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TOPICAL REVIEW

A change of heart: understanding the mechanisms regulating cardiac proliferation and metabolism before and after birth

Catherine G. Dimasi, Jack R. T. Darby and Janna L. Morrison

Early Origins of Adult Health Research Group, Health and Biomedical Innovation, UniSA: Clinical and Health Sciences, University of South Australia, Adelaide, SA, Australia

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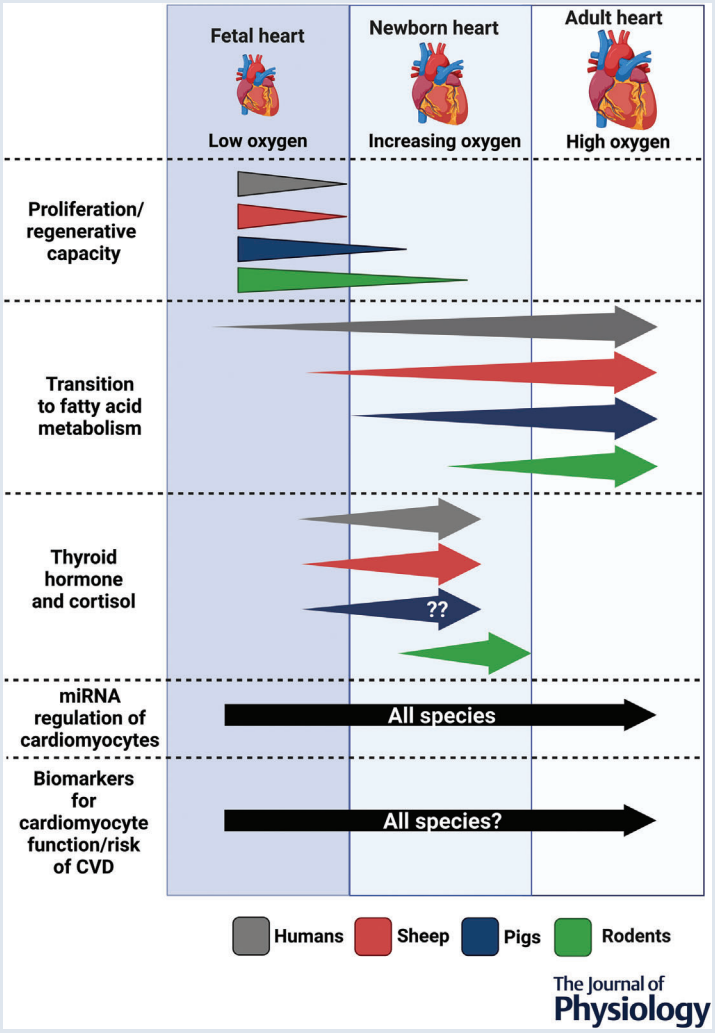

Abstract Mammalian cardiomyocytes undergo major maturational changes in preparation for birth and postnatal life. Immature cardiomyocytes contribute to cardiac growth via proliferation

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and thus the heart has the capacity to regenerate. To prepare for postnatal life, structural and metabolic changes associated with increased cardiac output and function must occur. This includes exit from the cell cycle, hypertrophic growth, mitochondrial maturation and sarcomeric protein isoform switching. However, these changes come at a price: the loss of cardiac regenerative capacity such that damage to the heart in postnatal life is permanent. This is a significant barrier to the development of new treatments for cardiac repair and contributes to heart failure. The transitional period of cardiomyocyte growth is a complex and multifaceted event. In this review, we focus on studies that have investigated this critical transition period as well as novel factors that may regulate and drive this process. We also discuss the potential use of new biomarkers for the detection of myocardial infarction and, in the broader sense, cardiovascular disease.

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Corresponding author Janna L. Morrison: Early Origins of Adult Health Research Group, Health and Biomedical Innovation, UniSA: Clinical and Health Sciences, University of South Australia, GPO Box 2471, Adelaide, SA 5001, Australia. Email: Janna.Morrison@unisa.edu.au

Abstract figure legend In the mammalian fetal (immature) heart, cardiomyocytes proliferate and can regenerate in a low-oxygen environment. In the lead up to and after birth, major changes occur to cardiomyocytes that result in regeneration no longer being possible; however, the timing of these events varies across species. Factors that regulate this cardiomyocyte transition include nutrient and oxygen availability, hormones and microRNAs. An emerging field of research is the use of biomarkers as a non-invasive detection method for cardiovascular disease.

Introduction

Cardiovascular disease (CVD), particularly myocardial infarction (MI), and subsequent heart failure (HF), remains the leading cause of death worldwide (WHO, 2022). Whilst significant advances in detection, treatment and management of CVD have resulted in an ∼70% decline in 5 ear mortality over recent decades, the rate of hospital admissions and healthcare expenditure has begun to rise again (Roth et al., 2020). This is due to several factors including the increasing incidence of type 2 diabetes mellitus (T2DM) and an ageing population.

The adult human heart is unable to replace damaged cardiac tissue with functional cardiomyocytes (CMs). As a result, any damage to the heart in the event of an MI is permanent. Non-functional scar tissue replaces functional units of the myocardium (the healthy CMs), which then impacts contractile function (Porrello & Olson, 2014). The limited regenerative potential of the adult myocardium is probably the result of CM quiescence (not actively proliferating cells, but still active in terms of generating contractile force); however, during gestation CMs of the mammalian fetal heart possess the ability to undergo proliferation (Herdrich et al., 2010). In large mammals such as humans, sheep and pigs, CMs progressively lose their proliferative capacity in late gestation and enter a non-proliferative state, as seen in the adult human heart (Fig. 1) (Botting et al., 2012; Burrell et al., 2003; Jonker et al., 2007, 2015; Lock et al., 2019; Mollova et al., 2013; Morrison et al., 2015; Velayutham et al., 2020).

An important aspect of this transition is the link between myocardial metabolism and cardiac function, as the heart is rapidly growing during this time and is the largest consumer of energy in the body. Glycolysis and oxidative phosphorylation are the main pathways that CMs use to produce energy in the form of ATP. Glycolysis can occur both aerobically or anaerobically depending on whether oxygen is available and involves multiple biochemical steps that involve the splitting of glucose into pyruvate within the cytoplasm of cells to produce ATP. Whereas mitochondrial fatty-acid β-oxidation breaks down fatty acids to produce acetyl-CoA, which is used in the citric acid cycle/tricarboxylic acid (TCA) cycle. Electron carriers produced from the citric acid cycle

Catherine Dimasi received her BSc (Biomedical) in Biochemistry and Honours in Obstetrics and Gynaecology at the University of Adelaide. She is currently pursuing a PhD at The University of South Australia in the Early Origins of Adult Health Research Group under the supervision of Professor Janna Morrison. Her research interests are in fetal development and lifelong health.

can then enter the electron transport chain (ETC) and undergo oxidative phosphorylation (OXPHOS). In contrast to glycolysis, OXPHOS requires oxygen and is much more efficient at producing ATP. OXPHOS occurs in the inner mitochondrial membrane and involves moving electrons from one transport chain to the next in a series of redox reactions. At complex V, or ATP synthase, ADP gets converted to ATP to produce energy. Oxygen is the ultimate factor in determining what pathway can be used, and oxygen availability changes dramatically between fetal and postnatal life.

Sir Joseph Barcroft was the first to coin the term 'Everest *in utero*' referring to the hypoxaemic conditions in which a fetus normally develops. This contrasts to *ex utero* when the fetus transitions into an oxygen-rich environment and begins air-breathing (Barcroft, 1933). At birth when oxygen availability increases dramatically, there is a switch in CM energy metabolism from carbohydrates such as glucose and lactate to OXPHOS in rodents (Fig. 1) (Ascuitto & Ross-Ascuitto, 1996; Lopaschuk et al., 1992). However, this switch in metabolism is hypothesised to begin prior to or around the time of birth in large mammals, before there is an increase in oxygen availability. It is unknown if a progressive change in energy metabolism instigates the switch from proliferative to quiescent CMs, or if the switch from proliferative to quiescent CMs drives the change in cardiac energy metabolism in large mammals. Moreover, it is not fully understood whether the regulation of these metabolic pathways occurs solely by 'sensing' the abundance of specific metabolic substrates or whether additional regulatory molecules play a role.

microRNAs (miRNAs) are known to regulate proliferation, hypertrophy and metabolism during development. However, the significance of miRNA regulation of CMs in humans and other large mammals is unknown. In this review, we aim to integrate recent research into CM transition before and after birth, and the role of miRNAs in cardiac regulation, their possible use as biomarkers and therapeutic potential in CVD.

