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STRUCTURAL CHANGES AND PATOLOGY OF TENDONS IN THE ELDERLY

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ABSTRACT:

Tendons are fundamental structures for maintaining posture and achieving movements.Tendons consist of compact connective tissue, they are slightly vascularized, receiving their blood supply primarily from the surrounding vessels. In sarcopenic patients we can see a reduction in strength, power and resistance. Muscle's performance is also affected by the lifestyle, environment, epigenetic mutations, editable factors and unchangeable factors such as age. In vitro studies have shown that age is associated with a reduction in the number of collagen fibers, an increase in elastin content, a reduction in extracellular water, a reduction of nutritive substances and reduction in diameter of collagen fibrils. Therapy can be divided in: conservative, infiltrative and surgical. There are also innovative infiltrative therapies like PRP, mesenchymal stem cells, autologous peripheral blood progenitor cells. In our experience we use, with ultrasound control, a multiple injections with a centrifugation of PRP on the Achilles tendon and early rehabilitation.

ANATOMY AND ULTRASTRUCTURE OF TENDONS

Tendons are structures that possess characteristics of elasticity, plasticity and viscosity and a function as transmitters of the muscle strength to the skeletal levers, allowing the maintenance of body posture and movement. Tendons are more rigid than muscles, having much greater tensile strength and being able to withstand very high loads with minimal deformities. [1]

Macroscopically they have various shapes in relation to their specific muscle: muscles performing fine movements have long and thin tendons, on the contrary short and strong tendons are associated with muscles that perform strength and resistance actions. To support these structures there are different structures, with important functional purposes, such as fibrous sheaths or retinaculae, synovial sheath, peritendon sheaths (or paratenon), reflection pulleys, tendon borsae[40].

Under the paratenon, the tendon is surrounded by a thin sheath of dense connective tissue, called epitenon.[2,3]; paratenon and epitenon are sometimes referred to as peritendon .[4]

Microscopically they consist of compact connective tissue, with cells and an extracellular matrix; the latter is made by collagen and elastic fibers, embedded in a hydrophilic amorphous substance, composed of proteoglycans, glycoproteins and glycosaminoglycans (GAGs). Collagen is the most abundant protein (75-90%) and provides strength to the structure[5,6,7,8]. Proteoglycans and GAGS retain a considerable amount of water forming a colloidal solution and a highly hydrated gel, which provides the tensile strength and elasticity.

The tendon has a poorer blood supply compared to other tissues, getting it primarily from the surrounding tissue vessels[9].

The tendon has three basic characteristics: elasticity, which is the material's quality to deform proportionally to the applied force and to come back to the initial conditions when the deforming force is ended; plasticity, ensured by collagen fibers, which is the tendon's feature not to be deformed any given force until it reaches the mechanical point break. Finally, the viscosity is the tendon's ability to deform, not only proportionally to the force to which it is subjected, but also to the time in which it is applied.

Ultra-structurally tendon bundles have a characteristic wavy module denominated crimps, well visible using electron microscope. Stretching from the beginning, these structures give the "shock-absorber" tendon typical biomechanical feature.

Diamant has proposed a model describing the crimps as triangular waves with a planar arrangement according to the tendon's axis [10], and identifies two important parameters: the angle θ and the period 1, which is equal to distance between the two wave peaks: 1 represents the length and θ the angle of the crimp.

Several authors [10,11,12,13,14] have shown that the appearance of crimps changes with age, through the increase of the length and a reduction of the angle, while the number remains constant.

Studies in animal models have shown that young tendons typically have greater compliance and extensibility than the older ones and also the deformation percentage required to determine the maximum elongation of crimps is considerably greater in young animals [10,12,15]. The crimps' angle is related to the function that the tendons have in relation of specific muscle's action[3,14,16,17]; the greater the load placed on the tendon, like the quadriceps tendon or the Achilles tendon, the greater is the crimps' angle and vice versa.

The crimps possess mechanical characteristics typical of the inert materials, namely the ability to deform and get up to rupture when subjected to stress. Some authors [18] summarized these

characteristics through the stress-train diagram, which relates the stress undergone by the tendon with the deformation that ensues.

The diagram is represented by a curve, in which the first part, when it's applied stress to the tendon, shows an initial crimps' relaxation and therefore the progressive recruitment and a new orientation of the bundles of fibers that are aligned in the direction of the applied load. This initial region, called non-linear or toe region, follows an almost linear, more rigid region, characterized by a sudden increase in the curve's slant. In fact the tissue's stiffness increases as the load increases , since the deforming force is applied on stretched collagen fibers, responding with an increased tensile strength[15,19]; therefore is required a progressively greater force in order to produce a precise elongation to that value. For excessive elongations the curve may stop suddenly or point down, as a result of irreversible changes in tissue's structure.

