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DIFFERENTIAL SCANNING CALORIMETRIC AND HISTOLOGICAL EXAMINATIONS OF THE LONG HEAD OF THE BICEPS IN CADAVERS

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Shoulder pain is a common presentation of the dysfunction of the glenohumeral joint. The long head of the biceps tendon has been proposed as a source of pain in rotator cuff pathologies. The rotator cuff is a dynamic stabilizer of the glenohumeral joint, and its tear can create different shoulder dysfunctions. The long head of the biceps has a special intraarticular localisation, so the arthricular destruction affects its tendon too. In the process of the rotator cuff degeneration and tear the structure of the biceps tendon pathological transforms. With foregoing studies authors have demonstrated the feasibility of DSC in the investigation of the musculoskeletal system.

The aim of this study was to establish the curves and the histological properties of the tendon of the long head of the biceps in different magnitudes of the rotator cuff tear on cadavers. The DSC results clearly proved that definitive differences are present between the structural state of the tendons in different magnitudes of the rotator cuff tears, which have also been demonstrated by the histological examination.

Keywords: *biceps tendon, DSC, histology, rotator cuff*

Introduction

The long head of the biceps tendon has been suspected as a source of clinically significant pathology and symptoms [1]. The biceps tendon is often involved in degenerative pathology in shoulder disorders. Lesions of the long head of the biceps tendon (LHB) are generally a component of a diffuse degenerative process involving the subacromial space including the rotator cuff, bursa, biceps tendon, and possibly the acromioclavicular joint [2, 3]. Murthi *et al.* found a significant relationship between the glenohumeral arthritis and pathologic changes of the LHB [3]. The extreme stress of the long head of the biceps tendon is defined by its specific intra-articular anatomic course [4].

The function of the biceps brachii at the elbow has been extensively studied, and it is well accepted that this muscle is a supinator of the forearm and a flexor of the elbow joint [5], while it is also known to be a flexor of the shoulder joint [6]. Hitchcock and Bechtol [7] have pointed out, that the LHB itself does not slide; rather, the humeral head moves on the fixed tendon during motion at the shoulder joint, an action well known to represent the bicipital glinding mechanism. The tendon of the long head of the biceps brachii is a stabilizer of the humeral head in the glenoid, especially in the abduction of the shoulder, as well as the anterior joint of the capsule itself. Moreover it is able to compensate for inadequate rotator cuff function, which results in extreme stress of the tendon [8]. The recurrent microtrauma resulting from continuous mechanical loading in the critical zones leads to degenerative changes in the tendon structure, it means in the tendons tissue [9].

Tendons tissue histological type is connective tissue. Connective tissue mechanical behavior is primarily determined by the composition and organisation of collagens. In tendons, type I collagen is the principial structural element of the extracellular matrix, which acts to transmit force between bones or bone and muscle, respectively [10]. Other elements of the matrix are the glycoaminoglycans and proteoglycans. Its function is to separate and lubricate collagen bundles as they move relative to each other during the motions. Arnesen *et al.* [11] found in tendons the increase in collagen cross linking and in total amount of collagen during the ageing. It leads to a decline in both its flexibility and its ability to heal after an injury [11]. The authors described the decline of the fibroblast function too during the ageing. It leads to increase of many age underlying pathologies of the musculoskeletal system [11], so this fact can explain the higher prevalence of the shoulder pathologies in aged.

Degeneration alters the morphology and the mechanical properties of the tendon and it leads to its gradual destruction and finally it leads to tendon rupture [12]. Degenerative changes of the biceps tendon

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occurred in the distal bicipital groove and near to the origin of the tendon from the superior part of the glenoid labrum [12]. Biceps tendon tear is usually due to impingement of the biceps and supraspinatus tendon in the area of the biceps sulcus. Biceps tendinopathy may be a result of an ongoing subacromial impingement syndrome and rotator cuff disease [13].

