The effects of deformation, ischemia, and reperfusion on the development of muscle damage during prolonged loading


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Deep tissue injury (DTI) is a severe form of pressure ulcer where tissue damage starts in deep tissues underneath intact skin. In the present study, the contributions of deformation, ischemia, and reperfusion to skeletal muscle damage development were examined in a rat model during a 6-h period. Magnetic resonance imaging (MRI) was used to study perfusion (contrast-enhanced MRI) and tissue integrity (T2-weighted MRI). The levels of tissue deformation were estimated using finite element models. Complete ischemia caused a gradual homogeneous increase in T2 (~20% during the 6-h period). The effect of reperfusion on T2 was highly variable, depending on the anatomical location. In experiments involving deformation, inevitably associated with partial ischemia, a variable T2 increase (17–66% during the 6-h period) was observed reflecting the significant variation in deformation (with two-dimensional strain energies of 0.60–1.51 J/mm) and ischemia (50.8–99.8% of the leg) between experiments. These results imply that deformation, ischemia, and reperfusion all contribute to the damage process during prolonged loading, although their importance varies with time. The critical deformation threshold and period of ischemia that cause muscle damage will certainly vary between individuals. These variations are related to intrinsic factors, such as pathological state, which partly explain the individual susceptibility to the development of DTI and highlight the need for regular assessments of individual subjects.

PRESSURE ULCERS ARE LOCALIZED areas of tissue degeneration that occur in bedridden or wheelchair-bound individuals as a result of sustained mechanical loading. For example, their occurrence is a major secondary complication for spinal cord injured individuals (16, 32), which can seriously affect their quality of life. In addition, the treatment of pressure ulcers and related complications represents a financial and human burden in terms of extended hospitalization and possible surgical interventions (3, 9).

Deep tissue injury (DTI) is a severe form of pressure ulcer where tissue damage starts in subcutaneous tissue layers, such as skeletal muscle, underneath intact skin (2, 4). DTI represents a serious problem, because tissue damage only becomes apparent at the skin surface at an advanced stage, at which time treatment becomes problematic and several complications can occur (9, 41). Therefore, early identification of DTI and subsequent treatment are critical to reduce morbidity and costs (9). This requires a better understanding of the etiology of DTI to develop appropriate risk assessment tools and early detection methods.

Compression-induced ischemia is traditionally considered to represent the most important factor in the etiology of DTI (13, 24). More recently, other theories have been proposed, including ischemia-reperfusion injury (35, 42), impaired lymphatic drainage (33), and sustained tissue deformation (8, 12, 17, 27, 39). For example, in a rat model, deformation was reported to be the primary determinant for skeletal muscle damage for loading periods up to 2 h (Fig. 1). This damage, however, was confined to specific regions of the muscle, despite the fact that the complete muscle was ischemic during loading (39). In addition, dedicated finite element (FE) models of Ceelen et al. (12) demonstrated that these regions of damage coincided with those regions subjected to the largest deformations and that damage only occurred if a specific deformation threshold was exceeded. Moreover, a correlation between the amount of damage and deformation was found, and recent observations have suggested that the deformation threshold for muscle damage appeared to be similar for a range of loading conditions (29).

In clinical practice, however, where individuals are often exposed to prolonged loading, it might be predicted that ischemia plays an increasingly important role in the damage process. In addition, repositioning strategies and pressure-relieving mattresses are employed to periodically unload and thereby stimulate reperfusion of tissue areas at risk of developing ischemic injury. It is generally accepted that reperfusion after an ischemic period is beneficial for the tissue to reverse the changes that occur during ischemia. However, reperfusion can also aggravate tissue damage due to the combined activation of reactive oxygen species, inflammation, and edema. Indeed, it was shown in a rat model that an increasing number of ischemia-reperfusion cycles was more damaging to the skin of rats than continuous ischemia alone (35). Moreover, a separate study (43) reported that muscle damage was less extensive after 2.5-h ischemia if gradual reperfusion was used compared with instantaneous reperfusion. Therefore, it may be hypothesized that the contributions of ischemia and reperfusion to the damage process increase with prolonged loading. To test this hypothesis, the effects of deformation, ischemia, and reperfusion on muscle damage development were investigated.
in a rat model for DTI during a 6-h period using magnetic resonance imaging (MRI).

