35th Annual International Conference of the IEEE EMBS
Osaka, Japan, 3 - 7 July, 2013

Investigation of Muscle Degeneration Process in Young Rats with Ischemia Injury Using MR Diffusion Tensor Imaging*

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Abstract – **Skeletal muscle is vulnerable to ischemia injury after direct trauma or indirect causes. Magnetic resonance diffusion tensor imaging (DTI) has been demonstrated to be a powerful tool to investigate muscle structures. However, most previous DTI studies that investigated ischemia muscle were performed on mature species, results of which may not apply on other age populations, such as young groups. In this study, age-related variation of muscle regeneration course after ischemia injury was investigated for the first time. Ten young and ten mature SD rats were induced ischemia in hindlimbs, and the evolutions of the skeletal muscle regeneration were longitudinally studied using DTI before and till 21 days after surgery. Results showed that the young group was more sensitive to ischemia and recovered more rapidly than the mature one. This study confirms the age-related variation of muscle regeneration process, and may provide supplemental information for better understanding of muscle repair evolution.**

I. INTRODUCTION

Skeletal muscle is vulnerable to ischemia injury after direct trauma or indirect causes, such as neuromuscular diseases, neurological dysfunction or innate genetic defects. Tremendous efforts have been made to advance the knowledge of the process of skeletal muscle regeneration with kinds of methods, such as molecular biology, sonography, and computed tomography and so on [1-5]. Recently, magnetic resonance diffusion tensor imaging (DTI) has emerged as a powerful tool to noninvasively probe biological structure at microscopic level. Muscle fiber architecture obtained using 2D or 3D DTI methods were reported to be in good agreement with histological measures [6-9], validating the DTI approach in characterization of skeletal muscle structure. Unlike T2-weighted imaging which usually reflects a wide range of pathological processes including edema, necrosis, and inflammation [10] without emphasizing on specific damage mechanisms [11], DTI has the superiority of delineating muscle intrinsic microstructural characteristics, such as fiber directional integrity, water molecule mobility [8] and even fiber diameter [12]. Proved to be an earlier, more specific and sensitive indicator than T2 in the evaluation of acute muscle injury [14, 15], DTI has been widely used in characterization of muscle microstructural alterations in pathological states. Typically, mean diffusivity (MD) was generally found to decrease during ischemia and increase upon reperfusion [11]. The severity of muscle degradation was observed to change over time [16]. Specifically, severe damage was usually exhibited during the first several days after ischemia injury, and subsequently the diffusion properties gradually returned to normal values owing to muscle regeneration ability.

However, it was noteworthy that most previous DTI studies of revealing microstructural alterations of ischemia muscle were performed on mature species. As biological components and physiological structures of muscle vary with age, the evolution course of skeletal muscle in response to ischemia may be different with age. Thus, the existing results obtained from mature species may not directly apply on other age populations. In this study, regeneration process of ischemia muscle was longitudinally explored in young species with mature animals as controls, and the age-related variation of muscle regeneration process was evaluated for the first time. The experimental findings may provide supplemental information for better understanding of muscle repair evolution.

II. MATERIALS AND METHODS

A. Animal model preparation

The animal experiments were approved by the local institutional ethics committee for animal research. Ten young (≈ 4 weeks) and ten mature (≈ 11 months, denoted as control) Sprague-Dawley (SD) rats were induced ischemia in hindlimbs with permanent ligation of abdominal aorta. Note that the ischemia was induced in both hindlimbs to avoid side limb circulation and ensure the successful rate of the surgery.

B. MR experiments

The imaging experiments were conducted on a whole body 3T Siemens MAGNETOM Trio scanner (Siemens Medical Systems, Erlangen, Germany) before, 2 hours after, and 2, 9, and 21 days after ischemia surgery. The animals were covered with a blanket to maintain their normal body temperature during imaging. The hindlimbs were fixed on a plastic plank and imaged with a custom-built 2 channel radio-frequency coil. A multi-slice readout-segmented echo-planar imaging diffusion tensor imaging (EPI-DTI) [17] was performed with the following parameters: TR = 5000 ms; TE = 55 ms; FOV = 128×128 mm^2; matrix size = 128×128; image resolution = 1.0×1.0×2.0 mm^3; slice gap = 0.2 mm; slice number = 10 with the imaging slab covering the gastrocnemius; number of readout segments per image = 7; echo-spacing = 0.5 ms; EPI factor = 64; diffusion direction number = 6; maximum b-value = 500 s/mm^2; and number of average = 4. Total DTI scan time was around 25 minutes per animal. For each time point, 6 rats were scanned and 1 was

*Resrach supported by NSFC 30900387, GIRTF-LCHT, BRPSZ JC201005270311A

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sacrificed after imaging for hematoxylin and eosin (H&E) histological analysis in each group.

