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.

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,
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
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 is mediated by the acetylation of histone deacetylase (HDAC) and histone acetyltransferase (HAT) proteins. The acetylation of these proteins results in the recruitment of transcription factors, such as p300/CBP, which can activate the expression of IGF-2 and IGF-2R genes.

![Figure 3](http://joe.endocrinology-journals.org)
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)-1α 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-1α 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 HIF1α 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 Gαq 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 Rh-associated, coiled-coil containing protein kinase 1 (ROCK1), phosphatase and tensin homolog (PTEN), Ca2+/calmodulin-dependent protein kinases II δ (CaMKIIδ), 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 midgestation 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-1α 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 & Abbellatif 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
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_{m} 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 (Friehs 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 & Pulikki 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<sub>L</sub>, neutralizing the protective effect of Bcl-X<sub>L</sub> 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<sub>L</sub> (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, Manning & Cantley 2007),...
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 increases basal glucose uptake and cardiac glycogen deposition (Matsui et al. 2006). Downregulation of peroxisome proliferator-activated receptor (PPAR) α and PPARγ 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**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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**References**


Botting KJ, McMillen IC, Forbes H, Nyengaard JR & Morrison JL 2014 Chronic hypoxia in late gestation decreases cardiomyocyte number but does not change expression of hypoxia-responsive genes. *Journal of the American Heart Association* **3** e000531. (doi:10.1161/JAHA.113.000531)


Li F, Wang X, Bunting PC & Gerdes AM 1997a Formation of binucleated cardiac myocytes in rat heart: I. Role of actin-myosin contractile...


McMullen JR, Sherwood MC, Tarnavski O, Zhang L, Dorfman AL, Shiou T & Izumo S 2004 Inhibition of mTOR signaling with rapamycin regresses established cardiac hypertrophy induced by prenatal hypoxia. Circulation Research 105 1698–1705. (doi:10.1161/01.HY.0000144292.69599.0c)


Porrello ER 2013 microRNAs in cardiac development and regeneration. Clinical Science 125 151–166. (doi:10.1042/CS20130001)

Porrello ER, D‘Amore A, Curi CI, Allen AM, Harrap SB, Thomas WG & Delbridge LM 2009 Angiotensin II type 2 receptor antagonizes angiotensin II type 1 receptor-mediated cardiomyocyte autophagy. Hypertension 53 1032–1040. (doi:10.1161/HYPERTENSIONAHA.108.128488)


Vijayakumar M, Fall CH, Osmond C & Barker DJ 1995 Birth weight, weight at one year, and left ventricular mass in adult life. British Heart Journal 73 363–367. (doi:10.1136/hrt.73.4.363)

Vu TH, Li T & Hoffman AR 2004 Promoter–restricted histone code, not the differentially methylated DNA regions or antisense transcripts, marks the imprinting status of IGF2R in human and mouse. Human Molecular Genetics 13 2233–2243. (doi:10.1093/hmg/ddh244)


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