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## CALL FOR PAPERS | *Mitochondrial Metabolism*

# Growth restriction in the rat alters expression of metabolic genes during postnatal cardiac development in a sex-specific manner

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**Wadley GD, McConell GK, Goodman CA, Siebel AL, Westcott KT, Wlodek ME.** Growth restriction in the rat alters expression of metabolic genes during postnatal cardiac development in a sex-specific manner. *Physiol Genomics* 45: 99–105, 2013. First published December 11, 2012; doi:10.1152/physiolgenomics.00095.2012.—This study investigated the impact of uteroplacental insufficiency and growth restriction on the expression of genes related to mitochondrial biogenesis, glucose transport, and antioxidant defenses in cardiac tissue at embryonic day 20 (E20) and postnatal days 1, 7, and 35 in male and female Wistar rats (8–10 per group). Bilateral uterine vessel ligation to induce growth restriction (Restricted) or sham surgery was performed at pregnancy day 18. In male and female Controls, expression of most cardiac genes decreased during postnatal life, including genes involved in mitochondrial biogenesis regulation such as PGC-1 $\alpha$ , NRF-2, and mtTFA and the glucose transporter GLUT-1 ( $P < 0.05$ ). However, the pattern of gene expression during cardiac development differed in male and female Restricted rats compared with their respective Controls. These effects of restriction were observed at postnatal day 1, with female Restricted rats having delayed reductions in PGC-1 $\alpha$  and GLUT-1, whereas males had exacerbated reductions in PGC-1 $\alpha$  and mtTFA ( $P < 0.05$ ). By day 35, cardiac gene expression in Restricted hearts was similar to Controls, except for expression of the antioxidant enzyme MnSOD, which was significantly lower in both sexes. In summary, during postnatal life male and female Control rats have similar patterns of expression for genes involved in mitochondrial biogenesis and glucose transport. However, following uteroplacental insufficiency these gene expression patterns diverge in males and females during early postnatal life, with MnSOD gene expression reduced in later postnatal life.

fetal programming; heart; development; metabolism

LOW BIRTH WEIGHT IN HUMANS is a risk factor for the development of cardiovascular disease and hypertension in adulthood (4, 24), with a major cause of fetal growth restriction in the Western world being uteroplacental insufficiency (4, 24). Using well-established rodent models of uteroplacental insufficiency, our group, and others, have shown that growth restriction in prenatal and/or postnatal life adversely impacts on later metabolic and cardiovascular health in adulthood, including hypertension (27, 31, 32), cardiac hypertrophy (32), myocar-

dial insulin resistance (26), impaired glucose tolerance (20), reduced cardiomyocyte number (5), and reduced expression of genes regulating mitochondrial biogenesis (synthesis) in skeletal muscle (29).

Mitochondrial oxidative metabolism provides ~90% of the energy requirements for a normal healthy heart. The master regulator of mitochondrial biogenesis, peroxisome proliferator-activated receptor (PPAR)- $\gamma$  coactivator-1 $\alpha$  (PGC-1 $\alpha$ ) (35), is critical to the regulation of mitochondrial content in the heart (3, 11). PGC-1 $\alpha$  coactivates transcription factors, such as nuclear respiratory factor (NRF)-1 and -2 and PPAR $\alpha$  (18, 35). These transcription factors activate genes encoding oxidative enzymes (i.e., cytochrome oxidase, COX) and mitochondrial transcription factor-A (mtTFA), which in turn promotes mitochondrial DNA transcription, increasing mitochondrial synthesis (18, 35). Therefore, the transient upregulation of the PGC-1 $\alpha$ -dependent mitochondrial biogenesis pathway is thought necessary to increase the energy-producing capacity of the heart during early postnatal development (11). However, there are conflicting data as to whether PGC-1 $\alpha$  mRNA is upregulated at all during cardiac development of the rat (11, 23); while very little is known about the expression of other mitochondrial biogenesis genes (i.e., NRF-2, mtTFA, and COX) during this time. We have reported that uteroplacental insufficiency, which results in restriction of both prenatal and postnatal growth (Restricted) (29, 32, 33), reduces PGC-1 $\alpha$  expression and other genes involved with mitochondrial biogenesis, including mtTFA and COX in the skeletal muscle of adult rats (29).

PGC-1 $\alpha$  also plays a key role in the regulation of antioxidant enzymes, such as superoxide dismutase (SOD) (21). The mitochondrial manganese form of SOD (MnSOD) is an important reactive oxygen species detoxifying enzyme since it is implicated in cardioprotection from ischemia-reperfusion injury (14). However, the gene expression of MnSOD during cardiac development or the impact that growth restriction may have on its expression are yet to be defined.

