Fetal origins of adult cardiac disease: a novel approach to prevent fetal growth restriction induced cardiac dysfunction using insulin like growth factor

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BACKGROUND: Fetal growth restriction (FGR) is a risk factor for adult cardiovascular disease. Intraplacental gene transfer of human insulin-like growth factor-1 (IGF-1) corrects birth weight in our mouse model of FGR. This study addresses long term effects of FGR on cardiac function and the potential preventive effect of IGF-1.

STUDY DESIGN: Laparotomy was performed on pregnant C57BL/6J mice at embryonic day 18 and pups were divided into three groups: Sham operated; FGR (induced by mesenteric uterine artery ligation); treatment (intraplacental injection of IGF-1 after uterine artery ligation). Pups were followed until 32 wk of life. Transthoracic echocardiography was performed starting at 12 wk.

RESULTS: Systolic cardiac function was significantly impaired in the FGR group with reduced fractional shortening compared with sham and treatment group starting at week 12 of life (20 ± 4 vs. 31 ± 5 vs. 32 ± 5, respectively, $n = 12$ for each group; $P < 0.001$) with no difference between the sham and treatment groups.

CONCLUSION: Intraplacental gene transfer of IGF-1 prevents FGR induced cardiac dysfunction. This suggests that *in utero* therapy may positively impact cardiac remodeling and prevent adult cardiovascular disease.

Fetal growth restriction (FGR) remains a significant cause of perinatal morbidity and mortality, with effects extending beyond the immediate perinatal period (1). In FGR the deficiency of necessary factors in the fetal environment will lead to fetal adaptive responses that involve structural, physiological, and metabolic changes influencing health later in life (2). Such adaptive responses have been termed "fetal programming" with the implication that environmentally driven changes in the fetal genetic program ultimately results in phenotypic changes that persist beyond fetal development (2).

In the late 1980s David Barker *et al.*, published his first study that demonstrated an association between FGR and the risk of obesity and cardiovascular disease in adults (3,4). Since that time, his epidemiologic observations of this relationship has been validated and extensively replicated (5). In infants and children FGR causes systolic cardiac dysfunction with decreased stroke volume and cardiac output (6–8). In adults, FGR is a risk factor for obesity, coronary artery disease, and heart failure (9). Multiple animal models have been developed to study the effect of FGR and fetal programming on the heart (10), with placental insufficiency via uterine artery ligation or embolization emerging as a reliable method for induction of FGR (10,11). Similar to its consequences in humans, FGR in mice also results in systolic dysfunction (12–14). Transthoracic echocardiography has been used to evaluate the consequences of FGR on cardiac function as this method is quantitative, noninvasive, and may be performed serially on the same animal allowing longitudinal assessment over the life of the animal (8,12,13).

The insulin like growth factor 1 (IGF-1) axis has been proposed as a potential mechanism linking FGR with adult cardiac dysfunction, as both conditions manifest abnormalities in this pathway. In FGR, multiple studies confirm that IGF-1 plays a central role in fetal and placental growth; furthermore, decreased levels of IGF-1 are found in growth restricted fetuses and newborns (15,16). IGF-1 induces physiological heart growth, enhances cardiac contractility and appears to be protective against cardiovascular disease due to its antiapoptotic and antifibrotic properties in the heart (15–17). In a porcine model of adult cardiac dysfunction, accumulating evidence suggests that IGF-1 treatment leads to sustained improvement in cardiac function after myocardial infarction (18,19). However, the potential role of IGF-1 in FGR induced cardiac dysfunction is unknown.

