MR study of postnatal development of left ventricular myocardium structure and function in rats

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Introduction
Development of heart is known to be essential for all organs growth [1]. Numerous studies have been performed to investigate the growth of cardiac myocytes during postnatal period [2-3]. However, development of myocardial fiber structure, which plays key role in cardiac function [4], remains to be explored. In current study, CMR and DTI study were performed to examine myocardium structure maturation concurrent with cardiac function development in postnatal rats.

Method
Imaging experiments were conducted on a 7T Bruker PharmaScan. SD rats were examined at 2, 4, 7, 14, 21, 28, 56 days after birth (N=6 for each time point). In vivo CMR study: ECG and respiratory triggered FLASH cine sequence was performed on four short-axis slices covering whole heart with parameters: TR/TE=24.5/2.3 ms, flip angle=30º, matrix size=192×192, cardiac frame was related with heart rate, FOV and slice thickness were heart size dependent with slice gap equal to 10% of slice thickness. Ejection fraction (EF), stroke volume (SV) and cardiac output (CO) were computed. Ex vivo DTI study: All animals were sacrificed and the excised hearts were fixed with formalin. DTI was performed at the same four short-axis slices using SE DTI. Imaging parameters were: TR/TE=1500/29 ms, diffusion b= 800 s/mm^2, 6 gradient directions, FOV=2.55 cm^2, matrix size=256×256, and NEX=10. The scan time is ~7hr per sample. FA, mean ADC, axial and radial diffusivities, and fiber orientation was measured and averaged among six samples at each time point. Three groups of fibers were categorized: left-handed helical fiber (LHF) with helix angle within -90º to -30º in epicardium, circumferential fiber (CF) within -30º to 30º in midwall, and right-handed helical fiber (RHF) within 30º to 90º in endocardium [5]. Histological analysis was performed using H& E stain after DTI study. Student's t-test was performed with p<0.05 was regarded as significance.

Results
Cardiac functional changes with age and t-tests with previous time point were summarized in Table 1. Heart rate increased substantially during the early postnatal period and then remained relatively constant into adulthood. EF changed significantly after birth and became stable around 70% after day 14. SV and CO increased significantly with body weight, mainly due to the augmentation of LV dimension. From ex vivo DTI study, FA was found to decrease during the whole period examined (Fig.1a), especially from day 14 to day 28. Mean ADC increased significantly from day 2 to day 4, then decreased till day 21, and became stable afterwards (Fig.1b). Both axial and radial diffusivities increased substantially from day 2 to day 4, suggesting significant morphological changes along both axial and radial directions. Axial diffusivity decreased afterwards, especially at day 21. But radial diffusivity exhibited no apparent change after day 4. Percentages of three groups of fibers at each time point were illustrated in Fig.1d. CF decreased substantially from day 2 to day 4, and became stable afterwards. RHF increased first, then decreased significantly from day 4 to day 14, then kept stable thereafter. No significant change was observed for LHF during the entire period. Helix angle maps were shown in Fig.2, and a clear double-helical structure was apparent as early as day 2. Fig. 3 reveals that intermyocyte distance increased from day 2 to day 14, and myocyte size increased from day 14 to day 56.

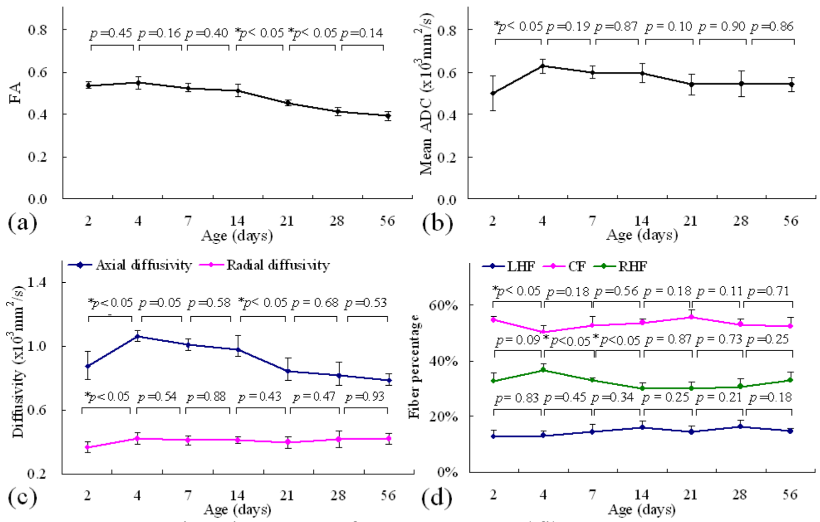

Fig.1 Time courses of DTI parameters and fiber percentages.

