Early origins of heart disease: Low birth weight and the role of the insulin-like growth factor system in cardiac hypertrophy

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Summary

1. Epidemiological studies indicate that poor growth before birth is associated with left ventricular hypertrophy and an increased risk of death from heart disease later in life.

2. In fetal life, the insulin-like growth factor (IGF) system has been implicated in physiological growth of the heart, while in postnatal life IGFs can be involved in both physiological and pathological cardiac hypertrophy.

3. A reduction in substrate supply in fetal life, resulting in chronic hypoxaemia and intrauterine growth restriction, results in increased cardiac IGF-1R, IGF-2 and IGF-2R gene expression; and there is also evidence for a role of the IGF-2R in the ensuing cardiac hypertrophy.

4. The persistent high level of cardiac IGF-2R gene expression from fetal to postnatal life may be due to epigenetic changes in key cardiac hypertrophy regulatory pathways.

Left ventricular hypertrophy and mortality

An increase in heart mass is known as cardiac hypertrophy and can be classified as either physiological, *i.e.* when cardiac enlargement is compensatory and reversible, or pathological, *i.e.* when cardiac enlargement is decompensatory and irreversible (see reviews Bernando *et al.*, 2010;[1] Adams *et al.*, 1998;[2] Lorell & Carabello, 2000;[3] Knöll *et al.*, 2011[4]). The main contributor to pathological hypertrophy is an increase in left ventricular weight. Left ventricular hypertrophy (LVH) is initiated as a physiological adaptation to compensate for an increased cardiac workload as a result of either pressure or volume overload[3] or in response to normal growth signals *in utero*. If maintained or excessive, LVH becomes pathological, and is associated with a significant increase in morbidity and mortality. For example, the Framingham Heart Study showed that patients with ST-T repolarization abnormalities, suggestive of left ventricular strain, were six times more vulnerable to cardiac death over a 20 year follow up period.[5] In addition, data from the Bronx Longitudinal Aging Study also suggested that the presence of LVH (based on electrocardiographic definition) is an independent predictor of adverse cardiovascular outcomes.[6] LVH has therefore been recognized as the strongest risk factor for cardiovascular disease.[5,7]

A number of physiological, environmental and life style factors have been linked with LVH. The prevalence of LVH is age dependent with only 6% of individuals diagnosed before 30 years and up to 43% of individuals >70 years diagnosed with LVH.[8,9] Women with LVH are more likely to have negative cardiovascular outcomes than men, when adjusted for age.[10] LVH is associated with a number of conditions such as obesity,[11] diabetes and myocardial infarction.[12] Hypertension is a risk factor for LVH; where a small rise in blood pressure is associated with an increased risk of LVH[13] and patients with LVH are 3 times more likely to have hypertension.[8] Another significant risk factor for LVH is reduced growth before birth[14-16] although the mechanisms underlying this relationship are complex and poorly understood.

Intrauterine growth restriction and mortality: cardiac hypertrophy

Intrauterine growth restriction (IUGR) is associated with cardiac hypertrophy in infants[14,17] and adults,[18-20] and results in an elevated risk of cardiovascular disease independent of blood pressure, smoking and cholesterol concentrations.[6,7] IUGR is defined clinically as having a birth weight below the 10th centile for gestational age[21-24] and can be caused by a range of factors including maternal environment,[24,25] maternal undernutrition,[26,27] placental insufficiency[28] or fetal gene defects, including chromosomal abnormalities; all of which can result in the fetus failing to achieve its genetic growth potential and exhibit asymmetric growth.[22,28-30] Epidemiological studies have demonstrated that IUGR fetuses are at increased risk of cardiovascular disease in later life; with birth weight[31,32], maternal body size and placental shape and size[33] determining the subsequent risk of cardiovascular disease.[32,34-39]

IUGR fetuses can be hypoxaemic, hypercapnic, hyperlacticaemic, acidotic,[40] hypoglycaemic,[41] hypertriglyceridaemic[42] and have increased plasma concentrations of cortisol and noradrenaline.[43] These

