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Abstract

Tendinopathies have a multifactorial etiology driven by extrinsic and intrinsic factors. Recent studies have elucidated the importance of thyroid hormones in the alteration of tendons homeostasis and in the failure of tendon healing after injury. The effects of thyroid hormones are mediated by receptors (TR)- α and $-\beta$ that seem to be ubiquitous. In particular, T3 and T4 play an antiapoptotic role on tenocytes, causing an increase in vital tenocytes isolated from tendons *in vitro* and a reduction of apoptotic ones; they are also able to influence extra cellular matrix proteins secretion *in vitro* from tenocytes, enhancing collagen production. From a clinical point of view, disorders of thyroid function have been investigated only for rotator cuff calcific tendinopathy and tears. In this complex scenario, further research is needed to clarify the role of thyroid hormones on the onset of tendinopathies.

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General Aspects and Epidemiology

The natural history of tendinopathies is difficult to define. Different factors, such as genetic background, biomechanics, intrinsic degeneration within the tendon itself and co-morbidities, are involved in the pathogenesis of tendon diseases. The etiology of tendon tears remains multifactorial and attempts have been made to unify intrinsic and extrinsic theories. Recent evidence strongly suggests that most of rotator cuff tears are caused by primary failed healing response [1], but it is not clear whether circulating hormones can act on tendons and which is the link between hormonal and metabolic diseases and the development of tendinopathy [2]. In this complex framework,

thyroid hormones (Ths) imbalance can deeply affect the structural setting and the homeostasis processes of tendons, but the precise mechanism is still unknown [3]. Thyroid hormones play an important role in the regulation of adult metabolism influencing genomic actions, such transcriptional processes and phosphorylation and non genomic actions such as thermogenesis, cellular growth and mitochondrial pathways [4]. In both cases, The start a transduction cascade with a strong impact on cellular metabolism by activation of multiple mechanisms [5]. These hormones are essential both in early and adult life and they strongly influence changes in oxygen consumption, protein, carbohydrate, lipid and vitamin metabolism [6]. In particular, T4 (thyroxine) is important for both collagen synthesis and extra cellular matrix (ECM) metabolism. Hypothyroidism causes accumulation of glycosaminoglycans (GAGs) in the ECM, which may predispose to tendon injury [7]. Tendinopathy can be the presenting complaint in hypothyroidism, and symptomatic relief can be obtained by appropriate management of the primary thyroid deficiency.

An epidemiological study recently showed the possible role of thyroid hormones on modifying and increasing the rate of non traumatic rotator cuff tear in male and female [8]. A higher frequency (63 %) of disorders of thyroid function was detected in females: according to these data, gender can be considered as an increased risk factor for rotator cuff diseases, enhanced by the higher incidence (56 % of the total female group between 60 and 80 year) of thyroid pathologies among the females. According to this study, even though aging or genetic predisposing factors or traumas are the main actors in the tendons tears, an important role is played by metabolic substrate. Thyroid disorders strongly influence the failed healing response in some subjects, causing the final symptomatic tears [9].

Basic Science

The effects of Ths are mainly mediated by T3, which regulates gene expression by binding the TH receptors (TR)- α and $-\beta$ that seem to be

ubiquitous [10]. The importance of Ths on the genesis of the tendinopathies has been confirmed by *in vitro* studies that have detected the presence of Ths receptors (TR α/β isoforms) on tenocytes and confirmed the essential role of T3 and T4 in regulating cell proliferation. Tenocytes were isolated from human tendon tissues obtained from healthy subjects and cultured for 72 h with or without Ths. As expected, both T3 and T4 induced cell growth and the higher increase was obtained by 72 h of hormone treatment, being 19 % for T3 and 10 % for T4. The addition of the Ths in the culture medium led to stimulation of cell growth with a reduction of the doubling time. These findings stress the physiological action of THs in the homeostasis of tendons: T3 and T4 play an antiapoptotic action on tenocytes, causing an increase in vital tenocytes isolated from tendons *in vitro* and a reduction of apoptotic ones in a dose- and time-dependent manner [11] (Fig. 12.1).

