Akt signaling as a mediator of cardiac adaptation to low birth weight

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Abstract

Intrauterine insults, such as poor nutrition and placental insufficiency, can alter cardiomyocyte development, and this can have significant long-term implications for heart health. Consequently, epidemiological studies have shown that low-birth-weight babies have an increased risk of death from cardiovascular disease in adult life. In addition, intrauterine growth restriction can result in increased left ventricular hypertrophy, which is the strongest predictor for poor health outcomes in cardiac patients. The mechanisms responsible for these associations are not clear, but a suboptimal intrauterine environment can program alternative expression of genes such as cardiac IGF-2/H19, IGF-2R and AT₁R through either an increase or decrease in DNA methylation or histone acetylation at specific loci. Furthermore, hypoxia and other intrauterine insults can also activate the IGF-1 receptor via IGF-1 and IGF-2, and the AT₁ receptor via angiotensin signaling pathways; both of which can result in the phosphorylation of Akt and the activation of a range of downstream pathways. In turn, Akt activation can increase cardiac angiogenesis and cardiomyocyte apoptosis and promote a reversion of metabolism in postnatal life to a fetal phenotype, which involves increased reliance on glucose. Cardiac Akt can also be indirectly regulated by microRNAs and conversely can target microRNAs that will eventually affect other specific cardiac genes and proteins. This review aims to discuss our understanding of this complex network of interactions, which may help explain the link between low birth weight and the increased risk of cardiovascular disease in adult life.

Key Words

- cardiac hypertrophy
- intrauterine growth restriction
- Akt
- IGFs
- epigenetics

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Association between low birth weight and cardiovascular disease

Cardiovascular disease is the world's leading cause of mortality, equating to ~17.5 million deaths per year, which is projected to increase to 22.2 million deaths per year by 2030 (WHO 2014). Large-scale epidemiological studies have shown that low birth weight is associated with an increase in the age-adjusted relative risk for cardiovascular disease (Barker *et al.* 1989, Osmond *et al.* 1993, Fall *et al.* 1995a,b,

Rich-Edwards *et al.* 1997). This association is independent of the recognized lifestyle-related cardiovascular disease risk factors, such as smoking, ethnicity, body mass index and socioeconomic grouping (Rich-Edwards *et al.* 1997).

In a cohort of 13,249 English men from Hertfordshire and Sheffield, those who had low birth weight and remained small at 1 year of age had 3 times the risk of death

from cardiovascular disease than males who were heavier at 1 year of age (Barker *et al.* 1989). Additional studies have confirmed that there is a significant and specific inverse relationship between birth weight and the risk of cardiovascular disease in adulthood (Osmond *et al.* 1993, Rich-Edwards *et al.* 1997, Leon *et al.* 1998, Huxley *et al.* 2007). In humans, reduced growth before birth is also associated with altered left ventricular mass (Vijayakumar *et al.* 1995), with intrauterine growth-restricted (IUGR) fetuses having a larger heart relative to their body weight (Veille *et al.* 1993). Furthermore, the altered left ventricular hypertrophy was transmitted to the F2 offspring in rats, indicating transgenerational programming effects of IUGR (Master *et al.* 2014). Left ventricular hypertrophy in adulthood is also associated with deleterious cardiovascular disease outcomes (Levy *et al.* 1988, 1989, 1990), prompting studies to understand the molecular basis of this association and the impact on cardiac growth and maturation. Some of the physiological mechanisms underlying the association between suboptimal growth during gestation or low birth weight and later life cardiovascular disease are known, but many questions remain. This review explores insulin-like growth factor 1 receptor (IGF-1R), insulin-like growth factor 2 receptor (IGF-2R) and angiotensin II type 1 receptor (AT₁R)-mediated Akt signaling as molecular foundations for the association between low birth weight and cardiovascular disease in adulthood.