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metabolic and endocrine changes can alter early cardiac growth and lead to a vulnerability to cardiac hypertrophy.[44-48] To date, the independent and relative impact of each insult has not been determined. We[18,49] and others[50-52] have shown that in animal models of IUGR, absolute heart weight is reduced compared to normally grown fetuses. In late gestation, IUGR sheep fetuses also have a reduction in cardiomyocyte proliferation[50] and a higher percentage of mononucleated cardiomyocytes,[49-51] indicating a delay in cardiomyocyte binucleation and maturation. Furthermore, IUGR due to placental growth restriction results in longer binucleated cardiomyocytes relative to heart weight[18,49] whilst maintaining normal arterial blood pressure.[53-55] It is, therefore, unlikely that the increase in cardiomyocyte size in the placentally restricted fetuses is due to changes in afterload.[55,56] The IUGR sheep fetus is more dependent on the renin-angiotensin system[54] and the sympathetic nervous system (SNS),[53] but not endothelial nitric oxide,[57] for the maintenance of basal arterial blood pressure. These studies suggest that there are a range of neuroendocrine adaptations in response to a decrease in substrate supply in the IUGR fetus, which maintain arterial blood pressure and this may impact on the growth and functional development of the heart.

Altered cardiac growth is also seen in postnatal life. The low birth weight (LBW) lamb, defined as a having a birth weight two standard deviations below the mean of a large cohort of normally grown fetuses,[18,58] which was induced either by placental restriction or spontaneous growth restriction, has a relatively larger heart[19] and left ventricular[18] weight (heart or left ventricle weight : body weight) compared to the average birth weight (ABW) lamb. Along with changes to cardiomyocyte growth, IUGR, due to maternal protein restriction in rats reduces the number of cardiomyocytes at birth.[52] Interestingly, in a genetic rat model of adult cardiac hypertrophy without hypertension, pups at 2 days of age also have fewer cardiomyocytes.[47]

Since IUGR can be caused by restriction of oxygen and/or nutrients, it is currently unclear if there is a common mechanism linking IUGR (by any means) to negative cardiac outcomes in adulthood, or if there are several mechanisms leading to a similar outcome. IUGR results in a range of neuroendocrine adaptations that may also lead to changes in cardiac growth.[55,59-65] Insulin-like growth factors (IGFs) are critical regulators of placental and fetal growth.[66,67] The IGF signalling pathway is a nutritionally sensitive pathway and its activation is altered in IUGR.[68,69] IGFs also have an important role in cardiac growth.

The role of IGF signalling in cardiac growth in late gestation

IGFs play an important role in cardiac growth in fetal life and are associated with both hyperplasic and hypertrophic cardiomyocyte growth. The expression of IGF-1, IGF-2, IGF-1 receptor (IGF-1R) and IGF-2R gene transcripts in the left and right ventricles has been confirmed at as early as 80 days gestation in the sheep (term, 150 days).[70] The amount of IGF-1 and IGF-1R gene expression is relatively constant across late gestation.[70] Many studies have shown that IGF-1 stimulates cardiac hypertrophy in adult life.[71,72] IGF-1 and IGF-2 can each bind to the IGF-1R, which stimulates downstream signalling pathways involved in cardiac proliferation[64,73] and hypertrophy.[74,75] IGF-1R downstream effectors include phosphoinositide 3-kinase [PI3-K, (p110α)],[74] protein kinase B (Akt),[76] mammalian target of rapamycin (mTOR)[77] and p70 ribosomal S6 kinase[74] (for review see Bernardo *et al.*, 2010).[1] In fetal life, the role of IGF-1 is less clear as some studies show that IGF-1 is involved in the proliferation of cardiomyocytes,[64] while others have shown an effect on hypertrophy.[78]

Cardiac gene expression of both IGF-2 and IGF-2R decreases with increasing gestational age[70] and IGF-2 can act on both IGF-1R and IGF-2R. The downregulation of IGF-2R, an IGF-2 clearance receptor, in late gestation is important to allow continued cardiac growth in response to IGF-2.[79] The IGF-2R is a multifunctional receptor that has been traditionally viewed as a clearance receptor for IGF-2 (Figure 1). The binding of IGF-2 to the IGF-2R results in this complex being endocytosed; while the extracellular domain of the IGF-2R binds IGF-2, the cytoplasmic tail sequence regulates traffic to different intracellular compartments.[80,81] In the acidic conditions of the endosome-lysosome system IGF-2 is dissociated from the receptor and the latter can be degraded by the constituent lysosomal hydrolases.[81,82] After IGF-2 dissociation, the IGF-2R can then be recycled back to the plasma membrane. The IGF-2R has been thought to act primarily as a degradative pathway to remove excess IGF-2 from the circulation. Downregulation of the IGF-2R in late gestation is normally important to allow continued cardiac growth in response to IGF-2,[79] resulting in increased cardiomyocyte proliferation and reduced apoptosis.[83] Embryonic mice that inherit mutated and non-functional *IGF-2R* through the maternal germ line had greater body weight and larger hearts due to cardiomyocyte proliferation compared to controls and die shortly before birth or at birth[84] due to congestive heart failure.[85]

