Maternal nutrient restriction during early to mid gestation up-regulates cardiac insulin-like growth factor (IGF) receptors associated with enlarged ventricular size in fetal sheep

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Abstract

Intrauterine undernutrition is associated with a high incidence of cardiovascular diseases in adulthood. We previously showed that maternal nutrient restriction during early to mid gestation produces ventricular enlargement, although the mechanism is unknown. We examined myocardial expression of insulin-like growth factor 1 (IGF-1), IGF-2, IGF binding protein 3 (IGFBP-3), IGF-receptor 1 (IGF-1R) and IGF-2R in fetal sheep with maternal undernutrition. Multiparous ewes were fed with 50% (nutrient-restricted, NR) or 100% (control-fed, C) of NRC requirements from day 28 to 78 of gestation. Some of NR and C ewes were euthanized on day 78, and the rest were fed 100% NRC requirements from day 79 to 135 of gestation. At necropsy on day 78 or day 135 of gestation, gravid uteri were recovered. mRNA expression of IGF-1 and IGF-2 in ventricles were measured with RT-PCR, and protein expression of IGF-1R, IGF-2R, IGFBP-3 was quantitated with Western blot. Crown-rump length was reduced and left ventricle was enlarged in NR fetuses on day 78. At day 135 after re-alimentation, ventricular weights were similar between the two groups although ventricular wall thicknesses were greater in NR than C fetuses. No difference was found in IGF-1, IGF-2 or IGFBP-3 levels between the NR and C groups at either gestational age. Protein expression of IGF-1R and IGF-2R in the left ventricle and IGF-1R in the right ventricle was significantly elevated in the NR group on day 78 of gestation. Only IGF-1R expression remained elevated after late gestational re-alimentation in association with increases in ventricular wall thickness. Our study suggest that maternal undernutrition from early to mid gestation may change the expression of IGF-1R and IGF-2R in fetal myocardium, and play a role in cardiac ventricular enlargement in fetal sheep.

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1. Introduction

Changes in maternal nutritional status during pregnancy often results in permanent structural and functional deficits in fetal as well as postnatal growth, which predisposes the fetus to detrimental pathological consequences in both intrauterine and extrauterine life [1–4]. Gestational undernutrition especially during the first to second trimesters of pregnancy may directly enhance the propensity of cardiovascular, metabolic and endocrine diseases in later postnatal life [5–7]. It was demonstrated that unsupplemented ewes on range-land lose a significant amount of weight during early
to mid gestation. Even after nutrient supplementation later in gestation, the overall health condition of their offspring is still compromised [7–10]. This gestational undernutrition-triggered pre- or postnatal defect is consistent with the critical role of the first half of gestation for fetal development [6,11]. Despite these clinical and agricultural observations, the precise mechanism responsible for abnormal fetal growth and physiological function as a result of maternal undernourishment remains poorly elucidated.

