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 has a special intraarticular localisation, so the arthicular 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

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].

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 rotator cuff 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.

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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...
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 lesion 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 vertebral 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 characteristic 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 lesions 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