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THREE-DIMENSIONAL RECONSTRUCTION OF THE RAT TRICEPS SURAE MUSCLE AND FINITE ELEMENT MESH GENERATION OF THE GASTROCNEMIUS MEDIALIS MUSCLE

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ABSTRACT

In order to simulate blood flow in skeletal muscle, our group has developed a finite element description of perfused skeletal muscle. This model requires input parameters concerning the vascular system, muscle contraction and the geometry of a muscle, including its aponeuroses. The objective of the present paper is to create a geometrical reconstruction of the rat gastrocnemius medialis muscle that can be incorporated in the finite element model. Since this muscle is connected to the plantaris and the gastrocnemius lateralis muscle, a detailed computer graphical reconstruction of the triceps surae muscle, based on histological cross-sections, has been accomplished first. Using this reconstruction, relevant sections were selected to create the finite element mesh of the gastrocnemius medialis muscle. Special attention was paid to the location of the aponeuroses. The mesh can be used in finite element simulations of perfused skeletal muscle.

KEYWORDS: reconstruction – gastrocnemius – muscle – geometry – perfusion

INTRODUCTION

Blood flow through muscle is important for oxygen supply and transport of metabolites. Petrofsky & Hendershot (1984) found that isometric endurance time of contraction increases by increasing perfusion pressure, showing the importance of mechanical variables for muscle perfusion. Several models concerning the mechanical behaviour of muscle during contraction have been developed (Woittiez et al., 1984; Huying & Woittiez, 1985; Van Leeuwen & Spoor, 1992; Van Leeuwen & Spoor, in press). However, these models do not take into account changes in

blood flow as a result of intramuscular pressure and muscle deformation. In our group, a finite element model of blood perfused skeletal muscle has been developed (Vankan et al., in press). With this model, deformation, intramuscular pressure, blood pressure and blood flow in different blood compartments can be calculated in contracting perfused skeletal muscle. This model is implemented in the finite element software package DIANA (DIANA analysis bv, Delft, The Netherlands). We choose to simulate perfusion of the gastrocnemius medialis muscle (GCM), because much research that can be used to validate the model has already been carried out regarding this muscle. Regional blood flow distribution during contraction was measured by Pendergast et al. (1985) and Piiper et al. (1989) and measurements of deformation during contraction were performed by Zuurbier et al. (1992) and Van Bavel et al. (in press). Accurate calculation of deformation and perfusion

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during contraction, calculated by means of the finite element method, requires incorporating mechanically important aspects of the geometry of the GCM into the model. This includes the geometry of the muscle belly and the aponeuroses as well as muscle fiber directions. Several geometries of the GCM have already been described in the literature (Holewijn et al., 1984; Woittiez et al., 1985; Van Leeuwen & Spoor, 1992; Van Leeuwen & Spoor, in press). However, their geometrical descriptions are not detailed enough for accurate finite element simulations. Therefore, a finite element mesh of the GCM had to be created. A finite element mesh is built with a number of nodes that are connected to each other, forming elements. The more details are to be included into a mesh, the more elements and nodes have to be used. However, calculation time increases with the number of elements. Calculation time of simulations on a SUN Spark Classic, using a mesh built with about 1000 elements, is still acceptable.

Since the GCM cannot be dissected from the gastrocnemius lateralis muscle (GCL) without damaging the muscle distally, a precise histological reconstruction of the rat triceps surae muscle, visualised by means of computer graphical techniques, was accomplished in advance. Outlines of the GCM and the geometry and thickness of accompanying aponeuroses were identified in this reconstruction and used to actually generate the finite element mesh. Although muscle fibers directions are important for the behaviour of the muscle during contractions, they have not been determined in this reconstruction yet. Magnetic Resonance Imaging might be a suitable means to accomplish this (Van Doorn et al., in press). The generated finite element mesh is used to calculate passive perfusion (Vankan et al., in press).

The aim of the present study is to generate a detailed geometrical reconstruction of the GCM to be used in finite element analyses. This is preceded by a detailed histological reconstruction of the triceps surae muscle, using computer graphical techniques.

