Role of ischemia and deformation in the onset of compression-induced deep tissue injury: MRI-based studies in a rat model

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Submitted 4 October 2006; accepted in final form 24 January 2007

Stekelenburg A, Strijkers GJ, Parusel H, Bader DL, Nicolay K, Oomens CW. Role of ischemia and deformation in the onset of compression-induced deep tissue injury: MRI-based studies in a rat model. J Appl Physiol 102: 2002–2011, 2007. First published January 25, 2007; doi:10.1152/japplphysiol.01115.2006.—A rat model was used to distinguish between the different factors that contribute to muscle tissue damage related to deep pressure ulcers that develop after compressive loading. The separate and combined effects of ischemia and deformation were studied. Loading was applied to the hindlimb of rats for 2 h. Muscle tissue was examined using MR imaging (MRI) and histology. An MR-compatible loading device allowed simultaneous loading and measurement of tissue status. Two separate loading protocols incorporated uniaxial loading, resulting in tissue compression and ischemic loading. Uniaxial loading was applied to the tibialis anterior by means of an indenter, and ischemic loading was accomplished with an inflatable tourniquet. Compression of the muscle tissue during uniaxial loading was measured using MR tagging. Compression of the tissues for 2 h led to increased T2 values, which were correlated to necrotic regions in the tibialis anterior. Perfusion measurements, by means of contrast-enhanced MRI, indicated a large ischemic region during indentation. Pure ischemic loading for 2 h led to reversible tissue changes. From the MR-tagging experiments, local strain fields were calculated. A 4.5-mm deformation, corresponding to a surface pressure of 150 kPa, resulted in maximum shear strain up to 1.0. There was a good correlation between the location of damage and the location of high shear strain. It was concluded that the large deformations, in conjunction with ischemia, provided the main trigger for irreversible muscle damage.

decubitus; pressure ulcer; etiology; skeletal muscle; tagging magnetic resonance imaging

PRESSURE ULCERS CAN ARISE when a prolonged mechanical load is applied to soft biological tissues, e.g., when subjects are bedridden or wheelchair bound for prolonged periods or when orthoses and prostheses are used to support soft tissues. Pressure ulcers represent a serious health problem to the individual and a financial burden to health services (54). They can interfere with quality of life, activities of daily living, and rehabilitation and, in some cases, may prove life threatening.

Pressure ulcers can start superficially or deep within the tissues, depending on the nature of the surface loading and the tissue integrity (9). Superficial ulcers form within the skin and may progress downward, whereas deep ulcers arise in muscle layers covering bony prominences and are mainly caused by sustained compression of the tissues. The former are easily detectable, and adequate treatment measures can be taken. In contrast, when a deep pressure ulcer finally becomes visible at the skin layer, effective clinical intervention may prove problematic, and prognosis is variable. The focus of the present study is therefore on deep pressure ulcers.

It is clear that the successful prevention and treatment of deep pressure ulcers require an improved knowledge of the temporal events associated with their etiology. Traditional theories on the mechanisms of ulcer formation have implicated localized ischemia as the primary cause of the onset of damage (14, 15). However, there has been recent interest in alternative theories, implicating the roles of reperfusion injury (26), impaired interstitial fluid flow and lymphatic drainage (35, 48), and sustained deformation of cells (8, 51).

There is much recent focus on deep pressure ulcers, which necessarily involve deep tissue injury, recently defined by the US National Pressure Ulcer Advisory Panel (NPUAP) as “a pressure-related injury to subcutaneous tissues under intact skin” (2). This definition instantly reveals one of the major problems associated with their early detection. Debate of tissue injury at the NPUAP meeting in 2005 revealed many contrasting viewpoints related to its causation, whether it can be truly considered a pressure ulcer, and, if so, how it can be classified in the current staging system (16). All NPUAP delegates agreed, however, that the underlying mechanisms of deep tissue injury require further investigation.

In past decades, different kinds of studies have been performed to understand the underlying mechanisms of pressure ulcers. There is considerable debate about the relative merits of in vitro cell-based model systems and in vivo animal models in pressure ulcer research. Although the in vitro cell-based model systems allow examination of the effects of cell deformation alone in the absence of ischemia (10, 45), their relevance to the clinical situation is necessarily limited. By contrast, it is inevitable that loading of animal tissues, which has been extensively investigated over several decades (7, 14, 15, 23, 29, 52), involves complex interactions. However, these in vivo experiments enable discrimination between the effects of pure ischemia on muscle tissue and the effects of tissue compression, including ischemia, large cell deformation, and possible disturbance of the metabolic equilibrium.