MATERIALS AND METHODS

Animal Model

A previously developed animal model was used to study the effects of both deformation and ischemia (37, 38). Sixteen 4- to 5-mo-old female Brown-Norway rats, weighing between 157 and 190 g, were used. The animals were housed under well-controlled laboratory conditions (12:12-h light-dark cycles) and maintained on standard chow and water ad libitum. At the start of the experiment, the animals were anesthetized with 0.6 l/min medical air with 3% isoflurane for induction and 1–2% for maintenance. Respiratory rate and rectal temperature were monitored and maintained within the physiological range. A heating pad was used to maintain body temperature between 36 and 37°C.

Hairs of the left hindlimb were removed by shaving, after which the lower leg was placed in a specially designed mold and fixed with plaster cast. A water-filled capillary was embedded in the plaster cast as a reference point for comparison between MRI acquisitions. A hole in the cast was created to enable compression of the tibialis anterior (TA) muscle with an MR-compatible cylindrical indenter (diameter of 3 mm, length of 6 mm, attached to a rod), as described previously (12, 29). A catheter was implanted in the jugular vein to enable the administration of contrast agent to investigate perfusion. Each rat was placed supine in the experimental setup, which consists of two concentric tubes, as detailed in Stekelenburg et al. (37, 38). The inner tube housed the animal, and the outer tube was used to position the animal in a 6.3 T MR scanner (Bruker BioSpin; horizontal bore, inner diameter of 120 mm) with a 400 mT/m gradient coil. The left foot was positioned within a special holder, and a birdcage radio-frequency coil was placed around the lower leg in a fixed position.

Four different loading protocols were applied, as illustrated in Fig. 2A. In group I (n = 4), 6-h ischemia was applied with a silicone vessel loop (Identi Loops supermaxi blue; Dispo Medical), which was applied around the thigh to restrict blood flow in the complete lower leg. In group II (n = 4), the vessel loop was removed after 4 h to enable reperfusion. Experimental data for this group have recently been reported separately as well (30). In group III (n = 4), the TA muscle was compressed with the indenter. After 4-h compression, a vessel loop was applied to cause complete ischemia for another 2 h and the indenter was removed. In group IV (n = 4), the TA muscle was compressed with the indenter for 4 h, after which the indenter was removed.

Fig. 1. A: T2 map obtained from T2-weighted magnetic resonance images (MRI) of a cross section of a rat leg, indicating that muscle damage [area with increased T2, high-intensity (white) pixels] due to 2-h compression only occurred in a small region of the muscle. B: internal strain energy density distribution estimated with finite element (FE) models showed an overlap with the damaged region in the tissue. C: total strain energy in the muscle clearly correlated with the amount of tissue damage once a specific threshold value was exceeded [adapted from Loerakker et al. (29)].

Fig. 2. A: overview of the combinations of deformation, ischemia, and reperfusion in the 4 experimental groups. Group I: 6-h ischemia; group II: 4-h ischemia followed by 2-h reperfusion; group III: 4-h deformation followed by 2-h ischemia; group IV: 4-h deformation followed by 2-h reperfusion. B: time schedule of the different MRI acquisitions: G, transversal scout images to assess the geometry of the leg; P, dynamic contrast-enhanced MRI to investigate the perfusion; T = T2-weighted MRI to monitor changes in tissue integrity. C: the time course of contrast agent C(t) was characterized by fitting the data of each voxel to a piecewise linear fit model consisting of 3 line segments with intersection points α and β. The first segment represents the baseline concentration (c), the second segment with slope b1 describes the rapid increase in C(t), and the third phase has slope b2, which can be either positive or negative [adapted from Loerakker et al. (30), with permission].
removed to enable reperfusion. The effect of reperfusion was examined by comparing the results of group I and II. The relative effects of deformation and ischemia were investigated by comparing the results of group I with group III and group II with group IV.

At the end of the 6-h investigation period in each loading protocol, the TA muscles were taken from both experimental and control legs, and the animals were euthanized. The tissues were snap-frozen in melting isopentane and stored at −80°C. Frozen samples were cut in transversal direction in 50-μm thick sections and stained with hematoxylin and cosin. The experimental protocol was approved and supervised by the Animal Care Committee of Maastricht University.