Although fatty acid oxidation is the major source of energy for cardiac muscle in the adult heart, glucose metabolism is an important source of energy for contraction during ischemia, hypoxia, exercise, and the late stages of fetal development (for review, see Ref. 22). The key glucose transporters (GLUT) in

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the heart are GLUT-1 and GLUT-4 (9). The influence of growth restriction following uteroplacental insufficiency on the expression of these key cardiac glucose transporters during development is unknown. Importantly, adult female rats exposed to maternal and postnatal undernutrition have lower adult cardiac GLUT-1 and GLUT-4 gene expression (9), which may result in an impaired ability for energy provision from glucose during critical periods, such as ischemia.

Since we have previously observed marked sex differences in a range of metabolic and cardiovascular outcomes in adult rats following uteroplacental insufficiency (20, 29, 31), it is possible there could be similar sex-specific effects on the expression of genes in the mitochondrial biogenesis pathway, GLUTs and antioxidant enzymes during cardiac development in growth restricted rats.

For these aforementioned reasons, a major aim of this study was to examine if uteroplacental insufficiency reduces the expression of genes that regulate mitochondrial biogenesis, MnSOD and GLUT-1 and GLUT-4 in the heart during postnatal development. A further aim of this study was to determine if the pattern of expression in these metabolic genes during cardiac development due to growth restriction was different in males versus females.

## METHODS

**Animals.** All experiments were approved by The University of Melbourne Animal Experimentation Sub-Committee. Wistar Kyoto rats (9–13 wk of age) were obtained from the Australian Resource Centre (Murdoch, Western Australia, Australia) prior to mating. On *day 18* of gestation, pregnant rats underwent bilateral uterine vessel (artery and vein) ligation to induce growth restriction, as previously described (16, 31, 33), or sham surgery, which was identical except the uterine vessels were not ligated. We have previously shown that (Restricted) pups from uteroplacentally restricted mothers are prenatally and postnatally growth restricted compared with pups from sham-operated mothers (Controls) (29, 32, 33).

**Developmental timeline.** At *day 20* of gestation mothers that underwent either bilateral uterine vessel ligation or sham surgery were

anesthetized by intraperitoneal injection of Ketamine (50 mg/kg) and Ilium Xylazil-20 (10 mg/kg), and the uterus was exposed. Fetuses were weighed, separated by sex, and then killed by decapitation, and whole hearts were removed and pooled within litters. Similarly at *day 1* and *7* after birth, offspring were weighed, separated by sex, and then killed by decapitation, and individual whole hearts were collected. At *day 35* individual male and female offspring were anesthetized by intraperitoneal injection of Ketamine (225 mg/kg) and Xylazil-20 (30 mg/kg), the chest cavity was opened, and individual whole hearts were collected and weighed. At the time of postmortem all samples collected were frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$ . For tissue extraction, due to the small mass of the E20 hearts, two whole hearts were pooled from one litter to represent one sample (i.e.,  $n = 1$ ). For *day 1*, *day 7*, and *day 35* each sample (i.e.,  $n = 1$ ) represents a single whole heart, each from a separate litter. Hearts were crushed into a powder in liquid nitrogen, and then 20–30 mg was extracted for RNA and SOD activity.

**Gene expression.** Total RNA was extracted from frozen heart with TRIzol and DNase on-column digestion (Invitrogen, Melbourne, Australia) and reverse transcribed as previously described (28). Real-time PCR using SYBR Green chemistry was performed as previously described (Rotor-Gene v6, Corbett Research, Sydney, Australia) (29). Primer sequences for PGC-1 $\alpha$ , mtTFA, NRF-2, MnSOD, GLUT-4, and COX III have been described previously (28, 29), as have the primer sequences for the commonly used “housekeeping” gene ribosomal 18S (31). Primer sequences for GLUT-1 (NM\_138827) were 5'-GGCATCAATGCTGTGTTCTACTAC-3' and 5'-CGAGCCGATGGTGGCATAAC-3' and for the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH, NM\_017008.3), were 5'-AGACAGCCGCATCTTCTTGT-3' and 5'-TGATGGCAACAATGTC-CACT-3'. Relative quantification of gene expression was initially performed by the comparative CT ( $\Delta\Delta\text{CT}$ ) method using the housekeeping genes ribosomal 18S and GAPDH. However, the expression levels for ribosomal 18S and GAPDH were found to vary significantly across ages in both male and female hearts ( $P < 0.05$ , see RESULTS and Fig. 1). Therefore, the mRNA of each gene was normalized to the absolute cDNA content in each sample that was determined using an OliGreen assay with an oligonucleotide standard (Invitrogen) as previously described (28). This is a suitable method of normalization