The objective of the present in vivo animal study was to distinguish between the different factors that contribute to
muscle tissue damage related to deep pressure ulcers that develop after compressive loading. In a previous study, an animal model and an MR-compatible loading device were developed (55). This approach allowed the simultaneous application of pressure to the tibialis anterior (TA) in a rat model and the measurement of muscle tissue status by T2-weighted MR imaging (MRI). In the present study, two separate loading protocols were used to distinguish between the effects of uniaxial loading, which produces tissue compression, and ischemic loading. In addition, the perfusion status in the leg during both forms of loading was measured by contrast-enhanced MRI. To determine the precise deformation of the muscle during loading, MR-tagging experiments, which allowed correlation of the location of damage to the location of maximum deformations during loading, were performed.

MATERIALS AND METHODS

Animal Model

Female Brown Norway rats (n = 11, 170–200 g body wt, 20 wk old) were housed under standard laboratory conditions (12:12-h light-dark cycles) and maintained on standard laboratory food and water ad libitum. Each rat was anesthetized for the preparation phase by injection of xylazine (1 µg/g body wt sc, 2 g/l) and ketamine (0.8 µg/g body wt im, 100 g/l). During the MR measurements, anesthesia was maintained with isoflurane inhalation (0.4–1.0% isoflurane with 1:1 N2O:O2). Vital signs (pulse and respiratory rate) were monitored and maintained within physiological values. The rat was placed on a heating pad to maintain body temperature at 35–37°C. Before the loading protocols, the hairs on the left TA region were removed by shaving. The leg was placed in a specially designed mold, and a plaster cast was applied to obtain a firm fixation in the setup. For loading protocols, the hairs on the left TA region were removed by shaving. The leg was placed in a specially designed mold, and a plaster cast was applied to obtain a firm fixation in the setup. For loading protocols, the hairs on the left TA region were removed by shaving. The leg was placed in a specially designed mold, and a plaster cast was applied to obtain a firm fixation in the setup. For

MR-Compatible Device

The experimental setup is described in detail elsewhere (55, 56). Briefly, the device consisted of two concentric tubes: an inner tube, which housed the animal, and an outer tube, which was used to position the whole arrangement in a 6.3-T MR scanner (Varian, Palo Alto, CA) operating at 270 MHz (horizontal bore, 95 mm diameter) with a 380 mT/m gradient coil. The anesthetized animal was placed supine in the loading device, with the animal’s foot positioned in a special holder, such that the leg was fixed in the setup. A birdcage radio-frequency (RF) coil was placed in a fixed position around the limb. A hole in the cast enabled application of a plastic indenter to the TA region. The indenter was fixed to a glass fiber-reinforced polymer (Ertalon 66GF30) loading beam to which strain gauges were attached to enable force measurements during loading. The loading beam could be attached to the outer tube of the setup. The indenter was applied by rotation of the bar that was attached to a cam mechanism. The cam was designed in such a way that, by rotation of the bar to the left, the indenter could be applied at a slow rate. If the bar was rotated to the right, the indenter was applied instantly (55). For the tagging measurements, custom software written in LabVIEW (National Instruments) was used to apply the indenter in a controlled and repetitive manner.

Loading Protocols

Uniaxial loading. Uniaxial loading (n = 6) was applied to the TA region by means of an indenter. The 3-mm-diameter indenter was curved at its edges to minimize high stresses. An 4.5-mm-deep indentation, applied slowly at a rate of 1.5 mm/s to avoid impact damage, was maintained for 2 h and then removed. This indentation resulted in surface pressures of ∼150 kPa. These experiments are referred to as the indenter experiment.

Ischemic loading. Ischemic loading (n = 3) was applied to the TA by an inflatable tourniquet, which was positioned above the knee of the animal. The tourniquet was inflated instantaneously up to 140 kPa, and after 2 h the pressure was released. These experiments were performed using the above-described loading device in the absence of the indenter and are referred to as the tourniquet experiment.

Rapid repetitive uniaxial loading. Rapid repetitive uniaxial loading (n = 2) was applied to the TA to allow determination of the deformation of the muscle during indentation by means of MR tagging. An indentation of 4.5 mm was applied instantly via the cam. From repeated measurements, local strain fields could be calculated. These strain fields were globally compared with the damage location measured in the indenter experiments. Successive applications of the indenter were separated by 4 s.

Experimental Protocol

The experimental protocol for all three loading protocols involved several distinct phases. The preparation phase consisted of sedation of the animal, insertion of a catheter in the jugular vein (loading protocols 1 and 2), casting, and fixation of the animal in the setup. This phase took ~60 min. Subsequent phases included MR measure-
ments, for which the time schedules are depicted in Fig. 1. The indenter experiments were divided into two groups: one (n = 3) involved perfusion and T2 measurements, and the other (n = 3) consisted of only T2 measurements. The loading protocol was the same for both groups.

