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Changes in thermal denaturation properties of the long head of the biceps during lifetime

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Abstract Long head of the biceps (LHB) is an intra-articular tendon component of the shoulder joint. The function of this tendon is complex. First, it is an origin of flexion in upper limb, and second it plays role in joint stabilisation during shoulder movements. Histological type of tendon tissues is connective tissue. The mechanical behaviour of connective tissue is primarily determined by the composition and organisation of collagens. In tendons, type I collagen is the principal structural element of the extracellular matrix, which acts to transmit force between bones or bone and muscle. Owing to the special localisation of this tendon, the intra-articular mechanical forces affect it to a considerable extent. The LHB is known as a source of pain in pathologic states of the shoulder joint. The goal of this study was to establish the calorimetric standards of the LHB in different ages, and to observe the changes of thermal properties of collagen during lifetime. LHB samples were taken from 38 cadavers (between ages 0 and 90 years) without macroscopic sign of shoulder pathology. DSC analyses were performed with SETARAM Micro DSC-II. The thermal denaturation parameters varied between T_m (°C): 57, ΔH (J/g): 0.26 (age: 0 year) and T_m (°C): 62.92, ΔH (J/g): 1.28 (age: 90 years). The ageing of collagenous tendon tissue can be clearly followed in changes of thermal denaturation properties. The knowledge of the ageing of normal collagen provides a good basis to analyse further the LHB pathology.

Keywords Long head of the biceps · Differential scanning calorimetry · Ageing · Collagen

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Introduction

The long head of the biceps (LHB) tendon is often involved in degenerative pathology in shoulder disorders. It has been suspected as a source of clinical symptoms [1]. Degenerative changes in the tendon occurred mainly in the distal bicipital groove and near to the origin of the tendon from the superior part of the glenoid labrum [2]. Age-related or degenerative changes seem to predispose lesions that are important source of shoulder pain. These could be detected mostly after middle age.

Histological type of tendons tissue is connective tissue. Connective tissue's mechanical behaviour is primarily determined by the composition and organisation of collagens. In tendons, type I collagen is the principal structural element of the extracellular matrix, which acts to transmit force between bones or bone and muscle [3]. The covalent cross-linking of collagen molecules in connective tissues, such as in tendon is essential for proper tissue function.

Increased cross-link concentration and a change in the quality and type of the cross-linking and tissue hydration are observed with maturation, and a further increase occurs with ageing of mature tissue by non-enzymatic glycation cross-linking [4]. Arnesen et al. 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 [5].

Differential scanning calorimetric (DSC) examination is a well-known method for the demonstration of thermal consequences of collagen ageing [6].

The purpose of this cadaveric study was to establish the changes in thermal characteristic of the LHB tendon during ageing.

Materials and methods

Sample preparation

All the samples were obtained during autopsy within 24 h post-mortem, with standard methods. We dissected the shoulder and the rotator cuff carefully, the rotator cuff lesions were evaluated, and the intra-articular portion of the LHB near to the bicipital groove was removed for the DSC examination. The cadavers with macroscopic signs of rotator cuff pathology, or systemic metabolic disease such as diabetes as well as anamnesis of systemic corticosteroid medication were excluded from this study. Samples from 41 dissected cadavers fulfilled these requirements (age: 0–90 years). Our activities were done under the proper law paragraphs and valid permission.

DSC measurements

For the DSC examination, the samples were washed three times in PBS (sterile phosphate-buffer saline, pH 7.4) to eliminate tissue remnants. Samples were then put into RPMI-1640 solutions (SIGMA) containing 10% foetal bovine serum (HYCLONE Laboratories), antibiotic solution (1 U/mL 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 48 h, before they were subjected to calorimetric measurements. The samples were monitored by a SETARAM Micro DSC-II calorimeter. All the experiments were conducted between 0 and 100 °C. The heating rate was 0.3 K/min in all the cases. Conventional Hastelloy batch vessels were used during the denaturation experiments with 850-µL sample volume (tendons plus buffer) in average. Typical tendon wet masses for calorimetric experiments varied between 200 and 250 mg (in the case of foetal samples between 70 and 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 was calculated from the area under the heat absorption curve by using two-point setting SETARAM peak integration.