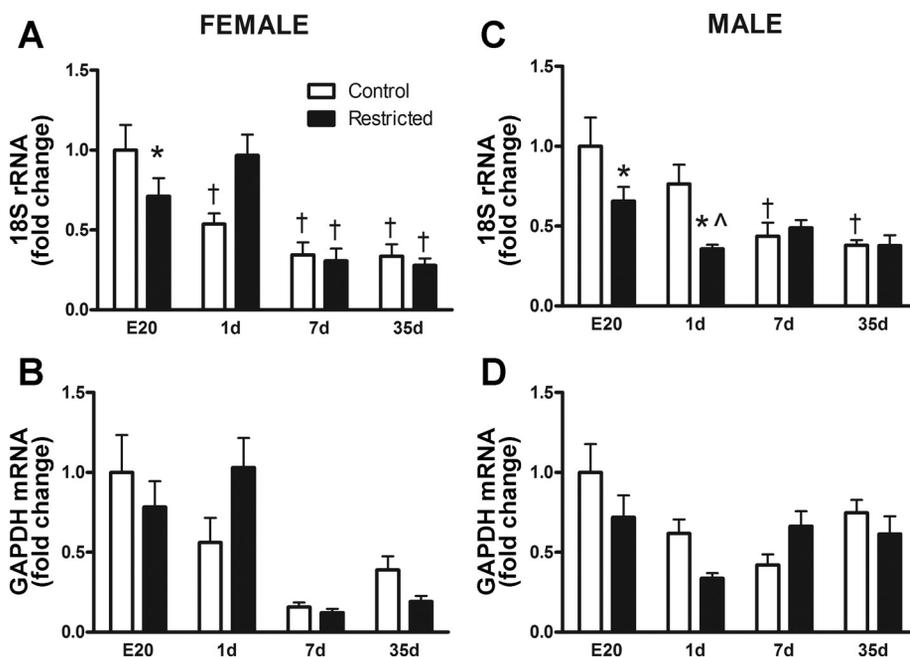


Fig. 1. Effect of uteroplacental insufficiency (Restricted) during cardiac development on expression of the housekeeping genes. Female ribosomal 18S (A), female GAPDH (B), male ribosomal 18S (C), male GAPDH (D). Values are means  $\pm$  SE;  $n = 8$ –10. E20, embryonic *day 20*; 1d, postnatal *day 1*. \* $P < 0.05$  vs. Control, † $P < 0.05$  vs. E20, ^ $P < 0.05$  vs. Female. Control, clear bars; Restricted, black bars.

that avoids the many problems associated with housekeeping genes (13, 28).

**Total SOD activity.** In the *day 7* and *35* hearts, 20  $\mu$ l per mg (volume/weight) of frozen tissue was homogenized in freshly prepared ice-cold buffer (100 mM NaH<sub>2</sub>PO<sub>4</sub>, at pH 7.0 containing 1 mM EDTA, 0.5% vol/vol Triton X-100). Tissue lysates were incubated on ice for 20 min and then spun at 16,000 g for 20 min at 4°C. Protein concentration was determined using a bicinchoninic acid protein assay (Pierce, Rockford, IL) with BSA as the standard. Just prior to analysis all samples were diluted to 100  $\mu$ g/ml of total protein in sample buffer (50 mM Tris-HCl pH 8.0). Total SOD activity was determined spectrophotometrically using a commercially available kit according to the manufacturer's instructions (Cayman Chemical).

**Statistical analyses.** Results were initially analyzed by a three-way ANOVA (treatment, sex, and time). If this analysis revealed a significant interaction, specific differences between mean values were located with Bonferroni post hoc analysis in SPSS. All gene expression data are presented as means  $\pm$  SE and expressed as a fold change relative to E20 Control. The level of significance was set at  $P < 0.05$ .

## RESULTS

**Growth profiles and heart mass.** Female and male Restricted rats had significantly lower body weight compared with Control rats at all ages studied, with no evidence of catch-up or accelerated growth ( $P < 0.05$ , Table 1). At E20 and postnatal *day 1*, there was no significant difference in absolute heart mass for male and female Restricted rats compared with Controls. Although relative heart mass was significantly higher in male and female Restricted rats at postnatal *day 7* compared with Control rats (Table 1), this was probably due to lower overall body mass since absolute heart mass was not different between Restricted and Control animals at *day 7* (Table 1).

**Expression of housekeeping genes.** The expression of ribosomal 18S and GAPDH in female and male rat hearts was generally highest at the late prenatal stage (E20) with a subsequent reduction in expression postnatally (Fig. 1,  $P < 0.05$ ). In female Restricted rats, however, cardiac ribosomal 18S was lower at E20 compared with Controls ( $P < 0.05$ , Fig. 1). For male Restricted rats, cardiac ribosomal 18S was significantly lower at E20 and postnatal *day 1* compared with Controls ( $P < 0.05$ , Fig. 1). In females, ribosomal 18S was significantly reduced from postnatal *day 1* onward in Control hearts and from postnatal *day 7* onward in Restricted rat hearts (Fig. 1). In

males, cardiac ribosomal 18S was significantly reduced from postnatal *day 7* onward in Controls (Fig. 1). Furthermore, at *day 1*, ribosomal 18S levels were significantly lower in Restricted males than females (Fig. 1). Since these commonly used endogenous reference or housekeeping genes were not stably expressed in the heart during early development in our study, cardiac gene expression was normalized to absolute cDNA levels, which was  $286 \pm 6$  ng/ml for all samples.

