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Adapting to a new environment: postnatal maturation of the human cardiomyocyte

Shatha Salameh[1,2], **Vanessa Ogueri**[3], **Nikki Gillum Posnack**[1,2,3,4]

[1]Department of Pharmacology & Physiology, George Washington University, Washington, DC, USA

[2]Sheikh Zayed Institute for Pediatric Surgical Innovation, Children's National Hospital, Washington, DC, USA

[3]Children's National Heart Institute, Children's National Hospital, Washington, DC, USA

[4]Department of Pediatrics, George Washington University, Washington, DC, USA

Abstract

The postnatal mammalian heart undergoes remarkable developmental changes, which are stimulated by the transition from the intrauterine to extrauterine environment. With birth, increased oxygen levels promote metabolic, structural and biophysical maturation of cardiomyocytes, resulting in mature muscle with increased efficiency, contractility and electrical conduction. In this Topical Review article, we highlight key studies that inform our current understanding of human cardiomyocyte maturation. Collectively, these studies suggest that human atrial and ventricular myocytes evolve quickly within the first year but might not reach a fully mature adult phenotype until nearly the first decade of life. However, it is important to note that fetal, neonatal and paediatric cardiac physiology studies are hindered by a number of limitations, including the scarcity of human tissue, small sample size and a heavy reliance on diseased tissue samples, often without age-matched healthy controls. Future developmental studies are warranted to expand our understanding of normal cardiac physiology/pathophysiology and inform age-appropriate treatment strategies for cardiac disease.

Graphical Abstract

Corresponding author Nikki Gillum Posnack: Sheikh Zayed Institute, 6th floor, 111 Michigan Avenue, NW, Washington, DC 20010, USA. nposnack@childrensnational.org.

Author contributions

All authors contributed to the conception, drafting and/or revising the work for critically important intellectual content. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Competing interests

The authors declare no competing interests.

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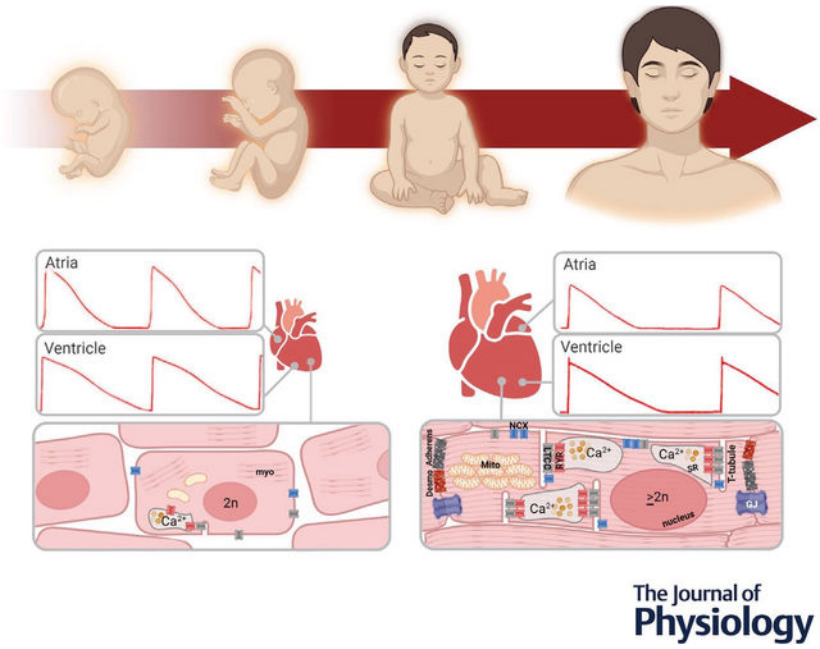

Abstract figure legend Conceptual image of postnatal cardiomyocyte maturation. Human heart development is associated with changes in ionic currents that mediate the cardiac action potential shape (red traces depict atrial and ventricular action potentials) and cardiomyocyte morphological characteristics that collectively influence cell-cell coupling, electrophysiology, metabolism, and contraction. Abbreviations: Adherens, adherens junction; Desmo, desmosome; GJ, gap junction; LTCC, L-type calcium channel; Myo, myofilament; NCX, sodium–calcium exchanger; RYR, ryanodine receptor; SR, sarcoplasmic reticulum. Action potentials are adapted with permission (Cohen & Lederer, 1993). Image created with Biorender.com

Keywords

age dependence; cardiomyocyte; cardiac electrophysiology; development; excitation–contraction coupling; human physiology; maturation

Introduction

During postnatal life, the human heart contracts >2 billion times during the average lifespan. Through coordinated activity, the four distinct chambers of the heart work together to deliver oxygenated blood to the body and return deoxygenated blood to the lungs. The cellular composition of the human heart is both heterogeneous and dynamic, and this cellular landscape can change in response to normal development, ageing or disease (Grandi et al., 2023; Litviňuková et al., 2020; Suryawanshi et al., 2020; Tucker et al., 2020). A variety of specialized cell types support heart function, including cardiomyocytes, which compose ~20–40% of the total numberofcells and ~70–85%oftotaltissuevolumeowing to their large size (Bergmann et al., 2015; Litviňuková et al., 2020; Zhou & Pu, 2016). These excitable muscle cells conduct electrical signals and generate contractile force, which pumps blood into the circulatory system in a coordinated rhythmic fashion. Throughout fetal and postnatal

development, cardiomyocytes adjust to haemodynamic demands by changing their size, structural organization, metabolism and gene/protein expression profile. These adaptations coincide with a shift in the electrophysiology and contractile properties of the cells.

Despite their critical role in sustaining perinatal life, our current knowledge of human cardiomyocyte physiology and maturation is hindered by the scarcity of healthy heart tissue for biomedical research. Donated biospecimens have provided some insight into cardiomyocyte proliferation, growth and cell structure, but with modest temporal and spatial resolution. Moreover, atrial and ventricular cardiomyocytes are likely to have different maturation trajectories owing to the distinct electrophysiological and mechanical properties of the individual cardiac chambers (Rumyantsev, 1991). Furthermore, fixed tissue specimens provide minimal information on the physiology of living cells. As such, insight into the cardiomyocyte maturation process has largely been extrapolated from either mammalian models (Swift et al., 2020; Velayutham et al., 2020) or human pluripotent stem cell-derived cardiomyocytes (hPSC-CM; Lundy et al., 2013). Although facets of myocyte maturation remain conserved between species, there are significant differences in electrophysiology, calcium handling, contraction and relaxation that can hinder the translational applicability of these findings to humans (Kannan & Kwon, 2020; Milani-Nejad & Janssen, 2014; Noujaim et al., 2004). This is to be expected, because cardiovascular systems have evolved differently across species to adapt to unique physiological demands (West et al., 1997). For example, smaller animals have faster heart rates (e.g. mice ~600 beats min^{-1} at rest), which require rapid rates of contraction and relaxation to maintain cardiac output, in comparison to larger mammals ((e.g. 75 beats min^{-1}) humans Bratincsák et al., 2020; Jann Hau, 2010; Janssen & Periasamy, 2007; Rudolph, 2009). These physiological differences are supported by both anatomical adaptations and underlying molecular mechanisms (Edwards & Louch, 2017; Milani-Nejad & Janssen, 2014; Odening et al., 2021; Ripplinger et al., 2022).

To bridge this gap, hPSC-CM have gained prominence because these cells have structural features and a gene/protein expression profile that is similar to primary human cardiomyocytes (Casini et al., 2017; Denning et al., 2016; Liang et al., 2013). Moreover, hPSC-CM can be used in the laboratory in large quantity, which is not possible with limited biospecimens that are collected from donated tissues or from patients undergoing corrective surgery. Nevertheless, hPSC-CM lack structural and phenotypical maturity and more closely resemble fetal than adult cardiomyocytes (Bedada et al., 2014; DeLaughter et al., 2016; Kannan & Kwon, 2020; Kannan et al., 2021; Lewandowski et al., 2018; van den Berg et al., 2015). Accordingly, tremendous efforts have been made to increase the maturity of hPSC-CM using biophysical and biochemical cues and to identify regulatory pathways that drive the maturation process (Murphy et al., 2021; Parikh et al., 2017; Ribeiro et al., 2015; Yang et al., 2019; Yoshida et al., 2018). Although animal models and hPSC-CM cannot serve as a replacement for human studies, they are an important resource for expanding our knowledge on postnatal cardiomyocyte maturation. In this article, we aim to highlight key studies that inform our current understanding of human cardiomyocyte maturation, which can help us to use animal or hPSC-CM models better, at appropriate developmental stages and with clear insight into their underlying developmental differences.

Transition from hyperplasia to hypertrophy

At birth, the neonatal heart is roughly 1/10th its adult size, and continued heart growth (e.g. heart weight, ventricular wall thickness and ventricular cavity size) is correlated with advancing age and increasing body size (Scholz et al., 1988; St. John Sutton et al., 1982). At first, fetal cardiac growth is accomplished through hyperplasia (cells proliferate and divide into two daughter cells), then later in development, growth occurs by cardiomyocyte hypertrophy (cell size increases without a change in the total number of cells; Günthel et al., 2018; Tan & Lewandowski, 2020). The fetal myocardium experiences a 6.7-fold increase in the total number of cardiomyocyte nuclei between 16 and 42 weeks of gestation (Mayhew et al., 1997; Table 1). A hallmark of cardiomyocyte maturation is the transition from hyperplasia to hypertrophy. A few studies have found indications that this process begins *in utero*, supported by the presence of binucleated cardiomyocytes (Kim et al., 1992) and a reduction in proliferative activity (Huttenbach et al., 2001), although it appears that this transition occurs in earnest with the first few months after birth. To date, the exact timing of this event is unknown and differs slightly between experimental studies, probably owing to differences in sample size, species and the experimental technique adopted (Ang et al., 2010; Austin et al., 1995; Botting et al., 2012; Burrell et al., 2003; Jonker et al., 2015; Mandarim-de-Lacerda et al., 1997; Soonpaa & Field, 1998). For example, Mayhew et al. (1997) measured an equivalent number of human cardiomyocyte nuclei at birth (42 gestational weeks) compared with 40 postnatal weeks old. Furthermore, Mollova et al. (2013) reported that the percentage of human cardiomyocytes in mitosis (0.1%) and cytokinesis (0.016%) was highest in infants <1 year old, which then decreased ~11-fold in older heart tissue samples (0.009% mitosis and 0.005% cytokinesis, 10–20 years old). Ye et al. (2016) also found that the window of postnatal proliferation is narrow in humans, because prominent cell cycle activity (0.47%) was observed in atrial myocytes isolated from the youngest congenital heart disease patients, <3 months old, which decreased 27-fold in older infants (0.02% at 7–12 months old).

