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Negative impact of disuse and unloading on tendon enthesis structure and function

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ABSTRACT

Exposure to chronic skeletal muscle disuse and unloading that astronauts experience results in muscle deconditioning and bone remodeling. Tendons involved in the transmission of force from muscles to skeleton are also affected. Understanding the changes that occur in muscle, tendon, and bone is an essential step toward limiting or preventing the deleterious effects of chronic reduction in mechanical load. Numerous reviews have reported the effects of this reduction on both muscle and bone, and to a lesser extent on the tendon. However, none focused on the tendon enthesis, the tendon-to-bone attachment site. While the enthesis structure appears to be determined by mechanical stress, little is known about enthesis plasticity. Our review first looks at the relationship between entheses and mechanical stress, exploring how tensile and compressive loads determine and influence enthesis structure and composition. The second part of this review addresses the deleterious effects of skeletal muscle disuse and unloading on enthesis structure, composition, and function. We discuss the possibility that spaceflight-induced enthesis remodeling could impact both the capacity of the enthesis to withstand compressive stress and its potential weakness. Finally, we point out how altered compressive strength at entheses could expose astronauts to the risk of developing enthesopathies.

1. Introduction

Throughout their lives, people may be exposed to chronic periods of inactivity and skeletal muscle disuse through a sedentary lifestyle, immobilization following an injury, bed confinement due to illness, aging, spinal cord injury, or certain chronic diseases. A striking but less common cause of reduced muscle activity is spaceflight, which places astronauts in a microgravity environment leading to hypokinesia and hypodynamia (Booth et al., 2017). Since mechanical stress is necessary to maintain the integrity of the musculoskeletal system, reductions in movement and mechanical loading lead to structural changes in the different components of the system and eventually to physiological and mechanical dysfunctions. Understanding the changes that occur in muscle, tendon, and bone is an essential step toward limiting or preventing the deleterious effects of inactivity. Space exploration, with its increasing long-term missions, requires this knowledge so as to anticipate the effects of a microgravity environment on astronauts, as well as to optimize post-flight rehabilitation strategies. Many studies have investigated the effects of inactivity and microgravity on muscle and

bone. Both factors not only result in muscle deconditioning, which translates mainly into decreased muscle mass and strength, and increased fatigability (Chopard et al., 2009; Dedley-Javoroski and Shields, 2008; Gao et al., 2018; Lang et al., 2017; Michael, 2000; Narici and De Boer, 2011), but also in bone remodeling characterized by losses in bone mass and bone mineral density (Bloomfield et al., 2016; Dudley-Javoroski and Shields, 2008; Lang et al., 2017; McGee-Lawrence et al., 2008; Sievänen, 2010; Vico and Hargens, 2018).

In contrast, although the tendon and its myotendinous and osteotendinous interfaces support force transmission from muscle to bone, considerably less research has explored how the tendon is affected by disuse. A few reviews have summarized these effects of unloading on the tendon (Bloomfield et al., 2016; Dideriksen, 2014; Magnusson and Kjaer, 2019; McCrum et al., 2018; Reeves et al., 2005) and the myotendinous interface (Curzi, 2016). However, none focused on the tendon enthesis, the attachment site of a tendon to bone. To address this gap, our review first looks at the relationship between entheses and mechanical stress, showing how mechanical stress influences the structure, composition, and function of this interface. The second part focuses on

the deleterious effects of disuse and unloading on enthesis structure, composition, and function. It discusses the consequences that spaceflight-induced remodeling of the enthesis could have on both its capacity to withstand compressive stress and its potential weakness. A parallel between the tendon and the tendon enthesis is drawn throughout this review to highlight the fact that, although it belongs to the tendon, the enthesis has different structural and molecular characteristics from the tendon, which probably explains why it does not necessarily respond in the same way to inactivity.

