

Magnetic Resonance Imaging and Histopathological Analysis of Experimental Muscle Injuries in a Rabbit

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Abstract

Objective To investigate the correlation of magnetic resonance imaging (MRI) with histopathological changes, and to evaluate T₂ mapping in assessing muscle trauma in a rabbit model of muscle injury.

Methods We divided 35 rabbits into seven groups that each represented a different time point after intramuscular hemorrhage and muscle injury. Hemorrhage was created by injecting autologous blood into the left legs, and muscle injury was created by scalpel incision of the biceps femoris of the right legs. At different time points, the rabbits underwent T₁-weighted imaging and T₂-weighted imaging (T₁WI and T₂WI) and T₂ mapping. T₂ relaxation times were measured, and the corresponding samples were evaluated for pathological changes.

Results After 2 h, the intramuscular hemorrhage model demonstrated an increased signal intensity on both T₁WI and T₂WI. Histological examination showed erythrocytes within the muscle bundle. On days 1 and 3, the MRI signals were decreased, and there were no significant changes after day 7. From 2 h to 3 days, the muscle-injury model showed a high signal on both T₁WI and T₂WI. Corresponding pathological changes included rupture and edema of muscle fibers, and inflammation. The abnormal signals were reduced on day 7. After day 14, the T₂WI intensity remained high. T₁WI showed no abnormal changes, but some models showed a high signal, representing fresh bleeding and fatty tissue. T₂ relaxation times were significantly different between the central and marginal regions, and between the marginal and normal regions.

Conclusion MRI clearly demonstrates intramuscular hemorrhage and muscle injury, which correlate well with histopathological changes. T₂ mapping is useful in assessing the extent of injury.

Key words: Muscle injury; Intramuscular hemorrhage; Magnetic resonance imaging; T₂ mapping

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INTRODUCTION

Muscle injury is one of the most common sports-related injuries encountered in clinical practice^[1].

Traditionally, the diagnosis mainly depends on clinical history and physical examination findings. Magnetic resonance imaging (MRI), with soft-tissue contrast and multiplanar capability superior to that of computed tomography, enables better evaluation

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of the extent and severity of injury^[2-6]. Only a few studies have dealt with experimental muscle injuries. In addition, no studies have found a consistent signal pattern for acute intramuscular hemorrhage^[7], and few studies have correlated signal changes in MRI with related pathological changes in experimental muscle injuries. Therefore, in an experimental rabbit model we investigated the correlation between MRI findings and pathological changes of intramuscular hemorrhage and muscle injuries, and evaluated T₂ mapping for assessment of the extent of muscle trauma.

MATERIALS AND METHODS

Animal Model

We randomly divided 35 male Beijing big-ear white rabbits with an average weight of 3.0 kg (range, 2.8-3.2 kg) into seven groups of five rabbits each. Within each group, four rabbits were assigned to serve as animal models and the remaining rabbit was assigned to serve as an experimental control. Each of the seven groups represented one the following post-injury time points: 2 h, 1 d, 3 d, 7 d, 14 d, 21 d, and 28 d.

Each rabbit was fixed on an operating table in the dorsal position after being anesthetized with an intramuscular injection of 0.1 mL/kg of *su mian xin* (a combination of dihydroetorphine hydrochloride, dimethylaniline thiazole, ethylenediaminetetraacetic acid, and haloperidol)^[7]. The precordial region and lateral region of the bilateral femurs were cleaned and disinfected. For the intramuscular hemorrhage model a syringe (10 mL) and needle were introduced into the left ventricle vertically on the left sternal border of the third intercostal space, and 8 mL of arterial blood was obtained. The arterial blood was then immediately injected into the biceps femoris muscle of the left leg. The injection site was approximately 4 cm over the lateral condyle of the left femur, at a depth of about 1 cm. The speed of injection was slow and uniform, and the syringe was kept in place for 10 min. The injected region was lightly compressed for 1 min with an aseptic cotton ball after the syringe was extracted, to prevent blood from streaming from the injection site. For the muscle injury model, the right bicep's femoris was exposed under aseptic conditions and an incision of 1 cm was made in the middle of the muscle belly by transecting the muscle fibers perpendicular to their course (not a complete transection). Finally, the skin was sutured. The control animals underwent the

same procedure, but blood was not injected on the left, and muscles were not transected on the right.

