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

Shatha Salameh, Vanessa Ogueri, and Nikki Gillum Posnack
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The following individual(s) involved in review of this submission have agreed to reveal their identity: Mitchell C Lock (Referee #2)

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Dear Dr Posnack,

Re: JP-TR-2022-283792 "Adapting to a new environment: postnatal maturation of the human cardiomyocyte" by Shatha Salameh, Vanessa Ogueri, and Nikki Gillum Posnack

Thank you for submitting your manuscript to The Journal of Physiology. It has been assessed by a Reviewing Editor and by 2 expert referees and we are pleased to tell you that it is acceptable for publication following satisfactory revision.

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We look forward to receiving your revised submission.

If you have any queries, please reply to this email and we will be pleased to advise.

Yours sincerely,

Professor Laura Bennet
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EDITOR COMMENTS

Reviewing Editor:

This is a comprehensive review of what is known about cardiomyocyte maturation in humans. As it is a large review, some references are missing and should be added. Clarity of some sections could be improved. Overall, a great effort.

It would be helpful to include a summary that clearly indicates if data is from atrial or ventricular cells, right or left. A summary figure for all developmental processes or several summary figures would also be useful to help the reader integrate what is known and what remains controversial due to several different findings.

Line 63 - Please explain what differences hinder translation.

There are errors in the text in the blue box. Is this an abstract? It is not standard to have such boxes in review in JP. Could this information be integrated into the main body of text.

Line 103 - Does study population mean different species? If yes, please cite papers that used other species(Burrell et al., 2003; Jonker et al., 2006; Jonker et al., 2007; Jonker et al., 2010; Botting et al., 2012; Jonker et al., 2015; Jonker & Louey, 2016; Jonker et al., 2018a; Jonker et al., 2018b).

Line 120 - Repair has been shown in other studies and in other species(Soonpaa & Field, 1997; Porrello et al., 2011; Porrello et al., 2013; Zgheib et al., 2014).

Line 160 - This sentence refers to rodents but the Bensley paper looks at sheep. Please expand the sentence to indicate the differences between rodents and sheep and include a greater variety of references(Li et al., 1996; Soonpaa et al., 1996; Burrell et al., 2003; Jonker et al., 2006; Jonker et al., 2010; Jonker et al., 2015).

Line 389 - The previous paragraph ends by saying that an increase in oxygen causes metabolic changes. This is shown in mice. This paragraph talks about changes in the human fetal heart. When oxygen levels are low. Thus, is it appropriate to argue that an increase in oxygen is the underlying cause? Please see (Lock et al., 2018).

Line 411 and 420 - Please be clear about what tissue source is being reported. Are these human cells are IPSCs?

Line 455 - ...compared to other....

Line 476 - Please indicate the species studied.

Line 482 - Are the age-dependent effects really species dependent effects?

Line 498 - Reference formatting needs correcting.

Line 503 - Please be clear about the tissue sources. ISPCs or species?

Line 631 - compared to when?

Line 634-35 - Please explain. What is the baseline to which the difference is compared?

Line 650 - ... fetal and postnatal development.....

Line 674 - The sample sizes are small but the ages studied are also limited. This should be indicated.

Invited reviews generally have a graphical abstract. This could be helpful.

JP guidelines indicate the Additional Information that should be included before the references section. Please update.

Botting KJ, Wang KC, Padhee M, McMillen IC, Summers-Pearce B, Rattanatray L, Cutri N, Posterino GS, Brooks DA & Morrison JL. (2012). Early origins of heart disease: low birth weight and determinants of cardiomyocyte endowment. Clin Exp Pharmacol Physiol 39, 814-823.

Burrell JH, Boyn AM, Kumarasamy V, Hsieh A, Head SI & Lumbers ER. (2003). Growth and maturation of cardiac myocytes in fetal sheep in the second half of gestation. Anat Rec 274A, 952-961.

Jonker SS, Faber JJ, Anderson DF, Thornburg KL, Louey S & Giraud GD. (2006). Sequential growth of fetal sheep cardiac myocytes in response to simultaneous arterial and venous hypertension. Am J Physiol Regul Integr Comp Physiol.

Jonker SS, Giraud MK, Giraud GD, Chattergoon NN, Louey S, Davis LE, Faber JJ & Thornburg KL. (2010). Cardiomyocyte enlargement, proliferation and maturation during chronic fetal anaemia in sheep. Exp Physiol 95, 131-139.

Jonker SS, Kamna D, LoTurco D, Kailey J & Brown LD. (2018a). IUGR impairs cardiomyocyte growth and maturation in fetal sheep. J Endocrinol 239, 253-265.

Jonker SS & Louey S. (2016). Endocrine and other physiologic modulators of perinatal cardiomyocyte endowment. J Endocrinol 228, R1-18.

Jonker SS, Louey S, Giraud GD, Thornburg KL & Faber JJ. (2015). Timing of cardiomyocyte growth, maturation, and attrition in perinatal sheep. Faseb j 29, 4346-4357.

Jonker SS, Louey S & Roselli CE. (2018b). Cardiac myocyte proliferation and maturation near term is inhibited by early gestation maternal testosterone exposure. Am J Physiol Heart Circ Physiol 315, H1393-h1401.

Jonker SS, Zhang L, Louey S, Giraud GD, Thornburg KL & Faber JJ. (2007). Myocyte Enlargement, Differentiation, And Proliferation Kinetics In the Fetal Sheep Heart. J Appl Physiol 102, 1130-1142.

Li F, Wang X, Capasso JM & Gerdes AM. (1996). Rapid transition of cardiac myocytes from hyperplasia to hypertrophy during postnatal development. J Mol Cell Cardiol 28, 1737-1746.

Lock MC, Tellam RL, Botting KJ, Wang KCW, Selvanayagam JB, Brooks DA, Seed M & Morrison JL. (2018). The role of miRNA regulation in fetal cardiomyocytes, cardiac maturation and the risk of heart disease in adults. J Physiol.