**Expression of mitochondrial biogenesis genes during cardiac development.** In general, the expression of genes involved with the regulation of mitochondrial biogenesis (PGC-1 $\alpha$ , NRF-2, mtTFA) in female and male hearts were significantly altered during early development, with expression being highest at E20 and a subsequent reduction in expression postnatally (Figs. 2 and 3, main effect for time,  $P < 0.05$ ).

Growth restriction also had a sex-specific effect in the expression pattern of several mitochondrial biogenesis genes in the heart. In particular, at *day 1*, cardiac PGC-1 $\alpha$  and mtTFA mRNA levels were significantly lower in Restricted males than females (Fig. 2 and 3, respectively). At *day 35*, cardiac mtTFA mRNA levels were significantly lower in male than female Controls (Fig. 3). There was also a tendency for an interaction between sex, treatment, and time for COX III ( $P = 0.061$ , Fig. 3, three-way ANOVA). In female hearts: compared with E20, PGC-1 $\alpha$  mRNA was significantly reduced from postnatal *day 1* onward in Controls and from postnatal *day 7* onward in Restricted rats (Fig. 2). Furthermore, in Restricted females, PGC-1 $\alpha$  mRNA was also significantly higher at *day 1* compared with Controls (Fig. 2). In male hearts: compared with E20, PGC-1 $\alpha$  was significantly reduced by postnatal *day 7* in Controls (Fig. 2C) and was significantly reduced from postnatal *day 1* in Restricted rats (Fig. 2C). Furthermore, in Restricted male rats, PGC-1 $\alpha$  and mtTFA gene expression levels were also significantly reduced at *day 1* compared with Control rats (Figs. 2 and 3).

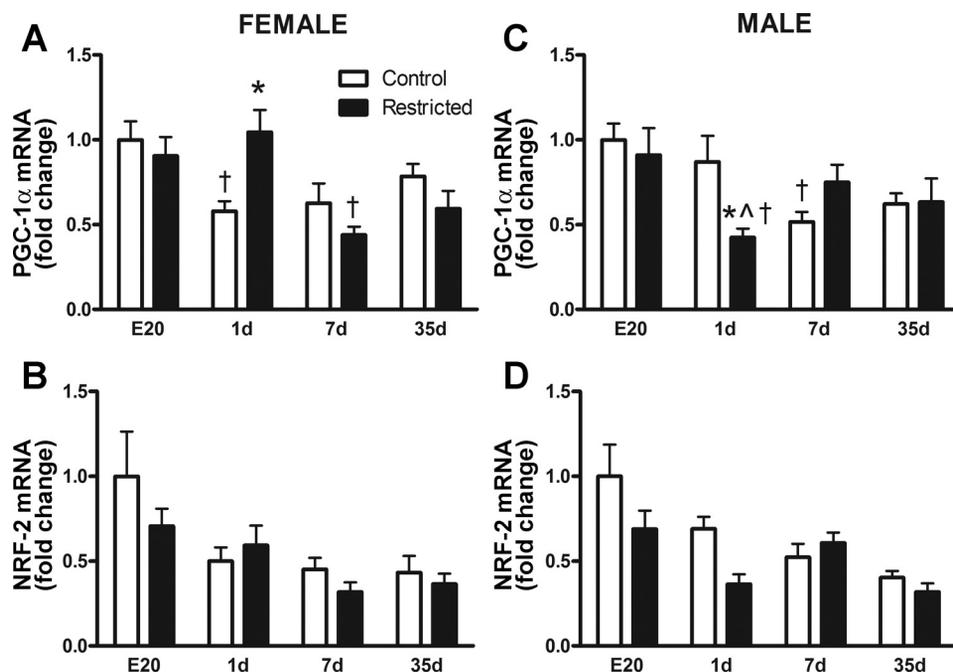
**MnSOD gene expression and total SOD activity during cardiac development.** In both male and female rats, MnSOD gene expression was significantly altered during cardiac development, with expression remaining low during late prenatal and early postnatal development and then increasing during late postnatal development on *day 35* ( $P < 0.05$ , Fig. 4, main effect for time). There was also a tendency for an interaction between sex,