A crimps' elongation up to 4% is considered physiological, after this point are observed partial tears in the structure; over the 8% the rupture of the tendon occurs.

TENDON AGING AND TENDINOPATHY

The sarcopenic muscle has a reduction in strength up to about 75%, a reduction in muscle power of 40% and a decrease of the resistance of 65%. Morphologically, the muscle of sarcopenic patients, at first presents serious alterations in cell turnover, partly dependent on the increased oxidative stress; the alterations depend on specific mitochondrial variations, vacuolation of the cell. Another typical feature of the sarcopenic tissue is a reduction in number of satellite cells, which results in a significant loss of muscle regenerative power. The muscle's aging process begins with the progressive denervation of the single motor unit, which is followed by the consistent reduction of the type II fast-twitch muscle fibers [20] partly replaced by type I slow-twitch muscle fibers, though most of the type II fibers will be replaced by adipose tissue. According to scientific literature We know that in patients there is a reduction in number of muscle fibers up to 50% after 80 years. While the hormonal influence on the muscular tone remains controversial, we know that lack of physical activity, lipid metabolism alteration, and protein reduction in alimentation badly affect the muscles' tropism [42]. The literature claims that lifestyle, environmental situations and epigenetic mutations, associated with modifiable factors such as activity or exercise performance and unchangeable factors as the age, contribute to determine the musculoskeletal structure of each individual [41].

Studies have considered the reactions of isolated materials, especially non-human, and they show that disuse and aging both affect collagen, muscles and tendon mechanical's characteristics. The ultrasound is critical to consider in vivo tendons mechanical properties, by this mean it was possible to determine that disuse has negative effects on the tendon's physiology, and on the aging of it as well. Moreover some studies [21] on the gastrocnemius tendon, have shown that after 90 days of disuse a reduction of 60% of the tendon's diameter at the ultrasound scan occurs. Considering the values obtained through the ultrasound measurement it was also found that the variation of the tendon's diameter, illustrated on a stress-strain graph, varies between two groups: one composed of individuals studied after 90 days of bed rest in total immobility (with spinal injury) and the other consisting of subjects that during this period performed isometric exercises of flexion-extension of the foot: it was noted, based on the Young's modulus concerning the elasticity of the materials, a substantial difference between the two. The group that performed exercises against resistance during the period of immobility, presented a lesser reduction in the mechanical properties compared to the other group.

Onambele et al.[22] examined the mechanical properties of the gastrocnemius tendon in young people aged 24 ± 1, in middle-aged subjects (46 ± 1) and in older aged 68 ± 1 and determined that both stiffness and Young's modulus decrease with age, with a difference between the group of young people and seniors that reaches 36% and 48% respectively. In other words, a tendon with more compliance?, as in the case of disuse and aging, theoretically determines a lower strength at equal length.

It was shown that the oldest tendons are about 15% more compliant than young ones and that this difference is due to changes in the tendon of the same materials, in particular as regards the collagen. These changes stem from a number of mechanisms that are activated and prevail in disuse and aging.[23]

In vitro analysis [24] have shown that age is associated with:
- Reduction in the number of collagen fibers;
- Reduction of the crimps;
- Increase in elastin content;
- Reduction in extracellular water and the content of mucopolysaccharides;
- Increase of collagen type III and V;
- Reduction of nutritive substance;
- Reduction in the diameter of collagen fibrils;
- Increase in intermolecular cross-linking.

Some of these mechanisms are related with the secretion of cytokines (IL-1β, TNF-α) which increases the activity of matrix's metalloproteinases leading to the collagen degradation.[25]

An alteration in the remodeling activity is associated with tendinopathy development; molecular changes include reduced expression of collagen III, tenascin C, biglycan and aggrecan. These changes are originated by a reparation, but it could also be an adaptive response during the mechanical stretching of the tendon.
The metalloproteinase enzymes play an important role in the tendon matrix, being responsible for the degradation of collagen and proteoglycans in patients both in good health and in those with diseases. Among these enzymes, those that increase expression in patients with tendinopathy include ADAM 12 and MP23 [26] The most frequent tendinitis are: patellar, Achilles and rotator cuff.

THERAPY

The therapy is divided in: conservative (which is based on eccentric exercise and use of vitamin D), infiltrative and surgical.

