New insights into the proximal tendons of adductor longus, adductor brevis and gracilis

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ABSTRACT

Objective The adductor muscles are implicated in the pathogenesis of groin strain, but the proximal morphology of this muscle group is poorly defined. The purpose of this study was to investigate the detailed anatomy of the entheses and proximal musculotendinous junctions (MTJs) of adductor longus (AL), adductor brevis (AB) and gracilis.

Methods The adductors were dissected bilaterally in 10 embalmed cadavers (7 females, mean age at death 79 years (range 57–93 years)), focusing on the type of enthesis, and architecture of the proximal tendons and MTJs. Histology was used to determine if the entheses contained fibrocartilage and to semiquantitatively assess the vascularity of each proximal tendon.

Results All entheses were fibrocartilaginous. The proximal tendons of AB and gracilis were fused, forming a common tendinous insertion in all specimens. AL and AB both contained extensive intramuscular tendons of variable length (AL 11.1 ± 1.5 cm; AB 5.4 ± 1.1 cm); this has not been recorded previously. The vascularity of AL and AB tendons decreased significantly towards the enthesis (p < 0.05), and their entheses were significantly less vascular than that of gracilis (p < 0.05).

Conclusions The proximal anatomy of AL, AB and gracilis is more complex than previously described. The arrangement and fusion of these muscles, their fibrocartilaginous entheses and differences in vascularity of their proximal tendons may be important anatomical considerations in the pathogenesis and pattern of adductor-related groin pain.

INTRODUCTION

Groin pain is a common complaint in athletes, and particularly prevalent in sports that involve repetitive twisting, kicking and rapid changes in direction, such as football, ice hockey and Australian football.1–6 Studies on professional football players quote a prevalence of up to 12%,3,7 and an incidence of two adductor injuries per 1000 player-hours.4 Differential diagnosis of this complex, multifactorial disorder is often challenging due to the lack of consensus regarding both nomenclature and diagnostic criteria.9 Despite this, it is generally accepted that groin pain can be classified as either acute or chronic.10 Acute injuries encompass a variety of potential pathologies such as adductor tendinitis, muscle tears and strains, and inguinal hernia.8 11 12 Acute rupture or avulsion of a proximal adductor tendon is rare.13 14 Chronic groin pain can result from conditions such as osteitis pubis, tendinopathy, enthesopathy, hip joint pathology and ‘sportsman’s hernia’.11 12 15–20 Diagnosis of adductor-related groin pain is usually based on clinical findings.19,21 Consequently, few studies have reported specifically what muscles are involved and their precise site of pathology. Although the adductors are often referred to as a group, several sources state that, from clinical examination, adductor longus (AL) is most often implicated in adductor-related groin pain.18–20,22–25 In some cases, AL pathology has been confirmed by MRI, which has shown pathology at the enthesis alone,2 20 25 or distributed across the enthesis, proximal tendon and muscle.25 Involvement of adductor brevis (AB) and gracilis in this condition has been reported less often.2 17 26–28 Surprisingly, no study appears to have systematically investigated all these muscles in affected individuals. Similarly, little attention has been given to specifying the precise anatomical location of adductor-related groin pain: the proximal enthesis,15 18 20 22 proximal tendon,11 12 15 16 musculotendinous junction (MTJ)16 18 27 and muscle11 have each been identified as actual or potential sites of injury. However, with the exception of one study15 no data exist on the relative frequencies of injury at these various sites. Accurate morphological description of the proximal adductor muscles is essential if we are to enhance our understanding of adductor-related groin pain in general and to improve clinical diagnosis, interpretation of imaging investigations, and injury prevention and treatment strategies in particular. Given the most commonly reported site of injury, surprisingly few studies have investigated the proximal anatomy of the adductors;14 29–31 only one study has considered this muscle group in its entirety.29 The MTJ of AL has been examined in just two studies, which reported on its outer appearance32 and cross-sectional area (CSA) of the proximal tendon.31 Detailed descriptions of the MTJ and proximal tendon of AB and gracilis are lacking. Proximal fusion of muscles could affect patterns of injury but the connection between AL and gracilis is controversial; some reports state that the tendons are fused,2 33 34 whereas anatomy reference texts typically maintain that they are separate.35–37 With regard to AB, it has been reported that only a small portion of its tendon is visible on the external surface of the muscle,30 which suggests the presence of an intramuscular tendon but this has yet to be confirmed. Since some cases of adductor-related groin pain may arise from an enthesopathy or tendinopathy,15 15–17 19 20 knowledge of enthesis composition and tendon vascularity also seem relevant. With the exception

