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Growth plasticity of the embryonic and fetal heart

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The developing mammalian heart responds to a variety of conditions, including changes in nutrient availability, blood oxygenation, hemodynamics, or tissue homeostasis, with impressive growth plasticity. This ensures the formation of a functional and normal sized organ by birth. During embryonic and fetal development the heart is exposed to various physiological and potentially pathological changes in the intrauterine environment which dramatically impact on normal cardiac function, tissue composition, and morphology. This paper summarizes the mechanisms employed by the embryonic and fetal heart to adapt to various intrauterine challenges to prevent or minimize postnatal consequences of impaired cardiac development. Future investigations of this growth plasticity might lead to new therapeutic strategies for the prevention of cardiac disease in postnatal life.

Keywords: cardiac growth; cardiomyocyte proliferation; fetal programming; heart development; heart regeneration

Introduction

The heart is the first internal organ system that needs to be functional during mammalian embryonic development. Therefore, both the early patterning events of the embryonic heart as well as growth and maturation of the various cardiac compartments during embryonic, fetal, and even postnatal life are imperative for survival of the organism. A constantly growing number of animal models have been found to present with developmental defects of the heart, shedding light on molecular and cellular processes necessary for proper cardiac patterning and maturation. However, these studies also highlight the intricacy of regulatory processes that convert the small pool of cardiac progenitor cells in the post-implantation embryo to the complex tissue network of highly specialized cell types that make up the four-chambered adult heart.

In humans, one of the most intensively studied conditions caused by impaired cardiac development are the various forms of congenital heart disease (CHD).[1] Information gleaned from these studies provides mechanistic insight into the plasticity of the heart during development. The identification of human mutations as well as generation of animal models have been instrumental in uncovering genes and pathways involved in the pathogenesis of CHD, although many mechanisms remain elusive.[2] For example, clinical phenotypic variability points to other important but as yet unknown factors that influence cardiac development and later cardiac outcomes. A better understanding of both endogenous as well as environmental factors that negatively influence normal heart growth is essential if we are to develop new prenatal therapeutic strategies to prevent CHD.

According to the so-called ''Barker hypothesis'' many human conditions, such as coronary artery disease (CAD), hypertension, or diabetes mellitus, have their origin during prenatal life and are significantly influenced by various maternal exposures during pregnancy.[3] Thus, disturbed intrauterine growth has a negative influence on the development of the cardiovascular system favoring the occurrence of CAD or hypertension in adult life.[4,5] This fetal programming in combination with an unhealthy lifestyle is thought to be the major determinant of the worldwide epidemic of some of the chronic diseases mentioned above. Therefore, the identification of factors and mechanism that induce fetal programming might be key for the prenatal prevention of some of the most prevalent human diseases.

This paper aims to provide an overview of some basic mechanisms of pre- and postnatal cardiac growth. Most importantly, it attempts to summarize recent research dealing with growth plasticity of the embryonic and fetal heart in response to various environmental and intrauterine conditions, including nutrient restriction, placental insufficiency, hypoxia, hemodynamics, and tissue damage.

Growth pattern of the pre- and postnatal heart

In mammals, a unique feature of embryonic and fetal cardiomyocytes in contrast to adult cardiomyocytes is their ability to rapidly proliferate. This proliferative potential, however, ceases in late gestation and is lost shortly after birth such that postnatal cardiac growth is mainly achieved by cardiomyocyte hypertrophy.[6] In rodents, the transition from

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proliferative to hypertrophic growth has been reported to occur within the first week of life, whereas even earlier in larger mammals.[7–10] Shortly after birth cardiomyocytes undergo a final round of DNA synthesis and nuclear mitosis without cell division (cytokinesis), which results in binucleation of the majority of cardiomyocytes in the adult heart.[11,12] The molecular signaling pathways mediating the switch from proliferative to hypertrophic growth are not well understood; however, changes in the expression pattern of cell cycle-regulating genes seem to have a significant impact.[13–15] For example, expression of the cell cycle promoting cyclin-dependent kinases (Cdk-2, -4, and -6) as well as their essential binding partners, the various cyclins (cyclin D1–D3, cyclin A, B, and E) is high in embryonic and fetal hearts but decreases in neonates and is barely detectable in adult hearts.[11,16–18] Consistently, combined inactivation of both Cdk-2 and -4[19] as well as cyclin D1, D2, and D3[20] in mice results in embryonic lethality with severe cardiac malformations including reduced global size and thin ventricular walls as well as reduced proliferation of cardiac cells. Interestingly, cardiomyocyte-restricted overexpression of D-type cyclins in adult mouse hearts leads to substantial cardiomyocyte DNA synthesis,[18,21] supporting a role of cyclin D downregulation in cardiomyocyte cell cycle withdrawal.