Cardiomyocyte development during fetal life

Cardiogenesis is the process in which the heart is formed from progenitor cells including looping and septation. During early gestation, after cardiogenesis, mononucleated

cardiomyocytes increase the mass of the heart by hyperplastic growth (Burrell *et al.* 2003, Jonker *et al.* 2007b, Botting *et al.* 2012, Jonker & Louey 2016). This is followed by a transition period during which there is a decline in the proportion of mononucleated cardiomyocytes and a corresponding increase in the proportion of cardiomyocytes that are binucleated or polyploid (Burrell *et al.* 2003, Jonker *et al.* 2007b). Binucleation occurs when mononucleated cardiomyocytes undergo DNA synthesis and nuclear mitosis without cytokinesis (Li *et al.* 1997a,b, Jonker *et al.* 2007a, Jonker & Louey 2016). These binucleated cardiomyocytes do not proliferate and subsequent increases in cardiac mass occur via hypertrophy of binucleated or polyploid cardiomyocytes (Smolich *et al.* 1989, Lumbers *et al.* 2005, Botting *et al.* 2012). Interestingly, this process begins before birth in some species, such as humans and sheep, but after birth in other species such as mice and rats (Botting *et al.* 2012). This is important because it is in late gestation when complications in pregnancy such as placental insufficiency may impact on cardiomyocyte endowment in the human (Fig. 1), and thus, studies in an animal model where the maturation of cardiomyocytes occurs at the same time as in the human are likely to be the most informative. Furthermore, although there is some capacity for renewal of cardiomyocytes during childhood and adolescence in humans (Bergmann *et al.* 2009, 2015, Mollova *et al.* 2013), the capacity to repair after damage is very limited after birth (Fratz *et al.* 2011). Therefore, any insults to the heart during this developmental transition period can have a lifelong effect, especially as cardiomyocytes begin withdrawing from the cell cycle at around the time of birth. In IUGR, there are fewer cardiomyocytes in the fetus and offspring (Stacy *et al.* 2009, Black *et al.* 2012,

Figure 1
IUGR decreases cardiomyocyte endowment and the reduced number of cardiomyocytes in the heart are forced to undergo hypertrophy to maintain cardiac function (Morrison *et al.* 2007, Wang *et al.* 2011, Black *et al.* 2012, Botting *et al.* 2014, Master *et al.* 2014).

Botting *et al.* 2014), which may limit the potential of the heart to adapt to changes in supply of nutrients and oxygen in fetal life or to changes in load after birth. Thus, to maintain homeostasis, the fetal heart adapts to continued environmental insults through compensatory hypertrophy that may begin as physiological (Bae *et al.* 2003, Morrison *et al.* 2007, Thornburg *et al.* 2008, Wang *et al.* 2011, Botting *et al.* 2014), but proceed to pathological later in life (Vijayakumar *et al.* 1995, Master *et al.* 2014), and thus, lead to health consequences in adult life.

Cardiomyocyte growth is determined by a range of hormones and signaling pathways. The insulin-like growth factor (IGFs) and renin–angiotensin system (RAS) signaling pathways play vital roles in heart growth and the overall growth of the fetus. In fetal life, the IGF-1R signaling pathway has been implicated in physiological growth of the heart, which can involve both proliferation and hypertrophy (Sundgren *et al.* 2003, Lumbers *et al.* 2009, Wang *et al.* 2011). The IGF-2R was once viewed as only a clearance receptor for plasma IGF-2 (Kornfeld 1992, Powell *et al.* 2006) but is now acknowledged to induce cardiac hypertrophy (Chu *et al.* 2008, Wang *et al.* 2011, 2012a,b). Additionally, the RAS also plays a role in cardiac hypertrophy (Ichihara *et al.* 2001) and regulates factors involved in capillary formation (Yazawa *et al.* 2011), autophagy (Porrello *et al.* 2009) and fibrosis (Domenighetti *et al.* 2005). The activities of both the IGF and RAS signaling pathways can be moderated by epigenetic mechanisms, such as DNA methylation, histone acetylation and microRNA (miR), which will be discussed later in this review. Furthermore, the activation

of both of these signaling pathways can regulate the activity of downstream proteins such as protein kinase B (Akt). Akt is a serine/threonine protein kinase that plays a central role in regulating different aspects of heart growth (Fig. 2), which determine the number (proliferation, apoptosis and autophagy), size (hypertrophy) and metabolic activity (Datta *et al.* 1997, Donthi *et al.* 2000, DeBosch *et al.* 2006, Chang *et al.* 2010, Hua *et al.* 2011) of cardiomyocytes. We will review the effects of each of these signaling pathways on cardiac growth and on downstream proteins, their possible regulation through epigenetic mechanisms, and their roles in mediating the effects of IUGR on cardiac development in the fetus and cardiac health in postnatal life.