Ths are also able to influence tenocyte secretion of ECM proteins *in vitro*. Tendons are fibrous connective tissues composed of cells surrounded by a complex extra cellular matrix rich of collagens, proteoglycans, glycoprotein and water [12]. When primary tenocyte-like cells were cultivated in the presence of T3 or T4 individually or in combination with ascorbic acid (AA), Ths together with AA significantly increase the expression of collagen I, the major fibrillar collagen of the tendon, in ECM. In addition, among the proteins, Cartilage Oligomeric Matrix Protein (COMP) was enhanced. This is an abundant glycoprotein, first identified in cartilage, particularly present in tendon exposed to compressive load. COMP belongs to the thrombospondin gene family with the ability to bind to type I, II and IX collagen molecules as well as fibronectin (see Chap. 2). COMP modulates the organization of collagen fibrils. In contrast, Ths do not affect the expression of collagen III, which is normally less abundant in tendon and increases only during the early phases of remodeling or in tendinopathies. Therefore, Ths play a role on the extra cellular matrix of tendons, enhancing *in vitro* the production of several proteins [13].

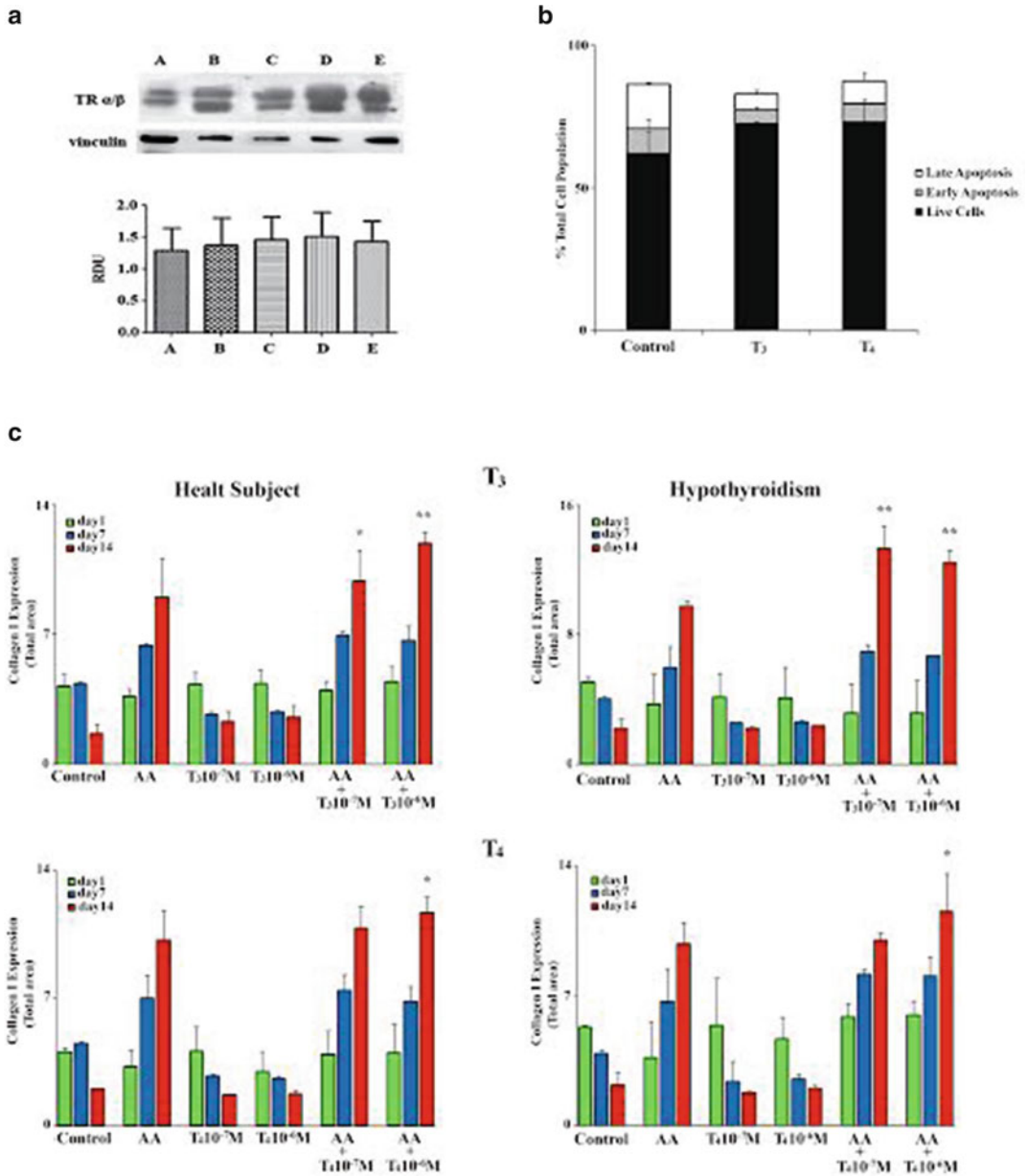


Fig. 12.1 (a) Western blot analysis of TR α/β isoforms. A indicates patients with healthy rotator cuff tendons; B–C indicate patients with rotator cuff tears without thyroid disease, D–E represent patients with rotator cuff tears and thyroid disease. The polyclonal antibodies against TRs α/β recognize two specific bands at 47 and 55 kDa, respectively. Densitometric absorbance values from three separate experiments were averaged (\pm SD), after they had been normalized to Vinculin for equal loading. Data relative to each protein are presented in the histogram of the Western Blot as Relative Densitometric Units (*y* axis). (b) Determination of apoptosis by Annexin V assay. At 48 h after culture, the tenocytes in serum deprived medium, double staining with Annexin V/PI, apoptotic cell population were evaluated by flow cytometry. Results from one