In the first two loading protocols (Fig. 1, A and B), the first injection of contrast agent was given to measure the perfusion index (PI) map before loading, which was followed, at 30 min after injection, by measurement of a T2 map. After these preloading measurements, the load was applied for 2 h, during which a PI map and T2 maps were measured. A sagittal image was recorded in the indenter experiment to determine the direction of indentation. Immediately after unloading, a PI map was measured, and then a series of T2 measurements were obtained. In the group without perfusion measurements, T2 maps were measured immediately after unloading. In the tourniquet experiment, an extra perfusion measurement was performed to examine the restoration of normal blood flow. In the final phase, 4 h after load removal, the animal was killed for histological examination.

The measurement protocol for the MR-tagging experiments is shown in Fig. 1C. Five slices, centered on the position of the indenter, were selected. Tag lines were applied in the two orthogonal directions, with two acquisitions for each direction, with a phase difference of 180°, requiring four measurements per slice. A motor rotated the bar attached to the cam with a constant frequency of 0.25 Hz.

**MR Measurements**

**T2-weighted MRI.** Changes in the transverse (spin-spin) relaxation time (T2) are generally accepted as a measure of tissue damage (20). The pathological features associated with pressure ulcers (27, 29) that may affect muscle proton density and relaxation times are inflammation, edema, necrosis, hemorrhage, fibrosis, and fatty infiltration. Of these, inflammation, edema, and hemorrhage can lead to an increased proton density, caused by an increase in intra- and extracellular free water. This will result in an increase in T1 (longitudinal relaxation time) and T2, because free water has longer relaxation times. T1 reflects structural aspects but is relatively insensitive to changes in the state of the muscle. In contrast, T2 is very sensitive to tissue changes.

To obtain T2 maps of the lower limb, a multiecho spin-echo sequence was used. Signal intensities (SI) of successive echoes were fitted, on a pixel-to-pixel basis, to the following equation to determine the direction of indentation. Immediately after unloading, a PI map was measured, and then a series of T2 measurements were obtained. In the group without perfusion measurements, T2 maps were measured immediately after unloading. In the tourniquet experiment, an extra perfusion measurement was performed to examine the restoration of normal blood flow. In the final phase, 4 h after load removal, the animal was killed for histological examination.

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**Contrast-enhanced MRI.** Contrast-enhanced MRI was used to determine the perfusion status of the muscle before, during, and after loading. This method, involving an injection of a contrast agent, has a high signal-to-noise ratio, which makes it suitable to determine perfusion status in resting muscle. It is based on the fact that, after an intravenous injection of a bolus of a contrast agent, in our case, gadolinium-diethylenetriaminepentaacetic acid (Gd-DTPA), its initial accumulation and distribution in tissues can be followed with T1-weighted fast MRI sequences. During the first pass, the contrast agent circulates through blood vessels into capillaries and then diffuses into the interstitial tissue space in a unidirectional fashion. This initial accumulation of the contrast agent into the tissue is largely dependent on tissue perfusion (32).

On the basis of a previous procedure (32), a T1-weighted gradient spoiled-echo multislice sequence was used to obtain a PI map in three selected transverse 1-mm-thick slices. For the indenter experiment, one slice was selected along the axis of the indenter, and the other two slices were selected 3 mm distal and proximal to the indenter position, respectively. For the tourniquet experiment, slices were selected at equivalent positions through the TA. After baseline image acquisitions, a bolus (0.2 mmol/kg) of Gd-DTPA (Magnevist, Schering, Berlin, Germany) was administered via a catheter in the jugular vein. Imaging acquisition was continued for 6.5 min with a time resolution of 5 s/slice. Imaging parameters are shown in Table 1.

It has recently been shown that the maximal relative signal intensity difference (ΔSI<sub>rel</sub>) with respect to a reference signal at the same location is a valid measure for perfusion status (33). ΔSI<sub>rel</sub> is defined as follows

\[
\Delta SI_{rel} = \frac{SI_{max} - SI_0}{SI_0} \times 100\% = PI
\]

where \(SI_0\) represents the average of 10 baseline values, measured before the injection of the contrast agent, and \(SI_{max}\) is the average of 10 measurements at maximal signal increase after injection.

The temporal resolution of sequential PI measurements is in the range of 60 min because of the relatively slow clearance of the contrast agent (32). In the present study, successive PI measurements were separated by ~60 min (Fig. 1). In addition, pilot studies showed that T2 measurements were not affected by the contrast agent when conducted ≥30 min after the injection (data not shown).

**MR tagging.** To determine the deformation of the muscle tissue during loading, MR-tagging measurements were used. The determination of local displacements within skeletal muscle is relatively new. Tagging and phase-contrast sequences are two established noninvasive MRI methods for tracking local displacements due to motion (47), but they are mostly used in the field of cardiac biomechanics (1). Much more recently, however, these techniques have been applied to skeletal muscle (17, 34).

