nature publishing group

The human fetal right ventricular myocardium appears without a sub-epicardial base-apex oriented layer of myocytes

Advance online publication 4 January 2017. doi: 10.1038/pr.2016.248

Dear Editor,

In our recent contribution to your journal, we investigated myocardial architectural changes in persistent pulmonary hypertension of the newborn (1). We found the normal ovine neonatal hearts to contain a sub-epicardial layer of myocytes, approximately 1 mm in thickness, confined to the right ventricle aligned strictly from apex-to-base. In this letter, we describe how we investigated the presence of this phenomenon in human specimens. We conclude that the sub-epicardial layer of myocytes is not present in humans. Our conclusions are mainly based on histological examinations and supported by diffusion tensor imaging analyses. Based on present literature, it has not been possible to confirm the presence of the apex-to-base sub-epicardial layer of right ventricular myocytes in humans. The adult human right ventricular myocardium have previously been described as a layered structure with the most sub-epicardial layers running circumferentially, and the sub-endocardial fibres running longitudinally from apex-to-base forming the trabeculae and the papillary muscles (2), but myocytes aligned from apex-to-base in the right ventricular sub-epicardium is never mentioned. The same myocardial architecture is found in the fetal and infant myocardium (3). As in our previous study in lambs, diffusion tensor imaging has been used to characterize the right ventricular myocardial morphology in swine (4). In this very recent study by Zhang and co-workers, the morphological changes were studied in the porcine heart during the transition from fetal to neonate circulation. Unfortunately, their tractography images do not allow us to fully evaluate the right ventricular sub-epicardium. Neither do they show histological images of the area, but a helical angle plot is provided where the sub-epicardial angles approach 90 degrees indicating that the alleged layer could be present in pigs. Collecting whole human fetal hearts for diffusion tensor imaging and histological examination, in an ethical acceptable manner within a reasonable time frame would be next to impossible. Our department of pathology, however, houses an extensive archive of histological specimens taken routinely from all performed autopsies including the relatively rare cases of autopsies on fetuses and children. Using these achieves, we have designed the present study to elucidate the existence of apex-to-base aligned myocytes in the fetal right ventricular sub-epicardium. We obtained all available histological slides of myocardial biopsies from 57 cases of routine autopsies on human fetuses and neonates performed from 2009 to 2015. All parents of diseased infants and children have provided informed consent to the processing of tissue for the archives. The identity of the patients was blinded to all investigators not involved in primary diagnostics and treatment. We selected only specimens from humans older than 38 wk of gestation thereby elucidating the myocardial architecture around birth as done previously in sheep (1). All cases of left ventricular or uncertain origin were discarded. The remaining 18 cases, comprising 23 histological slides, underwent further histological evaluation. All specimens were cut close to the transverse plane, stained with hematoxylin and eosin and subsequently evaluated using a conventional light microscope at 20 and 40 times magnification. Each slide was systematically examined identifying and following the epicardial border looking for abrupt changes in the orientation of the myocytes comparable to what was found in sheep. Tissue areas potentially comparable to our earlier findings were photographed and discussed within our research group. To support the histological findings, diffusion tensor imaging was performed on two larger myocardial short axis sections from a pair of preterm twins stillborn at 24 wk of gestation. At autopsy, neither of the twins exhibited any signs of heart disease or other illnesses apart from prematurity. The myocardial sections were obtained as a part of the routine autopsy procedure and were, subsequent to scanning, further processed for routine pathological examination. They were fixed in formalin for routine histological processing. During the scan, the biopsies were temporarily embedded in a MRI neutral polymer (histomer) to prevent evaporation and motion artifacts. We utilized the same diffusion tensor imaging protocol as described in our previous contribution (1). In 16 of 23 examined histological slides, we observed what could be interpreted as undeveloped layer of myocytes in the sub-epicardium, having a thickness between 200–500 μm with a different orientation than in the deeper myocardium. None of the hearts, however, exhibited a layer of myocytes completely covering the entirety of the right ventricle as seen previously in lamb. Diffusion tensor imaging revealed a right ventricle containing myocytes with a predominantly circumferential orientation and no myocytes aligned strictly from apex-to-base were found. Tractography in both ventricles shows a classical distribution of helical angulation of myocytes with a left-hand helix in the sub-epicardium changing progressively toward a right-hand helix in the sub-endocardium. Our histological investigations and diffusion tensor imaging concurrently indicate that the distinct epicardial layer of myocytes aligned from apex-to-base in sheep is not to be found in human hearts. We have confirmed that it is feasible to scan the heart of the human fetus using diffusion