Previous work in our laboratory demonstrated that intraplacental treatment with IGF-1 is an important regulator of placental growth (20). Prenatal treatment with IGF-1 to restore normal birth weight in FGR was used in animal models before with mixed results. This may be due to different routes of

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administration. Specifically intra-amniotic and intravenous routes had limited success and need for repetitive administration during gestation (21–23). Intraplacental gene transfer of adenoviral conjugated human IGF-1 (Ad-hIGF-1) is a novel and very appealing strategy that corrects placental insufficiency and restores birth weight in our mesenteric uterine artery ligation [AU: Please note that abbreviation "MUAL" used less than 5 times is deleted as per journal style.] model of mouse FGR (11,20). However, the effects of intraplacental IGF-1 transfer on postnatal growth and cardiac function over the life of animal are not known and accordingly were the focus of this study. We hypothesized that FGR mice will demonstrate cardiac dysfunction in adulthood and that this dysfunction will be prevented by intraplacental Ad-hIGF-1 therapy. We tested this hypothesis in our surgical mesenteric uterine artery ligation FGR model by characterizing postnatal growth trajectories and assessing cardiac status by serial postnatal transthoracic echocardiograms.

METHODS

Mouse Model of FGR

The Institutional Animal Care and Use Committee of Cincinnati Children's Hospital Research Foundation approved this study (3D02011). Time-mated pregnant C57/BL6J mice were obtained from Charles River Laboratories (Wilmington, MA), housed under standard conditions and allowed free access to standard chow and water.

We used a previously described surgical model of FGR by ligating one of the two branches of the uterine artery feeding a specific gestational unit, taking into consideration pup number per litter and the number per side of the uterine horn. Birth weight, IGF-1 expression and microarray gene profiling validated this model (11).

Study Groups

As previously described, on day 18 of pregnancy the first laparotomy was performed on time-mated pregnant C57/BL6J mice to mimic the effect of third trimester placental insufficiency in humans (11,24). Pups were divided into three groups: sham-operated, where pups were exposed to the stress of surgery with no intervention; FGR, where mesenteric uterine artery ligation was performed leading to FGR; and IGF-1, which underwent mesenteric uterine artery ligation followed by a trans-uterine intraplacental injection of adenovirus vector carrying Ad-hIGF-1. A standard 50 µl syringe (Hamilton Microliter Syringes) was used for transferring the human IGF-1 trans-gene (1×108 PFU). Dams in all three groups underwent cervical cerclage at the end of the procedure to prevent premature delivery.

Study Design

On day 20 of gestation a second laparotomy was performed and the litters were delivered. After documenting the birth and placental weights of each fetus, pups were cross-fostered to time-matched CD-1 surrogate dams. Litters were equalized to eight with limitation of four adopted pups per dam to normalize rearing. To reduce stress, pups were not handled during the first week of rearing. The different coat color (black C57/BL6J) from the recipient strain (white CD-1), helped in identification of the cross-fostered pups from recipient siblings. Pups were housed under standard conditions and during suckling, surrogate moms were provided standard chow ad libitum.

At week 4, pups were weaned and sorted by gender. All animals were subsequently individually housed under standard conditions with access to water and chow ad libitum.

Ad-hIGF-1 Construct[AU: Please check and confirm if the edit changes to the section heading is appropriate and do not alter the intended meaning.]

As previously described, all constructs used in this study were first generation recombinant replication defective, serotype 5 adenovirus vectors (25). All adenoviral genomes had E1 or both E1 and E3 regions deleted. All transgenes were driven by the cytomegalovirus promoter.

Postnatal Growth

At 4 wk of age, following weaning, offspring were individually housed and allowed food and water ad libitum. Offspring body weight was measured at birth and weekly up till 32 wk of age. All animals were housed in a facility with constant temperature and humidity and a controlled 12 : 12 h light/dark cycle.

Echocardiographic Evaluation

Transthoracic Echocardiograms were performed at postnatal week 12, 24, and 32. Cross-sectional, 2D, and Doppler transthoracic echocardiography was performed by experienced sonographers using a Visual Sonics Vevo 2100 Imaging System (Toronto, Canada) and a 30 MHz transducer (26,27). Off-line analyses included measurement of interventricular septum in diastole (IVSd) and left ventricular posterior wall in diastole thickness (LVPWd), the left ventricular (LV) end-diastolic and end-systolic internal dimensions (LVIDd and LVIDs, respectively). LV fractional shortening (FS) was calculated as $FS = ((\text{LVIDd} - \text{LVIDs}) / \text{LVIDd})*100$. LV volumes were calculated using the single-plane geometric model from the parasternal long axis view and the ejection fraction was calculated using the previously published methods (28). The echocardiographic measurements were done by single sonographer and read by a single attending pediatric cardiologist. Both were blinded to the study groups.

