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Mechanical behaviour and structural characterization of human fascia lata

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Abstract

Purpose: The fascia lata is a key structure determining the physiological functioning of knee and thigh muscles. At the same time it assures mechanical protection of the tissues situated beneath. This study aimed to clarify the comprehensive mechanical properties and microscopic structure of human fascia lata to understand its mechanical behaviour better. *Methods*: The pathologically unchanged fascia lata used in this study were collected *post mortem* from adult males of different age. The mechanical properties of the samples were determined under the uniaxial tensile test in two directions (longitudinal and transverse to collagen bundles arrangement). The structure observations were performed using a light and scanning electron microscope. *Results*: The mechanical tests have shown the anisotropic properties of the examined tissue. The values of the particular mechanical parameters were determined. The maximum failure force, stiffness coefficient, tensile strength, and Young's modulus values were higher for longitudinal than transversal samples. Before the uniaxial tensile test, the collagen bundles within each layer of examined samples were aligned parallel to each other, showing a regular, wavy architecture (crisped pattern). The elastin fibres within these layers were much less numerous than collagen fibres. The applied mechanical forces disrupted the intact, regular collagen pattern, making the majority of collagen bundles loosely and irregularly oriented. *Conclusions:* Knowledge of the mechanical and structural properties of the human fascia lata is an important issue in the context of clinical applications, tissue engineering as well as numerical modelling.

Keywords: *Human fascia lata, uniaxial tensile test, mechanical properties, collagen and elastin fibres, scanning electron microscopy*

Introduction

Despite crucial (mechanical and metabolic) functions it performs in the body, the fascia remains a tissue that is poorly known, especially when compared to other broadly understood musculoskeletal components such as bones, muscles, tendons or ligaments. In the past, the fascia was perceived as “junk tissue”, essentially useless, and research into it had been neglected for decades. This was, in part, caused by a lack of satisfactory research methods, and in part by a lack of understanding of the crucial role that the fascia plays in the body. It is only in the last decades that the development of research techniques (imaging and functional testing) of soft tissues, enabled studies to be carried out on different fascia behaviour [29]. This possibility has drawn scientists’ attention to the complexity of its structure and the functions it performs.

It is now known that fasciae are structures consisting of dense connective tissue, rich in the basic structural protein – collagen, ubiquitously present in the body. Fasciae form a three-dimensional mechanical and metabolic network ("having no beginning or end"), and this network extends in the planes of the body and forms a structural scaffolding surrounding muscles, bones, internal organs, nerves, vessels, and structures of the central nervous system.

In the body, the fascial tissue system has two main functions: mechanical and protective. These functions are mainly performed by deep fasciae, of which the fascia lata (FL), the subject of this study, is a typical representative. The protective function involves forming a "wrapping" for individual tissue structures (i.e. muscles, internal organs), maintaining their shape, and ensuring their appropriate spatial positioning in relation to each other. Fasciae separate tissues and organs while, simultaneously, ensuring communication between them. Protection against mechanical injury is ensured, in particular, by the deep fasciae, e.g. the fascia lata or the dura mater [32][36]. The mechanical function, on the other hand, involves loads transfer, coordination of motor units and connection of joints, and stabilisation, thus ensuring the correct posture and harmonious movement [18][32]. For this reason, the fascial system is sometimes referred to as the "soft skeleton". Less obvious functions of the fascia include interstitial signal transmission (due to the presence of cells called telocytes) and pain transmission (due to the presence of free nerve endings in the fascia [5][6][9][18][35]. In summary, the functions of the fascial system are summed up by the acronym "4Ps": passageway, posture, packaging, protection [17].