Akt is epigenetically regulated in several ways

Impact of DNA methylation of *IGF-2/H19*, *IGF-2R* and *AT₁R* genes on Akt

Epigenetic changes involve modifications that either interfere with the binding of transcription activators or repressors to specific gene promoters and/or changes in the structure of chromatin (Ptak & Petronis 2008). Epigenetic changes occur in response to environmental and behavioral influences, such as nutrition and smoking (Mathers *et al.* 2010) and are responsible for inducing stable inheritable changes in gene expression that are potentially reversible (Kiefer 2007), but may also promote a transgenerational phenotype (Hanson & Skinner 2016). A suboptimal intrauterine environment can lead

Figure 2

The impact of epigenetic regulation (miR, DNA methylation and histone acetylation) of IGF-1R, IGF-2R, AT₁R and Akt on cardiac growth and development (Datta *et al.* 1997, Brunet *et al.* 1999, Bartel 2004, Shiojima *et al.* 2005, Wang *et al.* 2015a,b).

to epigenetic changes in *IGF-2/H19*, *IGF-2R* in various tissues, such as the placenta (for further details, please read Young *et al.* 2001, Reik *et al.* 2003, Dolinoy *et al.* 2007) and *AT₁R* of the liver (Tosh *et al.* 2010). In human adults that were born growth restricted; for example, there is increased methylation at the differentially methylated region (DMR) 2 of the *IGF-2R*, in DNA from the peripheral blood lymphocytes of adults compared to individuals that were born a normal birth weight (Turner *et al.* 2010). It was hypothesized that the increased methylation at DMR 2 of *IGF-2R* in growth-restricted individuals resulted in increased *IGF-2R* gene expression, which could lead to a decrease in circulating *IGF-2* and thus decreased growth (Turner *et al.* 2010). Changes in the DNA methylation status of the *IGF-2/H19* and *IGF-2R* genes due to a suboptimal intrauterine environment have also been found in sheep adrenals (Zhang *et al.* 2010) and human HUVEC and endothelial cells (Ollikainen *et al.* 2010).

Although DNA methylation may be involved in cardiac pathogenesis, the significant increases in cardiac *IGF-2* and *IGF-2R* mRNA expression in low-birth-weight lambs before and after birth were not due to changes in the DNA methylation at the DMR within the intron 2 of *IGF-2R* (Wang *et al.* 2011). Rat models of left ventricular hypertrophy, including angiotensin II (ANGII) infusion or spontaneous hypertension, are also associated with

increased cardiac *IGF-2R* gene expression. However, this was not due to changes in DNA methylation either, at least not for the DMR within the promoter of intron 1 nor within intron 2 in the *IGF-2R* (Chu *et al.* 2012). In the IUGR fetus experiencing hypoxemia *in utero*, the increase in *IGF-2* and *IGF-2R* mRNA expression may be a response to chronic hypoxemia; however, this would not be the case in the low-birth-weight lamb after birth because it is normoxemic. In addition, we found decreased cardiac *AT₁R* protein in low-birth-weight lambs with no change in *AT₁R* promoter DNA methylation or *AT₁R* mRNA expression (Wang *et al.* 2015a). These findings suggested that DNA methylation was not involved in the cardiac hypertrophy in response to hypoxemia, but rather that other epigenetic mechanisms may be responsible for the increased expression of these genes before birth and that are maintained after birth.

Histone acetylation on *IGF-2* and *IGF-2R* gene expression and Akt

Hypoxia can induce histone acetylation, specifically at histone 3 lysine 9 (H3K9) and histone 4 lysine 8 (H4K8) (Johnson *et al.* 2008, Bouquet *et al.* 2011), opening the chromatin, making the DNA available for transcription and resulting in increased mRNA expression (Fig. 3). This effect

Figure 3

HIF1 α and reactive oxygen species induce cardiac protein acetylation (Grandjean *et al.* 2001b, Patterson *et al.* 2010, Bouquet *et al.* 2011, Guo *et al.* 2011, Wang *et al.* 2015b).