Use of vitamin D is important for the changes of tendons in the elderly , in fact Rodeo et al found that decreased vitamin D levels affect tendon healing as it relates to collagen remodeling and strength of the healing enthesis. Although their work should be considered preliminary, our findings offer insight into how the manipulation of vitamin D metabolism may improve the strength and organization of the healing enthesis.[27]

Several nonsurgical treatment options provide significant symptom relief. Eccentric squat-based physical therapy has good evidence for use as an initial conservative treatment [43]. David C. Flanigan et al, in their review recommend a standard treatment algorithm that begins with an initial nonsurgical treatment, lasting a minimum of 6 months. Patients undergo eccentric squat-based physical therapy as initial monotherapy, though there is evidence to support the use of shockwave therapy and, to a lesser extent, PRP, as an alternative. With eccentric squat therapy, although patients should experience significant symptom improvements for up to 12 weeks, evidence suggests that the rate of improvement will drop significantly by 6 months. For this reason we recommend waiting a minimum 6 months after the initiation of eccentric therapy before considering surgical therapy.[28]

Infiltrative therapy is based on the use of steroids and hyaluronic acid; according to Meloni et al. infiltration with hyaluronic acid under ultrasound guidance should not be used only as a lubricant but also to prevent cartilage degeneration and to protect the articular cartilage; furthermore sodium hyaluronate can reduce the inflammatory process[29].

The hyaluronic acid interacts with metal proteinases, regulating their activities, blocking the fragments of fibronectin action which inhibits degenerative processes and promotes regeneration and biosynthesis of tendon constituents.

A study performed by Hailic M. et al. [30] considered two groups of rabbits and showed that the use of hyaluronic acid results in a reduction of adhesion's tissue and an acceleration in the healing of the tendon after 6 and 12 weeks due to vascular proliferation.

According to Monica Wiing et al. [31] hyaluronic acid does not affect the cell's proliferation rate or matrix synthesis in favor of the tendon's or synovial sheath's healing. In addition, these results show that the cellular activities between tendon and synovial sheath are different during the healing process and that these activities are not affected by the hyaluronic acid.

Innovative infiltrative therapies rely on:
- PRP (Platelet Rich Plasma);
- Mesenchymal stem cells;
- Autologus peripherial blood progenitor cells;

The cell concentrates contain numerous important cellular growth factors able to stimulate various cellular mechanisms inherent to tissue growth, including angiogenesis, chemotaxis of macrophages, proliferation and migration of fibroblasts and collagen synthesis.

Stem cells are unspecialized cells and renew themselves through cell division for an indefinite period of time and they can become specialized cells of various tissues of the body. Because of these properties, stem cells have the ability to repair damaged organs replacing dead, or not working, cells; They are found in the embryo, in the bone marrow and in the umbilical cord and are totipotent or multi-potent cells.

Structurally, tendons and associated extracellular matrix are composed of nanostructured materials. For this reason , there has been a growing interest on new approaches for tendon regeneration based on nano-materials. Nanoparticles (NPs) which dimension are almost <100nm, represent a bridge between the conventional size materials, that are actually used in orthopedic surgery, and the atomic level tendon structures. Nanoparticles could play an important role in tendon healing: labeling TSCs, working as carrier for gene therapy and for drug delivery, allowing the fabrication of a new generation of bioactive scaffolds and modulating the cellular response.[32].

During the process of healing and repair of tissue damage, the platelets exert hemostasis function, capillary integrity , active transport, phagocytosis, repair, scarring and secretion.

PRP

Marcacci et al described in a review that, based on the current evidence, patellar tendons seem to benefit from PRP injections, whereas results in the Achilles tendon do not justify the application of the evaluated platelet concentrates, neither conservatively nor surgically. The findings on the conservative treatment of rotator cuff are still too limited; on the other hand, there is a more consistent literature with an overall agreement on the lack of substantial benefit of PRP surgical augmentation. Lateral elbow tendinopathy showed an improvement in most of the high-level studies, but the lack of proven superiority with respect to the more simple whole-blood injections still questions its use in the clinical practice.[33]
Leading and regenerative tissue stimulus is induced by growth factors, contained in the α-granules of platelets released during the clot formation process: these stimulate the replication of mesenchymal cells and exert a chemotactic action to polymorph nucleated cells, monocytes and macrophages. The growth factors are small fragments of biologically active proteins, belonging to the group of cytokines that bind to specific targets cell receptors and trigger a cascade of chemical reactions within the cytoplasm. This creates a series of chain effects with stimulation, recall, migration and proliferation of important cell lines involved in tissue regeneration.

The GFs are numerous and each one performs a specific function:
1. PDGF (Platelet Derived Growth Factor) stimulates cell replication (mitogenesis), the differentiation of pre-mitotic osteoprogenitors, replication of endothelial cells and promotes angiogenesis.

