Early origins of cardiac hypertrophy: Does cardiomyocyte attrition program for pathological 'catch-up' growth of the heart?

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Summary

1. Epidemiologic and experimental evidence suggests that adult development of cardiovascular disease is influenced by events of prenatal and early postnatal life. Cardiac hypertrophy is recognized as an important predictor of cardiovascular morbidity and mortality, but the developmental origins of this condition are not well understood.

2. In the heart, a switch from hyperplastic to hypertrophic cellular growth occurs during late prenatal or early postnatal life. Postnatal growth of the heart is almost entirely reliant on hypertrophy of individual cardiomyocytes and damage to heart muscle in adulthood is typically not reparable by cell replacement. Therefore, a reduced number of cardiomyocytes may render the heart more vulnerable in situations where an increased workload is required.

3. A number of different animal models have been used to study fetal programming of adult diseases, including nutritional, hypoxic, maternal/neonatal endocrine stress, and genetic models. Although studies investigating the cellular basis of myocardial disease in growth-restricted models are limited, a reduction in cardiomyocyte number through either reduced cellular proliferation or increased apoptosis appears to be a central feature.

4. The mechanisms responsible for the programming of adult cardiovascular disease are poorly understood. We hypothesize that cardiac hypertrophy can have developmental origin in excess cardiomyocyte attrition during a critical perinatal growth window. Findings which have directly assessed the impact of fetal growth restriction on the myocardium are considered, and cellular and molecular mechanisms involved in the potential pathologic 'catch-up' growth of the heart during later maturation are identified.

Introduction

Epidemiologic and experimental evidence suggests that adult development of cardiovascular disease is influenced by events of prenatal and early postnatal life.[1] Cardiac hypertrophy (an increase in heart size) is recognized as an important predictor of cardiovascular morbidity and mortality,[2] but the developmental origins of this condition are not well understood. Indeed, current understanding of the early mechanisms which drive the programming of adult diseases in general is limited. We hypothesize that cardiac hypertrophy can have developmental origin in excess cardiomyocyte attrition during a critical perinatal growth window. Findings which have directly assessed the impact of fetal growth restriction on the myocardium are considered, and cellular and molecular mechanisms involved in the potential pathologic 'catch-up' growth of the heart during later maturation are identified.

The developmental origins of adult disease

The developmental (or "early"/"fetal") origins of adult disease hypothesis postulates that environmental factors, particularly nutrition, act early in life to program the later occurrence of cardiovascular and metabolic disease and premature death in adulthood.[1] Correlations between infant mortality rates and cardiovascular and coronary heart disease incidence were first reported over 30 years ago.[3] The hypothesis that cardiovascular disease has origin *in utero* emerged following the seminal observations of Barker,[4] who found high rates of coronary heart disease occurred in populations with high neonatal mortality. Subsequent epidemiological investigations extended these initial findings to identify associations between low birth-weight and increased risk for hypertension, impaired glucose tolerance, type 2 diabetes, insulin resistance, obesity, and metabolic syndrome.[1]

There is now convincing epidemiological evidence to support the contention that impaired growth *in utero* is associated with increased cardiovascular risk in adulthood, and this has prompted a recent call to move beyond observation of association towards achieving an understanding of the underlying mechanisms.[5] Experimental studies have significantly advanced understanding of the consequences of intrauterine growth restriction (IUGR) on vascular, renal, and endocrine development, antecedent to the later occurrence of hypertension, renal disease, and type 2 diabetes, respectively (reviewed in McMillen, 2005[1]). Surprisingly, while left ventricular hypertrophy has been reported in growth restricted infants,[6] experimental investigation of the effects of early growth perturbation on later disease outcome in the heart has been extremely limited.