Morphologic changes in the long head of the biceps were described in association with rotator cuff diseases, yet mechanical significance of these changes remains unclear [14]. Sakurai *et al.* [4] analysed the morphologic changes in the LHB in rotator cuff dysfunction. They found in moderate cuff tears a relative stenosis of the bicipital groove induced by enlargement of the LHB. This relative stenosis can lead to the mechanical overuse and to pathologic structural conversion. Itoi *et al.* [15] revealed that LHB is widened in cuff deficient shoulders. Jarvinen *et al.* showed, that from overuse ruptured tendons had significant smaller diameter than normal tendons [16]. The mechanism of biceps laesion involvement in rotator cuff tear shoulders has not yet been fully elucidated, and we have found no previous studies about the thermal consequences of the structural changes in the biceps tendons.

Differential scanning calorimetric (DSC) examination allows to demonstrate the thermal consequences of local as well as global conformational changes in tissue elements. This technique has already proved to be applicable in the research of medical problems: pathology of human cartilage and vertebrate discs [17–20], abnormalities of human leg skeletal muscles [21] and dog trachea [22]. Present authors have already used DSC in the research of shoulder pathology [23].

The purpose of this study was to establish the thermal characteristic of the long head of the biceps tendon in different states of the shoulder, and to compare with its histological properties. After dissection and macroscopic observation of the state of the rotator cuff and the biceps tendon, the long head of the biceps tendon was removed for the analysis with differential scanning calorimetry and with histology. The DSC results clearly proved that definitive differences are present between the thermal caracteristic of the tendons with normal and torn rotator cuff, which have also been demonstrated by the histology.

Experimental

Materials and methods

Sample preparation

All samples were obtained during autopsy within 24 h postmortem, with standard methods. We dissected the shoulder and the rotator cuff carefully, the rotator cuff laesions were evaluated, and the intraarticular portion of the LHB near to the bicipital groove was removed to the histology and the DSC examination. We dissected 32 cadaveric shoulders to analyse their biceps tendons. This 32 tendons were divided in 4 groups. By group A and B (16 cases) we found no sign of rotator cuff pathology. 6 of them were from foetal cadavers (Group A) and 10 were from adults (Group B, age range 34–56 years, mean age: 37 years). In 8 cases we found small rotator cuff tears, it means the tear was smaller than 50 mm (Group C, age range 52–79, mean age: 59 years). In 8 cases we found massive cuff tears, it means they were larger than 5 cm (Group D, age range 61–93 years, mean age: 81 years).

DSC measurements

For the DSC examination the samples were tendon stripes with cc. 5×5×10 mm, and washed three times in PBS (sterile phosphate-buffer saline, pH 7.4) in order to eliminate tissue remnants. Samples were than put into RPMI-1640 solutions (SIGMA) containing 10% foetal bovine serum (HYCLONE Laboratories), antibiotic solution (1 U mL^{-1} penicillin, streptomycin, gentamycin and fungisone, GIBCO Laboratories), nonessential amino acids (GIBCO) and sodium carbonate. All the individual samples were stored separately at 4°C, no longer than 24 h, before they were subjected to calorimetric measurements.

The samples were monitored by a Setaram Micro DSC-II calorimeter. All experiments were conducted between 0 and 100°C. The heating rate was 0.3 K min^{-1} in all cases. Conventional Hastelloy batch vessels were used during the denaturation experiments with 850 μL sample volume (tendons plus buffer) in average. Typical tendon wet weights for calorimetric experiments varied between 200–250 mg (in case of foetal samples between 70–100 mg). RPMI-1640 solution was used as a reference sample. The sample and reference vessels were equilibrated with a precision of ±0.1 mg. There was no need to do any correction from the point of view of heat capacity between sample and reference vessels. The scan of RPMI-1640 solution was used as baseline reference, which was subtracted from the original DSC curve. Calorimetric enthalpy (ΔH) was calculated from the area under the heat absorption curve by using two-point setting Setaram peak integration.