Regulation of CM proliferation in different species

In the human heart, the first beats are detected by the fifth week of pregnancy and the heart and circulatory system becomes functional by the eighth week of pregnancy (Tan & Lewandowski, 2020). CMs make up 75–85% of total heart volume (Zhou & Pu, 2016). In the ventricular regions of the heart, CMs make up half (49.2%) of all cells and 30.1% in the atria. Endothelial cells, fibroblasts, leukocytes, immune cells and smooth muscle cells make up a large majority of the remaining proportion (Litviňuková et al., 2020; Quaife-Ryan et al., 2017). During this time, the cell cycle of the CM population is highly active as the heart grows, a process driven primarily by cyclin and cyclin-dependent kinase (CDK) families (Bishop et al., 2021).

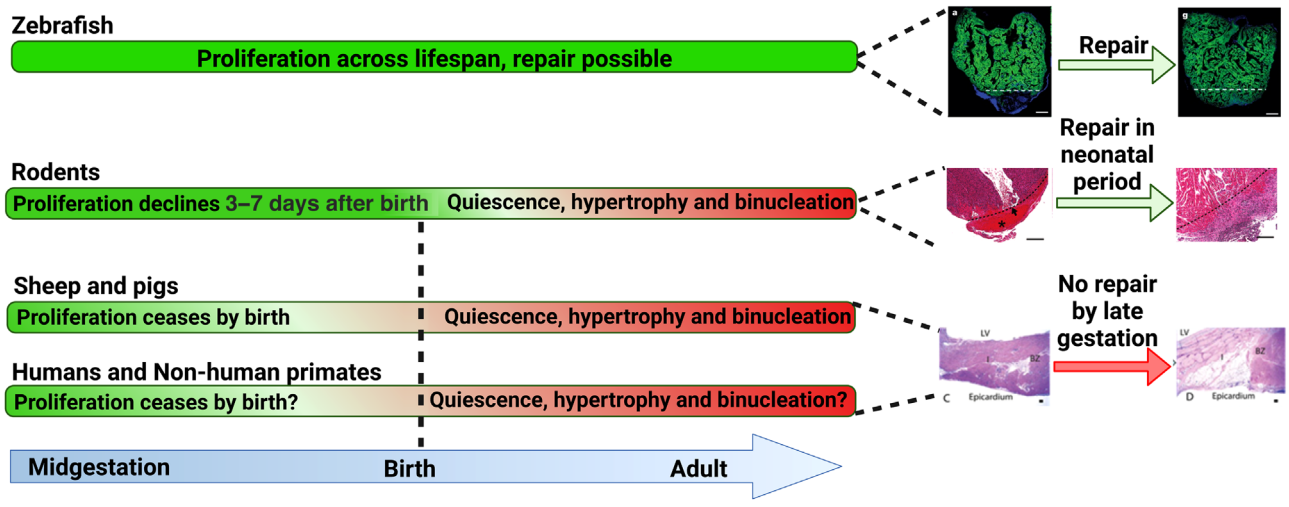

Figure 1. Cardiomyocyte transition from proliferation to quiescence occurs at different times in zebrafish, rodents and large mammals
During embryonic development, when oxygen is low (deep blue section of arrow), CMs undergo proliferation (green bars) and remain mononucleated. Postnatal CMs in zebrafish remain proliferative throughout life despite low oxygen, whilst rodent CMs exit the cell cycle and become non-proliferative (red bars) within 3–7 days after birth when oxygen increases (lighter blue arrow). In contrast, this transition event occurs during late gestation in large mammals when oxygen levels are low. Heart volume can only increase by hypertrophic growth of pre-existing CMs and mononucleated CMs become binucleated or in the case of pigs even multinucleated. Adapted from Lock et al. (2018).

Initially, the heart grows via the proliferation of mononucleated CMs (Burrell et al., 2003; Jonker et al., 2007; Li et al., 1996; Soonpaa et al., 1996). In large mammals during late gestation, CMs enter cell cycle arrest before birth (Bergmann et al., 2009; Jonker et al., 2007). In contrast, this transition begins late in the first week after birth in rodents (Fig. 1) (Li et al., 1996; Porrello, Mahmoud et al., 2011; Soonpaa et al., 1996). During this time, CMs transition from contributing to heart growth by hyperplastic to hypertrophic growth, and in many species, mononucleated CMs become bi- or even multinucleated. Binucleation occurs when CMs undergo karyokinesis (nuclear division) but not cytokinesis (cell division) (Li et al., 1996). However, there is also some degree of DNA endoreduplication in large mammals (replication of the nuclear genome without undergoing cell division) and CMs become polyploid. This is more so observed in humans (Derks & Bergmann, 2020), but has also been reported in sheep (Bensley et al., 2010). As polyploid, bi- and multinucleated CMs have limited capacity for cell division, they contribute to heart mass by increasing their size through physiological hypertrophic growth (Ahuja et al., 2007; Oparil et al., 1984).

The physiological significance of transitioning to bi- or multinucleated cells is unknown; however, there are multiple conflicting hypotheses suggesting why it may or may not be beneficial. One hypothesis is that multinucleation optimises cellular responses and enhances cell survival under stressful conditions (Anatskaya & Vinogradov, 2007). Another argument hypothesises that binucleation is an adaptive response to meet the high metabolic demand of CMs using OXPHOS for energy production. During this transition, the capacity to generate twice the RNA for protein synthesis may be beneficial (Ahuja et al., 2007). However, Gan et al. (2020) suggest polyploidy may not be an adaptive response. One theory suggests there is no need for proliferation, as the human heart can survive for decades (without injury) and therefore proliferation is simply not required for normal heart function. Another commonly mentioned hypothesis is that bi- and multinucleation specifically arose to inhibit CM proliferation. As adult CMs are elongated myofibres, cell division may cause the entire CM to lose contraction (as the sarcomeres must partially dissemble to allow cytokinesis). However, the counterargument exists that the fetal heart undergoes high amounts of proliferation and therefore extensive proliferation does not have a major impact on heart contraction (Gan et al., 2020). Thus, the role of polyploidy in CMs is controversial, and remains to be elucidated.

CM proliferation and transition events have been studied across multiple species and have been well characterised in small animals such as zebrafish and rodents, but less extensively studied in larger species such as sheep, pigs and non-human primates, in which findings may better recapitulate the human scenario (Fig. 1 and Table 1).

Zebrafish. Zebrafish are one of the most widely used animal models for the study of developmental biology and have a remarkable capacity for regeneration of injured and amputated tissues (Jopling et al., 2010). Early studies of CM regulation were first carried out in zebrafish, demonstrating that they can fully regenerate heart tissue within 2 months of a 20% ventricular resection (Poss et al., 2002). Additionally, their hearts retain proliferative capacity throughout life (Jopling et al., 2010; Yin et al., 2012a). However, the cardiac physiology of zebrafish is dissimilar to humans and other large mammals. They have a two-chamber heart, possess exclusively mononucleated and proliferative CMs, and remain in a hypoxic environment throughout life (Singleman & Holtzman, 2012).

Rodents. Due to their short reproductive cycle and gestation [mice, term ∼21 days gestational age (d GA), rats (term ∼21–23 days)] and relative ease of genetic manipulation, rodents have been used extensively to investigate regulation of CM growth. Initial studies by Soonpaa et al. (1996) involving mice observed CM proliferation significantly drop from 10% at embryonic day (E) E18 to 2.3% at birth, with no proliferation observed by postnatal day (P) 15. There was a spike in proliferation at P4.6, but this was attributed to a high degree of binucleation occurring simultaneously. Binucleation was first observed in mice at E18 and, by P15, 95% of CMs were binucleated (Soonpaa et al., 1996). In adult mice, there is a 5% yearly turnover in CMs, and a 2.6% turnover in old mice (Senyo et al., 2013). In rats by 3 days after birth, Li et al. (1996) observed exclusive mononucleation of CMs, but by P4 increases in cell volume and binucleation were observed. By P12 ∼90% of CMs were binucleated and there was a 2.5-fold increase in cell volume, with no proliferation of CMs observed after P4 (Li et al., 1996).

These initial profiling studies paved the way for future studies investigating cardiac regeneration after ventricular resection. In mammals, this was first observed in neonatal mice, where in P1 mice, new heart tissue was formed by proliferation of existing CMs, but this was not observed in P7 mice (Haubner et al., 2012; Porrello, Mahmoud et al., 2011). Thus, the regenerative capacity of the heart ends within 3−7 days after birth in mice and rats. At the same time, CMs enter cell cycle arrest and become binucleated, cardiac fibroblasts produce collagen-rich extracellular matrix (ECM) and OXPHOS becomes the main form of ATP production (Lopaschuk et al., 1992; Notari et al., 2018).