Neonatal cardiac growth and development

Cardiomyocyte hyperplastic and hypertrophic growth

The heart is the first organ to form during mammalian embryogenesis[7] and is the organ most affected by disease in childhood and in adulthood.[8] Prenatal growth of the heart is primarily due to hyperplasia of cardiomyocytes. A switch from hyperplastic to hypertrophic cellular growth occurs during late prenatal or early postnatal life.[9,10] This switch from hyperplastic to hypertrophic growth coincides with binucleation of cardiomyocytes and the transition to terminal differentiation (i.e. karyokinesis in absence of cytokinesis). The human equivalent of third trimester gestational development occurs postnatally in rodents, and the shift to terminal differentiation extends 3-4 days into the postnatal period.[9,10] In humans, there is evidence that this transition is more fully enacted prior to birth, but the switch from hyperplastic to hypertrophic cellular growth has not been tracked in detail. After the transition to terminal differentiation is completed, postnatal growth of the heart is almost entirely reliant on hypertrophy of individual cardiomyocytes and damage to heart muscle in adulthood is typically not reparable by cell replacement (Figure 1).
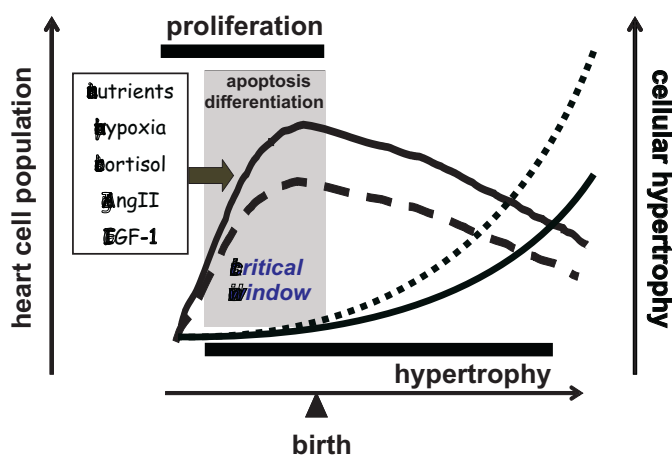
***Figure 1. Neonatal heart plasticity.** A hypothetical 'critical window' where proliferative, hypertrophic and apoptotic responses determine the extent of myocyte culling and define the cell population for ongoing hypertrophic growth. Perinatal insults such as poor nutrition, hypoxia and endocrine stress reduce the number of cells (dashed line) and limit the cell population available to support myocardial growth trajectory. Reduced cell number is hypothesized to be linked with increased cellular hypertrophy during maturation (dotted line).*

At the molecular level, cardiomyocyte number appears to be exquisitely controlled. Cardiac-specific deletion of survivin, an important regulator of cell division, leads to a reduced cardiomyocyte mitotic rate, without increased apoptosis, and a reduction in total cardiomyocyte number.[11] Survivin knock-out mice display cardiac dilatation, decompensated heart failure, and premature

mortality.[11] In hearts of these mice it was demonstrated that below a critical cardiomyocyte population threshold (5×10^6 cells per heart), cardiomyocyte size increased exponentially in association with a decrease in total myocyte number.[11] This finding suggests that a reduced number of cardiomyocytes may render the heart more vulnerable in situations where an increased workload is required (Figure 1). Despite these recent developments in the understanding of the complex regulation of the cardiac cell cycle, little is known about how physiological insults during early development (such as IUGR) can influence cardiomyocyte number at birth.

Cardiomyocyte apoptosis shapes the neonatal heart

Apoptosis is an important instrument in cardiovascular development and occurs at critical time points during major developmental processes.[12] While the importance of cardiomyocyte apoptosis during embryogenesis has been well documented,[12] less is known about the role of apoptosis during late gestation and early postnatal life. In rats, the number of cardiomyocytes in the right and left ventricle are approximately equal at birth.[13] Relatively abrupt changes in blood flow and circulatory resistance occur shortly after birth and the number of cardiomyocytes in the left ventricle becomes two-fold greater than the right ventricle.[14] Apoptotic activity in the postnatal rodent heart is highest at postnatal day 1 and declines thereafter.[15,16] Apoptosis affects the right ventricle more than the left ventricle during this critical postnatal period[15,16] and likely plays an important role in the cardiac adaptations that occur shortly after birth. The importance of apoptosis during the neonatal period was recently highlighted by a study in which the mitochondrial death protein Nix was conditionally over-expressed in either neonatal or adult mouse hearts.[17] While Nix over-expression in the early postnatal period resulted in apoptotic cardiomyopathy, Nix expressed at identical or higher levels in the adult heart had minimal effects on cardiomyocyte apoptosis, chamber size, or ventricular contractile function. Over-expression of Gαq under the control of the cardiac-specific alpha-myosin heavy chain (α-MHC) promoter in the post-natal heart is associated with apoptotic cardiomyopathy at maturity.[18] More recently, it was shown that conditional over-expression of Gαq was only associated with eccentric cardiac hypertrophy and systolic dysfunction following induction of the transgene in neonates, but not adults.[17] Clearly, apoptosis is critical for neonatal cardiac development and dysregulation of this process can result in cardiac disease. A more detailed understanding of how perinatal insults such as growth restriction impact myocardial apoptosis induction is required.