representative experiment of three independent experiments with similar finding are shown. Annexin V-PI- cells were considered as vital, Annexin V + PI- cells were considered as early apoptotic, Annexin V + PI+ cells as late apoptotic. The results are expressed as percentage of total cells. The percentage of control cells (untreated) and treated (T₃ or T₄) are presented in the histogram. All the data are presented as mean \pm SD, and are the results from five individual experiments. (c) Collagen I expression of primary tenocyte-like cells *in vitro* culture isolated from five healthy subjects and three hypothyroidism. Collagen I Intensity (Total Area was quantified by anti-collagen I) it was measured by Nikon software. F) Data are expressed as mean \pm SD for four independent experiments for samples run in triplicate (n = 4, *P < 0.05, **P < 0.01)

Tendons are affected by thyroid disorders because of the heterogeneous composition of their matrix: in fact, the T3-mediated up-regulation of collagen is reflected in a change of the cross-linking pattern, with a resulting improvement of collagen quality. Thyroid diseases compromise collagen quality affecting transduction mechanism involved in collagen maturation: in particular, in hypothyroidism genes coding for collagen I and collagen Va1 are poorly expressed, with a marked disruption of the architecture of the ECM [14]. This is in contrast with the endocrine theory according to which hyperthyroidism may be accompanied by an increased catabolism of proteins and collagen, probably because other intrinsic factors should be considered, such as other metabolic pathways and the global hormonal status.

Some studies have demonstrated that T3 negatively influence normal human skin fibroblasts by inhibiting the accumulation of glycosaminoglycan (GAGs) and the synthesis of fibronectin [15]. Skin fibroblasts and tenocytes have similar characteristics in terms of cell morphology and extracellular matrix components, and this may explain why hypothyroidism leads to accumulation of GAGs in the ECM, with a high risk of tendon calcification and nerve compression by mixedema as in the carpal tunnel syndrome [16].

The pattern of worsening hypoxia-induced apoptosis supports a continuous failure of the tendons in the rotator cuff; this is why it is important to determine the actual size of these effects on the functional recovery in tendon injuries. Disorders of thyroid metabolism are a most interesting aspect of calcific tendinopathy. One of the most reliable theories on the genesis of calcific tendinopathies concerns the hypothyroidism related reduction of oxygenation in the rotator cuff tendons with a consequent metaplasia that leads to calcium deposition [17].