Porrello ER, Mahmoud AI, Simpson E, Hill JA, Richardson JA, Olson EN & Sadek HA. (2011). Transient regenerative potential of the neonatal mouse heart. Science 331, 1078-1080.

Porrello ER, Mahmoud AI, Simpson E, Johnson BA, Grinsfelder D, Canseco D, Mammen PP, Rothermel BA, Olson EN & Sadek HA. (2013). Regulation of neonatal and adult mammalian heart regeneration by the miR-15 family. Proc Natl Acad Sci USA 110, 187-192.

Soonpaa MH & Field LJ. (1997). Assessment of cardiomyocyte DNA synthesis in normal and injured adult mouse hearts. Am J Physiol 272, H220-226.

Soonpaa MH, Kim KK, Pajak L, Franklin M & Field LJ. (1996). Cardiomyocyte DNA synthesis and binucleation during murine development. Am J Physiol 271, H2183-2189.

Zgheib C, Allukian MW, Xu J, Morris MW, Jr., Caskey RC, Herdrich BJ, Hu J, Gorman JH, 3rd, Gorman RC & Liechty KW. (2014). Mammalian fetal cardiac regeneration after myocardial infarction is associated with differential gene expression compared with the adult. The Annals of thoracic surgery 97, 1643-1650.

REFEREE COMMENTS

Referee #1:

Regulation of perinatal cardiac growth and maturation has been of scientific and medical interest for a long time, as demonstrated by a long publication history in the field. The basic biological mechanisms involved in creating a mature, functional heart are fascinating, as are interspecies differences affecting the form, function, and regenerative potential of the heart. But the area is also one of profound clinical importance. New surgical approaches have enabled infants with profound congenital defects incompatible with a mature circulation to live beyond parturition. However, medical advances to support or therapeutically regulate the maturing myocardium lag, primarily due to challenges that impede discovery in human fetuses and infants. The observations that have been made in humans create a fragmented and sometimes contradictory picture, due both to those populations from which samples can be drawn (usually those with disease, or who have died), as well as methodological limitations. What this manuscript contributes is a synthesis of the history of research in the perinatal human heart, as well as an up-to-date perspective on gaps in the field that demand attention. The authors are to be commended for this effort.

Throughout, studies using atrial cardiomyocytes are presented alongside studies using ventricular cardiomyocytes (or even right versus left ventricular cells). It is important to remember the differences in the relative roles of the different cardiac chambers. Mature cardiomyocytes from these different locations differ from one another in their number and size, their structure, their electrophysiological properties, and in excitation-contraction coupling (e.g. Growth and Hyperplasia of Cardiac Muscle Cells. Soviet Medical Reviews. Supplement Series. Cardiology: Vol.3. Chapter 6. Rumyantsev P.). Consequently, it is important for the reader to remember that their maturational trajectories will also differ. An initial reminder of these differences may aid the reader.

The purpose of this review is to present the state of the field in perinatal human cardiomyocyte maturation, not interspecies comparisons. Nevertheless, research in other animals has provided the foundational knowledge which serves as the encyclopedic reference on we rely for our interpretation of the human studies. We know about many basic physiological mechanisms, some different from the human, from studies in species as diverse as the fruit fly, the zebrafish and salmonids, and the newt. Our understanding of genetics and signaling pathways has been greatly aided by studies in mice. The animals with the most similar cardiovascular systems to humans, however, are generally those of a similar size (West GB, Brown JH, Enquist BJ. A general model for the origin of allometric scaling laws in biology. Science. 1997 Apr

4;276(5309):122-6.). Dogs and pigs have been very important for adult translational studies, while sheep are the cardiovascular developmental gold standard model in which critical discoveries and advances have been made (Rudolph AM. Congenital Diseases of the Heart: Clinical-Physiological Considerations, Third Edition. Wiley-Blackwell, Chichester, UK, 2009). Consequently, where animal data must be used in this manuscript to buttress or approximate understanding of the human condition, it would be best to draw on data, if it exists, from large mammals. Further, as the unique value of this manuscript is its focus on the human data, it should be clear where statements are supported only by animal data (e.g. 377-380).

There are a few instances in which description of biological mechanisms is confused, or additional citations add differing perspective:

• Line ~92 text box: Regarding the description of how fetal blood becomes oxygenated - maternal blood does not ever mix with fetal blood.

• 101-102: Please include discussion of evidence to the contrary, for instance appearance of binucleate myocytes in gestation (Kim et al., 1992), the decline in cell cycle activity (Huttenbach Y, Ostrowski ML, Thaller D, and Kim HS. Cell proliferation in the growing human heart: MIB-1 immunostaining in preterm and term infants at autopsy. Cardiovasc. Pathol. 2001. 10, 119-123) and other stereological estimates of cell number (Mandarim-de-Lacerda CA, das Santos MB, Le Floch-Prigent P, Narcy F. Stereology of the myocardium in human foetuses. Early Hum Dev. 1997 May 28;48(3):249-59.).

• 106-112: Although the possibility of DNA replication without subsequent karyokinesis is discussed later (144), it is essential in this discussion of proliferation to avoid conflation of cell cycle activity with an outcome of successful division into daughter cells. Alternative outcomes to cell cycle activity include replication of DNA both with karyokinesis (leading to formation of multi-nucleated cardiomyocytes), or without karyokinesis (leading to multiple chromosome number within one nucleus). In fact, cytological features of cytokinesis such as a cleavage furrow may appear in the process of binucleation (e.g. Li, F. , Wang, X., Bunger, P., Gerdes, A. M. Formation of binucleated cardiac myocytes in rat heart: I. Role of actin-myosin contractile ring. J Mol Cell Cardiol. 1997 Jun;29(6):1541-51.)

• 129: What is meant by "dedifferentiation" and is this a helpful biological insight? Some of the cited studies define "dedifferentiation" as activation of cell cycle regulators. Surely it is not surprising that proliferating cells have activation of cell cycle regulators. Please expand or clarify.