Histology

For the histological examination the specimens were fixed in 4% buffered formalin for a week. After fixation serial cross sections were cut and embedded in paraffine, and cut to a thickness of 2 μm. The sections

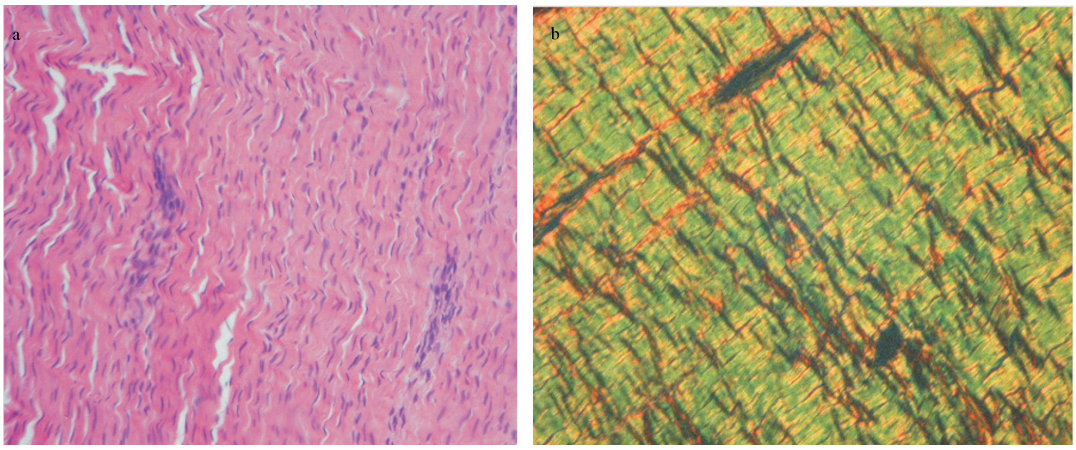

Fig. 1 Histological sections from the LHB of a foetal cadaver (Group A). Healthy cellular tendon tissue. a – Hematoxylin-eosin stained, b – picrosyrius-red stained

were stained with hematoxylin-eosin (HE) and picrosyrius-red. HE staining was done according routin histological protocols, picrosyrius-red staining is useful for demonstration of collagen fibers [24]. The histological analysis was performed with Nikon Eclipse E400 light microscope (usual magnification 100×) to examine the changes of the tendon structure. Our activities were done under the proper law paragraphs and valid permission.

Results and discussion

In group A (foetal sample as intact reference) we found macroscopically no sign of rotator cuff tear. The articular surfaces are intact. The surfaces of the biceps tendons are smooth, the cross sectional areas are oval. Microscopically on the HE stained sections (Fig. 1) we can see a healthy cellular fibrous tissue. The tendinocytes squeezzed between the fibers. Tendinocytes are elongated narrow, inactive cells. We can see on this picture tendinoblasts in a great number. Tendinoblasts are the active form of tendinocytes. They are larger basophilic cells with 3–4 processes and with looser chromatin in the nucleus. Tendinoblasts can proliferate and secrete collagen fibers and any other matrix element. So the large amount of the tendinoblasts indicate a great production of new collagen bundles. On the picrosyrius-red stained section we can observe the structure of the collagen bundles. We can see the parallel orientation of the bundles. This bundles are thicker (high water content of the foetal tissue), but regularly arranged.

The DSC scan (Fig. 2) shows a tipical collagen denaturation curve with a T_m~57±0.3°C and ~1.5±0.1°C half-width of melting temperature. The calorimetric enthalpy change (ΔH) was 0.26±0.02 J g^{-1} (mean±s.d., n=6). It was normalised for the total sample mass, because the precise mass of