Table 1. Known or estimated timepoints at which major CM remodelling occurs in various species

Species	Length of gestation/incubation (days)	Timing of bi-/multinucleation	CM quiescence	Transition to fatty acid metabolism	References
Zebrafish	3–4	Never	Never	Never	(Jopling et al., 2010; Poss et al., 2002; Raya et al., 2003; Yin et al., 2012a)
Mice	21	3–12 days postnatal	3–7 days postnatal	3–7 days postnatal	(Haubner et al., 2012; Porrello et al., 2013; Porrello, Johnson et al., 2011; Porrello, Mahmoud et al., 2011; Soonpaa et al., 1996)
Rats	21–23	4–12 days postnatal	4–7 days postnatal	∼7 days postnatal	(de Carvalho et al., 2017; Li et al., 1996; Wang et al., 2020)
Sheep	145–150	Begins at ∼110d GA	∼90–145d GA	Unknown before birth to 1 month	(Bensley et al., 2016; Burrell et al., 2003; Jonker et al., 2015; Thornburg et al., 2011)
Pig	115	Binucleation at 5−30 days postnatal, multinucleation occurs until 6 months	Unknown before birth to 2 months	Unknown before birth to 1 month	(Agnew et al., 2020; Gräbner & Pfitzer, 1974; Velayutham et al., 2020; Yin et al., 2020)
Human	280	∼224d GA to 1 year	Unknown before birth to 1 year	Unknown	(Amir et al., 2008; Bergmann et al., 2009; Bergmann et al., 2015; Huttenbach et al., 2001; Kim et al., 1992; Mollova et al., 2013; Schmid & Pfitzer, 1985; Silva et al., 2012)

d GA, days of gestation.

However, development of major organs including the heart is dissimilar to that in humans and cardiac proliferation is delayed until after birth (Morrison et al., 2018; Porrello & Olson, 2014; Porrello, Mahmoud et al., 2011). Therefore, the use of large animals such as pigs, sheep and non-human primates is necessary to better reflect human physiology. These large mammals are often used as preclinical models in cardiac physiology as they are anatomically and haemodynamically similar to humans (Morrison et al., 2018). Importantly, the timing of cardiac development in these large mammals aligns with that of humans, where CMs become terminally differentiated in late gestation, and cardiac endowment is set at birth (Fig. 1) (Botting et al., 2012; Burrell et al., 2003; Lock et al., 2018; Mollova et al., 2013)

Sheep. Studies in sheep have shown that proliferation ceases by birth. At 95d GA (term, ∼147–150d GA), ∼7% of CMs were active in the cell cycle, falling to 1.5% by 140d GA. From 95 to 140d GA, there is an approximate doubling in CM number (Jonker et al., 2007) with a peak at 136d GA followed by a steep decline in CM number until birth. By 1 week after birth, only ∼1% of CMs remain active in the cell cycle (Jonker et al., 2015).

Hypertrophy begins as early as 110−120d GA with cell volumes in the left ventricle increasing in cross-sectional area (Burrell et al., 2003; Jonker et al., 2015; Thornburg et al., 2011). Thus, both hyperplasia and hypertrophy contribute to CM growth in the late gestation fetal sheep heart.

The onset of CM binucleation in sheep occurs before birth, from 110d GA. At 131d GA, 87% of CMs in the left ventricle and 91% of CMs in the right ventricle were binucleated (Bensley et al., 2016). However, there is some variability in the literature reporting CM binucleation at birth (Burrell et al., 2003; Jonker et al., 2015). By P4, 83% of CMs are binucleated and by 1 month after birth this rises to almost 100% (Adler et al., 1996; Bensley et al., 2016). By 4−6 weeks after birth, the number of CMs compared to before birth is significantly lower due to increased

apoptosis (an important determining factor in CM endowment), but these CMs are much larger, indicating the cessation of proliferation and the induction of hypertrophic growth has been completed by this time (Burrell et al., 2003). By 9 weeks, 97% of CMs in both ventricles are binucleated (Bensley et al., 2016). Additionally, studies have shown fetal sheep hearts return to normal function 1 month after an MI, which was not seen in adult sheep (Allukian et al., 2013; Zgheib et al., 2014).

Pigs. In swine, CMs enter a proliferative burst at day 30 of gestation with a peak at day 55 (term, 115d GA). By P1, the majority of CMs have withdrawn from the cell cycle and proliferation diminishes dramatically. There is cell cycle activity postnatally between days 5 and 30, with a peak at P15, but this is largely attributed to longitudinal growth of CMs by binucleation and multinucleation rather than proliferation of CMs (Velayutham et al., 2020; Yin et al., 2020). CM maturation is largely completed within the first 6 months of life, with cell cycle arrest, loss of mononucleated CMs and concurrent binucleation/multinucleation, hypertrophy, sarcomeric maturation and collagen deposition occurring by 2−6 months of age (Velayutham et al., 2020). CMs exhibit extensive multinucleation, with up to 32 nuclei observed per CM. There is also increased oxidative stress from reactive oxygen species (ROS) in CMs arising as a by-product of OXPHOS from ∼P30, which may contribute to CM cell cycle arrest (Yin et al., 2020). Interestingly, studies have shown some regenerative potential in CMs after MI up to 2−3 days after birth in pigs, but there was no evidence of cell-cycle activity or regeneration after P3 (Agnew et al., 2020; Copeland et al., 2022; Ye et al., 2018; Zhu et al., 2018).

Humans and non-human primates. In humans and other non-human primates, information on CM regulation is lacking, with little consensus in the literature about the proliferative capacity of CMs postnatally. Non-human primates such as chimpanzees (*Pan troglodytes*), baboons (*Papio*) and gorillas (*Gorilla*) exhibit multinucleation at 4 years of age, whilst in rhesus monkeys (*Macaca mulatta*) 90% of CMs remain binucleated (Rumyantsev & Carlson, 1991). In humans, binucleated cardiomyocytes appear at 32 weeks of gestation (Kim et al., 1992). By birth, 8% of cardiomyocytes in the left and 11.7% in the right ventricle are binucleated, increasing to 56% and 42%, respectively at 1 year after birth (Schmid & Pfitzer, 1985). In contrast, another study found that CMs were mostly mononucleated in the left ventricle at 26 years of age (74% mononucleated, 25.5% binucleated, 0.4% trinucleated and 0.1% tetranucleated, respectively). However, regardless of the nucleation number, the polyploid mononucleated CM is terminally differentiated and therefore unable to divide (Olivetti et al., 1996).

Although some studies have provided evidence that human neonatal CMs may retain limited proliferative potential (∼0.04%) up to 1 year after birth, this is insufficient for repair after damage. In a study by Mollova et al. (2013), mitosis was detectable in 0.012% of CMs and cytokinesis in 0.003% at 1 year, but these were undetectable by 20 years of age. However, Bergmann et al. (2009) reports CMs possess an annual turnover of ∼1% in the first 25 years, which then reduced to 0.3% by 75 years of age. Neuregulin-1 was able to induce CM proliferation in intact cultured myocardium from infants with heart disease who were less than 6 months of age (Polizzotti et al., 2015). Other reports indicate that CMs can still proliferate at 10 years of age (Bergmann et al., 2015), with CM proliferation observed in 3-month-old hearts (Amir et al., 2008; Huttenbach et al., 2001; Silva et al., 2012; Ye et al., 2016).

Perspectives from studying various species. Researchers have reported the rise in atmospheric oxygen at birth in mice as the primary driver of CM cell cycle arrest (Nakada et al., 2017; Puente et al., 2014). As rodent lungs are immature at birth, rodents are still mildly hypoxaemic (Lock et al., 2013; Pringle, 1986), allowing proliferation for 3−7 days after birth. However, CMs exit the cell cycle in large mammals in fetal life when P_{O_2} is low, 20−25 mmHg in sheep (Duan et al., 2017; Orgeig et al., 2010) and 20−35 mmHg in humans (Soothill et al., 1986). In fact, P_{O_2} decreases slightly during this period of late gestation. In addition to this, pigs still retain proliferative capacity in their CMs 1 day after birth (Velayutham et al., 2020; Yin et al., 2020). Thus, it is unlikely that oxygen is the main driver and exposure has differing effects in different species. For these reasons, understanding the similarities and differences in the timing of the cessation of CM proliferation between species provides important information that will lead to a mechanistic understanding of the process, but this requires further investigation.

CM metabolism

A major determinant of CM growth and proliferation is the metabolic capacity of the heart. During embryonic development, the heart mainly relies on carbohydrates, primarily glucose and lactate, as the preferred sources for ATP production because oxygen is limited. However, there is a metabolic switch to oxygen-dependent ATP generation when oxygen becomes readily available. Therefore, there is a shift in substrate utilisation from pyruvate to fatty acids, as fatty acids are energetically favourable (Zheng, 2012).