**MR Measurements**

High-resolution transversal scout images [field of view (FOV) = 25 × 25 mm² and matrix size = 256 × 256] were obtained during the experiment to assess the geometry of the rat leg. Dynamic contrast-enhanced MRI (DCE-MRI) was used to calculate local contrast measurements. Dynamic series of T1-weighted FLASH (RF- and gradient-echo) were acquired using a multi-echo spin echo sequence (FOV 128 × 96 × 16, repetition time (TR) = 10 ms, echo time (TE) = 2.8 ms, and flip angle α = 15°). After four baseline image acquisitions, a 100-μl bolus of ProHance (0.2 mmol/kg) was administered via the catheter in the jugular vein, while image acquisition was continued for a further 45 scans. The local time profile of T1 was determined as described in Loerakker et al. (30), and the concentration of contrast agent C(t) in each voxel was calculated from the change in T1 according to:

\[ C(t) = \frac{1}{r_1} \left( \frac{1}{T_1(t)} - \frac{1}{T_1(\text{pre})} \right) \]  

where \( r_1 \) represents the T1 relaxivity of the contrast agent, equal to ∼3 mM/s at 6.3 T (6, 25).

To characterize the time course of contrast enhancement, a piecewise linear fit was applied to C(t) in each voxel (36). As illustrated in Fig. 2C, each curve was divided into three linear phases (30, 36): a constant baseline phase, a rapid initial rise in C(t), and a subsequent phase in which C(t) can be either increasing or decreasing:

\[ C_{\text{Inf}}(t) = \begin{cases} 
  c 
  & \text{for } t \leq \alpha \\
  c + b_1(t - \alpha) 
  & \text{for } \alpha \leq t \leq \beta \\
  c + b_1(\beta - \alpha) + b_2(\beta - t) 
  & \text{for } t \geq \beta 
\end{cases} \]  

where \( \alpha \) and \( \beta \) are the intersection time points between the line segments, \( c \) is the baseline concentration before contrast agent administration, and \( b_1 \) and \( b_2 \) are the slopes of the second and third line segment, respectively. Constants \( c, b_1, \) and \( b_2 \) were derived by fitting \( C_{\text{Inf}}(t) \) to C(t) using the least-squares method, and \( \alpha \) and \( \beta \) were varied until the difference between \( C_{\text{Inf}}(t) \) and C(t) was minimal. Parameter \( b_1 \) was chosen as perfusion index, since the initial rise in the signal intensity or concentration curve is often used to estimate perfusion (1, 23, 31). From the \( b_1 \) distribution in the initial DCE-MRI scan, the 25th (\( p_{25} \)) and 75th (\( p_{75} \)) percentile and the interquartile range \( r_{0.25} = p_{75} - p_{25} \) were calculated. For each experiment, a threshold equal to \( p_{25} - r_{0.25} \) was set to distinguish between ischemic (\( b_1 \), lower than threshold) and perfused areas in subsequent DCE-MRI acquisitions.

**T2-weighted MRI**

Changes in muscle integrity were monitored with T2-weighted MRI, using a multi-echo spin echo sequence (FOV = 25 × 25 mm², matrix size = 128 × 128, slice thickness = 1 mm, 11 slices, TR = 4 s, TE = 10–320 ms, 32 echoes, and fat suppression using a spectrally selective 90° pulse followed by a spoiler gradient). A quantitative T2 map was obtained by fitting the signal intensity \( S \) of the first eight echoes to:

\[ S = S_0 e^{-TE/T_2} \]  

T2 was corrected for the influence of ProHance, as recently detailed in Loerakker et al. (30).

**Comparison of contrast enhancement and T2.** The \( b_1 \) and T2 data were coregistered in four consecutive MR slices. The high-resolution scout images (Fig. 2B) were used to define the outer boundary of the leg and the tibia in each slice. Between these two boundaries, a grid was defined with the resolution of the DCE-MRI data (distance between grid points 25/128 and 25/96 mm in horizontal and vertical directions, respectively). Subsequently, this grid was mapped to the corresponding slices in the DCE-MRI data based on the contours of the leg, and the pixel values of \( b_1 \) were linearly interpolated onto the grid points. The same procedure was applied to the T2 maps, to enable comparison of \( b_1 \) with T2 at identical locations.