In contrast, expression of cell cycle inhibitory genes, such as the Cdk inhibitors (CdkI) p21/CIP1 and p27/KIP1 as well as the pocket protein retinoblastoma (Rb), increases in adult hearts compared to prenatal stages.[22–24] Consistent with the role of pocket proteins in cardiomyocyte cell cycle withdrawal and terminal differentiation, combined inactivation of the two family members Rb and p130 in mouse hearts results in cardiac enlargement and persistent cardiomyocyte cycling in the postnatal period.[23] Cardiomyocyte proliferation during heart development is furthermore influenced by Forkhead O (FOXO) transcription factors, which regulate expression of CIP/KIP family CdkIs. Overexpression of FOXO1 in the developing heart results in embryonic lethality at mid gestation caused by myocardial malformations and thin ventricular walls accompanied by premature activation of p21, p27, and p57 and decreased cardiomyocyte proliferation. Conversely, inhibiting FOXO1-mediated transcription reduces CdkI expression and increases cardiomyocyte proliferation, resulting in increased ventricular wall thickness prior to birth.[25] These studies establish an important role of FOXO in perinatal CdkI-dependent cardiomyocyte cell cycle withdrawal and terminal differentiation.

Interestingly, expression of p21 and p27 is downregulated in postnatal hearts upon hypertrophic stimulation accompanied by an induction of cell cycle promoting genes,[26,27] suggesting a role of this prenatal expression pattern in cell growth. Consistently, expression of the proto-oncogene c-myc, which controls cell proliferation and differentiation in various tissues, declines during embryonic and fetal heart development.[28] Overexpression of c-myc in the developing heart results in cardiac enlargement in adulthood caused by cellular hyperplasia and a doubling in the number of cardiomyocytes.[29] Activation of c-myc in the adult heart, however, induces cardiomyocyte hypertrophy as well as cell cycle reentry,[30] which was furthermore shown to be cyclin D2 dependent.[31] In contrast, inactivation of c-myc in the adult mouse heart attenuated cardiomyocyte hypertrophy in response to cardiac stress.[31] Thus, genes that regulate cardiomyocyte proliferation in the prenatal heart often seem to be involved in postnatal cardiomyocyte hypertrophy.

Morphogenesis of the ventricular myocardium

Morphologically the embryonic heart develops from a simple tube-like structure into a complex organ consisting of four contracting chambers. The early patterning events of cardiac development are beyond the scope of this paper and have been reviewed elsewhere.[32,33] However, after formation of the heart tube the ventricular myocardium exhibits various stages of myocardial organization and maturation throughout development.[34] The primitive heart tube is composed of a thin layer of cardiomyocytes that is separated from the lumen by extracellular matrix and an endocardial cell layer. After looping, the luminal appearance of the heart changes such that trabeculation of the ventricular myocardium becomes evident,[35,36] being composed of protrusions of cardiomyocytes lined by endocardial cells (Fig. 1A–C). In these early phases of myocardial development, the trabeculated layer contributes significantly to cardiac contraction and is much thicker than the outer layer of compact myocardium (Fig. 1A).[37] By building up a trabeculated layer the inner surface area of the embryonic heart increases so that nutritional needs of the growing myocardium can be met from the luminal blood flow in the absence of a mature coronary circulatory system. After septation of the heart is complete, the ventricular myocardium undergoes further remodeling, which largely occurs as a result of proliferative growth and thickening of the compact myocardium accompanied by compaction of the trabeculation.[35] Concomitantly, the coronary circulatory system is established to meet the increasing nutritional demands of the mature myocardium.[38] Furthermore, maturation of the ventricular myocardium depends on signaling from both the endocardium as well as the epicardium,[39,40] while the latter might even contribute cardiomyocytes.[41,42] In the adult heart the proportion of the compact myocardium further increases, especially in the left ventricle, while the trabeculation forms a thin layer of a honeycomb-like relief on the luminal surface of the ventricles.[34]

The state of cardiomyocyte differentiation as well as the rate of proliferation is not uniform across the embryonic and