Physiological and pathological cardiac growth signaling

The neonatal period is a developmental period during which the heart grows rapidly.[19] Myocardial growth during this period must not only accommodate the increasing demands of the rapidly growing animal but also post-natal

changes in the patterns of blood flow and circulatory resistance (i.e. right to left heart loading).[20] Physiological growth of the heart also occurs in response to regular physical activity or chronic exercise training.[21] At the molecular level, an insulin/insulin-like growth factor 1 (IGF-1)-responsive signaling pathway involving activation of phosphoinositide 3-kinase (PI3K) and Akt is associated with physiological hypertrophy of the heart.[21] In mice, ablation of the p85 regulatory subunit of class 1_A PI3K suppresses post-natal developmental cardiac growth and impairs the physiological hypertrophic response of the heart to exercise.[22] However, information regarding the ontogeny of PI3K/Akt expression is limited and the physiological importance of this signaling pathway during the neonatal period awaits further resolution.[23-25]

In contrast, cardiac hypertrophy in response to pathological stimuli (e.g. hypertension, myocardial infarction) ultimately results in heart failure, arrhythmia, and sudden death.[26] The molecular hallmarks of pathological cardiac hypertrophy are well described and typically involve G protein-coupled receptor activation, signaling through mitogen activated protein kinases (MAPKs) and calcineurin A, and reversion to a fetal gene program.[27] In the adult, MAPK pathways have been characterized as mediating pathological remodeling. However, MAPK pathways also support growth of the neonatal heart, and studies of genetically manipulated models indicate that these pathways are of physiological importance early in maturation.[17,28] Despite intensive interrogation of hypertrophic signaling pathways in the adult heart, an understanding of the contributions of MAPK *vs* PI3K/Akt signaling in defining 'physiological' *vs* 'pathological' growth programming in the neonatal heart is lacking.

Intrauterine growth restriction and myocardial growth

A number of different animal models have been used to study fetal programming of adult diseases. These include nutritional (caloric restriction, low protein diet), hypoxic (bilateral uterine artery ligation, utero-placental embolization, single umbilical artery ligation, hypoxic chambers), maternal/neonatal endocrine stress (glucocorticoid exposure, maternal diabetes), and genetic (Spontaneously Hypertensive Rat; insulin receptor, insulin gene, IGF-1 and IGF-2 knock-out).[29] While the impact of IUGR on nephrogenesis has been extensively investigated,[30] fewer studies have examined the influence of IUGR on cardiogenesis. Below, findings of altered myocardial development in different experimental models of IUGR (maternal nutrient restriction, fetal hypoxia, and glucocorticoid exposure) are summarized and contrasted (Table 1).

Maternal nutrient restriction

Reduced supply of nutrients during prenatal and early postnatal life interferes with cell proliferation in various organs, including the heart,[31] and has been proposed as a central mechanism driving the 'programming' of adult

cardiovascular and metabolic disease. The most common nutritional models of IUGR involve feeding pregnant rats or sheep either a reduced calorie diet (50% normal intake) or a low protein diet (8-10% normal intake) during gestation, but there is considerable variation in the duration and nature of dietary interventions utilized in these types of investigation.

Minimal information regarding the influence of maternal caloric restriction on the heart is available. In rats, maternal nutrient restriction (40% control diet) during mid- to late-gestation does not program cardiac hypertrophy in neonates[32] or in offspring at either 4 or 7 months of age.[33] In sheep, maternal undernutrition (50% control diet) during early- to mid-gestation is usually associated with right and left ventricular hypertrophy in the fetus,[34-36] but the long-term effects in adult offspring have not been examined. An investigation of the impact of maternal caloric restriction on cardiomyocyte hypertrophy, hyperplasia, or apoptosis in growth-restricted offspring has not been reported.