**Finite Element Model**

To estimate the deformation of the muscle tissue during indentation in groups III and IV, dedicated plane stress FE models were developed for four MR slices underneath the indenter for each animal, as described in Loerakker et al. (29). The outer boundary of the leg and tibia, as determined from the initial scout images, was used to create the mesh (Fig. 3A). The tibia was considered rigid, and the muscle tissue was modeled as an incompressible single-mode hyperelastic Ogden material with strain energy density \( W \):

\[ W = \frac{\mu}{\alpha} \left( \lambda_1^2 + \lambda_2^2 + \lambda_3^2 - 3 \right) \]  

where \( \lambda_i \) (\( i = 1, 2, 3 \)) are the principal stretch ratios, \( \mu = 3.6 \) kPa, and \( \alpha = 5 \) (29).

From scout images of the deformed leg, the movement of the tibia during indentation, and the angle and depth of indentation were derived (Fig. 3B). The displacements of the tibia and the indenter were prescribed as essential boundary conditions. Coulomb friction was assumed between the leg and the indenter, where the friction coefficient was adjusted between 0 and 1 for each FE model to optimize the correspondence of the outer contour of the leg during indentation between experiment and simulation, as determined by visual inspection. The plaster cast surrounding the leg was modeled as a rigid body in free-slip contact with the leg (Fig. 3C). The FE model was implemented in MSC.Marc (MSC Analysis Research, 2005).

A region of interest, covering the TA muscle, was selected for each model (29). To obtain a global measure of deformation, the two-dimensional (2-D) strain energy was calculated by integrating \( W \) over this area.

**Comparison of Experimental Groups**

For group I, the median T2 in the leg (in all four MR slices) was determined for each animal at each time point. For group II, this was done similarly for the T2 maps during the ischemic phase. Since reperfusion in this group was not homogeneous throughout the body, the median T2 was determined separately for areas with and without reperfusion, which were distinguished using a threshold for \( b_1 \). For group III, only the T2 values in the TA muscle region were considered because this area was subjected to deformation. During deformation, only the ischemic area of the TA muscle was selected. For group IV, the same approach was used during deformation. During the reperfusion phase, the median T2 in the TA muscle was determined separately for the areas with and without reperfusion, similar to the approach adopted for group II. For experiments in group III and IV, the strain energy as determined from the FE model was averaged over the four slices and compared with T2 at the end of the experiment and

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the level of ischemia in the leg during deformation. A one-way ANOVA was used to examine differences in $T_2$ between experimental groups and time points. Significant differences between pairs were determined using a Bonferroni post hoc test with $P < 0.05$ considered significant.

**RESULTS**

The main results of the four experimental groups are summarized in Table 1.

In Fig. 4, the $b_1$ and $T_2$ maps with time are shown for one animal of each experimental group. For all experiments, a homogeneous initial distribution of $b_1$ and $T_2$ was observed. For group I (Fig. 4A), the low values in the $b_1$ maps confirm the ischemic condition during application of the vessel loop. The $T_2$ maps show a homogeneous increase in $T_2$ with time throughout the leg. For group II, similar results were observed during ischemia (Fig. 4B). After removal of the vessel loop, heterogeneous $b_1$ and $T_2$ profiles were evident. There were areas in which contrast enhancement was absent, indicating no-reflow, which were associated with an increase in $T_2$. The locations of these distinct areas of no-reflow were similar across the experiments in group II. By contrast, a decrease in $T_2$ was observed in areas with reperfusion. The $b_1$ maps of group III indicate that part of the leg was ischemic during deformation and completely ischemic when the vessel loop was subsequently applied (Fig. 4C). $T_2$ increased in the TA muscle region during deformation and increased further in the complete leg during ischemia. For group IV (Fig. 4D), similar results were observed during the deformation period as for group III. After deformation, however, the $b_1$ maps indicate that reperfusion occurred in the complete leg of this animal. Reperfusion also occurred for two other animals in this group, while for one animal no-reflow was observed in the complete TA muscle region. For two of these three animals with reperfusion, $T_2$ increased further in parts of the TA muscle region during the reperfusion phase.

