Mini-review Article

Structure, function and adaptation of bone-tendon and bone-ligament complexes

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Abstract

Mechanical and physiological processes contribute to joint tissue adaptations during growth and exercise and after injury. Those adaptations are often in response to the mechanotransductive stimuli linked to the transmission of forces across these load-bearing structures. Muscle-tendon interactions have been explored during skeletal loading and describe the relation of sarcomere shortening at the expense of tendon lengthening[1,2]. The effects of load transmission through the bone-tendon and bone-ligament complexes, however, have not been studied as extensively, although both disuse and exercise will alter the stiffness of these significant structures. Recently, however, renewed interest has emerged about the pathogenesis underlying enthesopathies and enthesitis, and investigators are beginning to reveal the intricacies of bone-tendon and bone-ligament complexes[3,4,5]. Here, we summarize the structure and function of the types of entheses between bone-tendon and bone-ligament, and relate how mechanical loading leads to functional adaptation, and at times, entheseal pathophysiology.

Keywords: Bone-Tendon Complex, Enthesis, Ligament Insertion, Biomechanics

Introduction

Collagenous tendons and ligaments insert into bone, where they are secured in a calcified matrix. The transitional zones of these tissues, which comprise an insertion or enthesis, result in a structure whose material properties facilitate the transmission and dissipation of forces across the composite structure, enabling skeletal loading.

An enthesis is the insertion of a tendon, ligament, capsule, or fascia into bone[3]. These insertions are classified as either fibrous or fibrocartilaginous, depending on the cellular processes involved during their formation. Fibrous entheses form through the process of intramembranous ossification and have their calcified anchorage via perforating collagen (Sharpey's) fibers. An example of a fibrous enthesis is the distal medial collateral ligament insertion into the proximal tibia[6]. Fibrocartilaginous entheses arise through endochondral ossification and have a discontinuous cement line separating mineralized fibrocartilage and lamellar bone. An example of a fibrocartilaginous enthesis is the proximal medial collateral ligament insertion into the distal femur.

Structure of tendon and ligament entheses

During embryological development, tendons and ligaments appear as fibrous cellular outgrowths from the perichondrium of cartilaginous precursor to later bone. As the cartilaginous anlage (or primordium) ossifies, fibrocartilaginous elements of the ligament are cemented into the initial calcified cartilage, which is later replaced by mature bone. Only a thin layer of proliferative epiphyseal cartilage remains at the interface, and that later disappears. The resulting mature tendon or ligament insertion comprises four zones: collagenous ligament or tendon, fibrocartilage, mineralized fibrocartilage, and mineralized bone[7] (Figure 1).

Cooper and Misol examined the femoral attachment of the medial collateral ligament insertion in dogs using light and electron microscopic evaluations[8]. They separated the four zones using morphological criteria, and noted that the zones

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gradually merged into one another, providing a gradual change in structural composition from ligament through to mineralized bone. Their detailed zonal classifications were:

"**Tendon or ligament**: *Consisting of parallel collagen fibers with interspersed elongated cells.*

Fibrocartilage: *The same collagen bundles continue into this zone, which ranges from 150 to 400 μm in width. The cells are arranged in pairs or in rows, become round, and lie in lacunae of extracellular matrix between separated collagen fibers.*

Mineralized fibrocartilage: *This zone ranges from 100 to 300 μm in width and is separated sharply by a perpendicularly traversing line or the tidemark, demarcating mineralized matrix. The collagen bundles continue into this region, with cells retaining their structural features from the previous zone.*

Bone: *Organized lamellar bone contrasts to the irregular contour of adjacent mineralized fibrocartilage[8]*".