Table 1. Growth profile and heart mass data

	E20	1d	7d	35d
Body mass, g				
Female Control	1.74 $\pm$ 0.04	4.04 $\pm$ 0.06	9.53 $\pm$ 0.26	71.71 $\pm$ 2.55
Female Restricted	1.46 $\pm$ 0.05 $\dagger$	3.52 $\pm$ 0.02 $\dagger$	7.00 $\pm$ 0.39 $\dagger$	61.97 $\pm$ 2.95*
Male Control	1.87 $\pm$ 0.06	4.28 $\pm$ 0.09	10.28 $\pm$ 0.27	81.43 $\pm$ 2.35
Male Restricted	1.51 $\pm$ 0.07 $\dagger$	3.49 $\pm$ 0.14 $\dagger$	6.95 $\pm$ 0.50 $\dagger$	64.40 $\pm$ 3.64* $\dagger$
Heart mass, mg				
Female Control	8.6 $\pm$ 0.8	20.6 $\pm$ 0.8	57.2 $\pm$ 1.5	336.2 $\pm$ 13.6
Female Restricted	7.6 $\pm$ 0.7	17.9 $\pm$ 0.9	54.1 $\pm$ 3.0	294.0 $\pm$ 22.5*
Male Control	8.9 $\pm$ 0.8	21.4 $\pm$ 0.1	57.9 $\pm$ 3.9	352.3 $\pm$ 15.4
Male Restricted	7.2 $\pm$ 0.6	16.0 $\pm$ 0.9 $\dagger$	49.6 $\pm$ 4.5	281.3 $\pm$ 11.0* $\dagger$
% Heart mass/body mass, g/g				
Female Control	0.49 $\pm$ 0.04	0.51 $\pm$ 0.02	0.60 $\pm$ 0.01	0.47 $\pm$ 0.02
Female Restricted	0.52 $\pm$ 0.03	0.51 $\pm$ 0.02	0.78 $\pm$ 0.02*	0.47 $\pm$ 0.03
Male Control	0.48 $\pm$ 0.05	0.50 $\pm$ 0.02	0.57 $\pm$ 0.02	0.43 $\pm$ 0.01
Male Restricted	0.48 $\pm$ 0.03	0.46 $\pm$ 0.03	0.71 $\pm$ 0.04*	0.44 $\pm$ 0.01

Values are means  $\pm$  SE,  $n = 6-10$  for all groups. E, embryonic day; d, postnatal day. \* $P < 0.05$  vs. Control (2-way ANOVA);  $\dagger P < 0.05$  versus Control (1-way ANOVA).

Fig. 2. Effect of uteroplacental insufficiency (Restricted) during cardiac development on gene expression of mitochondrial biogenesis markers: female peroxisome proliferator-activated receptor- $\gamma$  coactivator-1 $\alpha$  (PGC-1 $\alpha$ ) (A), female nuclear respiratory factor (NRF)-2 (B), male PGC-1 $\alpha$  (C), male NRF-2 (D). Values are means  $\pm$  SE;  $n = 8-10$ . \* $P < 0.05$  vs. Control, † $P < 0.05$  vs. E20, ^ $P < 0.05$  vs. Female. Control, clear bars; Restricted, black bars.

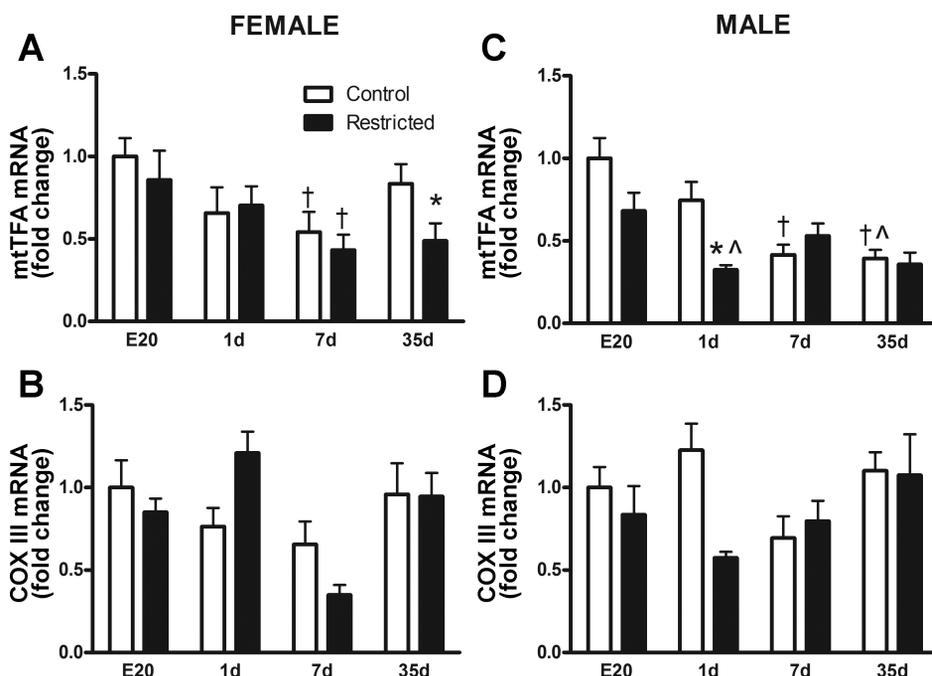


treatment, and time ( $P = 0.056$ , Fig. 4, three-way ANOVA). Furthermore, there was an overall reduction in cardiac gene expression of MnSOD at *day 35* for both Restricted male and female rats compared with Controls ( $P < 0.05$ , Fig. 4, treatment  $\times$  time). Consistent with the increased MnSOD gene expression at *day 35*, there was an overall increase in total SOD activity from *day 7* to *day 35* in both male and female hearts ( $P < 0.05$ , main effect for time, Fig. 4). Interestingly, Restricted female rats had higher overall cardiac total SOD activity compared with the Controls ( $P < 0.05$ , treatment  $\times$  sex). There was no significant difference in total SOD activity between male Control and Restricted hearts.