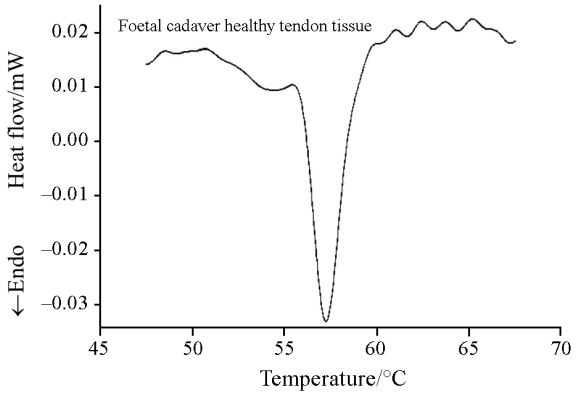

Fig. 2 Thermal denaturation of a foetal cadaver LHB (Group A)

components is unknown, and we were not able to resolve different thermal domains.

In group B we found normal anatomy in the dissection of the shoulder (Fig. 3). The rotator cuffs and the articular cartillages were intact. The biceps tendons were macroscopically normal too, with smooth surface and oval cross section. On the HE stained section we can see the basophilic nuclei of the tendinocytes, and homogen eosinophil collagen bundles. On the picrosyrius-red stained section we can observe the paralellly oriented, densely packed collagen bundles. There is no sign of irregularity or rupture. The thermal denaturation resulted in a well cooperative endotherm with T_m~66±0.2°C and ~1.2±0.1°C half-width of melting temperature (ΔH= 1.8±0.06 J g^{-1}, n=10), which is the sign of a densely packed structure with greater heat capacity (Fig. 4b).

In group C we found dissections of the shoulder tears of the rotator cuffs (Fig. 5). The size of the tear varied between 18–38 mm (mean size: 26 mm). The supraspinatus tendons were touched in these cases. On the surface of the articular cartilage we found same mild erosions. The biceps tendons were

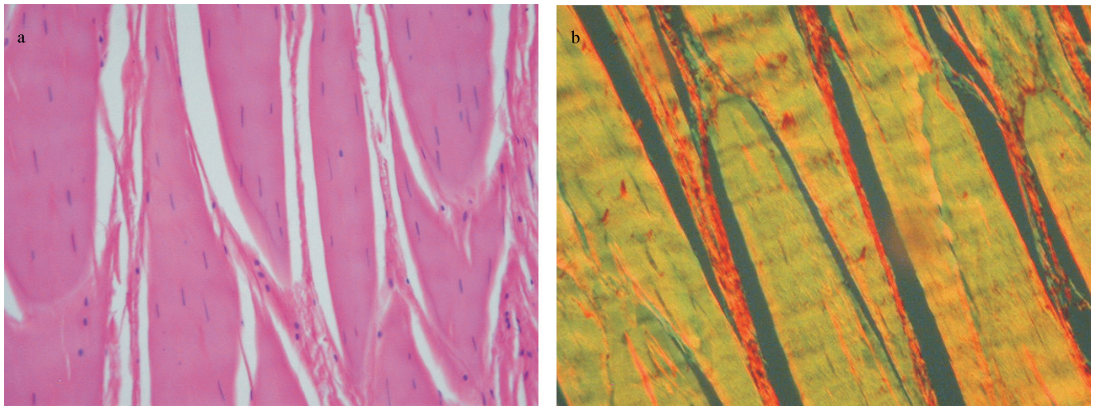

Fig. 3 Histological sections from the LHB of an adult cadaver without rotator cuff pathologies (Group B). Normal tendon structure. a – Hematoxylin-eosin stained, b – picrosyrius-red stained