• 146-151: In contrast, Adler finds more cardiomyocyte nuclei are tetraploid than diploid after age 8 (Adler CP, Costabel U. Cell number in human heart in atrophy, hypertrophy, and under the influence of cytostatics. Recent Adv Stud Cardiac Struct Metab. 1975;6:343-55.)

• 158-162: Although elsewhere issues of allosteric scaling are briefly mentioned as an important difference in interspecies comparisons, the impact of this consideration is largely inappropriately ignored where animal data is brought in to support or add to the human data. Here swine and rodents are mentioned, which are both born in large altricial litters with birthweights much lower than humans. Sheep typically have 1-2 offspring which are of more similar size to humans at birth (Burrell JH, Boyn AM, Kumarasamy V, Hsieh A, Head SI, Lumbers ER. Growth and maturation of cardiac myocytes in fetal sheep in the second half of gestation. Anat Rec A Discov Mol Cell Evol Biol. 2003 Oct;274(2):952-61.; Jonker SS, Louey S, Giraud GD, Thornburg KL, Faber JJ. Timing of cardiomyocyte growth, maturation, and attrition in perinatal sheep. FASEB J. 2015 Oct;29(10):4346-57.).

• 166-167: Not entirely unknown. This is addressed in some of Adler's papers, as well as Brodsky (Adler CP, Costabel U. Myocardial DNA and cell number under the influence of cytostatics. I. Post mortem investigations of human hearts. Virchows Arch B Cell Pathol Incl Mol Pathol. 1980;32(2):109-25; Brodsky VYa, Sarkisov DS, Arefyeva AM, Panova NW, Gvasava IG. Polyploidy in cardiac myocytes of normal and hypertrophic human hearts; range of values. Virchows Arch. 1994;424(4):429-35.)

• 173-174: It seems that the de Simone paper has been misinterpreted. Stroke work increases between 4- to 10-fold fold, not by 65%, between birth and adulthood (Fig 3). Unfortunately, in this paper stroke volume is reported as an average of all children. Their mean age was about 10 and body weight was about 36kg (Table 1), which is much greater than a normal birth weight of 3.3kg. Consequently, a conclusion cannot be made from this paper about change in stroke volume between birth and adulthood. The same authors did previously publish a paper looking more carefully at stroke volume from birth to adulthood (de Simone G, Devereux RB, Daniels SR, Mureddu G, Roman MJ, Kimball TR, Greco R, Witt S, Contaldo F. Stroke volume and cardiac output in normotensive children and adults. Assessment of relations with body size and impact of overweight. Circulation. 1997 Apr 1;95(7):1837-43.). If the shortest individuals in the study presumed to be the newborns, stroke volume increases about 6-12 fold between birth and adulthood (Fig 1). It is unclear why the Gilbert paper is cited.

• 229-232: The Reiser reference in not in the reference list. The summarization of this paper is confusing. a-MHC was the primary isoform found in the fetal atria. This did not change with gestational age, and a-MHC continued to predominate in the adult atria. B-MHC predominated in the fetal ventricles. The small amount of a-MHC in the fetal ventricles may decline with advancing gestational age (the author notes that the results are heavily influenced by two outlier samples).

• 237-240: It is worth noting that this is likely due to the difference in heart rate between those two species, and that in other large mammals (e.g. sheep) the MHC isoforms are similar to humans (Hodges MM, Zgheib C, Liechty KW. A Large Mammalian Model of Myocardial Regeneration After Myocardial Infarction in Fetal Sheep. Adv Wound Care (New Rochelle). 2021 Apr;10(4):174-190.).

• 336-338: What is the purpose of noting the finding in humans is different to the mouse, does it cast doubt on the finding in the human?

• 374: Glucose and lactate oxidation is by definition not anaerobic.

• 382-385: Care should be taken extrapolating mechanisms and timing of maturational events between the rodent and human due to the persistence in the rodent of an immature state after birth. Note also that the heart of the large mammalian fetus can take up and oxidize fatty acids (Bartelds B, Knoester H, Smid GB, Takens J, Visser GH, Penninga L, van der Leij FR, Beaufort-Krol GC, Zijlstra WG, Heymans HS, Kuipers JR. Perinatal changes in myocardial metabolism in lambs. Circulation. 2000 Aug 22;102(8):926-31.), and that the human is unique among placental mammals in the high level of placental transfer of fat to the fetus. It is, of course, unknown the extent to which the human fetal heart metabolizes the available fatty acids.

• 444-445: "right ventricular hypertrophy regresses over 2-3 months" Remodels would be more accurate than regresses, as the RV mass is in fact increasing in this period (but changing shape and decreasing relative to the LV or total heart).

• 592: They are not absent (lines 200-202).

• 596-599: It's not clear what the point is, there's variation across species (Growth of the heart in health and disease ed. Zak R. Raven Press, New York. 1984; etc).

• Other references for studies in humans that you may consider for their contribution to understanding in this topic include: Ball AJ, Levine F. Telomere-independent cellular senescence in human fetal cardiomyocytes. Aging Cell. 2005 Feb;4(1):21-30; Adler CP. Relationship between deoxyribonucleic acid content and nucleoli in human heart muscle cells and estimation of cell number during cardiac growth and hyperfunction. Recent Adv Stud Cardiac Struct Metab. 1975;8:373-86; Austin A, Fagan DG, Mayhew TM. A stereological method for estimating the total number of ventricular myocyte nuclei in fetal and postnatal hearts. J Anat. 1995 Dec;187 (Pt 3)(Pt 3):641-7.

Errata:

• Line ~92 text box: an extraneous "and" at the end of the sentence ending on the fifth line.

• 96-98: Please provide references for this statement.

• 96, 942, etc: Capitalize "St".

• 102: Sentence beginning "Although" is a fragment.

• 156-157: "Similar to humans" should be "Similar to human cardiomyocytes"?

• 175-176: >90% increase in mass in what period? Human newborn heart weights are typically less than 20g, normal adult heart weights are ~280-350g, which is much more than a doubling in heart weight.

• Figure 1. Kindly explain what is a z-tubule.

• 297-298: Sentence fragment.