*Expression of GLUT-1 and GLUT-4 during cardiac development.* Cardiac gene expression of GLUT-1 for both male and female rats were highest in late prenatal development and then gradually declined throughout postnatal development (Fig. 5,  $P < 0.05$ ). In contrast, the gene expression of GLUT-4 for both male and female rats remained low during late prenatal and early postnatal development and then increased by *day 35* (Fig. 5, main effect for time  $P < 0.05$ ).

Growth restriction also resulted in clear sex differences in the expression patterns of GLUT-1. At *day 1*, GLUT-1 gene expression was significantly lower in Restricted males than females (Fig. 5). In female hearts: GLUT-1 gene expression

Fig. 3. Effect of uteroplacental insufficiency (Restricted) during cardiac development on gene expression of mitochondrial biogenesis markers; female mitochondrial transcription factor-A (mtTFA, A), female COX (cytochrome oxidase) III (B), male mtTFA (C), male COX III (D). Values are means  $\pm$  SE;  $n = 8-10$ . \* $P < 0.05$  vs. Control, † $P < 0.05$  vs. E20, ^ $P < 0.05$  vs. Female. Control, clear bars; Restricted, black bars.



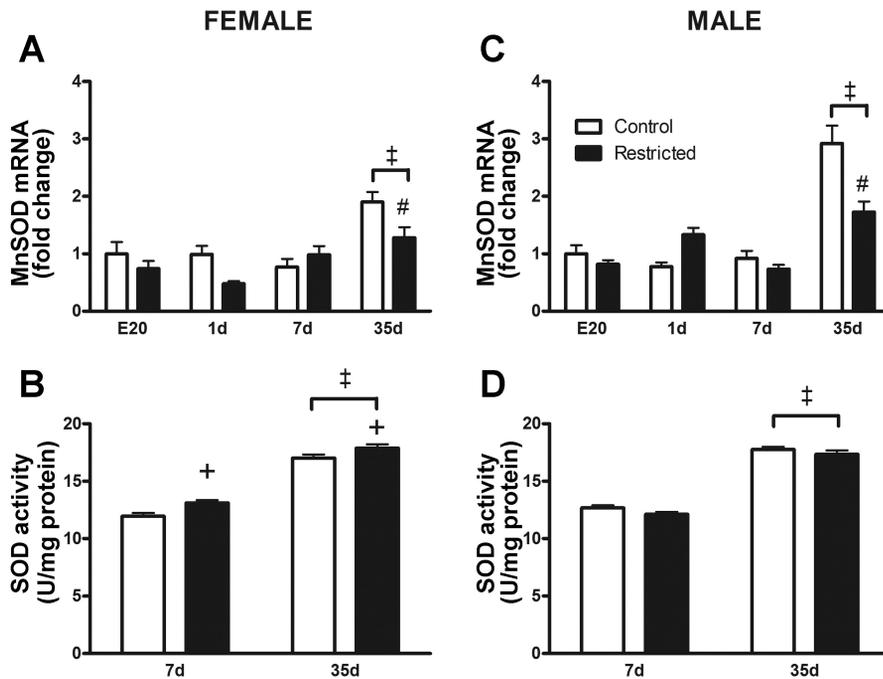


Fig. 4. Effect of uteroplacental insufficiency (Restricted) during cardiac development on gene expression of manganese form of superoxide dismutase (MnSOD) and total SOD activity in female (A and B, respectively) and male (C and D, respectively) offspring. Values are means  $\pm$  SE;  $n = 8-10$ . ‡ $P < 0.05$  main effect of time, # $P < 0.05$  treatment  $\times$  sex. Control, clear bars; Restricted, black bars.

was significantly higher in Restricted rats at *day 1*, although there were no significant differences in GLUT-1 gene expression at *day 35* (Fig. 5). In male hearts: GLUT-1 gene expression was significantly lower at E20 in Restricted rats (Fig. 5).