Several hormones and growth factors have been indicated to participate in the nutrition deficiency-related fetal and possibly postnatal physiological dysfunction. Insulin-like growth factors (IGF), namely IGF-1 and IGF-2, which possess metabolic, mitogenic and differentiative actions, play an important role in fetoplacental growth throughout the gestational period. Maternal undernutrition during periconceptual and gestational periods has been shown to cause significant falls in fetal insulin, IGF and IGF binding protein-3 (IGFBP-3) levels in conjunction with enhanced IGFBP-2 in sheep [4,12]. Such alterations of IGF cascade are speculated to play a significant role in intrauterine undernutrition-associated compromised fetal growth and fetal programming [4,12]. Nevertheless, conflicting results have indicated that birth weight rather than maternal nutrition may serve as the key determining factor for IGF-1 levels in sheep [13]. Both IGF-1 and IGF-2 genes (*Igf-1* and *Igf-2*) are expressed in fetal tissues from the earliest stage of pre-implantation development to the final phase of tissue maturation just prior to birth, with *Igf-2* gene expression being more abundant than expression of the *Igf-1* gene [14]. IGF-1 and IGF-2 have been shown to determine both normal and abnormal growth and development of many organ systems including heart and vasculature [15–19]. Mutation of the IGF-1 receptor or deletion of the *Igf-1* gene retards intrauterine and postnatal growth while overexpression of the *Igf-2* gene stimulates fetal overgrowth [20]. IGFs are believed to determine not only the growth of individual fetal tissues but also uptake/utilization of nutrients by fetal/placental tissues. Expression of IGF genes is developmentally regulated in a tissue-specific manner and may be altered by nutritional and endocrine conditions in utero [17,21]. In general, the *Igf-1* gene is more responsive to these stimuli than the *Igf-2* gene. In addition, the effects of IGFs on fetoplacental growth can be amplified or attenuated by the IGFBPs, which are themselves regulated by nutritional and endocrine signals [22]. The *Igf-2* gene appears to provide the constitutive drive for intrauterine growth via its placental and paracrine actions on fetal tissue while the *Igf-1* gene regulates fetal growth and development in relation to the nutrient supply [22]. It is possible that maternal undernutrition may impact the fetal IGF system and therefore affect organogenesis and fetal development during critical stages of gestation, the period of rapid cell division [6]. Although several reports have been seen with regards to gestational nutrient restriction in the ewe [23–25], no special attention has been given to ventricular development and cardiac IGF cascade. Therefore, the aim of this study was to analyze the impact of an early and prolonged nutrient restriction on fetal cardiac growth, development and expression of IGF-1, IGF-2, IGFBP-3 and receptors for IGFs.

2. Materials and methods

2.1. Experimental animals

All animal procedures were approved by the Animal Care and Use Committee at the University of Wyoming (Laramie, WY). On day 20 of pregnancy, 30 multiparous ewes (mixed breeding) were weighed to allow individual diets being calculated on a metabolic body weight basis (weight0.75). The diet consisted of a pelleted beet pulp (79.7% total digestible nutrients, TDN, 93.5% dry matter, DM and 10.0% crude protein). Rations were delivered on a DM basis to meet the total TDN required for maintenance for an early pregnant ewe (NRC requirements) [26]. A mineral–vitamin mixture [51.43% sodium triphosphate, 47.62% potassium chloride, 0.39% zinc oxide, 0.06% cobalt acetate and 0.50% ADE vitamin premix (8,000,000 IU vitamin A, 800,000 IU vitamin D₃ and 400,000 IU vitamin E per pound); amount of vitamin premix was formulated to meet the vitamin A requirements] was included with the beet pulp pellets to meet nutritional requirements. On day 21 of gestation, all ewes were placed in individual pens and fed with control rations. On day 28, ewes were randomly assigned to a control-fed (C) group [100% NRC requirements [26] which included 100% mineral–vitamin mixture] or a nutrient-restricted (NR) group (fed 50% NRC requirements which included 50% mineral–vitamin mixture). Beginning on day 28 of gestation, and continuing at seven day intervals, ewes were weighed and rations adjusted for weight gain (i.e., increased the amount of feed) or loss (i.e., decreased the amount of feed). On day 45 of gestation, the numbers of fetuses carried by each ewe was determined by ultrasonography (Ausonics Microimager 1000 sector scanning instrument; Ausonics Pty Ltd., Sydney Australia). Randomly selected C and NR ewes were euthanized on day 78, and the remaining C and NR ewes were fed the control diet from day 79 to day 135, and then euthanized (gestation length ~150 days). At sacrifice, each ewe was weighed and was given an overdose of sodium pentobarbitol (Abbott Laboratories, Abbott Park, IL) and exanguinated, and the gravid uterus quickly removed and weighed.
Fetal weight, crown-rump length (CRL), abdominal circumferences and sex were recorded. The weights and wall thicknesses of left and right ventricles of the heart were recorded, and samples of each were snap frozen in liquid nitrogen for later analysis. Total DNA was measured using the fluorescent dye Bisbenzimide (H33258) [27]. Total protein was measured with the Bradford protein assay (Bio-Rad).