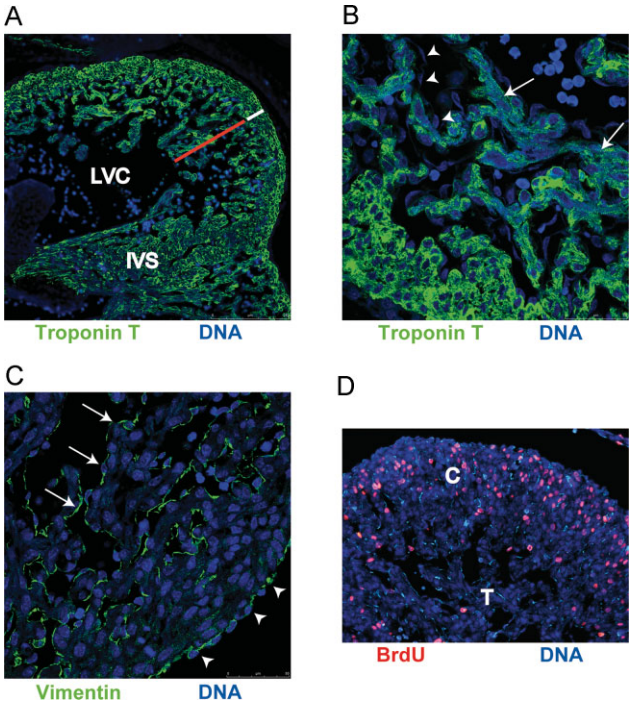

Figure 1. Morphology and proliferative potential of ventricular myocardium from 13.5 dpc mouse embryos. **A**: Immunofluorescence image of the left ventricle of a 13.5 dpc mouse embryo stained with an antibody against the sarcomere protein troponin T (green) to identify cardiomyocytes, and with the nuclear stain DAPI (blue). Note the thin myocardial compact layer (white bar) compared to the much wider trabeculation zone (red bar). LVC, left ventricular cavity; IVS, interventricular septum. **B**: Higher magnification of the left ventricular wall of a 13.5 dpc mouse embryo showing differentiated cardiomyocytes with cellular elongation and visible sarcomere cross-striation in the trabeculation zone (arrows), while cardiomyocytes in the compact layer are small and rounded. Note the lining of the trabeculation with a thin sheet of endocardial cells which are troponin T negative (arrowheads). **C**: Immunofluorescence staining with an antibody against the mesenchymal cell marker vimentin identifies non-myogenic cells in the ventricular myocardium of a 13.5 dpc mouse embryo. Note the lining of the myocardium with endocardial cells toward the ventricular cavity (arrows) and with epicardial cells toward the pericardium (arrowheads). **D**: Immunofluorescence image showing proliferating cells within the left ventricular myocardium after bromodeoxyuridine (BrdU) uptake. After administration, BrdU is incorporated into the DNA of replicating cells in S phase of the cell cycle and can later be detected using a BrdU-specific antibody. Note that proliferating cells (red) are predominantly located within the ventricular compact layer (C) and only sporadically appear in the trabeculation (T). (Images A–C are confocal fluorescence images, D is an epifluorescence image).

fetal heart but rather a gradient exists from the outer layers of the compact myocardium toward the luminal zone of trabeculation. The grade of differentiation increases from the outside to the lumen with elongated cardiomyocytes with visible cross-striation (reflecting mature sarcomere organization) located in the trabeculation, while cells in the outer compact zone have a small and round appearance with less mature sarcomere structures (Fig. 1B).[43,44] Also, proliferation rates are significantly higher in the outer compact zone compared to the slowly proliferating cardiomyocytes of the trabecular zone,[14,45] which might in part be explained by the high expression of cell cycle inhibiting genes like jumonji (which represses cyclin D1 expression)[45] and the CdkI p57/KIP2[46] in the trabeculated myocardium. Thus, proliferative growth of the embryonic heart mainly occurs by apposition from the periphery (Fig. 1D).

Consequences of variations in litter size on postnatal cardiac growth

First indications of a significant variation of postnatal cardiac growth in response to environmental factors come from studies reporting a negative correlation between litter size and postnatal heart weight and cardiomyocyte number in rats.[47–50] At 3 weeks of age slow-growing rats from large litters had significantly lower heart weights and smaller cardiomyocytes compared to fast-growing animals from small litters, although heart weight to body weight ratios appeared to be unchanged. Furthermore, a 21% reduction in cardiomyocyte number was observed in slow-growing rats compared to fast-growing animals.[48] This difference in cardiomyocyte number (but not cell size) persisted to adulthood, indicating that there is no significant compensatory "catch up" proliferation in these hearts after the age of 3 weeks.[48] Consistently, an increased proliferation rate of cardiac cells has been observed in rats from small litters compared to animals from large litters between postnatal days 1 and 21,[47] which might explain the difference in cell number described above. Investigation of the influence of litter size on cardiac changes as a result of postnatal cardiac stress induced by carbon monoxide (CO) inhalation showed that animals from small litters had the largest hearts with the highest DNA content and these differences were even more pronounced after CO exposure.[49] Although functional consequences of postnatal differences in cardiac cellular composition due to variation in litter size have been proposed based on blood pressure measurements after 3 weeks,[50] another study showed that differences in cardiac performance do not persist in the adult heart.[51]

Intrauterine growth restriction (IUGR) by modifying maternal diets

Various animal models have shown that IUGR by limiting maternal nutritional supplies during pregnancy has significant effects on growth of various organs including the heart which often persist after birth.[52,53] A low-protein diet fed to female rats throughout pregnancy results in lower body and heart

weights at birth compared to pups from dams on normal protein diet although heart weight to body weight ratios were not different between the groups. Importantly, hearts of newborns from mothers on low-protein diet showed a significant reduction in cardiomyocyte number.[54] Interestingly, however, the difference in heart weight seen in newborns from dams on low-protein diet was no longer evident at adulthood, suggesting postnatal compensatory growth. Cardiomyocyte size or proliferation rates were not examined in these studies, so it remains to be determined whether postnatal cardiac growth is mediated by hyperplasia or hypertrophy in this model. Further studies in rats also indicated that maternal low-protein diet during pregnancy results in increased left ventricular interstitial cardiac fibrosis in adult offspring (aged 6 months),[55] which might be caused by increased cardiac collagen synthesis and reduced activity of matrix metalloproteinases.[56] Additionally, offspring from nutrient-restricted dams developed diastolic dysfunction with left ventricular stiffening and impaired recovery of cardiac function after ischemia by 7 months of age.[56] These results strongly support the hypothesis that impaired embryonic or fetal development can have significant consequences on the heart in adulthood.