Francois and colleagues noted that the enthesis belongs to both the inserted structure and to the bone, in which it is secured[3]. Hence, the calcified fibrocartilage of the enthesis is contiguous with the surrounding mature bone, but each was formed and is remodeled differently. During entheseal development, the cartilaginous anlage to the developing enthesis is eroded on the inner surface by bone cells involved in bone formation, through the process of endochondral ossification. Osteoclasts erode the anlage, and in turn, osteoblasts deposit lamellar bone and remain as osteocytes or bone lining cells once the remodeling cycle has ceased. Similarly, metaplastic chondrocytes divide from the tendon or ligament side of the enthesis, to maintain the transitional fibrocartilage layer, with its deeper area becoming calcified[3]. This calcified fibrocartilage is visible in decalcified sections as a tidemark, especially by using histological stains (e.g., hemotoxylin and eosin or Safranin O).

When bone forms via intramembranous ossification, the vascular collagenous tissue is transformed into a vascularized, calcified matrix. The trapped fibroblasts or mesenchymal stem cells differentiate into osteoblasts, and begin the lamellar reorganization of the matrix, through osteoclast precursor recruitment and osteoclastogenesis. Unlike endochondral ossification, where the matrix is replaced by lamellar bone, intramembranous ossification cements the pre-existing collagenous tendon or ligament fibers into the lamellar bone. These collagen bundles—known as Sharpey's fibers or perforating fibers—originate from the tendon or ligament and periosteum, and penetrate into underlying bone[6].

In addition to the insertion of tendon and ligament into bone, occasionally, composite tissue specializations constitute an enthesis organ (e.g., at the tendon of the tibialis posterior). This organ consists of the enthesis itself, the superomedial portion of the calcaneonavicular ligament, the tendon sheath, and associated accessory bone[9]. These structures are rich in fibrocartilage and function to reduce stress concentrations in tissues at the insertion site during the trans-
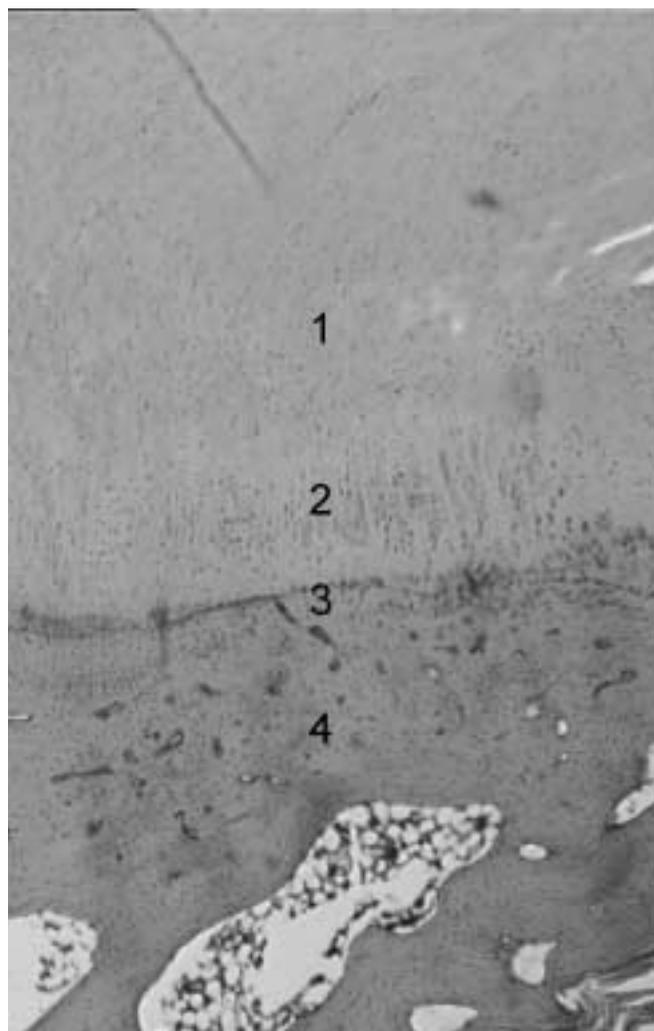

Figure 1. The 4 tissue zones of the fibrocartilaginous enthesis of the medial collateral ligament in rabbit. Zone 1 - ligament; zone 2 - fibrocartilage; zone 3 - mineralized fibrocartilage; zone 4 – bone. (H&E Stain, Mag. x10).

mission of force.