The time course of $T_2$ for all individual experiments is shown in Fig. 5. In group I, the increase in $T_2$ in the leg was relatively similar for each animal (Fig. 5A). For group II, larger differences between animals were present after removal of the vessel loop (Fig. 5B). $T_2$ remained elevated or even increased in areas with no-reflow, whereas a decreasing trend in $T_2$ was observed for areas with reperfusion. Considerably more variation was observed between animals in groups III and IV (Fig. 5, C and D). In group III, the overall increase in $T_2$ differed between animals, although the time profile was comparable. For animals in group IV, major differences were present after removal of the indenter. Reperfusion occurred in three of the four cases, accompanied by a variable increase in $T_2$. No-reflow in the complete TA muscle occurred in one animal (rat 4), associated with an elevated $T_2$ compared with the situation during loading.

For each of the experiments involving deformation (groups III and IV), the mean 2-D strain energy, percentage of ischemia in the leg, and median $T_2$ in the TA muscle are presented in Table 2. In addition to the variation in $T_2$ that was observed from Fig. 5D, also the levels of deformation and ischemia appeared to vary across the experiments.

For groups I and II, the mean group results were compared, where the significant differences in $T_2$ within and between these groups are presented in Table 3. For both groups, no significant difference in $T_2$ was found between the initial distribution and after 2-h ischemia. After 4-h ischemia, $T_2$ was significantly increased in both groups compared with the situ-

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**Table 1. Overview of the main findings from the four experimental groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>Main Findings</th>
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<tbody>
<tr>
<td>I</td>
<td>Perfusion was absent during application of the vessel loop. $T_2$ increased gradually in the complete leg.</td>
</tr>
<tr>
<td>II</td>
<td>Heterogeneous $b_1$ pattern after removal of the vessel loop. $T_2$ was larger in no-reflow areas when compared to reperfused areas.</td>
</tr>
<tr>
<td>III</td>
<td>Partial ischemia of the leg during compression followed by complete ischemia. $T_2$ increased mainly in large parts of the compressed TA muscle. Levels of ischemia, deformation, and $T_2$ varied considerably between experiments.</td>
</tr>
<tr>
<td>IV</td>
<td>Partial ischemia of the leg during compression. After compression, reperfusion of the TA muscle was present in 3 out of the 4 animals. $T_2$ increased in large parts of the compressed TA muscle. Levels of ischemia, deformation, and $T_2$ varied considerably between experiments.</td>
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</table>

TA, tibialis anterior.
ation after 2-h ischemia. In group I, T2 did not significantly increase further after 4-h ischemia. In the reperfused areas in group II, T2 at the end of the 6-h investigation period was not significantly larger than after 2-h ischemia, whereas T2 in the no-reflow areas was still significantly elevated. Moreover, a significant difference in T2 was found between the no-reflow and reperfused areas. No significant differences were found between groups I and II. Although there was a significant difference between T2 in the no-reflow and reperfused areas at 6 h in group II, both distributions were not significantly different from T2 after 6-h ischemia in group I.

In Fig. 6, comparisons between the appropriate experimental groups are shown. Due to the considerable variation between experiments in groups III and IV, the mean data of these groups were not used for comparison with other groups. In Fig. 6A, the individual results of group III are compared with the mean data of group I to investigate the relative effects of deformation and ischemia. Compared with the effect of complete ischemia
(group I), it can be observed that deformation combined with partial ischemia of the leg (group III) resulted in a slightly larger increase in T2 in two animals (rats 1 and 2) and to a smaller increase in T2 in the other two animals. The comparison of group II with the individual experiments of group IV shows that T2 in the group IV experiments with reperfusion increased considerably in rats 1 and 2, whereas a decrease in T2 was observed for the reperfused areas in group II (Fig. 6B). In the animal with no-reflow in the complete TA muscle region (rat 4), a considerable increase in T2 was present after removal of the indenter.