is persistent, but reversible (Jeppesen 1997, Smith *et al.* 2002, VerMilyea *et al.* 2009, Fernandez-Gonzalez *et al.* 2010), which is important from a therapeutic viewpoint, if this is the hypoxia-induced mechanism that leads to cardiac hypertrophy. Hypoxia-inducible factor (HIF)-1 α can activate the demethylase protein jumonji domain containing 1A (JMJD1a), which demethylates H3K9 residues and allows them to be acetylated by histone acetylases (Mimura *et al.* 2011). HIF-1 α also acts through Akt to increase the expression of GCN5, a histone acetyltransferase that targets genes with a specific H3K9 and H4K8 sequence (Kuo *et al.* 1996, Maeng *et al.* 2009, Guo *et al.* 2011, Jin *et al.* 2011). Hypoxia increases reactive oxygen species (ROS) generation (Patterson *et al.* 2010), and the accumulation of ROS under oxidative stress can also increase protein acetylation in the heart (Thompson & Al-Hasan 2012, Horton *et al.* 2016). The elements of these pathways that result in histone acetylation may therefore be responsible for the altered expression of genes encoding growth factors and receptors, which activate the signaling pathways associated with hypoxia-induced cardiac hypertrophy.

If gene methylation is not a factor in hypoxia-induced pathogenesis, then histone acetylation of cardiac *IGF-2*, *IGF-2R* and *AT₁R* may be the underlying mechanism linking low birth weight to poor cardiovascular health in adult life. There is evidence linking altered histone acetylation with increased cardiac expression of *IGF-2*/*IGF-2R* and Akt signaling in response to hypoxia. For example, ANGII-induced hypertrophy in the rat heart increases *IGF-2R* gene expression specifically through histone acetylation at H3K9 and histone 4, and not through the methylation of the DMRs in *IGF-2R* promoter, intron 1 or 2 (Chu *et al.* 2012). Furthermore, *IGF-2* and *IGF-2R* gene expression patterns are more closely related to the acetylation of H3K9 and H4K8 (Hu *et al.* 2000, Grandjean *et al.* 2001, Vu *et al.* 2004, Singh *et al.* 2010) than to methylation status (Yang *et al.* 2003). Interestingly, despite an increase in cardiac gene expression of *IGF-2* and *IGF-2R* in the chronically hypoxemic sheep fetus in late gestation (Wang *et al.* 2011), there is no evidence that the heart is hypoxic at this stage of gestation, as there was no change in gene expression of HIF1 α or its target genes (Botting *et al.* 2014). However, the heart may have been hypoxic earlier in gestation. Thus, a response to hypoxia in early gestation may result in altered gene expression that persists into postnatal life. Thus, it is important to understand at what stage in gestation the fetal heart has responded to hypoxia or ROS and if hypoxia or ROS are the underlying mediator of changes in cardiac *IGF-2*/*IGF-2R*

expression leading to an increased risk of left ventricular hypertrophy.

We have found increased acetylation of histone H3K9 of the *IGF-2R* promoter and *IGF-2R* intron 2 DMR in the hearts of low-birth-weight lambs compared to average-birth-weight lambs (Wang *et al.* 2015b). These observations may explain the increase in the gene expression of *IGF-2R* (Wang *et al.* 2011) and result in *IGF-2R* activation of the downstream G α q signaling pathway (Wang *et al.* 2015b) as well as left ventricular hypertrophy (Wang *et al.* 2011), which was observed in the low-birth-weight lamb. In addition, we reported decreased acetylation of the histone H3K9 *AT₁R* promoter, which may explain the decreased cardiac *AT₁R* protein abundance observed in low-birth-weight lambs (Wang *et al.* 2015a). Although histone acetylation may be important in altering the expression of cardiac *IGF-2*, *IGF-2R* and *AT₁R* of the IUGR fetus and/or low-birth-weight lamb, there is evidence that the *IGF-1R*, *IGF-2R* and *AT₁R* signaling pathways involving Akt, are also regulated by specific miR, which are short, noncoding RNA molecules that cause gene silencing by inhibiting mRNA translation or promoting mRNA degradation and have been implicated in cardiac development and disease (Porrello 2013). We have previously investigated the changes in sheep cardiac miRs across late gestation and early postnatal life that suggests a coordinated and complex role of multiple miRNA in the regulation of cardiomyocyte quiescence (Morrison *et al.* 2015). Such miRs may be an important component of the regulatory process contributing to an increase in cardiac *IGF-1R*, *IGF-2R*, *AT₁R* and Akt signaling in response to chronic hypoxemia in the IUGR fetus.