C. Data analysis

From the DTI data, the three major eigenvalues were calculated using a home-written MATLAB program. Axial diffusivity ($\lambda_{\parallel}$), radial diffusivity ($\lambda_{\perp}$), MD and fractional anisotropy (FA) were computed pixel by pixel. Note that $\lambda_{\parallel}$ and $\lambda_{\perp}$ were defined as the primary eigenvalue and the average of the secondary and tertiary eigenvalues, representing the water diffusivities parallel and perpendicular to the principle diffusion direction, respectively. The DTI indices were averaged within each slice with bones and large vessels excluded, and then averaged among slices and the same group at each time point.

One-way analysis of variance (ANOVA) with post hoc Bonferroni's multiple comparison tests were performed to evaluate the temporal evolutions of the DTI indices with $p<0.05$ indicated significance. Unless otherwise stated, all data were presented as means ± standard deviation (SD).

D. Histology analysis

For each group, one representative animal was selected at each time point for H&E histological analysis. The fixed samples were sectioned into 4 μm and stained with the H&E. Muscle structures were visualized at cross- and longitudinal-sections with ×400 magnification, respectively.

III. Results

Figure 1 shows the DTI index maps of both young and control groups before and after ischemia surgery. Note that each type of DTI index map was displayed in the same scale for all time points.

Sequential alterations of the DTI indices were shown in Figure 2. For young group, FA was observed to significantly increase from 0.35 to 0.39 at day 2 and last till day 9 after surgery, and then was renormalized at day 21 (0.33). MD, $\lambda_{\parallel}$, and $\lambda_{\perp}$ substantially decreased from the baseline values of 1.22×10^{-3}, 1.70×10^{-3}, and 0.98×10^{-3} mm^2/s to 1.09×10^{-3}, 1.52×10^{-3}, and 0.87×10^{-3} mm^2/s respectively 2 hours after ischemia induction, and gradually increase afterwards with recovery at day 21 (1.19×10^{-3}, 1.64×10^{-3}, and 0.96×10^{-3} mm^2/s for MD, $\lambda_{\parallel}$, and $\lambda_{\perp}$, respectively). For control group, significant change of FA only occurred at day 9 (0.30) with the value increased by 13.3% compared to that of 2 hours (0.27). MD, $\lambda_{\parallel}$, and $\lambda_{\perp}$ substantially reduced from the baseline values of 1.33×10^{-3}, 1.75×10^{-3} and 1.12×10^{-3} mm^2/s to 1.23×10^{-3}, 1.59×10^{-3} and 1.05×10^{-3} mm^2/s 2 hours after surgery and continuously dropped till day 9 (1.20×10^{-3}, 1.60×10^{-3} and 1.00×10^{-3} mm^2/s for MD, $\lambda_{\parallel}$, and $\lambda_{\perp}$, respectively). Then the values were almost renormalized at day 21 (1.28×10^{-3}, 1.69×10^{-3} and 1.08×10^{-3} mm^2/s for MD, $\lambda_{\parallel}$, and $\lambda_{\perp}$ respectively).

Table 1 summarizes the relative percentage change of DTI indices respective to the control time point of each group.

Figure 3 illustrates the histological observations of representative gastrocnemius structures with 400 magnifications at typical time points following ischemia surgery. When the most substantial structural alterations were exhibited (e.g. 2 hours for young and day 9 for control groups based on quantitative analysis above), swollen myocyte with shrinkage of extracellular space appeared, and more anisotropic fiber structure was found with increase of myocyte packing density. At day 21, the muscle microstructures presented to be similar with those at the time point before surgery.