• 376: What is meant by "increased blood volume" at birth?

• 435: Blue box: "observed ~6 months of life" should be "observed at ~6 months of life"?

• 436: What does "reorganization of the cardiovascular system" mean?

• 489-499: Missing close parens on citation?

• 961: Capitalization

Referee #2:

The narrative review titled "Adapting to a new environment: postnatal maturation of the human cardiomyocyte" by Salameh and colleagues aimed to examine the developmental maturation of the human heart including; structure, electrophysiology, metabolomic and contractile function. The review has a focus on studies from human tissue samples rather than animal models of cardiac development (though there are some helpful comparisons where necessary). The focus being on human studies grants a unique perspective and allows for a detailed exploration of the field due to the limited number of publications from human tissue samples. The review highlights this as a major limitation, with a strong conclusion section indicating that a thorough understanding of pediatric cardiomyocyte physiology can better inform clinical care as well as fill knowledge gaps regarding cardiomyocyte quiescence. I especially enjoyed the electrophysiology and excitation-contraction coupling sections that were comprehensive and informative, though I wonder if the review could benefit from the addition of a figure showing the generic components of excitation-contraction coupling (t-tubule with LTCC next to SR etc) to assist readers that aren't familiar with this area. The review could also potentially benefit from a summary diagram or table indicating the relative difference in expression of proteins examined throughout the paper.

Though the review is overall well written, there are a number of grammatical errors throughout the first section specifically that require attention (I have listed many of them in the minor comments section).

Line 100 - Though there is limited evidence in humans as the reviewers have indicated, there is some evidence to suggest that in large animals (for example sheep) the transition from proliferative growth to hypertrophic growth actually begins before birth (starts at 110 days - term = 150d). For references please see this review: https://doi.org/10.1152/ajpregu.00391.2017

Line 165-170 - This reviewer recognises that the review is centred around human studies, however, there are a number of studies in sheep that have looked at cardiomyocyte endowment in models of growth restriction and changes in the intrauterine environment that seem to have been overlooked. https://pubmed.ncbi.nlm.nih.gov/25085511/ for example.

Some additional suggested triggers for cardiomyocyte quiescence in rodent studies are also thyroid hormone (see Naqvi et al., 2014) and the transition from low PO2 to the postnatal oxygen rich environment, mitochondrial stress and ROS production (Puente et al., 2014). Though it is likely a combination of a number of factors rather than a single trigger.

Line 426 - Though not a study in humans, there is some evidence for the effects of developmental hypoxia on fetal cardiac mitochondrial function. See: https://doi.org/10.1111/jpi.12821

https://doi.org/10.1152/physiol.00022.2022

Minor corrections:

Line 49 - add a space between end of sentence and references.

Line 79 - missing period.

Line 90 - first box: I suggest rephrasing some of these sentences as the language here does not match the rest of the

review. There also seems to be a number of missing words and missing periods in here. Eg. "to support the metabolic needs of the growing fetus" "which is a tightly regulated process that has been well characterized".

Fetal hemodynamics are also slightly oversimplified in this box, but the full details may not be required for this review.

Line 104 - missing period

Line 162 - missing period

Line 196 & 198 - the units here are wrong. This reads as millimolar (a unit of concentration) where is should be m (micro meter).

Line 291 - Ehlamine et al., did not find...

Line 387 - There is something wrong with the references in this sentence.

Line 417, 418 - ATP synthase is the same as Complex V. Please be consistent with the naming.

Line 435 box - compared with healthy controls

Line 499 - Again there seems to be an error in the citation here.

REQUIRED ITEMS:

-Please include an Abstract Figure file, as well as the figure legend text within the main article file. The Abstract Figure is a piece of artwork designed to give readers an immediate understanding of the Review Article and should summarise the main conclusions. If possible, the image should be easily 'readable' from left to right or top to bottom. It should show the physiological relevance of the Review so readers can assess the importance and content of the article. Abstract Figures should not merely recapitulate other figures in the Review. Please try to keep the diagram as simple as possible and without superfluous information that may distract from the main conclusion of the Review. Abstract Figures must be provided by authors no later than the revised manuscript stage and should be uploaded as a separate file during online submission labelled as File Type 'Abstract Figure'. Please ensure that you include the figure legend in the main article file. All Abstract Figures will be sent to a professional illustrator for redrawing and you may be asked to approve the redrawn figure before your paper is accepted.

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considered to be good or compelling reasons to protect access to the data, for example commercial confidentiality or legitimate sensitivities around data derived from potentially identifiable human participants, these should be included in the statement.

Acknowledgements: Acknowledgements should be the minimum consistent with courtesy. The wording of acknowledgements of scientific assistance or advice must have been seen and approved by the persons concerned. This section should not include details of funding.

-Please upload separate high quality figure files via the submission form.

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END OF COMMENTS

Confidential Review **15-Nov-2022**

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Line 162 – missing period
Line 196 & 198 – the units here are wrong. This reads as millimolar (a unit of concentration) where is should be µm (micro meter).

Line 291 – Ehlamine et al., did not find…
Line 387 – There is something wrong with the references in this sentence.
Line 417, 418 - ATP synthase is the same as Complex V. Please be consistent with the naming.
Line 435 box - compared with healthy controls
Line 499 – Again there seems to be an error in the citation here.

Children's National

Dr. Laura Bennet
Senior Editor
The Journal of Physiology

February 16, 2023

Re: Manuscript Revision #JP-TR-2022-283792

We would like to thank the Senior Editor and the reviewers for taking the time to read our manuscript and provide invaluable feedback. We have addressed each of the suggestions line-by-line below. We have also incorporated these suggestions into the revised manuscript. Thank you again for the opportunity to revise and resubmit our improved manuscript!

Editor's Comments:

This is a comprehensive review of what is known about cardiomyocyte maturation in humans. As it is a large review, some references are missing and should be added. Clarity of some sections could be improved. Overall, a great effort.
Thank you. We have clarified and responded to the reviewer's comments line-by-line below. Additionally, missing references have been added.