MRI

MRI was performed with a 1.5T whole-body scanner (Signa Twin-Speed Excite, GE Healthcare, Waukesha, WI, USA) using a surface extremity coil. 1) Transverse T₁-weighted spin-echo images (T₁WI) were obtained (echo time, 15 milliseconds; repetition times, 300, 600, and 900 milliseconds in 3 separate scans; field of view, 18 cm × 18 cm; section thickness, 4 mm; intersection gap, 0 mm; number of excitations, 2) Transverse T₂-weighted fast spin-echo images (T₂WI) were obtained (repetition time, 3300 milliseconds; echo times, 60 and 90 milliseconds in 2 separate scans; field of view, 18 cm × 14 cm; section thickness, 4 mm; intersection gap, 0 mm; number of excitations, 3) We obtained T₂ mapping and T₂ relaxation time of the normal region before injury and of the central region (positive region seen by the naked eye) and the marginal region (negative region seen by the naked eye) after injury, using FuncTool software (GE Healthcare).

Histopathology

To conduct the histopathology studies, the rabbits were immediately euthanized following MRI. Muscle samples were kept in 10% formaldehyde for 24 h. Histological sections of 4-μm thickness were obtained from 4 mm samples that corresponded to transverse MRI scans. Hematoxylin and eosin histopathology was assessed by light microscopy.

Statistical Analysis

Comparison of T₂ relaxation times for the central versus marginal regions and for the marginal versus normal regions was conducted using the paired *t*-test and SPSS Statistics software (version 17.0; IBM, Armonk, NY, USA). Statistical significance was set at *P*<0.05.

RESULTS

MRI

Intramuscular Hemorrhage Model The injected region in the left legs became edematous and demonstrated a high, slightly lamellar T₁WI signal with an undefined margin 2 h after hemorrhage. The muscle bundle gap demonstrated a high T₁WI signal, producing a feathery appearance. On the first and third days after hemorrhage, the T₁WI signal

decreased with the decreasing extent of the abnormality. The margin of the region was still blurry after one day. However, on the third day, the margin sharpened, with a discernible boundary between the injected regions and adjacent normal muscle. On the seventh day, there was either almost no positive sign or only a somewhat confused tissue structure. On the 14th through to the 28th days, no abnormality could be seen in the region (Figure 1A and 1B).

Two hours after hemorrhage, the T_1 WI and the T_2 WI had a similar appearance. However, the feather-like pattern was more obvious on the T_2 WI (Figure 1C). On the first and third days after

hemorrhage (Figure 1D), the T_1 WI and T_2 WI signals appeared similar to the naked eye, but the T_2 relaxation time was reduced for the T_2 WI and the extent of abnormal signals was greater for the T_2 WI than for the T_1 WI. The margin was best defined on the first day after hemorrhage. On the seventh day, some regions had irregular, mild-high signals on the T_2 WI, and the T_2 relaxation time was much shorter. On the 14th through to the 28th days, the T_2 relaxation time was nearly normal and there was no abnormality (Figure 1E and 1F). Table 1 and Figure 2A show T_2 relaxation times before and after injections.