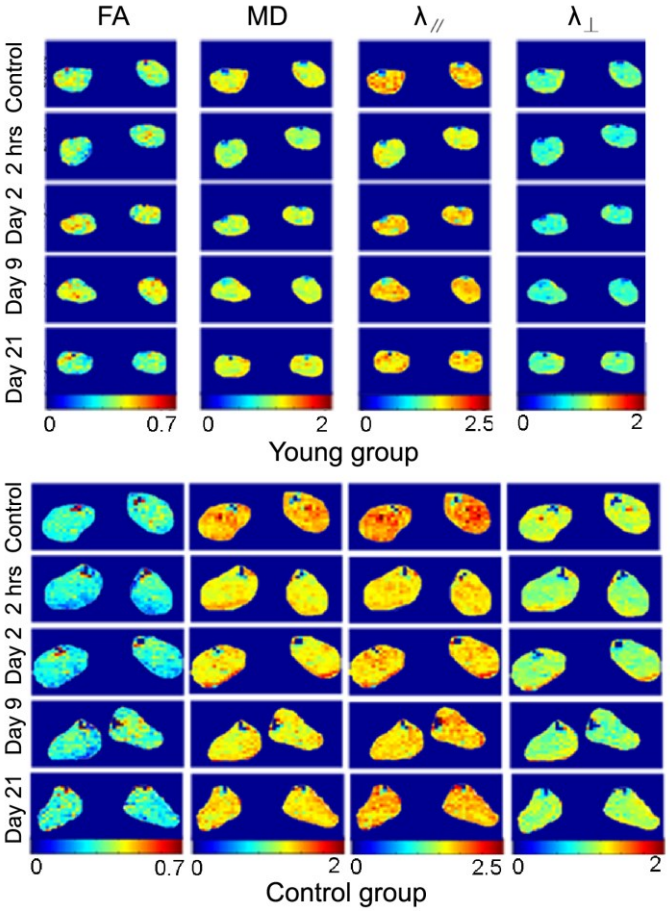

Figure 1 DTI index maps of young and control groups before and after ischemia surgery. Each type of DTI index map was displayed in the same scale for all time points. The unit of diffusivity is $\times10^{-3}$ mm^2/s. *$p<0.05$.

IV. Discussion and Conclusion

Owing to the remarkable capability of muscle regeneration, a working skeletal musculature could be maintained after injury. Typically, the regeneration process is characterized by two phases, namely degeneration and regeneration, respectively [1]. The degenerative phase was reported to happen at the early event following muscle injury when muscle fibers were necrotic and number of non-muscle mononucleate cells increased. Subsequently in regenerative phase, a muscle repair process was activated with myogenic proliferation for formation of new myofiber. In this study, both degenerative and regenerative stages were clearly observed in young and mature groups.

Some DTI indices, such as FA, were known to be sensitive to detrimental effects of noise and would be misestimated with noise contamination [18]. In the current study, the averaged SNRs of DWIs were typically ≈ 110 for