Given the important functions of the fascial system in the body, it is not surprising that pathological changes concerning the structure or function of the fascia result in a range of motor dysfunctions of the musculoskeletal system and, importantly, pain symptoms. Very often, this is the result of lack of physical activity, compressive lesions in the fascia, microtraumas, strains, or motor stereotypies (e.g. associated with the performed work). They lead to changes in the mechanical properties and architecture of fascial tissues, with formation of so-called densifications (an increase in the density of the intercellular matrix and impaired configuration of collagen fibres with a possible increase in their number) [23]. As a result, the fascia is no longer able to adapt to tensions transferred from the adjacent muscle fibres and its mobility is restricted, and this in turn can cause pain, limit muscle mobility and lead to compression of other tissues such as nerves, blood or lymphatic vessels. Therefore, diseases affecting fascial tissues may not only result in pain, postural disorders and motor dysfunctions, but may also disrupt functions of internal organs and the nervous system [18].

In clinical practice, the process of diagnosing and treating conditions related to fascial structures still poses many difficulties. A significant number of fascial dysfunctions are still considered idiopathic, with no causative treatment available [11]. This is primarily due to insufficient knowledge of the aetiopathogenesis of individual fascial syndromes and disorders and this, in turn, results from an insufficient understanding of mechanical, biochemical, structural and ultrastructural properties of the fascia. Knowledge of factors leading to fascial dysfunctions can contribute to development of new therapeutic and diagnostic methods, and the fascial research will result in the ability to treat pain and dysfunction associated with fascial disorders. The most common conditions involving fascial tissues include: myofascial pain syndromes, compartment syndromes (forearm compartment syndrome, erector spinae syndrome, carpal tunnel syndrome, or frozen shoulder), and fibromyalgia, as well as inflammatory diseases such as eosinophilic fasciitis. One of the more known examples of a condition affecting FL is iliotibial band syndrome (ITB).

Analysis of the literature may result in a conclusion that the research on determination of mechanical properties of FL is 'neglected'. This probably results from the fact that the majority of the research on deep fascia is carried out by clinicians and anatomists, so it mainly focuses on the tissue structure and behaviour during clinically relevant therapies (e.g. fascial manipulation, myofascial point therapy). Therefore, there is a clear need for this type of research and to expand its scope.

The aim of the conducted study was to determine the directional mechanical properties of the human fascia lata (in different age groups), together with a description of its microscopic structure, in order to better understand the behaviour of the studied tissue. Determination of the mechanical characteristics of the deep fascia in the context of its structure and ultrastructure can become the basis for developing new strategies for target treatment of fascial disorders.

2.Material and methods

2.1. Material

The study material consisted of pathologically unchanged fragments of the FL of 10x15cm, collected *post mortem* from 36 men aged between 18 and 69 years. The material was divided into three age groups: I up to 21 years of age; II from 40 to 50 years of age; III above 60 years of age. Tissue fragments were dissected at the Department of Forensic Medicine, Medical University of Wrocław, with the approval of the Ethics Committee (No. KB-262/2010). All samples were examined fresh, within 24 hours of their collection, without being subjected to freezing processes. The collected material was divided into three groups: 1) for mechanical

testing; 2) for examination under light microscopy; 3) for examination under scanning electron microscopy.

2.2. Mechanical properties

From each fascia fragment, rectangular specimens of 5x50 mm were cut with a special punch in two directions, longitudinal and transverse to the direction of collagen fibre alignment (Fig. 1a). The direction was selected according to the macroscopic direction of the fibre arrangement on the tissue surface. The number of samples cut in each direction (longitudinal and transverse) was 7, in total n = 504 samples were cut.

Prior to mechanical testing, the thickness (t_0) of the fascia specimens tested was measured using a digital micrometer (Mitutoyo, Digimatic IP65 0–25; 0.001 mm). Measurements were taken at several locations, and then the obtained results were averaged. Uniaxial tensile tests of the fascia specimens were carried out using the MTS Synergie 100 machine (Fig. 1b); the tensile speed was 5mm/min, and the specimen measurement length was (l_0) 15 mm. The number of pre-cycles was determined experimentally as 3 (up to 10% of the initial sample length). The fascia was kept moist in isotonic saline (0.9%) throughout the mechanical tests, to prevent the samples from drying out. All tests were conducted at room temperature. The successive steps of stretching of the specimen of the human FL, clamped in the grips, are shown in the figure (Fig. 1c).

