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ULTRASTRUCTURAL CHARACTERISTICS OF TENSIONAL REGIONS IN TENDONS FROM RATS OF DIFFERENT AGES

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ABSTRACT

The calcaneal tendon and the deep digital flexor tendon are collagen-rich structures which are adapted to resist tensile stress. Since during aging tendons undergo modifications in their mechanical properties and in collagen aggregation, an understanding of the structural changes involved is important. In this work, the ultrastructural organization of the tensile region of the calcaneal and deep digital flexor tendons was studied in male Wistar rats 30, 180 and 730 days old. Large quantities of rough endoplasmic reticulum and peripheral secretory microvesicles were observed in the calcaneal tendon of 30-day-old rats. In the case of the deep digital flexor tendon, this organelle remained well-developed up to 180 days. A marked decrease in rough endoplasmic reticulum was observed in both tendons in 730-day-old rats. Proteoglycans associated with collagen fibrils were visible in the two tendons of all age groups. The reduced amount of rough endoplasmic reticulum and secretory microvesicles may be correlated with the known lower turnover of extracellular matrix components during aging.

Key words: Aging, rat, tendon, ultrastructure

INTRODUCTION

Tendons are fibrous structures of the musculoskeletal system which attach muscle to bone and transmit the tensile stress generated during muscle contraction to the bones. Tendons are formed by a dense arrangement of collagen fiber bundles arranged parallel to elongated cells, which follow the major axis of the bundles [43]. The presence of large amounts of cross-linked collagen molecules confers tensile strength to tendons and makes them hight resistent to mechanical stress.

Tendons consist mainly of collagen type I, which accounts for 65-75% of the tissue dry weight, in addition to proteoglycans and non-collagenous proteins [20]. All of these components are produced by fibroblasts called tenocytes. The association between collagen and other extracellular matrix elements is reflected in the biomechanical properties of tendons. In addition to collagen type I, type III has also been detected [5,14], while type V [17] and types XII and XIV [6,36] have been observed only in fetal bovine tendon.

The ability of collagen molecules to undergo lateral self-aggregation depends on the molecules characteristics, and on the presence of intermolecular interaction sites located at regular intervals along the collagen molecules and along the fibrils themselves [23]. This interaction is determined by the primary amino acid sequence, with the sites involved being rich in polar and hydrophobic residues [15].

The structural characteristics of tendons are related to the fibrous extracellular matrix, and many events associated with the aging of these structures are related to the collagen molecule. In general, the total collagen content and collagen density increase with age [34], mainly because of decreased collagen turnover and a reduction in the synthesis of collagenolytic enzymes during aging [27].

Other components of the fibrous extracellular matrix of tendons include proteoglycans, in particular small proteoglycans, which show a great diversity in terms of size and molecular mass. Proteoglycans consist of a central protein skeleton and at least one covalently linked glycosaminoglycan chain. Dermatan sulfate-containing proteoglycans have been detected in tail tendons of newborn rats [32], and adult bovine tendon contains large and small proteoglycans, mainly decorin and fibromodulin [28,42]. Small proteoglycans such as decorin and fibromodulin interact with collagen fibrils in an ordered fashion to regulate fibrillogenesis *in vitro* [8,9,13,30] and to maintain the tendon's properties [30].

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The aim of this investigation was to analyze the morphology of the tensile region of the calcaneal and deep digital flexor tendons of rats at different ages.

MATERIAL AND METHODS

Male Wistar rats were reared in the Animal House of the Department of Cellular Biology until 30, 180 and 730 days of age, during which time they received food and water *ad libitum*. The rats were killed with sodium pentobarbital (40 mg/kg, i.p.; Hypnol®, Cristália, Espirito Santo do Pinhal, SP, Brazil). The calcaneal tendon (CT) and the deep digital flexor tendon (DDFT) were then removed from the hind limbs of three rats and only the tensile region was used for analysis.