Impact of a suboptimal intrauterine environment and miRs on Akt

microRNA-378 is abundant in the rat cardiomyocytes, and it can directly target *IGF-1R* and subsequent downstream signaling of Akt, which is associated with postnatal cardiac remodeling and cardiomyocyte survival during stress (Knezevic *et al.* 2012). We have also previously shown that the expression of miR-378 increased from 90-day gestation to 173 days after birth in sheep (Morrison *et al.* 2015). Many other miRs also target *IGF-1R* in other tissues (Huang *et al.* 2011, Gao *et al.* 2014, Wang *et al.* 2014). *In vitro* cardiac fibroblast studies have also shown that *AT₁R* can regulate various miRs (Jeppesen *et al.* 2011) and also appear to be a target of the miR-132/212 family (Eskildsen *et al.* 2015).

The significant increase of Akt in the hearts of low-birth-weight compared to average-birth-weight lambs in the absence of increased IGF-1R (Wang *et al.* 2011), but decreased AT₁R (Wang *et al.* 2015a), suggests that Akt may be epigenetically programmed in a suboptimal intrauterine environment. However, there are no data to suggest that hypoxia activates Akt through histone acetylation, but there is evidence that miR may be involved (Bartel 2004). miR plays important roles in cardiac development (Tatsuguchi *et al.* 2007, Porrello *et al.* 2011) and equally in heart failure (Ikeda *et al.* 2007, Thum *et al.* 2007).

Akt exists in different isoforms (Akt1, Akt2 and Akt3), which have been identified as a direct target of specific miRs (Fig. 2). For example, Lin and coworkers have shown that Akt1 is a direct target of miR-149* (Lin *et al.* 2010) and that miR-149* represses Akt1 to induce apoptosis *in vitro* in human cancer cells (Lin *et al.* 2010). It is more likely that miR regulates Akt2 rather than Akt1, as Akt2 has been demonstrated to be a direct target for a range of miR including miR-203 in a bladder cancer cell line (Saini *et al.* 2011) and miR-184 in neuroblastoma cell lines (Foley *et al.* 2010). Akt3 appears to be a direct target of miR-519d, as demonstrated in hepatocellular carcinoma cells (Fornari *et al.* 2012). Using a heart failure rat model, miR-133a was shown to improve cardiac function and reduce fibrosis via the inhibition of Akt (Sang *et al.* 2015). Thus, further work is required to establish if there is a direct link between miR and cardiac Akt expression in response to hypoxia.

miRs can also regulate Akt signaling indirectly by targeting genes that can inhibit or stimulate Akt (Fig. 2). For example, overexpression of cardiac miR-494 leads to decreased abundance of pro-apoptotic proteins such as Rho-associated, coiled-coil containing protein kinase 1 (ROCK1), phosphatase and tensin homolog (PTEN), Ca²⁺/calmodulin-dependent protein kinases II δ (CaMKII δ), as well as the anti-apoptotic proteins fibroblast growth factor receptor 2 (FGFR2) and leukemia inhibitory factor (LIF) (Wang *et al.* 2010). The overexpression of these proteins can increase phospho-Akt abundance and thus downstream targets of Akt to reduce ischemia/reperfusion injury (Wang *et al.* 2010). Thus, suppression of these proteins by the action of miR-494 reduces phospho-Akt abundance and ultimately the protective effects of Akt targets against ischemic injury. miR-378 can directly target IGF-1R resulting in its inhibition and thus a reduction in Akt signaling (Knezevic *et al.* 2012). PTEN, a phosphatidylinositol (PI)-3-kinase (PI3K)/Akt antagonist that acts through dephosphorylation of phosphoinositide-3,4,5-triphosphate, an upstream effector

of Akt, is a direct target of many miRs, resulting in the inhibition of Akt signaling (Shiojima & Walsh 2006). miRs that can target PTEN include miR-21 (Sayed *et al.* 2010, Dey *et al.* 2012, Kumarswamy *et al.* 2012b), miR-205 (Qu *et al.* 2012), miR-221/222 (Garofalo *et al.* 2009), miR-216a/217 (Kato *et al.* 2009), miR-26b, miR-128 (Palumbo *et al.* 2013) and miR-486 (Small *et al.* 2010), all of which were expressed in the sheep heart from mid-gestation to 173 days after birth (Morrison *et al.* 2015). These studies demonstrate the potential of manipulating Akt using a specific miR to either stimulate or inhibit Akt signaling.