Statistical Analysis

Experimental results are reported as mean ± SE. Data was stratified based on sexual dimorphism. Two-way ANOVA was used to test for FS, left ventricular dimensions and interaction between these variables in the three groups using the Prism Software (Graph Pad Software, San Diego, CA)[AU: Please provide the version of software if applicable.]. Two-way repeated measures ANOVA was used to compare the echocardiographic measurements for the same animal at multiple time points. When appropriate, a Bonferroni post hoc test was performed to look at statistical differences within the variables. Differences with $P < 0.05$ were considered significant.

RESULTS

Birthweight and Growth Trajectory

We previously established birth weight percentiles for C57/BL6J at gestational age day 20 and the mean weight at birth was 1.111 ± 0.08 g while the tenth percentile was 1.012 g (11). The birth weight in the FGR group was (0.91 ± 0.09 g, $n = 12$) which was significantly lower than the sham group (1.15 ± 0.12 g, $n = 12$) ($P < 0.001$) (**Figure 1a**, and **Figure 2**). Intraplacental IGF-1 normalized birth weight with no significant difference in birth weight between the sham group and the IGF-1 group (1.15 ± 0.12 g vs. 1.11 ± 0.15 g, $n = 12$) respectively.

Postnatally the FGR group had a period of catch up growth and reached similar weight compared with the sham group at 8 wk. When followed to adulthood the FGR group initially had a similar growth curve followed by a period of accelerated weight gain resulting in higher weight compared with the sham group starting at week 24 of life. There was no significant difference in weight between the sham and the IGF-1 groups at any time (**Figure 1a**).

When stratified by gender, FGR males had earlier catch up growth and reached the sham group weight at postnatal week 5 compared with week 8 in females (**Figure 1b**,**c**). In adulthood both males and females in the FGR group had significantly higher weight compared with the sham and IGF-1 groups (**Figure 1b**,**c**).

