Magnetic resonance elastography of skeletal muscle deep tissue injury

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The current state-of-the-art diagnosis method for deep tissue injury in muscle, a subcategory of pressure ulcers, is palpation. It is recognized that deep tissue injury is frequently preceded by altered biomechanical properties. A quantitative understanding of the changes in biomechanical properties preceding and during deep tissue injury development is therefore highly desired. In this paper we quantified the spatial–temporal changes in mechanical properties upon damage development and recovery in a rat model of deep tissue injury.

Deep tissue injury was induced in nine rats by two hours of sustained deformation of the tibialis anterior muscle. Magnetic resonance elastography (MRE), T2-weighted, and T2-mapping measurements were performed before, directly after indentation, and at several timepoints during a 14-day follow-up.

The results revealed a local hotspot of elevated shear modulus (from 3.30 ± 0.14 kPa before to 4.22 ± 0.90 kPa after) near the center of deformation at Day 0, whereas the T2 was elevated in a larger area. During recovery there was a clear difference in the time course of the shear modulus and T2. Whereas T2 showed a gradual normalization towards baseline, the shear modulus dropped below baseline from Day 3 up to Day 10 (from 3.29 ± 0.07 kPa before to 2.68 ± 0.23 kPa at Day 10, P < 0.001), followed by a normalization at Day 14.

In conclusion, we found an initial increase in shear modulus directly after two hours of damage-inducing deformation, which was followed by decreased shear modulus from Day 3 up to Day 10, and subsequent normalization. The lower shear modulus originates from the moderate to severe degeneration of the muscle. MRE stiffness values were affected in a smaller area as compared with T2. Since T2 elevation is related to edema, distributing along the muscle fibers proximally and distally from the injury, we suggest that MRE is more specific than T2 for localization of the actual damaged area.
Sustained deformation of skeletal muscle in the proximity of bony structures may lead to deep tissue injury, which is one of the subcategories in the pressure ulcer classification system by the National and European Pressure Ulcer Advisory Panel (NPUAP/EPUAP). Deep tissue injury commonly starts at the bone-skeletal muscle interface, and therefore is initially invisible and could remain undetected for days or weeks. As soon as the injury becomes visible as a purple or maroon discolored skin spot at the skin surface, it might progress rapidly into a severe and difficult to heal stage III or stage IV pressure ulcer. Intensive- and acute-care patients, hospice patients, as well as people that require long-term care, for example, after a spinal cord injury or stroke, are at particular high risk of developing a deep tissue injury. Deep tissue injury is related to increased morbidity and mortality and puts a significant cost burden on the healthcare system.

Palpation is one of the oldest ways by which medical doctors perform a physical examination to detect disease. Also, for the detection of pressure ulcers, experienced clinicians and wound nurses frequently use palpation. In fact, in the international pressure ulcer guidelines, palpation is included as one of the diagnostic tools. In the section describing deep tissue injury it reads: "The area may be preceded by tissue that is painful, firm, mushy, boggy, warmer or cooler as compared to adjacent tissue." However, the description is of a rather qualitative nature. A quantitative understanding of the changes in tissue biomechanical properties preceding and during wound development is therefore highly desired.

Most of the current knowledge on changes in biomechanical properties related to deep tissue injury and pressure ulcers in general were obtained from controlled indentation experiments in animals and finite element analysis (FEA). One of the well-established animal models of deep tissue injury involves the deformation of the rat tibialis anterior (TA) muscle with a custom loading device that can be placed in a MRI scanner for imaging during the development of the damage. Several MRI and histopathologic readouts were employed to provide a comprehensive understanding of the damage development, recovery, and regeneration in this model. It was found that the initial tissue response to deformation started at some distance from the center of indentation, affecting a relatively large area. Secondly, a single indentation of the TA muscle resulted in muscle damage that required at least two weeks to recover. Finally, MRI provided specific imaging parameters with diagnostic relevance for deep tissue injury-related muscle damage development and remodeling. T2-weighted and T2*-weighted MRI corresponded with edema, increased interstitial space, muscle cell damage, inflammatory onset, and inflammation. T2* provided a readout of tissue perfusion, hemorrhages, and inflammation. Diffusion-weighted imaging is sensitive to the integrity of the tissue microstructure and reflects muscle degeneration and edema, as well as extracellular matrix remodeling during the muscle remodeling processes. Time of flight MR angiography provided information on occlusion of blood flow during the indentation period. Furthermore, the use of an animal-specific FEA model, for which the geometry and loading conditions were derived from MRI, supplied estimations of the local tissue deformations. This combined experimental-numerical approach resulted in new understandings of the biomechanical conditions that contribute to the development of deep tissue injury. In those previous studies, the involved tissues were always considered to have homogeneous material properties (stiffness). Because the entire experiment was kinematically driven, with a described displacement of the indenter and a fixation of the leg of the animal, the local tissue deformations could be determined without accurate knowledge of the material properties. For the studies in the current paper it is hypothesized that, due to changes in the tissue as a result of the mechanical load, local changes in stiffness will occur leading to heterogeneous material properties. This requires a new method to determine these local properties.