Tags represent regions of tissue, the longitudinal magnetization of which has been altered before imaging, so that for ~1 s they temporarily appear dark in subsequent MR images. The tags move with the underlying tissue and serve as easily identifiable landmarks within the tissue. From the displacement of tag lines due to indentation, local tissue deformation and related strain distributions can be calculated.

To determine displacement during indentation in one direction, a one-dimensional tagging grid was applied perpendicular to that direction by complementary spatial modulation of magnetization.

### Table 1. Imaging parameters for measurement of T2-map, T2-weighted, contrast-enhanced, and tagged images

<table>
<thead>
<tr>
<th></th>
<th>TE, ms</th>
<th>TR, s</th>
<th>No. of Slices</th>
<th>Slice Thickness, mm</th>
<th>Orien</th>
<th>FOV, mm²</th>
<th>Matrix Size</th>
<th>Res, μm²</th>
<th>No. of SA</th>
</tr>
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<tr>
<td>T2 map</td>
<td>12–48</td>
<td>4.5</td>
<td>41</td>
<td>1</td>
<td>Trans</td>
<td>25×25</td>
<td>128×128</td>
<td>195×195</td>
<td>2</td>
</tr>
<tr>
<td>T2-weighted</td>
<td>25</td>
<td>4</td>
<td>31</td>
<td>1</td>
<td>Sag</td>
<td>60×30</td>
<td>128×128</td>
<td>469×234</td>
<td>2</td>
</tr>
<tr>
<td>PI map</td>
<td>2.5</td>
<td>0.035</td>
<td>3</td>
<td>1</td>
<td>Trans</td>
<td>25×25</td>
<td>64×64</td>
<td>391×391</td>
<td>2</td>
</tr>
<tr>
<td>Tagged</td>
<td>10</td>
<td>0.0025</td>
<td>5</td>
<td>1</td>
<td>Trans</td>
<td>30×30</td>
<td>128×64</td>
<td>234×469</td>
<td>2</td>
</tr>
</tbody>
</table>

TE, echo time; TR, repetition time; Res, in-plane resolution; Trans, transversal slices; Sag, sagittal slices; FOV, field of view; SA, signal averages; PI, perfusion index; Orien, orientation.
The tagging grid was applied with a Gaussian RF pulse of 200 µs with a flip angle of 45°, a gradient strength of 30 mT/m, and an effective gradient duration of 150 µs, which resulted in a grid line spacing of 14 pixels or 3.3 mm. A T1-weighted gradient spoiled-gradient echo sequence was used to image the tagging grid. Imaging parameters are indicated in Table 1. The read-out direction was chosen orthogonal to the tagging direction.

From the MR-tagging data, the tensile and compressive strains and the maximum shear strain were calculated in the muscle during indentation.

**MR Data Analysis**

**T2.** To obtain an enhanced contrast-to-noise ratio between normal and damaged tissues based on differences in T2, the individual images of the multiecho sequence were summed. This is comparable to using a fast spin-echo sequence, which also uses multiple spin echoes to reconstruct an image, but with the advantage that the images are not blurred by the T2 weighting and can be used to extract T2 values as well as to reconstruct a T2-weighted image with a higher contrast-to-noise ratio from one data set. The images are referred to as T2-weighted-sum (T2ws) images. For quantification of the changes in T2 values in the indenter experiments, three regions of interest (ROIs) were selected, each incorporating ~60 pixels. These ROIs are indicated in Fig. 2A, which shows a transverse slice of the lower limb. Thus 1) ROI 1 was chosen within the compressed area, as determined from the T2ws images taken during loading, 2) ROI 2 was selected outside the compressed region, but inside the region with hypoperfusion, as determined from the corresponding PI map during indentation, and 3) ROI 3 served as a control region.

In the indenter experiment, ROI 1 was compressed, and thus T2 values during loading could only be estimated in ROI 2 and ROI 3. In the tourniquet experiment, T2 values were estimated in ROI 1 to enable comparison between the effects of both loading protocols after unloading in the same region. For each experiment, T2 values were normalized to the preloading value. This normalization was used, since the measured T2 is sensitive to the position in the RF coil and precise MR settings, which were slightly different for each experiment; this normalization also minimized interanimal variations.

**Perfusion.** ΔSrel was calculated on a pixel-by-pixel basis to determine a PI map before, during, and after application of the indenter or tourniquet.