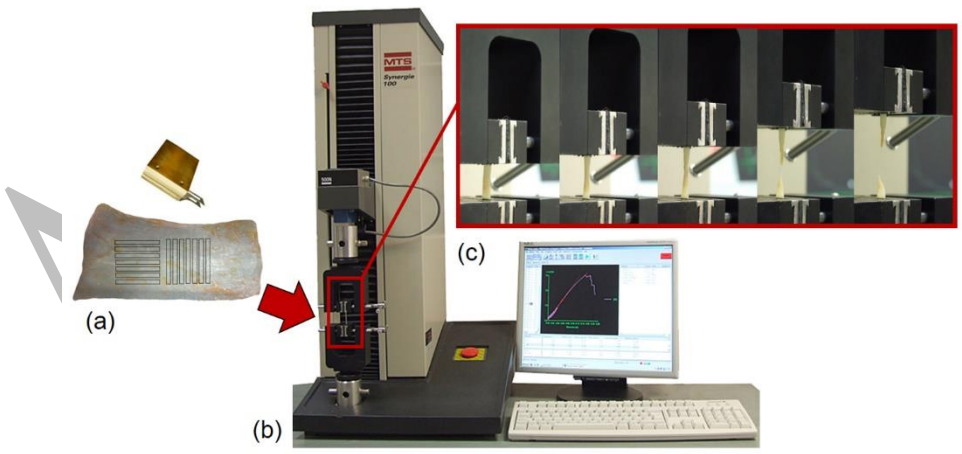

Fig. 1. Specimen of human fascia lata with visible lines of samples cutting (in two directions: longitudinal and transversal to the direction of collagen bundles arrangement) using a punch (a); the experimental setup – testing machine MTS Synergie 100 (b); fascia's specimen fixed in the clamp was subjected to a controlled tension until failure (c)

The following mechanical parameters were determined on the basis of the obtained force curves as a function of displacement and stress as a function of strain: the maximum failure force (F_{max}), conventional stiffness coefficient (k), maximum failure stress (σ_{max}), and conventional tensile modulus (E). Young's modulus was defined as the slope at the linear portion of the stress-strain curve, whilst the stiffness was defined as the slope of the linear section of the load-displacement curve. To adjust geometric changes of the cross-sectional area A [mm^2] of the sample connected with its elongation during the conducted uniaxial tensile test the following formula was utilised [36] (1):

$$A = \frac{l_0 \cdot w_0 \cdot t_0}{\Delta l + l_0} \quad (1)$$

where l_0 [mm] is the original length of the specimen, w_0 [mm] is the original width of the specimen, t_0 [mm] is the original thickness of the specimen, Δl [mm] is elongation.

The results of the obtained mechanical parameters were presented as mean values with standard deviation (SD). Statistical analysis was performed using the Origin-7 program. The normality of the distribution of the results was analysed using the Shapiro-Wilk test. On the other hand, in order to compare the means between the individual study groups, ANOVA was used with a significance level of 0.05.

2.4. Structure

Part of the material to be examined under the light microscope was fixed in 10% buffered formalin; after dehydration, the tissue sections were embedded in paraffin and cut into ca. 5-µm-thick sections. These sections were then stained with haematoxylin and eosin (H&E) (Sigma-Aldrich, Munich, Germany) to visualise the basic histological structure and special staining, with Wright's stain (Sigma-Aldrich, Munich, Germany) was used to selectively visualise elastic fibres. Microscopic analysis was carried out using the Axio Imager.M1m light microscope (Carl Zeiss Microscopy GmbH, Jena, Germany).