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of a small case series indicating that the enthesis of AL is fibrocartilaginous, no other information is available on this subject. The vascularity of these tendons could influence their susceptibility to injury and ability to repair. It is well known that tendons heal slowly after trauma and relatively poor vascularity could contribute to adductor pathology. While it has been hypothesised that the anterior region of the proximal tendon and MTJ of AL is poorly vascularised, the vascularity of the adductor tendons and their MTJs has not been formally investigated.

The aims of this exploratory study were to examine the proximal anatomy of AL, AB and gracilis specifically to determine the architecture of their tendons and MTJs, ascertain the extent of fusion between these muscles, establish the composition of their entheses and investigate the relative vascularity of their tendons and MTJs.

Figure 1  Schematic showing sites of transverse sections taken through the proximal tendons of AL, AB and gracilis. Slice A at 3 mm from enthesis, slice B at mid-tendon, and slice C at mid-MTJ. AB, Adductor brevis; AL, adductor longus; MTJ, musculotendinous junction.

Figure 2  (A–D) Representative images of proximal adductor fusion. (A) AB and gracilis fusion showing AB muscle fibres inserting into the deep aspect of the gracilis tendon (male specimens, n = 6/6). (B) Proximal fusion between the tendons of AB and gracilis (female specimens, n = 14/14). (C) Fusion of the proximal tendons of AL and gracilis (n = 2/20). (D) Decussation of the proximal tendons of gracilis (n = 12/20) and fusion between the tendons of AL and rectus abdominis (n = 20/20). AB, adductor brevis; AL, adductor longus.
MATERIALS AND METHODS
Using a combination of dissection and histology, the adductor muscles were examined by one investigator (JAD) in 10 embalmed cadavers (20 lower limbs), bequeathed to the University of Otago under the New Zealand Human Tissue Acts (1964 and 2008). Seven cadavers were female and three were male, with a mean age of 79 years (range 57–93 years) at death. Only specimens without evidence of previous surgery were included and all measurements were taken using electronic Tresna point digital callipers (Series SC02, Guilin, China; accuracy ±0.03 mm and resolution 0.01 mm).

Dissection
Skin and subcutaneous tissue were excised from the thigh to expose the inguinal ligament, femoral triangle and associated structures (AL, pectineus, the femoral artery, vein and nerve, sartorius and rectus femoris). Sartorius was separated and reflected superiorly to expose AL, AB and gracilis. The epimysium investing each muscle was removed, taking care not to damage any connections between tendons, and then each muscle was detached from its pubic enthesis using sharp dissection. The shape of each tendinous footprint was recorded along with its maximum height and width.

The dimensions of the tendons and MTJs of each muscle were documented. Tendon length was measured from the enthesis to the most distal extent of the MTJ (surface or intramuscular). The MTJ was defined as the region over which muscle fibres merged with the tendon.40 The extent of the MTJ was determined by examining the superficial and deep surfaces of the muscle, reflecting if necessary any superficial fascicles to expose an intramuscular tendon. In a small sample (n = 5) it was possible to measure the femur length, from the anterior superior iliac spine to the medial side of the medial knee joint;41 this measurement was used to standardise intramuscular tendon length by expressing it as a percentage of femur length.

Each musculotendinous complex was then divided into segments using a standardised approach: each tendon was divided 3 mm distal to the enthesis and at the midpoint of the MTJ; this segment was then bisected transversely, producing a proximal and distal tissue block for histological processing (figure 1).