**Tagging.** The applied method for quantification of the tag displacements was the HARmonic Phase (HARP) analysis, which is based on the method described by Osman et al. (40). From the displacement fields, the Green-Lagrange strains were estimated using a gradient deformation method proposed by Geers et al. (22). This method estimates the deformation tensor \( F \) at a certain point from the displacements of neighboring points. Nine neighboring pixels were used for each pixel in the image to calculate \( F \). With \( F \) in a point known and \( I \) the unity tensor, the Green-Lagrange strains followed from

\[
E = \frac{1}{2} (F^T \cdot F - I)
\]

The maximum shear strain \( \tau \) in a two-dimensional slice could be calculated from

\[
\tau = \frac{1}{2} (\lambda_1 - \lambda_2)
\]

with the principal strains \( \lambda_1 \) and \( \lambda_2 \) representing the eigenvalues of the Green-Lagrange strain tensor. For this method, it is assumed that, in the slice underneath the indenter, the strain in the direction perpendicular to the two-dimensional surface is also a principal strain.

**Statistical Analysis**

SPSS version 12.0.1 (SPSS, Chicago, IL) was used for statistical analysis. Differences between data and control were assessed by ANOVA. Dunnett’s post hoc test was then applied, and statistical significance was prescribed at a 5% level. Values are means ± SE.

**Histological Analysis**

Immediately after the MR measurements, each killed animal was perfusion fixed with 4% buffered formalin. The lower limbs were dissected and stored in formalin. Both TAs were excised ≥2 wk later to ensure complete tissue fixation. The unloaded muscle was used for control samples. The midportion of the muscle was dehydrated in a series of alcohol solutions and embedded in plastic (Technovit 7100, Kulzer). The muscle was cut in 5-µm-thick transverse slices, and individual samples were stained with toluidine blue.

**RESULTS**

**MRI**

T2ws images, determined from the multiecho sequence, for a typical indenter experiment are shown in Fig. 2, A–C, before, during, and after release of the applied indentation. T2ws images measured during loading showed some signal increase in the region near the indenter (Fig. 2B). A much more pronounced increase in signal intensity, however, was observed after indenter release, in an area extending from the skin to the tibial bone (Fig. 2C). The corresponding PI maps, determined from the relative signal increase after Gd-DTPA injection, are indicated in Fig. 2, D–F. Figure 2D shows a normal preloading PI map, which is indicative of a homogeneous PI. The PI map taken during loading demonstrated a large region with low PI values (Fig. 2E); after unloading, the...
PI values were high in this region (Fig. 2F1). On an expanded scale (Fig. 2F2), it is evident that the highest PI values were found in the previously compressed region. This region, with high apparent PI values, was located at approximately the same position as the region with high signal intensities in T2-weighted images (Fig. 2C).

PI maps measured during indentation in three slices through the lower limb are shown in Fig. 3. There are clear differences in the perfusion status in the three slices. For example, the PI maps measured in the slices underneath (Fig. 3B) and distal to (Fig. 3C) the indenter showed low PI values in the TA region, whereas the PI map proximal to the indenter showed no evidence of hypoperfusion. In the TA region, the PI values were even slightly increased in the slices distal to the indenter.

The T2ws images measured distal and proximal to the indenter position also demonstrated a distinct response to loading, as shown in Fig. 4, which depicts T2ws images collected before and during loading and 15 and 65 min after unloading. In the slice proximal to the indenter, the area with increased T2 was comparatively small 15 min after loading but increased thereafter (Fig. 4, C and D). Conversely, in the slice distal to the indenter, immediately after unloading, a large area was visible with increased T2 values, which decreased thereafter (Fig. 4, G and H).

The effect of ischemic loading was examined in the tourniquet experiment. The PI maps and T2ws images for a tourniquet experiment are shown in Fig. 5. During inflation of the tourniquet, the perfusion was strongly reduced in the whole lower limb (Fig. 5F). After 2 h, the tourniquet was deflated, which initially led to high PI values in the leg, presumably associated with a reactive hyperemia response (Fig. 5G). These PI values were restored to normal within 90 min (Fig. 5H). T2ws images obtained during inflation showed some global increase in signal intensity, but these values returned to preloaded values within 2 h.

Figure 6 illustrates the normalized T2 values determined from the indenter and tourniquet experiments in the slices at the level of the indenter. T2 in the TA region before loading was 21.5 ± 0.3 ms (n = 9). In the indenter experiments, T2 values during loading could only be calculated in ROI 2 and ROI 3. There was a small shift of tissue in ROI 2 during indentation. T2 values were signficantly increased in the compressed area (ROI 1) after unloading (P < 0.001). In ROI 2, the T2 values were increased (not significantly) during the ischemic period but, after unloading, were restored to preloaded values within 40 min. There were no significant changes in T2 values in ROI 3 throughout the indenter experiments. T2 values in the tourniquet experiment (ROI 1) showed a small decrease after 20 min of ischemia followed by an increase after 95 min of ischemia. T2 values were restored to preloaded values within 40 min after deflation of the tourniquet.