2.2. mRNA measurement of IGF-1 and IGF-2

To quantitate IGF-1 and IGF-2 mRNA levels, total RNA was extracted from the ventricles of fetal sheep using the guanidine thiocyanate method and was quantified spectrophotometrically with a 260/280 nm ratio of ~1.5. The total RNA was treated with DNase to prevent possible PCR amplification of chromosomal DNA contaminant. Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis was performed as follows: Total RNA (4 μg in d78 groups, 1.5 μg in d135 groups) was reverse transcribed at 42 °C for 50 min using 1 μl oligo(dt)12−18 (500 μg/ml) and 1 μl (200 U) super II reverse transcriptase (Invitrogen, Carlsbad, CA) in 20 μl reaction containing 0.1 M DTT 2 μl, 5 × RT buffer 4 μl, 10 mM of each dNTP 1 μl. The RT reaction was terminated by heating at 70 °C for 15 min. cDNA (1 μl) was amplified in 25 μl reaction containing: 2.5 μl 10 × PCR buffer, 0.75 μl 50 mM MgCl2, 0.5 μl 10 mM of each dNTP, 1 U of platinum Taq DNA polymerase (Invitrogen, Carlsbad, CA), and 0.5 μl of the respective oligonucleotide primer pair (25 pM/μl). The primers used in this study were synthesized by IDT integrated DNA technologies Inc. (Coralville, IA) as the following: sheep IGF-1: 5′ GAC AGG AAT CGT GGA TGA GTG 3′ (forward) and 5′ AAC AGG TAA CTC GTG CAG AGC 3′ (reverse) (Accession No. M30653, 270 bp); sheep IGF-2: 5′ CGT GGCTC TTG TGA GAG TGT 3′ (forward) and 5′ GTGGCTGACCTTCCAAGCTGAA 3′ (Accession No. X55638, 277 bp); β-actin: 5′ CAT GCC CTT CCT GCT TGA 3′ (Accession No. U39357, 420 bp) [28]. PCR for IGF-1 and IGF-2 consisted of initial denaturing at 94 °C for 2 min; then at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s; followed by 30 cycles with a final extension at 72 °C for 7 min. PCR was performed in a P×2 PCR System (Thermo Hybaid, Franklin, MA). PCR products were separated by electrophoresis on 1.5% agarose gels and stained with ethidium bromide. The intensity of bands was measured with a scanning densitometer (model GS-800; Bio-Rad, Hercules, CA) coupled with Bio-Rad PC analysis software. The relative levels of PCR products were normalized compared with mRNA for β-actin. Although starvation may affect many muscle proteins, β-actin was shown to be a good internal control for nutritional restriction or fasting states [29].