2. The Tendon and its Enthsis: role, structure and composition

Tendons are an integral part of the musculoskeletal system. They play a role in proprioception by constantly informing the central nervous system about the tensile state of the muscle-tendon complex, through their mechanoreceptors (Jozsa et al., 1993; Proske and Gandevia, 2012). In addition, tendons allow joint movement by transmitting forces generated by muscle to the bone with minimal energy dissipation and deformation (Franchi et al., 2007; Juneja and Veillette, 2013). The mechanical properties of tendons are determined by the biochemical composition and the structural organization of the tendon and its interfaces (Killian et al., 2012; Magnusson et Kjaer, 2019). The material properties of tendons and their high tensile strength ensure efficient force transmission with minimal risk of injury (Franchi et al., 2007; Magnusson and Kjaer, 2019; Reeves et al., 2005). The folded membrane of muscle fibers at the specific site of myotendinous junction reduces the stress applied on the interface (Charvet et al., 2012). Moreover, the enthesis fibrocartilage, which allows a gradual transition of mechanical properties between the tendon and bone, protects from the risks of wear and tear (Shaw and Benjamin, 2007; Zelzer et al., 2014).

The tendon is a dense connective tissue containing few cells and whose main extracellular matrix (ECM) component is collagen fibers (Magnusson and Kjaer, 2019). The dry mass of the tendon is composed of 86% collagen, as well as 2% elastin and 1–5% proteoglycans (Juneja and Veillette, 2013). In a healthy tendon, almost all the cells are fibroblasts, also called tenocytes (Juneja and Veillette, 2013), which are differentiated from tendon progenitor cells (Subramanian et schilling, 2015). These cells not only synthesize the various components of the

ECM (Juneja and Veillette, 2013), but also participate in its degradation (Leyden et al., 2019). The tendon has a very hierarchical structure. The collagens that are the main protein of the ECM assemble to form fibrils, and the latter are grouped into fibers, which assemble into bundles. Fibrils, fibers, and collagen bundles are mainly oriented along the force-producing axis (Leyden et al., 2019; Khayyeri et al., 2017). The collagen molecules that compose the fibrils of a healthy tendon are mainly type I collagen (Freedman et al., 2014; Killian et al., 2012). Type III and type II collagens have been described at the myotendinous insertion (Jakobsen et al., 2017; Kannus et al., 1992) and osteotendinous insertion (Friese et al., 2020; Jensen et al., 2018; Waggett et al., 1998), respectively. Inside the tendon ECM, collagen fibers are accompanied by elastic fibers that enable the tendon to recover its shape after being stretched (Killian et al., 2012; Leyden et al., 2019) and by small proteoglycans (SLRP) that ensure the cohesion of the fibrils and collagen fibers (Freedman et al., 2014; Leyden et al., 2019). The ECM also contains glycoproteins, water, and other large proteoglycans involved in the tendon's capacity to resist compressive forces (Leyden et al., 2019; Juneja and Veillette, 2013). Large proteoglycans are also reported to provide the force transmission between the fibrils (Franchi et al., 2007; Magnusson and Kjaer, 2019).

The tendon enthesis is the site where a tendon inserts into bone. There are two types of tendon enthesis : fibrous entheses and fibrocartilaginous entheses. In a fibrous enthesis, the tendon's collagen fibers attach to the periosteum of the long bone diaphysis by perforating mineralized collagen fibers, also called Sharpey's fibers (Bunker et al., 2014; Friese et al., 2020). In a fibrocartilaginous enthesis (Fig. 1), the tendon inserts into the long bone epiphysis through a fibrocartilaginous area (Friese et al., 2020). The present review only considers fibrocartilaginous entheses. The tendon enthesis transmits forces from the tendon (soft tissue) to the bone (hard tissue), thereby exposing it to high tensile stress (Fig. 1). Due to the disparity in the mechanical properties of the two tissues it joins, this interface is a region where mechanical stress is highly concentrated, making it vulnerable (Benjamin et al., 2006; Derwin et al., 2018; Killian et al., 2012). However, tissue organization and molecular composition at a fibrocartilaginous enthesis provide protection against wear and tear from tensile, shear, and compressive stress (Shaw and Benjamin, 2007). In addition to the tensile stress that the