However, it is important to consider that cell cycle activity is not only indicative of cell proliferation; cells can replicate DNA with or without karyokinesis, resulting in the formation of multinucleated cardiomyocytes or multiple chromosomes within a single nucleus. In the aforementioned studies, the time line of cell cycle withdrawal is in agreement with work by Bergmann et al. (2015), who used a specific marker for cardiomyocyte nuclei to calculate that the final number of cardiomyocytes in the heart (~3.2×10^9) was established within 1 month of birth (the youngest age assessed). Although the total number of myocytes remained relatively constant between 1 month and 73 years old, the number of endothelial cells and mesenchymal cells continued to increase, by 6.5- and 8.2-fold, respectively.

The mechanisms driving cardiomyocyte cell-cycle arrest are still poorly understood in humans but remain an active area of investigation (Ball & Levine, 2005; Lock et al., 2018), because identifying strategies to ‘restart’ myocyte proliferation could lead to new therapeutic interventions to regenerate and repair heart tissue after injury, as recently demonstrated in several animal models (Abouleisa et al., 2022; Porrello et al., 2011, 2013; Soonpaa & Field, 1997; Zgheib et al., 2014). Further, a newborn child with severe myocardial infarction

rapidly recovered left ventricular function within weeks after injury (Haubner et al., 2016). Additional studies are needed to understand the timing and mechanistic regulators of cell-cycle arrest in humans, in order to evaluate potential heterogeneities within different regions of the heart and to assess the functional outcomes of this process.

As cardiomyocytes develop and acquire structural maturity [e.g. sarcomere assembly and expansion, intercellular coupling via intercalated discs and invaginations of the sarcolemma to form transverse tubules (T-tubules)], it can become disadvantageous to undergo cell proliferation as a means of continued growth, because proliferation requires dedifferentiation, a process by which sarcomeres disassemble and neighbouring cardiomyocytes become uncoupled (Jopling et al., 2010; O'Meara et al., 2015; Zhu et al., 2021). Accordingly, in the perinatal period, human cardiomyocytes undergo hypertrophy to increase their overall size and expand the number of connected sarcomeres (the contractile unit of the cardiomyocyte). Mayhew et al. (1998) reported that the total cell volume per cardiomyocyte nucleus remained constant during fetal development (16–35 weeks gestation). Thereafter, Mollova et al. (2013) demonstrated that cardiomyocyte size begins to increase after birth, with an average cardiomyocyte volume increasing 8.6-fold from infants (0–1 year) to older individuals (10–20 years). Cardiomyocyte hypertrophy was also demonstrated by a 6.6-fold decrease in the density of nuclei within myocardial tissue, from 2.4×10^8 nuclei cm^{-3} in infants to 0.36×10^8 nuclei cm^{-3} in older individuals. This is in agreement with the study by Bergmann et al. (2015), who reported an even greater (15-fold) decrease in myocyte nuclei density per volume of ventricular tissue, from birth to adulthood. Cardiomyocyte hypertrophy occurs in tandem with increased myofibrillar density and alignment, allowing the heart to adapt and respond to the increasing workload that accompanies rapid postnatal growth.

With increased age, a substantial number of human cardiomyocytes also undergo polyploidization (Adler, 1975; Adler et al., 1996; Eisenstein & Wied, 1970), a process in which DNA content increases without subsequent karyokinesis and cytokinesis. Given the large size of adult cardiomyocytes, increasing the DNA content might help to support transcriptional needs for protein biosynthesis. Takamatsu et al. (1983) estimated that 94.3% of infant cardiomyocytes are diploid ($2n$), with an increased incidence of hyperploidy ($>2n$) at later stages of development (13.6% at 1–9 years, 26.7% at 9–22 years and 31.4% at 22–75 years). A comparable trend was reported by Mollova et al. (2013), whereby the percentage of hyperploid cardiomyocytes increased steadily during development, from 16.3% in the first year of life to 54.2% in individuals >40 years old. Likewise, Bergmann et al. (2015) reported that almost all cardiomyocyte nuclei were diploid within the first few years of life, with polyploidization occurring much later in development, during the second and third decade of life, although other studies suggest that polyploidization occurs even earlier in life, particularly in cases of congenital or acquired heart diseases (Adler, 1975; Adler & Costabel, 1975; Brodsky et al., 1994). Overall, the majority of human cardiomyocytes remain mononucleated (~63–80%) throughout development, with a smaller proportion (20–37%) becoming multinucleated (karyokinesis without cytokinesis), although there is no discernible difference between age groups (Bergmann et al., 2015; Takamatsu et al., 1983). Similar to human cardiomyocytes, hPSC-CM are primarily mononucleated, with a smaller percentage of cells becoming multinucleated (Kong et al., 2017; Laflamme & Murry, 2011).

This is in stark contrast to many experimental mammalian models, thus highlighting the risk of inaccuracy when extrapolating animal data to humans. For example, ~50% of sheep cardiomyocytes become binucleated *inutero* (Jonker et al., 2015; Jonker, Zhang et al., 2007), ~90% of rodent cardiomyocytes become binucleated within the first 2 weeks after birth (Bensley et al., 2016; Clubb & Bishop, 1984; Li et al., 1996, 1997; Soonpaa et al., 1996), and swine cardiomyocytes become multinucleated within 6 months and often present with 8–16 nuclei per cell (Velayutham et al., 2020). The mechanisms triggering these adaptations are understudied, but are likely to be influenced by changes in haemodynamics, hormones and oxygen levels (Jonker & Louey, 2016; Jonker, Faber et al., 2007; Jonker, Louey et al., 2018; Naqvi et al., 2014; Puente et al., 2014). Of interest, differences in cell nucleation have been shown to alter cardiomyocyte physiology, with mononucleated rabbit cardiomyocytes exhibiting a faster beating rate, larger calcium transients and increased ryanodine receptor (RYR) density, compared with binucleated cells (Huang et al., 2012). The functional outcomes of multinucleation and polyploidization are still incompletely understood, as is the impact of congenital heart defects, preterm delivery and intrauterine growth restriction on these maturation patterns (Adler & Costabel, 1980; Botting et al., 2014; Brodsky et al., 1994; Jonker, Kamna et al., 2018). Furthermore, the mechanisms underlying species-specific differences remain understudied, which can limit the human applicability of experimental animal models in developmental research (Kannan & Kwon, 2020).

Structural maturation of the human cardiomyocyte

Given the rapid rate of body growth, postnatal development is accompanied by an increase in metabolic demand, cardiac workload and cardiac output, culminating in a 6- to 12-fold increase in stroke volume from birth until adulthood (De Simone et al., 1998). To adapt, cardiomyocytes undergo developmental hypertrophy (discussed above), resulting in a significant increase in left ventricular mass. Concurrent with this increase in cell size, myocytes acquire structural adaptations to improve the efficiency and capacity of muscle contraction. Key components of the cardiomyocyte architecture have been reviewed in detail previously (Ehler, 2016; Gautel & Djinovic-Carugo, 2016;´ Henderson et al., 2017). Briefly, human adult cardiomyocytes are large in size, rod shaped and contain densely packed myofibrils, the contractile machinery of cardiac muscle (Gerdes et al., 1992). Myofibrils are composed of actin and myosin filaments that are intricately arranged to facilitate cross-bridge formation, sarcomere shortening (contraction) and subsequent relaxation (Ehler, 2016) and are composed of multiple sarcomeres in series, defined at the ends by Z-lines or lateral boundaries. In adult cardiomyocytes, the sarcolemma is invaginated to form T-tubules near these Z-lines (Richards et al., 2011). The T-tubules are a key structural component of excitation–contraction coupling, localizing L-type calcium channels in close proximity to ryanodine receptors (RYR) on the junctional sarcoplasmic reticulum (SR), which supports synchronized activation of the myocyte (Jayasinghe et al., 2012). In turn, the SR is an intracellular membrane network that serves as a source for Ca^{2+} release during excitation–contraction coupling.

Progressive changes to the cardiomyocyte architecture have been documented during human fetal growth, although less information is available on the dynamic changes that occur postnatally. Early in fetal development (17–24 weeks), Kim et al. (1992) described

cardiomyocytes that were smaller, more rounded and contained scattered myofibrils near the periphery of the cell (Fig. 1 and Table 2). Sarcomeres were identifiable but lacked some distinguishing features, and myofibrils were loosely packed, with limited alignment. Racca et al. (2016) reported similar observations in early fetal development (52 days of gestation), including wavy Z-bands, narrow myofibrils (0.36 μM) and low myofibril density. With continued fetal development (127 days of gestation), Racca et al. visualized Z-band alignment with adjacent myofibrils, which were noticeably wider (0.97 μM). Kim et al. also found more densely packed myofibrils with greater alignment at 32–40 weeks gestation, although some prominent features were still largely absent in sarcomeres (e.g. H zone, M line, crosslinking of thick filaments). In late gestation, T-tubules and the SR began to develop near sarcomere Z-lines, although these structures were immature in comparison to the adult myocardium (Crossman et al., 2011; Kim et al., 1992; Lyon et al., 2009). Wiegerinck et al. (2009) reported that T-tubules were still sparse in newborn hearts (<3 weeks old), but continued to develop throughout the first postnatal year (3–14 months).