METHODS

1. Reconstruction of the triceps surae muscle. To obtain a triceps surae muscle for a reconstruction, the distal part of the tibia and the foot of an anaesthetised 12 weeks old male Lewis rat were fixed. The angles of both the knee and the ankle were set at

90 degrees. The rat was perfusion fixated with a buffered formaldehyde solution (pH 7.2), injected into the left ventricle. X-ray images of the hind limb were obtained after the perfusion fixation to verify the angle of the knee and the ankle joint. The triceps surae muscle, including the GCM, GCL, plantaris (PL) and soleus muscle (SOL), was dissected free from its surroundings. The distal tendon was cut near the calcaneus and the individual muscles were carefully removed from their origins. The muscle was dehydrated and embedded in a solution of Technovit 7100 with 0.2% polyethyleneglycol. It was longitudinally surrounded by 5 parallel graphite bars (0.3 mm, 2B) that were attached in small plastic containers, positioned at both sides of the muscle. 80 out of 8000 cross-sections (4 μ m) through the complete length of the muscle, which were obtained with a microtome (2218 HistoRange), were coloured with trichrome Gomori for 3 hours, to discriminate between collagen of aponeuroses and muscle fiber tissue. As a result of the mentioned graphite bars, reference holes remained in each slide.

Photographic slides were taken from the coloured sections through a microscope (Zeiss Tessovar, camera: Contax). These were projected on a table through a slide projector (Kindermann AV100, lens: Isco-Göttingen Projar 1:2.8/60). The reference holes of the first slide were drawn on the table, while those of the other projections were fitted on these drawings. In this way, the magnification factor of each slide was the same. The outlines of the 4 muscles as well as their aponeuroses and the reference holes of each section were drawn on natural tracing paper. The sections were thoroughly examined under a microscope and relevant histological aspects that could not be seen properly in the projection were added to the drawings, using a drawing prism (Zeiss). Finally, 80 detailed drawings of the outlines of the triceps surae muscle, including the aponeuroses, tendons and large structures of loose connective tissue were obtained.

The outlines of the 4 different muscles, their aponeuroses and the 5 reference holes were digitized separately, using an xy-tablet (Summagraphics ID). Using the coordinates of the reference holes, the slide numbers and the 2 dimensional registration of the outlines in each plane, a 3 dimensional reconstruction of the muscle complex could be produced. The reconstruction was visualized by a computerized drawing program that allows separate drawing of select-

ed outlines and presentation from any viewpoint or in any perspective (Van Mameren *et al.*, 1985).

2. Generation of the finite element mesh. After examination of the detailed reconstruction, 14 sections of the GCM, representing the total length of the muscle, have been selected. These included the section containing the largest cross-sectional area of the GCM (18.2 mm from the proximal origin), and the ones containing the most proximal part of the distal aponeurosis (5.8 mm from the proximal origin) and the most distal part of the proximal aponeurosis (16.2 mm from the proximal origin).

In order to generate 3D elements for the finite element mesh, 28 nodes were indicated in each section and connected with each other as follows (Fig. 1). First, two nodes were placed on the muscle's outline, indicating its largest diameter(*). Then, two points (^) were used to mark the edges of the aponeuroses on the outline, to eventually locate the aponeuroses in the finite element mesh. If there was another aponeurosis, two more points were used. After that, 14 (or 12 in case of two aponeuroses) points were evenly distributed along the outline of the muscle fiber tissue. Care was taken to indicate the points in such a way that connective lines between the nodes followed the outlines of the muscle accurately. Using these 18 points, representing the muscle's outline, the muscle area was divided into 18 (3*6), approximately equally sized quadrangles with 28 nodes.

They were fed into the computer, using the digitizer tablet. Corresponding nodes between successive sections were connected. This resulted in $13 \times 3 \times 6$ hexahedral elements, each of which was divided into 5 tetrahedral elements. The aponeuroses were represented by 2D triangular elements. These were attached to the sideplates of the tetrahedral elements at the surface of the muscle where aponeurosis was seen in the cross-sections. The local thickness of the aponeuroses, measured from the digitizer tablet, was assigned to corresponding elements. A total of 54 and 76 triangular elements, for the proximal and distal aponeurosis respectively, were used to describe the aponeuroses. Thus, a finite element mesh of the GCM, consisting of 1170 ($5 \times 13 \times 3 \times 6$) 3D tetrahedral elements (muscle fiber tissue), 130 2D triangular elements (aponeuroses) and 392 nodes, resulted.