The aim of this study was therefore to quantify the spatial-temporal changes in skeletal muscle mechanical properties upon damage development and (partial) recovery in the rat model of deep tissue injury. For this purpose, we employed the recently introduced rat magnetic resonance elastography (MRE) setup, which facilitates direct quantification of muscle shear modulus values. This setup enables MRE estimations of local muscle tissue mechanical properties pre, during (not shown), and post-indentation of the rat TA muscle in the MRI scanner.

2 | METHODS

2.1 | Animal model

A total of nine Sprague-Dawley rats (♀, 11-week-old, Charles River, Paris, France) were included. Animals were housed under standard laboratory conditions with a 12-h light/dark cycle and were maintained on a standard diet and with access to water ad libitum. All animal experiments were approved by the Animal Care and Use Committee of Maastricht University (protocol 2013–047, Maastricht University, Maastricht, The Netherlands) and performed in accordance with Directive 2010/63/EU for animal experiments in the European Union.

Figure 1 shows the timeline of the longitudinal study. MRE, T2-weighted, and T2-mapping measurements were performed pre and post-2 h damage-inducing deformation of the TA muscle at Day 0, as well as at Day 3, 5, 7, 10, and 14. The T2-weighted and T2-mapping measurements
were also performed during the period of deformation. The rat was placed in supine position in the MR compatible indentation and MRE setup and anesthetized with isoflurane (4.0 vol.% for induction, 1.0–2.0 vol.% for maintenance) in 0.6 L/min medical air. To review briefly, the setup consisted of an indentation and MRE part (Figure 2). The indenter rod was positioned on the rat's TA muscle using a movable indenter holder and rotatable half arch. The MRE transducer piston is brought into motion via a drive rod attached to an electromagnetically driven shaker (LDS V201, Bruel and Kjaer, Royston, UK) and cantilever. Buprenorphine (0.05 mg/kg subcutaneously) was administered for analgesia. Eye ointment was applied to prevent eye dehydration. Body temperature was maintained at 35–37°C with a heating blanket and monitored with a rectal temperature sensor. Respiration was monitored with a balloon pressure sensor placed on the abdomen and kept stable by adjusting the anesthesia. The right leg of the rat was shaved and positioned in a u-shaped profile filled with alginate molding substance for firm fixation and susceptibility matching. The TA muscle in the rat hindleg was deformed by manually pushing the indenter rod into the muscle. The extent and severity of damage caused by deformation of the TA and the time course of recovery is variable among rats and depends on various factors, including the individual rat leg anatomy, the depth and angle of indentation, the induced strain in the muscle as well as the extent of ischemia. The duration of 2 h deformation was in line with previous studies, in which different durations of deformation, ischemia and reperfusion were studied. During the deformation period, part of the alginate was removed to make the TA muscle assessable for the indenter. The MRE transducer piston was coupled to the alginate, close to the tendon at the distal side of the TA muscle. After the last measurement at Day 14, the rats were sacrificed by means of exsanguination from the inferior vena cava. This procedure was performed under anesthesia and after administration of analgesia.

### 2.2 MRI

Measurements were performed with a 7.0 T small animal MRI scanner (Bruker BioSpin MRI GmbH, Ettlingen, Germany) equipped with a 660 mT/m, 4570 T/m/s gradient coil (BGA-125 HP, Bruker BioSpin MRI GmbH). An 86-mm-inner-diameter quadrature transmit coil was used in combination with a 20 or 30 mm receive surface coil, placed on top of the TA muscle inside the indentation device.