The proteomic composition of the sarcomere also changes during development, as myofilament proteins, regulatory proteins and cytoskeletal proteins shift from fetal to adult isoforms, which change the inherent contractile properties of the cardiomyocyte. Muscle contraction occurs as myosin and actin myofilaments rachet or slide over one another, which results in sarcomere shortening. Myosin is comprised of light chains (MLCs) and heavy chains (MHCs); human cardiomyocytes express α-MHC and β-MHC, with the latter being associated with slower contractile kinetics (Bouvagnet et al., 1989; Mercadier et al., 1983). Reiser et al. (2001) found that fetal atrial samples (47–110 days) primarily express α-MHC (96–97%), whereas fetal ventricular samples express β-MHC (90–93%). In ventricular samples, the small amount of α-MHC expression declined even further between ~12 weeks of gestation and adulthood. In atrial samples, Cummins & Lambert (1986) reported an increase in β-MHC expression during gestation, reaching adult levels soon after birth. Other groups have detected only the β-MHC isoform in ventricular samples collected from fetal, paediatric and adult tissues, suggesting that any slight change in MHC isoform expression is likely to occur either before or near birth (Auckland et al., 1986; Elhamine et al., 2016; Racca et al., 2016; Schier & Adelstein, 1982). This is remarkably different from rodent models, wherein the fetal ventricle predominately expresses β-MHC, with α-MHC expression beginning to increase after birth, with nearly complete turnover to this isoform in young adult animals (Lompre et al., 1981; Lompré et al., 1984). This is one example of key species-specific differences, wherein larger animals with slower heart rates are more similar to humans and follow a similar pattern of MHC isoform switching (Hodges et al., 2021; Jann Hau, 2010; Janssen & Periasamy, 2007).

Although human cardiomyocytes have limited age-dependent MHC isoform switching, there is a significant developmental difference in the expression of MLCs in the human myocardium. Cummins et al. (1980) and Price et al. (1980) identified a unique MLC (ALC-1, atrial essential myosin light chain) expressed in fetal, but not adult ventricular tissue samples. Moreover, Schier and Adelstein (1982) reported that expression of this fetal isoform in infant tissue coincided with 50% less myosin enzymatic activity, in comparison to adults. Further study by Auckland et al. (1986) revealed a steady decline in ALC-1 expression, representing 46.5% of total MLC expression at 18–21 weeks of gestation and

rapidly decreasing thereafter to 18.3% at birth, 5.5% in infants <2 years old, and negligible expression at older ages (only expressing VLC-1 and VLC-2, ventricular essential light chains). It is estimated that ALC-1 represents ~42% of total MLC expression in neonates after birth, declining monoexponentially with a half-time of 5.8 months that coincides with replacement by VLC-1 and VLC-2 (Elhamine et al., 2016). This isoform switch was associated with an age-dependent change in myofibrillar biomechanics, including contraction kinetics and maximal force. Conversely, infants and children with tetralogy of Fallot have persistently higher levels of ALC-1, probably owing to haemodynamic influences, which further highlights the effect of cardiac disease in postnatal maturation (Auckland et al., 1986).

Troponin I (TnI) is a regulatory protein that inhibits actin–myosin interactions at basal Ca^{2+} levels, and its isoforms are expressed in an age-dependent manner. Specifically, the slow isoform (ssTnI) is abundantly expressed in fetal and infant cardiomyocytes, with negligible amounts in adult tissue, which predominately expresses the cardiac isoform (cTnI) (Bhavsar et al., 1991; Elhamine et al., 2016; Hunkeler et al., 1991; Racca et al., 2016; Sasse et al., 1993). Bhavsar et al. (1991) reported that 8-week-old fetal atrial tissue expressed only ssTnI, whereas both ssTnI and the cTnI isoforms were readily detected later in gestation (33 weeks) and in the early postnatal period (15 days). Only cTnI was detected in atrial tissue from a 9-month-old specimen, suggesting that this switch occurs within the first year of life. Sasse et al. (1993) reported a similar temporal pattern in ventricular tissue, wherein ssTnI was the predominant isoform during fetal development (90% of total expression), with slightly reduced levels at birth (80%) and early postnatal development (60%), but undetectable levels by 9 months of age. As ssTnI declined, cTnI expression increased in an age-dependent manner, with a similar expression profile observed between 9 months of age and adulthood. In agreement, Elhamine et al. (2016) found that ssTnI declined monoexponentially within the first 10 months after birth and was replaced by the cTnI isoform with a half-time of 4.3 months, although there was variability between patients in this developmental decline, which might be attributed to the type of congenital heart disease. Racca et al. (2016) confirmed that ssTnI is expressed early in fetal development, which is associated with slower rates of force development and relaxation. A progressive increase in cTnI was seen with gestational age (up to 134 days), which coincided with increased force and faster activation/inactivation kinetics, probably owing to reduced Ca^{2+} sensitivity. Although maximal force increased 6-fold with increasing gestational age (7.8 mN mm^{-2} at 58 days *vs.* 49.9 mN mm^{-2} at 130 days of gestation), this was still considerably less than values reported for adult ventricular samples (90–110 mN mm^{-2}; Piroddi et al., 2007). This is likely to be influenced by inefficient strain transmission caused by underdeveloped and unaligned myofibrils.

Developmental changes in cardiac troponin T (cTnT; a regulatory protein that binds the troponin complex to tropomyosin and modulates Ca^{2+} concentration-dependent ATPase activity) are largely limited to fetal development. Anderson et al. (1991, 1995) detected four cTnT isoforms in fetal ventricular tissue (14–15 weeks of gestation), but only two isoforms in adult ventricular tissue. Furthermore, myofibrillar ATPase activity was related to the ratio of cTnT isoform expression in adult tissue (healthy *vs.* heart failure), highlighting disease-associated changes. Similar to observations in adult heart failure tissue, Saba et al.

(1996) reported an increase in $cTnT_4$ expression in congenital heart disease patients with increased haemodynamic loading. In all atrial samples, $cTnT_3$ was the predominant isoform (6 days to 35 years), but there was an age-dependent increase in $cTnT_3$ expression as $cTnT_1$ expression decreased. Racca et al. (2016) also found that $cTnT_3$ was the predominant isoform in ventricular tissue samples, with increased protein density observed later in gestation (59–134 days). However, in samples collected postnatally (4 days to 11 years), Elhamine et al. (2016) did not find a significant difference in cTnT isoforms, suggesting that this age-dependent switch might occur around the time of birth. Additional work is needed to understand fully the dynamic shifts in myofilament, cytoskeletal and regulatory protein expression during postnatal development and the impact on myocardial stiffness and contractility. For example, Narolska et al. (2005) demonstrated that MHC isoform expression alters myocardial energetics and performance in adults with atrial fibrillation, whereas Bond et al. (2018) highlighted significant changes in the proteomic profile of the right ventricle in infants with cyanotic *versus* acyanotic heart disease. Specifically, acyanotic patients with a ventricular septal defect had increased ventricular stiffness and reduced expression of key sarcomeric proteins (e.g. tropomyosin, myomesin and obsecurin). Future studies are warranted to investigate differences in cardiomyocyte development and contractile function between healthy and diseased tissue.

Establishment of intercellular connections

The heart is composed of billions of individual cardiomyocytes, yet it functions as a syncytium, with synchronized contraction and relaxation. This coordination of electrical and mechanical activity is facilitated by specialized structures, called intercalated discs, which develop between neighbouring cardiomyocytes. Classically, the intercalated disc has been described to contain three main complexes: adherens junctions, desmosomes and gap junctions (Vermij et al., 2017). Desmosomes and adherens junctions are adhesion structures that tightly couple neighbouring cardiomyocytes. Desmosomes provide structural support through connections with intermediate filaments, whereas adherens junctions connect to the actin cytoskeleton and serve to anchor myofibrils. Gap junctions provide electrical continuity through intercellular connexon channels (composed of connexin proteins), which allow small molecules to flow freely between neighbouring myocytes. Nearly 200 different proteins have been found to localize to the intercalated disc (Estigoy et al., 2009), including the cardiac voltage-gated sodium channel (Nav1.5), which is responsible for the action potential upstroke and fast electrical propagation in atrial and ventricular cardiomyocytes.

Fully developed intercalated discs are absent at birth, but these specialized structures develop postnatally to maintain electromechanical function while cardiomyocytes undergo hypertrophy and remodelling. Across multiple species, mechanical junctions are established earlier in life than electrical junctions, although the timing varies by species (Hirschy et al., 2006; Vreeker et al., 2014). In a foundational study, Peters et al. (1994) monitored the postnatal development of intercalated discs in ventricular tissue specimens from paediatric patients (4 months to 15 years) (Table 3). In neonatal hearts, gap junction- (e.g. connexin-43) and adherens junction-specific proteins (e.g. N-cadherin) were punctuated indiscriminately throughout the myocyte surface until ~6 years of age, when these proteins co-localized and became confined to intercalated discs. This developmental time

frame is in line with other age-related adaptations, including the maturation of heart rate and ventricular mechanics (Bratincsák et al., 2020; Colan et al., 1992). Likewise, Vreeker et al. (2014) reported age-dependent changes in the spatiotemporal localization of intercalated disc proteins in ventricular specimens (15 weeks to 11 years). Proteins found in adherens junctions (e.g. N-cadherin and Zonula occludens-1 (ZO-1)) and desmosomes (e.g. plakoglobin, desmoplakin and plakophilin-2) were diffuse with lateral labelling in fetal samples, but with postnatal development, these proteins moved progressively to the myocyte termini, displaying a mature, adult phenotype by ~1–2.5 years after birth. Notably, adherens junction and desmosome proteins were co-localized throughout all stages of fetal and postnatal development. Sodium channels (Nav1.5) followed a similar spatiotemporal pattern, with prominent detection at the intercalated disc at ~2.5 years old. In the study by Vreeker et al. (2014), connexins were scarce during fetal development, then diffuse with lateral localization ~5 months after birth. Connexin proteins did not co-localize with adherens junctions at the intercalated disc until much later in life (~7–11 years). In agreement, Salameh et al. (2014) reported an age-dependent change in connexin-43 distribution, whereby the ratio of polar/lateral localization to intercalated discs increased in children from 2.9 (0–2 years) to 8.5 (>12 years). Immunostaining revealed that connexins were largely confined to the intercalated disc ~8.5 years old, which is consistent with the described time line produced by Vreeker et al. (2014). However, it is important to note that these developmental time lines are only an approximation, because many of these human studies have a relatively small sample size. Further study is warranted, because developmental differences and interactions between cardiomyocyte size, cell–cell coupling and ion channel expression can contribute to arrhythmias with age-dependent penetrance (Nowak, Poelzing et al., 2021).