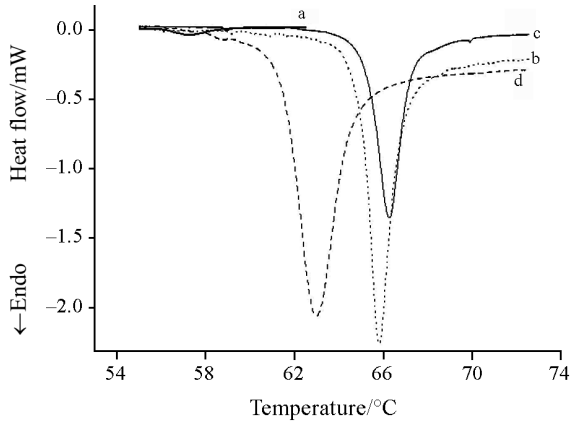

Fig. 4 DSC scans of LHB from different (A–D) stages of rotator cuff (see more in text)

softly widened and flattened, on the surfaces there were mild scabrous erosions. On the HE stained section we can see the tendinocytes and some tendinoblasts. It can indicate the reparation of the damaged collagen structure. On the picrosyrius-red stained section we can observe the moderate irregularity of the collagen fibres. In some areas we can see there is early separation and minimal disruption of the fibres. The thermal denaturation resulted in a less cooperative endotherm a case of C with T_m~66.5±0.2°C and ~1.2±0.05°C half-width of melting (ΔH= 1.2±0.05 J g^{-1}, n=8), which is the sign of a destructed structure compared with B (Fig. 4c).

In group D we found in the dissections of the shoulders massive rotator cuff tear (Fig. 6). The size of the tear varied between 53–76 mm (mean size: 67 mm). The supraspinatus and the infraspinatus tendons were touched in different magnitude. The articular surfaces were severe erodated. The biceps tendons were progressively damaged, flattened and widened, and there were hard scabrous erosions on its surface. On the HE stained section we can see the desorganised collagen fibres and large mucoid deposits. The tissue is hypocellular with more collagen than by the others. On the picrosyrius-red stained section we can better observe the desorganised collagen bundles with decreased collagen fibre thickness. On this picture we can see severe damage of the tendon with multiple disruption, separation and staining irregularity and staining variation of fibers. The thermal results are the mirror of histologic pictures (Fig. 4d). We have got a less cooperative denaturation (half-width of melting is 2±0.04°C) with smaller T_m

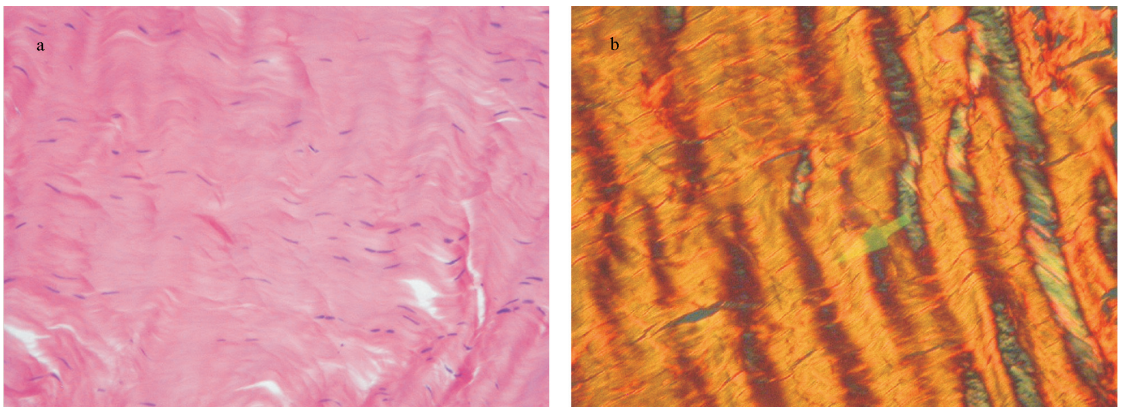

Fig. 5 Histological sections from the LHB of an adult cadaver with rotator cuff tear (<50 mm, Group C). Moderate degenerated tendon structure. a – Hematoxylin-eosin stained, b – picrosyrius-red stained

