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Implications of the calf musculature and Achilles tendon architectures for understanding the site of injury

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ABSTRACT

Clinically the sites of Achilles Tendon (AT) overuse conditions can be divided into the tendon mid-portion and osteotendinous attachment. *Purpose*: We propose an anatomical analysis of the triceps surae musculotendon unit that could provide a possible anatomic explanation for these 2 sites of injury. *Method*: Twelve cadavers (age 74 ± 7 years) were studied. In both legs, calf muscles (lateral gastrocnemius (LG), medial gastrocnemius (MG) and soleus) were dissected and their volumes measured. Fine saw cuts were made in the sagittal plane, either side of the midline of the calcaneus. Each strip contained the distal part of the tendon and its insertion, together with the superior tuberosity of the calcaneus. Trabecular architecture was analyzed from X-rays taken with Faxitron radiography. Histological sections of the enthesis and the thickness of the uncalcified fibrocartilage and the subchondral plate were evaluated. A finite element model of tendon coupled to a rupture index was developed to investigate the AT response to mechanical load. *Results*: Muscle volume was highest for the soleus, followed by the MG, and LG. Within the AT, the soleus fibers occupy the antero-medial parts, the MG fibers form the posterior lateral layer, yet the LG head fibers retain the antero-lateral part. The quantity of bone and the apparent trabecular thickness at the enthesis were greatest in the central part of the enthesis. Thickness of calcified fibrocartilage tissue was significantly greater in the central part than medially ($P=0.04$) and laterally ($P=0.03$). Uncalcified fibrocartilage was significantly thicker medially than laterally ($P=0.02$). Finally, finite element analysis showed that AT mechanical stress increased with muscle load and converged at 4.6–7.9 cm of the enthesis. *Conclusion*: Our data suggest that the triceps surae musculotendon unit is composed of anatomically distinct parts that undergo non-uniform mechanical loading. There are two sites where potentially tendon mechanical stress increases, the medial/central portions of the enthesis and the tendon midportion.

1. Introduction

It is well recognized that repetitive strain is likely a major cause of overuse injuries to the AT. The enthesis is also a region of high-stress concentration that is commonly affected by overuse injuries in sport (Shaw and Benjamin, 2007).

When affected, both the tendon and its enthesis can no longer endure the imposed mechanical stresses and consequently its structure begins to change microscopically, and inflammation, edema and pain result (Kannus et al., 1991).

Anatomically, the AT constitutes the distal insertion of the gastrocnemius–soleus musculotendinous unit. The contribution of fibers from the gastrocnemius and soleus to the AT is variable. In most individuals, the soleus (SL) contributes more fibers than the gastrocnemius (Cummins and Anson, 1946). As both muscles travel distally, the AT rotates internally, consequently the initially posterior fibers of the SL insert mainly on the medial aspect of the AT enthesis whereas those of the gastrocnemius insert mainly on the lateral aspect of the enthesis (Cummins and Anson, 1946).

The muscles contributing to the formation of the AT also have different functions and different physiological properties. The SL plantar flexes the ankle joint and contains a high proportion of

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type I (slow-twitch) fibers, which facilitates its role as a postural muscle, preventing the body from falling forward when standing (Carr and Norris, 1989). However, the gastrocnemius also flexes the knee joint, and contains a greater number of type IIB fibers (fast twitch). This complex architecture, paired with independent loading from the SL and gastrocnemius muscles, (Arndt et al., 1999) contributes to the potential for development of non-uniform deformation within the free AT.

Biomechanical studies based on finite element (FE) analyses have shown that local strains within the AT near its insertion to the calcaneus are not uniform (Maganaris et al., 2008). Furthermore, it has been proposed that the AT undergoes non-uniform deformation under *in vivo* loading conditions (Slane and Thelen, 2014). Recently, Shim et al. (2014) showed that both tendon geometry and material properties are highly subject-specific (Shim et al., 2015, 2014). This subject-specificity was also evident in their rupture predictions as the locations and loads of tendon rupture were different for all specimens tested. Tendon rupture locations were more dependent on tendon geometry than tendon material properties.

To our knowledge, there is no single comprehensive study on the anatomy and biomechanics of the AT that has investigated the association and impact of the lateral and medial gastrocnemius and the SL muscles on the structure and mechanical load transmitted to the tendon-bone units. Calf muscle group insertions and orientations could generate different tendon loads that could in turn help to explain the different sites of AT injury. The main objective of this study was to investigate the full structure of the triceps surae musculotendon unit paying particular attention to the calf muscles and their effects on tendon loading. We constructed a FE model to simulate the effects of the calf muscle loads on rupture profiles of the AT. Our goal was to understand more about the effects of the various components of the gastrosoleus muscle AT complex as a first step to understanding more about the role of muscle and tendon geometry on injury patterns.

2. Materials and methods

The study was approved by the NHS South Glamorgan Ethics Research Committee (Agreement number GHK/MM1425) and was conducted in accordance with the principles of the Declaration of Helsinki. Twelve dissecting room cadavers (6 male, 6 female, ages 67–79 years) donated to Cardiff University for anatomical examination under the provision of the 1984 Anatomy Act and the 1961 Human Tissue Act were used. Cadaveric preservation was achieved by perfusion for 72 h with an embalming fluid containing 4% formaldehyde and 25% alcohol. This fixative is the most widely used, can effectively prevent autolysis and provides excellent preservation of tissue and cellular morphology. Cadavers were also selected according to the quality of preservation and the absence of gross abnormalities in the ankle and the absence of any calcaneal spur. The presence of spur was verified by a Standard X-rays using a Faxitron Specimen Radiography System (Model MX-20, Wheeling, IL, USA). Medical histories (other than the cause of death) were not available.