RESULTS

1. Reconstruction of the tricep surae muscle

Figs. 2 and 3 show the result of the detailed reconstruction. The names of the muscles are given in the figures. The dorsal (Fig. 2a) and ventral view (Fig. 2b) of the triceps surae muscle show the orientations of the different muscles. Proximal, the PL can be seen at the surface of the triceps surae muscle (Figs. 2a and 3a), while the remaining part is covered by the CGM and the GCL. The distal aponeuroses of

Fig. 1. FIRST, 2 NODES (*) ARE PLACED ON THE MUSCLE'S OUTLINE TO INDICATE ITS LARGEST DIAMETER, WHILE 4 NODES (^) MARK THE TRANSITIONS OF THE APONEUROSSES INTO LOOSE PERIMYSIAL CONNECTIVE TISSUE. The thickness of the aponeuroses is exaggerated in the figure, to clearly show its presence. Next, 13 nodes are evenly distributed along the muscle's outline. After connecting these nodes, a 4×7 grid results. The intersections form 10 more nodes.

Fig. 2. DORSAL (A) AND VENTRAL (B) VIEW OF THE TRICEPS SURAE MUSCLE. The muscles, ordered from dark to light, are the m. gastrocnemius medialis (GCM), m. soleus (SOL), m. gastrocnemius lateralis (GCL) and m. plantaris (PL). Aponeuroses and the Achilles tendon are white.

the 4 muscles join distally, forming the Achilles tendon.

From the cross-sections (Fig. 3) it is clear that the GCM only has one distal and one proximal aponeurosis. The GCL contains 2 distal and 2 proximal aponeuroses. One of the distal aponeuroses divides the GCL into a lateral and a medial part (Fig. 3b). The medial part is more pronounced distally, and shares a proximal aponeurosis with the PL. The GCM and GCL are connected to each other distally, where they share an aponeurosis. Fig. 3b shows an invagination of the distal aponeurosis into the GCM dorsal. Such invaginations are also present in other aponeuroses (Fig. 3c). Fig. 2b shows the large neurovascular bundle, accompanied by loose connective tissue, in between the GCL and the PL. This structure extends towards the GCM, while it remains located between the PL and the medial part of the GCL.

The place where the neurovascular bundle enters the GCM can be identified on the ventrolateral view of the GCM (Fig. 4a), near the insertion at the femur. The distal aponeurosis of the GCM (Fig. 4a) encloses 62% of the length of the muscle, while the proximal aponeurosis (Fig. 4b) is 78% of the length of the GCM. The thickness ranges from 0.02 mm to

0.56 mm. The complete length of the GCM is 26.2 mm from the most proximal attachment to the bone to the most distal section where muscle fibers can be seen in the cross-sections. Both a cross-section and a longitudinal section in the ventromedial to dorso-lateral plane through the GCM show that the muscle is falcated. The maximum diameter of the muscle in a cross-section is 10.0 mm in the ventromedial to dorsolateral direction, and in the direction perpendicular to that one 4.2 mm (Fig. 3b).

2. Generation of the finite element mesh

A view of the final mesh of the GCM, created by the program Femview (Femview Limited, Leicester, LE2 1GF, England), is shown in Fig. 4c. Viewpoints in Figs. 4b and 4c are the same. The Achilles tendon starts at the lower end of the mesh. Since the exact geometry of this tendon is beyond the scope of this study, it is not included. The muscle consists of 1170 tetrahedral elements for the muscle geometry, which can be identified in Fig. 4c, and 54 and 76 triangular elements for the proximal and the distal aponeurosis respectively. These triangular elements are attached to the triangular sideplanes of the tetrahedral elements, but cannot be identified in the figure. The

Fig. 3. PROXIMAL (a), MEDIAL (b) AND DISTAL (c) CROSS-SECTION THROUGH THE TRICEPS SURAE MUSCLE. The muscles, ordered from dark to light, are the m. gastrocnemius medialis (GCM), m. soleus (SOL), m. gastrocnemius lateralis (GCL) and m. plantaris (PL). Aponeuroses and the Achilles tendon are white.