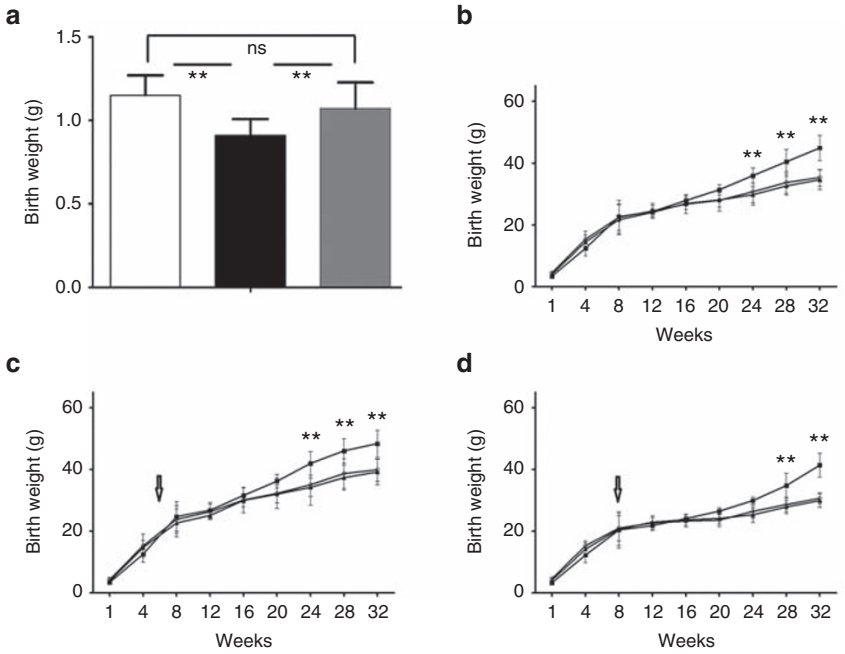

Figure 1. Postnatal growth trajectory. (**a**) Total population birth weight. White: Sham, black: fetal growth restriction (FGR), gray: insulin like growth factor 1 (IGF-1). (**b**) Total population growth from birth until week 32 of life. Circles represent Sham, squares represent FGR, and triangles represent IGF-1. (**c**) Male growth from birth to week 32 of life. Catch up growth is noted at 5 wk of life (arrow). Circles represent Sham, squares represent FGR, and triangles represent IGF-1. (**d**) Female growth from birth until week 32 of life. Catch up growth is noted at 8 wk of life (arrow). Circles represent Sham, squares represent FGR, and triangles represent IGF-1. ***P*-value < 0.005.

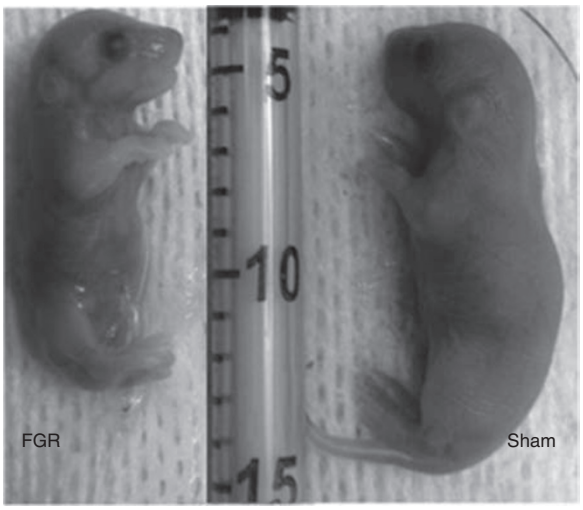

Figure 2. The size of the newborn pups in the fetal growth restriction (FGR) group compared with the SHAM group.

Table 1. Echocardiographic parameters at 12 wk of gestation for the three groups

Parameter	Sham ($n = 12$)	FGR ($n = 12$)	IGF-1 ($n = 12$)	*P*-value
FS (%)	31 ± 5	20 ± 4*	32 ± 5	<0.001
EF (%)	59 ± 7	40 ± 7*	61 ± 7	<0.001
IVSd (mm)	0.66 ± 0.06	0.65 ± 0.05	0.68 ± 0.04	NS
IVSs (mm)	1.07 ± 0.12	0.94 ± 0.08	1.09 ± 0.07	NS
LVPWd (mm)	0.72 ± 0.14	0.68 ± 0.17	0.75 ± 0.16	NS
LVPWs (mm)	1.05 ± 0.19	0.86 ± 0.22	1.10 ± 0.09	NS
LVIDd (mm)	4.50 ± 0.40	4.60 ± 0.44	4.39 ± 0.40	NS
LVIDs (mm)	3.09 ± 0.49	3.76 ± 0.39*	2.99 ± 0.45	<0.001
LV mass (mg)	91.5 ± 10.7	93.3 ± 27.9	90.2 ± 18.3	NS

SHAM group is the reference group. EF, ejection fraction; FGR, fetal growth restriction; FS, fractional shortening; IGF-1, insulin like growth factor 1; IVSd, interventricular septum in diastole; IVSs, interventricular septum in systole; LVPWd, left ventricular posterior wall in diastole; LVPWs, left ventricular posterior wall in systole; LVIDd, left ventricular internal dimension in diastole; LVIDs, left ventricular internal dimension in systole; NS, nonsignificant; * $P \leq 0.001$ FGR vs. Sham and vs. IGF-1.