Histology
Each tissue block (n = 180 from 20 limbs) and its associated enthesis (n = 60 from 20 limbs) were postfixed in 10% neutral-buffered formalin and processed through to paraffin. Four 4-μm thick sections were obtained. The first was taken from the enthesis itself and stained with H&E and Alcian blue to identify fibrocartilage;42 the fibrocartilage appears as rows of chondrocytes embedded within parallel bundles of collagen fibres.43 The remaining three sections (slices A–C) were taken at 3 mm distal to the enthesis (A), mid-MTJ (C) and halfway between these two points (mid-tendon, B) (figure 1) and stained with Verhoeff–van
Gieson.\textsuperscript{58, 42} Stained sections were mounted in DPX, coverslipped and examined under an Olympus AX70 light microscope (Olympus, Tokyo, Japan).

QCapture software (QCapture 3.0; QImaging, Surrey, Canada) and a digital camera (MicroPublisher 5.0 RTV; QImaging) were used to take photomicrographs, which were then assembled into a montage using Photoshop CS3 Extended Version 10.0 (Adobe Systems Incorporated, San Jose, California, USA), and analysed using ImageJ (National Institutes of Health, Bethesda, Maryland, USA). The ‘polygon section tool’ was used to draw a line around the perimeter of the entire section and, within sections of the MTJ, around the tendinous portion; in this way, both the total CSA of the slice and the proportion occupied by tendon could be calculated. Within the tendinous area, all blood vessels with a maximum diameter of 20 \( \mu \text{m} \) or more were identified and their total combined CSA was calculated.\textsuperscript{59} Blood vessel CSA was then expressed as a percentage of tendon CSA.

Statistical analysis
Measurement reliability of CSA and vessel count was assessed by reanalysing 10 randomly selected sections. Intraclass correlation coefficients were calculated (SPSS version 18; SPSS Inc, Chicago, Illinois, USA) and interpreted using the criteria of Landis and Koch (1977).\textsuperscript{44} To compare vascularity between the three tendon levels, data for mean total CSA, tendon CSA and blood vessel CSA from each slice (A–C) were entered into Microsoft Excel (2008) and compared using paired (within samples) or unpaired (between samples) \( t \) tests. \( p \) values less than 0.05 are considered statistically significant.

RESULTS
Dissection
The proximal attachments of AL and AB extended from the anterior surface of the pubic body inferior to the pubic tubercle. For AL this was in a superior direction partially covering the insertion of AB while the attachment of AB extended down the inferior pubic ramus where it fused with the proximal tendon of gracilis. The shapes and dimensions of each entheseal footprint varied (table 1).

AB and gracilis were fused proximally in all specimens. In males, AB muscle fibres inserted into the deep aspect of the gracilis tendon (figure 2A), whereas in females, the tendons fused to form a common tendon that inserted into the inferior pubic ramus (figure 2B). The proximal tendons of AL and gracilis were fused in one male specimen (figure 2C); in all others they were separate. The pattern of fusion was symmetrical in all individuals.

Some of the proximal anterior fibres of the large AL tendon inserted into the capsular tissues of the pubic symphysis and were continuous with rectus abdominis (figure 2D). Little substantial tendon was evident on the surface of AB and many of its muscle fibres inserted directly into bone. The proximal tendon of gracilis was short, wide and thin (table 1); in six specimens it decussated and fused with the capsular tissues of the pubic symphysis (figure 2D).

An extensive intramuscular tendon was present in all AL and AB muscles. With the exception of AL in one specimen, these intramuscular tendons extended further distally than the superficial tendon (table 1). The intramuscular tendons of AL and AB measured 23\% ± 3\% and 11\% ± 2\% of femur length, respectively. The length of the MTJs corresponded to tendon length such that the MTJ of AL was the longest, while that of AB and gracilis were substantially shorter (table 1). Gracilis had no intramuscular tendon.