Fibrous, load-bearing connective tissues (e.g., ligaments and tendons) are sparsely vascularized, mostly in their outer epitendinous and epiligamentous layers of loose connective tissue. Few vessels, if any, traverse the osteotendinous junction, largely because the mineralized front acts as a barrier to vascular invasion. Instead, blood vessels branch and form a surrounding vascularized mesh, which encapsulates the joint. Blood vessels branch from the vascularized mesh (i.e., the *circulus articuli vasculosus*) to supply all joint structures[10], except the articular cartilage. On a similar note, entheses are innervated with proprioceptive and pain receptors[11], and hence, neuromuscular feedback from ligaments influences subsequent joint loading. For example, creep (i.e., the time-dependent inelastic strain under sustained load) in the anterior cruciate ligament (ACL), resulting from sustained static loading of the viscoelastic ACL, has an

effect on the reflexive activation of associated musculature and may increase the risk of joint injury[12].

Load transmission at tendon-ligament entheses

Tendons and ligaments encounter loads in everyday activities, as they transmit forces from muscles or bone to the skeleton. The fundamental mechanical quantities that influence connective tissues are force, deformation, and time. Under the action of external forces, connective tissues change shape (i.e., deform or strain). Tensile loads cause elongation between the original tissue ends, while compressive loads result in a contraction between ends. Similarly, forces parallel to the cross-section can create shear loads and result in tissue strains.

Tendons and ligaments and their entheses are viscoelastic materials, which exhibit time-dependent properties, such as strain-rate sensitivity, load relaxation, and creep. The rate of change of force with deformation defines the structural stiffness of the tissue. When these load-deformation curves are adjusted by dividing force by the original cross-sectional area of the tissue, and deformation by initial length, the material parameters of load (i.e., stress), deformation (i.e., strain), and stiffness (i.e., elastic modulus) are obtained (Figure 2). The stress-strain material behaviours of tendons and ligaments are non-linear when plotted against each other. During early loading, the "toe" region of the curve demonstrates little stress relative to the strain applied, and is thought to correspond to the straightening out of collagen fibers. The linear region, where collagen fibers take up force follows, and then flattens out as the failure of fibers progresses to complete rupture.

Along the length of the enthesis, for each transitional zone of connective tissue, a different material medium surrounds the collagen fibers, and affects tissue stiffness and modulus accordingly. The transition of tendon or ligament through different zones of increasing stiffness may offer a mechanical advantage during loading, by minimizing the effects of stress concentration, and permitting gradual changes from the compliant ligament to the stiffer bone[13].

Tendons and ligaments often contain fibrocartilage in regions subjected to compression. That occurs principally as they wrap around bony pulleys and where they insert into bone[4]. At the enthesis, the zone of calcified fibrocartilage interdigitates with the underlying bone and increases the cross-sectional area of interface between the two structures[14]—which decreases stress concentrations during load transmission from the compliant ligament to the stiffer bone.

Three principal modes of failure have been reported in bone-ligament complexes[15]:

1. Ligamentous failure, through the ligament body, resulting in a characteristic *mop end* appearance,

2. Bone avulsion fracture, where failure occurs in the bone beneath the enthesis, or

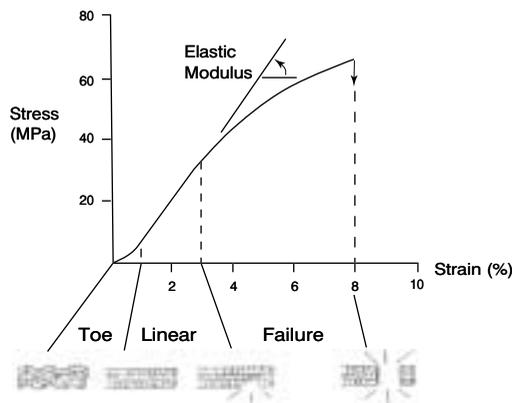3. Cleavage at the ligament-bone interface, often through the mineralized fibrocartilage zone.