The material for scanning electron microscopy was fixed in 2.5% glutaraldehyde solution on phosphate buffer, and dehydrated in increasing acetone concentrations (from 30% to 100%). The test material was then dried, glued on to metallic stubs and coated with gold. Samples prepared in this way were examined under the EVO 15 LS scanning electron microscope (Carl Zeiss Microscopy GmbH, Jena, Germany).

3. Results

3.1. Mechanical properties

The thicknesses of the individual specimens of the human FL were measured prior to the strength tests; the mean value of the measured thickness was 0.58±0.13 mm. Force-displacement and stress-strain curves were plotted from the data obtained from the uniaxial tensile tests. The mechanical parameters of the test material were then determined, i.e.: the maximum failure force (F_{max}), conventional stiffness coefficient (k), maximum failure stress (σ_{max}), and conventional Young's modulus (E), as described by other authors in earlier studies [40][41]. All parameters were measured in the longitudinal and transverse direction in relation to the collagen fibre alignment. The obtained characteristics were non-linear, and the example of a stress-strain curve is shown in Fig. 2.

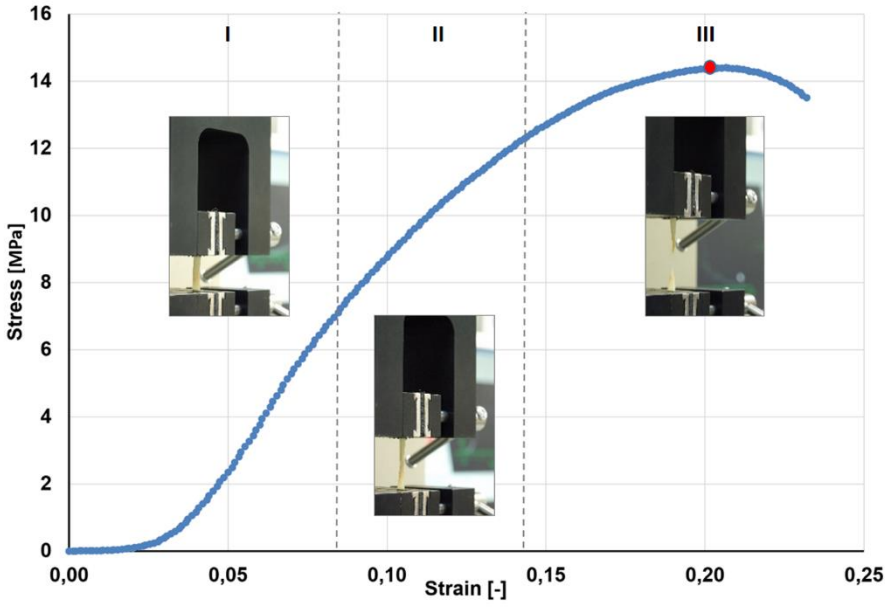

Fig. 2. Representative non-linear stress-strain curve obtained for the human fascia lata specimen with three areas (I – toe, II – linear, III – yielding and failure)

In order to compare the mechanical parameters of FL specimens cut longitudinally and transversely, the obtained results were summarised in column diagrams (Fig. 3a–d). For most of the samples, significant differences were observed between the values obtained for the longitudinal and for the transverse samples ($p<0.05$). It was noted, moreover, that for the longitudinal samples, the values of all parameters tested were lowest in the youngest age group.

The maximum failure force (F_{max}) values obtained for the longitudinal specimens were higher (even up to five times) than for the transverse specimens, and their average values ranged

between ca. 19–23N. For transversely cut samples, F_{max} values varied the least in the first age group (approx. 46%), and the most in the second age group (approx. 80%). Their mean values ranged between ca. 4.5–10N. In the case of the conventional stiffness coefficient (k), the values obtained ranged between ca. 9–12N/mm for longitudinally cut specimens; for transversely cut specimens, the values obtained were even up to nine times lower, and were ca. 1–2.5N/mm. For the transversely cut samples, the highest k values obtained for the youngest age group were observed to be 2.5 N/mm, and were 55% higher than the lowest k value obtained for the oldest age group. The maximum failure stress (σ_{max}) values obtained for the longitudinal samples were higher than for the transverse samples, and their average values ranged between ca. 6–11 MPa. For the transversely cut samples, σ_{max} values varied the least in the first age group (ca. 16%), and the most in the second age group (ca. 80%); their mean values ranged between ca. 2–5MP.