The impact of maternal gestational protein restriction on fetal/neonatal myocardial development has been studied more extensively. The low protein diet model typically involves feeding pregnant rats an isocaloric low protein diet (8-10% protein *vs* 20% protein in normal diet) from the first day until the end of gestation. A low protein diet consistently programs hypertension and insulin resistance (reviewed in Vuguin, 2007[29]) and was linked with reduced survival at 11 months of age in rats.[37] While maternal protein restriction is associated with cardiac enlargement at 3 and 11 months of age in the rat,[37,38] a reduced heart weight is often found at younger ages.[31,39,40] Maternal protein restriction (9% protein) in rats is associated with increased rates of cardiomyocyte apoptosis[38] and a reduction in the total number of cardiomyocytes per heart at birth.[31] Interestingly, in these animals, a low protein diet significantly depresses ejection fraction and is associated with a thinner left ventricular wall in offspring during the first two weeks of life.[38] The early depression of cardiac function was followed by a gradual recovery and normalization of ejection fraction and a progressive increase in the thickness of the left ventricle wall.[38] At 40 weeks of age, these rats had significant left ventricular hypertrophy and displayed signs of cardiac dysfunction, indicative of pathological remodeling.[38] These studies indicate increased cardiomyocyte apoptosis during early development and post-natal 'catch-up' growth of the heart may be important for the programming of pathological cardiac hypertrophy.

Fetal hypoxia and placental restriction

Maternal chronic hypoxia exposure during gestation (10.5% O_2 from E15-21) increases the incidence of cardiomyocyte apoptosis almost 3-fold in the fetal rat heart.[41] The increased incidence of apoptosis in these hearts is associated with reduced expression levels of the pro-survival protein Bcl-2 and a concomitant increase in the apoptotic inducer Fas.[41] The size of binucleated cardiomyocytes is also increased in the fetal hypoxic rat

Table 1. *Analysis of hypertrophic, hyperplastic and apoptotic phenotypes in animal models of intrauterine growth restriction.*

Model	Species	Heart Size		Cardiomyocyte hypertrophy	Cardiomyocyte hyperplasia	Cardiomyocyte apoptosis	References
		Fetus/ Neonate	Adult				
Caloric	Rat	↔	↔	?	?	?	32,33
restriction	Sheep	↑	?	?	?	?	34-36
Protein	Rat	↓	↑	?	?	↑	31,37,38
restriction							
Hypoxia/	Rat	↑	↑	↑	?	↑	32,33,41
placental	Sheep	↓ or ↑	?	↔ or ↓	↔	?	42-44
restriction							
Glucocorticoid	Rat	↑	↑ or ↓	↑	↓	↔	50,53,54
exposure	Sheep	↑	↑	↑	↓ or ↑	?	48,49,51,55

Effect of intervention: ↑ increased effect, ↓ decreased effect, ↔ no effect, ? unknown effect.

heart, consistent with the interpretation that myocyte hypertrophy compensates for myocyte loss in maintaining tissue growth. The effect of fetal hypoxia on cardiomyocyte proliferation was not reported in this study. Studies of ovine models of placental restriction have indicated that cardiomyocyte proliferation (Ki67 staining) is unaffected by fetal hypoxia,[42] but have not yet addressed the question of whether apoptosis might be implicated.

Despite the implication of an important role for apoptotic cell death in the hearts of hypoxic rats with IUGR, the impact of fetal hypoxia on cardiac hypertrophy predisposition is still unclear. Studies in placental-restricted, hypoxic, ovine models of IUGR have reported reduced[42,43] or increased[44] heart size and delayed cardiomyocyte development in the growth-restricted fetus.[42,43] In contrast, maternal gestational hypoxia in rats induced cardiac hypertrophy in the fetus,[41] neonate,[32] and in offspring at 4 and 7 months of age, with associated increases in pathological hypertrophic gene expression, cardiac fibrosis and diastolic dysfunction.[33] Interestingly, maternal hypoxia in the rat was shown to increase predisposition to ischemia-reperfusion injury in adult offspring at 4, 6, and 7 months of age.[33,41] However, Li *et al.* could not detect any differences in the recovery from ischemia-reperfusion injury in offspring at 2 months of age.[45] Although there were minor differences in the ischemic protocols between these studies, these discrepancies suggest that increased vulnerability to ischemia-reperfusion injury develops relatively late in this model. While the effect of maternal hypoxia on hypertrophic predisposition in offspring awaits further resolution, it is clear from these studies that myocardial development at the cellular, whole organ, and functional level is significantly affected by chronic fetal hypoxia. A systematic analysis of the effects of fetal hypoxia on cardiomyocyte apoptosis, proliferation, and maturation throughout cardiac development is required.