To determine the deformation of the muscle tissue during loading, MR tagging was used. In the MR-tagging experiment, the tagging grid was applied in two directions. The tagged images at the level of the indenter with the lines approximately perpendicular to the direction of indentation are shown in Fig. 7, A and B, before and after indentation, respectively. From the displacement of the tag lines, the indentation corresponding to 4.3 mm was calculated (Fig. 7C). As shown in Fig. 7C, the indentation is correctly calculated from the displacement of the tagging lines. The calculated distribution of the tensile and compressive strains are depicted in Fig. 7,D and E, respectively. In a localized area extending from the skin and the bone, high values (1−1.7) of tensile strain were found. In the whole TA region, values of the compressive strain varied between −0.11 and −0.37. The calculated maximum shear strain distribution is depicted in Fig. 7F. A region with high maximum shear strain values extended from the skin toward the tibial bone, with peak values on the order of 1.0. The distribution of the tensile strain and maximum shear strain showed close resemblance, because the tensile strains were higher than the compressive strains.

**Histological Findings**

Transverse histological slices of muscle tissue from the TA (ROI 1) from indenter and tourniquet experiments are shown in Fig. 8. A control slice, in which the normal rectangular shape of muscle fibers with small interstitial spaces is visible, is shown in Fig. 8A. Slices of the compressed muscle tissue exhibited large necrotic regions. Necrotic fibers with disorganization of the internal structure are shown in Fig. 8B. Slices of muscle tissue from the tourniquet experiments demonstrated some small regions with increased interstitial space and somewhat rounder fibers (Fig. 8C), although the major part of the TA had a normal histological appearance (explaining the normal T2 values after unloading in the tourniquet experiments in Fig. 6). No necrotic fibers were observed.

**DISCUSSION**

Ischemia and deformation have been implicated in the development of deep tissue injury after compressive loading. In the present study, two loading protocols, designed to distinguish between these two factors, were applied for 2 h to the lower limb of Brown Norway rats. An inflatable tourniquet positioned above the knee of the animal was applied for ischemic loading, and uniaxial loading was applied to the TA region by means of an indenter to study the combined effect of ischemia and deformation. Spatial, as well as temporal, information on the tissue perfusion was obtained using contrast-enhanced MRI. Changes in tissue status, during and after loading, were measured with T2-weighted MRI. Both MR measurements were performed consecutively for up to 6 h in

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**Fig. 3.** PI maps measured during indentation in 3 slices of lower limb in the sagittal image at left. A: 3 mm proximal to the indenter position (slice 1). B: underneath the indenter (slice 2). C: 3 mm distal to the indenter position (slice 3).
In this study, large indentations, equivalent to surface pressures uniformly, tissue distortion can lead to localized tissue damage. By contrast, if external pressures are applied non-uniformly, tissue distortion can lead to localized tissue damage. This occurs, for example, during deep sea diving. The nature of the loading is an important determinant in the damage of soft tissues. Indeed, it is well established that body tissues can support high levels of hydrostatic pressure with no resulting tissue damage. This occurs, for example, during deep sea diving. By contrast, if external pressures are applied non-uniformly, tissue distortion can lead to localized tissue damage. In this study, large indentations, equivalent to surface pressures of ~150 kPa, simulating the indentation of muscle tissue by bony prominences, were applied (18, 39). However, the surface pressure measurement in this study does not relate to the interface pressure measurements as reported in clinical studies. This is caused by the loading geometry, which was such that the muscle tissue is compressed against the tibial bone by an indenter. It must be accepted that the nature of the indenter, in terms of its curvature and stiffness, is also equivalent to a bony prominence. The applied surface pressure in this study can therefore not be compared with the interfacial pressures commonly measured in seated individuals by arrays of sensors embedded in a mattress. This arrangement highlights the importance of the internal mechanical state and the limited value of surface pressures. Indeed, several authors have reported on the variable relation between surface pressure and internal, or interstitial, pressure, which is a function of internal geometry, including the proximity of bony prominences (4, 31, 39, 49). Recently, Linder-Ganz and colleagues (31) demonstrated that interface pressure measurements in sitting humans evaluate loading only at the least-loaded tissue, i.e., skin, and that the most highly loaded tissues, i.e., muscle and fat, are ignored.

The applied pressure in the present study resulted in internal maximum shear strains of 70–100% in the deformed region (37% maximum principal compressive strain and 170% maximum principal tensile strain). Such large strains are considered to be relevant, in particular, for sitting spinal cord injury (SCI) subjects with muscle atrophy and/or flaccid muscle paralysis (50); however, parameters may be different for animal and human tissues (e.g., rats are less sensitive than humans to damage, and SCI subjects are more susceptible than healthy subjects). To determine internal stresses and strains in sitting subjects, Linder-Ganz et al. (31) combined measurements performed in a double-donut MR scanner, which allowed imaging of sitting subjects, and the results of subject-specific finite-element models. From their measurements in six healthy sitting humans, they found maximum principal compressive strains of 70–84% and tensile strains of 68–83%. By adding 5 kg of extra weight, they found that strains increased to >90%. It has been shown that properties of muscle tissue change after SCI (53). One of the effects is severe muscle atrophy, which increases the local strains in these tissues. Castro et al. (12) reported 27–56% atrophy in a group of SCI subjects, and Modlesky et al. (36) described comparable numbers.