The tendons were fixed in 2% glutaraldehyde and 0.1% tannic acid dissolved in 0.1 M cacodylate buffer, pH 7.3, for 2 h at room temperature. The material was washed in the same buffer and post-fixed in 1% osmium tetroxide at 4°C for 1 h. The specimens were then washed in glucose saline, treated with 1% uranyl acetate at 4°C for 18 h, washed again in glucose saline, and dehydrated in an increasing (70-96%) ethanol series. Tendon fragments were also stained with cuprolinic basic blue to detect proteoglycans [31]. After fixation, the material was processed and embedded in Epon resin. Ultrathin sections were cut with an Ultracut UCT (Leica) ultramicrotome, counterstained with uranyl acetate [29] and analyzed with a LEO 906 transmission electron microscope (Zeiss).

RESULTS

Cross-sections of the CT (Fig. 1) and DDFT (Fig. 2) from all age groups showed tenocytes immersed in collagen fibers in the tensile region. The cells had an irregular surface, and emitted processes that extended to and interacted with the surrounding collagen fibrils (Fig. 2C). In the CT (Fig. 1A) and DDFT (Fig. 2A) of 30-day-old rats, the fibroblasts had a similar morphology consisting of a well-developed rough endoplasmic reticulum and a large number

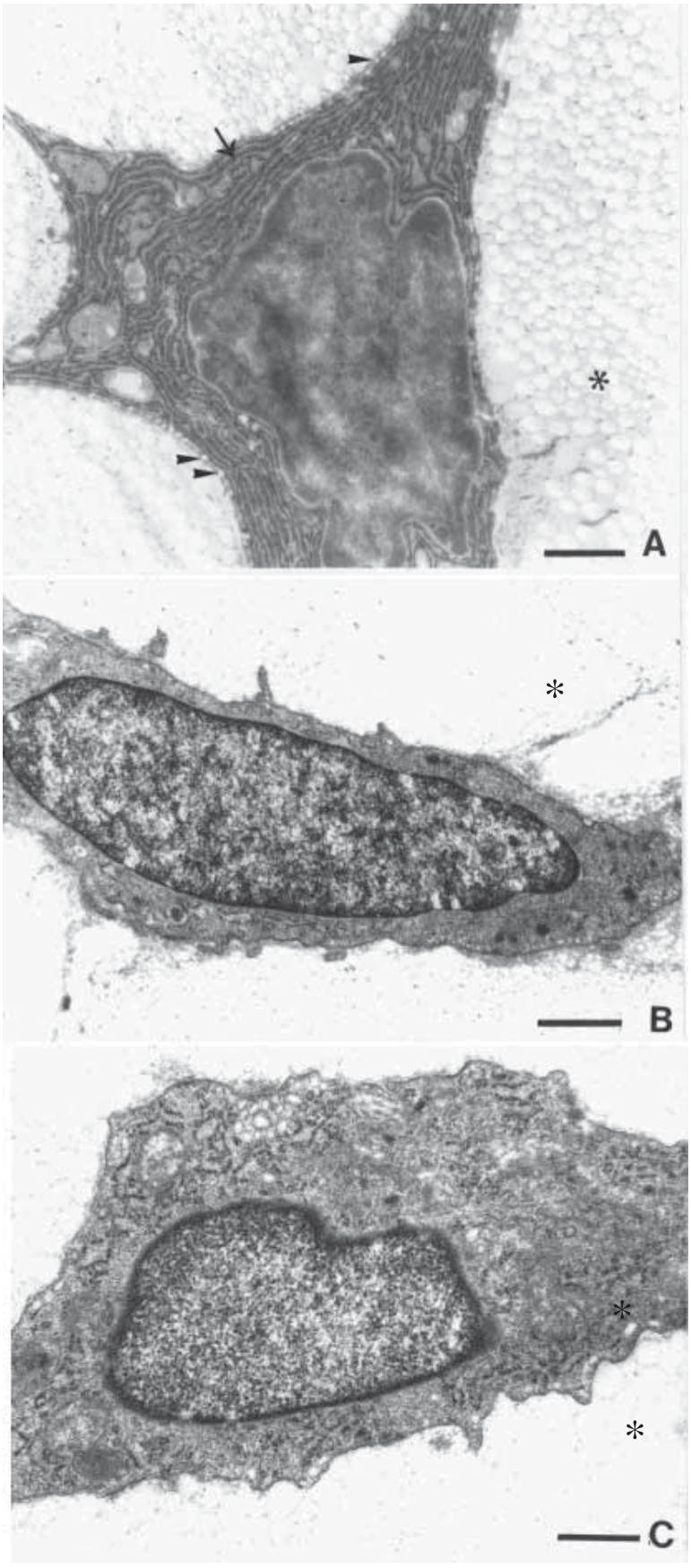

Figure 1. Transmission electron micrograph of the calcaneal tendon region of rats 30 (**A**), 180 (**B**) and 730 (**C**) days old. Fibroblasts immersed in a collagen fibril-rich extracellular matrix were observed in all age groups (*). In **A**, note the presence of large quantities of rough endoplasmic reticulum (➔) and vesicles at the cell periphery (▶). A decrease in the number of these organelles was observed at 180 (**B**) and 730 (**C**) days. Bar = 5 μm.