It is important to note that human cardiomyocytes express multiple gap junction proteins (e.g. connexin-40, -43 and -45), each with distinct channel properties (e.g. voltage and chemical gating and permeability) that support cardiac electrophysiology in a chamber-related and age-dependent manner (Verheule & Kaese, 2013). In the adult heart, the faster-conducting bundle branches and Purkinje fibres have large gap junction plaques that contain a large quantity of connexin-43, -40 and -45, whereas slower-conducting nodal regions (sinus and atrioventricular) predominately express connexin-45 and -40 in lesser amounts (Chandler et al., 2009; Davis et al., 1995; Greener et al., 2011). All three major connexin isoforms are found in atrial and ventricular myocytes, although atria have a higher density of connexin-40, in comparison to connexin-43 in the ventricles (Davis et al., 1995; Greener et al., 2011; Vozzi et al., 1999). In the fetal heart (9 weeks of gestation), Coppen et al. (2003) detected connexin-40 and -43 most prominently in trabeculated ventricular tissue, with reduced expression in the compact myocardium. The authors also detected connexin-45 in developing conduction tissues, although this differs from a study by Chen et al. (1994), who reported that connexin-45 was inconspicuous in fetal hearts (74–122 days of gestation) but readily detected in cardiac tissue from older children (0.5 months to 3 years of age) and adults. In a study of patients with tetralogy of Fallot, Salameh et al. (2014) noted an age-dependent decline in connexin-43 protein expression between infants (0–2 years), older children (2–12 years) and those nearing adulthood (>12 years). This progressive decline in expression might be offset by dense connexin-43 localization to the intercalated discs in

older children, wherein gap junctions facilitate fast electrical conduction along the fibre axis in the longitudinal direction. Collectively, the expression and spatial distribution of connexin proteins modulates electrical coupling within different regions of the heart, which changes dynamically during postnatal heart development (Nowak, Veeraraghavan et al., 2021; Spach et al., 2000).

Adaptations in myocardial metabolism

The heart has a high rate of energy production and utilization, with ~70% of ATP used for contraction and ~30% for specialized ion pumps (Gibbs, 1978; Suga, 1990). Given the hypoxic intrauterine environment, fetal cardiomyocytes rely heavily on glycolysis for energy production. Animal studies have shown that the transition to the extrauterine environment brings about sudden changes in circulating nutrients, oxygen levels and haemodynamics, which reshape cardiomyocyte bioenergetics (Girard et al., 1992; Makinde et al., 1998). At the cellular level, metabolic maturation includes alterations in mitochondrial content, membrane transporters and enzymatic activity to support the postnatal transition of the myocytes from glycolytic to oxidative metabolism (Bartelds et al., 1998; Lopaschuk & Jaswal, 2010; Lopaschuk et al., 1991; Mdaki et al., 2016). Metabolic remodelling becomes necessary to support the increased demand on the developing heart, as fatty acid oxidation yields more ATP (per unit substrate) compared with glycolysis. The time frame and mechanisms underlying this metabolic switch are underexplored in humans, but in rodent models, studies suggest that this process is influenced by the increase in ambient oxygen at birth and the subsequent reduction in hypoxia signalling via oxygen-sensitive transcription factors [e.g. hypoxia-inducible factor-1α (HIF1α) and HAND1; Liu et al., 2021; Neary et al., 2014]. Chronic hypoxic conditions have been shown to dysregulate metabolic remodelling in rodents and impair heart development in sheep by reducing the total number of cardiomyocytes (Botting et al., 2014; Smith et al., 2022; Song et al., 2021). In humans, cyanotic congenital heart disease can disrupt postnatal maturation, because chronic hypoxia results in elevated HIF1α and persistent glucose-dominated cardiac metabolism in paediatric patients (Horikoshi et al., 2019; Liu et al., 2021; Piccoli et al., 2017; Porter et al., 2011; Quing et al., 2007). Collectively, cardiomyocyte maturation and metabolic remodelling are likely to be influenced by a number of factors, including changes in oxygen levels, hormone levels and metabolic stress associated with intermittent feeding (Lock et al., 2018). Species-specific differences should also be considered, because larger mammals can use alternative energy sources (e.g. fatty acids and lactate) *in utero* (Bartelds et al., 1999, 2000).

Dramatic changes in mitochondrial morphology are observed throughout cardiomyocyte maturation, as these small, fragmented organelles expand and grow into larger networks with an increased density of cristae (Feric & Radisic, 2016; Horikoshi et al., 2019; Porter et al., 2011) and myofibril alignment (Kim et al., 1994; García-Pérez et al., 2008; Piquereau et al., 2010; Table 4). Kim et al. (1992) reported that human cardiomyocyte mitochondria are circular, scarce and scattered throughout the sarcoplasm during early fetal development (17–24 weeks of gestation). With further maturation (≥30 weeks gestation), mitochondria become more elongated, increase in number and begin to aggregate between myofibrils. Fetal cardiomyocytes also contain large pools of glycogen (stored carbohydrates), which are used for glycolytic metabolism. This is in agreement with mammalian studies, wherein

glycogen occupies ~30% of the fetal myocyte cell volume and only ~2% of the adult cell volume (Shelley, 1961). As glycogen reserves become depleted, mitochondrial density increases to occupy ~22–25% of the human adult cardiomyocyte volume (Barth et al., 1992; Schaper et al., 1985). In an age comparison study, Marin-Garcia et al. (2000) reported a ~4-fold increase in mitochondrial DNA levels in human heart tissue between early gestation (45–65 days) and neonates (1–30 days). Furthermore, Pohjoismäki et al. (2013) reported a ~5-fold increase in mitochondrial DNA and ~2-fold increase in mitochondrial mass between fetal and adult cardiomyocytes. The impact of cyanotic heart conditions was highlighted in a study by Ye et al. (2020), wherein mitochondrial DNA was significantly less in tetralogy of Fallot patients with moderate *versus* mild hypoxia (75–85% *vs.* >85% arterial oxygen saturation).

Metabolic maturation is supported further by human developmental studies, which demonstrate age-dependent changes in membrane transporters, mitochondrial protein expression and enzymatic activity. With increased gestational age, human fetal cardiomyocytes gradually upregulate the expression of genes involved in fatty acid metabolism (Dai et al., 2017; Iruretagoyena et al., 2014). For example, Dai et al. (2017) reported a surge in mitochondrial activity between early (50–60 days) and late (100–115 days) gestation, with heightened respiratory complex activity (6.4-fold complex I, 3.6-fold complex II, 2.1-fold complex III and 3.1-fold complex IV). Respiratory complex activity was increased even further in adult tissue (3.4–11.3-fold) compared with early gestation. Marin-Garcia and colleagues also noted an age-dependent increase in key enzymes involved in the Krebs cycle and electron transport chain (Marin-Garcia & Baskin, 1989; Marin-Garcia et al., 2000). Between early gestation (45–65 days) and the neonatal period (1–30 days), a ~4-fold increase in citrate synthase, ~3-fold increase in complex IV and ~2-fold increase in ATP synthase was measured in ventricular tissue samples. ATP synthase was undetectable in cardiac tissue during early gestation, but increased ~3-fold between late gestation (85–110 days) and the neonatal period. Furthermore, cytochrome *c* oxidase content increased ~10-fold and oxidase activity ~8-fold across age groups (15–21 weeks of gestation *vs.* adults). Yatscoff et al. (2008) also reported an age-dependent (0.2–10 months) increase in proteins associated with fatty acid oxidation (e.g. malonyl CoA decarboxylase) and a decrease in inhibitory regulators of fatty acid oxidation (e.g. acetyl CoA carboxylase, 5′-AMP-activated protein kinase).

Collectively, these studies demonstrate an age-dependent shift in cardiac metabolism from glycolysis to fatty acid oxidation in humans, but the regulatory mechanisms and time line of this transition are unclear. Moreover, the impact of persistent hypoxia on this developmental process is understudied in humans. For example, metabolic maturation was impaired in patients with cyanotic heart conditions, because cardiac tissue samples had different metabolomic profiles (e.g. increased lactate and pyruvic acid, accumulation of Krebs cycle intermediates) compared with acyanotic myocardial samples (Dong et al., 2021). In a separate study, cyanotic patients also had higher myocardial lactate concentrations, and younger patients had a lower ATP/ADP ratio (Modi et al., 2004). Patient age and cyanosis are important factors affecting basal myocardial metabolism, which could also influence ischaemia–reperfusion injury and postoperative outcomes (Imura et al., 2001).

Age-dependent changes in electrophysiology

Transition from the intrauterine to extrauterine environment results in changes to blood circulation and myocardial maturation. These developmental adaptations are detected in the ECG, including changes in heart rate, the electrical axis, waveform morphology and ECG interval durations (Chan et al., 2008). During fetal development, blood is shunted away from the lungs, and the systemic circulation is driven by the right side of the heart. Accordingly, the electrical heart axis of the fetus is oriented to the right, owing to right ventricular dominance that is responsible for ~60% of cardiac output (Lempersz et al., 2021; Mielke & Benda, 2001). With birth, the placental circulation is halted, the ductus arteriosus closes, and venous return to the left atrium increases, culminating in an increase in left ventricular output (Agata et al., 1991). During infancy, the right ventricle remodels over 2–3 months, and the left ventricle increases in muscle mass, shifting the heart axis to the left (Jurko Jr, 2004). Normative data for paediatric ECG values are readily available, although ECG variables are heavily influenced by gestational age, sex and race (Bratincsák et al., 2020; Rijnbeek et al., 2001; Saarel et al., 2018; Schwartz et al., 2002; Sharieff & Rao, 2006). Broadly, postnatal age is associated with heart rate slowing from infancy (~150 beats min^{-1}) to adulthood (~75 beats min^{-1}), which is driven by changes in sinus node automaticity and increased vagal tone. Atrioventricular conduction is shorter in infants and children (~100 ms PR interval), probably owing to a smaller muscle mass, which lengthens during adolescence and adulthood (~150 ms). Likewise, ventricular depolarization (QRS complex) is also faster in infants and young children (~60 ms) compared with adults (~90 ms) (Bratincsák et al., 2020). The corrected QT interval, representing ventricular depolarization and repolarization, is longer in infants <6 months of age (upper limit of 490 ms) compared with other older age groups (upper limit 440 ms) (O'Connor et al., 2008).