Fig. 4. VENTROLATERAL (a) AND DORSOMEDIAL (b) VIEW OF THE DETAILED RECONSTRUCTION OF THE M. GASTROCNEMIUS MEDIALIS. The thick white lines represent the aponeuroses. THE RESULTING FINITE ELEMENT MESH (c) IS SHOWN FROM THE SAME VIEWPOINT AS USED IN (b). The grey values in (c) are a result of 3D-shading.

grey levels in Fig. 4c are a result of 3D shading. Despite the large number of elements, the mesh contains no more than 392 nodes, resulting in acceptable calculation times for finite element simulations.

DISCUSSION

1. Reconstruction of the triceps surae muscle

The perfusion fixation of the muscle was established under well controlled conditions. During the fixation the angle of the knee and the ankle were both

held at 90 degrees to minimize deformation of the muscle as a result of the perfusion fixation. This was confirmed by X-ray images. After complete fixation, the muscle was carefully dissected free, dehydrated and embedded in plastic. Care was taken not to deform the muscle during these procedures. The next steps in the reconstruction included the production and the photographing of the cross-sections and the drawings of the slide projections on the tracing paper. Since the reference holes on the drawings fitted to each other properly, it can be concluded that errors, resulting from the projections or the produc-

tion of the sections, were minor. The resulting drawings at this point include the rough outer geometry and the large tendinous structures of the muscles. Although the use of the drawing prism introduced projection errors, the fine-tuning of the drawings did not produce systematic errors, since the structures involved were always centered and fitted to the structures that were drawn on the sheet.

The invaginations of the thin parts of the aponeuroses affect muscle fibers attachment in two ways. First, more fibers can attach, since the upper side as well as the lower side of aponeuroses can be used. Second, the angle of attachment of the fibers on the lower side, which tends to decrease near the thinner part of the aponeurosis (Van Leeuwen & Spoor, 1995), is increased.

Because of tissue-shrinkage during the fixation of the muscle and the production of the sections, gaps occurred at some places in the intra- and intermuscular aponeuroses. Therefore, it is possible that the thickness of these aponeuroses was sometimes overestimated. Aponeuroses situated on the surface of the triceps surae muscle hardly show gaps.

2. Generation of the finite element mesh

In order to generate the anatomical reconstruction of the triceps surae muscle geometry, as many details as possible were included. In the contrary, concerning the finite element mesh generation, we were restricted in the number of points that could be used to describe the muscle's outline. Therefore, a manner to define nodes on characteristic sites of the outline, which has been described in the methods section, was developed. Afterwards, these nodes were used to generate the finite element mesh. Special attention was paid to the location of the aponeuroses by indicating its transition to loose connective tissue by nodes in each section. The resulting mesh includes the geometry of the GCM and its aponeuroses, the distal one ending just above the Achilles tendon.

The local thickness of the aponeuroses was measured for each relevant node. Although this can result in an overestimation of the thickness at some parts of the proximal aponeurosis, the largest part could be measured properly. When necessary, the local thickness was estimated, based on the thickness at the surrounding nodal points.

Since the GCM, GCL and the PL are attached to each other, and because of the muscle's specific geometry, analysis of the separate GCM is questiona-

ble. Suppression of deformation of the ventrolateral side of the muscle could improve the simulations in the present finite element model.

CONCLUSIONS

We were able to make a detailed histologic reconstruction of the geometry of the triceps surae muscle, including muscle geometry, aponeuroses, tendons and large bundles of connective tissue. From this detailed reconstruction, a finite element mesh of the GCM was derived, including muscle geometry and the two aponeuroses of the GCM. This mesh can be incorporated into the finite element description of perfused skeletal muscle that is formulated by our group to calculate deformation, intramuscular pressure, blood pressures and blood flows in different blood compartments.

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