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STRAIN DISTRIBUTION ON RAT MEDIAL GASTROCNEMIUS (MG) DURING PASSIVE STRETCH

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Abstract—Deformation of the surface of passive medial gastrocnemius muscle (MG) was measured in vivo while performing a hysteresis test. The gastrocnemius muscle of male rats were dissected free and the distal tendon was cut. The lateral head was separated from the medial head. The muscle origins were left intact. 60-70 fluorescent polystyrene spheres (diameter 0.7 mm) were attached to the surface of the MG. During the experiment, two-dimensional video recordings of the movements of the MG were made. The coordinates of the marker centroids were obtained by computer processing of digitized images and marker displacements as a function of time were calculated. Green-Lagrange strains in two principal directions were calculated ($e_1$, $e_2$) for three specimens. $e_1$ had approximately the same direction as the muscle fibers. The longitudinal strain of the fibers (20-30%) was larger than the strain of the aponeurosis (1-5%); $p < 0.001$. No significant difference was found between the values of the transverse strains of muscle fibers and aponeurosis; the value of $e_2$ was -6 to -9% for both tissue structures.

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Keywords: Transverse strain; Longitudinal strain; Aponeurosis; Gastrocnemius muscle.

INTRODUCTION

Quantifying material properties of biological tissues involves many difficulties because (1) biological tissues often possess inhomogeneous structures and morphology and (2) the condition of in vitro tissue samples is difficult to maintain. Skeletal muscle consists of three components: the muscle fibers with their contractile properties, the aponeurosis at which the muscle fibers insert and free tendon. Each component exhibits different material behaviour, and within these components inhomogeneities may occur (Butler et al., 1990; Zernicke et al., 1984; Zuurbier et al., 1994). In a study of the regional behaviour of the proximal aponeurosis of rat m. gastrocnemius medialis in both passive and active conditions, Zuurbier et al. (1994) found that longitudinal extension of the proximal aponeurosis of the rat gastrocnemius medialis muscle was heterogeneously distributed along its length, with the largest extension occurring in the most distal part of the aponeurosis. In muscle modelling however, homogeneous lengthening of the aponeurosis is often assumed (van Leeuwen, 1992; Otten, 1988).

Knowledge concerning the elastic behavior of aponeurosis is essential for a better understanding of its role as a force transmitter within a muscle-tendon complex, as tendon sheets can take up a considerable part of the length changes imposed to the muscle-tendon complex. Recently, the length behaviour of aponeurosis has been studied in order to explain the discrepancies between length changes of muscle fibers and their parent muscle (Hoffer et al., 1992; Scott and Loeb, 1993). In a muscle-tendon complex, the muscle fibers insert to the aponeurosis over its entire area and exert forces in more than one direction, so a detailed understanding of its mechanics requires three-dimensional deformation analysis. However, to our knowledge, even two-dimensional deformation measurements are not yet available in the literature. Therefore, we developed a two-dimensional optical marker tracing method to quantify the local material deformation of a muscle-tendon complex. Two-dimensional measurements, using an optical marker following system, have been performed earlier on heart tissue (Delhaas et al., 1993; Prinzen et al., 1989, 1986), and skin (van Ratingen et al., 1993); local strains were calculated from these data.

Experiments on in vitro samples of skin tissue have indicated that local deformations in a nonhomogeneous material can be determined with the use of an image analysis system. Subsequently, with the use of a numerical model, local material characteristics can be calculated (van Ratingen et al., 1993).

In the present paper a method for two-dimensional quantification of strain fields of rat MG in vivo during passive extension is presented. The purpose of this study was twofold: (1) we examined whether strains are uniformly distributed on the aponeurosis surface of the MG during loading and; (2) strains of muscle fibers and aponeurosis in both principal directions were compared. A realistic description of the constitutive behavior of skeletal muscle tissue necessitates a finite-element model in which the contractile properties of the sarcomeres must be included. Future research includes deformation measurements during both isometric and dynamic contraction of the m. gastrocnemius.
METHODS