Interestingly, Akt can also regulate its downstream targets through epigenetic mechanisms, creating several steps for epigenetic regulation of the activity of Akt. For example, in breast cancer cell lines with higher Akt1 activity, there is epigenetic silencing of PI3K/Akt-regulated loci through either trimethylation of H3K27 or DNA methylation (Zuo *et al.* 2011). Akt regulates miR-210 through a HIF-independent mechanism in hypoxic cultured cardiomyocytes (Mutharasan *et al.* 2011). Furthermore, Akt can also upregulate miR-21, resulting in the inhibition of pro-apoptotic genes such as Fas ligand and PTEN in these hypoxic cardiomyocytes (Sayed *et al.* 2010). In addition, constitutively active Akt was induced in cultured cardiomyocytes by a 70% reduction in miR-199a-5p expression. This inhibition of miR199a-5p resulted in an increase in the expression of its target genes, HIF-1 α and Sirt1, both of which promote cardiomyocyte survival (Rane *et al.* 2010). Therefore, Akt plays a major role in modulating anti-apoptotic pathways in cardiac ischemia preconditioning (Sayed & Abdellatif 2010). Akt phosphorylation in chronic failing hearts is accompanied by FoxO3a phosphorylation, which is responsible for decreased miR-1 expression (Kumarswamy *et al.* 2012a). The repression of miR-1 in failing hearts can be normalized by AAV9.SERCA2a treatment via reversal of the phosphorylated Akt–FoxO3a axis (Kumarswamy *et al.* 2012a). In HeLa and 293T cell lines, Akt can also modulate the stability of DNA methyltransferase (DNMT) 1 protein (Sun *et al.* 2007), a protein that maintains methylation at genomic imprints during preimplantation development (Branco *et al.* 2008). In addition, Akt enhances DNMT 1 stability and maintains DNA methylation and chromatin structure in HeLa and 293T cell lines (Sun *et al.* 2007). Collectively, this shows that Akt is integrally involved in epigenetic regulation both of its own activity and as a consequence of its impact on targets through different epigenetic mechanisms. Consequently, there

are many targets for programming a lifelong change in the expression of specific genes, which can regulate the growth of cardiomyocytes not only in fetal life but also after birth, potentially influencing cardiovascular risk in adult life.

Cardiac Akt has roles in multiple signaling pathways that mediate multiple effects in the heart of the IUGR fetus

Impact of IUGR on Akt-mediated cardiomyocyte growth

Activation of Akt results in downstream activation of hypertrophic factors such as P70S6K, which is dependent on the mammalian target of rapamycin (mTOR; Fig. 2). Activation of mTOR leads to the activation of angiogenic factors, vascular endothelial growth factor-A (VEGF-A) and angiopoietin-2 (Ang-2) (Shiojima *et al.* 2005). In terms of the impact of IUGR on cardiomyocyte growth, we have shown a delay in binucleation of cardiomyocytes in IUGR fetuses. Associated with this delay in binucleation, we have observed an increase in the expression of molecules involved in hypertrophy in the IUGR fetus that persists in the low-birth-weight lamb, which have increased left ventricle relative to body weight (Wang *et al.* 2011). We have speculated that in fetal life, IGF-1R mediates the delay in binucleation, whereas IGF-2R mediates the increase in hypertrophy of cardiomyocytes (Wang *et al.* 2011). In average-birth-weight lambs, IGF-2R appears to play the traditional clearance role. However, in low-birth-weight lambs, there was increased Akt (Wang *et al.* 2015a), and this IGF-2R G_{aq} signaling may be responsible for the increased left ventricular hypertrophy (Wang *et al.* 2011, 2015b). These data show that a suboptimal *in utero* environment alters the expression of different receptors with the subsequent activation of signaling pathways that may alter the phenotype of the heart.