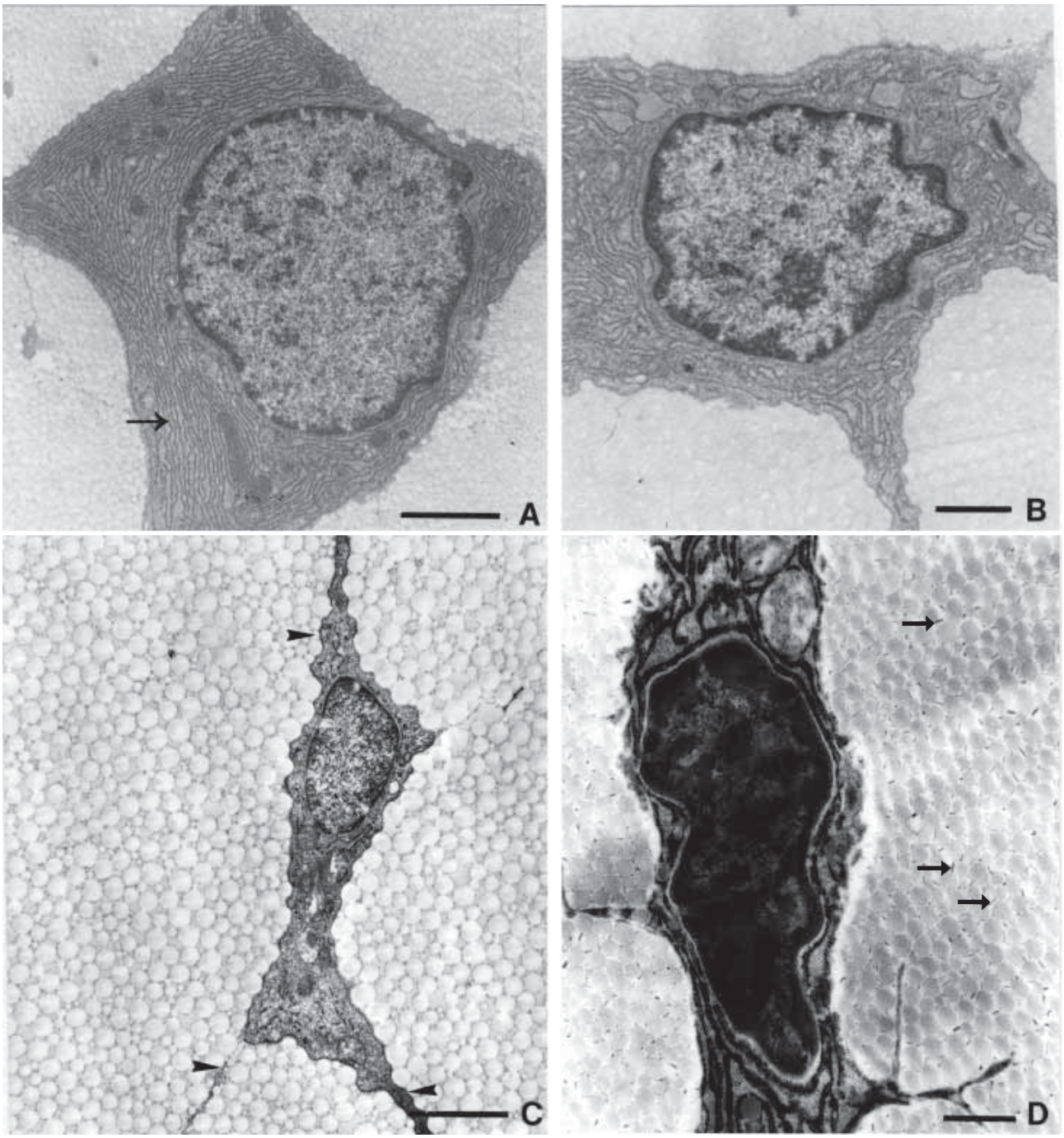

Figure 2. Electron micrograph of the tensile region of the deep digital flexor tendon of rats 30 (**A**, **D**), 180 (B) and 730 (**C**) days old. The rough endoplasmic reticulum (➔) was well-developed in rats aged 30 (**A**) and 180 (**B**) days old. The tenocytes had processes (➤) that extended among the collagen fibrils. At 730 days (**C**), the rough endoplasmic reticulum and the cytoplasm volume were markedly reduced compared to 30- and 180-day-old rats. Proteoglycans (➞) were associated with collagen fibrils (**D**), but there was no difference in proteoglycan distribution among the different ages. The calcaneal tendon had a similar structural aspect (not shown). Bar = 5 μm.

of microvesicles at the cell periphery. The CT of rats 180 (Fig. 1B) and 730 (Fig. 1C) days old showed a marked reduction in the rough endoplasmic reticulum and in the number of microvesicles. In contrast, in the DDFT of 180-day-old rats (Fig. 2B), the rough endoplasmic reticulum was still pro-nounced, whereas by 730 days this organelle had practically disappeared (Fig. 2C). Cuprolinic blue staining showed proteoglycans associated with the collagen fibrils of the two tendons in all age groups. There was

essentially no difference in the cuprolinic blue staining between the tendons CT and DDFT or age groups. The image was the same as that shown for the DDFT from rats 30 days old (Fig. 2D).

DISCUSSION

The physiological function of tendons within the musculoskeletal system is to transmit muscle-generated stress to bones to produce, in most cases, joint movement. The ability of tendons to resist stress, torsion, friction and compression is directly related to the structural organization of the extracellular matrix [41]. However, functional adaptation is not restricted to sites where different mechanical stresses occur since analogous regions of different tendons can show structural variations, depending on the mechanical requirements imposed on these tendons. The CT is a tendon with a laminar structure which originates from the triceps surae muscle and is inserted in the calcaneus. The DDFT, which possesses a cylindric structure, originates from the deep digital flexor muscle and is inserted into the plantar side of the fingers. In addition to anatomical considerations, comparison of the postnatal organization of the extracellular matrix of these tendons also should take into account the physical activity of the animals. In the present study, the rats were not submitted to any additional physical exercise and were therefore considered sedentary.

