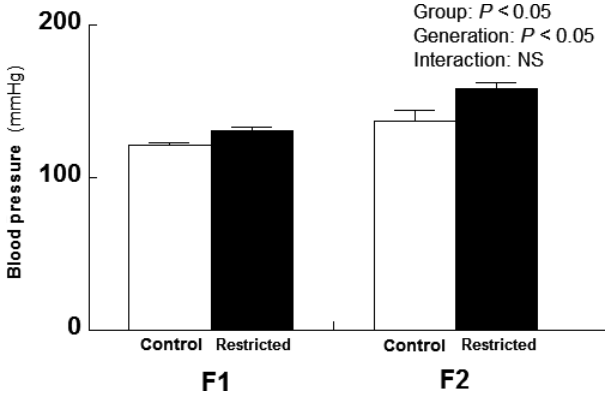


**Fig. 4** First generation (F1) and second generation (F2) male 6-month blood pressure. All data are expressed as mean ± SEM; $n$ = 6/group/generation. $P < 0.05$ versus controls (main group effect) and $P < 0.05$ versus generation (main generation effect).

decrease in the density of cardiomyocytes in the myocardium (the number of cardiomyocytes per volume of myocardium) of both the F1 and F2 offspring, which likely leads to impaired contractility of the cardiac muscle. The F2 restricted male offspring seem to show a greater degree of left ventricular hypertrophy compared with the F1.

The question therefore arises: how is the left ventricular hypertrophy mediated in the F1 and F2 restricted offspring? Given the lack of stimulatory effects on cardiac muscle growth, it appears that the left ventricular hypertrophy could be mediated by extracellular matrix deposition. In support of this suggestion, an increase in collagen deposition and altered biochemical composition of the myocardium has been reported in rat offspring (F1 progeny) that were growth restricted in early life as a result of maternal protein restriction.[19] Also, in the rodent model of maternal protein

restriction, there is induction of left ventricular hypertrophy by middle age in the growth-restricted F1 offspring, even though blood pressure is not always elevated.[19,27,38] In future studies, it would be beneficial to compare the extracellular matrix deposition in the myocardium of our F1 and F2 restricted offspring. Indeed, we have previously reported, at 6 months-of-age in F1 restricted male offspring, an increase in COLL3, transforming growth factor-$\beta$ and $\beta$-myosin heavy chain, which certainly implicates cardiac ventricular remodelling and deposition of extracellular matrix within the myocardium of the F1 offspring.[26]

It is possible that the induction of left ventricular hypertrophy observed in the F1 and F2 progeny is an adaptive response of the heart to an increase in haemodynamic load. Certainly, when blood pressure was measured at 6 months-of-age, there was an increase in the F1 and F2 restricted offspring consistent with other transgenerational studies where blood pressure is increased in adult life in two generations of offspring.[8,13–15] To the contrary, however, there was no detectable increase in blood pressure before 6 months-of-age in both generations of restricted offspring, which does not support the concept that left ventricular hypertrophy is haemodynamically mediated at day 35. Hence, the present data suggest ventricular remodelling events might be occurring first, impacting on blood pressure later in life. Importantly, the transgenerational effects observed in the present study appear to be mediated by the maternal line, and might be linked to heritable changes as a result of an altered *in utero* environment. In order to decipher between germ-line changes or abnormal pregnancy adaptations, as mechanisms of transmission, assessment of the paternal line is necessary. In future studies, this should be explored.

Our current findings relating to the number of cardiomyocytes in the hearts of restricted offspring appear to be in conflict with our previous report where we reported a significant reduction in the number of cardiomyocytes in F1 restricted offspring. In our previous study, we examined cardiomyocyte number in F1 restricted male offspring at 7 days-of-age.[27] Interestingly, in that study when we quantified the nuclearity of the cardiomyocytes, we found that approximately 30% were still mononucleated in both the restricted offspring and control offspring at postnatal day 7.[27] Given that mature, terminally differentiated cardiomyocytes are binucleated, this implies that there was a subset of cardiomyocytes at postnatal day 7 that were still capable of division. Hence, if this is the case, it provides a plausible explanation of how the complement of cardiomyocytes was not different between groups at 35 days-of-age in the present study, but was reduced at 7 days-of-age in our earlier study. In support of this idea, we have previously observed an apparent 'catch-up' hyperplasia in F1 offspring exposed to maternal protein restriction, where maternal nutrition was normalized at 2 weeks postnatally.[18,19] At birth, there was a significant reduction in the number of cardiomyocytes in the growth restricted offspring, whereas at 4 weeks-of-age there was no difference compared with the controls.[18,19] Hence, the current findings, in combination with recent publications, suggest that F1 male and female restricted offspring in the first week of life present with a reduction in overall cardiomyocyte number with the complement of cardiomyocytes restored to normal during the postnatal suckling period.[18,19,27] If cardiomyocyte number was assessed at birth, or shortly after birth, in the F2 restricted male offspring, it can then be determined whether or not cardiomyocyte number was also restored in the same way as F1 restricted offspring. The mechanisms underpinning this catch up cardiomyocyte hyperplasia are not well understood, but might involve alterations in the renin–angiotensin–aldosterone system, extracellular matrix, vascular responsiveness or sympathetic innervation, which we and others have reported in similar models.[8,28,39–41] It was not possible to carry out molecular or protein analysis in the current study, as the whole heart was used for cardiomyocyte number estimation and, as a result, no frozen tissue was available.

Importantly, the transgenerational effects observed in the present study appear to be mediated by the maternal line, and might be linked to heritable epigenetic changes as a result of an altered *in utero* environment. As the F2 offspring were derived from F1 restricted females, the transgenerational effects occurred through the maternal line. We have also previously shown that F1 restricted female rats exhibit changes in the reactivity of the uterine artery, and experience altered adaptations to pregnancy such that they become glucose intolerant during late pregnancy.[29] This suggests that offspring born from growth restricted mothers develop in a suboptimal environment; this provides an alternative mechanism for the growth restriction phenotype in the next generation. Future studies should be aimed at examining whether there is also transmission of an adverse cardiovascular phenotype by the paternal line of transmission. This would help to identify if the maternal environment alone or epigenetic mechanisms, or a combination of the two are responsible for transgenerational programming of diseases to the next generation.

In conclusion, this is the first study to describe transgenerational programming of left ventricular hypertrophy and hypertension as a result of intrauterine growth restriction caused by uteroplacental insufficiency. The transmission of hypertension and left ventricular hypertrophy to both the F1 and F2 generations early in life is of utmost clinical importance, given that hypertension is a major risk factor for cardiovascular disease, and left ventricular hypertrophy is the most important prognostic indicator of adverse cardiovascular events.

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