Developmental changes in ECG variables are well documented (Bratincsák et al., 2020; O'Connor et al., 2008; Rijnbeek et al., 2001; Saarel et al., 2018; Schwartz et al., 2002; Sharieff & Rao, 2006), but the associated changes in action potential morphology and adaptations in cardiac ionic currents are vastly understudied in young (human) cells. Given that normal healthy myocardial tissue is unavailable, electrophysiological studies are performed using isolated cells from small pieces of atrial or ventricular tissue collected from patients undergoing palliative or corrective heart surgery. As such, patient age, disease and course of treatment are important confounding factors that can influence data interpretation. Although action potential recordings in neonatal paediatric human cardiomyocytes are limited (Escande et al., 1985; Cohen & Lederer, 1993; Schaffer et al., 1999; Wang et al., 2003; Table 5), a few distinguishing features have been noted; namely, young atrial or ventricular cardiomyocytes tend to have a more pronounced overshoot (peak amplitude at higher positive membrane potential), a less pronounced notch (early repolarization), and a plateau phase that persists at more positive membrane potentials, resulting in a longer action potential duration at 20–30% repolarization (Fig. 2).

Atrial and ventricular action potentials are characterized by a resting membrane potential that is stabilized by potassium currents, including I_{K1}, which helps to suppress automaticity (phase 4 of the action potential; for a review, see Bartos et al., 2015). Across different species (e.g. rodents, hPSC-CM), neonatal and immature cardiomyocytes frequently display

spontaneous activity that coincides with reduced I_{K1}, increased I_f (pacemaker current) and/or increased (reverse-mode) Na^+–Ca^{2+} exchanger (NCX) activity (Carmeliet, 2019; Cerbai et al., 1999; Giannetti et al., 2021; Goversen et al., 2018; Hoekstra et al., 2012; Kim et al., 2015; Masuda & Sperelakis, 1993; Vaidyanathan et al., 2016; Wahler, 1992; Wang et al., 2013). Experimental studies have reported similar resting membrane potentials, ranging from −71 to −85 mV in both paediatric and adult cardiomyocytes, isolated from either atrial or ventricular tissue (Cohen & Lederer, 1993; Escande et al., 1985; Schaffer et al., 1999; Wang et al., 2003). However, Schaffer et al. (1999) identified a barium-sensitive I_{K1} inward current in paediatric ventricular myocytes (16–91 months old), with a peak current density that was ~50% less (−22.8 pA pF^{-1}) than the reported value for adult ventricular myocytes (−45 pA pF^{-1}). This suggests an age-dependent difference in I_{K1} repolarizing current, which could alter the resting membrane potential and increase automaticity, akin to observations in immature hPSC-CM (Goversen et al., 2018; Hoekstra et al., 2012). In contrast, Crumb et al. (1995) found a comparable I_{K1} density in paediatric (−3.19 pA pF^{-1}, 1 day to 2.5 years) and adult atrial cardiomyocytes (−2.5 pA pF^{-1}, 11–68 years), with the caveat that these recordings at room temperature were significantly smaller in magnitude compared with the values reported by Schaffer et al. (1999) at physiological temperatures. Schaffer et al. (1999) also detected a slowly activating inward current in young ventricular myocytes that resembled the pacemaker current (I_f). In follow-up studies by the same group, Zorn-Pauly et al. (2003) detected this pacemaker current in 88% of paediatric right ventricular myocytes (mean age 15.4 months) but did not find an age-dependent difference in the current–voltage relationship or half-maximal activation, in comparison to adult left ventricular myocytes. Admittedly, the sample size for this study was small, and the I_f density in paediatric myocytes (−2.0 pA pF^{-1}) was slightly larger than previously reported for adult myocytes (−1.18 pA pF^{-1}; Hoppe et al., 1998). In experimental models, cardiomyocyte immaturity can alter I_f activation threshold, peak density and/or the expression of associated genes (Giannetti et al., 2021; Qu et al., 2001; Robinson et al., 1997), but additional studies are necessary to characterize postnatal developmental changes in human cardiomyocytes. Finally, spontaneous activity can be enhanced in immature cells through increased NCX expression and/or reverse-mode activity (Kim et al., 2015). NCX is localized to the cell periphery in neonatal human ventricular myocytes (preceding T-tubule formation), and NCX gene and/or protein expression is highest during the fetal–neonatal period (<2 weeks), declining thereafter with increasing postnatal age (Qu et al., 2000; Wiegerinck et al., 2009). In immature hPSC-CM, increased NCX activity enhances spontaneous activity (Kim et al., 2015), but the functional outcomes of NCX overexpression in human neonatal–infant cardiomyocytes have not been evaluated thoroughly.

Atrial and ventricular cardiomyocyte depolarization is driven by fast sodium current, I_{Na} (phase 0 of the action potential). Using isolated human atrial cardiomyocytes, Cai et al. (2011) measured a significantly smaller peak I_{Na} amplitude in paediatric cells (−31.8 pA pF^{-1}; mean age 3.4 years old) compared with adult cells (−46.7 pA pF^{-1}; mean age 42.5 years old). Furthermore, paediatric atrial myocytes had slower time-dependent properties (activation and inactivation time) and a shift in voltage-dependent inactivation towards a negative potential. A similar age-dependent shift in the voltage-dependence of I_{Na} inactivation was observed by Sakakibara et al. (1993) using paediatric and adult ventricular

myocytes. Counterintuitive to the reduced I_{Na} peak amplitude observation by Cai et al. (2011), Wang et al. (2003) reported a significantly faster maximal rate of depolarization in paediatric (462 V s^{-1}) *versus* adult (203 V s^{-1}) atrial myocytes. A faster rate of depolarization is generally an indicator of cardiomyocyte maturation, because slower rates are commonly observed in neonatal myocytes or immature hPSC-CM (Agata et al., 1993; Hoekstra et al., 2012; Pacioretty & Gilmour, 1995).

Following rapid depolarization, early repolarization (or 'notch') is driven primarily by the transient outward current, I_{to} (phase 1 of the action potential). In an influential study, Escande et al. (1985) documented developmental changes in the atrial action potential of paediatric cells (2–22 months of age), with an immature repolarization pattern that suggested a lack of I_{to}. Paediatric atrial cells were less responsive to an I_{to} blocker (4-aminopyridine), and the shape of the paediatric action potential changed to become more comparable to an adult action potential after prolonged periods of rest, suggesting that I_{to} might be present but non-functional at physiological stimulation rates. Furthermore, paediatric atrial action potentials were more triangular, never displayed a prominent 'notch', and had a shorter (sometimes unidentifiable) plateau phase that hovered near a positive potential.

Follow-up studies strongly suggest a developmental change in I_{to} in the human atria, but details are inconsistent. Mansourati & le Grand (1993) reported that I_{to} was completely absent in atrial cells isolated from children (2 months to 5 years of age), but tissue samples were described as 'clearly dilated and diseased', which was found to reduce I_{to} markedly in adult cells. Using young atrial cells from haemodynamically normal children (1 day to 2.5 years of age), Crumb et al. (1995) detected I_{to} in only 67% of paediatric samples (compared with 100% of adult cells). Moreover, in younger atrial cells, I_{to} recovery was twice as slow (recovery time constant: 294 ms paediatric *vs.* 138 ms adult), with significant frequency-dependent inhibition and a significantly smaller peak current density (8 pA pF^{-1} young *vs.* 12.4 pA pF^{-1} adult). Notably, the greatest age-related change in peak current density occurred between the ages of 10 months and 2 years, suggesting that developmental adaptations occur early in life, within a narrow time frame. Wang et al. (2003) noted a similar action potential morphology in infant atrial cells (2.5–10.5 months of age), including a less prominent notch, more positive plateau phase, and a longer action potential duration at 30% repolarization (28.6 ms infant *vs.* 4 ms adult). However, in disagreement with earlier reports, Wang et al. (2003) found an increase in I_{to} peak density in infant atrial cells, but also noted faster inactivation, a slower recovery from inactivation (32 ms infant *vs.* 17 ms adult) and a more significant frequency-dependent inhibition of I_{to} peak current at physiological rates (23.7% infant *vs.* 8.9% adult inhibition at 3 Hz). Slower I_{to} inactivation in adult atrial cells would lead to more current during the early phase of depolarization, which shortens repolarization time at APD_{30}. However, many of these findings were not observed in a study by Gross et al. (1994), in which the authors reported no discernible age-dependent differences in I_{to} density or recovery and only a slightly slower inactivation in younger atrial cells (59.3 ms, *vs.* 43.3 ms in older children). Measurements in paediatric ventricular cardiomyocytes are more limited. Cohen and Lederer (1993) reported that paediatric ventricular cardiomyocytes had a less prominent notch and a more positive plateau phase, consistent with many of the atrial myocyte findings described above. Paediatric action potentials also appeared significantly longer, at 30% or 50% repolarization, although average

values were not reported in the study. Schaffer et al. (1999) detected I_{to} in paediatric ventricular myocytes (16–91 months of age), but peak density measurements were variable even within the same patient, ranging from 0.3 to 8.6 pA pF^{-1}. Furthermore, the authors noted a rate-dependent reduction in I_{to} amplitude (11–65%) when ventricular myocytes were stimulated at 4 Hz, but with notable variations between individual cells.