Echocardiographic Analysis

Cardiac morphometry and function at 12 wk of age. The FGR group demonstrated systolic dysfunction with significantly decreased FS compared with the sham and IGF-1 groups, respectively (20 ± 4% vs. 31 ± 5% vs. 32 ± 5%; $P < 0.001$) (**Table 1** and **Figure 3**). The ejection fraction was similarly decreased in the FGR group compared with sham and IGF-1 (**Table 1**). Additionally, the FGR mice had increased LVIDs compared with the sham and IGF-1 groups (3.7 ± 0.4 vs. 3.1 ± 0.5 vs. 3.0 ± 0.5; $P < 0.001$) (**Table 1**). There was no significant difference in LVIDs or FS between the sham and the IGF-1 groups (**Table 1**). There was no significant difference among the groups in LVIDd, LVPW or IVS thickness in systole or diastole (**Table 1**).

Changes in cardiac morphometry and function are persistent at 24 and 32 wk of age. FGR group continued to have systolic dysfunction evidenced at follow up echocardiograms at 24 and 32 wk of age, with significantly higher LVIDs and lower FS compared with the sham group (**Figure 4**). There were no significant differences between sham and IGF-1 mice in any

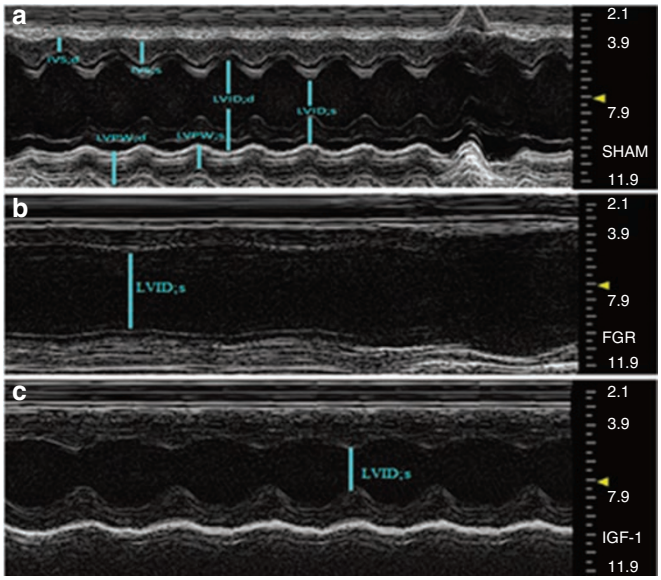

Figure 3. M-Mode for the left ventricle in the three study groups. Note that the LVIDs has increased in the fetal growth restriction (FGR) group and was back to normal in the insulin like growth factor 1 (IGF-1) group. (**a**) M-Mode for the Sham mice. (**b**) M-Mode for FGR, and (**c**) M-Mode for the IGF-1 group. EF, ejection fraction; FS, fractional shortening; IVSd, interventricular septum in diastole; IVSs, interventricular septum in systole; LVPWd, left ventricular posterior wall in diastole; LVPWs, left ventricular posterior wall in systole; LVIDd, left ventricular internal dimension in diastole; LVIDs, left ventricular internal dimension in systole.

parameters at 24 and 32 wk. Interestingly, while the FS was lower at 12 wk in the FGR group, the impaired systolic function remained stable over time with no evidence of further deterioration or recovery with age (**Figure 4**).[AU: Please check that the hierarchy of head levels are correct.]

Sexual Dimorphism Effect on Cardiac Morphometry and Performance

When data was stratified by gender, both males and females in the FGR group had significantly lower FS with higher LVIDs as compared with sham and IGF-1 mice (**Figure 5**). Likewise, when males and females were compared within each study group (sham, FGR and IGF-1), we found no significant differences in any morphometric or functional parameters (data not shown).