Results

All of the dissected samples showed a typical one peak denaturation curve of collagen connective tissues depending on whether they were prepared from healthy or degenerated samples (Figs. 1, 2). The melting points varied from 56.43 to 66.41 °C. Figure 3 shows the changes of the melting temperatures (T_m) in function of the age (years). We could fit on the T_m–years data a sigmoid curve. The melting temperatures rise boldly from 56.43 to 63.43 °C (No. of samples: 8) in the age interval of 0–35 years. From 35 to 90 years, an increasing spread is characteristic for the main transition temperatures (No. of samples: 33). Figure 4 represents the calorimetric enthalpy changes (J/g) during the denaturation as the function of the age. The enthalpies show high spreading. A polynomial of fourth degree could be fit to the measured values, with a peak at 51 years, with calorimetric enthalpy of 6 J/g (normalised to the wet mass). Taking into consideration the water and dry material content of the tendon samples, this value is in a good coincidence with calorimetric enthalpies measured in case of dried collagens. Our calorimetric enthalpy, at first look, seems to be very

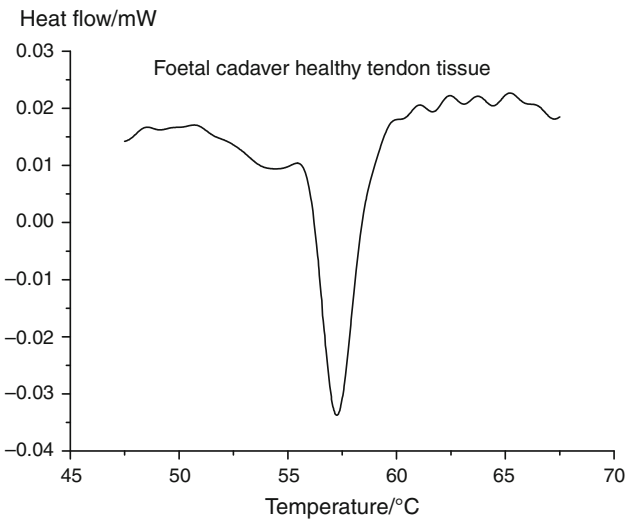

Fig. 1 Typical denaturation curve of collagen (dissected from a foetal cadaver)

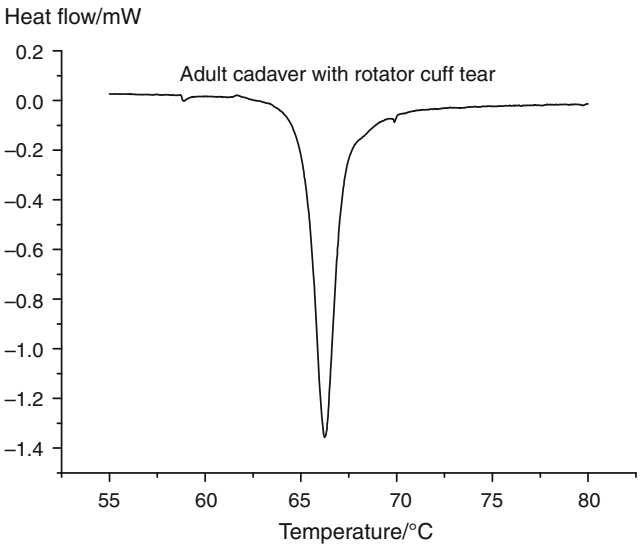

Fig. 2 Denaturation scan of collagen, dissected from degenerated sample

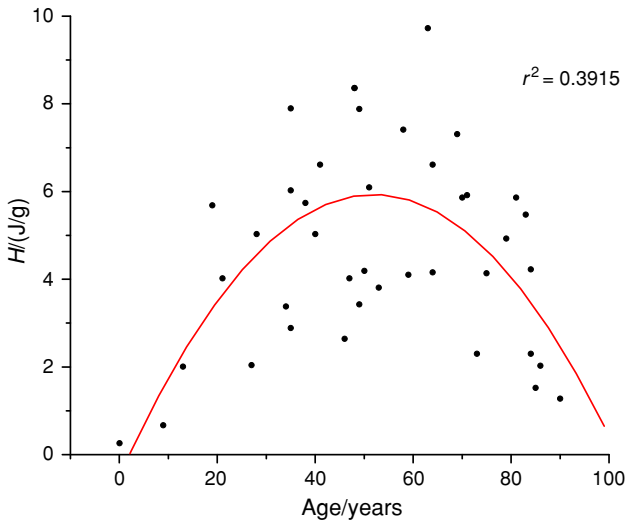

Fig. 4 ΔH (J/g) calorimetric enthalpy change (normalised on the wet sample mass) in gear to the age (years)