It would be helpful to include a summary that clearly indicates if data is from atrial or ventricular cells, right or left. A summary figure for all developmental processes or several summary figures would also be useful to help the reader integrate what is known and what remains controversial due to several different findings.
Excellent suggestion. We have added summary tables to each major section of the manuscript, which indicates the cell type for the referenced study, age range, and the key findings to help the reader interpret the differences between studies.

Line 63 - Please explain what differences hinder translation.
Added to the text: "…significant differences in electrophysiology, calcium handling, and contractile function that can hinder the translational applicability…"

There are errors in the text in the blue box. Is this an abstract? It is not standard to have such boxes in review in JP. Could this information be integrated into the main body of text.
We included these call out boxes to provide more general information, as they were used in another JPhysiology publication we recently participated in (Grandi, et al. 2023: White Paper: Diversity of Cells and Signals in the Cardiovascular System). Nevertheless, we have removed them from the revision to coincide with standard JP format.

Line 103 - Does study population mean different species? If yes, please cite papers that used other species(Burrell et al., 2003; Jonker et al., 2006; Jonker et al., 2007; Jonker et al., 2010; Botting et al., 2012; Jonker et al., 2015; Jonker & Louey, 2016; Jonker et al., 2018a; Jonker et al., 2018b).
We tried to limit our discussion to humans only – but in some cases, it was unavoidable. We have modified the sentence from "study population" (humans) to "species" and included some of the additional references that were directly applicable and support this claim.

Line 120 - Repair has been shown in other studies and in other species(Soonpaa & Field, 1997; Porrello et al., 2011; Porrello et al., 2013; Zgheib et al., 2014).
Thank you, the text has been modified to clarify that repair is poorly understood in humans but has been seen in other species. The citations have also been added to strengthen this point.

Line 160 - This sentence refers to rodents but the Bensley paper looks at sheep. Please expand the sentence to indicate the differences between rodents and sheep and include a greater variety of references(Li et al., 1996; Soonpaa et al., 1996; Burrell et al., 2003; Jonker et al., 2006; Jonker et al., 2010; Jonker et al., 2015).
Thank you for the comment. Bensley et al 2016 provides information on sheep, rodents, and rabbits. Our goal here was to highlight the distinction between small rodent models and larger mammals, thus, we have kept the Bensley 2016 citation that includes rodent studies. Per the editor's suggestion, we have also included the additional suggested references.

Line 389 - The previous paragraph ends by saying that an increase in oxygen causes metabolic changes. This is shown in mice. This paragraph talks about changes in the human fetal heart. When oxygen levels are low. Thus, is it appropriate to argue that an increase in oxygen is the underlying cause? Please see (Lock et al., 2018).
Thank you, the paragraph has been edited to include other potential underlying causes of metabolic maturation and also includes Lock et al. reference.

Line 411 and 420 - Please be clear about what tissue source is being reported. Are these human cells are IPSCs?
We apologize for any confusion. The previous sentence specifies "human fetal cardiomyocytes" and does reference the correct paper. The editor is correct that the paper is titled "Mitochondrial Maturation in Human Pluripotent Stem Cell Derived Cardiomyocytes" – but this specific study includes human tissue samples, and compares them to hiPSC-CM. To avoid confusion – we have specifically added "human developmental studies" to the first sentence of this paragraph.

Line 455 - ...compared to other....
Changed, thank you.

Line 476 - Please indicate the species studied.
Added, thank you.

Line 482 - Are the age-dependent effects really species dependent effects?
This sentence reads "The latter suggests an age-dependent difference in Ik1 repolarizing current …"

Line 498 - Reference formatting needs correcting.
Reformatted, thank you.

Line 503 - Please be clear about the tissue sources. ISPCs or species?
We specify …"in immature hPSC-CM increased NCX activity enhances spontaneous activity, but the functional outcomes of NCX overexpression in human neonatal-infant cardiomyocytes have not been thoroughly evaluated"

Line 631 - compared to when?
Modified – "NCX expression also changes during development in the human heart … "

Line 634-35 - Please explain. What is the baseline to which the difference is compared?
Thank you. Modified to note the comparison is to early gestation.

Line 650 - ... fetal and postnatal development.....

We opted for the term "age-dependent studies" and added "neonatal and adult myocytes" because the referenced paper examines changes after birth to adulthood. Fetal measurements are not included in the Kumar et al. study.

Line 674 - The sample sizes are small but the ages studied are also limited. This should be indicated.
This is an important point – now included!

Invited reviews generally have a graphical abstract. This could be helpful.
Thank you! A graphical abstract has been included.

JP guidelines indicate the Additional Information that should be included before the references section. Please update.
Thank you. The competing interests, author contributions, funding, and acknowledgement sections have been added. Since this is a topical review and we do not have data to share – that information/statement was omitted.

Referee #1 comments:

Regulation of perinatal cardiac growth and maturation has been of scientific and medical interest for a long time, as demonstrated by a long publication history in the field. The basic biological mechanisms involved in creating a mature, functional heart are fascinating, as are interspecies differences affecting the form, function, and regenerative potential of the heart. But the area is also one of profound clinical importance. New surgical approaches have enabled infants with profound congenital defects incompatible with a mature circulation to live beyond parturition. However, medical advances to support or therapeutically regulate the maturing myocardium lag, primarily due to challenges that impede discovery in human fetuses and infants. The observations that have been made in humans create a fragmented and sometimes contradictory picture, due both to those populations from which samples can be drawn (usually those with disease, or who have died), as well as methodological limitations. What this manuscript contributes is a synthesis of the history of research in the perinatal human heart, as well as an up-to-date perspective on gaps in the field that demand attention. The authors are to be commended for this effort.
Thank you for this kind endorsement.