Four male adult Lewis rats (350–375 g) were anaesthe-
tized with ketamin (Nimatek, A.U.V., 40–60 mg kg\(^{-1}\)
body weight s.c.) and xylazin (Sedalun, A.U.V.,
3–8 mg kg\(^{-1}\) body weight s.c.). The m. gastrocnemius
was dissected free and the lateral head of the muscle was
carefully separated from the medial head. The distal
head was cut loose from the calcaneus and the muscle
origins were left intact. The epimysium lying loosely on
the surface of the proximal muscle fibers, the achilles
tendon and the proximal aponeurosis were carefully re-
moved with a pair of tweezers and scissors. Following
this, 60–70 fluorescent, polystyrene spheres (diameter
0.7 mm) were approximately evenly attached to the sur-
face of the distal muscle fibers and the proximal aponeuro-
osis of the MG with histoacryl tissue glue (Histoacryl
blau, Braun, Melsungen) with an interdistance of
1–2 mm. To be certain that the epimysium was removed
sufficiently it was checked for each marker that it could
not be moved over the muscle surface. The rat was
positioned in a uniaxial tensile testing apparatus (Zwick
1445, Zwick GmbH, Ulm, Germany) (Fig. 1). The distal
tendon was clamped and connected to the force and
displacement transducer of the upper traverse of the
tensile testing apparatus (TTA), which has a sensitivity of
0.001 N and 0.01 mm. The femur was kept in a fixed
position by a stainless-steel clamp which was introduced
into the leg from the lateral side. This clamp was connec-
ted to a rigid Plexiglass plate that was attached to the
lower traverse of the TTA. A thin layer of paraffine oil
was used to prevent the muscle from drying out during
the experiment. The attachment area of the markers was
inspected post-experimentally by SEM.

Experimental procedure

A hysteresis test on the passive muscle–tendon com-
plex was performed using the TTA:

1. The muscle was stretched to a resting length, \( L_0 \), at
a speed of 50 mm min\(^{-1}\). This resting length was defined
as the length at which the force that the muscle–tendon
complex exerted on the force transducer equalled ap-
proximately the wet weight of MG (0.085 N). This weight
was determined earlier with gastrocnemius muscles of
other rats of the same weight.

2. Immediately following this, the muscle–tendon
complex was stretched three times from 102 to 110% of
\( L_0 \) at a speed of 8% of \( L_0 \) per minute.

The percentages of the resting length were chosen
arbitrarily, while the velocity of the movement was
chosen to simulate a quasi-static situation. No spontan-
eous contractions occurred in the sequences we analyzed.

Data processing

Two-dimensional recordings of the movements of the
distal muscle fibers and the proximal aponeurosis of the
MG were made, using a 50 Hz CCD video camera (MO,
HTH) and a video recorder (Panasonic VHS, NV-D80).

Fig. 1. The experimental setup used in the present study. The rat was placed in the tensile testing apparatus
(TTA). The achilles tendon was clamped and connected to the upper traverse of the TTA. The femur was
kept in a fixed position. A CCD camera was placed in front of the medial view of the MG; video recordings
of the movements of the markers attached to the distal muscle fibers and the proximal aponeurosis were
made. VCR, video recorder; TIM, image analysis system.
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To make optimal use of the fluorescence, a BG12 filter (Schott) was placed in front of the light source (Fiberoptic A G., L 100) and a KG1 filter (Schott) was placed in front of the camera. Digitized images of the recordings were obtained by sampling images at a frequency of 0.5 Hz with the use of a frame grabber (PCVISIONplus, Imaging Technology Inc.). Image analysis was performed by computer processing of the digitized images. The coordinates of the marker centroids in each frame were obtained and marker displacements as a function of time (marker tracks) were calculated. Subsequently, local deformations (Green–Lagrange strains) of each marker were computed as described by Peters (1987). A strain group was defined by choosing an area (radius ± 3 mm) surrounding one marker and allocating all markers within this area to the strain group. Each strain group consists of a minimum of three markers. The strain tensor was calculated assuming constant deformation within each strain group. The principal strains \( e_1 \) and \( e_3 \) are ranked in descending order, from most positive to most negative.

Due to the curvature of the muscle the lateral parts are more subject to projection errors than the central part. Therefore, we defined an area in the length direction on the central third part of the muscle. We only used the results of markers located within this area. Three sub-regions were defined: muscle fibers, aponeurosis and a transition zone in between. Strains for both muscle fiber tissue and aponeurosis were calculated and compared. The transition zone was defined post-hoc. This choice is argued in the discussion.