Akt increases angiogenesis to match cardiomyocyte endowment

A transient increase in Akt activity has been shown to increase protein levels of VEGF-A and Ang-2, coupled with an increase in capillary density (Shiojima *et al.* 2005). However, prolonged expression of Akt, as observed in pathological hypertrophy, does not increase either of these angiogenic growth factors and decreases capillary density (Shiojima *et al.* 2005). This suggests that angiogenesis can initially support hypertrophy, but is not maintained with

the advent of excessive hypertrophy, and this absence of compensatory angiogenesis appears to be due to increased Akt activity (Shiojima *et al.* 2005). In rats, prenatal glucocorticoid-induced IUGR resulted in increased Akt activity in 24-week offspring (Langdown *et al.* 2001). Ten-week-old rats that were exposed to chronic hypoxia *in utero* had decreased capillary density and impaired cardiac relaxation, but their cardiac contractile performance was increased (Hauton & Ousley 2009). On the other hand, transgenic mice with overexpression of cardiac ANGII have decreased capillary density (Xu *et al.* 2007). In 21-day low-birth-weight lambs with left ventricular hypertrophy, there is a decrease in total capillary length compared with average birth weight lambs due to increased cardiac ANGII and increased Akt signaling (Wang *et al.* 2015a).

Studies in models of pathological hypertrophy have investigated mechanisms to correct the mismatch between cardiomyocyte size and capillary density. Rapamycin administration, which blocks Akt activity through mTOR, attenuates the hypertrophy induced by pressure overload and subsequently improves contractile function (McMullen *et al.* 2004). Furthermore, angiogenic growth factor VEGF-A treatment of newborn rabbits that have pressure overload-induced hypertrophy decreases apoptosis of cardiomyocytes, preserves contractile function and reduces the incidence of death due to heart failure (Friebs *et al.* 2006). Consequently, by reducing the volume of cardiomyocytes or increasing the volume of coronary capillaries, contractile function is improved and apoptosis is reduced. These studies highlight the importance of maintaining the optimum ratio of cardiomyocyte to capillary volume, but this optimum is lost in pathological hypertrophy and can be induced either by ANGII or low birth weight.

Role of Akt in mediating IUGR-induced apoptosis

There is increased vulnerability to ischemia/reperfusion injury in rats exposed to prenatal hypoxia, which may be due to decreased protection against apoptosis (Li *et al.* 2003). Apoptosis is a programmed cellular death that can be activated by a wide range of physiological and pathophysiological events, including an extrinsic or intrinsic pathway (for further details, please read Saraste & Pulkki 2000, van Heerde *et al.* 2000, Botting *et al.* 2012). Akt can influence apoptosis through its actions on at least three different molecules (Fig. 2), some of which are also altered in IUGR. Akt is responsible for the phosphorylation of Bad (Datta *et al.* 1997,

del Peso *et al.* 1997), a member of the Bcl-2 family and promotes apoptosis through heterodimerization with anti-apoptotic Bcl-X_L, neutralizing the protective effect of Bcl-X_L in blocking cytochrome C release from the mitochondria. Phosphorylation of Bad, however, results in Bad dimerization with 14-3-3, preventing its association with Bcl-X_L (Zha *et al.* 1996). Activation of Akt also results in the phosphorylation of a member of the FKHRL1, which promotes the expression of Fas (death ligand) and other genes that are critically involved in apoptosis. Phosphorylation of FKHRL1 leads to its association with 14-3-3 proteins and retention in the cytoplasm (Brunet *et al.* 1999). Akt also phosphorylates pro-caspase 9 directly, preventing its cleavage by cytochrome C and subsequent 'effector' caspase activity (Cardone *et al.* 1998).

In the absence of phosphorylation of these pro-apoptotic factors by Akt, apoptosis will be increased, which has direct implications for cardiomyocyte endowment in the developing heart because cardiomyocyte endowment, the complement of heart cells that an individual will have for life, is determined before or around birth in humans and sheep, but after birth in rodents. We have previously shown that in sheep fetuses exposed to chronic hypoxemia throughout gestation, there was a reduced number of total cardiomyocytes in the IUGR fetuses, but no change in the percentage of apoptotic cardiomyocytes or Bcl-2 and Bax mRNA expression (Botting *et al.* 2014).

In contrast, fetal rats exposed to maternal hypoxia in the last week of gestation had a greater percentage of apoptotic cardiomyocytes (Bae *et al.* 2003). Therefore, it seems that the timing of gestational insult may play a bigger role in programming cardiac apoptosis.