DISCUSSION

Numerous cohort studies and animal models show that FGR predisposes to adult overweight and cardiovascular disease, which is the number one cause of mortality in the United States (10,29,30). Given the high prevalence of FGR there is a tremendous need to develop interventions to prevent FGR and subsequently modify the risk of cardiovascular disease (30). In our surgical mouse model, FGR led to accelerated weight gain and overweight in adults. The FGR group also developed systolic cardiac dysfunction that was detected in both males

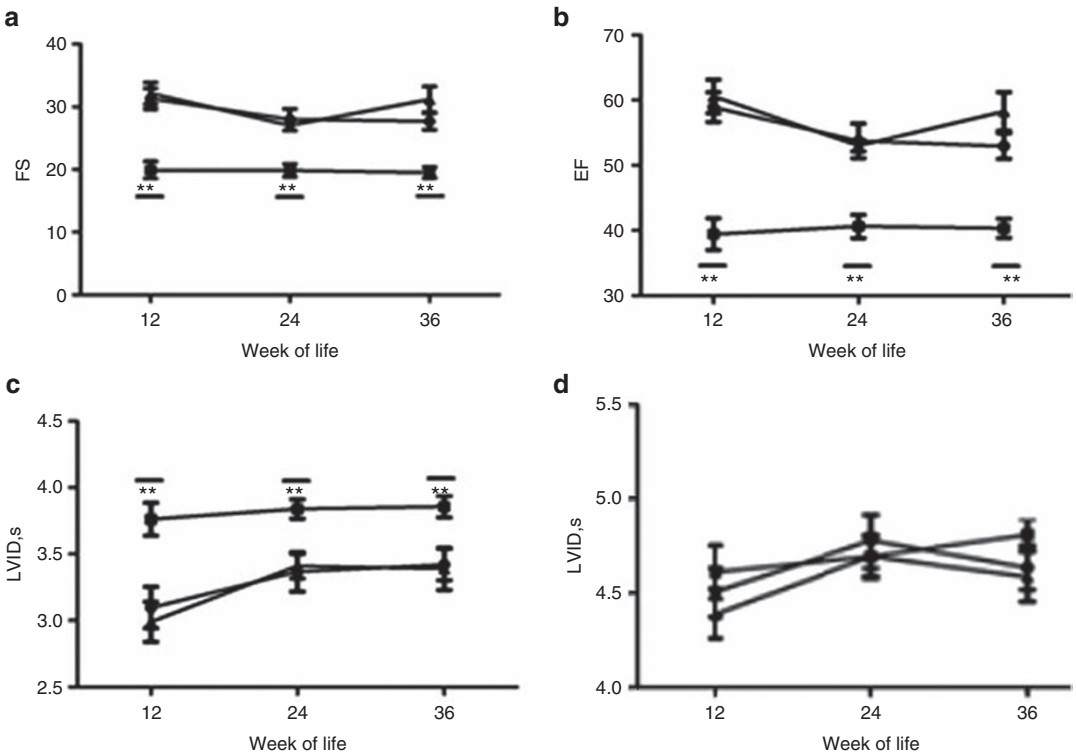

Figure 4. Changes in cardiac morphometric and performance persist at 24 and 32 wk of age. (**a**) Fractional shortening (FS) for the three groups at 12, 24, and 32 wk. The fetal growth restriction (FGR) group showed persistent impairment of the FS. (**b**) Ejection Fraction (EF) for the three groups at 12, 24, and 32 wk. The FGR group showed persistent impairment of the EF. (**c**) Left ventricular internal dimension in systole showing persistent increase of the LVIDs. (**d**) Left ventricular internal dimensions in diastole with no difference at 12, 24, or 32 wk of age. Circles represent FGR, squares represent Sham, and triangles represent IGF-1. ***P*-value < 0.001.