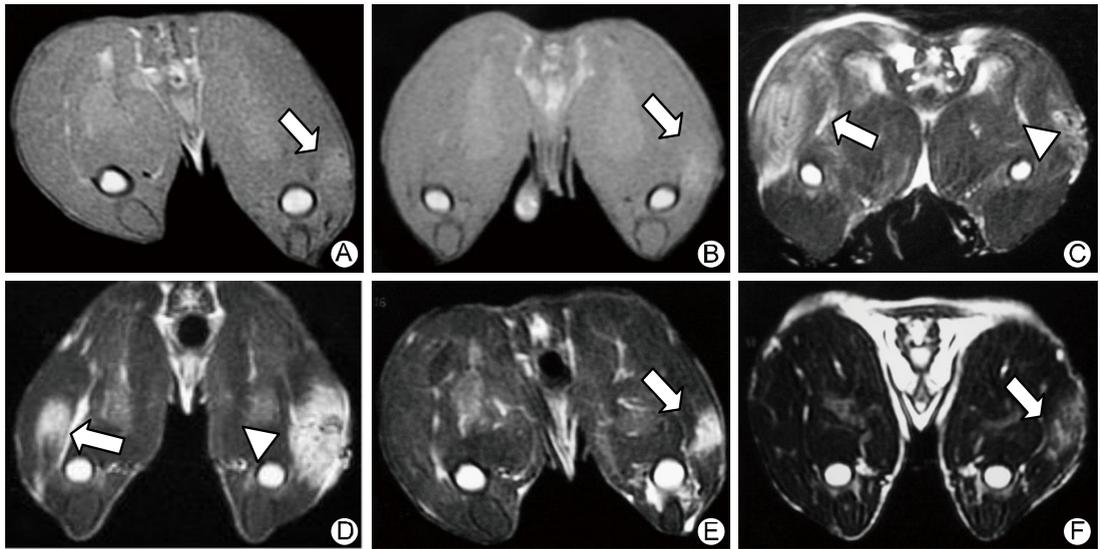


Figure 1. A. On the 14th day, the area affected of the left leg no longer had an abnormal appearance. A uniform, slightly high signal was apparent on T_1 -weighted images in the right leg (*arrow*); B. On the 21st day, there was no positive sign on T_1 -weighted images in the left leg. The uniform slightly high signal was still apparent in the right leg (*arrow*); C. At 2 h, the left leg showed an extensive high signal and a typical feathery appearance (*arrow*). The signal intensity increased in the central area of the right leg, and its margin was relatively sharp (*arrowhead*); D. At the third day, the margin of the high-signal area had become sharp in the left leg (*arrow*). In the right leg, the extent of the high signal area enlarged and its margin was blurry and showed a typical feathery appearance (*arrowhead*); E. On the 14th day, the area affected of the left leg no longer had an abnormal appearance. On T_2 -weighted images, the extent of the high signal was small and the gap had disappeared in the right leg (*arrow*); F. On the 28th day after injury, T_2 -weighted images showed slight amorphous high signal that was lower than the former in the right leg (*arrow*).

Table 1. T_2 Relaxation Times (milliseconds) for the Intramuscular-hemorrhage Model

| Parameters | Time Point after Hemorrhage | | | | | |
|---|----------------------------------|--------|--------|--------|--------|--------|
| | 2 h | 1 d | 3 d | 7 d | 14 d | 21 d |
| Mean value before injecting blood | 36.849 | 37.294 | 36.682 | 37.571 | 36.985 | 34.792 |
| | 94.194 | 70.175 | 57.026 | 47.801 | 37.175 | 31.047 |
| | 83.817 | 68.329 | 50.179 | 43.350 | 40.889 | 32.813 |
| Individual values after injecting blood | 87.630 | 72.756 | 55.532 | 45.386 | 39.258 | 37.190 |
| | 91.975 | 64.883 | 52.337 | 46.713 | 38.448 | 35.323 |
| | Mean value after injecting blood | 89.404 | 69.036 | 53.769 | 45.813 | 38.943 |

Muscle Injury Model For the T_1 WI, the right thigh showed a slightly amorphous high intensity signal 2 h after injury. There was an irregular low T_1 WI signal around the gap of the muscle tear at the injury site. The margin of the injured muscle was blurry. On the first day after injury, there was no signal within the gap. The boundary between injured muscle and normal muscle could not be identified. On the third day, no signal could be seen in the muscle gap, but the gap narrowed, with the extent of the high T_1 WI signal getting smaller and the signal intensity becoming lower. On the 7th day, the T_1 WI signal intensity was much lower and the gap signal was slightly low. On the 14th to 28th days (Figures 1A and 1B), the gap signal was nearly normal, but in some specimens, there was a slightly high, uniform T_1 WI signal in the injured region (there were 3 on the 14th day and 2 on both the 21st and 28th days) whose margin was undefined. The rest had no positive sign.