Little is known about molecular changes in the fetal and newborn heart as a response to IUGR. It has been shown in sheep that maternal undernutrition induces expression of various genes associated with cardiac hypertrophy and tissue remodeling.[57] Interestingly, however, in contrast to most of the rat models described above, in sheep maternal nutrient restriction results in increased heart weight to body weight ratios of affected offspring[57] (although measurements were performed prior to birth instead of postnatally as in the rat studies). This might rather resemble the situation in humans where intrauterine growth retardation has been shown to cause cardiac hypertrophy in affected infants.[58,59] Molecular examination of hearts from IUGR fetal sheep suggested that insulin-like growth factor (IGF) signaling might be involved in the hypertrophic response.[60]

Intrauterine growth restriction due to placental insufficiency

The majority of studies using animal models of placental insufficiency were done by placental embolization in fetal sheep. This technique requires surgical implantation of a permanent catheter placed in the abdominal aorta of the fetus, which is used for repeated injections of microspheres to occlude small blood vessels within the placenta. Embolization is done under tight control of arterial blood oxygenation to ensure comparable levels of hypoxemia between different fetuses. Using placental embolization for 21 days in late gestation sheep fetuses, it has been shown that IUGR in combination with hypoxemia induces myocardial hypertrophy, which was evident by both increased left and right ventricular wall thickness and increased cardiomyocyte cell size.[61] It was proposed, however, that these effects are not primarily due to IUGR but caused by other changes that result from placental embolization, namely increased cardiac afterload due to arterial hypertension and increased plasma norepinephrine levels.[61] Interestingly, a recent study using placental embolization for 10 or 20 days on late gestation sheep fetuses reported induction of fetal hypoxemia without arterial hypertension.[62] In the absence of hypertension, placental embolization does not cause cardiomyocyte hypertrophy and results in normal heart weight to body weight ratios. Strikingly, however, a reduced cardiomyocyte cell cycle activity and proliferation rate accompanied by suppressed terminal differentiation of right and left ventricular cardiomyocytes was shown after 20 days of placental embolization.[62] Another study confirmed this delayed cardiomyocyte maturation after IUGR in fetal sheep evident as a larger proportion of mononucleated cardiomyocytes accompanied by a reduction in binucleated cardiomyocytes in the absence of hypertrophy in the left ventricle.[63]

Another reported method to experimentally induce placental insufficiency in sheep is to surgically remove the majority of uterine caruncles in ewes prior to conception.[64,65] In contrast to placental embolization, which results in acute placental insufficiency, this method induces placental insufficiency from conception throughout embryonic and fetal development. Using this technique in sheep resulted in an increased proportion of mononucleated cardiomyocytes in the right and left ventricle of late gestation fetuses with no effect on cardiomyocyte proliferation, a result similar to those described above for placental embolization.[65] Furthermore, there was no indication of cardiomyocyte hypertrophy (cardiomyocytes were actually smaller in width and length in IUGR fetuses compared to controls), although both bi- and mononucleated cardiomyocytes were larger in IUGR fetuses when cell size was correlated with absolute heart weight.

Taken together, both acute placental insufficiency in late gestation as well as chronic placental insufficiency throughout pregnancy seem to delay cardiomyocyte maturation in the fetal heart, whereas only acute placental restriction decreases fetal cardiomyocyte proliferation (Table 1, Fig. 2). A possible hypertrophic response of fetal cardiomyocytes to placental insufficiency is controversial and might be influenced by several other factors induced by IUGR.

Consequences of hypoxia on fetal heart growth

To specifically study the influence of fetal hypoxia on the heart, female rats and sheep have been held under hypoxic

Table 1. Alterations in pre- and postnatal cardiac growth due to different manipulations affecting normal heart development