As noted above, paediatric atrial and ventricular myocytes display a more positive plateau phase (phase 2 of the cardiac action potential), suggesting sustained Ca^{2+} influx and/or reduced K^+ efflux (Cohen & Lederer, 1993; Escande et al., 1985; Wang et al., 2003). Cohen and Lederer (1993) reported a significant reduction in I_{Ca} density in paediatric (5.3 mA cm^{-2}, 3–18 months of age) *versus* adult ventricular myocytes (10.1 mA cm^{-2}), but did not find a comparable developmental difference in atrial cells. However, both paediatric atrial and ventricular myocytes displayed a shift in steady-state activation and inactivation to more positive potentials(+5to+17mVshift),resulting in a prominent I_{Ca} window current. This is consistent with the adult action potential plateau phase occurring at more negative potentials, in comparison to paediatric cells. Pelzmann et al. (1998) also observed a large Ca^{2+} window current between −45 and +40 mV in paediatric ventricular myocytes, which can prolong Ca^{2+} influx and increase the incidence of early after-depolarizations. Notably, two studies that used tissue samples from patients with tetralogy of Fallot recorded significantly longer action potential duration (APD) at 90% repolarization with a high incidence of afterdepolarizations [Pelzmann et al. (1998): 853 ± 167 ms; Schaffer et al. (1999): 794 ± 99 ms] compared with a mixed population of congenital heart disease patients [Cohen and Lederer (1993): 292 ± 84 ms]. Accordingly, it is difficult to discern whether developmental changes in I_{Ca} might be exaggerated further with specific pathologies. Minimal experimental data are available on the presence and kinetics of repolarizing K^+ currents in phase 2 or 3 of the cardiac action potential. Schaffer et al. (1999) measured I_K in ventricular myocytes from children with tetralogy of Fallot, but detected this current in only 2 of 34 myocytes. The time course of activation was fast (within 250 ms), suggesting that the current was probably attributable to the rapid component of the delayed rectifier (I_{Kr}) (Koumi et al., 1995). Developmental changes in cardiac delayed rectifier K^+ current have been observed in other species (Obreztchikova et al., 2003; Wang et al., 1996), but postnatal adaptations remain underexplored in humans.

Maturation of excitation–contraction coupling

Excitation–contraction coupling is the process by which an action potential triggers contraction and subsequent relaxation. In adult ventricular cardiomyocytes, sarcolemmal L-type calcium channels (LTCCs) are localized to T-tubules in close proximity to RYRs on the junctional SR. These LTCCs open during phase 2 of the cardiac action potential, allowing Ca^{2+} ions to flow into the cardiomyocyte and increase local [Ca^{2+}], which triggers Ca^{2+} release from the SR in a process known as calcium-induced calcium release (CICR). This increase in intracellular [Ca^{2+}] saturates the troponin complex, resulting in a conformational change that permits actin–myosin interaction and contraction. Following contraction, cardiac relaxation requires a decline in intracellular [Ca^{2+}], largely through the SR calcium-ATPase (SERCA) and NCX. This process is tightly regulated, both temporally and spatially, permitting fast, coordinated cycling of Ca^{2+} across the large-sized adult cardiomyocyte.

Developing cardiomyocytes have contractile activity, but anatomical immaturity limits excitation–contraction coupling efficiency in these cells. This is attributable, in part, to structural adaptations that are still missing during early postnatal life, because T-tubules are underdeveloped in the newborn human heart (<3 weeks) but are well developed later in infancy (3–14 months) (Crossman et al., 2011; Kim et al., 1992; Lyon et al., 2009; Wiegerinck et al., 2009). The T-tubules are extensions of the sarcolemma that penetrate into the cardiomyocyte; these structures are protein rich (e.g. LTCC and NCX), and subsections of the T-tubule network participate in dyadic coupling with the SR, which facilitates the CICR process. In rodents, T-tubules and dyads are also absent at birth, but begin to form and mature early in life between postnatal days 10 and 20 (Chen et al., 2013; Ziman et al., 2010). Consequently, fetal–neonatal rodent cardiomyocytes have fewer calcium sparks and less SR Ca^{2+} content and exhibit smaller calcium transients, with a slower upstroke and decay time (Seki et al., 2003; Ziman et al., 2010). As the cellular organization matures, excitation–contraction coupling becomes more efficient, with less dependence on trans-sarcolemmal Ca^{2+} influx (via NCX and T-type calcium channels), increased SR Ca^{2+} content and greater reliance on SERCA reuptake, resulting in prominent calcium transients (Bassani & Bassani, 2002; Cohen & Lederer, 1988; Escobar et al., 2004; Reppel et al., 2007; Swift et al., 2020).

Measurements of calcium require the use of live cardiomyocytes, and as a result, these studies are extremely limited in fetal, neonatal or infant human cardiomyocytes. Using single cells isolated from myocardial specimens obtained intraoperatively, Cohen and Lederer (1993) reported that paediatric ventricular myocytes (3–18 months of age) had reduced I_{Ca} density and a prominent Ca^{2+} window, in comparison to adults (Table 6). Likewise, Pelzmann et al. (1998) reported a large window current in paediatric ventricular myocytes (16–91 months of age), which favoured the occurrence of delayed after-depolarizations. A comparable shift in I_{Ca} steady-state inactivation and a prominent window current have also been observed in infant *versus* adult atrial myocytes (Cohen & Lederer, 1993). However, I_{Ca} density might be similar (Cohen & Lederer, 1993; Roca et al., 1996) or only slightly reduced in infant *versus* adult atrial cells (Hatem et al., 1995; Tipparaju et al., 2004). Tipparaju et al. (2004) reported that infant atrial myocytes had lower basal L-type calcium current amplitude (1.2 pA pF^{-1} at room temperature; 3–10 months of age) compared with adults (2.6 pA pF^{-1}; >50 years of age). Additional work by Wagner et al. (2005) found that infant atrial myocytes (3–8 months old) had a shorter calcium transient amplitude when adult action potentials were applied as voltage-clamp waveforms, but the amplitude increased when infant action potentials were applied (with a longer early repolarization phase). This finding suggests that infant atrial cells are more dependent on trans-sarcolemmal Ca^{2+} influx for contraction, whereby a prolonged early repolarization phase helps to increase Ca^{2+} entry and intracellular Ca^{2+} levels (with perhaps, less dependence on CICR and SR Ca^{2+} release). Hatem et al. (1995) also reported age-dependent effects, whereby infant atrial cells (<1 month old) had a slower Ca^{2+} upstroke velocity compared with infant and adolescent cells (>1 month old), which is probably attributable to structural immaturity and inefficient coupling between LTCCs and RYRs. However, the authors did find evidence of CICR and SR Ca^{2+} release beginning at a young age (3 days to 4 years of age), because Ca^{2+} transient amplitudes in atrial myocytes were smaller when the SR Ca^{2+} load was depleted by caffeine or ryanodine.

Age-dependent differences in excitation–contraction coupling are influenced by the variable expression of key calcium-handling proteins during development. Given that I_{Ca} density is reduced and CICR is inefficient in immature mammalian myocytes, these cells have a greater reliance on NCX for modulating intracellular [Ca^{2+}] (Artman, 1992; Chin et al., 1997; Escobar et al., 2004). Age-dependent changes in NCX expression are also observed in the heart, wherein peak NCX expression is observed in the fetal heart, with reduced expression at birth or later in adulthood (Qu et al., 2000; Wiegerinck et al., 2009). The human heart also displays an age-dependent increase in LTCCs, SERCA and phospholamban expression and a decrease in T-type calcium channel expression at birth and later in adulthood, in comparison to early gestation (Qu & Boutjdir, 2001; Wiegerinck et al., 2009). It is unclear whether these developmental differences occur continuously after birth, because human tissue samples are scarce, and results are almost always compared with adult tissue. Accordingly, our knowledge of perinatal cardiomyocyte development (e.g. fetal, infant and adolescent) is extremely limited. One exception is a study by Wiegerinck et al. (2009), which found that newborn ventricular muscle strips (<2 weeks of age) had reduced phospholamban expression, reduced phospholamban:SERCA ratio and increased NCX expression compared with slightly older infants (3–14 months of age). In tandem, newborn muscle strips displayed a blunted force–frequency response, suggesting that the SR Ca^{2+} load was decreased, possibly through increased Ca^{2+} extrusion (via NCX) or reduced Ca^{2+} reuptake (via SERCA). However, a blunted force–frequency response might also be attributed to differences in troponin isoform expression and sensitivity (Bhavsar et al., 1991). Finally, the newborn myocardium was less responsive to adrenergic stimulation; immature atrial cells responded to isoprenaline application with increased I_{Ca} peak and Ca^{2+} transient amplitude, but relaxation was unchanged, which can be attributed to reduced troponin sensitivity, phospholamban activity and/or SERCA reuptake (Bhavsar et al., 1991; Hatem et al., 1995). Decreased sensitivity to isoprenaline stimulation has also been attributed to increased expression of inhibitory G proteins in infant atrial cells (Tipparaju et al., 2004), which is in agreement with age-dependent studies performed using rabbit neonatal and adult myocytes (Kumar et al., 1996). As noted in the electrophysiology section, slight discrepancies between studies might be attributable to differences in recording temperature, small sample size, variable age groups and/or underlying pathology. All the described studies were conducted using cells obtained intraoperatively from neonates or children with congenital heart defects. Similar studies using ‘normal’ cardiac tissue are not feasible.

Conclusion

Here, we have highlighted key foundation studies that have informed our understanding of human cardiomyocyte maturation during postnatal development. Based on existing knowledge, it is evident that human atrial and ventricular myocytes undergo significant age-dependent changes in size, structure, electrophysiology and contractile phenotype as they transition from the intrauterine to extrauterine environment. Collectively, these studies suggest that human myocytes evolve rapidly within the first few months to 1 year, but do not reach a fully mature adult phenotype until the first decade of life (e.g. polyploidy and intracellular coupling). However, several limitations should be considered. Notably, human developmental studies rely on the availability of tissue biospecimens that are

collected either post-mortem or as medical waste from patients undergoing palliative or corrective heart surgery. Biospecimens collected during surgery are dependent on clinical diagnosis; neonatal patients (<1 month of age) are likely to have more severe cyanotic heart conditions that require early intervention, whereas older infants or children are more likely to have acyanotic conditions. Furthermore, the type of tissue available will depend on the corrective surgery performed; atrial appendage tissue might be procured during cannulation for cardiopulmonary bypass, whereas ventricular tissue might be resected in select patient groups (e.g. tetralogy of Fallot, truncus arteriosus or patients receiving a ventricular assist device). As such, patient age, disease, course of treatment, medication use and the anatomical location of tissue samples are all confounding factors that can influence data interpretation. It is also important to note that most of the studies described in this review article estimate the timing of cardiomyocyte maturation based on a relatively small sample size, with a limited age distribution; therefore, temporal patterns are only a rough approximation. Experimental animal models and/or hPSC-CM can help to fill in knowledge gaps regarding postnatal maturation into adulthood, but this should be pursued with caution, because species-specific differences and/or the lack of maturity beyond a fetal/neonatal phenotype have been well documented.