Throughout, studies using atrial cardiomyocytes are presented alongside studies using ventricular cardiomyocytes (or even right versus left ventricular cells). It is important to remember the differences in the relative roles of the different cardiac chambers. Mature cardiomyocytes from these different locations differ from one another in their number and size, their structure, their electrophysiological properties, and in excitation-contraction coupling (e.g. Growth and Hyperplasia of Cardiac Muscle Cells. Soviet Medical Reviews. Supplement Series. Cardiology: Vol.3. Chapter 6. Rumyantsev P.). Consequently, it is important for the reader to remember that their maturational trajectories will also differ. An initial reminder of these differences may aid the reader.
Thank you, excellent suggestion. We have added this resource and included additional text in the introduction. Since human atrial and ventricular cardiomyocyte studies are limited, so we chose to include both cell types to cover as much pertinent information as possible. But, to clarify the cell type for each study, we have included summary tables in each major section of the manuscript.

The purpose of this review is to present the state of the field in perinatal human cardiomyocyte maturation, not interspecies comparisons. Nevertheless, research in other animals has provided the foundational knowledge which serves as the encyclopedic reference on we rely for our interpretation of the human studies. We know about many basic physiological mechanisms, some different from the human, from studies in species as diverse as the fruit fly, the zebrafish and salmonids, and the newt. Our understanding of genetics and signaling pathways has been greatly aided by studies in mice. The animals with the most similar cardiovascular systems to humans, however, are generally those of a similar size (West GB, Brown JH, Enquist BJ. A general model for the origin of allometric scaling laws in biology. Science. 1997 Apr 4;276(5309):122-6.). Dogs and pigs have been very important for adult translational studies, while sheep are the cardiovascular developmental gold standard model in which critical discoveries and advances have been made (Rudolph AM. Congenital Diseases of the Heart: Clinical-Physiological Considerations, Third Edition. Wiley-Blackwell, Chichester, UK,

2009). Consequently, where animal data must be used in this manuscript to buttress or approximate understanding of the human condition, it would be best to draw on data, if it exists, from large mammals. Further, as the unique value of this manuscript is its focus on the human data, it should be clear where statements are supported only by animal data (e.g. 377-380).
Thank you! Yes, the intention of this manuscript was to focus on human data (as much as possible). In the revision, we have included references to larger mammals (e.g., sheep, swine) to help contextualize the developmental process. References to smaller rodents are included when species-specific differences should be considered (or, if larger mammalian data is lacking). We have attempted to clarify statements where the statement is supported by animal work – including the example listed above (377-380). We have also included the recommended West et al. 1997 and Rudolph 2009 references.

• Line ~92 text box: Regarding the description of how fetal blood becomes oxygenated - maternal blood does not ever mix with fetal blood.
All text boxes have been excluded to keep in line with Journal standard.

• 101-102: Please include discussion of evidence to the contrary, for instance appearance of binucleate myocytes in gestation (Kim et al., 1992), the decline in cell cycle activity (Huttenbach Y, Ostrowski ML, Thaller D, and Kim HS. Cell proliferation in the growing human heart: MIB-1 immunostaining in preterm and term infants at autopsy. Cardiovasc. Pathol. 2001. 10, 119-123) and other stereological estimates of cell number (Mandarim-de-Lacerda CA, das Santos MB, Le Floch-Prigent P, Narcy F. Stereology of the myocardium in human foetuses. Early Hum Dev. 1997 May 28;48(3):249-59.).
Thank you for this recommendation. We have included references that document an earlier switch from hyperplasia to hypertrophy occurring during gestation. We have included other stereological estimates of cell number.

• 106-112: Although the possibility of DNA replication without subsequent karyokinesis is discussed later (144), it is essential in this discussion of proliferation to avoid conflation of cell cycle activity with an outcome of successful division into daughter cells. Alternative outcomes to cell cycle activity include replication of DNA both with karyokinesis (leading to formation of multi-nucleated cardiomyocytes), or without karyokinesis (leading to multiple chromosome number within one nucleus). In fact, cytological features of cytokinesis such as a cleavage furrow may appear in the process of binucleation (e.g. Li, F. , Wang, X., Bunger, P., Gerdes, A. M. Formation of binucleated cardiac myocytes in rat heart: I. Role of actin-myosin contractile ring. J Mol Cell Cardiol. 1997 Jun;29(6):1541-51.)
Thank you for the suggestion. We have included an earlier discussion of alternative outcomes to cell cycle activity, as describe by the reviewer.

• 129: What is meant by "dedifferentiation" and is this a helpful biological insight? Some of the cited studies define "dedifferentiation" as activation of cell cycle regulators. Surely it is not surprising that proliferating cells have activation of cell cycle regulators. Please expand or clarify.

Dedifferentiation refers to the process by which large-sized cardiomyocytes disassemble sarcomeric structures and physically/electrically uncouple from one another – reverting these cells back to an “immature state” before reentering the cell cycle. We have briefly defined this term and added an additional reference (Zhu Y, Do VD, Richards AM, Foo R. What we know about cardiomyocyte dedifferentiation. J Mol Cell Cardiol. 2021 Mar;152:80-91. doi: 10.1016/j.yjmcc.2020.11.016. Epub 2020 Dec 1. PMID: 33275936.)

• 146-151: In contrast, Adler finds more cardiomyocyte nuclei are tetraploid than diploid after age 8 (Adler CP, Costabel U. Cell number in human heart in atrophy, hypertrophy, and under the influence of cytostatics. Recent Adv Stud Cardiac Struct Metab. 1975;6:343-55.)

Thank you, we have included this additional Adler reference to the sentence. We used the terms “polyploidization” or “hyperploid” to indicate >2N

• 158-162: Although elsewhere issues of allosteric scaling are briefly mentioned as an important difference in interspecies comparisons, the impact of this consideration is largely inappropriately ignored where animal data

is brought in to support or add to the human data. Here swine and rodents are mentioned, which are both born in large altricial litters with birthweights much lower than humans. Sheep typically have 1-2 offspring which are of more similar size to humans at birth (Burrell JH, Boyn AM, Kumarasamy V, Hsieh A, Head SI, Lumbers ER. Growth and maturation of cardiac myocytes in fetal sheep in the second half of gestation. Anat Rec A Discov Mol Cell Evol Biol. 2003 Oct;274(2):952-61.; Jonker SS, Louey S, Giraud GD, Thornburg KL, Faber JJ. Timing of cardiomyocyte growth, maturation, and attrition in perinatal sheep. FASEB J. 2015 Oct;29(10):4346-57.).
Thank you, we took this opportunity to emphasize once more the importance species-specific differences. These important references are now included in the revised manuscript.