For anatomical information, axial T2-weighted MRI was performed with a 2D rapid imaging with refocused echoes (RARE) sequence. The TA muscle was imaged by 16 1-mm-thick slices with field of view (FOV) = 40 x 40 mm², a 512 x 512 reconstruction matrix (MTX), number of averages (NEX) = 5, RARE factor = 8, effective echo time (TEeff) = 40 ms, repetition time (TR) = 2500 ms, chemical-shift selective (CHESS) fat suppression, and acquisition time 13 min.

MRE images were acquired with an in-house-developed spin echo echo-planar-imaging (SE-EPI) MRE sequence with CHESS fat suppression in coronal orientation. Motion encoding gradients (MEG) were placed symmetrically around the SE inversion RF pulse. The other parameters were: number of slices = 18, slice thickness = 0.3125 mm, FOV = 30 x 60 mm², MTX = 96 x 192, TE = 26.2 ms, TR = 1000 ms, actuator and MEG
frequency = 900 Hz, MEG-shape = sinusoidal, number of MEG cycles = 5, MEG amplitude = 660 mT/m, number of EPI segments = 4, NEX = 8, number of MRE phase offsets = 8, number of encoding directions = 3 (slice, phase, frequency encoding) plus 1 reference, and acquisition time ~16 min.

For indication of the location of skeletal muscle damage, $T_2$-mapping MRI was performed with a 2D multi-slice multi-echo (MSME) sequence in coronal orientation. The other acquisition parameters were: CHESS fat suppression, number of slices = 6, slice thickness = 0.9375 mm, FOV = 60 x 30 mm², MTX = 512 x 256, 33% zero filling, 20 echoes (TE = 10.18–203.5 ms), TR = 3200 ms, and acquisition time ~10 min, covering the same imaging volume as the MRE acquisition.

2.3 Data analysis

The MRE acquisitions provide phase data for the harmonic vibrations which are proportional to the harmonic displacements. The displacement data can be converted to maps of viscoelastic properties with a local inversion algorithm, i.e., images representing the local tissue linear elastic dynamic shear modulus $G_d$, loss shear modulus $G_l$, magnitude of the complex shear modulus $|G^*|$, and phase angle $\phi = \arctan \left( \frac{G_l}{G_d} \right)$. The inversion process assumes linear (visco) elasticity, isotropy, and local homogeneity for all tissues, and aims to solve the partial differential equation: $-\rho \omega^2 q(x) = G^2 \nabla^2 q(x)$, where $G^2$ is the complex shear modulus, $\rho$ is the density, $q$ is the curl of the complex displacement vector $(q(x) = \nabla \times u(x))$ derived from the MRE phase data, and $\omega$ is the known angular frequency.

Full details of the inversion algorithm implemented in the ROOT data analysis framework (ROOT 5.34/17, CERN, Meyrin, Switzerland) were previously described by Sinkus et al. To review briefly, the slice, phase, frequency and reference encoded MRE phase images were unwrapped and filtered using a three-dimensional Gaussian filter with $3 \times 3 \times 3$ support and $\sigma = 1$ voxel. To remove the compressional wave component the curl was calculated on a stencil of $3 \times 3 \times 3$ pixels, followed by reconstruction of the viscoelastic maps using the direct inversion method.

Quantitative $T_2$-maps were obtained by pixel-wise fitting the MR signal to $S(TE) = S(0)e^{-TE/T_2}$ (Mathematica 10, Wolfram Research, Champaign, IL, USA). Pixels with $R^2 < 0.9$ were excluded from subsequent analysis.