Use of contrast-enhanced MRI to measure the perfusion status in resting skeletal muscle was first reported by Luo and colleagues (32). In this study, a maximum uptake rate of the contrast agent was estimated to provide a quantitative PI. In the present study, however, this approach was not possible because of the relatively low signal-to-noise ratio attributed, in part, to a small selected slice thickness of 1 mm. As an alternative parameter, the maximal signal intensity difference was selected as a relative PI. The method did provide sufficient spatial resolution for the detection of low-perfusion areas, which were identified as regions with a relative PI of 0.25 or less.

![Fig. 4. T2-weighted-sum images 3 mm proximal (A–D) and distal (E–H) to indenter position before (T2p) and during (T2L) indentation and 15 min (T2o) and 65 min (T2) after its release. *Wrap-around artifact (backfolding).](image)

![Fig. 5. T2ws images (A–D) and PI maps (E–H) of lower limb for a typical tourniquet experiment. A–D: T2ws images measured before (A) and during (B) tourniquet application and 40 min (C) and 115 min (D) after tourniquet deflation. E–H: PI maps before (E) and during (F) tourniquet application and 5 min (G) and 90 min (H) after tourniquet deflation.](image)
resolution to distinguish regions with normal perfusion, hypo-perfusion, and reactive hyperemia (Figs. 2 and 3). The temporal resolution was limited to 90 min because of the relatively slow clearance of the contrast agent but was sufficient to measure a relative PI before, during, and after loading.

The deformation of the muscle during loading was measured using MR-tagging experiments. This technique is mostly used in cardiac imaging (1) and, more recently, in studying contracting skeletal muscle (34). In the present study, we have demonstrated that MR tagging can also be used to determine strain fields in skeletal muscle during compressive loading. The most important restriction was the requirement that the indenter be applied rapidly to allow measurement of the deformed tagging grid, which fades within 1 s because of T1 relaxation. The tagging experiments required rapid repetitive indenter applications, which caused inevitable damage to and swelling of the tissue. Therefore, these experiments could not be combined with the indenter experiments and were performed separately. Accordingly, only a global comparison could be made between the location of damage, as determined by T2-weighted MRI, and the location of high shear strains. All loading protocols were, however, performed inside the MR scanner; therefore, the precise indentation in each experiment was recorded. As a result, experiments with similar indentation could be selected for valid comparison.

Perfusion status in the lower limb during and after loading was measured using contrast-enhanced MRI. The PI map taken during uniaxial loading showed an extensive region with low PI values (Fig. 2E). This clearly indicates hypoperfusion, probably due to a compression-induced obstruction of a large

![Figure 6](image_url)

Fig. 6. Time course of normalized T2 values before and during loading and after unloading for indenter \((n = 6)\) and tourniquet \((n = 3)\) experiments. Shaded area, loading period. ROIs are indicated in Fig. 3A. During indenter application, no values could be calculated in ROI 1 because of compression of the tissue. Dashed lines, trends for results of the tourniquet experiment. Values are means ± SE. Time 0, moment of unloading. ***Statistical significance relative to initial value \((P < 0.001)\).

![Figure 7](image_url)

Fig. 7. C-SPAMM images with undeformed \((A)\) and deformed \((B)\) tagging grid. \(C\): indentation calculated from displacement of tagging lines. \(D–F\): calculated distributions of strain [tensile \((D)\), compressive \((E)\), and maximum shear \((F)\)]. Dashed lines, deformed and undeformed contour lines.
vessel adjacent to the tibial bone. This finding was observed in all three experiments. In the compressed region, in association with the high T2 values, very high PI values were found after unloading (Fig. 2F). It is proposed that these high PI values are a result of a combination of reactive hyperemia and possible enhanced leakage of the contrast agent into the damaged region as a direct result of damaged capillaries. This possibility might be further examined by administration of Evans blue dye, which is widely used to study blood vessel and cellular membrane permeability (24).