Figure 2. Example of a typical normalized stress-strain curve for collagen. (Adapted from Butler et al., 1978).

Both failure strain and the location of bone-MCL-bone complex failure change during maturation, with exclusively tibial avulsion in immature rabbits to predominantly mid-substance failure in skeletally mature rabbits[16]. Researchers from our group investigated the relation between surface strain and the site of failure in rabbit ligaments at various ages of skeletal maturity[17]. With *in vitro* testing, bone-MCL-bone complexes from NZW rabbits were tested to failure. The highest strains occurred at the femoral insertion, in the anterior portion of the MCL insertion. Strain decreased in the MCL midsubstance.

Gao and co-workers also showed that avulsion fracture was the most common mode of MCL failure in skeletally mature rabbits[14]. They noted a few cases of avulsion at the cement line between uncalcified fibrocartilage and the ligament. Nonetheless, they concluded that the subchondral bone beneath the femoral insertions was weaker than the fibrocartilaginous transitional zone between soft tissue and hard bone[12]. That would be analogous to the difference in strength between reinforced concrete (i.e., fibrocartilage) and plain concrete (i.e., bone cement). Avulsion fracture of bone is prevalent with underlying knee injury, where periarticular bone adaptation leads to an initial structural osteopenia, with the predisposition for fracture avulsion[18,19].

While studying muscle-tendon interactions during loading, Lieber and colleagues measured strains at the tendon, bone-tendon junction, and aponeurosis of the frog semi-tendinosis complex[2]. They partitioned the three regions with dye lines (i.e., an elastin stain) and measured average load-strain functions for the three distinct structures. They noted the greatest compliance in the aponeurosis (8% strain), with lower strain values for the tendon (2% strain) and bone-tendon junction (3% strain), indicating deformation at the fibrocartilaginous enthesis (Figure 3). Using a similar approach, Thomopoulos and co-workers applied stain lines along the length of the rat supraspinatus-humeral head bone-tendon insertion and measured the variation in peak
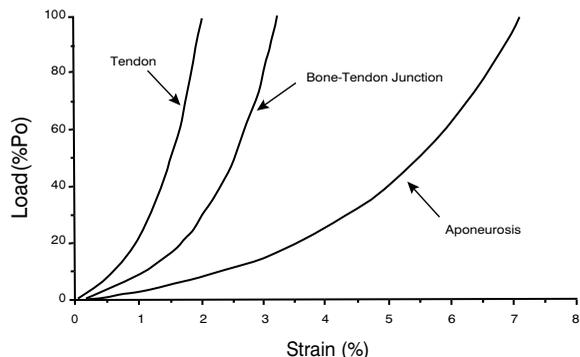
Figure 3. Average load-strain relation for tendon, bone-tendon junction, and aponeurosis. (Adapted from Lieber et al., 1992).

Figure 4. The metaphyseal depression in bone at the proximal MCL insertion of the distal femur (arrow) of the skeletally mature New Zealand White rabbit, by μCT.

strain (%)[20]. They concluded that the tendinous portion of the insertion was more extensible than the bony portion. Thus, in both investigations, the decreasing strain gradients from compliant muscle to non-compliant bone facilitated the physiological transmission of forces across the muscle-tendon-bone structure.