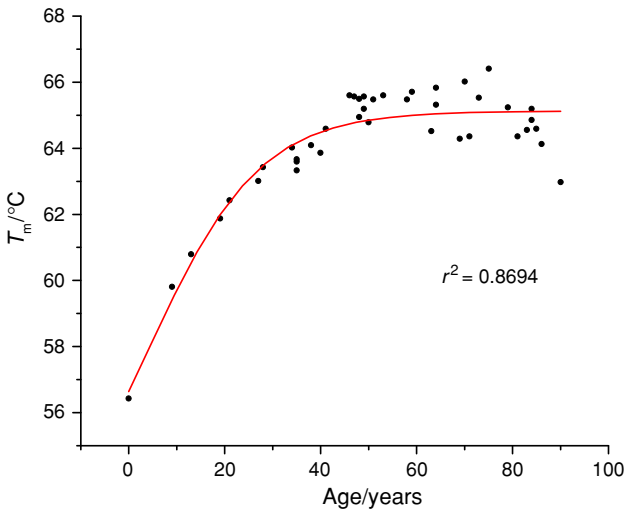

Fig. 3 T_m (°C) main transition temperatures (or melting temperature) as the function of the age (years)

small in case of control tendons compared with the recent data of literature [7]. This could be the consequence of the different heating rates at first (in our experiments it is 0.3 K/min, while in [7] it is 5 K/min), and secondly, we have normalised on the wet sample mass. If one takes into consideration that the intact Achilles tendon contains about 60% water and the collagen content is only 80% of dry mass [8], our results lay in the range of the published data.

Discussion

There is a powerful evidence to show that shoulder disorders occur with age [9]. DePalma showed that the frequency of shoulder pathology increased with age [10]. Ozaki et al. showed a direct correlation between age and incidence of shoulder disorder; 38% of disorders in case of cadavers could be observed in the sixth decade, and it is rising to 80% in the ninth and 10th decades [11]. Sher and Uribe found a rising prevalence of shoulder disorder with ageing in the asymptomatic living population using MRI scanning [12]. Bunker et al. showed that many of pathologic states are asymptomatic, and it is still not clear why some are painful and some are not [13]. Chard et al. showed that degenerative changes are commonly found in many cadavers with no prehistory of shoulder pain [14]. 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 [2].

Arnesen et al. found in tendons the increase in collagen cross-linking and in total amount of collagen during the ageing [5]. The covalent cross-linking of collagen molecules in connective tissues, such as tendon, is essential for proper tissue function. The intermolecular and intramolecular cross-links make the fibre suitable to transmit force between bones or bone and muscle, respectively [3]. Increased cross-link concentration and a change in the quality and type of the cross-linking and tissue hydration are observed with maturation, and a further increase occurs with ageing of mature tissue due to non-enzymatic glycation cross-linking [4]. The accumulation of increased concentration of mature cross-links with age is associated with a marked increase of thermal stability [15]. The experiment of Horgan et al. has demonstrated that increased temperature stability of collagen in cross-linked fibres is determined mainly by the intrafibrillar water content. Cross-linking causes dehydration of the fibres, and

it is the reduced hydration that caused the increased temperature stability [15]. The increase in denaturation temperature following cross-linking of collagen is caused by dehydration of the fibres [16]. The high enthalpy of unfolding of collagen is thought to derive mainly from the breaking of the hydrogen bonds forming the hydration network around the collagen molecule [17].

To the best knowledge of the authors, there is no published study about the thermal characteristic of LHB ageing. These authors' data demonstrate the effect of ageing on the thermal properties of LHB. Although LHB is often affected in pathological processes because of its special anatomical course, the ageing of intact LHB shows typical thermal property change of collagen ageing. From 0 to 35 years, we can see that the melting temperatures rise boldly from 56.43 to 63.43 °C. This may be a result of maturation and increasing concentration of mature collagen cross-links. From 35 to 90 years, an increasing spread in the melting temperatures is characteristic, which could be the structural consequence of the rising prevalence of shoulder disorders, which could be manifested as pain too (Fig. 3). The observed enormous spread in the calorimetric enthalpy (see Fig. 4) appears also above 35 years, so it could be the manifestation of the same effect which could be responsible for the 'saturation' and spread of melting temperatures. During the ageing of the collagen tissue, there is an appearance of Type III collagen, and its melting temperature and enthalpy differ from the normal collagen data. On the contrary, in the ageing of the connective tissue, there is a clear mucoid and lipoid degeneration (which could demonstrate with histological examination too), which could influence these thermal parameters.

The limitations of this study are that there are a low number of samples under the third decade (this is a consequence of the age distribution of cadavers), and the interpretation of thermal changes is not confirmed by biochemical analysis.

The established data of thermal properties of normal LHB collagen ageing serve a basis for a comparison and interpretation of the different pathological states.

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