For the T_2 WI, the signal intensity and T_2 relaxation time for the injured central region had increased remarkably 2 h after injury. The other appearances were similar to those for T_1 WI, but the extent of the feathery appearance was different from that of T_1 WI because of the difference in bleeding volume. The margin was relatively clear, but the T_2 relaxation time had increased in the marginal region (negative region seen by the naked eye; Figure 1C). On the first day after injury, the

affected muscle was swollen and deformed. The boundary between the injured and the normal muscle was blurry, and the margin where the muscle had torn was sharp. The injury extent was bigger than that shown on the T_1 WI at same time. On the third day (Figure 1D), the swelling was more significant and the T_2 WI showed an extensive and marked high signal. The gap became narrower, with an amorphous high signal. The typical feathery appearance was observed, and the margin of the affected muscle was blurrier. The T_2 relaxation times of the injured central region and marginal region increased significantly. On the seventh day, swelling continued to decrease, and the extent of affected muscle had decreased with a narrower gap. The boundary between injured and normal muscle was clear. On the 14th (Figure 1E) through to the 21st days, the extent of high signal was limited and the gap disappeared. This indicates that the ruptured muscle had reconnected, except in some specimens (including specimen 1 on the 14th and 21st days)^[8]. On the 28th day (Figure 1F), the T_2 WI showed a slight, amorphous high signal, but which was less than seen at earlier points, and the T_2 relaxation time for the injured marginal region was nearly normal. All T_2 relaxation times, including those for the central region (positive region seen by the naked eye), the marginal region (negative region seen by the naked eye), and the normal region before injury are shown in Table 2 and Figure 2B.

Table 2. T_2 Relaxation Times (milliseconds) for the Muscle-injury Model

| Parameters | Time Point after Injury | | | | | | |
|---|-------------------------|---------|--------|--------|--------|--------|--------|
| | 2 h | 1 d | 3 d | 7 d | 14 d | 21 d | 28 d |
| Normal region | 37.692 | 37.033 | 36.997 | 37.597 | 36.008 | 37.089 | 35.989 |
| | 37.052 | 36.507 | 37.677 | 37.074 | 37.920 | 37.537 | 37.348 |
| | 37.208 | 37.549 | 36.124 | 36.973 | 35.995 | 36.880 | 36.684 |
| | 36.883 | 37.394 | 36.242 | 36.855 | 36.766 | 36.196 | 36.712 |
| Central region (positive region seen by the naked eye) | 67.511 | 90.195 | 96.871 | 90.423 | 82.276 | 76.975 | 68.152 |
| | 78.046 | 86.523 | 99.223 | 88.643 | 79.857 | 71.118 | 66.032 |
| | 70.741 | 92.875 | 99.960 | 92.624 | 83.442 | 71.651 | 65.887 |
| | 73.661 | 101.315 | 89.913 | 93.631 | 80.958 | 75.396 | 66.842 |
| Marginal region (negative region seen by the naked eye) | 50.511 | 52.674 | 55.392 | 50.622 | 47.445 | 45.404 | 48.302 |
| | 47.222 | 50.259 | 57.898 | 49.665 | 46.375 | 46.681 | 45.311 |
| | 49.851 | 53.983 | 56.756 | 51.067 | 46.015 | 43.336 | 46.458 |
| | 50.868 | 54.439 | 49.177 | 50.231 | 45.559 | 44.581 | 41.234 |
| P value ^a | 0.004 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 |
| P^* value ^b | 0.001 | 0.000 | 0.002 | 0.000 | 0.001 | 0.001 | 0.013 |

Note. Central region: positive region seen by the naked eye. Marginal region: negative region seen by the naked eye. ^aObtained by comparing T_2 relaxation times for the central region versus the marginal region, using the paired t -test. ^bObtained by comparing T_2 relaxation times for the marginal region versus the normal region, using the paired t -test. All P and P^* values were <0.05 and thus were statistically significant.