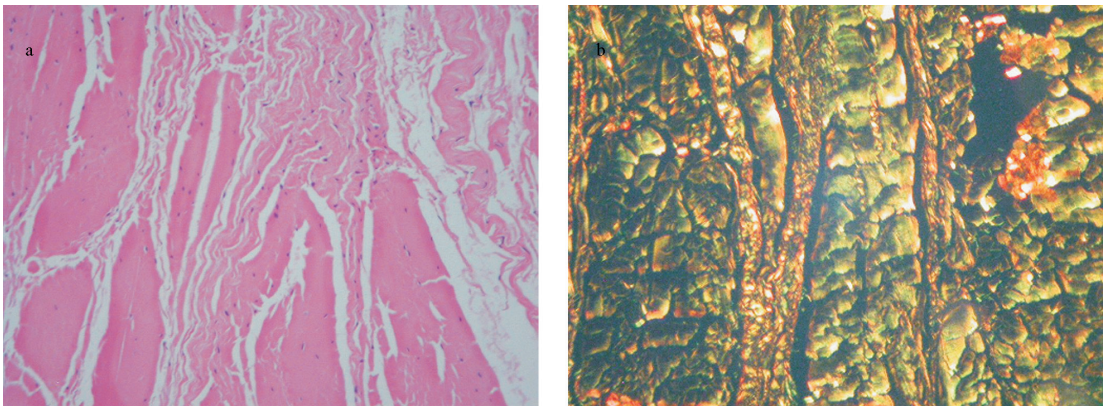

Fig. 6 Histological sections from the LHB of an adult cadaver with massive rotator cuff tear (>50 mm, Group D). Severe degenerated tendon structure. a – Hematoxylin-eosin stained, b – picrosyrius-red stained

(63±0.3°C). The significant increase of the calorimetric enthalpy (ΔH= 2.2±0.07 J g^{-1}, n=8) correlate well with the increased amount of collagen, and with the incresed amount of secondary bindings between the collagen fibres.

Recent biomechanical and clinical studies [15, 25–28] have clarified the role in stabilizing the humeral head in the glenoid during abduction of the shoulder. Bicipital laesions are an important cause of shoulder pain [1]. Codman [29] stated that supraspinatus lesions were the primary defect uncovering the biceps tendon, allowing it to slip at the top of the bicipital groove and become caught between the tuberosity and the acromion. DePalma and Callery [30], on the other hand, reported that bicipital tenosinovitis was the most common reason of stiff and painful shoulder. Indeed, many authors believe that bicipital tendinitis occurs with rotator cuff tears, most suggesting [31, 32] it to be secondary to a primary impingement syndrome. One of the present authors [33] found, in a cadaveric study, that flattening and degeneration of the LHB occurred in shoulders with incomplete cuff tears and that these changes progressed in association with the extent of the cuff tear. Sakurai *et al.* [4] found the decrease of the volume of the LHB by full thickness cuff tears; which appear to be due to tendon degeneration. It is well known that the LHB is often flattened and widened in shoulders with rotator cuff tear [34].

On the basis of these data, we can say that the structure of the LHB pathologically transforms because of the cuff tear. DSC examination is a well-established method for the demonstration of thermal consequences of local and global conformational changes in biological systems. We investigated histological changes and thermal consequences in the LHB in shoulders of cadavers with and without rotator cuff tear. We analysed the thermal consequences of the structural changes of the LHB occurred by the cuff tear. To our knowledge, there have been no such studies reported in the literature. With foregoing studies authors have demonstrated the feasibility of DSC in the investigation of the shoulder pathology [28]. The DSC results clearly proved that definitive differences are present between the tendons with normal and torn rotator cuff, which have also been demonstrated by the histology.