Topic	Procedure	Species	Gestational age[a]	HW	HW/BW ratio	CM number	CM size	CM prolif.	CM apoptosis	CM maturation	Comments	Ref.
Postnatal growth restriction	Variation of litter size	Rat	P1 to weaning[b]	↓↓	↔	↓↓	↓↓[c]	↓↓[c]	ND	↔[c]	CM size normalizes till adulthood[c]	(47,48)
IUGR	Restriction of maternal diets	Rat	Throughout pregnancy	↓↓	↔	↓↓	ND	ND	ND	↔	Hearts examined at P1	(54)
		Sheep	Early to mid gestation	↔	↑↑	ND	ND	ND	ND	ND	Hearts examined at mid gestation	(57,60)
	Placental embolization	Sheep	Late gestation	↑↑	↑↑	ND	↑↑	ND	ND	ND	Induction of arterial hypertension	(61)
		Sheep	Late gestation	↓↓	↔ (62) ↓↓ (63)	ND	↔	↓↓[c]	ND	↓↓	No arterial hypertension	(62,63)
	Removal of uterine caruncles	Sheep	Throughout pregnancy	↓↓	↔	ND	↓↓	↔	ND	↓↓	CM size ↑↑ when normalized to HW	(65)
Intrauterine hypoxia	Maternal hypoxia	Rat	Late gestation	↔	↑↑	ND	↑↑	ND	↑↑	↑↑	size ↑↑ in binucleated CM only	(66)
		Mouse	Mid gestation	ND	ND	ND	ND	↓↓	ND	ND	Hypoplastic hearts	(72)
Embryonic heart regeneration	Tissue mosaic for mitochondrial dysfunction	Mouse	Mid to late gestation	ND	ND	ND	ND	↑↑	↔	ND	Proliferation ↑↑ in healthy cells only	(91)

↓↓, significantly reduced; ↔, unchanged; ↑↑, significantly increased.
[a]Gestational age when procedure is applied/effective.
[b]Variations in methodology between referenced studies.
[c]Not determined in all referenced studies.
Abbreviations: BW, body weight; CM, cardiomyocyte; HW, heart weight; LV, left ventricle; ND, not determined; P, postnatal day; RV, right ventricle.

conditions (~50% reduction of oxygen in the air, mimicking high altitude) for a defined time during pregnancy. However, hypoxia also induces a reduction of maternal nutritional intake[56] such that there might be an overlap of effects between these experimental procedures. This aside, exposure of pregnant rats to hypoxic conditions for the final quarter of pregnancy resulted in increased heart weight to body weight ratios in the fetuses prior to birth.[66] Adult rats exposed to hypoxia during fetal life still exhibit left ventricular hypertrophy both on a cellular[69] as well as morphological level,[67] although the latter seemed to affect mainly males. Additionally, fetal hearts after hypoxia during late gestation showed an increased proportion of binucleated cardiomyocytes as well as hypertrophy of binucleated but not mononucleated cells, indicating premature terminal differentiation of cardiomyocytes.

Fetal hypoxia has been reported to have significant consequences for cardioprotection later in life, evident as increased susceptibility to cardiac ischemia and reperfusion injury.[68] Furthermore, heat stress-mediated cardioprotection and preconditioning protected adult control hearts from severe injury following ischemia and reperfusion but not hearts that were exposed to fetal hypoxia.[69] The authors propose that this loss of cardioprotection is at least in part mediated by fetal programming leading to aberrant expression of heat shock protein 70 in response to myocardial ischemia.[69] Another study, however, suggested that the high susceptibility to ischemic damage of adult hearts after fetal hypoxia could also be caused by decreased capillary density.[70] Interestingly, a study of perinatal hypoxia in rats (hypoxic conditions for 7 days before and 10 days after birth) reported increased tolerance of the adult heart to ischemia and reperfusion when exposed to perinatal hypoxia, although this effect was gender dependent and more pronounced in females.[71]

In contrast to the effect of late gestation hypoxia on fetal and adult rat hearts, another study investigated early fetal