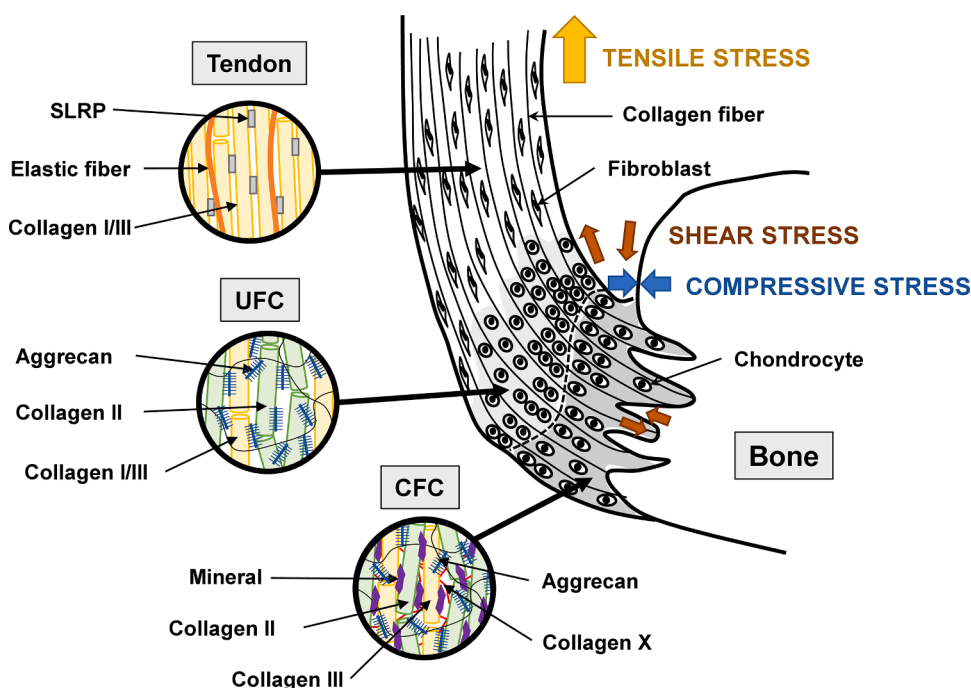


Fig. 1. Schematic representation of a fibrocartilaginous enthesis exemplified by the Achilles tendon enthesis. The fibrocartilage of the enthesis consists of an unmineralized zone (UFC) and a calcified zone (CFC). The latter is distinguished from the UFC by the tidemark (dotted line) and forms the osteochondral interface with the bone. At the tendon-to-bone interface, tendon fibroblasts are replaced by chondrocytes within the fibrocartilage. The tendon zone of the enthesis consists mainly of well-aligned type I collagen fibers as well as some type II collagen, elastin, and SLRP (small leucin-rich proteoglycan) (Apostolakis et al., 2014). The UFC zone contains multiple types of collagen, predominantly type II and type III (Waghray et al., 2015), as well as aggrecan associated with hyaluronic acid (Friese et al., 2020). In the CFC zone, although collagen III is expressed (Cury et al., 2016), collagen II is the predominant type of collagen. There are significant amounts of aggrecan as well as type X collagen, a non-fibrillar collagen believed to stimulate tissue mineralization (Jensen et al., 2018). The yellow, blue, and red arrows represent respectively the tensile, compressive, and shear stresses applied on the enthesis.

enthesis is subjected to, entheses also experience local compressive stresses, especially when the tendon fibers insert into the bone at an oblique angle. During movement of the articulation, the deep part of the enthesis and the bone are therefore pressed against each other, as occurs at the Achilles tendon enthesis during dorsiflexion of the foot (Benjamin et al., 2006). In addition, according to some authors, internal shear forces are created when the joint changes its position (Almekinders et al., 2003) not only in the deep part of the enthesis (Apostolakos et al., 2014; Benjamin and McGonagle, 2009) but also at the osteochondral interface (Benjamin and Ralphs, 1998; Milz et al., 2002).