Statistics

A two-way ANOVA (cycle, tissue) was performed on the strain data for each muscle. Strains at 110% of \( L_0 \) were compared with the reference situation at 102% of \( L_0 \). Markers which were missing in one or two cycles, were excluded from further analysis.

RESULTS

The results of the recordings of the medial side of the MG of three rats could be used. The results of one animal were excluded from further analysis, because marker tracks could be reconstructed for only one loading cycle.

Inspection of the SEM photographs showed that the diameter of glue was about 80% of the marker diameter (0.7 mm) (Fig. 2). The directions of the principal strains were obtained for each individual marker. Visual inspection indicated that the principal strain \( e_3 \) had approximately the same direction as the muscle fibers (Fig. 3). In the following sections, principal strains \( e_1 \) and \( e_3 \) will be referred to as ‘longitudinal’ and ‘transversal’ strains, respectively.

Figure 4(B) shows the results of the longitudinal strain for both muscle fibers and aponeurosis for three cycles. \( e_1 \) of the muscle fibers and aponeurosis are homogeneously

![Fig. 2. SEM microphotograph of a marker (M) on the muscle surface. Note the small diameter of glue (G).](image-url)
distributed. Between both tissue structures, a clear difference in \( e_1 \) can be seen. Figure 4(C) suggests that the second principal strain of each tissue structure was also homogeneously distributed. Furthermore, \( e_2 \) showed no difference between muscle fibers and aponeurosis; the average value of \( e_2 \) was in between \(-6\) and \(-9\)% (Table 1). Unlike what was intuitively expected, in aponeurosis the absolute values of the second principal strains were larger than the absolute values of the first principal strains.

Table 1 gives a summary of the average values of the principal strains, \( e_1 \), of muscle fiber tissue has an average strain value that is approximately 18 times larger compared to the value of aponeurosis; 22–29% and 1–5%, respectively. The second principal strain has a ratio fiber tissue/aponeurosis of approximately 1.

In Table 2 the results of the statistical analysis are presented. The difference between the \( e_1 \) of fiber tissue and that of aponeurosis was highly significant (\( p < 0.001 \)) (Table 2). For \( e_2 \) this was only so for one case (\( p < 0.05 \)), the \( e_2 \) of the muscle fibers being larger.

**DISCUSSION**

Although the present study was performed on only three specimens, it clearly shows that during passive extension of the MG the principal strain \( e_1 \) is homogeneously distributed for both the distal fibers and the proximal aponeurosis. Homogeneous strains have already been assumed in skeletal muscle modelling (van Leeuwen, 1992; Otten, 1988). A combination of experimental results and numerical calculations has shown that it is fair to assume fiber strains to be homogeneous; across the cardiac wall the fiber strains of the inner layers and the outer layers were uniformly distributed (Arts et al., 1982). This uniformity of strain has been observed for frog gastrocnemius muscle (Trestik and Lieber, 1993). Strains at \( P_B \) ranged from 1.8 to 2.5% except for the most proximal region of the proximal aponeurosis where strain was over 6%. In contrast, Zuurbier et al. (1994) observed a nonuniformity along the aponeurosis for both passive and active MG: strains in both end regions of the aponeurosis were larger than in the middle region. The thickness of the proximal aponeurosis decreases from proximal to distal; the forces exerted on the aponeurosis also decrease from proximal to distal. It seems that these changes are proportional, resulting in uniformly distributed stresses and strains over the aponeurosis, as is assumed in muscle models (van Leeuwen, 1992).

The longitudinal strains of the proximal aponeurosis were significantly lower than those in the distal muscle fibres, suggesting that aponeurosis has a greater stiffness in the longitudinal direction than muscle fibers. No significant differences in second principle strain were found between the aponeurosis and the muscle fibers. This can be explained by assuming that the aponeurosis behaves like a membrane, and therefore has no stiffness in compression. In this case, under negative strain, the magnitude of the strain is determined by the strain of the underlying muscle fiber tissue.