2.3. Western analysis of IGFBP-3, IGF-1 receptor and IGF-2 receptor

For Western blot analyses, tissue from fetal sheep ventricles were homogenized and lysed in RIPA Lysis buffer: 20 mM Tris (pH 7.4), 1 mM EDTA, 1 mM EGTA, 150 mM NaCl, 1% Triton, 0.1% SDS, Protease inhibitor cocktail (Sigma Chemicals, St. Louis, MO) 1:100 dilution. Lysates were sonicated and clarified by centrifuging at 13,000g for 25 min at 4 °C, protein concentrations were determined using the Bio-Rad protein assay reagent (Bio-Rad Laboratories, Inc., Richmond, CA). Protein samples (40 μg/lane) were then mixed 1:2 with Laemmli sample buffer with 5% 2-mercaptoethanol and heated at 95 °C for 5 min. SDS-PAGE was performed on a 7% (for IGF-1R and IGF-2R) and 15% (for IGFBP-3) acrylamide slab gels. IGFBP-3 is the major form of IGFBP in mammals [30]. SeeBlue plus2 Prestained markers (Invitrogen, Carlsbad, CA) were used as standards. Electrophoretic transfer of proteins to Nitrocellulose membranes (0.2 μm pore size, Bio-Rad Laboratories, Inc., Hercules, CA) was accomplished in a transfer buffer consisting of 25 mM Tris–HCl, 192 mM glycine, and 20% methanol for 60 min at 288 mA. Membranes were blocked for 60 min at room temperature in TBST (0.1% Tris-buffered saline Tween-20) with 5% non-fat dry milk. Membranes were incubated overnight with primary antibody at 4 °C. Primary antibodies anti-IGF-1R and anti-IGF-2R antibody (Cell Signaling, Beverly, MA), anti-IGFBP-3 (Upstate, Lake placid, NY) were used at a dilution of 1:1000. After incubation with the primary antibody, blots were incubated with either anti-mouse or anti-rabbit IgG HRP-linked antibodies at a dilution of 1:5000 for 120 min at room temperature. After three washes in TBST, immunoreactive bands were detected using the Super Signal west Dura Extended Duration Substrate (Pierce, Milwaukee, WI). The intensity of bands was measured with a scanning densitometer (model GS-800; Bio-Rad, Hercules, CA) coupled with Bio-Rad PC analysis software. Band density of all eight groups (always fit onto the same gel) was then normalized to that of the control left ventricle group.

2.4. Statistics

Data are presented as means ± SEM. Mean differences between groups were assessed using a two-way analysis of variance (ANOVA). When an overall significance was determined, a Dunnetts post hoc analysis was incorporated. A p value of less than 0.05 was considered significant.
3. Results

3.1. General features of ewes and fetuses under nutrition restriction

On day 28, prior to the onset of nutrient restriction, the weight of the ewes did not differ between the C (93.90 ± 4.66 kg, n = 15) and NR groups (91.56 ± 5.35 kg, n = 15). On day 78 of gestation following a 50-day nutrition restriction, the live weight of NR ewes was significantly less than that of C ewes (85.83 ± 5.01 kg vs. 102.08 ± 4.98 kg, respectively, p < 0.05), reflecting a 7.51 ± 1.29% body weight gain in C ewes and a 7.38 ± 0.87% body weight loss in NR ewes. On day 135, after re-alimentation (returning to full nourishment) of NR ewes, weights of both C and NR ewes were similar averaging ~122.45 kg.

The number of fetuses gestated by each ewe on day 45, as determined by ultrasonography, was identical to that on day 78 and 135, demonstrating no fetal loss during the subsequent maneuver of nutrient restriction or re-alimentation. Additionally, the lack of fetal loss was confirmed by counting the number of corpora lutea on the ovaries of each ewe at tissue collection (data not shown). The control-fed group had seven singleton and eight twin pregnancies, while the nutrition-restricted group had nine singleton and six twin pregnancies.

Fetal weight was not significantly different between C and NR groups on either day 78 or day 135. CRL was significantly reduced (p < 0.05) in NR ewes compared to C ewes on day 78, but was similar on day 135 following re-alimentation (Table 1). The left but not right ventricular weights were significantly increased in NR fetuses compared to C fetuses on day 78. The ratio of left but not right ventricular weight-to-fetal weight was elevated in NR fetuses compared to C fetuses. Left but not right ventricular wall thickness was significantly increased in NR fetuses compared to C fetuses. Interestingly, re-alimentation between days 78 and 135 abolished the differences in ventricular weight and ventricular weight-to-fetal body weight ratio between C and NR fetuses. Left ventricular wall thicknesses, however, remained markedly greater (p < 0.01) in fetuses from NR ewes compared to C group at day 135. Right ventricular wall thickness, which displayed a trend of increment but never reached significance at day 78, was also significantly greater in NR fetuses compared to C fetuses at day 135 (Table 1). Cardiac cell size appeared unaffected, as the protein-to-DNA ratio was unaltered in both the right and left ventricles of C vs. NR fetuses on day 135 (0.479 ± 0.058 and 0.413 ± 0.056 mg/μg vs. 0.478 ± 0.030 and 0.500 ± 0.087 mg/μg, respectively). Information of fetal ventricular wall thickness or protein-to-DNA ratio was not collected on gestation day 78 due to its small size and the quantity of tissue required for protein/DNA quantitation.