After inversion of the acquired MRE data, a stack of three middle slices—matching one $T_2$-mapping slice—were averaged. The MRE-averaged stack was interpolated to match the $T_2$-mapping resolution. Masking of the background was applied to all images. Region-of-interest (ROI)-based analysis of this selected volume was performed (Mathlab R2016a, The Mathworks, Inc., Natick, MA, USA). The ROI was defined by manually outlining the whole TA muscle, and by selecting a circular ROI of 2 $\times$ the indenter’s diameter around the center of indentation, on the first echo of the $T_2$-mapping dataset. Representative positioning of the circular ROI and whole TA ROI is shown in Figure 3E. Mean values of $T_2$, $G_d$, $G_l$, $|G^*|$, and $\varphi$ were determined in both ROIs at all timepoints. A repeated measures analysis using a linear mixed model with Bonferroni correction was conducted on both ROIs to test for significant differences of all timepoints with respect to baseline (pre-deformation) (SPSS 23, IBM, Armonk, NY, USA). MRE shear moduli with a mean nonlinearity >50% in the ROI were excluded from statistical analysis. The nonlinearity is a measure of the quality of the delivered MRE shear wave in the tissue and is defined as the ratio of the amplitude of the second harmonic of the vibrational frequency to the base frequency. Therefore, a perfect shear wave without any noise will lead to a nonlinearity of 0. Contrarily, if the second harmonic is as powerful as the base frequency, the ratio will be 1, i.e., 100%.

3 RESULTS

Anatomical $T_2$-weighted MRI was used for planning of the coronal $T_2$-mapping and MRE imaging volume. Representative axial $T_2$-weighted MR images of a central slice acquired pre, during, and post-2 h of deformation are shown in Figure 3A, B and C, respectively. In Figure 3A, the TA muscle, tibia bone, and planned coronal imaging volume are indicated with arrows. The central coronal slice location used for all analysis is indicated with a dashed line. The indenter, filled with a liquid solution of CuSO$_4$, was clearly visible (indicated by an arrow in Figure 3B). Post-deformation, the whole TA compartment was hyperintense (Figure 3C). A movie looping over all acquired slices, from ankle to knee, of pre, during, and post-2 h of deformation, is included in the supporting information for this article (Supplemental 1).

The corresponding coronal $T_2$-maps, MRE phase-image snapshots, and dynamic shear modulus $G_d$-maps of pre and post-deformation of the planned central coronal slice are shown in Figure 3D-1. The $T_2$-maps post-deformation showed a large region of elevated $T_2$ values in a muscle-fiber-like pattern, with somewhat lower values in the center of indentation compared with the immediate surroundings (Figure 3E, green arrow). The MRE phase-image snapshot post-deformation showed a distinct change in wave pattern compared with pre-deformation. A movie of all MRE phase-images of pre and post-2 h deformation can be found in the supporting information for this article (Supplemental II). A hotspot with increased shear dynamic shear modulus $G_d$ values was observed near the center of indentation, which colocalized with the area of somewhat lower $T_2$ values (Figure 3E, green arrow).

$T_2$, $G_d$, $G_l$, and $|G^*|$-maps during the full time course of damage induction and recovery of one representative animal with extensive damage of the TA muscle are shown in Figure 4. Maps at Day 0 pre were from healthy muscle to which all other timepoints were compared. Similar to the
example shown in Figure 3 at Day 0 after indentation resulted in elevated $T_2$ values in the whole TA muscle body and a hotspot with locally increased shear modulus values and lower $T_2$ values. The local hotspot persisted up to five days after damage induction. From Day 7 onwards $T_2$ returned to baseline values whereas shear modulus values became regionally somewhat lower compared with baseline at the later timepoints.

Individual time courses of the mean $T_2$ and $G_d$ values of the nine animals, determined in the circular ROI of 2 x the diameter of the indenter, are shown in Figure 5. A detailed summary of the mean shear modulus, phase angle, and $T_2$ values, including statistics, in the whole TA ROI and circular ROI at all timepoints, is given in Table 1. Distinctly different $T_2$ and $G_d$ time courses were observed. Whereas $T_2$ initially increases with a peak at Day 3, followed by a gradual decrease over time towards baseline values, $G_d$ showed a peak of elevated stiffness directly after end of deformation at Day 0, subsequently continued with a drop below baseline values from Day 3 up to Day 10, followed by normalization to baseline at
Day 14. Interestingly, the phase angle $\varphi$ remained constant over time. The time courses of all quantified MRE shear modulus parameters ($G_d$, $G_l$, and $|G^*|$) and $T_2$ are included in the supporting information for this article (Supplemental III).

Figure 5 also illustrates that not all animals had the same response in time after indentation. As an example, we discuss two distinctly different cases, RAT-001 (Figure 5, green line) and RAT-002 (Figure 5, magenta line). The quantitative parameter maps of RAT-001 and RAT-002 are shown in Figures 4 and 6, respectively. In RAT-002, injury from indentation was relatively mild, with $T_2$, $G_d$, $G_l$, and $|G^*|$ values not significantly different from baseline. By contrast, indentation in RAT-001 resulted in extensive TA muscle damage.