Hematoxylin and eosin stainings of the TA muscles of both control and experimental legs are presented in Fig. 7. Cross-sections of the control leg showed a normal polygonal appearance of muscle fibers with few interstitial spaces (Fig. 7A). After 6-h ischemia (group I), only minor alterations were observed, consisting of areas with somewhat larger cells and a little increase in interstitial spaces (Fig. 7B). In group II, more severe histological changes were observed after 4-h ischemia and 2-h reperfusion, although there were regional differences in the severity of these changes (Fig. 7, C and D). In some regions, only minor alterations were present, whereas in other areas rounded hypertrophic fibers and considerably increased interstitial spaces were evident. In the deformation experiments (groups III and IV), also regions with enlarged fibers and severely increased interstitial spaces were observed adjacent to areas with minor changes in the tissue (Fig. 7, E–H).

DISCUSSION

In the present study, the effects of deformation, ischemia, and reperfusion on muscle damage development were investigated. This involved the use of a previously developed rat model for DTI, with the animals subjected to different combinations of deformation, ischemia, and reperfusion during a 6-h period (Fig. 2A). Perfusion was examined using DCE-MRI, and tissue integrity was monitored with T2-weighted MRI. Local tissue deformations were estimated using animal-specific FE models. Complete ischemia of the leg caused a homogeneous increase in T2 in the leg (group I). Reperfusion after 4

<table>
<thead>
<tr>
<th>Animal</th>
<th>Mean 2-D Strain Energy, ( \times 10^{-4} ) J/mm²</th>
<th>Percent Ischemia, %</th>
<th>Median T2, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group III rat 1</td>
<td>1.29</td>
<td>50.8</td>
<td>44.5</td>
</tr>
<tr>
<td>Group III rat 2</td>
<td>1.23</td>
<td>67.6</td>
<td>44.0</td>
</tr>
<tr>
<td>Group III rat 3</td>
<td>0.78</td>
<td>32.7</td>
<td>38.6</td>
</tr>
<tr>
<td>Group III rat 4</td>
<td>1.47</td>
<td>80.4</td>
<td>41.5</td>
</tr>
<tr>
<td>Group IV rat 1</td>
<td>1.51</td>
<td>99.8</td>
<td>50.5</td>
</tr>
<tr>
<td>Group IV rat 2</td>
<td>1.39</td>
<td>55.5</td>
<td>43.9</td>
</tr>
<tr>
<td>Group IV rat 3</td>
<td>0.60</td>
<td>55.2</td>
<td>38.4</td>
</tr>
<tr>
<td>Group IV rat 4</td>
<td>1.29</td>
<td>91.6</td>
<td>44.0</td>
</tr>
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2-D, two-dimensional.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Significant Differences</th>
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<tbody>
<tr>
<td>Within group I</td>
<td>( T_{2_{pre}} &lt; T_{2_{4h}}, T_{2_{sh}}, T_{2_{6h}} )</td>
</tr>
<tr>
<td>Within group II</td>
<td>( T_{2_{pre}} &lt; T_{2_{4h}}, T_{2_{5h}}, T_{2_{6h}}, T_{2_{6hN}}, T_{2_{26h}}, T_{2_{26hR}}, T_{2_{6hN}}, T_{2_{26hR}} )</td>
</tr>
<tr>
<td>Between groups I and II</td>
<td>( T_{2_{26h}} &gt; T_{2_{5h}} )</td>
</tr>
</tbody>
</table>

N and R, areas with no-reflow and reperfusion, respectively.
Ischemia was absent in distinct areas, associated with larger T2 values compared with T2 in reperfused areas (group II). T2 increased in large parts of the TA muscle in all experiments involving deformation (groups III and IV), although large variations were observed between the individual experiments.

Local perfusion in the rat leg was examined using an intravenous bolus injection of contrast agent. A local perfusion index was derived from the initial slope of the local time profile of the contrast agent concentration. Although the initial slope of the signal intensity or concentration curve is often used to study changes in local perfusion (1, 23, 31), this parameter also reflects the permeability of the blood vessels and is consequently not a direct measure of perfusion alone. In the present study, however, it was still considered a suitable measure to distinguish between regions with and without perfusion, since complete absence of contrast enhancement can only be caused by a lack of perfusion. Changes in tissue integrity were monitored with T2-weighted MRI, where an increase in T2 was considered a measure of tissue damage involving edema, necrosis, or inflammation (15). Biopsies of the TA muscle were taken at the end of the 6-h investigation period to distinguish between the different pathological processes.