The enthesal fibrocartilage has an uncalcified zone (UFC) in continuity with the tendon, and a calcified zone (CFC) that inserts into the bone (Friese et al., 2020; Jensen et al., 2018; Lu and Thomopoulos, 2013). There is no clear boundary between the tendon and the fibrocartilaginous area (Benjamin et al., 1986), but rather a gradual transition from tendon fibrous tissue to enthesis fibrocartilage (Cooper and Misol, 1970; Friese et al., 2020; Thomopoulos et al., 2010). The boundary between the UFC and the CFC (also called the tidemark) is the enthesis mineralization front (Friese et al., 2020; Juneja and Veillette, 2013), which can be observed under light microscopy when using basophilic stains (Apostolakos et al., 2014). At the osteochondral interface, the collagen fibers whose cross-sectional areas decrease towards the enthesis, continue from the tendon and insert into the bone (Sartori and Stark, 2020). In transmission electron microscopy, collagen fibrils in mineralized fibrocartilage intermingle with those of lamellar bone (Cooper and Misol, 1970). Fibrocartilaginous and bone tissues are embedded in a puzzle-like fashion, which increases the mechanical adhesion between the fibrocartilage and the bone (Apostolakos et al., 2014; Bunker et al., 2014; Milz et al., 2002). It appears that the frequency and the depth of fibrocartilage invaginations into the underlying bone is determined by the force transmitted (Gao and Messner, 1996).

The fibrocartilage cells of the enthesis are chondrocytes with low proliferation capacity (Schwartz et al., 2015). They are located in lacunae in the ECM (Benjamin and Ralphs, 2004) and organized in columns parallel to the collagen bundles (Benjamin et al., 1986; Cury et al., 2016; Ralphs et al., 1992). There are fewer cells in the CFC, probably due to deposits of calcium salts that lead to cell death (Benjamin et al., 1986). Modulated by mechanical stress pre- and post-natally, the enthesis fibrocartilage cells are derived from enthesis progenitor cells, differing from the tendon midsubstance progenitors and primary cartilage progenitors in that they co-express Scx and Sox9 as well as Gli-1, a transcription factor activated through the Hedgehog signaling pathways (Dyment et al., 2015; Jensen et al., 2018; Schwartz et al., 2015).

3. Deleterious effects of inactivity on tendons and enthesis

Like muscle and bone, the tendon is a mechanosensitive structure. Its molecular composition, structure, and mechanical properties change according to the level of mechanical stress to which it is subjected (McCrum et al., 2018; Magnusson and Kjaer, 2019). In both humans and animals, excepted for Botox-induced muscle paralysis, most experimental situations that induce a reduction in mechanical stress lead to a loss in tendon elastic properties. Over periods of less than a month of inactivity, most ex- and in-vivo studies, respectively carried out on animals and humans, reported a decrease in tendon stiffness and/or Young's modulus (Almeida-Silveira et al., 2000; Couppé et al., 2012; De Boer et al., 2007; Kubo et al., 2000; Matsumoto et al., 2003). For example in rats, the Achilles tendon Young's modulus, calculated from tensile test data, decreased by 41.5% after 21 days of hindlimb suspension (Almeida-Silveira et al., 2000). In humans, using an ultrasound-based technique assessing the elongation of the tendon during an isometric test associated with force measurements, Kubo et al. (2000) reported a 32% decrease in stiffness after 20 days of bed-rest. The longer the bed-rest, the greater the impact on stiffness, which decreased by 58% after 90 days of bed-rest (Reeves et al., 2005). From a functional

point of view, the loss in tendon elastic properties may not only reduce the force output of the muscle-tendon unit but also reduce the speed of contractile force transmission to the skeleton, making the muscle-tendon unit less efficient (Narici and De Boer, 2011; Reeves et al., 2005; De Boer et al., 2007).