Ultrastructural analysis of the tensile regions of the CT and DDFT revealed differences in the cellular phenotype between young and old rats. These differences were related to the mechanical adaptation to which these two tendons were exposed during aging. Tendon fibroblasts are characterized by the presence of a well-developed rough endoplasmic reticulum and Golgi complex, free ribosomes and a large number of vesicles [12]. The rough endoplasmic reticulum and the Golgi complex are particularly prominent at the beginning of development, when these cells produce a large number of collagen fibrils, but become less evident with age [7,25]. The tendon cells examined here showed characteristics similar to those described for rabbits [22], in which elongated cells and cytoplasmic processes in intimate contact with collagen fibrils were observed.

Our data demonstrated a large amount of rough endoplasmic reticulum in the DDFT of 30- and 180-day-old rats, while in the CT an abundance of this organelle was observed only in rats up to 30 days old. This difference could reflect biomechanical particularities of these two tendons. Both tendons transmit tensile stress, but the DDFT apparently experiences stresses of a more variable nature due to movement of the fingers, whereas the CT has a more static behavior and experiences more continuous tensile stress. In chondrocytes, continuous mechanical stress leads to a marked decrease in the synthesis of matrix components when compared to the application of intermittent stress [19]. In tendons, a smaller variation in the presence of mechanical stress may lead to lower cellular activity in a given tendon compared to the activity in a tendon with more work. This variation is reflected in an increased or decreased content of cell organelles such as the rough endoplasmic reticulum and Golgi complex.

The tendon cell population undergoes changes at the molecular level, and there is a decrease in the cell density per unit of tendon surface [24]. The tenocytes become longer and more slender, and the nucleus/cytoplasm ratio of these cells increases, with most part of the cell volume being occupied by the nucleus. The chromatin becomes increasingly condensed and protein synthesis is reduced [4,16,24]. In addition, aerobic metabolic routes involved in the production of ATP also show a marked reduction with age [4].

Confocal microscopy of adult rat tendons has emphasized the complexity of tenocyte shape [21]. Tendon cells have numerous processes that extend laterally to create an elaborate system of segmented tunnels along which collagen fiber bundles run. The presence of numerous cell-collagen fiber and cell-cell contacts through gap junctions leads to the formation of a communication network within tendons [3]. The cell-matrix interaction involves a physical connection between the extracellular matrix and the cell surface and elements of the cytoskeleton in such a way as to permit the transmission of mechanical stress between the matrix and the cell, as well as to provide sites for the localized activity of signaling molecules [1].

The morphological aspects of the CT and DDFT in aged rats showed characteristics that were probaly associated with a reduction in metabolism. The synthesis and deposition of collagen molecules [10,18], as well as collagen remodeling [26], are more intense in young rats. With aging, collagen molecules become more compacted and the fibers become more crystalline [2,35], while at the same time there is a decrease in the proteoglycan and water content [4,16,33,37].

In tendons, the association between proteoglycans and collagen bundles occurs in an ordered fashion, to promote and maintain tissue integrity [30]. Although, an association between proteoglycans and collagen fibers was also observed here, no difference was detected among the different age groups.

Reduction in the amount of rough endoplasmic reticulum and in the number of vesicles in the CT and DDFT during the aging may reflect a cumulative effect of age and biomechanical properties which differ between these two tendons. Tissue modifications and interactions between the different extracellular matrix components serve to explain the morphological, biochemical and biomechanical characteristics of tendons [38,40]. The biological and mechanophysiological properties of the fibrous matrix of tendons also depend on the degree of aggregation and molecular order of the components involved [39]. Thus, tenocytes can detect alterations in the load to which the fiber surface is submitted. Any alteration in the ordered association of extracellular matrix components in response to mechanical stress exposes the cell to a load imbalance [40], and activates the electrochemical mechanisms involved in monitoring the matrix [11].

In conclusion, although no major ultrastructural differences were seen in the tensional region of the CT and DDFT in aging rats, there was a marked decrease in the amount of rough endoplasmic reticulum and secretory vesicles in old animals.

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