Quantifying pressure sore-related muscle damage using high-resolution MRI

E. M. H. Bosboom, C. V. C. Bouten, C. W. J. Oomens, F. P. T. Baaijens, and K. Nicolay. Quantifying pressure sore-related muscle damage using high-resolution MRI. J Appl Physiol 95: 2235–2240, 2003. First published June 20, 2003; 10.1152/japplphysiol.01023.2001.—To obtain insight into the etiology of deep pressure sores, understanding of the relationship between prolonged transverse loading and local muscle damage is required. To date, the amount and location of muscle damage have been determined by histological examination. In the present study, we determined whether T2-weighted high-resolution magnetic resonance imaging (MRI) can also be applied to evaluate muscle tissue after prolonged transverse loading. The tibialis anterior muscle and overlying skin in the right hindlimbs of five rats were compressed between an indenter and the tibia. The in vivo magnetic resonance images of the loaded and contralateral hindlimbs were obtained 24 h after load application. The tibialis anterior muscles were then processed for histological examination. In the magnetic resonance images of all five loaded hindlimbs, signal intensity appeared higher in the loaded regions of the muscle compared with the unloaded regions. The location of the higher signal intensity coincided with the location of damage assessed from histology. Also the amount of damage determined with MRI was in good agreement with the amount of damage assessed from histological examination. Because MRI is nondestructive, it is a promising alternative for histology in research on pressure sore etiology, especially in follow-up studies to evaluate the development of muscle damage in time and in clinical studies.

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damage in the tissue, it should be possible to validate the above-mentioned hypothesis.

Another drawback of previous animal studies is that histological examination was used for the evaluation of the tissue. This has two main disadvantages. First, it is labor intensive and thus hampers experiments on a larger scale. In addition, histology requires destruction of the tissue and therefore largely excludes clinical studies as well as follow-up studies to investigate the development of tissue damage in time. Magnetic resonance imaging (MRI) is considered a promising alternative, because it is nondestructive and less time consuming. Although MRI has proven to be a valuable tool in the planning of therapy for large pressure sores (10) as well as in the evaluation of nonhealing pressure sores (19), it was not applied to assess muscle damage related to pressure sores in an early stage. It has been shown in the past that MRI can be used to assess damage in different types of soft tissue (2, 8, 14, 16).

In the present study, the ability of MRI to quantify local muscle damage after prolonged compressive loading is studied. Earlier histological examination revealed that the localized damage in the developed rat models showed complete lysation of muscle fibers (see Fig. 4 in Ref. 4). It is expected that this alteration in the macromolecular makeup of the tissue results in changes of the T2 relaxation time. This implies that we should be able to detect the localization of damage at a resolution comparable to a few cells (150 × 150 μm). High-resolution T2-weighted MRI is thus applied to evaluate the muscle tissue, and histological examination is used to evaluate the MRI results. The magnetic resonance (MR) images are obtained 24 h after the loading, as histological examination demonstrated that at this point the lysation of muscle fibers can be clearly observed.

METHODS

The animal experiments consisted of three parts. First, the tibialis anterior muscle in the right hindlimb of a rat was loaded. This loading procedure took place outside the MRI setup. Second, 24 h after loading, in vivo MR images were obtained of the loaded and contralateral hindlimb. Third, after the MRI, the tibialis anterior muscles were excised and processed for histological examination. Both the animal model and the histological techniques have been presented previously (4) and will only be described here in short. The experimental protocol was approved by the Animal Care Committee of the Maastricht University.

Loading procedure. Experiments were performed on five male Brown Norway rats weighing 200–230 g. The rats were anesthetized with the use of a combination of ketamine (Nimatek, 0.1 ml/100 g ip) and xylazine (Sedamun, 0.05 ml/100 g ip). When required, supplemental doses of 0.1 ml ketamine were supplied. Body temperature of the rats was measured with a rectal probe and kept at 35–37°C by use of a heating pad. Before the loading, hairs on the right tibialis anterior region were cut off, with care taken not to damage the skin. The rat was then placed supine in a 4.7-Tesla Varian 200/400 system, operating at 200 MHz. A solenoidal radio frequency coil (inner diameter 16 mm, 3 windings) was placed around the hindlimb, and the foot was taped to a footplate so that the knee angle was ∼180° and the ankle angle ∼90°. Longitudinal scout images of the hindlimb were made to plan the transverse imaging perpendicular to the proximal aponeurosis of the tibialis anterior muscle. A T2-weighted spin echo sequence was then applied (echo time = 45 ms, repetition time = 5 s) to collect 51 consecutive 0.5-mm-thick transverse slices. The images had a field of view of 30 × 30 mm and a resolution of 256 × 256 pixels and were the average of two acquisitions. Both the loaded and the contralateral hindlimb were imaged. The acquisition time was 45 min per hindlimb. Immediately after the in vivo MRI, the rat was killed and perfusion fixed with 4% buffered formalin. Both hindlimbs of the rat were excised and stored in formalin. T2-weighted MR images were also obtained from these fixed hindlimbs.