3.2. Expression of fetal cardiac IGF-1, IGF-2, IGFBP-3 and IGF receptors following nutrition restriction

The mRNA levels for cardiac IGF-1 or IGF-2 were not significantly different between C and NR fetuses in either left or right ventricle at gestation day 78 or 135 (Fig. 1). The protein expression of IGFBP-3, was similar in both fetal groups in either ventricle at gestation day 78 or day 135 (Fig. 2). Fetal protein expression of IGF-1R but not IGF-2R was less (p < 0.05) in the right ventricle compared to the left ventricle at gestation day 78 in C fetuses (Fig. 3B). Interestingly, this difference in IGF-1R protein expression was reversed in the day 135 C fetuses, with IGF-1R expression being greater (p < 0.05) in the right than the left ventricle (Fig. 3C). At day 78, the protein expression of both IGF-1R and IGF-2R were both increased (p < 0.05) in the left ventricle of the NR group compared with the left ventricle of the C group (Fig. 3B). In the right ventricle, IGF-1R protein levels were increased (p < 0.05) in the day 78 NR group compared with the C group. In contrast,

Table 1
Fetal organ weights from male and female conceptuses on days 78 and 135 of gestation from control-fed and nutrient-restricted ewes

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Day 78</th>
<th>Day 135</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Control (n = 10)</td>
<td>Restricted (n = 8)</td>
</tr>
<tr>
<td>Fetal weight (g)</td>
<td>278.3 ± 7.3</td>
<td>266.9 ± 7.6</td>
</tr>
<tr>
<td>CRL (cm)</td>
<td>21.20 ± 0.30</td>
<td>20.19 ± 0.27*</td>
</tr>
<tr>
<td>LV (g)</td>
<td>0.86 ± 0.03</td>
<td>0.99 ± 0.07*</td>
</tr>
<tr>
<td>RV (g)</td>
<td>0.64 ± 0.03</td>
<td>0.67 ± 0.05</td>
</tr>
<tr>
<td>LV/FW (×100)</td>
<td>0.31 ± 0.01</td>
<td>0.37 ± 0.02*</td>
</tr>
<tr>
<td>RV/FW (×100)</td>
<td>0.23 ± 0.01</td>
<td>0.25 ± 0.02</td>
</tr>
<tr>
<td>LV thickness (mm)</td>
<td>3.71 ± 0.11</td>
<td>4.03 ± 0.11†</td>
</tr>
<tr>
<td>RV thickness (mm)</td>
<td>2.83 ± 0.15</td>
<td>3.09 ± 0.14</td>
</tr>
</tbody>
</table>

FW, fetal weight; CRL, crown-rump length; LV, left ventricle; RV, right ventricle, means ± SEM.
* p < 0.05.
†, p < 0.01.
** p < 0.001 differ from respective control within a measure, LV thickness is the average of three values at separate points on the ventricle.
while the IGF-2R levels in the NR fetuses increased numerically, they failed to reach significance ($p > 0.10$; Fig. 3B). Left ventricular IGF-1R but not IGF-2R protein expression remained elevated ($p < 0.05$) in NR fetuses from ewe's re-alimentation during late gestation when compared to fetuses from C ewes on day 135 of gestation (Fig. 3C). As observed on day 78, a numerical increase in right ventricular IGF-1R protein expression was seen on day 135 in NR fetuses vs. C fetuses, but again, significance was not achieved ($p > 0.10$). In association with the increased IGF-1R protein expression, ventricular wall thickness of NR fetuses was greater ($p < 0.01$) than that of C fetuses on day 135 of gestation (Table 1). No significant differences were found in expression of IGF-2R protein in the left and right ventricular tissues of NR and C fetuses on day 135 of gestation.