In most cases, loss in tendon elastic properties was not correlated with tendon atrophy, suggesting that the material properties of the tendon were primarily affected by the unloading. In other words, alterations to the mechanical properties were attributable to changes in the tendon itself (Almeida-Silveira et al., 2000; Couppé et al., 2012; De Boer et al., 2007; Kubo et al., 2000). Unfortunately, these authors did not conduct a tendon structural study in addition to their mechanical investigations, which would have allowed this hypothesis to be verified. However, it has been demonstrated that most unloading situations lead to collagen remodeling, with both a disorganization of collagen fibrils (Nakagawa et al., 1989; Yamamoto et al., 1993) and a decrease in their number and diameter (Nakagawa et al., 1989). This collagen remodeling could explain the loss in tendon elastic properties in response to unloading, since these properties are related to collagen structure (Svensson et al., 2016; Magnusson and Kjaer, 2019). In this context, the increased rat Achilles tendon stiffness reported after 5–6 weeks of Botox-induced muscle paralysis could be explained by increased collagen content (Eliasson et al., 2007; Khayyeri et al., 2017).

In addition to loss in tendon elastic properties, reduced muscle activity and/or unloading alter the tendon's resistance to tensile stress, resulting in functional weakness (Almeida-Silveira et al., 2000; De Boer et al., 2007; Yamamoto et al., 1993). As fibrillar collagens play a role in the mechanical resistance of the tendon (Banos et al., 2008), the alterations to collagen content, structure, and organization could be responsible for the lower tensile strength (Nakagawa et al., 1989; De Boer et al., 2007; Matsumoto et al., 2003). This was observed in simulated microgravity conditions where a 37.4% decrease in maximum stress was reported after 3 weeks of hindlimb suspension in rats (Almeida-Silveira et al., 2000).

While an increasing number of studies address the effects of inactivity on the tendon, very few consider the enthesis, even though the idea that its structure is determined by mechanical stress is widely supported in the literature. Some studies investigating mechanical strain at the human entheses in vivo (Chimenti et al., 2016, 2017) and ex vivo (Almekinders et al., 2002; Bah et al., 2016; Bey et al., 2002; Huang et al., 2005; Lyman et al., 2004) showed that the physiological load at the enthesis is a combination of tensile and compressive load. The latter seems to determine and influence the structure and the composition of the enthesis. At tissue level, the stress applied per unit area is mitigated by the flaring of the Achilles tendon when it approaches the calcaneus, the irregular CFC/bone interface, and the fibrocartilage (Milz et al., 2002; Shaw et Benjamin, 2007). Moreover, the helical fiber courses of the Achilles tendon could contribute to homogeneous stress distribution among collagen fibers. The highest collagen fiber curvature, found within the UFC, seems to be the result of compressive loads (Sartori et Stark, 2020). UFC thickness not only varies according to the mobility of the tendon and the angle of its insertion into the bone, but also varies with position in the enthesis. The tendons that are the most mobile and have a large insertion angle are those where the enthesis fibrocartilage is the thickest. In addition, UFC is thicker in the deep part (joint side) of the enthesis (Benjamin et al., 1992; Evans et al., 1991; Frowen and Benjamin, 1995). This probably reflects the non-uniform distribution of compressive strain at the enthesis. For example, in the Achillean enthesis, where UFC is thicker in the area near the bursa, greater compressive strain was described in the deep region of the enthesis compared to the superficial region, due to tendon compression against the calcaneum during ankle dorsiflexion (Chimenti et al., 2016). Broadly speaking, these data suggest that the higher the compressive stress, the thicker the UFC (Benjamin et al., 1992; Frowen and Benjamin, 1995).