In rodents, this switch to quiescence occurs in the first week after birth at the onset of maternal milk consumption and air-breathing (Ascuitto & Ross-Ascuitto, 1996;

Lopaschuk et al., 1991; Lopaschuk et al., 1992). In large mammals, information is lacking, but recent studies hypothesise there is a switch occurring before or around the time of birth (Velayutham et al., 2020) (Table 1). It appears that in both rodents and large mammals, this metabolic switch coincides with the transition of CMs from a proliferative to non-proliferative state (Lock et al., 2018; Morrison et al., 2018; Velayutham et al., 2019).

The role of glucose in the fetal heart is multifunctional. Not only is it an energy substrate, but it is also involved in nucleotide biosynthesis and the pentose phosphate pathway. Glucose dose-dependently inhibits cardiac maturation and promotes CM proliferation in human embryonic stem cell-derived CMs through nucleotide biosynthesis, suggesting that glucose is a negative regulator of fetal CM maturation, and a positive regulator of mitotic activity (Nakano et al., 2017). However, elevated glucose may not affect fetal CMs in the same way as stem cell-derived CMs, and results should be further corroborated.

The high levels of glycolytic activity observed in the fetal heart are the result of relatively higher circulating glucose and lactate compared to fatty acids *in utero*, due to poor placental transfer of fatty acids (Piquereau & Ventura-Clapier, 2018) and the low availablity of oxygen. Changes in the availability of substrates in the developing fetus play an essential role in energy metabolism as lactate inhibits the oxidation of lipids, whilst lipids can repress carbohydrate metabolism (Hue & Taegtmeyer, 2009). However, the healthy adult heart is flexible, and can metabolise carbohydrates, lipids, amino acids and ketone bodies to produce energy (Piquereau & Ventura-Clapier, 2018).

As the heart and body continue to grow rapidly after birth, there is increased contractile demand to maintain cardiac output, and thus energy production must also increase, which can only be achieved through OXPHOS (Kolwicz et al., 2013). It is imperative that fetal CMs retain the capacity to switch metabolic profiles to satisfy cellular demands or changes in substrate availability. Loss in flexibility and a reliance on glucose postnatally for ATP production is detrimental to heart health and contributes to pathological cardiac hypertrophy and eventually may lead to congestive HF in adults (Lopaschuk & Jaswal, 2010; Tham et al., 2015).

Animal studies have highlighted that many pathways involved in mitochondrial biogenesis increase in expression and activity in the first few weeks of postnatal life. In rodent studies, the glycolysis pathway is still markedly active in P1 mice, with cardiac metabolic reprogramming from glycolysis to OXPHOS occurring by the end of the first week of life (Lalowski et al., 2018; Velayutham et al., 2019). However, in large mammals, the timing of transition to OXPHOS has recently been challenged and is hypothesised to occur in late gestation when CMs cease to proliferate. In the neonatal heart, there is a marked increase in triglycerides and oxidation of palmitate (Ascuitto et al., 1989; Werner et al., 1989). In postnatal swine, there is no evidence of a postnatal switch to OXPHOS (Velayutham et al., 2020), suggesting the transition may occur before birth. In contrast, Li et al. (2021) observed significant levels of carbohydrate metabolism until P28 in pigs. In humans, energy metabolism in the fetal and postnatal heart is largely unknown. One transcriptomic study observed expression of fatty acid oxidation (FAO) genes as early as 10 weeks of gestation, with expression of these genes increasing with fetal age (Iruretagoyena et al., 2014). Mills et al. (2017) also report FAO to be a key driver during CM transition, including exit from the cell cycle via the shutdown of the β-catenin and YAP signalling pathways in a human cardiac organoid model. Whilst there have been some studies in rodents investigating metabolic manipulation as an approach to re-activating cell cycle activity (Cardoso et al., 2020; D'Uva et al., 2015; Magadum et al., 2020), large animal studies are lacking, but these are necessary to validate whether findings from rodents can be translated to humans.

Whilst the general consensus is that cardiac fetal metabolism is anaerobic, it is also important to consider newer hypotheses suggesting that the fetal heart uses aerobic glycolysis. One can argue that despite fetal hypoxaemia, the fetal CM in large mammals is not deprived of oxygen. This is because coronary blood flow to the fetal myocardium is approximately twice that of the adult heart to compensate for lower arterial oxygen saturation. Furthermore, oxygen content is lower in fetal arterial blood compared to adults; both fetal and adult hearts consume roughly the same amount of oxygen per gram of tissue (Fisher et al., 1980; Fisher et al., 1981). This suggests that oxygen in the fetal cardiac environment is adequate for aerobic glycolysis before birth.

In summary, the consensus on the timing of metabolic changes associated with cardiac proliferation in large mammals is not settled, and the dogma that FAO only occurs after birth may not be true. We hypothesise that some of the metabolic 'machinery' may be upregulated in late gestation in large mammals, ready for activation when lactation and air breathing begins. This may be a benefit in species where preterm birth occurs (sheep, pigs, humans, etc.) By contrast, preterm birth in rodents would not be compatible with survival and thus a transition to FAO and OXPHOS birth may make sense.

Hormonal regulation of proliferation in CMs

Whilst oxygen and nutrient availability play a crucial role in CM proliferation and metabolism (Puente et al., 2014), they are not the only contributing factors. The hormonal

environment changes in late gestation and after birth and this may play a role in the regulation of CM growth (Fig. 2) (Chattergoon et al., 2007; Naqvi et al., 2014; Thornburg et al., 2011). Glucocorticoids are key hormones that affect cardiac growth (Rog-Zielinska et al., 2013; Thomas et al., 1978), and have been linked to proliferation (Feng et al., 2013; Giraud et al., 2006; Reini et al., 2008), enlargement (Lumbers et al., 2005) and apoptosis (Feng et al., 2013; Reini et al., 2008). The major glucocorticoid in rats is corticosterone whereas cortisol is the major glucocorticoid in humans and sheep (Buckingham, 2006). In rats, the timing of plasma corticosterone concentration is contested, with Holt & Oliver (1968) reporting a sharp rise at E19 before dropping at E20 and E21, before it rises again within 1 h after birth. However, Rog-Zielinska et al. (2013) report an earlier surge at E15.5 with a peak at E17.5. Cortisol concentrations in sheep begin to rise at ∼134 days (Phillips et al., 1996), and cortisol is also responsible for stimulating deiodination of thyroxine (T4) to triiodothyronine (T3), and thus there is a prepartum increase in T3 (Chattergoon et al., 2012). Whilst information in humans is limited, cortisol concentrations begin to rise at 30 weeks of gestation (Hillman et al., 2012), with a peak at birth (deM Fencl et al., 1980) and then declines postnatally (Sippell et al., 1978).

In postnatal rats there is a surge in thyroid hormone at P15 followed by a burst in CM proliferation (Naqvi et al., 2014). However, in humans and sheep, the spike in thyroid hormone occurs before birth, when proliferation begins to decline in late gestation (Fig. 2) (Chattergoon et al., 2007; Forhead & Fowden, 2014; Nwosu et al., 1978). In contrast to Phillips et al. (1996), Forhead et al. (2006) reported an earlier prepartum cortisol surge at ∼120–130d GA in sheep; however, this may be due slight differences in gestation between sheep models (145 *vs.* 150 days). Concentrations of thyroid hormones from 110−125 to 135−145d GA increase 4-fold, with a doubling in thyroid hormone production rate and decreased clearance of T3