A correlation plot of $T_2$ versus $G_d$ for all animals at all timepoints is shown in Figure 7. Each timepoint is indicated with a different symbol. One rat with extensive damage of the TA muscle (RAT-009) is depicted with green symbols to highlight the $T_2$ and $G_d$ values of this representative animal. Overall, there exists no linear relation between $T_2$ and $G_d$. Instead, the initial muscle damage is characterized by increased $T_2$ and $G_d$ values (Figure 7, red arrow), whereas during recovery $G_d$ decrease precedes $T_2$ normalization (Figure 7, green arrow). For most animals $G_d$ was temporarily lower than baseline values during recovery.
TABLE 1  Quantitative MRE and T2-mapping parameter values of whole TA ROI and indentation center ROI

|                | Day 0 pre | Day 0 after | Day 3 | Day 5 | Day 7 | Day 10 | Day 14 |
|----------------|-----------|-------------|-------|-------|-------|--------|-------|
| **Whole TA**   |           |             |       |       |       |        |       |
| $T_2$ [ms]     | 38 ± 1    | 55 ± 9*     | 50 ± 11* | 44 ± 11 | 41 ± 5 | 41 ± 2* | 38 ± 4 |
| $G_d$ [kPa]    | 3.29 ± 0.07 | 3.32 ± 0.32 | 2.97 ± 0.23* | 3.01 ± 0.25 | 2.82 ± 0.23* | 2.68 ± 0.23*** | 3.09 ± 0.24 |
| $G_i$ [kPa]    | 2.11 ± 0.06 | 2.24 ± 0.26 | 1.90 ± 0.14* | 1.96 ± 0.17 | 1.83 ± 0.17* | 1.70 ± 0.12*** | 1.97 ± 0.20 |
| $|G'|$ [kPa]     | 3.94 ± 0.08 | 4.04 ± 0.41 | 3.55 ± 0.27* | 3.63 ± 0.30 | 3.39 ± 0.28* | 3.20 ± 0.25** | 3.69 ± 0.31 |
| $\varphi$ [rad] | 0.57 ± 0.01 | 0.59 ± 0.02 | 0.57 ± 0.02 | 0.58 ± 0.01 | 0.58 ± 0.02 | 0.57 ± 0.03 | 0.56 ± 0.02 |
| **Indentation center** |           |             |       |       |       |        |       |
| $T_2$ [ms]     | 37 ± 1    | 57 ± 10*    | 60 ± 15** | 53 ± 15* | 45 ± 9 | 41 ± 2* | 37 ± 4 |
| $G_d$ [kPa]    | 3.30 ± 0.14 | 4.22 ± 0.90 | 3.31 ± 0.47 | 3.09 ± 0.25 | 2.97 ± 0.30 | 2.86 ± 0.38 | 3.30 ± 0.36 |
| $G_i$ [kPa]    | 2.15 ± 0.08 | 2.90 ± 0.66 | 2.17 ± 0.27 | 1.98 ± 0.24 | 1.99 ± 0.27 | 1.84 ± 0.18 | 2.20 ± 0.40 |
| $|G'|$ [kPa]     | 3.97 ± 0.13 | 5.17 ± 1.13 | 4.00 ± 0.54 | 3.70 ± 0.33 | 3.60 ± 0.38 | 3.43 ± 0.41 | 4.01 ± 0.51 |
| $\varphi$ [rad] | 0.58 ± 0.03 | 0.60 ± 0.03 | 0.58 ± 0.02 | 0.57 ± 0.03 | 0.59 ± 0.04 | 0.58 ± 0.03 | 0.58 ± 0.05 |

Mean ± SD $T_2$, $G_d$, $G_i$, and $\varphi$ values of whole TA ROI and indentation center (2 x indenter's diameter) ROI. The asterisk (*) indicates a significant difference (** for $P < 0.001$, * for $P < 0.01$, * for $P < 0.05$) versus baseline value at Day 0 pre.
DISCUSSION

The response of skeletal muscle deep tissue injury is known to follow a well-known pathway with multiple overlapping pathological processes. Several studies have concluded that a single MRI contrast cannot capture all of the pathological processes involved in skeletal muscle damage, which therefore warrants a multi-parametric approach using several complementary MRI readouts to characterize different aspects of injury and recovery. Furthermore, thus far there is limited knowledge on the spatio-temporal changes in the biomechanical properties of the muscle tissue following deformation-induced muscle damage. We therefore employed a multi-parametric approach, involving $T_2$-mapping for damage quantification and MRE for quantification of mechanical properties, to monitor changes in the muscle stiffness in relation to deformation-induced muscle damage.