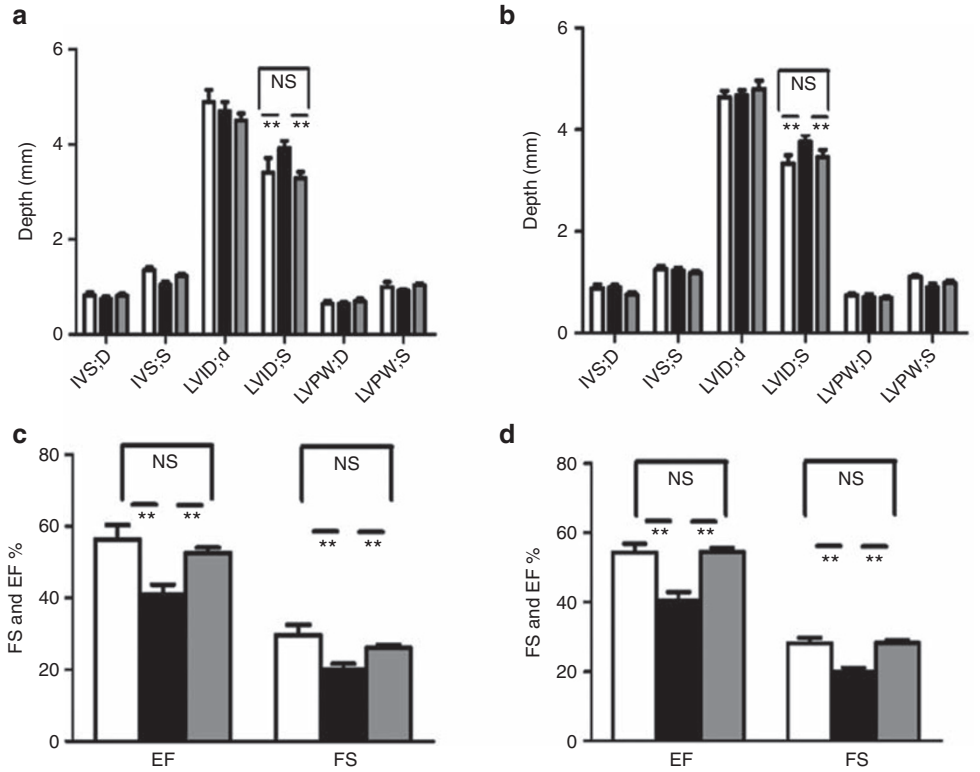

Figure 5. Gender effect at 12 wk of life. White represents Sham group, black represents fetal growth restriction (FGR) and gray represents insulin like growth factor 1 (IGF-1) group. (**a**) Left ventricular dimensions M mode in males for the three study groups. (**b**) Left ventricular dimensions M mode in females for the three study groups. (**c**) Left ventricular ejection fraction and fractional shortening in males for the three study groups. (**d**) Left ventricular ejection fraction and fractional shortening in males for the three study groups. NS, nonsignificant; ***P*-value < 0.005.

and females early in adult life by echocardiogram done at week 12 and persisted through week 32 of life with no evidence of cardiac recovery. This study provides the first evidence that intraplacental IGF-1 treatment reprograms fetal growth, normalizes postnatal growth, and prevents the long term cardiac dysfunction induced by FGR.

Postnatal growth following FGR was studied extensively in animals and humans. In rodents Ozanne *et al.* and Zambrano *et al.* induced FGR by low protein diet in pregnancy in mice and rats respectively and showed an accelerated period of catch up growth followed by overweight in childhood (31,32). In humans Barker *et al.* studied the postnatal growth in FGR by looking at the growth trajectories of patients with coronary artery disease who also had low birth weight in a large cohort study (Helsinki Birth Cohort Study). The patients had accelerated growth pattern to reach average weight by age 10 y then became overweight by early adolescence (33). This growth pattern was strongly linked to the later development of adult cardiovascular disease (33,34). Interestingly the FGR group in our study had accelerated weight gain in childhood to reach average weight by adolescence then become overweight in adulthood.

The link between FGR and cardiovascular disease has been made by many previous studies involving a number of species (12,35). Menendez-Castro *et al.* studied rats with FGR induced by maternal low protein diet and showed a significant decrease of ejection fraction (EF) in adults (35). Tintu *et al.* induced FGR by hypoxia during pregnancy in chicken and followed offspring into adulthood. FGR chicken had systolic cardiac dysfunction with decreased EF (12). Similarly, in our study the FGR group developed significant impairment in systolic function in early adult life (12 wk) that persisted at least up until 32 wk of age.