The strain \( e_1 \) was homogeneously distributed within each subregion [Fig. 4(B)], which can be interpreted as a similarity between the material properties within each tissue structure. In between these subregions there was an area which showed a strain gradient. This area was defined as a 'transition zone'. The use of strain groups to calculate strains causes high gradients in the strain field to spread over a larger area than in reality (Fig. 3). This results in a low pass filter effect (Peters, 1987). The extension of the gradient is approximately equal to the diameter of a strain group [Figs 4(B) and (C)]. In our case, the high gradient is produced by markers within the same strain group belonging to two different tissues. We assume that the longitudinal strain of the aponeurosis converts inhomogeneously into muscle fiber strain. An extension nonuniformity along the aponeurosis with the largest extension for the most distal part was demonstrated by Zuurbier et al. (1994). Detailed measurements using smaller markers will be needed to determine the course of the strain field of the area surrounding the myotendinous junction. The use of markers attached to the muscle surface proved to be a useful method, although with this method conclusions about strain fields can be drawn only for the outer surface of the muscle, which in our case were represented by the most distal fibers and the proximal aponeurosis. After removal of the epimysium, the markers were firmly attached to the muscle surface and the area of glue was smaller than the diameter of the markers (Fig. 2).

Two-dimensional analysis of curved surfaces causes projection errors. Rotation of strain groups along axes other than the optical axis of the camera may lead to strain artifacts because of the projection of the three-
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Fig. 4. (A) Medial view of the muscle–tendon complex with markers, strain group size (circle) and the contours of the proximal aponeurosis. The dotted lines represent the central area where strains were calculated. (B) $\varepsilon_1$ plotted against the y-position of the markers on the muscle. Results are given for each loading cycle. (C) $\varepsilon_2$ plotted against the y-position of the markers on the muscle. Results are given for each loading cycle.

dimensional strain field onto a plane. Therefore, strains were only calculated in the central, longitudinal part of the muscle. Nevertheless, errors in strain calculations could not be entirely avoided. As the muscle–tendon complex is lengthened, the muscle surface flattens in the longitudinal direction. This results in a fictitious longitudinal strain; a rough estimation of its value showed us that this can amount to $\pm 3\%$. The same phenomenon occurs for $\varepsilon_2$; the size of this error increases in lateral direction. The radius of the muscle–tendon complex altered from an average of approximately 11 mm at 102% of $L_0$ to approximately 6 mm at 110% of $L_0$. This caused a mean strain error of 2% in the central band which was approximately 3.5 mm wide. This means that future research should include three-dimensional measurements in order to exclude strain errors. Successive cycles yielded
Fig. 5. An illustration of the low pass filter effect on a high gradient caused by the size of a strain group (from: Peters, 1987).

### Table 1. Principal strains of aponeurosis and muscle fibers. \( \varepsilon_a \) and \( \varepsilon_b \) are the first and second principal strains for aponeurosis (mean ± S.D.). \( \varepsilon_f \) and \( \varepsilon_b \) are the first and second principal strains for muscle fibers (mean ± S.D.)

<table>
<thead>
<tr>
<th>Rat #</th>
<th>( \varepsilon_a )</th>
<th>( \varepsilon_f )</th>
<th>( \varepsilon_a )</th>
<th>( \varepsilon_b )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.01 ± 0.01</td>
<td>0.22 ± 0.10</td>
<td>-0.06 ± 0.02</td>
<td>-0.06 ± 0.02</td>
</tr>
<tr>
<td>2</td>
<td>0.01 ± 0.01</td>
<td>0.25 ± 0.10</td>
<td>-0.07 ± 0.02</td>
<td>-0.09 ± 0.03</td>
</tr>
<tr>
<td>3</td>
<td>0.05 ± 0.04</td>
<td>0.29 ± 0.06</td>
<td>-0.09 ± 0.06</td>
<td>-0.07 ± 0.02</td>
</tr>
</tbody>
</table>

Similar results for \( \varepsilon_f \) [Fig. 4(B)] indicating that random errors were small. The results for the second principal strain distribution however, were more noisy, so care must be taken in interpreting these data.

In summary, our data indicate that both distal muscle fibers and the proximal aponeurosis extend homogeneously under passive loading. As expected, we found that in the longitudinal direction the aponeurosis possesses a larger stiffness than the muscle fibers. No predictions could be made about the transition zone, although it was clear that the high gradient in strain was caused by the use of strain groups; further research is required to learn more about the behavior of this characteristic region.

### References