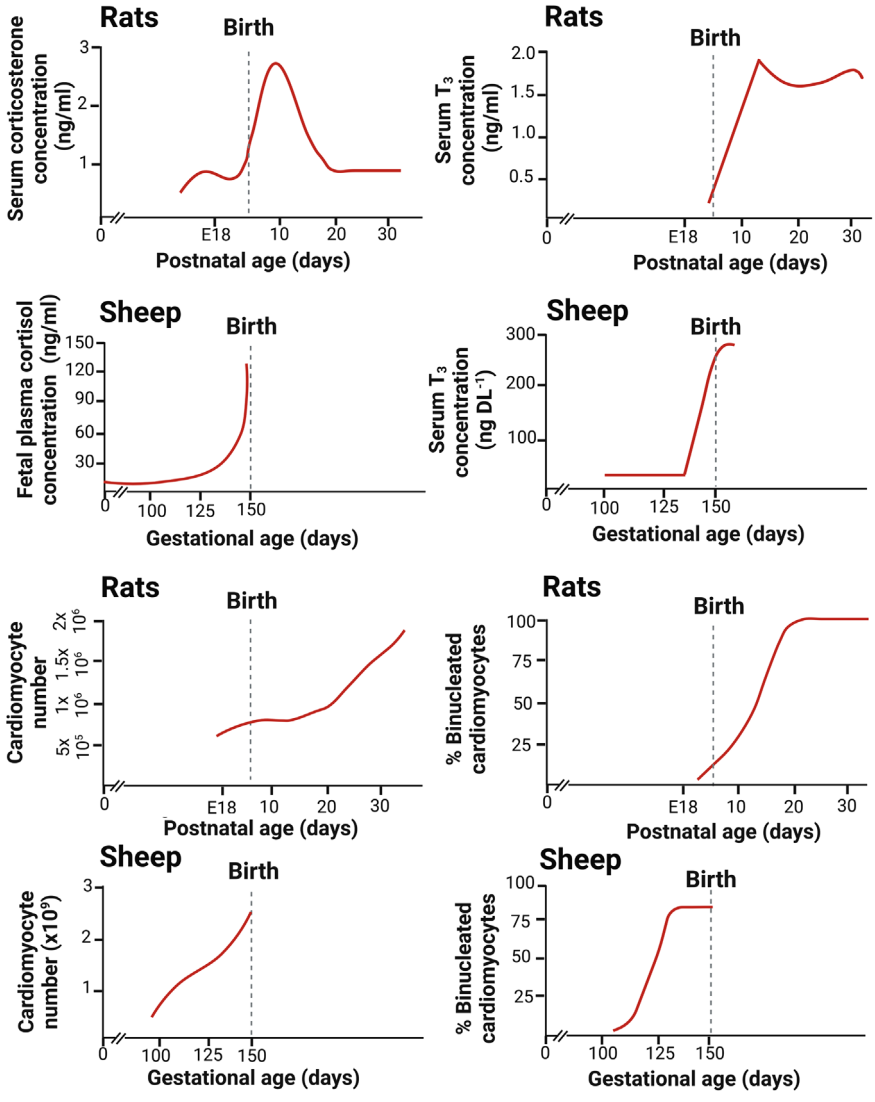

Figure 2. Cortisol and thyroid hormone concentrations and resulting cardiomyocyte number and binucleation after birth in rats and in late-gestation sheep fetuses
In rats, there is a steep surge in circulating corticosterone and thyroid hormone (T_3) postnatally at P10, leading to a proliferative burst of CMs at P15. In sheep, the surge in cortisol and circulating T_3 occurs during late gestation (∼135–145d GA), which coincides with the cessation of proliferation and rise in binucleation in CMs.

from the body (Fowden & Silver, 1995; Nathanielsz et al., 1973). Concurrently, when fetal sheep CMs transition from proliferative to hypertrophic growth, the thyroid hormone concentration is beginning to rise, with no evidence of a burst in CM proliferation during this time period (Chattergoon et al., 2012). T3 can also exert a broad impact on CM maturation, including induction of SERCA (SR Ca^{2+}-ATPase) expression, hypertrophy, isoform switching of the myosin heavy chain and cell polyploidization (Chattergoon et al., 2012; Hirose et al., 2019).

Other factors that influence fetal CM maturation

There is also a wealth of research investigating other factors that influence fetal CM maturation, such as IGF-1, angiotensin (Ang) II, atrial natriuretic peptide (ANP) and Meis1.

IGFs. IGF1 and IGF2 regulate important processes in cardiac development such as CM proliferation, hypertrophy, apoptosis and ageing (Díaz Del Moral et al., 2021). IGF2 is highly expressed during fetal development and is a potent mitogen for CMs, through activation of tyrosine kinase receptors and IGF1R (Li et al., 2011), and activation of IGF-2R stimulates CM hypertrophy in both cultured fetal CMs and the late-gestation sheep fetus (Wang et al., 2015; Wang, Brooks, Botting et al., 2012; Wang, Brooks, Thornburg et al., 2012). Inhibition of the *Igf2* gene results in a significant decrease in ventricular wall proliferation and hypoplasia (Li et al., 2011), whereas overexpression induces pathological hypertrophy, but not proliferation (Meganathan et al., 2015). It has also been reported that IGF1 and neuregulin-1β work synergistically not only to promote growth and proliferation, but also to stimulate OXPHOS and metabolic maturation (Rupert & Coulombe, 2017).

Ang II. Ang II has also been implicated in stimulation of CM hypertrophy (Sil Subha Sen, 1997) via the AT_1 receptor and the extracellular regulated kinase (ERK) signalling pathway. It also has roles in proliferation (Fukuda & Izumo, 1998), contractility, cardiac remodelling (Mello & Danser, 2000) and apoptosis (in mature myocytes only with p53 activation) (Kajstura et al., 1997). In a rat model of MI, angiotensin-converting enzyme inhibitors (ACEi) and AT_1 antagonists have cardioprotective effects (Liu et al., 1997). However, this research has been carried out in rodents, and in a study with fetal sheep CMs, Ang II was found to stimulate hyperplasia, but not hypertrophy as previously reported (Sundgren et al., 2003). This further highlights the need for more research using large mammals.

ANP. ANP is a cardiac hormone that aids in the regulation of blood pressure and has anti-hypertrophic and proliferative functions in the heart (Becker et al., 2014; Horio et al., 2000). ANP has been shown to decrease proliferation of fetal ovine CMs (O'Tierney et al., 2010). ANP activation also stimulates cGMP production through a series of downstream pathways inhibiting Ang II and the AT_1 receptor, thereby antagonising hypertrophic responses in CMs (Kinoshita et al., 2010). One study also indicated the ANP-mediated cGMP increase modulates cardiac contractility though enhancing β_1-adrenergic receptor/cAMP signalling (Perera et al., 2015).

Meis1. Meis1 is a transcription factor that regulates CM proliferation and is essential for cardiac development (Mahmoud et al., 2013; Porrello & Olson, 2014) and promotes glycolysis in haematopoietic stem cells (Lindgren et al., 2019). It has been found that Meis1 is a critical regulator of the CM cell cycle. Deletion of *Meis1* allows for an extension of the proliferative window in mice after birth at P14 and can re-activate CMs to enter the cell cycle in adult mice (Mahmoud et al., 2013). Interestingly in fetal sheep CMs, down-regulation of Meis1 increased mitochondrial activity and expression of oxygen consumption genes and decreased expression of glycolytic genes (Lindgren et al., 2019).

Sex differences. There are many processes during cardiac development where there are baseline sex differences, including lipid metabolism, rhythmicity and fibrosis (Coronado et al., 2017; Norheim et al., 2019; Ventura-Clapier et al., 2017). Biological sex is also an important factor in the development of CVD, with younger women typically exhibiting cardioprotection, but this is lost later in life (Blenck et al., 2016). Traditionally, sex differences in the heart were solely attributed to the influence of sex hormones (Clegg et al., 2017); however, more recently, studies have reported notable amounts of transcriptomic and epigenetic variability between males and females that may account for the broader phenotypic differences observed (Kararigas et al., 2012; Singmann et al., 2015). *Prdm14* is thought to be a crucial regulator in establishing sex-specific gene networks during cardiac development in embryonic stem cells, with differential expression between males and females at every stage of development (Deegan et al., 2019). The embryonic origins of sexual dimorphism, especially in CM development, have not been well considered by the research community and represents a gap in the literature that should be addressed.

miRNA regulation of CMs

CM proliferation and metabolism are a tightly regulated, highly complex symphony involving many cellular networks and signalling pathways. They collectively work to ensure the correct development of the heart whilst

simultaneously maintaining appropriate cardiac function. Whilst the molecular mechanisms for this process are well established, regulation of these genes by non-coding RNAs, such as miRNAs, remain ill-defined. By studying the many dynamic events that CMs experience across gestation and postnatally, miRNAs have emerged as crucial determinants for many aspects of cardiac development (van Rooij, 2011). miRNAs are highly conserved small RNA molecules (∼22 nucleotides long) that mediate gene silencing through targeted degradation, repression of translation or deadenylation by binding to the 3′ untranslated region (UTR) of the target mRNA. Through these mechanisms, miRNAs are involved in the fine-tuning of a wide range of biological processes, including cell proliferation, differentiation, migration, angiogenesis and apoptosis (Hwang & Mendell, 2006; Lock et al., 2017, 2018).

miRNA regulation of cardiac proliferation

Recent studies have demonstrated that miRNAs regulate genes essential in CM differentiation, proliferation, metabolism and physiological hypertrophy (Cordes & Srivastava, 2009).

miRNA-1. Previous studies have shown that serum response factor (SRF) is responsible for the regulation of miRNA-1, with highest expression of SRF in neonatal hearts (Zhao et al., 2005). As such, in P2 mice, miRNA-1 is lowly expressed, but by P13 (after mice lose their proliferation capacity) miRNA-1 is significantly upregulated. It is hypothesised that miRNA-1 suppresses proliferation by inhibiting *CCND1* and therefore represses the G1/S phase transition (Gan et al., 2019). miRNA-1 also targets insulin growth factor 1 (IGF-1) and IGF-1 receptor and as such can regulate cardiac hypertrophy (Elia et al., 2009; Sayed et al., 2007), with downregulation of miRNA-1 leading to pathological hypertrophy as seen in human hearts (Elia et al., 2009).