IUGR, IGFs and cardiac hypertrophy

In humans, IUGR term placentas have lower IGF-1,[86] but higher levels of IGF-2 and IGF-1R gene expression[87,88] compared to those from normal pregnancies. In sheep, there is a decrease in IGF-1 mRNA expression in the muscle, lungs and kidneys[89] as well as decreased plasma IGF-1 and IGF-2 concentrations in IUGR fetuses.[90] There are conflicting results in fetuses of ewes who were undernourished from 28-78d gestation with either an enlarged left ventricle, increased relative left ventricle weight and increased cardiac IGF-1R and IGF-2R protein expression[27] or no change[91] reported. In late gestation (135d), fetuses of undernourished ewes had increased cardiac IGF-1R protein expression and wall thickness.[27] Interestingly, fetuses of ewes who were overnourished over the same period of gestation had a similar increase in plasma cortisol as observed in fetuses of undernourished

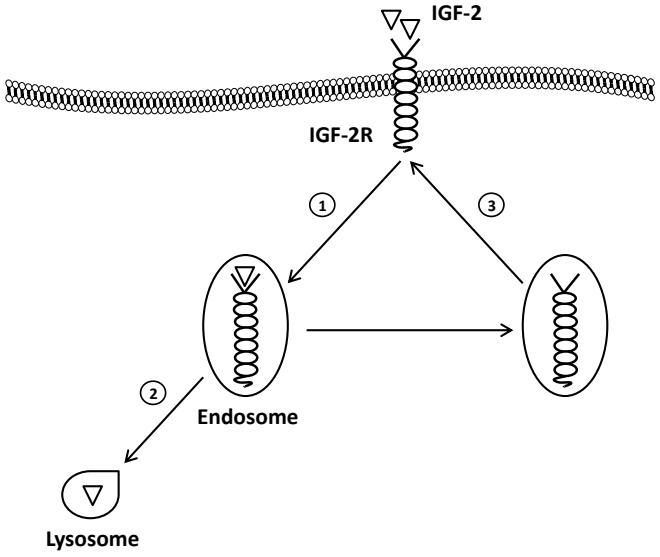

Figure 1. IGF-2R as a clearance receptor. *IGF-2R reduces the bioavaibility of IGF-2 by internalizing IGF-2 into the endocytic system ➀ and transporting IGF-2 to the lysosome to be degraded ➁, while the receptor, IGF-2R, is recycled back to the cell membrane ➂.*

ewes, but had an increased plasma IGF-1 concentrations, greater heart weight and ventricular weight, but there was no difference in relative heart or ventricular weight or cardiac IGF-1R protein abundance at 78d gestation.[91] In a sheep model of fetal anemia with fetal hypoxaemia, there was an increased relative left ventricular weight with no change in the expression of downstream cardiac IGF-1R signalling proteins Akt or total extracellular signal-related kinase (ERK)1/2, however, there was a decrease in active ERK1/2.[92] Placental restriction leading to fetal hypoglycaemia, chronic hypoxaemia and IUGR increases the size of cardiomyocytes relative to heart weight,[49-51] coupled with an increased cardiac IGF-2, IGF-1R and IGF-2R mRNA expression at 139 days of gestation.[18]

IGF-2R: a clearance or an activation receptor?

In adult life, IGF-1 has been implicated in the initiation of ventricular hypertrophy;[93] and in a range of *in vivo*[78] and *in vitro*[94] experimental models, IGF-1 has been shown to act on the IGF-1R to increase cardiomyocyte size.[47,95] Both IGF-1and IGF-2 can act on the IGF-1R to mediate effects on cardiomyocyte growth. However, when the IGF-1R signalling pathway is blocked *in vitro*, the addition of IGF-2 still results in an increase in the size of cardiomyocytes.[95] This indicated that IGF-2 may also act on the IGF-2R to stimulate heart cell growth and would be consistent with the activation of a signalling pathway.