Adaptation of entheses during disease and repair

Enthesopathy occurs when degenerative changes (after injurious stimuli) result in the tissues of the enthesis or enthesis organ. When examining bony heel-spur formation in the subcalcaneal enthesis, Kumai and Benjamin noted degenerative changes in entheseal fibrocartilage that preceded bone spur formation[21]. The changes included cartilage cell clustering with abundant extracellular matrix, longitudinal fissure formation deep into the fibrocartilage, and the local erosion of subchondral bone, in association with highly vascularized loose connective tissue.

The role of the blood supply in the pathogenesis and resolution of enthesopathy is significant. Many of the fibrous connective tissues (e.g., ligaments and tendons) are sparsely vascularized, mostly in the outer epiligamentous layers of loose connective tissue. Microtrauma and disruption of collagen fibrils result in an inflammatory stimulus in regions of densely packed, mineralized tissues with cartilaginous elements. Cartilage-derived inhibitors of vascularization are known to inhibit angiogenesis, and potentially need to be overridden for angiogenesis to proceed. Thus, enthesopathies have been treated with surgical debridement, adjacent incision, and possibly, aggressive bone (e.g., patellar) forage, to stimulate sufficient angiogenic stimuli capable of overriding the avascular environment of entheses[22].

Our group examined the changes in calcium mineral at the proximal medial collateral ligament (MCL) insertion into distal femoral bone in an osteoarthritis model. Using the rabbit anterior cruciate ligament-deficient (ACLX) model of post-trau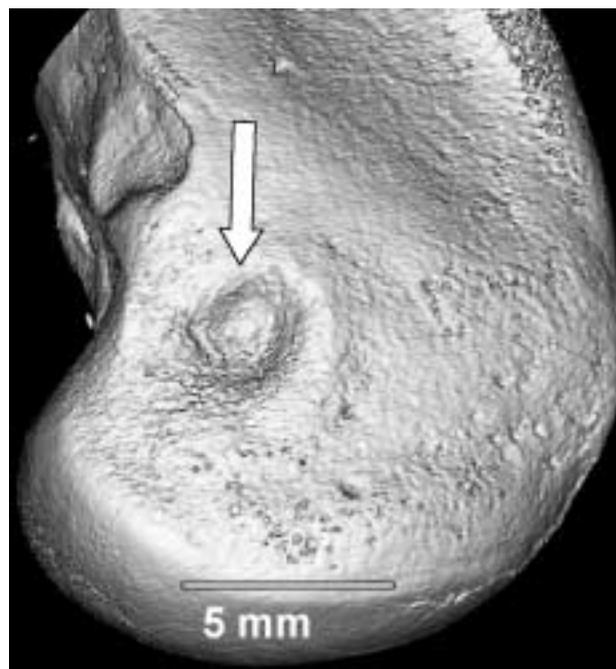matic osteoarthritis, we measured MCL insertional bone geometry (μCT [Figure 4]) and related it to changes in laxity of the MCL-complex. Early alterations of periarticular bone mineral after ACLX revealed significant loss at the MCL insertion, with concomitant increases in ligament laxity[23]. MCL insertional volume was significantly greater in the ACLX cohort at 6 weeks, compared to normal non-injured controls (Figure 5). Subsequently, we investigated whether short-term anti-resorptive therapy (daily bisphosphonate dosing) would alter periarticular bone mineral density and biomechanics. With daily doses of a bisphosphonate (risedronate) for a 6-week period, we found that ACLX animals had MCL insertional bone morphology not significantly different from normals[24,25]. Those adaptations likely altered tissue compliance and strain distributions at the enthesis, and may have contributed to the pathogenesis of osteoarthritis.