Histological analysis. The loaded tibialis anterior muscle was excised at least a month after the perfusion fixation, to ensure a completed tissue fixation. Next, the muscle was dehydrated in a series of alcohol solutions and embedded in plastic (Technovit 7100, Kulzer). To obtain a reference system three parallel holes (diameter of 0.5 mm) were drilled in the plastic around the muscle. Next, the muscle was cut longitudinally, perpendicular to the direction of load application (section thickness of 3 μm). Every 50th section was saved and mounted on an objective slide. The samples were stained with toluidine blue to visualize both the cross-striated appearance of the muscle fibers and the cell nuclei.

The histological sections were digitized by use of a Leica DMRA automated microscope. By using an image processing program in Matlab, the contour of the muscle was automatically detected. The damage was indicated manually from evidence of loss of cross striation of the muscle fibers and/or the infiltration of inflammatory cells. When a muscle fiber was damaged, at least every 30 μm a mark was placed in the center of the damaged fiber. In this way, a resolution of ∼30 μm, which is better than the in-plane resolution of the MRI (∼120 μm) and thus suffices for our purposes, i.e., the comparison of histology and MRI. The longitudinal histological sections were recompiled by using the reference system, so that a three-dimensional reconstruction of the damaged area in the muscle was obtained.

J Appl Physiol • VOL 95 • DECEMBER 2003 • www.jap.org
Comparison MRI and histology. A comparison between the 0.5-mm-thick transverse MR images and the 3-μm-thick longitudinal histological sections cannot be made directly. To enable a comparison, the three-dimensional histological reconstruction of the muscle was divided in transverse slices with a thickness of 0.5 mm. By projection of this 0.5-mm-thick transverse slice in a two-dimensional plane, a histological image was obtained. This histological image was compared with the MR image.

Qualitatively, the correspondence in damage location was determined by a visual comparison of the MR image and the histological image. For this purpose, the MR image and the histological image positioned at the middle of the indenter were taken.

Quantitatively, the amount of damage in the MR image and the amount of damage in the histological image were compared. Again, the images positioned at the middle of the indenter were taken. The excision and dehydration of the muscle for histology could give rise to a small variation in muscle volume. Therefore, the damage in the MR image and the damage in the histological image were expressed as a percentage of the muscle area. In the MR image, first the mean signal intensity and standard deviation of undamaged muscle tissue were determined in an unloaded region. Next, the tibialis anterior muscle was manually traced. Finally, the damaged area in the muscle was assessed by applying a threshold level of the mean signal intensity plus thrice the standard deviation. The percentage of MRI damage was then calculated by dividing the damaged area by the muscle area. In the histological image, the marks are so densely placed that they completely cover the damaged area. The percentage of histological damage was calculated by dividing the area covered by the marks, i.e., the damaged area, by the muscle area. The damaged areas were determined for all loaded muscles and for one control muscle.

Finally, linear regression analysis was performed on the six data points to determine the correlation between the MRI and the histology.

RESULTS

In the MR images of all five loaded hindlimbs, a higher signal intensity was visible in the loaded regions of the tibialis anterior muscle compared with the unloaded muscles (Fig. 1B). The regions with a higher signal intensity showed a patchy appearance. In the MR images of the contralateral hindlimbs no abnormalities in signal intensity were noted (Fig. 1A). Hence, the regions with a higher signal intensity were considered to be damaged. Despite the strictly controlled experimental and loading conditions, there was a large deviation in the amount of damage (Fig. 2), ranging from a highly localized small volume (rat 1) to a more diffuse large volume (rat 5). However, in all rats muscle damage ran from superficial to deep muscle layers.

For one rat (rat 3), next to the regions with high signal intensity, there was also a region with a lower
signal intensity compared with unloaded control tissue. When excising the tibialis anterior muscle of this rat, we noted a small hemorrhage, which is known to lead to signal hypointensities in T2-weighted MRI (2). No abnormalities were seen on excision of the muscles of the other rats. In the MR images of two rats (rats 3 and 5), the extensor digitorum longus muscle appeared to be damaged as well. For these two rats, the extensor digitorum muscle was also included in the histological analysis as well as in the comparison between MRI and histology.