Impact of IUGR on Akt-mediated metabolism

Glucose is the substrate predominantly utilized by the fetal heart for energy metabolism, with ATP production being derived mainly from anaerobic glycolysis (Lopaschuk *et al.* 1991). Akt mainly regulates glucose transporter (GLUT)-1 gene expression (Fig. 4), particularly by Akt-dependent activation of mTOR complex 1 (Manning & Cantley 2007). On the other hand, cardiac Akt is important for the translocation of GLUT-4 as Akt can also be activated by the insulin receptor (IR), leading to the inhibition of Akt substrate 160 (AS160), which then stimulates the translocation of GLUT-4-containing vesicles to the sarcolemma for the transport of glucose into cardiomyocytes (Sano *et al.* 2003). However, a rapid shift from glucose to fatty acid metabolism occurs in the postnatal period, where thereafter fatty acids contribute ~70% of the total ATP generated (Lopaschuk *et al.* 2010).

In animal models of fetal growth restriction (Langdown *et al.* 2001, Tappia *et al.* 2005, Chan *et al.* 2009) and ventricular hypertrophy (Iemitsu *et al.* 2003,

Figure 4

Myocardial uptake of glucose via facilitated diffusion is mediated by glucose transporters located on the sarcolemma. Akt activation results in the translocation of GLUT-1 and GLUT-4 to the sarcolemma for the transport of glucose into cardiomyocytes (Sano *et al.* 2003, Manning & Cantley 2007).

Nascimben *et al.* 2004), there is a reversion to the fetal phenotype of glucose metabolism in postnatal life. This has been attributed to a decrease in the expression of proteins involved in fatty acid uptake (Binas *et al.* 1999, Hajri *et al.* 2001). Akt has been shown to play an important role in mediating this shift toward increased glucose metabolism. For example, chronic activation of Akt in transgenic mice increased basal glucose uptake and cardiac glycogen deposition (Matsui *et al.* 2006). Downregulation of peroxisome proliferator-activated receptor (PPAR) α and PPAR γ coactivator 1 (PGC-1) was also reported in transgenic mice with cardiac-specific expression of constitutively activated Akt (Cook *et al.* 2002). In the hearts of IUGR sheep fetuses, there was increased glucose uptake and metabolic response to insulin to maintain myocardial energy supply and subsequent myocardial function and growth (Barry *et al.* 2016). There is also increased IR, GLUT-1 and phospho-Akt abundance in the hearts of low-birth-weight lambs at day 21, all of which suggest increased reliance on glucose metabolism for cardiac function (Wang *et al.* 2013). In addition, in the low-birth-weight lamb, there was increased pyruvate dehydrogenase kinase-4 (PDK-4) abundance, which may indicate impaired pyruvate conversion and decreased ATP production from glucose as a result of inhibiting pyruvate dehydrogenase (PDH) (Wang *et al.* 2013). In the context of low birth weight, if a switch to the fetal metabolic phenotype in the heart does occur after birth and is permanent, this may be one mechanism leading to increased vulnerability to developing cardiovascular diseases in adult life in individual born low birth weight.

Conclusion

miR can regulate Akt activation or function as downstream effectors of Akt (Sayed & Abdellatif 2010), and this has an important role in cardiomyocyte development, with direct consequences for cardiac hypertrophy in response to chronic hypoxemia in the fetus. Although Akt is a target for 'programming', directly blocking Akt is not an ideal therapeutic approach (Buss *et al.* 2012), because Akt is central to multiple signaling pathways that control homeostasis, including survival, energy production, contractility and response to pathological stress in the heart (Sussman *et al.* 2011). However, investigating cardiac miRs that can be regulate Akt in the context of low birth weight, may lead to new therapeutic agents to modulate Akt signaling in response to chronic hypoxemia. This may allow reversal

of the negative effects of this *in utero* environment before or shortly after birth. Importantly, the differential effects of cardiac IGF-2R and the effect of Akt on downstream pathways under conditions of decreased substrate supply *in utero* warrants further investigation. This new knowledge about the development of cardiac hypertrophy in the IUGR and chronically hypoxemic fetus will lead to improved identification and intervention strategies for individuals at risk of cardiovascular disease in later life and have direct significance for global heart health.

Declaration of interest

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