There is ample evidence that the effect of FGR on the cardiovascular system starts in prenatal life and persists to affect adults (36). Tintu *et al.* showed that FGR induced measurable systolic cardiac dysfunction in the fetus that continued into adult life in chicken (12). In human children Crispi *et al.* and Altin *et al.* found similar effects of FGR early in life with subclinical systolic dysfunction in neonates and children with FGR detected by echocardiogram. However, we can find no examples in the literature of published animal or human studies that evaluated serial echocardiograms into adulthood. Interestingly, we found that despite abnormally depressed values, systolic function appears to be stable in the FGR group at least up to 32 wk of age which correlates with a human age of 35 y (37). Our study demonstrates that the effects of fetal programming are detectable early in life and persists to adult life, albeit with no apparent progression or recovery, at least up to 32 wk of age. Our work opens up the possibility of successful fetal intervention to prevent the long-term effects of FGR targeting the IGF-1 axis (38,39).

Multiple studies recently showed the importance of the IGF-1 axis in cardiac embryonic development and cardiac

contractility in different animal models (17,40,41). In a mouse model of dilated cardiomyopathy due to *SRF* gene loss which leads to myocardial fibrosis, Touvron *et al.* showed that IGF-1 treatment resulted in a significant improvement in cardiac function (42). This was attributed to the significant reduction in fibrosis with the IGF-1 treatment (42). Ellison *et al.* induced acute myocardial infarction in pigs by percutaneous left anterior descending artery occlusion and simultaneously administered a single dose of IGF-1 through the infarct-related artery and found that IGF-1 therapy decreases infarct size and improved cardiac function post myocardial infarction (19).

Previous work in our laboratory using a rabbit FGR model and intraplacental IGF-1 to restore normal birth weight shows no *IGF-1* gene transfer in pups or maternal organs with altered protein expression in the placenta (43). Our previous work in mice compared intraplacental injection of Ad-hIGF-1 with controls that had Adenovirus *Lac-Z* gene (encoding beta-galactosidase) intraplacental injection. The *IGF-1* gene was overexpressed in the placenta and caused placental growth as compared with adenovirus *Lac-Z* controls (20). The placental growth was attributed to IGF-1 and not to the inflammatory reaction to Adenovirus as controls did not show any evidence of placental growth. Only minimal gene transfer to the fetus was noted (20). Histological exam of the placentas injected with IGF-1 showed an increase in both vascular area and labyrinth volume (43). This suggests that the potential mechanism of intraplacental IGF-1 effect may be due to normalizing placental growth and maturation (43). This study shows that intraplacental IGF-1 therapy not only results in a normal birth weight but also prevents the long term cardiac dysfunction induced by FGR. Studying the mechanistic effect of IGF-1 on the adult heart will be the target of future research.

In regards to possible sexual dimorphism interactions in FGR, Rueda-Clausen *et al.* showed that FGR rats have increased susceptibility to cardiac ischemia-reperfusion injury, with male and female rats being equally affected (44). Similarly, in human studies the Helsinki Birth Cohort Study showed an increase in coronary heart disease and similar growth pattern in both men and women with FGR (38). In our study we found no persistent gender influence on the long-term systolic dysfunction caused by FGR, although catch up growth and overweight happened earlier in male mice.

Our study is limited in that we have not assessed in our FGR model the effects of other risk factors for cardiovascular disease, such as diabetes and high fat diet. This is an area of ongoing investigation. Furthermore, we did not assess cardiac function in very young mice nor in senescence, as the echocardiograms were done at 12, 24, and 32 wk of life. The molecular basis for fetal programing and IGF-1 mechanism of action is to be investigated.

Despite the above limitations our mouse model of FGR faithfully recapitulates multiple characteristics of placental insufficiency, including long term cardiac sequelae. Furthermore our study supports the concept of fetal reprograming and shows the effectiveness of prenatal preventive therapy with IGF-1 to prevent FGR and its long term cardiac sequelae. This may open a new window of opportunity for early intervention to prevent fetal programming and all the detrimental effects linked to it.

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