When tendons transfer the force generated by muscles to bones, tensile load is placed on the tendon insertion. However, tensile strain

within the enthesis is not uniform. Most studies report the lowest tensile strain on the joint side of the enthesis and the highest tensile strain on the superficial side (Almekinders et al., 2002; Bey et al., 2002; Huang et al., 2005; Lyman et al., 2004), although only with certain angles of shoulder abduction in the case of the supraspinatus tendon enthesis (Bey et al., 2002; Huang et al., 2005). These results strongly suggest that tensile force is primarily transmitted through the superficial fibers of the tendon. Once again, the distribution of tensile stress seems to dictate the structure of the enthesis. Thus, the anterior side of the patellar tendon enthesis (Almekinders et al., 2002), which exhibits the highest tensile strain, has more calcified tissue in its superficial region (Evans et al., 1991). At the Achilles tendon enthesis, contrary to the deep region, the superficial region of the tendon displaying higher tensile strain during ankle dorsiflexion (Lyman et al., 2004) also shows a higher density of bone trabeculae aligned and oriented along the force transmission axis (McGonagle et al., 2008; Milz et al., 2002). Moreover, this difference in tensile strain between deep and superficial zones of the enthesis suggests that internal shear forces are created when the joint changes its position (Almekinders et al., 2003), which could also explain the presence of fibrocartilage (Apostolakis et al., 2014; Benjamin et al., 1992; Frowen and Benjamin, 1995). Finally, the progressive mineralization of the enthesis (Cooper and Misol, 1970; Genin et al., 2009; Killian et al., 2012) and the presence of fibrocartilage ensure a gradual transition of the mechanical properties between tendon and bone (Liu et al., 2011; Zelzer et al., 2014).

The structure-function relationship at the enthesis is also expressed at a molecular level. The entheses are enriched in type II collagen as well as in proteoglycans and glycoaminoglycans such as aggrecan and hyaluronic acid, which have a high water-binding capacity (Friese et al., 2020; Jensen et al., 2018; Waggett et al., 1998). These molecules expressed in the enthesis fibrocartilage (Fig. 1) are characteristic of other fibrocartilage tissues, such as meniscus or intervertebral discs, where compressive stress is high (Chen et al., 2017). Fibrillar collagens such as collagen I and collagen III were also found at the enthesis, reflecting the tensile stress that the interface is subjected to (Cury et al., 2016; Friese et al., 2020; Jensen et al., 2018). Additional findings from studies related to the development of the enthesis show that the mechanical load imposed on the enthesis also plays a role in its postnatal development and functionality. In the tendon of the supraspinatus muscle, enthesis fibrocartilage appears a few days after birth (Jensen et al., 2018; Thomopoulos et al., 2007). Furthermore, when the supraspinatus muscle was paralyzed by pharmacological agents in neonatal mice, collagen fibers were disorganized, fibrocartilage formation and mineralization of the ossification front were delayed, and osteoclastic activity increased at the osteochondral interface (Schwartz et al., 2013; Tataru et al., 2014; Thomopoulos et al., 2007; Zelzer et al., 2014). These structural changes were shown to result in alterations to the mechanical properties of the enthesis characterized by a decrease in stiffness and maximum force at failure (Schwartz et al., 2013; Zelzer et al., 2014).

While all these studies strongly support the idea that the enthesis structure is determined by mechanical stress, very few assessed enthesis plasticity, i.e. its ability to adapt to chronic variations in mechanical load. Of these studies, most investigated the effects of a chronic reduction of mechanical load and all were on animals. The reduction in mechanical load was induced by different experimental situations such as immobilization by plaster cast, deviation of mechanical strain, muscular paralysis by botulinum toxin injection, but also by real and simulated microgravity.