tensor imaging at a gestational age of just 24 wk and that the appearance of the tractography closely resemble what has been found in humans by others (5). Our diffusion tensor imaging data confirms existing knowledge. Like in our previous study in lamb (1), we find that both ventricles show the classical transmural change of myocyte orientation with a left-hand helix in the sub-epicardium changing progressively toward a right-hand helix in the sub-endocardium as seen in all diffusion tensor imaging studies of mammal hearts. In literature, it is generally presumed that mammalian hearts are very similar and that findings on sheep, pigs, and rodents can be readily translated into the human setting. Our study questions this notion and points toward a difference between species that could potentially have physiological consequences. Our results thus breathe new life into the common question if the human heart is at all comparable to hearts of other mammals. Sheep has a gestation period of 150 d, compared with 280 d in humans, but the birth weight of a sheep is around 2 to 5 kg, which is largely identical to that in humans of 2.5 to 4.5 kg. This considerable difference in gestation period relative to birth weight could indicate that the circulatory demands *in-utero* are different between the species. It may very well be speculated that the higher growth rate in sheep requires a different anatomical build of the right ventricle than in humans. Anatomical differences between porcine and human hearts are known to exist (6) and significant differences in angulation of left ventricular cardiomyocytes compared between hearts from mice, rabbits, and sheep have, moreover, been found (7). This difference was attributed to the different size of the animals. There are very few gross anatomical differences between hearts among the mammalian species including humans and it is widely accepted, although it is difficult to prove scientifically, that these differences are minuscule. All studies, to date, directly comparing hearts of mammalian species to the human heart have been conducted using dissection and gross anatomy description (6,8,9). Being a two-dimensional technique histology makes determination of the true orientation of the myocytes difficult. This is also a limitation of our present study. Histological examination was, furthermore, performed on specimens not specifically collected for the purpose of this study. Thus, the investigators had no control over biopsy sites in the fetal hearts. Hence, the estimation of the directionality of the myocytes was difficult in many cases. In order to obtain proper three-dimensional information on the orientation of the cardiomyocytes, three-dimensional imaging techniques such as diffusion tensor imaging are needed. To our knowledge, there has yet to be published a diffusion tensor imaging-based study specifically comparing the similitudes between human and other mammalian hearts. We were only able to preform diffusion tensor imaging on two twin hearts. Hence, detailed assessments and statistics are impossible. Explicit conclusions based on the presented diffusion tensor imaging data are, therefore, avoided. For obvious reasons, it was impossible to determine the contraction states of the hearts at histological section. It is known that cardiomyocytes rearrange to some extent during the cardiac cycle (10), thus differences in contraction states between the specimens can potentially confound the results. We consider it unlikely, however, that the discrepancies between the present study and our previous can be explained only by differences in contraction states.

In conclusion, we have described a new mammalian interspecies difference in cardiac anatomy. Our current data suggest that the right ventricular sub-epicardial layer of myocytes aligned from apex-to-base is not an omnipresent mammalian phenomenon. Because cardiac anatomy is the foundation of function, it emphasizes the importance of choosing the optimal animals for animal experimental studies that are supposed to simulate human conditions. Further studies into the differences in heart structure between humans and other mammals seem warranted.

STATEMENT OF FINANCIAL SUPPORT
This study is funded by a grant from The Danish Children Heart Foundation.

Disclosure: None.

Peter Agger[1,2], Daniel M. Gleesborg[1,2], Mette Ramsing[3] and Vibeke Hjortdal[2]

[1]Department of Cardiothoracic and Vascular Surgery, Aarhus University Hospital, Aarhus, Denmark; [2]Department of Clinical Medicine, Aarhus University Hospital, Aarhus, Denmark; [3]Department of Pathology, Aarhus University Hospital, Aarhus, Denmark.

Correspondence: Peter Agger (peter.agger@clin.au.dk)

REFERENCES

1. Agger P, Lakshminrusimha S, Laustsen C, et al. The myocardial architecture changes in persistent pulmonary hypertension of the newborn in an ovine animal model. Pediatr Res 2016;79:565–74.
2. Ho SY, Nihoyannopoulos P. Anatomy, echocardiography, and normal right ventricular dimensions. Heart 2006;92 Suppl 1:i2–13.
3. Sanchez-Quintana D, Garcia-Martinez V, Climent V, Hurle JM. Morphological analysis of the fish heart ventricle: myocardial and connective tissue architecture in teleost species. Ann Anat 1995;177:267–74.
4. Zhang L, Allen J, Hu L, Caruthers SD, Wickline SA, Chen J. Cardiomyocyte architectural plasticity in fetal, neonatal, and adult pig hearts delineated with diffusion tensor MRI. Am J Physiol Heart Circ Physiol 2013;304:H246–52.
5. Pervolaraki E, Anderson RA, Benson AP, et al. Antenatal architecture and activity of the human heart. Interface Focus 2013;3:20120065.
6. Crick SJ, Sheppard MN, Ho SY, Gebstein L, Anderson RH. Anatomy of the pig heart: comparisons with normal human cardiac structure. J Anat 1998;193 (Pt 1):105–19.
7. Healy LJ, Jiang Y, Hsu EW. Quantitative comparison of myocardial fiber structure between mice, rabbit, and sheep using diffusion tensor cardiovascular magnetic resonance. J Cardiovasc Magn Reson 2011;13:74.
8. Rowlatt U. Comparative anatomy of the heart of mammals. Zoological Journal of the Linnean Society 1990;98:73–110.
9. Jensen B, Agger P, de Boer BA, et al. The hypertrabeculated (noncompacted) left ventricle is different from the ventricle of embryos and ectothermic vertebrates. Biochim Biophys Acta 2016;1863(7 Pt B):1696–706.
10. Ferreira PF, Kilner PJ, McGill LA, et al. *In vivo* cardiovascular magnetic resonance diffusion tensor imaging shows evidence of abnormal myocardial laminar orientations and mobility in hypertrophic cardiomyopathy. J Cardiovasc Magn Reson 2014;16:87.