Glucocorticoid exposure

Exposure to natural or synthetic glucocorticoids throughout pregnancy or during early post-natal life consistently programs adult cardiovascular and metabolic disease in animal models (reviewed in McMillen & Robinson, 2005[1]). In humans, there is evidence that dexamethasone treatment in preterm infants at risk of chronic lung disease is associated with various long-term adverse outcomes,[46] including hypertrophic cardiomyopathy.[47] Cardiac hypertrophy is also commonly observed in animal models of maternal, fetal, or neonatal glucocorticoid exposure,[48-51] but the underlying cellular mechanism is controversial (Table 1). High-dose cortisol infusion into the near-term ovine fetus has been associated with increased left ventricular cardiomyocyte size, but no change in myocyte number.[51] In contrast, neonatal dexamethasone treatment in rats was reported to be accompanied by a marked suppression of cardiomyocyte proliferation on post-natal days 2 and 4[52] and by cardiomyocyte hypertrophy at 50 weeks of age.[53] Administration of dexamethasone to pregnant rats during late gestation was observed to decrease total myocardial DNA content in offspring suggesting decreased cardiomyocyte proliferation.[54] Similarly, direct infusion of cortisol into near-term fetal sheep was found to reduce left ventricular DNA content.[55] Glucocorticoids are commonly elevated in growth-restricted models.[56] Collectively, these studies indicate that in such a perturbed endocrinologic environment, reduced cardiomyocyte proliferation and induction of cardiomyocyte hypertrophy occurs during late pre-natal and early post-natal life.

One major difficulty in interpreting the impact of pre- and post-natal glucocorticoid administration on cardiac development is the potential confounding influence of blood pressure changes in these typically hypertensive models.[51] The fetal heart is particularly responsive to alterations in hemodynamic loading, which can affect cardiomyocyte size, number, and maturation.[57] In order to analyse the direct effects of cortisol on the fetal heart,

Giraud *et al.* infused a sub-pressor dose of cortisol (0.5 μg/kg.min, 7d) into the circumflex coronary artery of fetal sheep.[49] While sub-pressor doses of cortisol were sufficient to induce cardiac hypertrophy in the fetal lamb, this was not accompanied by an increase in cardiomyocyte size and was surprisingly associated with an increased percentage of cells which stained positive for the cell proliferation marker Ki67.[49] This study suggested that the increase in cardiomyocyte size that is often reported in glucocorticoid treatment models may be a consequence of loading influence. However, *in vitro* evidence that corticosterone stimulates a hypertrophic response in cultured neonatal cardiomyocytes, indicates that glucocorticoids can directly induce cardiomyocyte growth.[58] While it is evident that glucocorticoids can induce cardiac hypertrophy *in vivo*, the underlying cellular mechanism awaits further resolution.

Angiotensin II, IGF-1 and cardiac development

Numerous growth factors have been identified as regulators of cardiomyocyte hyperplasia, hypertrophy, and apoptosis. In particular, angiotensin II (AngII) and IGF-1 both play an important role in early cardiac development and have been implicated in the programming response to IUGR.

Angiotensin II

All components of the renin-angiotensin system (RAS) are expressed in the heart from early gestation. The major effector peptide of the RAS is AngII. Local cardiac production of AngII has been implicated in the pathogenesis of cardiac hypertrophy.[59] However, AngII is also important for neonatal cardiac growth. In animals and humans the activity of the RAS increases around birth and is thought to exert a major influence in regulating cell growth and organ differentiation.[60,61] Treatment of neonatal piglets with an angiotensin-converting enzyme (ACE) inhibitor has been shown to interfere with the normal physiological hypertrophy of the left ventricle[62] and to suppress cell proliferation and apoptosis in the neonatal rat heart.[60]

The circulating RAS is up-regulated in the growth-restricted fetus.[63] AngII signaling in the neonate may therefore be of crucial importance in defining organ plasticity and capacity for long term maturational growth. Increased RAS activity is involved in the development of hypertension associated with IUGR. Treatment of IUGR rats exposed to a low protein diet *in utero* with an ACE inhibitor or an AT_1 receptor antagonist from 2-4 weeks after birth was observed to prevent the development of hypertension.[64,65] Similarly, treatment of the Spontaneously Hypertensive Rat, a genetic model of essential hypertension and growth restriction,[66] with an ACE-inhibitor for a 4 week period early in life (6-10 weeks of age) was found sufficient to prevent genetic hypertension and cardiac hypertrophy in this model.[67] Collectively, these studies demonstrate that AngII is important in the development of hypertension and cardiac hypertrophy during early life. IUGR is also associated with altered expression patterns of the AngII receptor subtypes (AT_1 and AT_2) in the kidney, adrenals, and liver.[68] However, there is little information regarding the expression levels of AngII receptor subtypes in the hearts of growth-restricted animals. Decreased fetal cardiac protein levels of AT_1 and AT_2 receptors, but unchanged mRNA expression, has been observed with maternal nutrient restriction.[34] Maternal cortisol administration was found not to significantly affect cardiac AT_1 or AT_2 gene expression,[51,69] however, protein expression was not assessed in these studies. Given that AT_2 receptor expression is largely restricted to embryonic, fetal and neonatal tissues,[70] and that the involvement of this receptor in fetal/neonatal heart development and growth has not been defined, more detailed investigations into the role of cardiac AngII receptors in the programming of cardiac hypertrophy are required.