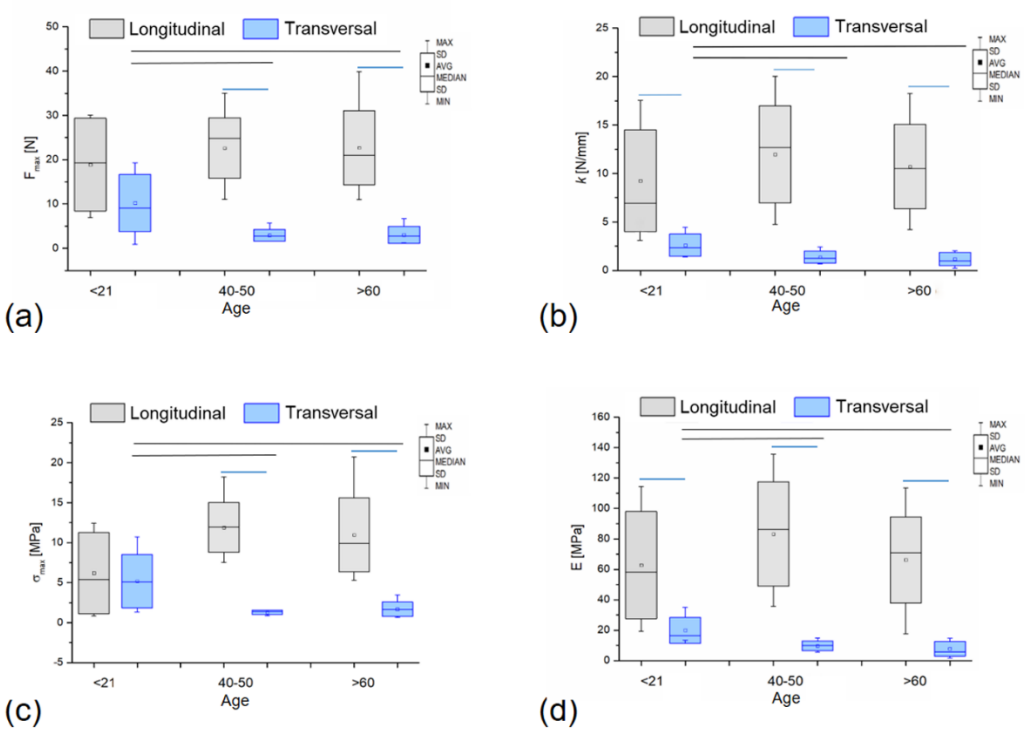

Fig. 3. The mechanical parameters of the human fascia lata tested longitudinally (grey) and transversally (blue) to collagen bundles arrangement: (a) maximum failure force (F_{max}); (b) conventional stiffness coefficient (k); (c) maximum failure stress (σ_{max}); (d) conventional Young's modulus (E). Statistical significance ($p < 0.05$)

In the case of the conventional Young's modulus (E) the values obtained were more than eight times higher for the longitudinal than transverse samples (in the second and third age groups); the smallest difference between the values obtained for the different directions of excision was

found in the group of men under 20 years of age, and was about 68%. Average *E* values ranged between ca. 63–83MPa for the longitudinal specimens and between ca. 8–20MPa for the transverse specimens.