Studies in cultured H92c cardiomyoblasts show that the IGF-2R can bind to G protein-coupled receptors with αq subunits (Gαq: Figure 2). This is an important discovery because Gαq pathways are associated with cardiac remodelling,[2,96] cardiac hypertrophy with a phenotype of increased cardiomyocyte size and heart weight relative to body weight.[96-98] Gαq can reactivate embryonic genes that are markers of pathological cardiac hypertrophy, such as atrial natriuretic peptide (ANP), α skeletal actin and β-myosin heavy chain.[96] Specific activation of the IGF-2R has been associated with pathological cardiac hypertrophy; Gαq mediated phosphorylation of protein kinase C-α (PKC-α) and Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), which results in the production of natriuretic peptides.[99] The IGF-2R has also been implicated in apoptosis[99,100] and myocardial extracellular matrix remodelling *via* Gαq.[101] IGF-2 and IGF-2R dose-dependently correlated with the progression of pathological hypertrophy and heart failure following abdominal aorta ligation.[102] Furthermore, it has been shown that in addition to IGF-2 other factors such as angiotensin II (ANGII), lipopolysaccharide, inomycin, and tumor necrosis factor-α[103] can also activate IGF-2R. Thus, there is emerging evidence for specific signalling that is mediated by the IGF-2R.

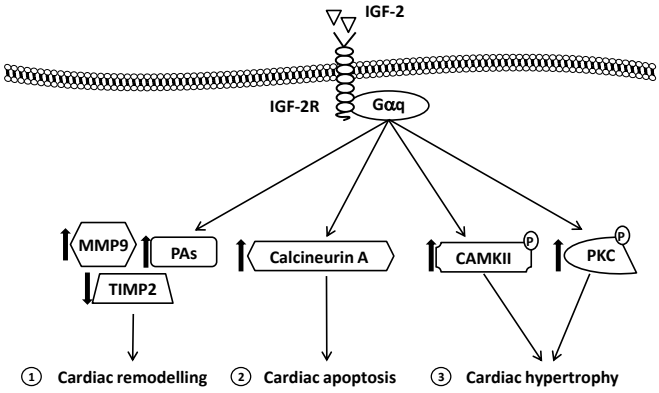

Figure 2. Signalling molecules affected by IGF-2R. *IGF-2R couples with Gαq leading to ➀ cardiac remodelling,* via *imbalance in the MMP-9/TIMP-2 expression levels and increases plasminogen activator (PAs) expression, ➁ apoptosis,* via *calcineurin A pathway, and ➂ hypertrophy* via *increased CaMKII and PKC protein phosphorylation.*

Lambs that were born LBW had an increased cardiac IGF-2 and IGF-2R gene expression at 21 d of age compared to ABW lambs.[18] In the ABW lamb, an increase in cardiac IGF-2R gene expression is related to a relatively smaller left ventricle.[18] In contrast there was a positive relationship between IGF-2R protein abundance and relative left ventricular weight in the LBW lamb, suggesting that IGF-2R may signal a cardiac hypertrophic pathway in the LBW lamb (Figure 3). The IUGR-induced increase in cardiac IGF-2 and IGF-2R gene expression persists from fetal to postnatal life and may be epigenetically programmed to result in activation of a hypertrophic signalling pathway rather than a clearance pathway (Figure 4).