In group A, by the foetal LHB the histology shows a normal cellular tendon tissue. Foetal tissues have higher water content than the others from the adults, which is demonstrated in the calorimetric curve too (Fig. 1). The differences between the foetal and the normal adult (group B) tendons in the total calorimetric enthalpy are presumably due to the different ratio of collagens and proteoglycans and their structure. The LHB from the adults (group B) have morphologically more compact collagen bundles than from the foetus, and in order to decompose its more compact structure, significantly more energy is needed; thus, it results in significantly higher enthalpy changes (p<0.05; Table 1). As a result of the degenerative process, characteristic changes can be observed in the structure, composition and function of the LHB especially in group C and D (Fig. 4). Repeating mechanical strain results the degradation of the original structure of collagens and proteoglycans. As a consequence, the LHB starts to disintegrate and several different morphological changes develop. Due to this changes the LHB lose the ability to transmit forces between bone and muscle physiologically. Presumably, due to mechanical overload and during the ageing secondary bindings (intra- and intermolecular hydrogen bridges) develop in the disintegrated structure [11]. Thus, the entire structure becomes more tightly 'packed'. For the disintegration of this compact structure, extra energy was necessary, thus the structural phase transformation began at a higher temperature (Table 1). These changes reduce the ability of the originally flexible tendon to bend in the process of the physiological shoulder movements,

Table 1 Calorimetric results of the tendon of the long head of the biceps. Symbols: T_m stands for the main transition temperature (or melting temperature) in °C, ΔH is the calorimetric enthalpy change in J g^{-1}

Group	No. of samples	Avr. age/year	Thermal parameters
A	6	0	T_m=57±0.3 ΔH=0.26±0.02
B	10	37	T_m=66±0.2 ΔH=1.8±0.06
C	8	59	T_m=66.5±0.2 ΔH=1.2±0.05
D	8	81	T_m=63±0.3 ΔH=2.2±0.07

and this brittle tendon is less able to supply its function. The decrease of the enthalpy of this structure along the degeneration is attributed to the loss of thermal cooperation of the components (Table 1). It was also supported by the thermal transition period and the asymmetry of the curves themselves. The drop of the main transient temperature in the degenerated tendons is mostly due to the loss of the immensely hydrated proteoglycans (Table 1).

Conclusions

With our study, we would demonstrate that DSC is an applicable method for the demonstration of thermal consequences of local as well as global conformational changes in the human LHB too, similarly in case of other medical problems [35–37]. The DSC scans of the LHB from different stages of the degeneration are very characteristic for the actual functional and structural state. It comes from the inherent nature of this method that we can not assign any thermal event to any molecular process directly, but our results suggest that definitive differences exist between the stages of the LHB degeneration in calorimetric measurements going either on local or global level. By the application of additional biochemical and histological methods, components of LHB responsible for the demonstrated findings could be identified in the future, which could be a molecular background of our thermal and histological data. Thus, revealing further details of the pathomechanism of the degeneration procedures can help in the surgical or conservative medical treatment of the shoulder diseases.