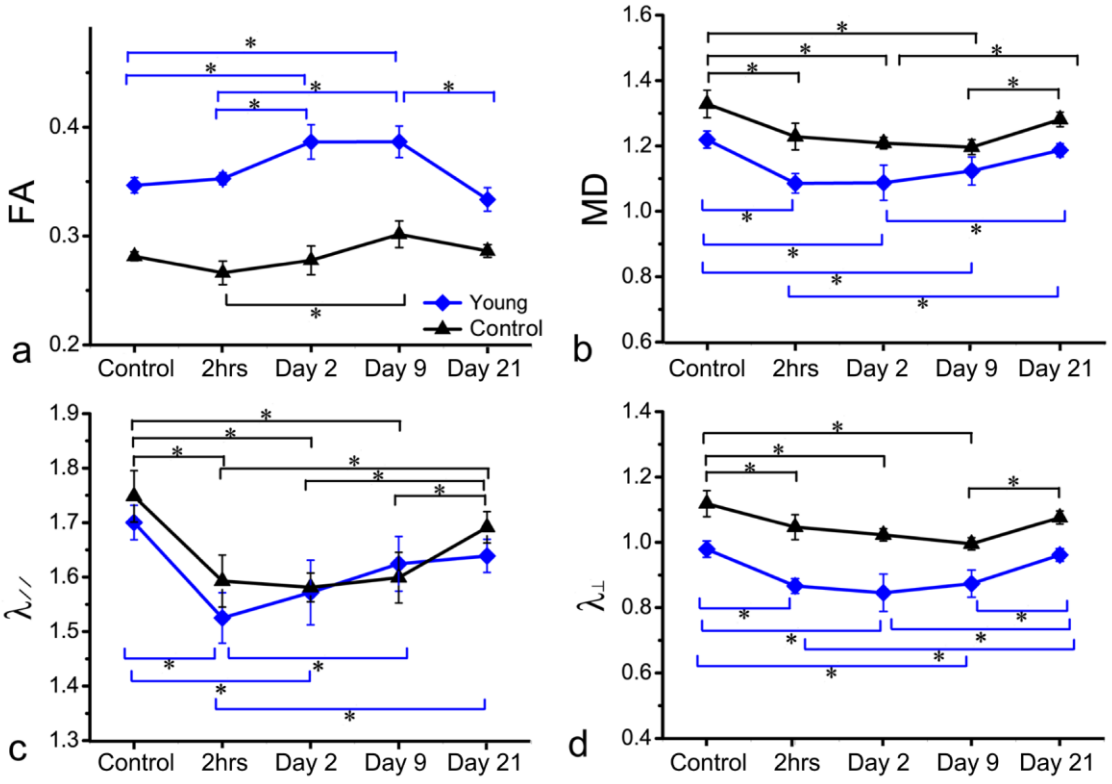

Figure 2 DTI index alterations of skeletal muscle in young and control groups. The unit of diffusivity is $\times 10^{-3}$ mm^2/s. *p<0.05.

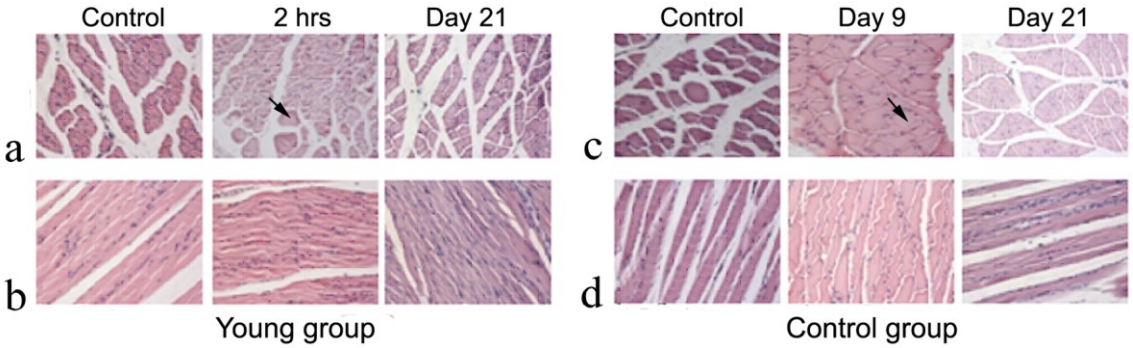

Figure 3 H&E histology of gastrocnemius in young and control groups at cross- (a, c) and longitudinal- (b, d) section views with ×400 magnification at three typical time points. Swollen myocyte were indicated by black arrows.

Table 1 Relative change percentage of DTI indices compared to the values at respective control time points of each group.

	FA		MD		$\lambda_{//}$		$\lambda_{\perp}$	
	Young	Control	Young	Control	Young	Control	Young	Control
2 hrs	1.8±1.6	-5.5±3.9	-11.0±2.5	-7.5±3.1	-10.3±2.7	-8.9±2.7	-11.5±2.3	-6.4±3.4
Day 2	11.5±7.4	-1.4±4.7	-10.8±4.4	-9.0±1.3	-7.6±3.5	-9.6±1.5	-13.6±5.8	-8.6±1.7
Day 9	11.5±4.2	7.2±7.9	-7.8±3.5	-9.9±1.7	-4.5±3.0	-8.5±2.7	-10.8±4.3	-11.0±1.7
Day 21	-3.8±3.1	1.7±2.0	-2.7±1.6	-3.6±1.7	-3.6±1.8	-3.2±1.6	-1.8±2.0	-3.8±1.8

control group and ≈ 100 for young group, which were high enough for reliable DTI index quantification [19]. Increase of FA was observed after ischemia injury, which may be probably due to increase of myocyte packing density or augmentation of intracellular space arising from myocyte swelling or inflammation. Concurrently, shrinkage of extracellular space was found, which resulted in decrease of mean and directional diffusivities. The DTI indices were found to be renormalized at day 21 due to the capability of muscle regeneration [1]. However, change of FA in young group (day 2) preceded that in mature group (day 9). In addition, recovery of mean and directional diffusivities in the young group occurred earlier (2 hours) than that of the mature group (day 9). The observed sensitive response and rapid recovery in young rats may be associated with the strong ability of myogenic proliferation for new myofiber formation

during growth stage.

In conclusion, muscle regeneration course was evaluated in young and mature rats with ischemia injury. Results showed that the young group was more sensitive to ischemia and recovered more rapidly than the mature one. These experimental findings confirmed the age-associated variation of muscle regeneration process, which may provide supplemental information for better understanding of muscle repair evolution.

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