miRNA-133a. miRNA-1 and miRNA-133a are transcribed together, and appear to have similar targets (Ohanian et al., 2013). miRNA-133a has known roles in differentiation, proliferation, hypertrophy and cardiac remodelling (Li et al., 2018). In zebrafish where CMs can proliferate across the lifespan, miRNA-133 is downregulated during CM proliferation but, when overexpressed, it inhibited proliferation and regeneration in an injured myocardium (Yin et al., 2012b). Likewise, when miRNA-133a is inhibited, there is aberrant proliferation of CMs by activation of SRF (Liu et al., 2008). In the fetal sheep heart, miRNA-133a increases in late gestation at the time when there is a decline in CM proliferation (Morrison et al., 2015).

miRNA-15 family. The miRNA-15 family is known to have broad roles in regulation of the cell cycle and cell survival (Fig. 3). A comparison of miRNAs across the CM transition period in mice revealed that miRNA-195 (a member of the large miRNA-15 family) is significantly upregulated at P10 when CMs have withdrawn from the cell cycle, implicating an important role for miRNA-195 in the inhibition of CM proliferation (Porrello et al., 2013; Porrello, Johnson et al., 2011). It is suggested that miR-195 may function through suppression of *CHEK1*, which promotes progression of the G2 to M phase in the cell cycle. In mice, multiple gene targets of miRNA-195, critical in cell cycle progression, were downregulated in non-proliferating CMs (Porrello, Johnson et al., 2011). Moreover, in P1 mice (an age when mouse CMs normally have regenerative capacity), overexpression of miRNA-195 suppressed CM proliferation and thus inhibited cardiac regeneration after MI (Porrello et al., 2013). In neonatal mice, inhibition of the miRNA-15 family can induce mitosis of CMs at P1, P7 and P14, leading to increased proliferation of pre-existing CMs in the adult mouse and overall improved cardiac function after MI induced at P21 (Porrello et al., 2013). Furthermore, inhibition of the miRNA-15 family after MI in 3-month-old pigs reduced hypoxia-mediated CM apoptosis (Hullinger et al., 2012).

Other members of the miRNA-15 family such as miRNA-15a/b, miRNA-16-1/2 and miRNA-497 also regulate many cell cycle genes such as *CRM1*, *CHEK1*, *CDC2A*, *BIRC5*, *SPAG5*, *MPS1*, *PGAM1*, *HAND1*, *CDK9*, *C-MYC*, *CYCLIN D1*, *CYCLIN D2*, *SRF* and *CONNEXIN-43* in rodents (Hullinger et al., 2012; Liu et al., 2008; Porrello et al., 2013; Porrello, Johnson et al., 2011; Yin et al., 2012a). Taken together, these studies suggest the miRNA-15 family negatively regulates CM proliferation, and upregulation of miRNA-15 family members shortly after birth may contribute to cell cycle arrest seen in mice. However, expression of the miRNA-15 family was variable with age in sheep, although target genes of the family *CHEK1*, *CDC2A*, *BIRC5* and *SPAG5* decreased with age, with a plateau after 140d GA. Given these genes are involved in mitosis and cell cycle progression, decreased expression with age suggests that they may be involved in cell cycle exit in large mammals (Morrison et al., 2015).

Let-7 family. Let-7 is one of the most highly upregulated miRNA families during CM maturation (Kuppusamy et al., 2015). Members of the Let-7 family of miRNAs regulate CM metabolism, proliferation, hypertrophy and contractility and are required for maturation, but not early differentiation into CMs. Overexpression of the Let-7 family triggers an increase in cell size, sarcomere lengthening, greater contractile ability and oxidative

respiration. It is hypothesised that downregulation of the phosphoinositide 3 kinase (PI3K)/AKT protein kinase/insulin pathway and an increase in FAO trigger greater expression of Let-7 to drive CM maturation (Kuppusamy et al., 2015). Inhibition of the Let-7 family following MI has been shown to attenuate cardiac remodelling and improve cardiac function. It also results in increased expression of pluripotency genes *Sox2* and *Oct4* in epicardial fibroblasts and increased neovascularisation, and as such may be a potential target for cardiac regeneration (Seeger et al., 2016; Tolonen et al., 2014).

miRNA-34a. Members of the miRNA-34 precursor family (miRNA-34a/b/c) have known roles in regulation of cardiac maturation. miRNA-34a is crucial in regulating apoptosis, inflammation, autophagy and fibrosis and as such its inhibition is a potential therapeutic in CVD (Hua et al., 2021). miRNA-34a is upregulated in CMs that have undergone cardiac injury (Bernardo et al., 2012) and in patients with heart failure (Matsumoto et al., 2013). In a rat MI model, overexpression of miRNA-34a promotes apoptosis by targeting Sirtuin 1 (SIRT1) (Dong et al., 2019), and in a chronic heart disease (CHD) model, miRNA-34a is a negative regulator of the Notch signalling pathway leading to increased pathogenesis in CHD (Wu et al., 2018). As a target of p53, miRNA-34a also targets Bcl2 in conjunction with SIRT1 to exacerbate myocardial injury by increasing apoptosis and autophagy (Fu et al., 2017) and cyclin D1 to promote cell cycle arrest (Yang et al., 2015).

miRNA-199a-3p and miRNA-590-3p. miRNA-199a-3p and miRNA-590-3p possess pro-proliferative effects in CMs through DNA synthesis and increased cytokinesis (Fig. 3). They are also involved in reducing fibrotic scar size and can improve cardiac function following MI in neonatal mice (Eulalio et al., 2012). A common target of