IGF-1

IUGR is associated with reduced fetal serum and tissue levels of IGF-1.[39,71] Consistent with an important role for insulin-like growth factors and IGF-binding proteins in fetal growth, genetic knock-out of components of the IGF axis commonly results in growth restriction (reviewed in Vuguin, 2007[29]). IGF-1 also plays a critical role in the normal physiological growth of the heart. Over-expression of IGF-1 in the mouse heart causes an increase in heart weight, which is apparently due to cardiomyocyte hyperplasia rather than hypertrophy.[72] However, other studies of IGF-1 and IGF-1 receptor transgenic mice have attributed cardiac hypertrophy in these models to an increase in cell size, even though cardiomyocyte proliferation was not concurrently assessed in these studies.[73,74] Gene expression levels of IGF-1 are reduced in the hearts of fetal rats exposed to gestational hypoxia.[75] Therefore, the reduction in cardiomyocyte number observed in different models of IUGR may be at least in part related to suppressed levels of IGF-1.

Interestingly, in rats which undergo post-natal 'catch-up' growth, IGF-1 levels are increased.[39] It has been suggested that rapid post-natal growth in the context of reduced cell numbers within key body organs may produce detrimental outcomes for organ function.[1] The IGF-1 signaling axis has been intensively interrogated in the adult heart and is essential for normal physiological growth processes.[21] However, increased expression of IGF-1 signaling components has been reported in the hearts of growth-restricted offspring in adulthood.[76] Thus it could be speculated that heightened activation of this 'physiological' growth signaling axis in the setting of reduced cellular endowment may actually contribute to adverse pathological remodeling associated with IUGR. The relative influence of 'physiological' and 'pathological' hypertrophic signaling during cardiac development in growth-restricted models should be an area of focus for future investigations.

Growth restriction targets the cardiac cell cycle

Studies employing unbiased gene expression profiling in experimental models of IUGR have revealed

new molecular targets in the programming of myocardial disease. Of particular interest is the identification of dysregulated expression of cell cycle proteins in the growth-restricted fetal heart. Maternal gestational hypoxia is associated with down-regulation of a number of cell cycle regulatory genes, including cyclin-dependent kinase 5, cyclin D2, cyclin D3 and cell division cycle 25B.[75] Similarly, maternal undernutrition results in a marked increase in the expression of the RNA helicase CHAMP (cardiac-specific helicase activated by MEF2) in the ovine fetal heart.[35] CHAMP is specifically expressed in the heart during development and adulthood.[77] Interestingly, at embryonic day 15, when ventricular cardiomyocytes form trabeculae, CHAMP appears to be expressed preferentially in the trabecular region where the proliferative rate is diminished relative to the adjacent compact zone, suggesting that CHAMP might play an active role in cell cycle arrest.[77] These findings indicate that IUGR induces global gene expression changes in the heart which act in concert to reduce cardiomyocyte proliferation. More detailed studies of the importance of cell cycle dysregulation in the growth-restricted fetal heart will provide new insight into the mechanisms responsible for programming of adult cardiovascular disease.

Conclusion and perspectives

The mechanisms responsible for the programming of adult cardiovascular disease are poorly understood. Cardiac hypertrophy, an important predictor of cardiovascular morbidity and mortality, can have developmental origins. Although studies investigating the cellular basis of myocardial disease in growth-restricted models are limited, a reduction in cardiomyocyte number through either reduced cellular proliferation or increased apoptosis appears to be a central feature. A reduced number of cardiomyocytes at birth may render the heart more vulnerable in situations where an increased workload is required and may subsequently increase susceptibility to cardiac hypertrophy and ischemic heart disease in adulthood. Future studies which allow molecular dissection of the key genes involved, and which generate targeted *in vivo* models of IUGR will advance our understanding of the mechanistic bases for the programming of adult cardiovascular disease.

Acknowledgements

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