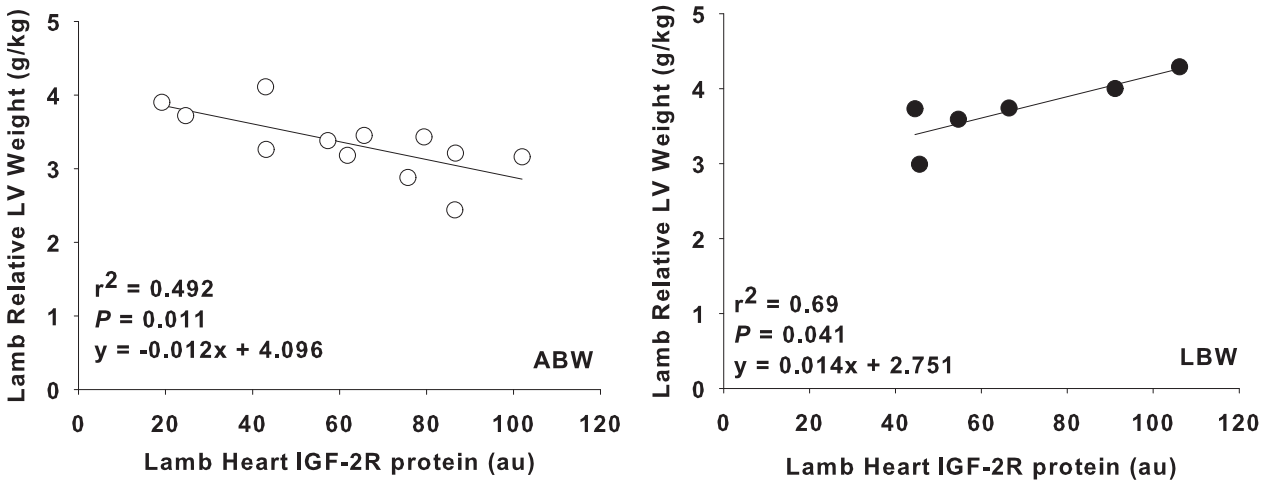

Figure 3. *In the normally grown average birth weight lamb (open circles) left ventricular weight relative to body weight is inversely correlated to IGF-2R protein abundance; suggesting IGF-2R is acting to clear IGF-2. In the low birth weight lamb, however, (filled circles) left ventricular weight relative to body weight is positively correlated to IGF-2R protein abundance; suggesting that IGF-2R is causing hypertrophy.*[18]

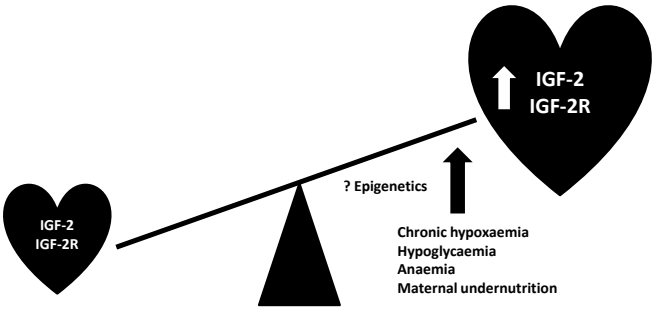

Figure 4. Intrauterine growth restriction and cardiac hypertrophy. *IUGR, as a result of a range of fetal and maternal insults, is associated with cardiac hypertrophy in fetal and postnatal life. This hypertrophy is associated with increased cardiac IGF-2 and IGF-2R gene expression, which emerging evidence suggests may be epigenetically regulated.*

Programming cardiac IGFs

IGF-2 and *IGF-2R* are parentally imprinted genes. *IGF-2* is expressed from the paternal allele, and *IGF-2R* is expressed from the maternal allele.[104-106] The imprinting at these loci involves epigenetic modification at regions within, or adjacent to the gene, and it is thought that these epigenetic modifications may be vulnerable to changes in the intrauterine environment.[107-111] It has been shown that *in vitro* culture of the sheep embryo results in epigenetic modifications at *IGF-2R*.[106] More recent studies raise the possibility that more subtle or physiological insults, such as IUGR, may result in epigenetic modifications of *IGF-2R*.[112,113] The major epigenetic processes include DNA methylation, acetylation, methylation or phosphorylation of histones, the proteins that are required for packaging DNA into chromatin, and small non-coding RNAs. These epigenetic modifications act either by interfering with the binding of transcription activators and repressors to specific gene promoters, and/or changing the structure of chromatin itself.[114] In the heart, IUGR did not change the degree of methylation of the 3 CTCF binding sites within the differentially methylated region (DMR) of *IGF-2/H19* or DMR within intron 2 of *IGF-2R*.[18] ANGII-induced hypertrophy *in vivo* and *in vitro* increases cardiac IGF-2R gene expression but there is no difference in the DNA methylation within the IGF-2R promoter compared to controls.[103] Interestingly, using inhibitors to individually block histone acetyltransferase (HAT) and histone deacetylase (HDAC) activity, it was demonstrated that histone acetylation was essential for ANGII-induced IGF-2R gene expression.[103] Furthermore, chronic hypoxia and maternal undernutrition results in epigenetic modification of other genes including PKC- ε, ANGII receptor 2 and peroxisomal proliferator-activated receptor-α in the heart.[115-117] Additional investigations are required to better understand the epigenetic regulation of IGF-2 and IGF-2R in the heart.

Conclusion

IUGR is associated with LVH and an increased risk of death from heart disease later in life. The IGFs and more specifically the IGF-2R have been implicated in pathological hypertrophy *via* Gαq signalling. Interestingly, the IGF-2R was traditionally viewed as a clearance receptor, internalising IGF-2 to prevent it from activating physiological hypertrophy through the IGF-1R signalling pathway. IUGR is associated with an increase in IGF-2R and its ligand IGF-2 in fetal life and this effect persists into postnatal life. Data presented in this review suggest that the

IGF-2R may contribute to the adverse adult cardiac outcomes in IUGR infants. It is clear that further studies are required to understand the regulation and programming of the IGF-2R and to determine whether or not intervention strategies to suppress the IGF-2R are likely to be beneficial in improving lifelong cardiac outcomes after IUGR.

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