Local deformations in the TA muscle during indentation were estimated using FE models in which the geometry and boundary conditions were adapted for each experiment. The original FE model was developed by Ceelen et al. (11) and validated with MR-tagging experiments. The applied strains in this animal model have previously been reported by Ceelen et al. (12), revealing compressive strains up to ~50% and maximum shear strains up to ~100%. These strains are similar to those in the buttocks of seated individuals. Indeed, an estimation of the internal strains during sitting by Linder-Ganz et al. (28) revealed compressive strains up to ~80% and maximum shear strains up to ~100%, particularly in spinal cord injured subjects. The model was further improved by Loerakker et al. (29) and showed good correspondence with the contours of the deformed leg in the MR images. The muscle tissue in the leg was modeled with a hyperelastic Ogden law using material properties partly based on experiments by Bosboom et al. (7). Consequently, the strain energy values calculated from the simulations should not be interpreted as absolute values but rather as a relative measure of the level of deformation to compare experiments.

In group II, reperfusion was noticeably absent in specific regions of the leg, where T2 remained elevated or even increased further after removal of the vessel loop (Fig. 5B). No-reflow is a feature of ischemia-reperfusion injury that can be caused by microvascular thrombosis, vasospasm, or edema (5, 44). The two major regions of no-reflow were located in the anterior tibial and superficial posterior compartments of the leg, corresponding with the results of Morikawa et al. (34), who performed similar experiments with ischemia and reperfusion in the rat hindlimb. The locations of no-reflow in the present study may be due to either the occurrence of acute compartment syndrome or by the specific architecture of blood vessels in the rat hindlimb (30). Only minor histological alterations were evident in group I animals after 6-h ischemia. Therefore, the T2 increase in this group may not be indicative of sustained structural damage but was more likely related to the intracellular metabolic changes and cell swelling that are both associated with ischemia. There were discrete regions of more severe swelling in parts of the TA muscle in group II, presumably coincident with the no-reflow areas that exhibited a further increase in T2. Similar morphological features involving edema were observed after ischemia and reperfusion in previous studies (10, 21). Hatoko et al. (22) also reported that interstitial edema after 6-h ischemia occurred gradually with reperfusion, which might explain the minor alterations in group I observed in the present study. Thus ischemia alone caused only minor changes in the muscle tissue in group I that could probably be reversed by reperfusion after 4-h ischemia in group II. However, tissue swelling could also be aggravated in group II in areas with no-reflow, indicating the complex effect of reperfusion on damage development in skeletal muscle. In addition, this swelling of the tissue may lead to a substantial increase in muscle stiffness, which will increase the load on the adjacent healthy tissue and can thereby contribute to the progression of tissue damage (18). Whether the effect of reperfusion is beneficial or not depends not only on the duration of ischemia, but also on the anatomical location of the insult, and the rate of the load removal (43).

The overall increase in T2 with time varied between the animals in group III. For example, compared with the results after 6-h ischemia in group I, the total T2 increase in group II was slightly larger in two animals and smaller in two other animals (Fig. 6A). These results indicate that deformation in combination with partial ischemia of the leg can lead to a smaller T2 increase in the TA muscle as a whole than complete ischemia of the leg. Locally, however, deformation in combination with partial ischemia can still be more damaging than complete ischemia (Fig. 7, E and F). In group IV, complete reperfusion of the TA muscle was observed for three of the four animals. During the reperfusion phase, T2 remained comparable to the situation after 4-h deformation in one animal and
increased considerably further in large parts of the TA muscle in the other two animals (Fig. 6B), this latter increase being considerably larger than in the group II experiments. A possible explanation for these results is that the deformation in these two experiments may have caused substantial structural damage in the muscle, which is manifested in the reperfusion phase by increased swelling in the tissue, or even aggravation of tissue damage.