Control Group there were no abnormalities on either the T_1 WI or the T_2 WI in the control group.

Histopathology

Intramuscular Hemorrhage Model Two hours after the hemorrhage (Figure 3A), there was a large amount of erythrocytes within the muscle bundle gap, called the bleeding string, which was more significant in the central region than on the margin.

These blood cells were intact, and the adjacent muscle tissue was compressed. On first and third days after hemorrhage, the histological section showed infiltration of a few inflammatory cells and edema of the marginal muscle of the bleeding string because of compression. These blood cells were morphologically intact. The edema was not apparent by the third day. By the seventh day, there were a large number of ruptured blood cells, but the width

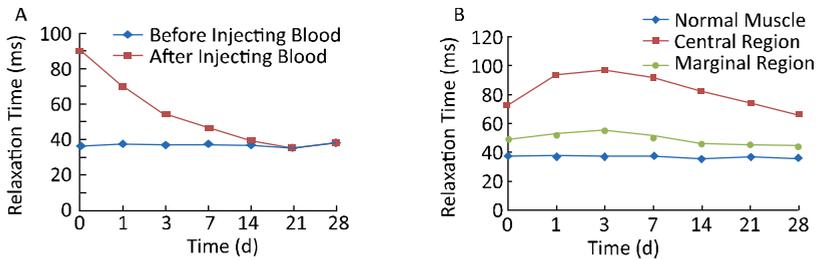


Figure 2. A. T_2 relaxation time of the intramuscular-hemorrhage model at every time point; B. T_2 relaxation time of the muscle-injury model at every time point.