Similar to the MRI, the histological examination revealed damage in all five loaded muscles. Damage consisted of a loss of cross striation of muscle fibers predominantly accompanied by infiltration of mononuclear cells. Cell infiltration without loss of cross striation was not observed. An example of the three-dimensional reconstruction of the histological data is shown in Fig. 3. Furthermore, from histological examination it was found that in some regions only single fibers were affected, whereas in other regions fiber damage involved all fibers in a zone of >1 mm in cross-fiber direction.

Qualitatively, the location of damage in the MR image coincided well with the location of damage determined with histological examination (Fig. 2). This holds for all rats, despite the large variations in damage, which is a strong argument to support the assumption that a higher T2 signal correlates with damage. There were some small differences in the shape of the tibialis anterior muscles between MRI and histology, but these were likely due to the excision of the muscle.

Quantitatively, the correlation between the damaged area assessed in the MR images and the damaged area determined from histological examination was high (Fig. 4, $R^2 = 0.926$). The lowest correspondence is found for the muscle that had a small hemorrhage. This hemorrhage resulted in a decrease in signal intensity on the MR images and is thus not included in the determination of the amount of damage with MRI. In the histological slices, however, the area with the hemorrhage showed the normal signs of damage and is therefore included in the determination of the amount of damage.

The results of the ex vivo MRI did not correspond to the results of the in vivo MRI or the histology. Moreover, results differed largely for the five rats. In three muscles, damage led to a decrease in signal intensity in the ex vivo MR images, in one muscle an increase in signal intensity was seen, and in the last muscle no abnormalities were visible. Most likely, this variation in results is caused by the formalin fixation, which alters the water compartmentation.

DISCUSSION

To obtain insight into the etiology of pressure sores, understanding is required of the relationship between external mechanical loading and the location and amount of tissue damage. To date, tissue damage induced by external loading was determined by histological examination, requiring tissue destruction and elaborate measurements. In this study we demonstrated that T2-weighted high-resolution MRI is a promising alternative to evaluate muscle tissue after prolonged compressive loading. Although it has been
demonstrated before that T2-weighted MRI can be used to find damage in muscle or other tissues, a detailed comparison of the location and magnitude assessed using MRI and histology at this high resolution (150 × 150 μm) has not been described before.

In the MR images a higher signal intensity is visible in the loaded tibialis anterior muscle compared with the unloaded muscles in the same hindlimb. The increase in signal intensity on the MR images is found in a localized zone near the place of indentation and clearly coincides with the damage found with histology. Increasing signal intensity on T2-weighted MR images of muscle can reflect a range of pathologies, including edema, necrosis, inflammation, and fatty infiltrations (8). Hence, the imaging method applied in the present study is nonspecific and does not give insight into the damage mechanisms. However, improved insights could be acquired in the same experimental setup by using other imaging methods.

The area of damage determined in the MR images correlates well with the area of damage assessed with histological examination, i.e., >90% of the variation in histological damage can also be detected by employing MRI. The comparison of histology with MRI was limited to two-dimensional transverse slices. However, because these slices were representative for the whole muscle, we were convinced that the conclusions remain valid for the three-dimensional situation.

The correlation was determined from linear regression analysis based on only 6 data points. The number of rats studied was that limited because of the fact that a histological study at this resolution is very labor intensive, which was the major reason to switch to using MRI. However, the agreement in the amount and location of damage found in both the MRI study and the histology study, together with the large spread found between rats, strongly supports the hypothesis that T2-weighted MRI can be used. Despite the small number of experiments, we still thought it to be advisable to employ a regression analysis, because the damaged areas induced in the present study cover a large range, from 0 to 70%, and are rather evenly spread over this range. The large range of damaged areas has the additional advantage that the relation between MRI and histology can be employed in future research, as newly induced damage likely falls within the present range.

The resolution of the MRI is lower than the resolution of the histology, i.e., every pixel in the MRI comprises a number of fibers whereas histological examination can be performed on the subfiber level. Hence, histological techniques remain preferred when subfiber accuracy is required or when very small amounts of damage are involved. However, the somewhat lower accuracy, which is still on the submillimeter level, does not outweigh the enormous benefits of MRI, being less labor intensive and nondestructive. MRI is thus a promising alternative in research on pressure sore etiology, especially in large-scale and clinical studies. Moreover, MRI enables follow-up studies to evaluate the development of muscle damage in time. In addition, a large variety of imaging techniques have been developed that can be applied to assess structure, function, and metabolism of skeletal muscle. For example, using contrast agents could enhance skeletal muscle fiber lesions (23), the components of the multiequponential T2 relaxation decay reflect the water content and compartmentation in the damaged area (21), and 31P-NMR diffusion spectroscopy gives insight into changes in the metabolism for damaged tissue (7).

We gratefully acknowledge Gerard van Vliet for technical assistance and Erwin Blezer and Boudewijn van der Sanden for help with the animal experiments.

REFERENCES