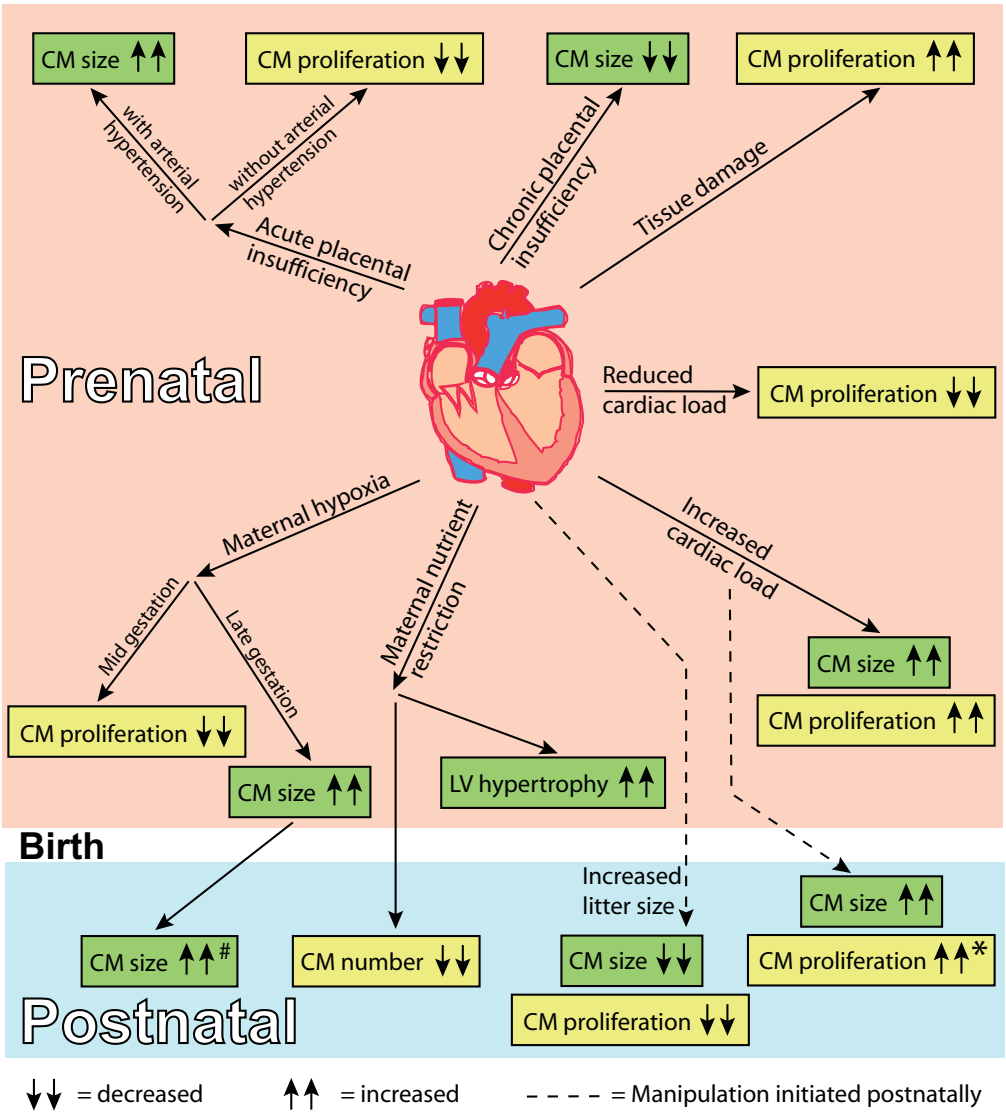

Figure 2. Consequences of various pre- and postnatal changes in the fetal or perinatal environment on cardiac growth. Note that this illustration is a broad overview summarizing results from numerous studies on different species and gestational ages using different experimental protocols. Also, the figure only depicts changes affecting hypertrophic or hyperplastic growth (*i.e.*, cardiomyocyte cell size and proliferation). For a more detailed overview including other cellular processes see Table 1 and 2. (*, only when manipulation is applied in the neonatal period but not thereafter; #, increase in cardiomyocyte size persists into adulthood).

hypoxia in mice.[72] Strikingly, 24 h of maternal hypoxia was lethal in a significant proportion of mid-gestation mouse fetuses. However, sublethal hypoxia in mid-gestation fetuses resulted in dilation of ventricular chambers, thinning of ventricular walls as well as signs of fetal heart failure. The changes in myocardial morphology were caused by decreased proliferation of cardiac cells, which reduced myocardial mass by almost 50%.[72] The study, however, did not investigate whether these cardiac changes can be reversed or at least attenuated in late gestation once fetuses were returned to normoxia, a question of interest in regard to the regenerative capacity of the embryonic and fetal heart (see below).

Effect of hemodynamic changes on fetal heart growth

It has long been recognized that intracardiac blood flow within the embryonic and fetal heart is an important stimulus for normal cardiac development and morphogenesis.[73,74] Impairing atrial contractility during heart development in

zebrafish[75] and mice[76] by mutating atrium-specific sarcomere components results in morphogenic defects of the ventricular myocardium secondary to atrial dysfunction. Increasing intracardiac pressure by directly manipulating the embryonic chick heart through outflow tract (conotruncal) constriction, and therefore increasing cardiac afterload, results in elevated ventricular weight due to hyperplastic growth of the myocardium.[77] Conversely, decreasing cardiac afterload by chronic drug treatment in chick embryos results in smaller hearts that are composed of fewer yet normally sized cells.[78] These data suggest that the embryonic or fetal heart responds to altered loading conditions by changes in proliferation rates of myocardial cells, which is in stark contrast to the hypertrophic response of the adult heart.

A proliferative response of the fetal myocardium to increased intracardiac pressure has also been shown in mammals where banding of the ascending aorta in late gestation guinea pig fetuses resulted in increased heart weight to body weight ratio, increased left ventricular wall thickness and increased proliferation of myocardial cells, while cell size was unaffected.[79] Interestingly, increasing cardiac afterload in neonatal rats by banding of the abdominal aorta on postnatal day 2 also resulted in an immediate hyperplastic response of the myocardium at postnatal day 3 followed by left ventricular hypertrophy evident both on a cellular as well as morphological level.[80] These data suggest that in rodents the neonatal heart, like the fetal heart, primarily responds to increased cardiac pressure *via* hyperplasia within the first days of life until transition from hyperplastic to hypertrophic growth normally occurs. After this time, and as seen in adult hearts, hypertrophy is the main adaptive mechanism.