Clinical Aspects

It is important to underline the similitude between skin fibroblast and tenocytes. The latter are specialized fibroblasts that regulate the

homeostasis of ECM in mesenchymal tissues. Recent studies used laboratory-amplified tenocyte-like cells to manage lateral elbow tendinopathy [18], while ultrasound-guided injections of autologous skin-derived tendon-like cells have been used to treat patellar tendinopathy [19]. Proliferation of fibroblasts is related to thyroid status. The complex interactions between thyroid hormones and fibroblast growth factors regulate cells proliferation and differentiation [20]. This deficiency also leads to a decrease of tendon fibroblasts activity, with down-regulation of the mechanism involved in ECM/cytoskeletal remodeling. The ECM structure becomes weaker, and tenocytes reduce their migration under mechanical stress stimuli.

Hypothyroidism leads to hypoxia and apoptosis: these two conditions are greatest in mild impingement and in partial, small, medium and large rotator cuff tears with macroscopic deterioration of the tendons [21]. The pattern of worsening hypoxia-induced apoptosis supports a continuous failure of the rotator cuff and, for this reason, it is important to determine the real size of these effects on the functional recovery in tendon injuries.

In addition, disorders of thyroid function are most interesting aspects of calcific tendinopathy [22]. Tendon healing includes many sequential processes, and disturbances at different stages of healing may lead to different combinations of histopathological changes, shifting the normal healing processes to an abnormal pathway. Patients with associated endocrine and thyroid disorders present earlier onset of symptoms, and longer natural history, and they undergo surgery more frequently compared to a control population. Hypothyroidism induces accumulation of glycosaminoglycans (GAGs) in the extracellular matrix, which may, in turn, predispose to tendon calcification [3, 23]. One of the most reliable theories on the genesis of calcific tendinopathies concerns the reduction of oxygenation in the rotator-cuff tendons with consequent metaplasia that leads to calcium deposition. The association between hypothyroidism and tendinous calcium deposits was first analyzed in myxedematous patients who showed synovitis of the wrists, metacarpal joints, and flexor tendon sheaths

thickening. Synovial fluid analysis demonstrated the presence of intra- and extracellular crystals, and the presence of chondrocalcinosis was distinguished from acute attacks of pseudogout, which was not observed in most patients [24]. In addition, a significantly increased expression of tissue transglutaminase (tTG) 2 and its substrate, osteopontin, was detected in the calcific areas: this shows that a variation in expression of different genes could be peculiar in calcific tendinopathy [25]. The etiopathogenesis of the mechanism whereby thyroid disease could contribute to enhance the influx of calcium deposits into tendons and joints is still unknown.

Conclusions

The relationship between thyroid hormones and tendons disorders is clinically relevant. The presence of high levels of thyroid receptors isoforms, their protective action during tenocytes apoptosis and their ability to enhance tenocyte proliferation *in vitro* in healthy tendons enhance the idea of a physiological action of THs in the homeostasis of tendons, but does not allow to clarify the role of THs in the pathogenesis of the rotator cuff tears. There is increasing recognition of the prevalence of autoimmune thyroid diseases in patients with connective tissue disorder, highlighting a common mechanism for the pathogenesis of these conditions [26]. THs may have a substantial role in failing the healing response during tendinopathies [27]. Much research remains to be performed to clarify the exact role of the THs in tendon tissue and their implications in tendon tears, tendinopathies and tendon healing after injury. Common tendinopathies affecting different sites could be associated to disorders of thyroid function, but the relationship has not been clearly defined. If this association is further confirmed, assessment and treatment of patients with tendon pathologies may have to be revisited and integrated. The natural history of tendon tears is to progress with time. These may well produce tendon retraction and fatty degeneration, which make it more difficult to successfully repair a tear. Tendons, including rotator cuff tears, seem

able to heal after surgical repair, but many factors influence their healing: age, sex and co-morbidities, including metabolic and endocrine disorders. If orthopaedic surgeons plan surgical repair of ruptured tendons, it is necessary to address the underlying conditions, because co-morbidities increase the probability of failure of surgical repair [28]. The knowledge of the mechanisms that underlie the onset of tendon pathology provides a comprehensive and multi-disciplinary approach to the patient and leads to a better outcome of the therapeutic strategy.

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