Most studies show that the tendon-to-bone interface is sensitive to a chronic reduction in mechanical load. This reduction induced a decrease in the area of fibrocartilage and its proteoglycan content (Frizziero et al., 2011; Järvinen et al., 1999; Mutsuzaki et al., 2015; Roffino et al., 2018), a decrease in the number and proliferation of chondrocytes (Järvinen et al., 1999; Mutsuzaki et al., 2015), as well as a modification of morphological characteristics of the fibrocartilage cells (Järvinen et al., 1999; Roffino et al., 2018). As a consequence of decreased mechanical

stress, the cells at the enthesis site lost their normal rounded appearance (Järvinen et al., 1999). In the ECM, collagen disorganization (Frizziero et al., 2011; Järvinen et al., 1999; Roffino et al., 2018) and a decrease in the expression of collagen II in the UFC (Roffino et al., 2018) were described. These changes indicate that the enthesis tends to lose its fibrocartilaginous characteristics in an environment where the mechanical load is reduced. Furthermore, the mineral component of enthesis was affected. After 3 weeks of paralysis of the supraspinatus muscle, the size, the alignment, and the composition of CFC hydroxyapatite crystals were altered (Deymier et al., 2019). Unloading induced by paralysis decreased bioapatite crystal size and crystallographic alignment in the enthesis, in addition to causing bone loss in the humeral head. This latter study is one of the few examining the functional consequences of unloading on the enthesis. Muscle paralysis was found to increase the risk and the extent of avulsion failure at the supraspinatus tendon enthesis level, without modifying its elastic properties (Deymier et al., 2019). On the same enthesis, the same authors studied the effects of short spaceflights (Deymier et al., 2020) and found, contrary to their previous study, that the structure and the mechanical properties of the enthesis of the supraspinatus tendon were not altered by microgravity. However, in contrast to the effects of paralysis, the supraspinatus was not subject to muscle atrophy after a 7-day spaceflight (Shen et al., 2017), showing that the unloading induced by weightlessness is not sufficient to trigger muscle deconditioning. Under 7-day weightlessness, the tension generated by the supraspinatus appears to maintain the structural and functional integrity of the supraspinatus tendon enthesis. Thus, the magnitude of the load reduction seems to be a factor in the mechanical response of the enthesis. It is therefore highly likely that entheses of tendons transmitting force from muscles that atrophy during spaceflights will undergo structural remodeling, with possible functional consequences. Further studies are needed to support this hypothesis. Finally, it is interesting that the enthesis is able to retrieve its characteristics during the recovery period after inactivity. Eight weeks of low- or high-intensity treadmill running restored the fibrocartilage thickness of the enthesis of the quadriceps tendon in rats after 3 weeks' immobilization by plaster cast (Järvinen et al., 1999). However, recovery seems to involve a stage where fibrocartilage synthesis is exacerbated. Only a few days after reloading, mice unloaded by hindlimb suspension for 2 weeks showed greater UFC and CFC surface areas than control entheses, an overexpression of type II collagen in the ECM, and a greater number of chondrocytes (Roffino et al., 2018). In addition, the magnitude of the load applied on the musculoskeletal system during remobilization could be an important factor to optimize enthesis recovery. A recent study in healthy rabbits investigated the effects of electrostimulation of the quadriceps muscle on the patellar tendon enthesis. The authors reported a structural remodeling of the enthesis at low electrostimulation intensity, reflecting an adaptive response to the increase in mechanical stimulation. However, when the electrostimulation induced a peak tetanic force of the quadriceps muscle beyond 18%, structural deleterious changes were observed at the enthesis (Fan et al., 2020).

The mechanisms underlying enthesis structural remodeling in response to a chronic variation in mechanical loading have yet to be elucidated. However, a very recent study (Shen et al., 2020) provides some explanation, highlighting a possible role for connexin 43 in these mechanisms. Connexin 43 is a gap junction protein largely expressed in skeletal tissues like tendon or bone and thought to be involved in mechanotransduction (Ralphs et al., 1998; Banes et al., 1995; Buo and Stains, 2014). Shen et al. (2020) studied the response of wild-type mice and mice lacking connexin 43 to a treadmill exercise. They demonstrated that, unlike the enthesis anabolic response of wild-type mice, which increased with exercise, the anabolic response of connexin KO mice was attenuated. This indicates that connexin 43 is necessary for tendon enthesis responses to mechanical stimulus. In addition, since hedgehog/Gli1 are sensitive to connexin 43 deletion, the authors hypothesized that enthesal mechanoresponsiveness is linked to activation

of the hedgehog/Gli1 signaling pathway, and that Cx43 is required for this response.