Human cardiomyocyte maturation is a dynamic process that warrants further investigation for a variety of reasons. First, defining the normal developmental process can help us to understand better the cardiac diseases that develop during gestation or shortly after birth. Second, a number of cardiac medications have been designed for adults, with off-label use in neonates, infants and paediatric patients (Bates et al., 2012; Pasquali et al., 2008). A more thorough understanding of paediatric cardiomyocyte physiology can better inform clinical care decisions and the use of age-appropriate drug therapies for young patients. Third, human adult cardiomyocytes are considered non-proliferative, because cell-cycle arrest is observed within the first year of life. Identifying key regulators of cell-cycle arrest can identify new strategies to ‘restart’ cell proliferation, with a focus on regenerating and repairing injured muscle tissue (Patterson et al., 2017; Porrello et al., 2011; Puente et al., 2014; Velayutham et al., 2019). Finally, experimental animal or hPSC-CM models will continue to play a prominent role in cardiac research. By characterizing the temporal patterns of human cardiomyocyte maturation, we can optimize the use of these experimental models at developmentally appropriate stages and improve the applicability of our findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Biographies

Shatha Salameh is a PhD student in the Institute for Biomedical Sciences programme at The George Washington University School of Medicine and Health Sciences. She received her Master's degree in Pharmacology from Georgetown University. Her research is focused on human postnatal cardiac maturation.

Nikki Gillum Posnack is an associate professor at the Children's National Hospital, with faculty appointments in the Sheikh Zayed Institute for Pediatric Surgical Innovation and Children's National Heart Institute. She also maintains academic appointments at The George Washington University School of Medicine and Health Sciences in the Department of Pediatrics and the Department of Pharmacology and Physiology. Researchers in Dr Posnack's laboratory are studying the impact of age and disease on cardiac physiology.

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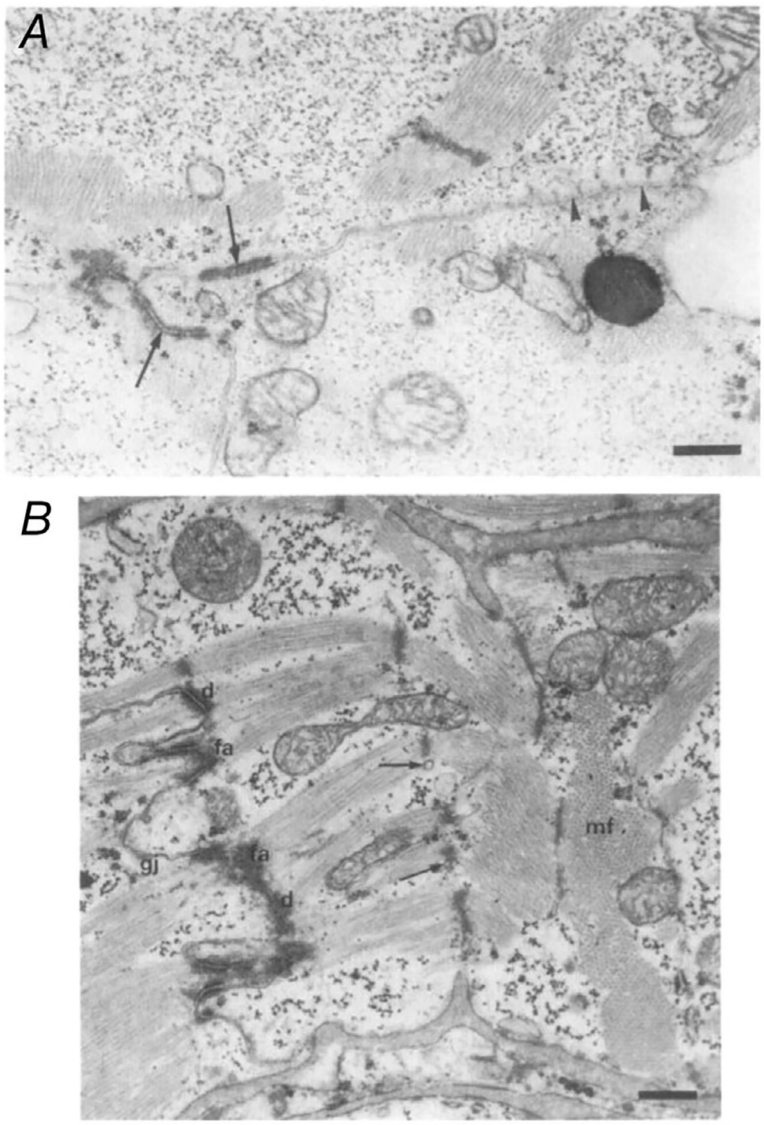

Figure 1. Electron microscopy of fetal hearts

A, fetal hearts at 20 weeks of gestation, with some desmosomes forming at intercalated disc (arrows). Note scattered myofibrils. *B*, fetal heart at 30 weeks of gestation, with more abundant desmosomes at the intercalated disc and more densely packed myofibrils. Arrows indicate sarcoplasmic reticulum tubules at the Z-line, which were not present in the earlier period. Abbreviations: d, desmosomes; fa, fascia adherens; gj, gap junctions; mf, myofibrils. Scale bars: 0.53 μM. Adapted with permission from Kim et al. (1992).

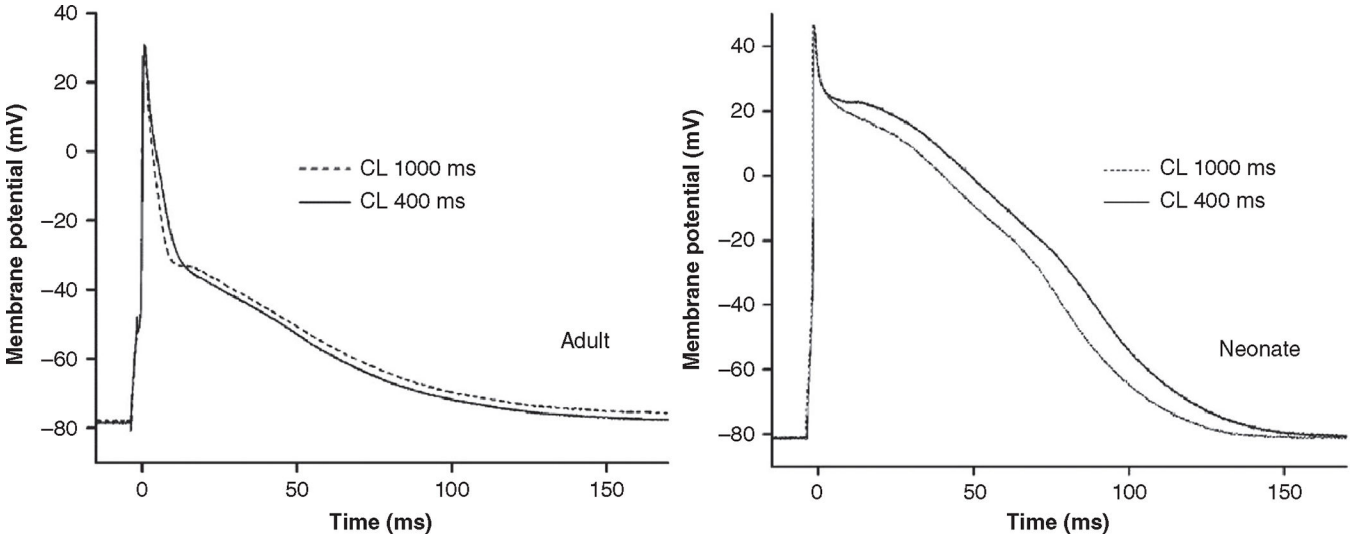

Figure 2. Action potential morphology differs between neonatal and adult human atrial cardiomyocytes

Action potential waveforms recorded at stimulation cycle lengths (CL) of 1000 and 400 ms. Adapted with permission from Wang et al. (2003).

Table 1.

Cardiomyocyte transition from hyperplasia to hypertrophy

Age range	Cardiomyocyte type	Key points	Citation
<0 weeks	Ventricular	**Hyperplasia contributes to increased heart size before birth** Total cell volume per cardiomyocyte nucleus remains consistent before birth	Mayhew et al. (1998)
≤16 weeks	Ventricular	**Hyperplasia ceases soon after birth** Number of cardiomyocyte nuclei increases throughout gestation	Mayhew et al. (1997)
≤1 year	Atrial	**Narrow window of postnatal proliferation** Postnatal cardiomyocyte proliferative potential is highest in patients <3 months old	Ye et al. (2016)
0–59 years	Left ventricular	**Hypertrophy begins after birth** Infants have highest rates of cardiomyocyte mitosis and cytokinesis. Cardiomyocyte volume is eight times larger in adults	Mollova et al. (2013)
0–73 years	Left and right ventricular	**Transition to hypertrophy occurs almost immediately** Total number of cardiomyocytes is established postnatally within 1 month. Volume of myocyte nuclei decreases, whereas hypertrophy increases from birth to adulthood	Bergmann et al. (2015)
0–75 years	Left ventricular	**One nucleus per cardiomyocyte** Cardiomyocytes remain mononucleated; nuclear content increases with age	Takamatsu et al. (1983)
0–90 years	Left ventricular	**DNA synthesis occurs with hypertrophy** Myocytes are diploid at birth, but increased polyploidy is observed with age	Eisenstein & Wied (1970)

Table 2.