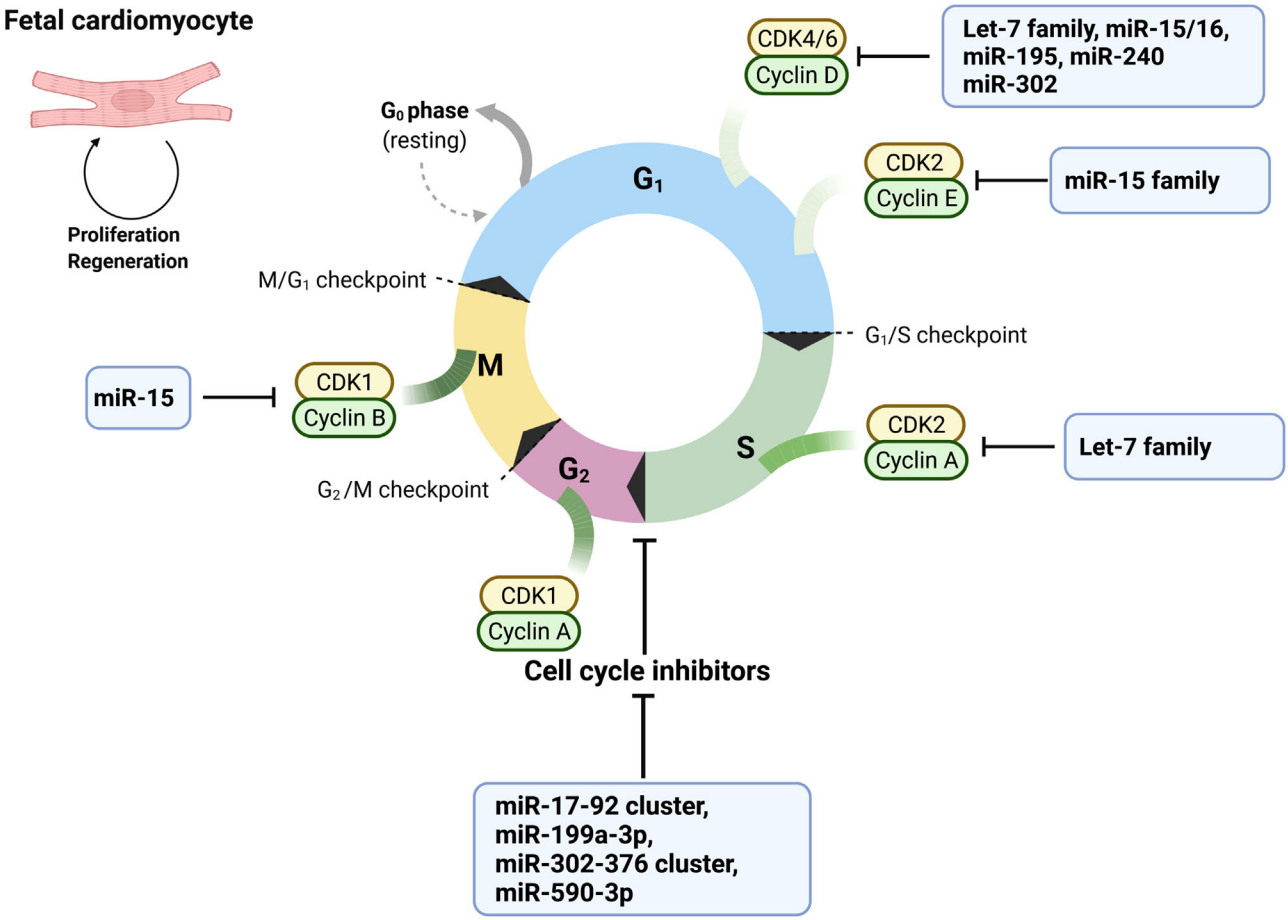

Figure 3. Regulation of the fetal cardiac cell cycle by miRNAs
There is a complex interplay of miRNA regulation and the cell cycle. The miRNA-15 family, the Let-7 family, miRNA-240 and miRNA-302 are miRNAs that inhibit CDK/cyclin complexes at various points in the cell cycle. The miRNA-17/92 cluster, miRNA-302/376 cluster, miRNA-199-3p and miRNA-590-3p negatively regulate cell cycle inhibitors allowing for proliferation. Note many more miRNAs interact with the cell cycle and this figure represents a simplified version.

miRNA-199a-3p and miRNA-590-3p is *HOMER1*, which encodes a protein that modulates Ca^{2+} signalling in the heart (Fill & Copello, 2002). In adult rodents treated with miRNA-199a and miRNA-590 mimics, CMs re-entered the cell cycle with promotion of cardiac regeneration (Eulalio et al., 2012).

In a pig model of MI, overexpression of miRNA-199a resulted in almost complete recovery of cardiac function parameters such as improvements in global and regional contractility, increased muscle mass and reduced scar size. However, prolonged, uncontrolled expression of miR-199a led to sudden death by ventricular arrhythmia. This may be the result of cardiac infiltration of proliferating cells displaying a poor, de-differentiated myoblast phenotype (Gabisonia et al., 2019). This highlights the need for further work in establishing the optimal duration and concentration of miRNAs as well as investigating any off-target side effects. In sheep, expression patterns of miRNA-199a and miRNA-590 were inconsistent with direct involvement in CM proliferation, suggesting a more complex role of miRNA regulation of CMs between large mammals that warrants further investigation (Morrison et al., 2015).

miRNA-558 and miRNA-1538. miRNA-558 and miRNA-1538 may also play important roles in the repair of a damaged heart with evidence coming from fetal sheep. Little is known about the roles of miRNA-558 and miRNA-1538 in CM regulation (Lock et al., 2020). They are uniquely expressed after MI depending upon age. In the adolescent lamb with CMs that do not proliferate, these miRNAs are upregulated in response to cardiac damage. However, in a fetus with CMs that can proliferate, these miRNAs are downregulated in response to cardiac damage. Furthermore, *in vitro* inhibition of these miRNAs in cardiomyoblasts shows that they are effective targets for increasing cell proliferation (Lock et al., 2020).

miRNA regulation of cardiac metabolism

Aside from the clear influence of miRNAs on the CM cell cycle, there is strong evidence for miRNAs also regulating CM energy metabolism through glucose and fatty acid metabolism (Fig. 4).

Glucose and lactate metabolism. Overexpression of miRNA-223 promotes glucose uptake in CMs through

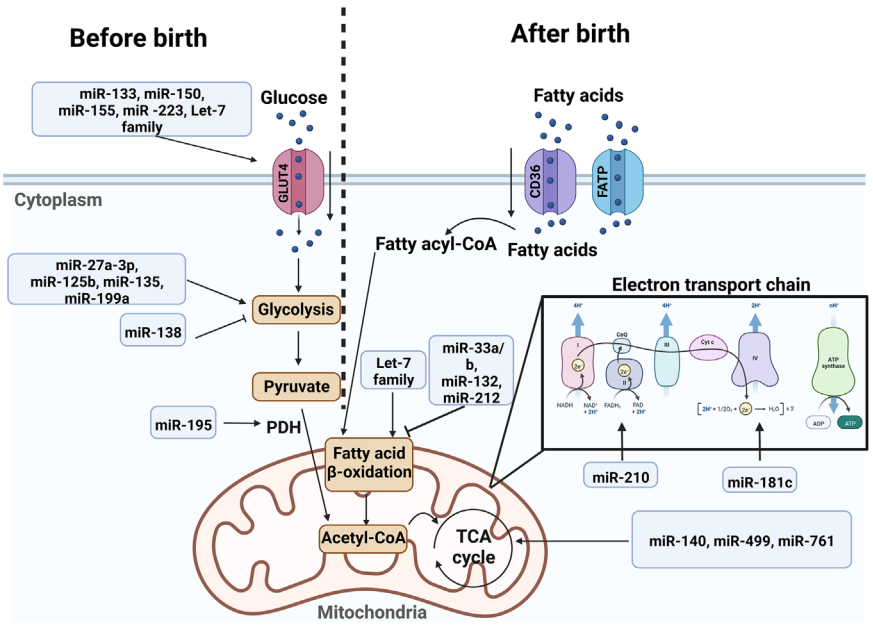

Figure 4. Regulation of cardiac metabolism by miRNAs before and after birth
miRNAs regulate many aspects of glucose and lipid metabolism in the heart. miRNA-133, -150, -155 and -223 and the Let-7 family regulate glucose transport via modulating the expression of GLUT4. miRNA-27a-3p, -125b, -135 and -199a promote glycolysis whilst miRNA-138 has been shown to inhibit the glycolysis pathway. miRNA-195 increases acylation of pyruvate dehydrogenase (PDH) to promote conversion of pyruvate to acetyl-CoA. The Let-7 family promotes FAO whilst miRNA-33a/b, -132 and -212 repress various enzymes involved in the pathway. miRNA-140, -499 and -761 regulate the TCA cycle inside the mitochondria, and miRNA-181c and miRNA-210 are involved in ETC remodelling via suppression of complex 2 (SDHB) and 4 (MTCO1). Note many more miRNAs interact with the metabolic pathways and this figure represents a simplified version.

upregulation of glucose transporter type 4 (GLUT4) (Lu et al., 2010) and miR-155 promotes glucose consumption by targeting GLUT1 (Jiang et al., 2012). miR-1 also regulates glucose flux in the heart through the pentose phosphate pathway (Singh et al., 2013). miRNA-378 inhibits lactate dehydrogenase A (*LDHA*) expression to maintain the balance between glycolysis and OXPHOS in CMs (Mallat et al., 2014). Interestingly, the miR-199a/214 cluster regulates the metabolic switch from FAO to glycolysis in response to heart failure by inhibiting *PPARβ*, an important regulator of energy metabolism in the rodent heart (el Azzouzi et al., 2013).

Fatty acid and mitochondrial metabolism. miRNA-1 possesses a dual role, as it not only regulates glycolysis but also fatty acid uptake in CMs by inhibiting fatty acid binding proteins and uptake in the heart (Singh et al., 2013; Varrone et al., 2013). In the mitochondria, miRNA-33a/b, miRNA-132 and miRNA-212 play important roles in regulation of mitochondrial FAO by inhibiting fatty acyl-carnitines involved in mitochondrial metabolism (Soni et al., 2014; Zhang & Schulze, 2016), and miRNA-138 can promote mitochondrial respiration and inhibit glycolysis by directly targeting PDK1. There is much evidence to suggest CM metabolism plays a key role in driving both OXPHOS and glycolysis that lead to improved cardiac function by targeting the Wee1/CyclinB-CDK1 complex (Borden et al., 2019). Additionally, the Let-7 family of miRNAs is highly upregulated during human cardiac maturation, with multiple members of the Let-7 family regulating both glucose homeostasis and FAO (Frost Robert & Olson Eric, 2011; Kuppusamy et al., 2015; Zhu et al., 2011).

Linking the role of miRNAs in cardiac proliferation and metabolism. Despite these studies, little is known about the role of miRNAs in cardiac metabolism in large mammals. Moreover, studies aimed at understanding the role of miRNAs on CM metabolism have done so without then aligning miRNA-induced alterations of metabolism to changes involved with CM growth profiles. As the CM transition period involves many fundamental changes in both growth and metabolism, and miRNAs often have multiple targets that play diverse roles, these events should be considered in conjunction if we are to understand precisely how they are linked. Future studies are needed to identify and investigate if any miRNAs are involved in both processes and if manipulation of one pathway will affect the other. Additionally, miRNAs can be detected in blood samples and, given their roles in CM proliferation and metabolism, they may play an important role in developing new therapeutic strategies.