Histology
All entheses were fibrocartilaginous (figure 3). A representative series of histological sections through the proximal portion of AB (slices A–C) is shown in figure 4. Data on tendon CSA and relative vascularity are shown in table 2. Intraclass correlation coefficients ranged between 0.63 and 1.00, indicating substantial to perfect agreement between repeated measures.\textsuperscript{44}

The CSA of the proximal tendons of AL, AB and gracilis decreased significantly from slices A to C (\( p \leq 0.001 \)). The only significant gender difference was in AL tendon morphology: in male specimens, 80\% of slice A (3 mm from the enthesis) comprised tendon with muscle constituting most of the remaining CSA at this point, whereas in female specimens 99.9\% of slice A was tendon (\( p < 0.05 \)). The vascularity of the proximal tendons of AL and AB decreased significantly at each level nearer

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Table 2 Adductor tendon and blood vessel CSA

<table>
<thead>
<tr>
<th>Muscles</th>
<th>Slices</th>
<th>Total section CSA (mm(^2))</th>
<th>Tendon CSA (mm(^2))</th>
<th>Blood vessel CSA (mm(^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Raw</td>
<td>% of total CSA</td>
<td>Raw</td>
</tr>
<tr>
<td>Adductor longus</td>
<td>Slice A</td>
<td>56.6 ± 23.1</td>
<td>50.8 ± 18.5</td>
<td>93.9 ± 18.0</td>
</tr>
<tr>
<td></td>
<td>Slice B</td>
<td>220.2 ± 120.9</td>
<td>20.8 ± 5.9</td>
<td>12.5 ± 7.7</td>
</tr>
<tr>
<td></td>
<td>Slice C</td>
<td>383.0 ± 234.1</td>
<td>11.5 ± 7.3</td>
<td>3.4 ± 2.3**</td>
</tr>
<tr>
<td>Adductor brevis</td>
<td>Slice A</td>
<td>65.0 ± 32.1</td>
<td>48.7 ± 19.7</td>
<td>82.8 ± 23.7</td>
</tr>
<tr>
<td></td>
<td>Slice B</td>
<td>226.7 ± 82.1</td>
<td>27.8 ± 10.6**</td>
<td>13.1 ± 5.1**</td>
</tr>
<tr>
<td></td>
<td>Slice C</td>
<td>319.4 ± 134.8</td>
<td>16.1 ± 11.3**</td>
<td>5.5 ± 3.5**</td>
</tr>
<tr>
<td>Gracilis</td>
<td>Slice A</td>
<td>71.3 ± 28.1</td>
<td>61.1 ± 22.0</td>
<td>88.4 ± 14.7</td>
</tr>
<tr>
<td></td>
<td>Slice B</td>
<td>72.4 ± 25.7</td>
<td>41.9 ± 13.5**</td>
<td>65.9 ± 28.8**</td>
</tr>
<tr>
<td></td>
<td>Slice C</td>
<td>154.3 ± 65.7</td>
<td>18.9 ± 15.3**</td>
<td>18.7 ± 24.6**</td>
</tr>
</tbody>
</table>

*Statistically significant values (\( p < 0.05 \)).
**Statistically significant values (\( p < 0.005 \)) within muscles. Slice A was taken 3 mm distal to the enthesis, Slice B at mid-tendon and slice C at mid-MTJ.

CSA, cross-sectional area.
the enthesis (AL p < 0.001; AB p < 0.05). In contrast, the vascularity of the proximal tendon of gracilis was greater in the mid-tendon compared to proximal and distal levels (proximal p = 0.0017; distal p = 0.057).

Near the enthesis (slice A), AL and AB were both significantly relatively less vascular than gracilis (AL p < 0.001; AB p < 0.05). There was no significant difference between the relative vascularity of AL and AB at this site. At the mid-tendon (slice B), AL was relatively less vascular than gracilis (p<0.005) and at the mid-MTJ (slice C), AB was significantly more vascular than gracilis (p<0.05) (figure 5).

DISCUSSION
This study reveals several novel features about the proximal segments of AL, AB and gracilis, which may be relevant to the pathogenesis of groin strain. In all specimens, entheses were found to be fibrocartilaginous, either the muscle (in males) or the tendon (in females) of AB was fused to the proximal tendon of gracilis, and an intramuscular tendon was identified within both AL and AB. In addition, we found statistically significant differences in the vascularity of each proximal tendon; the vascularity of the proximal tendons of AL and AB decreased significantly nearer the enthesis and both were relatively less vascular than gracilis at this site.