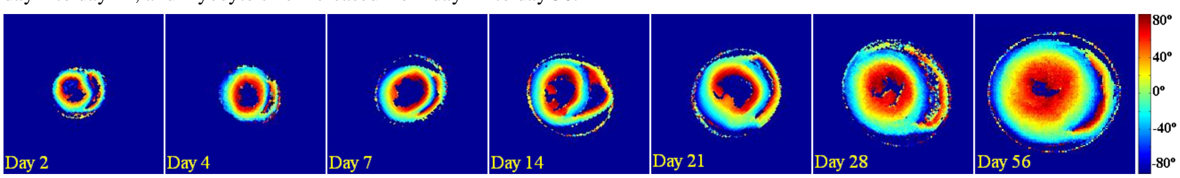

Fig.2 Helix angle map of a midventricle slice at different time point.

Table 1 Cardiac functional changes with age.

	Day 2	Day 4	Day 7	Day 14	Day 21	Day 28	Day 56
Body weight (g)	7.8±0.4	10.3±0.5*	16.2±1.3*	26.7±1.2*	45.8±3.7*	99.3±6.1*	346.6±13.9*
Heart rate (bpm)	213±13	284±9*	315±24*	377±28*	392±32	400±13	363±31
EF (%)	61.3±7.3	73.7±2.5*	66.2±7.1*	71.0±7.9	74.7±4.4	78.8±1.9	71.4±7.1
SV (ml)	0.008±0.002	0.016±0.003*	0.019±0.001*	0.029±0.003*	0.047±0.010*	0.093±0.007*	0.193±0.023*
CO (ml/min)	1.63±0.36	4.43±0.72*	5.81±0.53	10.84±1.68*	18.19±3.28*	37.16±3.85*	69.51±6.74*

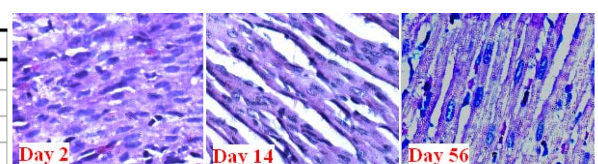

Fig.3 H&E stain of myocardium at ×200 magnification.

Discussion
In current study, postnatal development of cardiac function and myocardium structure were investigated. SV and CO increased with body weight to respond to the increasing mechanical load. Myocardial fiber quality was assessed by FA, mean ADC, axial and radial diffusivities. FA was found to decrease after birth to day 56, which may arise from decrease of myocyte density with age [6]. Between day 2 and day 4, increase of axial and radial diffusivities and mean ADC were observed, likely resulting from the increase of intermyocyte distance [6] and the ongoing transition from hyperplasia to hypertrophy [3]. After day 14, axial diffusivity decreased significantly, suggesting changes mainly occurring along fiber direction. This may arise from the continuing hypertrophy, i.e., shrinking extracellular space (with dominant morphology along fiber direction as seen in Fig. 3) [3]. Current study also confirmed the presence of helical structure of myocardial fibers at a very early stage, as reported by others [4]. Significant change of double-helical structure was found from day 2 to day 14 with increased CF and decreased RHF. This may be related with the concurrent cardiac functional alterations that can be further investigated with cardiac tagging in the future study. In conclusion, postnatal development of heart structure and function was characterized using DTI and CMR. The study reveals that significant changes in myocardial fiber quality and helical structure mostly occur during the first 28 days. Furthermore, DTI analysis provides a potentially valuable tool to assess the microscopic structural changes in heart.

References
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