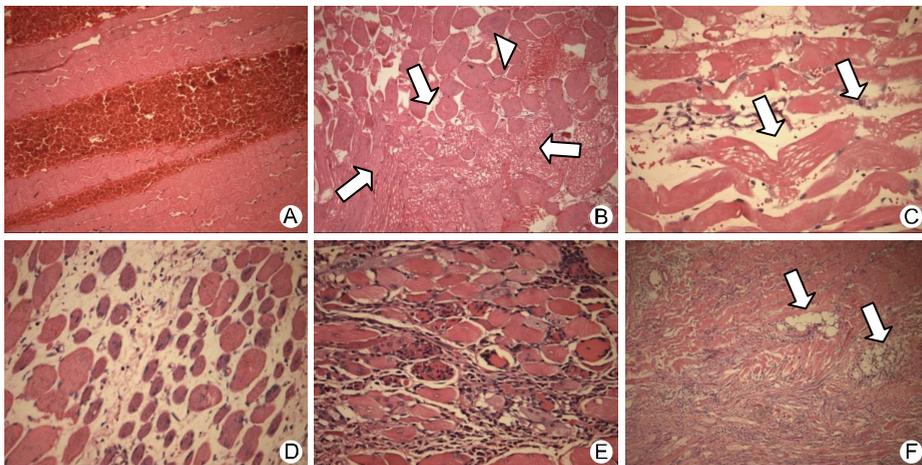


Figure 3. A. At 2 h after hemorrhage, there was a large amount of erythrocytes within the muscle-bundle gap, and these blood cells were intact in pathological section of the left leg (hematoxylin and eosin; original magnification $\times 40$); B. At 2 h after injury, there were muscular fibers, connective tissue edema (arrow), and lots of erythrocytes (arrowhead) in pathological section of the right leg (hematoxylin and eosin; original magnification $\times 20$); C. At the third day after injury, the degeneration and rupture of muscular fibers was apparent (arrow), and there were mainly lymphocytes in the pathological section of the right leg (hematoxylin and eosin; original magnification $\times 40$); D. The seventh day after injury represented the transition from the acute reaction to the repair stage, including hyperplasia of the fibers and blood vessels. Sporadic myoblasts were seen, and the inflammatory cells mainly included lymphocytes and plasmocytes in the pathological section of the right leg (hematoxylin and eosin; original magnification $\times 40$); E. On the 14th day after injury, the hyperplasia was quite evident, including a large number of myoblasts in the pathological section of the right leg (hematoxylin and eosin; original magnification $\times 40$); F. On the 28th day after injury, a large amount of fiber tissue was seen and the adipose tissue had collected in the marginal region (arrow) in the pathological section of the right leg (hematoxylin and eosin; original magnification $\times 40$).

of the bleeding string was unchanged. The outline of the adjacent muscle fiber was still vague. By the 14th day, the width of the bleeding string had become obviously narrower where there were only a few fragmented blood cells. The marginal muscle had become normal. On the 21st and the 28th days, no abnormality was seen under microscope.

Muscle Injury Model Two hours after the injury (Figure 3B), there was rupture and edema of the muscle fibers, edema of the connective tissue, and a high number of erythrocytes. The muscle fibers had thickened, and their gap had widened. On the first day after injury, the muscle had the characteristic appearance of an inflammatory reaction, including a large amount of neutrophils and a few eosinophilic granulocytes. The muscle fibers had agglutinated and lightened in color. On the third day (Figure 3C), the extent of injury had enlarged, with the inflammatory reaction peaking as the fibers degenerated and ruptured. Hyperplastic granulation tissue and a few myoblasts were visible. On the seventh day, the transition from the acute reaction to the repair stage was apparent. There was necrosis of some muscle fibers, along with hyperplasia of the fibers and blood vessels. Sporadic myoblasts were seen. The inflammatory cells mainly included lymphocytes and plasmocytes (Figure 3D). On the 14th day, the changes of repair predominated (Figure 3E). There was active hyperplasia, with a large number of myoblasts. Some neogenetic immature fiber tissue and a few erythrocytes could be seen. By the 21st day, the fasciculated neogenetic fiber tissue had become ripe. At the 28th day (Figure 3F), repair was complete and the edges of the rupture had been reconnected by fibrous tissue. Some fatty tissue in the marginal region and some sporadic lymphocytes and myoblasts were seen.

DISCUSSION

Muscle injury with intramuscular hemorrhage has been extensively studied in laboratory animals and in clinical settings. De Smet et al.^[7] reviewed eight studies of intramuscular hemorrhage that had not found consistent signal patterns for intramuscular blood. Therefore, we established an animal model to conveniently analyze the MRI signal features, and to compare with the histopathology.

In our study, we injected arterial blood from the heart into the left leg to simulate intramuscular hemorrhage. Subsequently, we studied the correlation of MRI findings and the corresponding

pathological appearance across multiple time points. The MRI findings for intracranial hemorrhage depend mainly on extensive investigation of hemoglobin changes, but there are differences in MRI findings for muscle hemorrhage. Two hours after hemorrhage in our specimens, a slightly high signal was demonstrated for both the T_1 WI and the T_2 WI. By the first and third days after hemorrhage, the signal decreased on the T_1 WI and the T_2 WI and its margin had sharpened. Beyond the seventh day after hemorrhage, there was nearly no abnormality. Similar findings were demonstrated in a study by Spielmann et al.^[8] in which a high signal on the T_2 WI was observed after a blood injection, but the signal started to decrease after a few minutes, which is similar to our findings.