Circulating miRNAs as diagnostic biomarkers of heart disease

Early detection of CVD is imperative for optimal long-term management or, preferably, reduction of risk to delay/prevent onset of disease. As such, there is a need for diagnostic biomarkers for non-invasive detection of CVD. The discovery of miRNA transportation in extracellular vesicles (EVs) or circulating in plasma has led to an interest in the potential utility of miRNAs as biomarkers, which has become an emerging field of research across several diseases such as CVD and pregnancy complications (Fig. 5) (Kalluri & LeBleu Valerie, 2020).

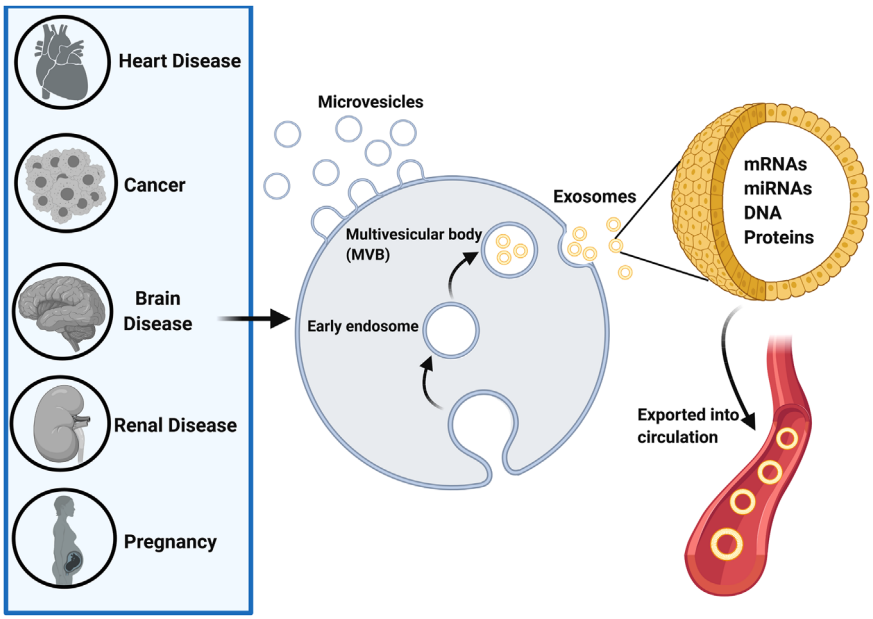

Figure 5. Overview of the formation and release of exosomes from cells and their use as biomarkers of disease
Numerous diseases including heart disease, brain/neurodegenerative disease, renal disease, cancer and even physiological alterations such as pregnancy can be detected via miRNAs packaged into circulating exosomes released into the bloodstream or other bodily fluids such as saliva and urine. Circulating exosomal miRNAs are an attractive diagnostic target due to their non-invasive nature.

EVs can be classified into either exosomes or ectosomes. Exosomes are ∼30–120 nm long spheroid vesicles of endosomal origin and are secreted by various cell types including CMs (Johnstone, 1992), whilst ectosomes are bigger vesicles ∼100–500 nm in diameter and released by budding from the plasma membrane (E et al., 2018). Both exosomes and ectosomes can contain a diverse range of biological constituents including nucleic and amino acids, proteins, metabolites, lipids and miRNAs, which may reflect their cell origin (Kalluri & LeBleu Valerie, 2020). Secreted exosomes can be taken up into proximally or distally located cells, where they can modulate biological function within recipient cells. Importantly, exosomes can also be released into bodily fluids such as blood, urine, saliva and breast milk (Zhang et al., 2019). Of particular interest are exosomal miRNAs, where exosomes can take up miRNAs in a tissue before being released into the circulation, where their expression is maintained in a stable fashion (Kosaka et al., 2010; Pegtel et al., 2010). The expression of exosomal miRNAs is impacted by both physiological conditions such as pregnancy (Gilad et al., 2008; Sadovsky et al., 2020; Smith et al., 2021) and pathological conditions including CVD (Zhou et al., 2018), cancer (Sun et al., 2018), infection (Alipoor et al., 2016) and neurodegenerative disease (Fig. 5) (Wang & Zhang, 2020). Owing to their robust stability and relative non-invasive ease of detection in the bloodstream, it is no surprise that circulating miRNAs have emerged as an attractive therapeutic and diagnostic biomarker (Kalluri & LeBleu Valerie, 2020).

The expression of circulating exosomal and ectosomal miRNAs can reflect cardiovascular pathologies including HF, fibrosis, arrythmias, atherosclerosis and MI (E et al., 2018; Gilad et al., 2008; Lu et al., 2019). Multiple studies have reported rapid release of cardiac enriched miRNAs, miRNA-1, miRNA-133a/b, miRNA-208a/b and miRNA-499, into the circulation after the onset of acute myocardial infarction (AMI) and acute coronary syndrome (ACS). In a study utilising plasma samples from ST-elevated myocardial infarction (STEMI) patients, traditional markers of MI such as cardiac troponin T peaked in plasma 3 h later than miRNA-1 and miRNA-133a/b, which were detected almost immediately after the onset of MI symptoms (D'Alessandra et al., 2010). Cardiac specific miRNA-208a is also an attractive diagnostic target of AMI in both rats (Ji et al., 2009) and humans (Wang et al., 2010). miRNA-208a is typically expressed exclusively in the heart. However, miRNA-208a was detectable in the plasma of 91% of patients within the first hour after AMI and in 100% of patients after 4 h. Importantly, miRNA-208a expression remained undetectable in plasma from those who had not suffered AMI (Wang et al., 2010). miRNA-1 and miRNA-133a were detected in human serum as quickly as 15 min after AMI (Liebetrau et al., 2013). A further study has validated that release of miRNA-133a, miRNA-208a and miRNA-499 was proportional to the degree of myocardial injury in ACS, demonstrating that damaged myocardium was the probable source of these miRNAs (De Rosa et al., 2011).

In CVD, studies investigating ectosomes are very limited; however, they play an important role in miRNA transfer and communication. Upregulation of TNF-α after injury triggers the release of ectosomes (or microparticles) with suppressed miRNA-126-3p and miRNA-21-5p, but upregulated miRNA-155-5p (Alexy et al., 2014).

Taken together, these findings suggest that miRNAs can be released into the circulation from damaged CMs, with release of specific miRNAs dependent on the nature and severity of the cardiac insult. Nevertheless, characterisation of a single reliable miRNA as a biomarker of AMI or ACS remains controversial, with further study needed to fully characterise the sensitivity and reliability of circulating miRNAs as biomarkers of CVD. Even more generally, a suitable biomarker for non-invasively detecting changes in CM proliferation and metabolism does not, to the best of our knowledge, yet exist. Identification of a biomarker specific to changes in CM proliferation and/or metabolism during late gestation may not only serve to monitor the ontogeny of the developing heart, but also identify any risk factors for later development of CVD.

There is also evidence from preclinical models of human pregnancy that complications in pregnancy such as intrauterine growth restriction and maternal under-nutrition can impact these regulatory pathways and therefore impact fetal cardiac development (Botting et al., 2012; Darby et al., 2018; Lie et al., 2013, 2014; Wang et al., 2011, 2013, 2017). Thus, early biomarkers of changes in cardiac development from as early as in the womb may be used to implement therapeutic strategies to reduce the risk of CVD later in life as well as to promote and monitor repair after an MI.

Conclusion

CMs undergo major changes during fetal development and in the transition to postnatal life. It is imperative that they retain metabolic flexibility during and after this transition event to sustain proper cardiac development and function not only in this highly vulnerable period but also throughout life. There is much evidence to suggest that CM metabolism plays a key role in driving CM maturation and that proliferation and metabolism are intrinsically linked. Key factors such as oxygen, nutrients and hormone concentrations play roles in triggering the metabolic, proliferative and subsequent morphological changes in CMs; however, their exact interactions remain unclear. miRNAs may play a key role in coordinating CM

proliferation and metabolism in preparation for postnatal life and possess many exciting diagnostic and therapeutic targets for cardiac disease; however, much of this research has only been performed in zebrafish and rodents. It is essential to study miRNAs in large preclinical animal models in which cardiac development better corresponds to humans. Thus, more work is required to fully elucidate the mechanisms by which miRNAs interact with genes involved in CM maturation and their potential role as biomarkers of altered fetal cardiac development in late pregnancy. These insights will allow for more effective and timely translation of potential treatments into the clinic.

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Additional information

Competing interests

The authors have no conflicts of interest.

Author contributions

C.D., J.R.T.D. and J.L.M. were responsible for the conception and design of the article, involved in analysis and interpretation of the data, drafted the article, and contributed to the final version and approved the manuscript submission.

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Supporting information

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