• 166-167: Not entirely unknown. This is addressed in some of Adler's papers, as well as Brodsky (Adler CP, Costabel U. Myocardial DNA and cell number under the influence of cytostatics. I. Post mortem investigations of human hearts. Virchows Arch B Cell Pathol Incl Mol Pathol. 1980;32(2):109-25; Brodsky VYa, Sarkisov DS, Arefyeva AM, Panova NW, Gvasava IG. Polyploidy in cardiac myocytes of normal and hypertrophic human hearts; range of values. Virchows Arch. 1994;424(4):429-35.)
The wording has been modified to "incompletely understood" – and these additional references have been included in this paragraph.

• 173-174: It seems that the de Simone paper has been misinterpreted. Stroke work increases between 4- to 10-fold fold, not by 65%, between birth and adulthood (Fig 3). Unfortunately, in this paper stroke volume is reported as an average of all children. Their mean age was about 10 and body weight was about 36kg (Table 1), which is much greater than a normal birth weight of 3.3kg. Consequently, a conclusion cannot be made from this paper about change in stroke volume between birth and adulthood. The same authors did previously publish a paper looking more carefully at stroke volume from birth to adulthood (de Simone G, Devereux RB, Daniels SR, Mureddu G, Roman MJ, Kimball TR, Greco R, Witt S, Contaldo F. Stroke volume and cardiac output in normotensive children and adults. Assessment of relations with body size and impact of overweight. Circulation. 1997 Apr 1;95(7):1837-43.). If the shortest individuals in the study presumed to be the newborns, stroke volume increases about 6-12 fold between birth and adulthood (Fig 1). It is unclear why the Gilbert paper is cited.
Thank you! We have modified the text according to the reviewer's excellent suggestion. The Gilbert paper has also been removed and replaced by the de Simone study.

• 229-232: The Reiser reference in not in the reference list. The summarization of this paper is confusing. a-MHC was the primary isoform found in the fetal atria. This did not change with gestational age, and a-MHC continued to predominate in the adult atria. B-MHC predominated in the fetal ventricles. The small amount of a-MHC in the fetal ventricles may decline with advancing gestational age (the author notes that the results are heavily influenced by two outlier samples).
We apologize for any confusion and have edited the text for clarity. We have also added this reference to the bibliography.

• 237-240: It is worth noting that this is likely due to the difference in heart rate between those two species, and that in other large mammals (e.g. sheep) the MHC isoforms are similar to humans (Hodges MM, Zgheib C, Liechty KW. A Large Mammalian Model of Myocardial Regeneration After Myocardial Infarction in Fetal Sheep. Adv Wound Care (New Rochelle). 2021 Apr;10(4):174-190.).
Yes, the reviewer is correct. We mentioned heart rate differences earlier in the manuscript, but we agree that it is important to make the connection again in this section. We have also added the Hodges reference.

• 336-338: What is the purpose of noting the finding in humans is different to the mouse, does it cast doubt on the finding in the human?
Removed to avoid confusion.

• 374: Glucose and lactate oxidation is by definition not anaerobic.
Thank you, the sentence is modified.

• 382-385: Care should be taken extrapolating mechanisms and timing of maturational events between the rodent and human due to the persistence in the rodent of an immature state after birth. Note also that the heart of the large mammalian fetus can take up and oxidize fatty acids (Bartelds B, Knoester H, Smid GB, Takens J, Visser GH, Penninga L, van der Leij FR, Beaufort-Krol GC, Zijlstra WG, Heymans HS, Kuipers JR. Perinatal changes in myocardial metabolism in lambs. Circulation. 2000 Aug 22;102(8):926-31.), and that the human is unique among placental mammals in the high level of placental transfer of fat to the fetus. It is, of course, unknown the extent to which the human fetal heart metabolizes the available fatty acids.
Excellent points. We have modified the text to highlight the model from which information has been extrapolated. We have also included a cautionary statement about metabolic maturation in different species.

• 444-445: "right ventricular hypertrophy regresses over 2-3 months" Remodels would be more accurate than regresses, as the RV mass is in fact increasing in this period (but changing shape and decreasing relative to the LV or total heart).
This is a valuable point – the text has been modified.

• 592: They are not absent (lines 200-202).
This sentence is referencing results of a specific paper – but for accuracy, we have modified "absent" to "underdeveloped" and referenced relevant studies.

• 596-599: It's not clear what the point is, there's variation across species (Growth of the heart in health and disease ed. Zak R. Raven Press, New York. 1984; etc).
There is a lack of data/information regarding intracellular calcium handling/sparks/transients in human cardiomyocytes, but we have provided some guidance based on experimental models.

• Other references for studies in humans that you may consider for their contribution to understanding in this topic include:
Ball AJ, Levine F. Telomere-independent cellular senescence in human fetal cardiomyocytes. Aging Cell. 2005 Feb;4(1):21-30; Adler CP. Relationship between deoxyribonucleic acid content and nucleoli in human heart muscle cells and estimation of cell number during cardiac growth and hyperfunction. Recent Adv Stud Cardiac Struct Metab. 1975;8:373-86; Austin A, Fagan DG, Mayhew TM. A stereological method for estimating the total number of ventricular myocyte nuclei in fetal and postnatal hearts. J Anat. 1995 Dec;187 (Pt 3)(Pt 3):641-7.
Thank you, we have included citations to these important studies.

• Line ~92 text box: an extraneous "and" at the end of the sentence ending on the fifth line.
Text boxes were removed, based on Editor suggestion.

• 96-98: Please provide references for this statement.
References added.

• 96, 942, etc: Capitalize "St".
Fixed.

• 102: Sentence beginning "Although" is a fragment.
Modified.