4. Discussion

The major findings of our study are that early to mid gestational nutrition restriction significantly reduced fetal CRL in conjunction with an enlarged left ventricular size (ventricle-to-body weight ratio and ventricular wall thickness). Further, our results are consistent with an enhanced expression of IGF receptors, but not IGF-1, IGF-2 or IGFBP-3 in association with increased
ventricular size in response to early to mid gestational nutrition restriction. In addition, our results indicated that ventricular IGF-1 receptors remained elevated in NR fetuses following re-alimentation during late gestation (day 79–135), in association with an elevated ventricular wall thickness when compared to C fetuses.

The IGFs, namely IGF-1 and IGF-2, possess key roles in the regulation of fetal development throughout gestation by exerting metabolic, mitogenic and differentiative actions in a wide range of fetal tissues including the heart [15,16,18,19]. It has been suggested that both IGFs may act as progression mediators in cell cycle, DNA synthesis and cell differentiation in embryos and fetal cell lines [31,32]. The levels of IGFs in the fetus are positively correlated with fetal birth weight in a number of species such as human, primates, sheep, rabbit, pig and rodents [22]. Maternal gestational undernutrition has been demonstrated to reduce circulating levels of IGF in fetal sheep [4,12], suggesting a likely link between fetal IGF cascade and fetal development, especially under gestational nutritional deficiency. Our present study found reduced CRL following maternal nutrient restriction. Intriguingly, our study revealed that fetal left ventricle was significantly larger following maternal nutrient restriction compared with control group, similar to our earlier observations [25]. Several speculations may be considered for the maternal nutrient restriction-induced ventricular enlargement. First, an increase in placental vascular resistance was suggested in response to maternal nutrition restriction, which may result in an increased ventricular afterload [33]. Enhanced placental vascular resistance may lead to a reduction in umbilical vein blood flow, which was reported in intrauterine growth-restricted fetuses [34]. Peripheral vascular resistance and blood viscosity have been demonstrated to be elevated in intrauterine growth-restricted human and sheep pregnancies [35,36], leading to an elevated afterload and a likely compensatory cardiac hypertrophy. Another scenario may be that IGF receptor expression from fetal ventricles following maternal nutrition restriction is elevated. IGF receptors are present in fetal as well as adult human cardiac myocytes, and are related to cardiac growth and development of myocyte hypertrophy in hypertrophic cardiomyopathy [37]. Evidence has suggested that both IGF-1 and IGF-2, by binding to their specific high affinity membrane receptors, activate multiple signal transduction pathways, which turn on muscle-specific genes and facilitate protein synthesis of myofibrils [17,21,38]. Therefore, the IGF receptors are essential and “rate-limiting” in the ultimate determination of myocardial cell mass and volume [17]. Our results revealed that maternal nutrient restriction directly enhanced the expression of IGF-1R and IGF-2R in the left ventricle and IGF-1R in the right ventricle whereas only IGF-1R expression remained elevated after late gestational re-alimentation in association with increases in ventricular wall thickness. These results, in conjunction with the unchanged expression of IGF-1, IGF-2 and IGFBP-3 observed in our current study, suggested a role of IGF receptors in facilitating fetal ventricular enlargement following intrauterine nutrient restriction.