A marked difference was found between the effects on T2 after 2 h of compressive and pure ischemic loading. For example, in the indenter experiment, T2 values in the compressed region were significantly increased after unloading (Fig. 6). Increased T2 values after compression were correlated to necrotic regions and regions with different stages of muscle fiber damage, as shown by histological examination (7, 55, 56). By contrast, ischemic loading led to a small initial decrease followed by an increase in T2 during loading (Fig. 6). After deflation of the tourniquet, the T2 values returned to normal within 40 min. The increase in T2 after 90 min of ischemia can be explained by osmotic shifts of water caused by an accumulation of waste products of anaerobic metabolism. Indeed, it has been shown in a human study that a 90-min period of ischemia leads to an accumulation of lactate (41). These increased lactate levels indicated accumulation of products of anaerobic metabolism, which was initiated by severe hypoxia caused by tourniquet inflation. An associated decrease in intracellular pH after occlusion of blood flow has been reported in several studies (5, 37). Appell et al. (3) demonstrated that relatively short periods (15–90 min) of ischemia, without reperfusion, already cause pathological alterations, particularly affecting metabolically important organelles. The T2 values that were increased during ischemic loading returned to pre-loading values within 40 min after deflation of the tourniquet (Fig. 7), indicating a rapid restoration of the normal water balance.

In our previous experiments (56), we observed a striking difference in temporal development of the affected area between slices proximal and distal to the indenter. Similarly, in the present study, in the slices proximal to the indenter, T2 values started to increase after unloading, and the affected volume increased in the 1st h and then slowly decreased (Fig. 4, A–D). In the slices distal to the indenter, however, higher T2 values were already observed during and directly after load removal. However, the affected area decreased in the 1st h (Fig. 4, E–H). The difference was hypothesized to be caused by differences in perfusion status during loading. The PI maps in the present study did indeed show a different perfusion status distal and proximal to the indenter position (Fig. 3), which suggests that the T2 differences can be explained in terms of regional differences in perfusion status during loading.

The combined effect of compression and ischemia on muscle tissue has previously been studied by examination of tissue beneath and distal to a tourniquet (43, 44). These studies were performed to define “safe” durations of tourniquet application in surgery on the extremities. A specially designed curved tourniquet applied to the hindlimb of a rabbit was used to produce a uniform distribution of tissue pressure on cuff inflation (42). Histological abnormalities were more severe in compressed thigh muscles than in ischemic leg muscles. In the compressed muscles, considerable intra- and interindividual variability in damage was noted. This might be explained by our present hypothesis concerning the importance of local deformation at the onset of damage. A 2-h period of distal ischemia induced minor histological abnormalities, which were also observed in the present study.

Compared with other tissues, such as cardiac and cerebral tissues, resting skeletal muscle has been described to be resistant to ischemia, with cessation of metabolic activity after >5 h (13, 25). However, the rate of metabolic processes is increased if, for example, twitch stimulation is imposed during ischemia and reperfusion (58). This increases the rate of energy depletion and accelerates the associated damage processes. If, instead, large deformations are imposed during ischemia and there is already damage from the large strains on the fibers, the damage processes due to ischemia might also be accelerated. Accordingly, 2 h, as applied in the present study, might be sufficient for ischemia to have an aggravating effect. This has
implications in the clinical setting, in which a 2-h period of ischemia is still used as a standard for manual pressure relief.

Reperfusion is one of the theories that has been mentioned to explain pressure ulcers, although few studies (26, 46, 57) have actually provided evidence for this theory. On the basis of the recovery of T2 values to preloading values after deflation of the tourniquet, no damaging effect of reperfusion was observed after 2 h of ischemia. In the indenter experiment, the effect of reperfusion was more difficult to determine. However, if damage is initiated during loading of the muscle tissue, the reperfusion phase might cause additional damage.

The present study is the first in which an experimental setup allows the measurement of the precise deformation, the local internal strains, and the perfusion status during compressive loading and the location of damage after unloading in skeletal muscle. This allows the comparison, although globally, since the tagging experiments required separate measurements, between locations with high strains and locations with damage. From comparison of the location of damage indicated in Fig. 2C, the region of ischemia (Fig. 2E), and the region with high shear strain values (Fig. 7F), it is evident that the location with damage correlated well with the region with high shear strains during loading. This strongly supports our proposal that strain is critically important in the initiation of damage.

As stated above, SCI subjects have an increased susceptibility for the development of deep tissue injury. The larger deformations and reduced perfusion in their muscles (53) may contribute to this. Improving one or both of these conditions, e.g., by the intervention of electrical stimulation, might reduce the risk of developing deep tissue injury (6, 28). Indeed, in a recent single case study by Bogie et al. (6), the positive effect of neuromuscular electric stimulation on muscle thickness, blood flow, and tissue tolerance was demonstrated.

In summary, the present study has demonstrated that a 2-h period of compressive loading leads to irreversible damage to the muscle tissue, whereas ischemic loading results in reversible tissue changes. This implies that large deformation, in conjunction with ischemia, provides the main trigger for irreversible muscle damage. This was further confirmed by the indirect correlation between the location of damage and the location of high shear strain values.

Acknowledgments

We are grateful to Jo Habets for help with the animal experiments and Joost Mulders for analyzing the MR-tagging data.

References