Studies on fetal sheep have shown that increasing right ventricular load by ligation of the pulmonary artery induces an increase in cardiomyocyte size and maturation in the right ventricle.[81,82] Based on a mathematical approach it was suggested that increased proliferation might also contribute to the adaptive response resulting in a combination of hypertrophy and hyperplasia.[82] Consistent with this, altering cardiac load in fetal sheep by either induction of combined arterial and venous hypertension using repeated intravascular plasma infusions[83] or banding of the aortic arch[84] results in increased heart weight to body weight ratios mediated by both hyperplasia and hypertrophy. Interestingly, however, in volume overload-treated fetuses the proliferative response of the myocardium precedes the increase in cell size, while increased afterload initially induces cardiomyocyte hypertrophy followed by hyperplasia. Also, the percentage of binucleated cardiomyocytes as a measure of maturation was greater after volume overload compared to controls[83] but decreased after aortic banding.[84] These seemingly contradictory results highlight that the fetal heart exhibits quite remarkable growth plasticity in response to hemodynamic changes, which is not uniform and probably depends on multiple endogenous as well as environmental factors.

Many studies examining the effect of alterations in hemodynamics on heart development in animal systems have related their findings to the human heart condition called hypoplastic left heart syndrome (HLHS). This relatively rare but severe congenital heart malformation exhibits an underdevelopment of most structures on the left side of the heart.[85] In chick embryos, a hypoplastic left ventricle can be experimentally induced by ligation of the left atrial appendix (left atrial ligation), which results in redistribution of blood flow preferentially to the right ventricle reducing left ventricular preload.[86,87] These changes in intracardiac hemodynamics result in significantly decreased cardiomyocyte proliferation in the left ventricular myocardium, suggesting a causative role in left ventricular underdevelopment.[87]

In accordance with data observed for left atrial ligation, clipping of the right atrial appendix in chick embryos results in increased blood flow to the left ventricle. This increased preload causes compensatory hyperplasia within the left ventricular and atrial myocardium.[88] Strikingly, however, left ventricular hypoplasia induced by left atrial ligation can be rescued by subsequent right atrial ligation restoring left ventricular proliferation rates as well as left ventricular mass.[88] This raises the exciting possibility that in humans prenatal surgical interventions restoring normal cardiac blood flow might prevent certain types of CHD such as HLHS.

Regeneration of the embryonic and fetal heart

The intrinsic regenerative capacity of the postnatal heart is clearly limited and does not allow substantial regeneration of acute injury to large myocardial areas, as *e.g.*, after myocardial infarction.[89,90] Until recently, little was known about the potential of the prenatal heart to repair damaged myocardium. Using cardiac-specific tissue mosaicism for mitochondrial dysfunction in mice, recent data have shown that the embryonic and fetal heart has a quite dramatic regenerative capacity.[91] This model is based on inactivation of the X-linked gene encoding holocytochrome *c* synthase (Hccs), an enzyme necessary for the activation of the two mitochondrial electron transport chain components cytochrome c and c1,[92] specifically in the developing heart. Loss of Hccs activity results in cellular energy starvation causing disturbed cardiomyocyte differentiation and ultimately cellular degeneration.[91] Because the *Hccs* gene is located on the X chromosome, allelic expression is subject to X chromosome inactivation, a stochastic process resulting in the transcriptional silencing of one of the two X chromosomes in female cells.[93] Therefore, in females heterozygous for the heart-

specific Hccs inactivation, ~50% of cardiac cells silence the defective X chromosome and remain healthy, while the other 50% keep the defective X chromosome active and develop mitochondrial dysfunction.

After inactivation of Hccs in cardiac progenitor cells during early heart development, hemizygous males as well as homozygous females die at mid gestation.[91] In contrast, heterozygous females survive to adulthood with only minor morphological and functional changes within the heart, which is unexpected considering a predicted 50:50 mosaic of healthy and Hccs-deficient cells. Detailed analyses of cardiac development revealed that the contribution of Hccs-deficient cells to the cardiac tissue is minimized from 50% at mid gestation to 10% at birth. Strikingly, this regeneration of the embryonic and fetal heart is mediated by increased proliferation of the healthy cardiac cell population. Hccs-deficient cells, however, are not actively eliminated and are still detectable in postnatal hearts of heterozygous females, suggesting that they might contribute to disturbance in cardiac function later in life.[91] Taken together, these data reveal that the embryonic heart can compensate for an effective loss of 50% of cardiac tissue at mid gestation to enable formation of a functional heart by birth.

The findings of this study argue that impairment of heart development or tissue damage in the embryonic heart can be restored providing that the pathological conditions are either locally or temporarily restricted. It remains to be determined, however, whether there is a window of opportunity for this regeneration after which damage to the fetal heart cannot be compensated for by birth resulting in postnatal consequences for cardiac morphology or function.