As mentioned earlier, gestational undernutrition may lead to reduced levels of IGF in fetus [4,12]. It may be postulated that a reduction in IGFs expression or loss of their function may play a role in the adverse effects of malnutrition since reduced protein or energy intake is known to decrease IGF expression in many tissues [39,40]. Hepatic IGF-1 mRNA level is extremely sensitive to reduced nutritional status [39]. Since liver is the major source of circulating IGFs, malnutrition may dramatically reduce circulating IGFs. Nevertheless, our current study displayed unaltered expression of cardiac IGF-1 and IGF-2 following intrauterine under-nutrition. The relative contribution of circulating vs. local IGFs in the anabolic and growth-promoting effects has recently been addressed [41]. It is concluded that although hepatic IGF is the major contributor to circulating IGF levels, it is not crucial for normal postnatal growth. It may be premature to apply this circulating vs. local IGF contribution scenario to pre-natal growth. However, the autocrine/paracrine role for IGF may certainly provide an explanation for the malnutrition-induced discrepant effects in hepatic vs. cardiac IGF.
expression. The IGFBPs serve not only to transport IGFs in the circulation but also prolong the half-lives of IGFs. In addition, IGFBPs may modulate the biological effect of IGFs in a variety of tissues [17]. Our current experimental results did not favor any role of IGFBP-3 in the maternal under-nutrition-induced bilateral ventricular enlargement. Circulating IGFBP levels may be affected by nutritional and catabolic states. Calorie-restriction has been shown to increase IGFBP-1 and IGFBP-2, whereas decrease IGFBP-3 levels [42]. However, our results suggested that local ventricular expression of IGFBP-3 may not be affected in the same manner as the circulating IGFBP-3. Since undernutrition may cause a significant reduction in serum IGF-1 levels [42], the upregulation of ventricular IGF receptor may be a compensatory mechanism to “make up” for the heart growth at early stage of gestational nutrient deficiency. However, with persistent nutrient deficiency and over expressed IGF receptor, the effect of IGF cascade on heart development may switch from a compensatory beneficial effect to the detrimental hypertrophic one.

The heart is the very first organ formed to function in the embryo. The structure and function of the heart usually determine the capacity (due to its oxygen delivery nature) of many other organs during either
embryogenesis or postnatal life. Abnormalities of heart or congenital heart diseases, afflicts 1% of the newborns and this frequency is much higher in aborted or poor gestational nutrition [43]. Certain forms of congenital heart diseases have been shown to be closely associated with right ventricular hypertrophy (e.g., triology of Fallot, atrial septal defect) or left ventricular hypertrophy (e.g., aortic stenosis). Hypertrophic factors such as endothelin-1 (ET-1) have been indicated to contribute to the development of congenital heart diseases [44]. However, the role of IGF and IGF-induced cardiac hypertrophy in congenital heart diseases are not clearly elucidated. Both growth hormone and IGFs play a role in cardiac development and cardiac physiology. Congenital lack of growth hormone and IGFs are associated with defective cardiac growth, ventricular wall thinning and impaired systolic function [45]. On the other side of the coin, increased IGF-1 level was found in cardiac hypertrophy in aortic regurgitation and stenosis [46]. Nevertheless, reduced serum IGF-1 levels were also seen in congenital heart disease [47], which may lead to a compensatory upregulation of IGF receptor and amplified IGF signaling en route to hypertrophy-associated congenital heart diseases. Due to the uncontrolled nature of these studies and the relatively few cases reported, conclusions with regards to the role of IGFs in the hypertrophic congenital heart diseases must await for further scrutinized study.

In summary, our findings depicted, for the first time, that early to mid gestational nutrient restriction-induced left ventricular enlargements may be associated with enhanced expression of IGF-1R receptors in both ventricles and that of IGF-2R in the left ventricle. Our results did not favor a role for locally expressed IGF-1, IGF-2 or IGFBP-3, in ventricular enlargement or hypertrophy-associat ed congenital heart diseases. Due to the uncontrolled nature of these studies and the relatively few cases reported, conclusions with regards to the role of IGFs in the hypertrophic congenital heart diseases must await for further scrutinized study.

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References