Stekelenburg et al. (39) showed that 2-h deformation, inevitably combined with partial ischemia, caused highly localized regions of irreversible muscle damage, whereas 2 h of complete ischemia only caused reversible changes in the tissue. The locations of tissue damage clearly coincided with the areas subjected to the highest deformations (12, 29). For loading periods up to 2 h, these results imply that ischemia has a negligible effect on muscle damage development and deformation is the primary trigger for muscle damage. In the extended experiments of the present study involving deformation, T2 increased in a large part of the TA muscle and not only in a narrow region as observed after 2-h deformation. Therefore, ischemia is probably responsible for part of the T2 increase, indicating that deformation and ischemia are both
involved in the damage process. In addition, a larger variation was observed between the experiments in which deformation was applied as those not involving deformation. This could be attributed to the significant variation in deformation between experiments as reflected in the broad range of values for the mean 2-D strain energies (Table 2) in both groups III and IV. This can be expected to cause a variation in size of the area with T2 increase caused by deformation (Fig. 1) (29). On the other hand, the percentage of ischemia in the leg also varied between experiments (Table 2), implying that ischemia could have had a variable influence on the increase in T2 in the different experiments as well. Therefore, deformation may have both a direct and an indirect effect on the damage process. The consequence of the former is that a larger overall deformation causes a larger area of T2 increase (29). The indirect effect arises from the fact that deformation partly determines the level of ischemia in the leg and thereby would subsequently influence the increase in T2.

Due to the large variation between the experiments involving deformation and partial ischemia, it is difficult to definitively prove this hypothesis. However, since the applied levels of deformation and ischemia will always be different in every experiment due to differences in leg geometry and load application, increasing the number of experiments would not reduce this variation in the deformation groups. To obtain more insight into the quantitative contributions of deformation and ischemia, the experiments could be classified into specific subgroups of deformation, ischemia, and reperfusion. However, due to the large variation between experiments, the complex effect of reperfusion, and the fact that there are probably interactions between the effects of deformation and ischemia, this approach will require a very large number of animals before significant differences between subgroups can be found. Furthermore, the large variation in T2 increase due to differences in load application and geometry between animals is also an important observation, as it may explain in part the differences in susceptibility to DTI development between different tissue sites on the same individual.

Based on the results of the present paper and previous studies, an hypothesis for the development of DTI can be proposed (Fig. 8). Deformations exceeding a specific threshold will directly cause muscle damage, where the degree of damage will be determined by the level of deformation and its exposure time (Fig. 8A). As the loading period increases, ischemia and reperfusion will also contribute to the damage process. Reperfusion will be beneficial for the tissue only for a limited period of ischemia. When this period is exceeded, reperfusion will aggravate existing tissue damage (Fig. 8B). The time point after which reperfusion causes additional damage depends on several factors, including the anatomical location and temperature of the involved tissue. Thus, for prolonged loading, deformations exceeding a threshold directly cause muscle damage that is subsequently aggravated by ischemia and reperfusion with increasing exposure time. The level of ischemia and thereby its damaging effect, are partly influenced by the level of deformation. Ischemia and subsequent reperfusion may ultimately contribute more to muscle damage development than deformation, as their damaging effects significantly increase with the exposure time. In summary, the damage process in skeletal muscle tissue is dominated by the level of deformation during short loading periods, corresponding to the initial plateau phase in the sigmoid pressure-time threshold for muscle damage as proposed recently (20, 19, 26, 40). However, during prolonged loading, ischemia and reperfusion will gradually take over and ultimately play a more important role than deformation.

For clinical practice, this hypothesis would imply that the rapid initiation and subsequent progression of DTI can be prevented by using appropriate cushioning to keep internal tissue deformations below the deformation threshold for damage. For prolonged loading, it is indeed important to limit the period of ischemia by means of repositioning strategies and pressure-relieving mattresses to prevent tissue damage related to ischemia or ischemia-reperfusion injury. The deformation threshold for damage as well as the time point after which ischemia starts to cause tissue damage will inevitably vary between individuals and also for a specific individual in time. These variations are probably related to pathologies, especially those related to spinal cord injury, that influence tissue properties (14, 28), the circulation, and the immune system. These factors will partly explain the individual susceptibility to the development of DTI and highlight the need for regular assessments of individual subjects.

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Fig. 8. Hypothesis for the development of DTI. A: deformation directly causes muscle damage if a critical deformation threshold \( d_c \) is exceeded. The amount of damage is determined by the exposure time. B: for long periods, ischemia and reperfusion become involved in the damage process. Reperfusion can reverse the damage process after a short ischemic period. However, if a critical time point \( t_c \) is exceeded, reperfusion will aggravate tissue damage. When the loading period increases, it is expected that ischemia will ultimately play a more important role in the damage process than deformation.
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GRANTS

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