#### DISCUSSION

A major finding of the present study was that in normal (nongrowth-restricted) rats the cardiac metabolic gene expression profile of male and female rats follows a very similar pattern during cardiac development. In these Control rats, genes involved in mitochondrial biogenesis (PGC-1 $\alpha$  and NRF-2) and basal glucose transport (GLUT-1) were highly

expressed in late prenatal development (E20), with a subsequent reduction in expression thereafter. Other genes involved in antioxidant defenses (MnSOD) and insulin/contraction stimulated glucose transport (GLUT-4) had low expression levels at E20 and then increased by later postnatal development (*day 35*) in Controls. However, in contrast to the similar patterns of gene expression during development in male and female Control rats, a major finding of this study was that growth restriction resulted in divergent responses in cardiac gene expression for male and female Restricted rats, particularly at postnatal *day 1*. Female Restricted rats had a significant delay in the reduction of PGC-1 $\alpha$  and GLUT-1 gene expression, whereas

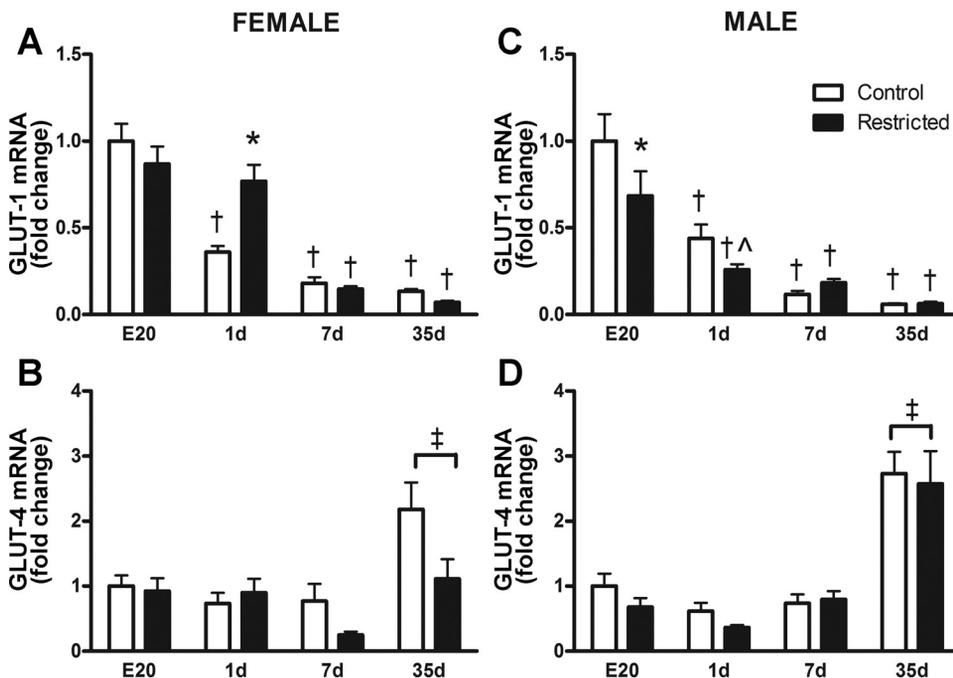


Fig. 5. Effect of uteroplacental insufficiency (Restricted) during cardiac development on gene expression of glucose transporters; female glucose transporter (GLUT)-1 (A), female GLUT-4 (B), male GLUT-1 (C), male GLUT-4 (D). Values are means  $\pm$  SE;  $n = 8-10$ . \* $P < 0.05$  vs. Control, † $P < 0.05$  vs. E20, ^ $P < 0.05$  vs. Female. ‡ $P < 0.05$  main effect of time. Control, clear bars; Restricted, black bars.

the males tended to have an earlier reduction in cardiac gene expression during development.

The present study observed that MnSOD gene expression was relatively stable in late prenatal and early postnatal life, with both MnSOD gene expression and total SOD activity increasing after 7 days of age. Furthermore, the present study found lower MnSOD gene expression in Restricted rats of both sexes at 35 days, although total SOD activity was not impaired. We have previously reported a tendency ( $P = 0.09$ ) for a 25% increase in cardiac oxidative stress in the hearts of 6 mo old Restricted (versus Control) male, but not female rats (30). Surprisingly, the present study found female Restricted rats actually had higher cardiac total SOD activity than Controls, suggestive of a compensatory protective effect in the female Restricted heart. Collectively, these findings suggest that despite significantly reduced gene expression of MnSOD in the hearts of young Restricted rats, the expression is not sufficiently reduced to impair total SOD activity, nor does it lead to the significant development of cardiac oxidative stress in early adulthood (6 mo old) (30). However, the normal aging process significantly increases oxidative stress in the hearts of rodents from 15 mo of age (25). Thus, any underlying impairment(s) in cardiac antioxidant defenses could likely exacerbate the cardiac oxidative stress normally observed with aging. Therefore, further studies using 15 mo old Restricted rats are now required. Furthermore, changes in cardiac MnSOD gene expression are linked to functional outcomes, since reductions in MnSOD gene expression are closely matched by reductions in enzyme activity during congestive heart failure in rodents (10). It is also well established that reduced antioxidant defenses in the heart leave it vulnerable to augmented ischemia-reperfusion injury (6, 7). Therefore, the findings of impaired MnSOD gene expression in the present study highlight the potential susceptibility of the heart to reduced antioxidant defenses following growth restriction at a much later age of life. Further work is now required to investigate the functional consequences for postnatal and adult heart health.