MRE revealed a local hotspot of elevated $G_0$, $G_r$, and $|G^*|$ near the center of deformation directly after load release at Day 0, whereas $T_2$ was elevated in a much larger area. This difference in spatial extend can be explained by higher specificity of $T_2$ for fluid accumulation, which diffuses
proximally and distally along the muscle fibers after injury. Similar to our results, Lv et al found elevated shear stiffness in skeletal muscle of rabbits up to 72 hours after crush injury with ultrasound elastography, which was attributed to swollen muscle cells and increased interstitial space. During recovery there was a clear difference in the time course of the shear modulus and $T_2$. Whereas $T_2$ showed a gradual normalization towards baseline over a 14-day time span, $G_d$, $G_l$, and $|G^*|$ shear moduli dropped below baseline from Day 3 up to Day 10, followed by normalization at Day 14. Decrease in shear stiffness values associated with diseased skeletal muscles, eg in myositis and hyperthyroid myopathy, was reported previously. With the histopathological remodeling processes of deep tissue injury in mind, as previously reported by Nelissen et al, the lower shear modulus values likely originate from the moderate to severe degeneration of the TA muscle with massive necrosis, hemorrhage, edema, and inflammation. The macroscopic MRE shear wave has shown to be highly sensitive to these changes in tissue microstructure based on the distribution and presence of microscopic scatterers. However, the constant phase angle $\phi$ over time implies no change in the ratio of elastic to viscous properties of the TA muscle, whereas a reduction of the phase angle due to disorganization of the damaged muscle fibers was expected. A possible explanation could be the precisely orchestrated process of skeletal muscle remodeling after damage, which is capable of preserving the muscle architectural organization.

For the inversion of the MRE data, linear (visco) elastic isotropic material properties were assumed for skeletal muscle. Although this approach is used by several groups, it is a simplification for skeletal muscle which generally exhibits nonlinear viscoelastic anisotropic material behavior. Others proposed the use of a linear (visco) elastic transverse isotropic material approach as an intermediate solution. Using this approach, Qin et al found a decrease in anisotropic ratio, related to necrosis, in an MDX mouse model of diseased skeletal muscle. However, to date the transverse isotropic MRE inversion is still rarely used, as muscle fiber directions obtained, for example by diffusion tensor imaging (DTI), are needed as input. Skeletal muscle DTI can be challenging in small animals and DTI measurements are lengthy. Nevertheless, a combination of MRE and DTI acquisitions in one measurement would facilitate a transverse isotropic MRE inversion. This might be achieved in a time-efficient manner by the use of advanced acquisition acceleration techniques for DTI and MRE such as multi-band imaging and compressed sensing.

From the clinical perspective concerning prevention of (recurrence of) deep tissue injury, the observed lower stiffness during the recovery process of the wound has a very relevant and practical implication. It was shown by several groups that the critical strain threshold for the development of injury is reached at lower indentation force when muscle stiffness is lower. This means that after initial injury the patient could be at higher risk of developing another deep tissue injury. Repeated muscle loading should therefore be avoided and preventive measures taken. In addition, the observed initial increase in shear stiffness direct after damage induction might be used for early diagnosis of deep tissue injury.

To conclude, in a rat model of deep tissue injury, a single 2 h indentation of the TA muscle caused extensive damage, which required at least 14 days to recover. We measured an initial increase in muscle shear modulus values directly after the end of a period of damage-inducing deformation, which was followed by decreased shear modulus values from Day 3 up to Day 10, and subsequent normalization. MRE stiffness values were affected in a smaller area compared with $T_2$, suggesting that MRE might be more specific as $T_2$ for identification of the actual damaged area. Reloading of the affected muscle should be avoided at all times in order to allow recovery and decrease the risk of new injury.
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