Control of cardiac organ size

In numerous animal model systems described above, especially those for fetal undernutrition, the treatment applied results in reduced fetal heart and body weight. The heart weight to body weight ratio, however, in most cases does not change compared to untreated controls (see Table 1). This strongly argues for some tight control mechanisms regulating heart growth in response to body size. Relatively little is known about organ size control during embryonic and fetal development, although basic mechanisms like cell growth, proliferation and cell death are necessarily involved.[94,95] Also, for the heart various components of the IGF/phosphatidylinosite-3-kinase/Akt pathway seem to be essential for the regulation of organ size.[96,97] However, studies on pancreatic development have shown that the pancreas relies on a certain number of progenitor cells to reach its final size and depletion

Table 2. Consequences of altered cardiac hemodynamics on pre- and perinatal heart growth

Procedure	Species	Gestational age[a]	HW	HW/BW ratio	CM number	CM size	CM prolif.	CM apoptosis	CM maturation	Comments	Ref.
Increasing LV afterload	Chicken	HH stage 21	↑↑	↑↑	ND	↔	↑↑	ND	ND		(77)
	Guinea pig	Late gestation	↑↑	↑↑	ND	↔	↑↑	↔	ND		(79)
	Sheep	Mid gestation	↑↑	↑↑	ND	↑↑	↑↑	ND	↓↓	Hypertrophy precedes hyperplasia	(84)
	Rat	P2 to P21	↑↑	↑↑	ND	↑↑	↑↑	↔	ND	Hyperplasia precedes hypertrophy	(80)
Increasing RV afterload	Sheep	Late gestation	↑↑	↑↑	ND	↑↑	ND	ND	↑↑[b]	Cellular changes in RV only	(81,82)
Increasing volume load	Sheep	Late gestation	↑↑	↑↑	ND	↑↑	↑↑	ND	↑↑	Hyperplasia precedes hypertrophy	(83)
Increasing LV preload	Chicken	HH stage 34	ND	ND	↔	↔	↑↑	ND	ND	Proliferation in RV unchanged	(88)
Reducing LV preload	Chicken	HH stage 24	ND	ND	ND	ND	↓↓	↔ in myocardium	↓↓	Hypoplastic LV	(86,87)

↓↓, significantly reduced; ↔, unchanged; ↑↑, significantly increased.
[a]Gestational age when procedure is applied/effective.
[b]Not determined in all referenced studies.
Abbreviations: see Table 1, HH, Hamburger and Hamilton.

of this progenitor cell pool results in a hypoplastic organ.[98] In contrast, the developing liver primarily relies on proliferation of hepatocytes to reach its final size and can tolerate a reduction of the progenitor cell pool by compensatory proliferation without reduction in organ size.[98] For the heart, similar mechanisms are not fully established yet; however, studies on embryonic heart regeneration indicate that compensatory proliferation in response to cardiomyocyte loss is possible, at least in the second half of gestation.[91] At the same time, reducing the number of cardiac progenitor cells during early heart development has severe consequences for cardiac morphogenesis and size, with a reduction in the cardiac progenitor pool resulting in smaller and misshapen hearts.[99–101] Therefore, the heart seems to rely on a sufficient number of progenitor cells during the initial stages of heart morphogenesis but can compensate for a loss of cardiac cells after mid gestation to reach its final size.

Conclusions

Various techniques altering cardiac growth conditions *in utero* have shown that the embryonic and fetal heart has quite a remarkable ability to adapt to both endogenous (*e.g.*, intracardiac hemodynamics) as well as environmental (*e.g.*, nutritional supply or hypoxia) challenges (for summary see Table 1 and 2, and Fig. 2). The predominant mechanism of prenatal cardiac growth plasticity is cardiomyocyte hyperplasia, in contrast to a hypertrophic adaption to cardiac stress in the adult heart, although in late gestation and in the perinatal period a combination of both might occur. Given that the fetal heart has been shown to have an impressive regenerative capacity in response to tissue damage, it is tempting to speculate that impairment of embryonic or fetal heart development might be partially reversible until birth if intrauterine cardiac stress is temporarily or locally restricted.

Certainly, accumulating evidence indicates that the intrauterine environment determines the incidence of cardiovascular disease in adult life. It therefore needs to be investigated whether correction of disturbed embryonic or fetal heart development will prevent postnatal cardiovascular disease, potentially by inhibiting fetal programming. In this regard, future prospects for prenatal surgical intervention for certain cardiac or vascular malformations (*i.e.*, to correct intracardiac blood flow and pressure) and new insights into the regenerative capacity of the fetal heart raise the hope that combined therapeutic approaches might prevent or at least attenuate acute postnatal clinical consequences of certain types of CHD. Finally, molecular mechanisms and signaling pathway mediating the various responses of the prenatal heart to different challenges in the intrauterine environment, *e.g.*, causing changes in cardiomyocyte cycling, size, or maturation, are almost completely unknown. Therefore, identification and characterization of molecular and cellular mechanisms involved in fetal cardiac growth plasticity and regeneration might uncover promising new strategies for regeneration in the adult heart after acute or chronic tissue damage.

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