The surgical repair of ruptured ligaments and tendons after acute trauma is often undertaken to restore function, and in most cases, to facilitate the healing response. Ruptures of the Achilles tendon, medial tendon of the rotator cuff in the shoulder, and ACL in the knee are all common traumatic injuries which heal poorly, if at all, without surgical intervention. Physical reattachment facilitates the return of function, but the source of blood vessels and cells that facilitate much of the healing do not originate from the ruptured tendon/ligament "stump" and complementary enthesis, but from adjacent connective tissues such as the subacromial bursa in the shoulder[26], and fibroblastic and/or
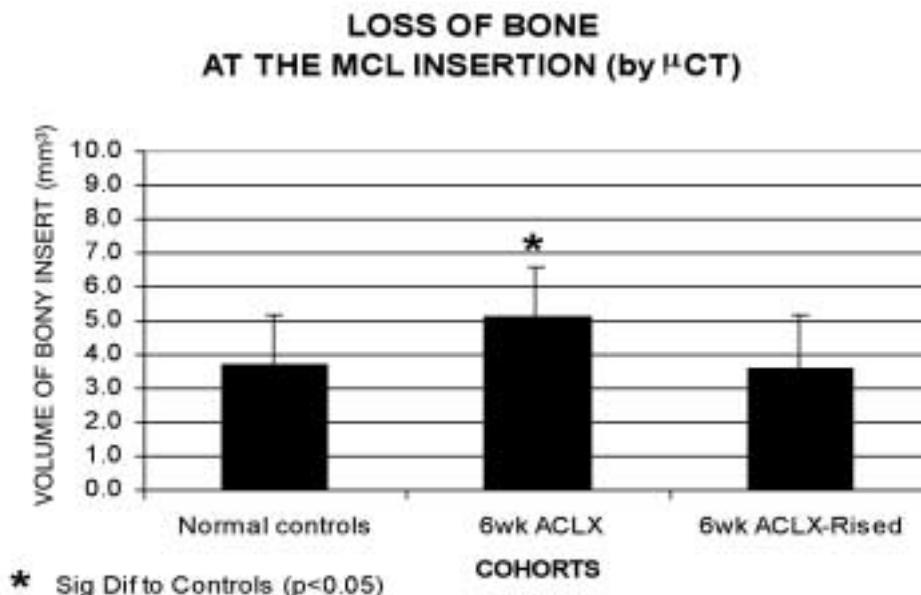
LOSS OF BONE
AT THE MCL INSERTION (by μCT)

Figure 5. Volume of the metaphyseal depression at the MCL insertion (by μCT). Values are means ± SD. ACLX, anterior cruciate ligament transected. Rised, risedronate-dosed.

pluripotent stem cells in adjacent soft tissues for ligaments[27]. Thus, successful repair depends not only upon functional restoration, but also on the reapposition and preservation of soft tissues surrounding the site of tearing.

The importance of immobilization and early loading after surgical reattachment of ligaments and tendons is also injury and site-specific. Joint immobilization after midsubstance ligament rupture and during subsequent repair is known to produce healed ligament of smaller fibril diameter and poorer functional quality[28,29]. Thus, midsubstance tears are preferentially treated with partial immobilization casts and progressive remobilization. The treatment of avulsional failures (e.g., after shoulder tendon rupture and reattachment, or ACL-reconstruction) of tendons and autogenous grafts are often reimplanted into bony troughs. In these surgical models, early immobilization can result in superior structural, compositional and some mechanical properties of the tendon-complex compared to those that were exercised early[30-32]. The net beneficial effect may avoid muscle and bone atrophy, while allowing fibrocartilaginous extracellular matrix elements to reestablish and cement themselves in the remodeling enthesis.

Conclusion

Mechanical loading of the bone-tendon and bone-ligament complexes is facilitated by the unique structural and material adaptations at their insertional interface. The enthesis reduces strain concentrations at the interface of these compositionally distinct tissues during the transmission of forces associated with skeletal loading. Adaptations and alterations of the enthesis in response to injury are contributing factors in the development of enthesopathy. Such adaptive responses in connective tissues can be linked to maintenance or repair of these injured structures. They, however, also significantly alter enthesis architecture and structure, and as such, may influence mechanical function and disease pathogenesis, with sequelae of sub-clinical injury and degradation.

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