2.1. Anthropometric analysis of the calf muscles

Careful layer-by-layer dissection included separation of the three muscles (SL, LG and MG) superiorly to their proximal bony insertion and distally within the AT as far as possible without destroying existing decussations between the tendons of the individual muscles. The tendinous fibers of the three muscles were followed layer-by-layer as far as possible toward their bony insertions under a Perfex Sciences oculaire microscope 10× wf and a Camiris camera (5 MP). During this procedure, macroscopic and digital magnification photographs were taken for anatomical and FE analysis.

The AT enthesis was next removed using a fine saw by cutting the calcaneus in the sagittal plane, either side of the midline, so that approximately 8-mm-wide strips of tissue were sampled from the insertion site and the AT approximately 1 cm from the bone. Each strip contained the distal part of the tendon and its insertion, together with the superior tuberosity of the calcaneus.

Each muscle was then placed in a 5 L graduated cylinder filled with tap water. Muscle volume was calculated as the difference between the pre- and post-volumes. Measurements were performed independently by two blinded technicians and average values are reported.

2.2. Faxitron radiology analysis

Standard X-rays were taken of the AT enthesis tissue on a Faxitron Specimen Radiography System (Model MX-20, Wheeling, IL, USA) with a high-resolution algorithm. The imaging parameters were identical for all specimens and the following settings were used for taking the contact radiographs: 300 s, 0.3 mA and 35 kV; pixel matrix, 1024 × 1054; resolution 200 pixels mm^{-1}. Images were transferred to a personal computer and structural analyses of the bone were performed. Algorithms used to characterize bone architecture were all developed in our laboratory using Matlab Software (Version 6.10.450, release 12.1) (Toumi et al., 2014).

2.3. Matlab treatment

Bone structure analysis was performed at all three parts (medial, central and lateral) of the enthesis and at three regions (superior tuberosity, central and distal) of the enthesis (Toumi et al., 2014, 2006, 2012). The boundary between the thin cortical bone and the underlying trabecular bone was defined using an automatic contour detection algorithm. Subsequently, a segmentation process permitted separation of spicules from bone marrow. The segmentation was made with an edge detection using a LaPlacian-Gaussian filter. This includes both a smoothing filter (which convolutes the image by a Gaussian filter) and a second-order derivative filter. Tuning of this combined filter addresses not only the size of the smoothing window, but also the variance of the convolutive Gaussian filter. Zero-crossing detection in the resulting image provides a binary image in which dark regions represent the bone marrow and light regions represent trabeculae. Several variables were calculated on the binary images based on two-dimensional (2D) analysis (Cortet et al., 2004). These were (1) apparent bone volume (BV)=trabecular bone (TB)/tissue volume (TV), (2) apparent trabecular number (TN)=(BV/TV)/TB, (3) trabecular separation (TS)=(1/TN)−TB and (4) apparent trabecular thickness (TH)=2/(TS/BV). It should be noted that these variables were obtained by analyzing 2D X-ray images. As this cannot equate to the real properties of 3D bone, it is the comparative rather than the absolute values of each parameter in different regions of the patella which are important in our study. In recognition of this, we have followed the convention of previous studies in referring to the various bone parameters as ‘apparent’ rather than ‘real’ (Cortet et al., 2004, 2002). This was validated previously by comparing TH, number and separation in histological sections of the patella with those obtained from X-ray images. The coefficient of correlation was always greater than 0.95.

2.4. Histology

For examining the AT enthesis, tissues were post-fixed in 10% neutral buffered formalin, decalcified with 5% nitric acid, dehydrated through a graded alcohol series, cleared in xylene and embedded in paraffin wax. Serial longitudinal sections were cut at 8 μm throughout the medial, central and lateral thirds of the enthesis and 12 sections were mounted on glass slides at 1-mm intervals. Slides were stained with Hall and Brunt's quadruple stain, Masson's trichrome (for photography) and toluidine blue (for fibrocartilage metachromasia).

2.5. Morphometric analysis of histological sections

For all three parts (medial, central and lateral) and three regions (superior tuberosity, midportion and distal portion) of the enthesis, the thickness of the calcified fibrocartilage (CF) was assessed at each 1-mm sampling point by measuring the distance from the tidemark to the distal part of the plate at 15 sites, equally spaced at approximately 500 μm along the section (from proximal to distal). Zone thickness of uncalcified fibrocartilage (UF) was estimated by measuring the distance from the tidemark to the furthest recognizable chondrocyte within the tendon, following a protocol adopted previously (McGonagle et al., 2008). Measurements were made with a micrometer eyepiece at a magnification of ×100. Two such measurements were taken at equal intervals in the proximal (I), central (II) and distal (III) thirds of the AT insertion.

2.6. Numerical models based on FE simulations

A FE model of tendon coupled to a rupture index was developed to investigate the AT response in terms of rupture zone. The AT was represented as a structural fiber-reinforced hyperelastic transversely isotropic hyperelastic material coupled to a fracture index using Abaqus code (ABAQUS, Abaqus documentation version 6.11-1, 2011). Tendon material properties were assigned based on a recent study of Shim et al. (2014). AT rupture was modeled based on a von Mises criteria, *i.e*, when the local applied stress exceeds the critical value of 100 MPa, local rupture occurs (Kongsgaard et al., 2005; Wren et al., 2001). The procedure and the accuracy of the