We believe that radiographic and pathological findings are relative to the muscle tissue itself. Muscle is made up of both fibers and bundles, which produces fasciculation^[9-12]. We found gaps not only among muscle fibers and bundles but also among muscles. When the muscle is injured, blood diffuses along these gaps, which produces a feathery appearance. This is different from the hematoma commonly seen with intracranial hemorrhage. Swensen et al.^[13] proposed that hematoma originates from the collection of a large amount of blood in a space or potential space. In intramuscular hemorrhage, the blood is diffused between the muscular fibers and thus reduces the impact of hemoglobin changes on MRI. In our preliminary experiment, there was an instance of hematoma with signals similar to those in intracranial hemorrhage. In the hemorrhagic region (except for blood cells), normal muscle and degenerative muscle and edema affected the MRI signal feature.

Signal intensity can be affected by many factors. We found that in intramuscular hemorrhage, the T_2 relaxation time of liquid (blood and edema) was longer than that of normal muscle, so a high signal was demonstrated on the T_2 WI. The long T_2 relaxation time affects the signal intensity on the T_1 WI^[14-17]. Muscle has a relatively high tolerance for hypoxia, in contrast to brain tissue. Therefore, the reaction in our experiment was trivial when muscle was compressed by blood. The high signal on the T_2 WI showed a little edema in adjacent muscle, while the margin was blurry and the boundary was unclear between injured muscle and normal muscle.

In the intracranial hemorrhage model, injection of arterial blood from the heart into the brain has

been extensively used, but this practice has many shortcomings in modeling intramuscular hemorrhage. In addition to the reasons already mentioned, rabbits have good recovery capability, but the injury and inflammatory reaction obtained by injection of blood was slight, which is different from muscle injury in the human body.

We created a muscle-injury model by cutting the biceps femoris, resulting in muscle rupture. Our study confirmed that the MRI signal changes of intramuscular hemorrhage were different from those of hematoma. From two hours to the third day after injury, both the T_1 WI and the T_2 WI showed a high signal, which is pathologically characterized by the degeneration and edema of muscle tissue, hemorrhage in the injury region, and an inflammatory reaction. During this period, the margin of the affected muscle was blurry. The histopathology indicates that the stress reaction was followed by edema of the adjacent tissue with a widened tissue space, which resulted in the indirect injury of marginal muscle. The extent of abnormality was larger on the T_2 WI than on the T_1 WI. Since the T_2 WI is more sensitive for demonstrating tissue edema, it better depicts the affected region. On the third day after injury, we found the histopathology reaction to be the most significant, which was consistent with the large extent of injury shown on the T_2 WI at that point. By the seventh day, histological repair was apparent and the extent of injury had become smaller on the T_1 WI and the T_2 WI. The high signal on the T_1 WI and the T_2 WI was associated with ectocytic methemoglobin.

There were some interesting phenomena from the 14th through to the 28th days. There was no positive sign on T_1 WI in some specimens, yet plenty of fiber tissue histology. A persistent, slightly high signal was observed in the other five specimens, and histological examination showed a large collection of erythrocytes in the local region. One reason for this may be because of recurrent hemorrhage, whereby new vessels ruptured in unhealed areas when the rabbit was in motion. Another reason, also proposed by Boutin et al.^[18] proposed might be because of a collection of adipose tissue, which was observed by histology in the marginal region on the 28th day.

We performed T_2 mapping of the normal region before injury and of the central region and marginal region after injury, and then measured and statistically analyzed the T_2 relaxation time at every time point. T_2 mapping is quite sensitive to microscopic hydrogen. In our study, T_2 relaxation

time changed in the negative region, as seen by the naked eye. T_2 relaxation time reached a peak on the third day (Table 2), which corresponds to the pathological appearance of severe edema. We believe that as a quantitative measurement, T_2 mapping is a better tool than conventional T_2 WI and ferucarbotran-enhanced T_2 WI for assessing the severity and the extent of muscle trauma. A more accurate assessment is helpful for clinicians in choosing appropriate treatments and determining prognosis. MRI can clearly depict intramuscular hemorrhage and muscle injury, and its findings correlate well with histopathological changes. T_2 mapping is a useful tool in assessing the extent of injuries, and MRI is the best choice for visualizing muscle hemorrhage and injuries.

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DECLARATION

The authors declare that they have no conflict of interest.

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