Cardiomyocyte structural maturation

Age range	Cardiomyocyte type	Key points	Citation
<0 weeks	Left and right ventricular	**Structural development continues after birth** Increase in cell size, elongation and contractile force. Increase in myofibril organization, density and width.	Kim et al. (1992)
<2 years	Left and right ventricular	Increase in SR formation, T-tubule formation and Z-band alignment	Wiegerinck et al. (2009)
<0–67 years	Left ventricular		Racca et al. (2016)
<0–68 years	Left atrial, right and left ventricular	**Myosin heavy chain expression, kinetics change with age (tissue specific)**	Reiser et al. (2001)
<0–81 years	Left and right atrial, left and right ventricular	Decrease in α-MHC. Increase in β-MHC. Increase in myosin enzymatic activity	Cummins and Lambert (1986)
0–80 years	Left ventricular		Schier and Adelstein (1982)
<0–11 years	Right ventricular	**Myosin light chain isoform switching occurs during perinatal development**	Elhamine et al. (2016)
<0–52 years	Atrial and ventricular	Increase in VLC-2.	Cummins et al. (1980)
<0–71 years	Left and right atrial, left and right ventricular	Increase in VLC-1. Decrease in ALC-1	Price et al. (1980)
<0–81 years	Right ventricular		Auckland et al. (1986)
< 1 year	Atrial	**Myofilament isoform switching occurs during perinatal development**	Bhavsar et al. (1991)
<1 year	Atrial		Sasse et al. (1993)
<0–11 years	Right ventricular	Decrease in number of cTnT isoforms. Decrease in ssTnI and cTnT1.	Elhamine et al. (2016)
0–35 years	Right atrial	Increase in cTnI and cTnT3	Saba et al. (1996)
0–58 years	Left ventricular		Anderson et al. (1991, 1995)
0-adult	Ventricular		Hunkeler et al. (1991)

Table 3.

Maturation of intercellular connections

Age range	Cardiomyocyte type	Key points	Citation
<0 years	Right and left ventricle	**Intercalated discs begin to form *in utero*** Immature intercalated discs are identifiable by 20 weeks of gestation; sparse gap junctions are identifiable between neighbouring cells by 32 weeks of gestation	Kim et al. (1992)
<1–15 years	Right ventricle	**Intercalated discs develop with postnatal age** Punctuated connexin pattern in neonatal heart tissue shifts progressively to transverse terminals of the cardiomyocyte with age. Gap junctions and adherens junctions show a mature pattern by ~6–7 years of age	Peters et al. (1994)
<0 years	Atria, ventricles and conduction tissue	**Regional specificity of connexin proteins begins *in utero*** Fetal connexin-40, -43 and -45 expression varies across tissues	Coppen et al. (2003)
<0–11 years	Left ventricle	**Intercalated discs develop with postnatal age** Increased adherens junctions, desmosomes, gap junctions and sodium channel localization to the intercalated discs during paediatric development	Vreeker et al. (2014)
<0–38 years	Atria, left and right ventricle	**Gap junction proteins develop with age** Connexin content and distribution vary between fetal, paediatric and adult tissue	Chen et al. ((1994)
<0–66 years	Right ventricle	**Density and localization of gap junction proteins develop with age** Connexin-43 protein expression decreases, whereas localization to intercalated discs increases	Salameh et al. (2014)

Table 4.

Developmental changes in cardiomyocyte metabolism

Age range	Cardiomyocyte type	Key points	Citation
<0 years	Right and left ventricle	**Mitochondrial mass increases throughout life** Sparse, circular mitochondria in early fetal period; with variable shapes, increased size, increased mitochondrial density, aggregation near nuclei and myofibrils later in fetal development.	Kim et al. (1992)
<0–90 years	Left ventricle	Mitochondrial mass and DNA content are increased in adult cardiomyocytes	Pohjoismaki et al. (2013)
5–45 years	Left ventricle	**Correlation between mitochondrial density, heart rate and oxygen consumption** Mitochondrial density increases, occupying ~25% of adult cardiomyocyte volume	Schaper et al. (1985); Barth et al. (1992)
<0 years	Whole heart	**Metabolic genes upregulated throughout gestation** Genes involved in fatty acid metabolism (*FABP4*, *FABP2* and *RBP*) are upregulated between first and second trimester	Iruretagoyena et al. ((2014a)
<0–22 years	Ventricles	**Increased mitochondrial activity during perinatal period** Mitochondrial DNA content and mitochondrial enzymes (citrate synthase, complex IV, ATP synthase, cytochrome *c* oxidase) increase with gestational and postnatal age	Marin-Garcia et al. (1989); Marin-Garcia et al. (2000)
<1 year	Right ventricle	**Age-dependent maturation of fatty acid oxidation** Increase in proteins associated with fatty acid oxidation (malonyl coA decarboxylase); decrease in inhibitory regulators of fatty acid oxidation (acetyl CoA carboxylase, 5'-AMP-activated protein kinase)	Yatscoff et al. (2008)
<2 years	Left atria	**Cyanotic congenital heart disease disturbs metabolic maturation** Cyanotic heart samples have reduced protein synthesis and downregulation of aerobic energy metabolism, in comparison to acyanotic heart samples	Dong et al. (2021)
0–9 years	Right ventricle	**Age and disease impact metabolic maturation** Younger patients had lower ATP/ADP ratio. Cyanotic heart samples had higher lactate levels	Modi et al. (2004)

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Table 5.

Developmental changes in cardiomyocyte electrophysiology

Age range	Cardiomyocyte type	Key points	Citation
<2 years	Right ventricular	**Age-dependent difference in I_{K1}** I_{K1} density ~50% less than previously reported for adult cells	Schaffer et al. (1999)
<1–68 years	Right atrial	**No age-dependent difference in I_{K1}** I_{K1} density is comparable in paediatric and adult cells (room temperature)	Crumb et al. (1995)
<2 years	Right ventricular	**No age-dependent differences in I_f**	Schaffer et al. (1999);
<1–53 years	Right and left ventricular	I_f detected in ~90% of cardiomyocytes; I_f density only slightly higher in paediatric cells *versus* values previously reported for adult cells	Zorn-Pauly et al. (2003)
<3.4–42.5 years 2–63.5 years	Right atrial Ventricular	**Age-dependent difference in I_{Na}** I_{Na} density is lower, with slower activation/inactivation; negative shift in voltage-dependent inactivation in paediatric *versus* adult cells	Cai et al. (2011); Sakakibara et al. (1993)
<1–74 years	Right atrial	**Age-dependent difference in I_{Na}** Faster depolarization in paediatric *versus* adult cells, suggests enhanced I_{Na} current	Wang et al. (2003)
<1–54 years	Right atrial	**Age-dependent difference in transient outward current (I_{to})** Paediatric action potential has a smaller notch and more positive action potential plateau phase; suggests age-dependent increase in I_{to}	Escande et al. (1985)
<1–57 years	Right atrial	**I_{to} is absent in children** I_{to} is absent in cardiomyocytes from children aged 2–5 years. Samples were all dilated, which could also reduce I_{to}	Mansourati and Le Grand (1993)
<1–68 years	Right atrial	**Age-dependent difference in I_{to}** I_{to} is detected in 67% of paediatric cells and 100% of adult cells. Reduced I_{to} density, slower recovery time and frequency-dependent inhibition in paediatric cells *versus* adult cells	Crumb et al. (1995)
<1–74 years	Right atrial	**I_{to} is present in neonatal cells, but properties are age dependent** Greater I_{to} density, faster inactivation, slower recovery from inactivation, and frequency-dependent inhibition in neonatal *versus* adult cells; neonatal action potential has a less prominent notch, a more positive plateau phase and longer action potential duration at 30% compared with adult cells	Wang et al. (2003)
<1–13 years	Right atrial	**Similar I_{to} properties across different age groups** No difference in I_{to} density or recovery, but slightly slower inactivation in younger cells	Gross et al. (1994)
<2 years	Right ventricular	**Frequency-dependent inhibition of I_{to} amplitude in young cells** No observed age difference in I_{to} density, but measurements were variable even within same patient	Schaffer et al. (1999)
<1–75 years	Right atrial and ventricular	**Age-dependent difference in action potential morphology** Paediatric atrial myocytes have a prominent overshoot and longer action potential duration at 30–50% compared with adult cells. Paediatric ventricular myocytes have a less prominent notch, more positive plateau phase and longer action potential duration compared with adult cells	Cohen and Lederer (1993)

Age range	Cardiomyocyte type	Key points	Citation
<1–75 years	Right atrial and ventricular	**Age-dependent difference in I_{Ca}** Reduced I_{Ca} density in paediatric *versus* adult ventricular myocytes (not atrial). Paediatric atrial and ventricular myocytes have a positive shift in steady-state activation and inactivation, resulting in a prominent calcium window current	Cohen and Lederer (1993)
1–8 years	Right ventricular	**Prominent calcium window current in paediatric cells** Window current might contribute to longer action potentials and after-depolarizations in paediatric cells. Results might also be linked to the disease state of paediatric cells	Pelzmann et al. (1998)

Table 6.

Developmental changes in excitation-contraction coupling

Age range	Cardiomyocyte type	Key points	Citation
<1–75 years	Right ventricular	**Age-dependent increase in I_{Ca} density**	Cohen and Lederer (1993)
<1–75 years 1–8 years	Right atrial and ventricular Right ventricular	**Age-dependent decrease in calcium window current**	Cohen and Lederer (1993); Pelzmann et al. (1998)
<1–79 years	Right atria	**Age-dependent increase in I_{Ca} amplitude** Lower basal L-type calcium current amplitude and increased expression of inhibitory G proteins is seen in infant atrial cells, compared with adults	Tipparaju et al. (2004)
<1–79 years	Right atria	**No age-dependent difference in I_{Ca}** No evidence for age-related changes in I_{Ca} density, steady-state inactivation or recovery from inactivation	Roca et al. (1996)
<2 years <0–40 years	Left and right ventricle Ventricular	**Age-dependent increase in calcium-handling proteins** Age-dependent increase in NCX, SERCA, phospholamban and L-type calcium channel; age-dependent decrease in T-type calcium channel expression	Wiegerinck et al. (2009); Qu et al. (2000)
<1–4 years	Right atria	**Excitation-contraction coupling matures with postnatal age** Slower Ca^{2+} upstroke velocity in infant cells is likely to be attributable to structural immaturity and inefficient coupling between calcium channels and ryanodine receptors	Hatem et al. (1995)
<1 year	Right atria	**Paediatric action potential waveform modulates excitation-contraction coupling** Prolonged early repolarization in paediatric cells is correlated with increased dependence on trans-sarcolemmal Ca^{2+} influx for contraction	Wagner et al. (2005)
<2 years	Left and right ventricle	**Age-dependent increase in force-frequency relationship** At faster frequencies, newborn ventricular muscle strips have less developed force compared with slightly older infant muscle strips; results are likely to be linked to developmental changes in protein expression	Wiegerinck et al. (2009)