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References

1 K. S. Paynter, Phys. Med. Rehabil. Clin. N. Am., 15 (2004) 511.
2 R. F. Warren, Instr. Course Lect., 34 (1985) 204.
3 A. M. Murthi, C. L. Vosburgh and T. J. Neviaser, J. Shoulder Elbow Surg., 9 (2000) 382.
4 G. Sakurai, J. Ozaki, Y. Tomita, Y. Nakagawa, T. Kondo and S.Tamai, J. Orthop. Sci., 3 (1998) 137.
5 J. V. Basmajian and A. Latif, J. Bone Joint Surg. Am., 39-A (1957) 1106.
6 J. Furlani, Acta Anat. (Basel), 96 (1976) 270.
7 H. H. Hitchcock and C. O. Bechtol, J. Bone Joint Surg. Am., 30 (1948) 263.
8 J. Strunk, U. Lange, B. Kurten, K. L. Schmidt and G. Neeck, Arthritis Rheum., 48 (2003) 1828.
9 H. Resch and H. Breitfuss, Etiol. Pathogen. Ther. Orth., 24 (1995) 209.
10 P. P. Provenzano and R. Vanderby Jr., Matrix Biol., 25 (2006) 71.
11 S. M. Arnesen and M. A. Lawson, Mech. Ageing Dev., 2006. Jun 14; [Epub ahead of print].
12 H. J. Refior and D. Sowa, J. Shoulder Elbow Surg., 4 (1995) 436.
13 N. Sethi, R. Wright and K. Yamaguchi, J. Shoulder Elbow Surg., 8 (1999) 644.
14 J. E. Carpenter, J. D. Wening, A. G. Mell, J. E. Langenderfer, J. E. Kuhn and R. E. Hughes, Clin. Biomech. (Bristol, Avon), 20 (2005) 162.
15 E. Itoi, N. E. Motzkin, B. F. Morrey and K. N. An, J. Shoulder Elbow Surg., 3 (1994) 135.
16 T. A. Jarvinen, T. L. Jarvinen, P. Kannus, L. Jozsa and M. Jarvinen, J. Orthop. Res., 22 (2004) 1303.
17 P. Than, Cs. Vermes, B. Schäffer and D. Lőrinczy, Thermochim. Acta, 346 (2000) 147.
18 P. Than and D. Lőrinczy, Thermochim. Acta, 404 (2003) 149.
19 I. Domán, Gy. Tóth, T. Illés and D. Lőrinczy, Thermochim. Acta, 402 (2003) 117.
20 I. Domán and T. Illés, J. Biochem. Biophys. Methods, 61 (2004) 207.
21 I. Gazsó, J. Kránicz and Á. Bellyei, Thermochim. Acta, 402 (2003) 117.
22 Z. Szántó, L. Benkő, B. Gasz, G. Jancsó, E. Rőth and D. Lőrinczy, Thermochim. Acta, 417 (2004) 171.
23 I. Szabó, G. Bognár, I. Magda, R. Garamvölgyi, G. Czóbel, L. Nőth, L. Kereskai and P. Bogner, Thermochim. Acta, 445 (2006) 179.
24 F. Sweat, H. Puchtler and S. I. Rosenthal, Arch. Path. (Chicago), 78 (1964) 69.
25 R. Glousman, F. Jobe, J. Tibone, D. Moynes, D. Antonelli and J. Perry, J. Bone Joint Surg. Am., 70 (1988) 220.
26 V. P. Kumar, K. Satku and P. Balasubramaniam, Clin. Orthop. Res., 244 (1989) 172.
27 M. W. Rodosky, C. D. Harner and F. H. Fu, Am. J. Sports Med., 22 (1994) 121.
28 J. J. Warner and P. J. McMahon, J. Bone Joint Surg. Am., 77 (1995) 366.
29 E. A. Codman, The shoulder, Malabar: Robert E. Krieger, (1984) 241-3.
30 A. F. DePalma and G. E. Callery, Clin. Orthop., 3 (1954) 69.

31 T. J. Neviaser, Orthop. Clin. North Am., 18 (1987) 383.
32 P. Paavolainen, P. Slatis and K. Aalto, Surgery of the shoulder, J. E. Bateman and R. P. Welsh, Eds, Decker Philadelphia: BC 1984, pp. 313–318.
33 Y. Nakagawa, Nippon Seikeigeka Gakkai Zasshi, 63 (1989) 75.
34 W. Z. Burkhead, M. A. Arcand, C. Zeman, P. Habermeyer and G. Walch, C. Rockwood Jr. and Fa III Matsen, Eds, 2nd Ed., The Shoulder, Saunders, Philadelphia, PA 1998, p. 1032.
35 T. Sillinger, P. Than, B. Kocsis and D. Lőrinczy, J. Therm. Anal. Cal., 82 (2005) 221.
36 L. Benkő, J. Danis, M. Czompo, R. Hubmann, A. Ferencz, G. Jancsó, Z. Szántó, A. Zólyomi, F. Könczöl, Á. Bellyei, E. Rőth and D. Lőrinczy, J. Therm. Anal. Cal., 83 (2006) 715.
37 Zs. Biró, V. Nemes, G. A. Molnár, I. Wittmann, A. Ludány and D. Lőrinczy, J. Therm. Anal. Cal., 85 (2006) 261.

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