2. EGF (Epidermal Growth Factor) is responsible for cell differentiation and stimulates the re-epithelialization, angiogenesis and the collagen's activities.

3. IGF (Insulin Growth Factor) important in wound healing and stimulates the proliferation and the different functions of osteoblasts.

4. VEGF (Vascular Endotelium Growth Factor) implicated in processes such as inflammation, angiogenesis of the ischemic cells. It induces an increase in the permeability of blood capillaries, and edema formation.

5. FGF (Fibroblast Growth Factor) growth factor foe the fibroblast.

6. TGF (Transforming Growth Factor) regulates the proliferation and differentiation of numerous cell lines: osteoblasts, fibroblasts, endothelial cells, and chondroprogenitors stem cells.

Platelet rich plasma (PRP) is derived from the centrifugation of the whole blood, it has a superior concentration of platelets than pure blood, it is the plasma's cell component that is deposited after centrifugation, and contains numerous growth factors.

According to Donan et al. There are several classes of PRP (architecture of fibrin and content of leukocytes)

1. Pure PRP (PRP-P)
2. PRP rich in leukocytes (L-PRP)
3. PRP Pure Rich Fibrin (PRF-P)
4. PRP rich in leukocytes and Fibrin (L-PRF)

The first two classes are liquid and become gel with the activation of thrombin-calcium, thus they have biological activation; the other two are solid only in the active form and so they have mechanical activation.

The PRP has been used extensively in recent years also in Sports Medicine, both in clinical and surgical procedures and muscular, ligaments, tendons and joints diseases.

According to G. Ferrero et al. [34] the use of PRP leads to significant and early results on clinical symptoms and to the healing of the tendon matrix by decreasing the possibility of a degenerative lesion.

Another study [35] states that aspects of PRP use that have yet to be determined are (1) injection volume / application, (2) the more effective preparation, (3) buffer / activation, (4) injection technique (1 depot vs. multiple depots), (5) the injection times for injuries, (6) single application compared series of injections, and (7) the more effective rehabilitation protocol to use after injection of PRP.

According to studies by J.F. Kaux et al [36] PRP must be used as a new therapy of chronic tendinopathy, in fact it is easy to prepare and has a relatively low cost and minimal invasiveness. In addition, the therapy with PRP is not associated with any side effects. Despite the proven efficacy of PRP on tissue regeneration in experimental studies, the clinical efficacy in chronic tendon diseases is still not obvious. Considering various types of preparation of PRP and different injection methods and protocols, according to these

authors the ideal PRP should not contain red and white blood cells that may hinder the effectiveness of the preparation because of their pro-inflammatory activity; it is important to have an adequate concentration of platelets and a good quality of PRP. To determine the effectiveness of PRP for chronic tendon injuries, however randomized trials with placebo groups are required.

According to Brian C. there is a rational basis regarding the use of PRP to enhance the healing of tissue, but the efficacy for many skeletal-muscle level uses remains unproven[37]. This treatment is seen as a rescue. Some questions on the PRP remained unsolved, especially regarding the optimal concentration of platelets, which types of cells should be present, the ideal frequency of application or the optimum rehabilitation for the repair of the tissue and the return to full function.

We used the PRP in particular in patients with degenerative tendinopathy of the Achilles tendon, performing a preliminary ultrasound which justifies the tendon degeneration and shows a thickening of the tendon and the presence of a wide hypoechogenic area within the tendon.

CONCLUSION

Aging is inevitable, but the structural changes may be delayed and improved with exercise and integrative therapies. It was shown that the oldest tendons show about 15% more compliance than in young and that this difference is due to changes in the tendon's components (especially when it relates to the collagen).

Potential mechanisms of aging are:
Reduction in the number of collagen fibers aligned longitudinally;
Reduction of crimps
Increase in elastin content;
Reduction in extracellular water and polysaccharides' content;
Increase of collagen type III and V;
Reduction of nutrients;
Reduction in the diameter of the collagen fibrils;
Increasing intermolecular cross-linking.

Some of these mechanisms are related with the secretion of cytokines (IL-1β and TNF-α) which increase the activity of matrix metalloproteinases leading to the degradation of collagen.[38]

These changes are caused by various mechanisms that are activated and prevail in a similar way in disuse and aging[39].

The tendinopathies find fertile ground in aged tendons amplifying the degenerative phenomena until the break.

There are many new infiltrative therapies, but currently there is little clinical evidence, and surgery is the definitive treatment for breaks or tendinopathies, when conservative therapy fails.

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