While the enthesis of AL is understood to be fibrocartilaginous,\(^\text{34}\) the nature of the entheses of AB and gracilis has not been previously examined. Nevertheless, our findings are not unexpected since fibrocartilaginous entheses are more common than fibrous types; their rows of collagen bundles oriented in the direction of stress to provide additional strength.\(^\text{45-46}\) Further, the amount of fibrocartilage at an enthesis directly correlates with the degree of compressive stress, the fibrocartilage helping to protect the tendon from compression and the bone from excessive shear. Fibrocartilage may also protect the tendon vasculature.\(^\text{46}\)
A distinctive intramuscular tendon was present in both AL and AB. A deep tendinous extension within the muscle belly forming an elongated MTJ has been demonstrated in other limb muscles, including the hamstrings, but we could not find any previous descriptions of intramuscular tendons within the adductors. Functionally, an intramuscular tendon may provide extra strength and stability during muscle contraction and could be especially important in muscles such as AL whereby the MTJ is subjected to large mechanical stresses. From a clinical perspective, adductor injury can occur at the proximal tendon or MTJ, awareness of the existence of an intramuscular tendon could therefore be important in diagnostic imaging. It is likely that previous reports of tendon and MTJ injuries affecting AL and AB have been limited to their surface tendons, and pathology affecting the intramuscular tendinous component may have been overlooked.

To our knowledge, this is the first study to investigate the relative vasculature of the proximal adductor tendons. AL and AB were less vascular nearer the enthesis, a factor which may adversely influence the capacity or rate of tendon repair in AL and AB. In contrast to AL and AB, the mid-tendon of gracilis was found to be significantly more vascular than its enthesis or mid-MTJ region, and gracilis was relatively more vascular than AL and AB at its enthesis. It has previously been hypothesised that poor entheseal blood supply could contribute to adductor-related groin pain, but, conversely, it has been shown that vascularity increases with enthesopathy. If vascularity is an important factor in proximal adductor tendon injury and repair, this could have implications for adductor-related groin strain. However, further research is needed to verify our observations, ascertain the topographical patterns of injury within the proximal segments of the adductor muscles, and determine if vascularity is a significant factor in the pathogenesis and/or prognosis of this condition.

The proximal anatomy of gracilis has been studied before as the muscle is often used for reconstructive surgery, but its relationship to adjacent muscles remains controversial. Fusion between the proximal tendons of gracilis and AL was evident in only one of our ten cadavers. This finding is consistent with most descriptions in anatomical texts, but is at odds with two MRI studies which stated that gracilis and AL fused to form a converging aponeurosis. These disparities may reflect individual anatomical variation or differences in study methods. An interesting finding in our study, documented only once before in the literature, was the discovery of fusion between proximal tendons of gracilis and AB in all specimens. In a study of seven cadavers, Budinoff and Tague (1990) found that AB and gracilis shared a common insertion site, although they made no comment on possible gender differences in this union. While our findings must be tentative because of the imbalanced male specimens, since adductor-related groin pain is more common in male athletes, moderately large variations in muscle bulk were evident between specimens, and this was reflected by the SDs of the data. In addition, some muscle shrinkage occurs during or after embalming, but whether tendon shrinks to the same degree is unknown. Further research including larger numbers of younger cadavers would be useful to confirm our findings. While our method of assessing tendon vascularity was similar to a previous study, it only assessed the relative proportion of the CSA of the tendon occupied by blood vessels above a threshold size. We did not attempt to separately quantify arteries and veins, and we do not know whether the total blood vessel CSA we calculated necessarily reflects blood flow in vivo. Finally, our novel observations require validation using imaging techniques in both healthy individuals and patients with groin strain. It would be particularly interesting to investigate the latter to determine if the intramuscular tendons in AL and AB show evidence of pathology and whether, using colour Doppler ultrasound imaging, there are demonstrable changes in blood flow to the tendon in the region of the proximal enthesis.

CONCLUSIONS

This study describes the anatomy of the proximal portions of the adductor muscles, AL, AB, and gracilis. We have shown that all three muscles have a fibrocartilaginous enthesis and that intramuscular tendons are a constant feature of AL and AB, but not gracilis. In addition, the proximal tendons of AB and gracilis are fused. The proximal tendons of AL and AB are less vascular towards the enthesis. These anatomical findings need to be considered as possible factors contributing to the pathogenesis and pattern of injuries in adductor-related groin strain.

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