• 156-157: "Similar to humans" should be "Similar to human cardiomyocytes"?
Modified.

• 175-176: >90% increase in mass in what period? Human newborn heart weights are typically less than 20g, normal adult heart weights are ~280-350g, which is much more than a doubling in heart weight.
Thanks for catching this mistake. The sentence was modified.

• Figure 1. Kindly explain what is a z-tubule.
We have edited this to say "SR tubules at the Z-line" for clarity.

• 297-298: Sentence fragment.
Modified.

• 376: What is meant by "increased blood volume" at birth?
Removed and simplified.

• 435: Blue box: "observed ~6 months of life" should be "observed at ~6 months of life"?
Text boxes were removed, based on Editor suggestion.

• 436: What does "reorganization of the cardiovascular system" mean?
Modified to “blood circulation” for clarity.

• 489-499: Missing close parens on citation?
Parentheses added

• 961: Capitalization
Fixed.

Referee #2 comments:

The narrative review titled "Adapting to a new environment: postnatal maturation of the human cardiomyocyte" by Salameh and colleagues aimed to examine the developmental maturation of the human heart including; structure, electrophysiology, metabolomic and contractile function. The review has a focus on studies from human tissue samples rather than animal models of cardiac development (though there are some helpful comparisons where necessary). The focus being on human studies grants a unique perspective and allows for a detailed exploration of the field due to the limited number of publications from human tissue samples. The review highlights this as a major limitation, with a strong conclusion section indicating that a thorough understanding of pediatric cardiomyocyte physiology can better inform clinical care as well as fill knowledge gaps regarding cardiomyocyte quiescence. I especially enjoyed the electrophysiology and excitation-contraction coupling sections that were comprehensive and informative, though I wonder if the review could benefit from the addition of a figure showing the generic components of excitation-contraction coupling (t-tubule with LTCC next to SR etc) to assist readers that aren't familiar with this area. The review could also potentially benefit from a summary diagram or table indicating the relative difference in expression of proteins examined throughout the paper.
Thank you for this suggestion. As also recommended by the Editor, we added a graphical abstract to the review article to help clarify key maturational changes.

Line 100 - Though there is limited evidence in humans as the reviewers have indicated, there is some evidence to suggest that in large animals (for example sheep) the transition from proliferative growth to hypertrophic growth actually begins before birth (starts at 110 days - term = 150d). For references please see this review: https://doi.org/10.1152/ajpregu.00391.2017 [doi.org]
Thank you for your comment! We have included examples where evidence of this transition may occur before birth.

Line 165-170 - This reviewer recognises that the review is centred around human studies, however, there are a number of studies in sheep that have looked at cardiomyocyte endowment in models of growth restriction and changes in the intrauterine environment that seem to have been overlooked.
https://pubmed.ncbi.nlm.nih.gov/25085511 [pubmed.ncbi.nlm.nih.gov]/ for example.
Thank You! This influential study has been included.

Some additional suggested triggers for cardiomyocyte quiescence in rodent studies are also thyroid hormone

(see Naqvi et al., 2014) and the transition from low PO2 to the postnatal oxygen rich environment, mitochondrial stress and ROS production (Puente et al., 2014). Though it is likely a combination of a number of factors rather than a single trigger.
Thank You! We included these additional factors.

Line 426 - Though not a study in humans, there is some evidence for the effects of developmental hypoxia on fetal cardiac mitochondrial function. See: https://doi.org/10.1111/jpi.12821 [doi.org] https://doi.org/10.1152/physiol.00022.2022 [doi.org]

Thank You! Another sentence has been added to reflect experimental studies in rodents and sheep.

Minor corrections: *Thank you for the minor comments related to grammar/typos, which have all been corrected in the revised manuscript.*

REQUIRED ITEMS:

-Please include an Abstract Figure file:
Graphical abstract is now included.

-Your MS must include a complete "Additional information section":
Other information is now included.

-Please upload separate high quality figure files via the submission form.
Reproduced figures are uploaded as separate files.

-Author profile(s) must be uploaded via the submission form.
Short author biography is now included.

Dear Dr Posnack,

Re: JP-TR-2023-283792R1 "Adapting to a new environment: postnatal maturation of the human cardiomyocyte" by Shatha Salameh, Vanessa Ogueri, and Nikki Gillum Posnack

We are pleased to tell you that your paper has been accepted for publication in The Journal of Physiology.

TRANSPARENT PEER REVIEW POLICY: To improve the transparency of its peer review process The Journal of Physiology publishes online, as supporting information, the peer review history of all articles accepted for publication. Readers will have access to decision letters, including Editors' comments and referee reports, for each version of the manuscript, as well as any author responses to peer review comments. Referees can decide whether or not they wish to be named on the peer review history document.

The last Word (or similar) version of the manuscript provided will be used by the Production Editor to prepare your proof. When this is ready you will receive an email containing a link to Wiley's Online Proofing System. The proof should be thoroughly checked and corrected as promptly as possible.

Authors should note that it is too late at this point to offer corrections prior to proofing. The accepted version will be published online, ahead of the copy edited and typeset version being made available. Major corrections at proof stage, such as changes to figures, will be referred to the Editors for approval before they can be incorporated. Only minor changes, such as to style and consistency, should be made at proof stage. Changes that need to be made after proof stage will usually require a formal correction notice.

All queries at proof stage should be sent to: TJP@wiley.com

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Yours sincerely,

Professor Laura Bennet
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EDITOR COMMENTS

Reviewing Editor:

Thank you for revising the paper in line with the reviewers comments. The figures and table are a fabulous addition. I'm sure that this will be an influential paper.

Senior Editor:

Thank you for this timely review. I know our readers will enjoy the overview of an important area of physiology.

REFEREE COMMENTS

Referee #1:

Well done on drawing out a comprehensible narrative of human cardiomyocyte development from a complex and fragmentary body of literature.

Referee #2:

The authors have made suitable revisions to the manuscript creating a thorough and impactful review. I have no further comments.

1st Confidential Review **27-Feb-2023**