4. Conclusion and perspectives

This review indicates that, like muscles and bones, tendons are sensitive to a chronic decrease in mechanical load. However, the tendon response to unloading varies according to the anatomical site of the tissue. While the tendon midsubstance shows a decrease in both tensile stiffness and tensile strength, impairment to the tendon-to-bone interface, or enthesis, seems to concern resistance to compression. Fibrocartilage gives the enthesis specific mechanical properties, notably its capacity to withstand compressive and shear stresses. Although studies on how disuse affects the enthesis are scarce, all agree that the fibrocartilaginous characteristics of the enthesis disappear with chronic reduction in mechanical load. Because fibrocartilage not only ensures gradual transition of mechanical properties between a soft tendon and a hard bone but also affords protection against compressive and shear stresses, it seems reasonable to assume that fibrocartilage loss weakens the enthesis when loading returns to normal. This weakness could be expressed by premature wear and tear. Deymier et al. (2019) showed that muscle paralysis increases the risk of avulsion failure at the enthesis, which supports this hypothesis. However, it remains to be corroborated by further studies, firstly because the authors were unable to confirm it in another study (Deymier et al., 2020), and secondly because the authors only tested enthesis resistance to tensile stress. In view of the role played by fibrocartilage and its remodeling under unloading, future work should also address the resistance of the enthesis to compressive stress.

Altered capacity to withstand compression could thus threaten the structural integrity of the enthesis when loading returns to normal. Here again, there is a lack of research, with only two studies investigating the effects of remobilization after a period of unloading (Järvinen et al., 1999; Roffino et al., 2018). Järvinen et al. (1999) showed that 3 weeks of exposure to normal loads is not sufficient to restore the structural characteristics of the enthesis, but that recovery is possible when low- or high-intensity exercises are performed. Moreover, significant structural remodeling was described a few days after a return to normal loads (Roffino et al., 2018). Unfortunately, these studies did not investigate the effects of remobilization on the mechanical properties of the enthesis and its potential fragility. It is therefore not known whether an unloaded enthesis is mechanically weakened during remobilization, nor whether an alteration in its mechanical properties alters its mechanical response. Future studies will need to address these questions. In the field of space-related medical research, this information would provide guidance not only for countermeasures but also for post-flight rehabilitation strategies.

Spaceflight's effects on the tendon attracted attention when astronauts complained of tendinopathy-type pain (Shackelford et al., 2004). Recently, Lang et al. (2017) pointed out the need for extended investigation of the enthesis in spaceflight in order to identify the key risk factors for injury. Although these studies did not specify whether astronauts' tendinopathies were enthesopathies, pain was reported in the shoulder and knee, where the supraspinatus, quadriceps, and patellar tendons are known to be almost exclusively affected by insertional tendinopathies. Whereas the role of excessive and repeated tensile loads in the etiology of enthesopathies is widely accepted, compressive stress also seems to be associated with the development of these pathologies (Maganaris et al., 2004). The study by Chimenti et al. (2016) strongly supports this hypothesis, since it shows that the deep region of the Achilles tendon enthesis (a common enthesopathy site) is subject to the greatest compressive stress during ankle dorsiflexion. Thus, astronauts' enthesopathies could partly be related to compressive stress induced by countermeasure exercises on an enthesis whose fibrocartilage is altered by microgravity.

Not only spaceflight, but all situations featuring transient inactivity,

involve a return to normal load conditions. Future scientific investigations of the biological and mechanical responses of the enthesis, therefore, should consider not only the effect of reduced mechanical stress but also the effect of a return to normal load. Another useful avenue of research would be to explore the as yet unknown mechanisms of fibrocartilage remodeling.

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