Interestingly, ribosomal 18S and GAPDH, which are commonly used housekeeping genes, followed an overall pattern whereby expression levels were highest in late prenatal life (E20) and are then reduced during postnatal development. This reduction in ribosomal 18S is consistent with a postnatal decline in cardiac ribosomal biogenesis in the rat (12). The decline in GAPDH expression during postnatal cardiac development is most likely due to the reduction in glycolysis, which is an important source of energy for the heart during fetal development and at birth, with glycolysis then declining to very low levels within a few days of birth (reviewed in Ref. 22). Nevertheless, these findings highlight the limitation of many commonly used housekeeping genes for normalizing gene expression across the prenatal and postnatal stages of cardiac development. Because of this we used the well-established method of normalizing to the cDNA levels in each sample (13, 28).

The biological significance of the differential transcriptional response pattern to growth restriction in male and female hearts is unclear. The sex-specific impact of growth restriction on genes involved in cardiac metabolism is largely unknown, although the developmental programming of adult disease following uteroplacental insufficiency or maternal undernutrition consistently show males to be more adversely affected than females (1, 31, 32, 34). Although speculative, a delayed reduction in transcription of

genes involved with metabolism at birth may allow the females to minimize any adverse effects of growth restriction in the heart. Indeed, our group and others have previously shown that there are sex-specific effects of prenatal and postnatal growth restriction in the adult heart, with males exhibiting hypertension (1, 15, 31, 32, 34) and left ventricle hypertrophy (32), while females do not.

Estrogen is considered a candidate to explain sex-specific effects of fetal programming, although its exact role remains poorly defined. Bolus administration of estrogen to female rats in the first few hours following birth programs for skeletal muscle insulin resistance and lower GLUT-4 mRNA in adulthood (2); however, little is known about its effects on the Restricted female heart. Also, there are no differences in fetal plasma estrogen and progesterone levels in the low protein model of growth restriction, despite maternal sex steroid levels being elevated with restriction (8). Furthermore, although estrogen can also protect against the onset of adult hypertension in the female growth-restricted rat (17), much of this protective effect can be attributed to estrogen production following puberty (17), which occurs much later than the time-course under investigation in the current study. Therefore, it's unlikely that estrogen is playing a significant role in the sex-specific differential responses during these prenatal and postnatal developmental stages.

Glucose uptake, primarily for glycolysis, is a significant source of energy during the fetal and early postnatal (*day 1*) stages of cardiac development (22). Consistent with previous findings in normal (nongrowth-restricted) rats (19), GLUT-1 gene expression during cardiac development was highest during prenatal life and progressively diminished to stable levels within several days following birth, whereas GLUT-4 gene expression was relatively stable during prenatal and early postnatal life and then increased several days after birth (19). The impact of growth restriction on GLUT-1 and GLUT-4 during cardiac development in males has not been previously investigated. Our findings of relatively normal GLUT-1 and GLUT-4 gene expression pattern in Restricted males, but not females, during cardiac development highlight clear sex differences and suggest that the transcriptional regulation of glucose transporters is more vulnerable to Restriction in the female than the male developing heart.

In summary, the expression of genes involved with mitochondrial biogenesis and basal glucose transport (GLUT-1) progressively decline, while GLUT-4 increases during postnatal cardiac development with similar responses, irrespective of sex. However, superimposing growth restriction results in divergent sex-specific effects in the expression pattern of genes involved with mitochondrial biogenesis and glucose transport, with females having a delayed reduction and males an accelerated reduction in these genes during postnatal cardiac development. Growth restriction following uteroplacental insufficiency also programs reduced cardiac gene expression of the key antioxidant enzyme MnSOD in male and female offspring. These findings may have implications for later cardiovascular health for offspring born small and those susceptible to ischemia, hypoxia, and oxidative stress.

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## DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

## AUTHOR CONTRIBUTIONS

Author contributions: G.D.W., G.K.M., A.L.S., and M.E.W. conception and design of research; G.D.W., C.A.G., and K.T.W. performed experiments; G.D.W., C.A.G., and A.L.S. analyzed data; G.D.W., G.K.M., C.A.G., A.L.S., K.T.W., and M.E.W. interpreted results of experiments; G.D.W. prepared figures; G.D.W., G.K.M., and M.E.W. drafted manuscript; G.D.W., G.K.M., C.A.G., A.L.S., K.T.W., and M.E.W. edited and revised manuscript; G.D.W., G.K.M., C.A.G., A.L.S., K.T.W., and M.E.W. approved final version of manuscript.

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