3.2. Morphological results

H&E staining confirmed that at the histological level, the tissue examined showed features typical for fibrous (dense) connective tissue characterised by a clear dominance of fibrous structural proteins. Among them, collagen fibres, were the most prevalent and constitute main structural material of the fascia. Bundles of collagen fibres gave the studied tissue a distinctly layered character. In the majority of the specimens examined, three main collagenous layers could be distinguished and each of those layer consists of packed, wavy and regularly arranged collagen bundles (Fig. 4a). The collagen bundles in a given layer ran parallel to each other, while the collagen bundles in adjacent layers were arranged in a different direction, at an angle in relation to the bundles of the layer in their vicinity.

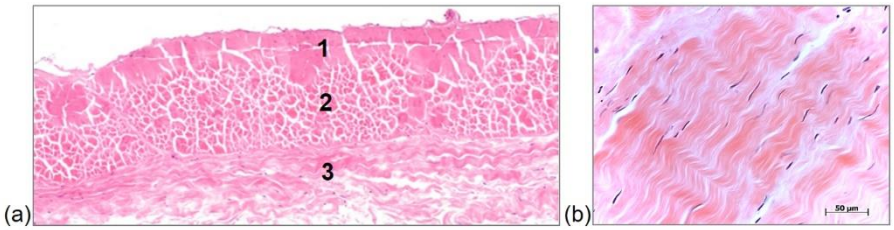

Fig. 4. Layering of collagen fibres in the human fascia lata; note the different direction of collagen fibre bundles in adjacent collagen layers (a) (H&E staining); parallel arrangement of collagen bundles within one of the layers (b) (H&E staining)

In the fascia samples examined in their native state (before mechanical testing), the collagen bundles were characterised, as mentioned above, by a regular arrangement, essentially running parallel to each other (Fig. 4b). Collagen fibres were arranged in bundles and did not branch out or anastomose, whereas in the fascia samples assessed after stretching, the collagen fibre bundles still showed a parallel arrangement, but they were characterised by wider spaces between their individual groups and a loss of the characteristic undulation (Fig. 5a). In some areas, partial “shrinkage” of the collagen fibre bundles after rupture was visible (Fig. 5b).

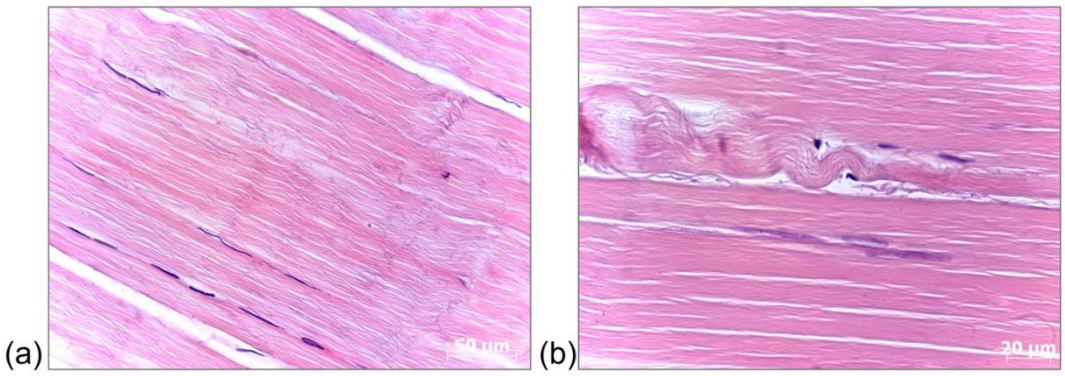

Fig. 5. Histological image of the human fascia lata after uniaxial tensile test: (a) loss of undulation of collagen fibres and widening of the space between individual groups of collagen bundles (H&E staining); (b) partially ruptured, shrunken bundle of collagen fibres (H&E staining)

Histochemical special staining i.e. Wright's stain was used to visualise the elastic fibres. This allowed a more effective visualisation of the elastin (Fig. 6a). The elastic fibres were stained dark blue and characterised by varying lengths and thicknesses. They were found both within the individual – main collagen layers and between them – in loose connective tissue areas, separating them, often forming a dense irregular network there (Fig. 6b).

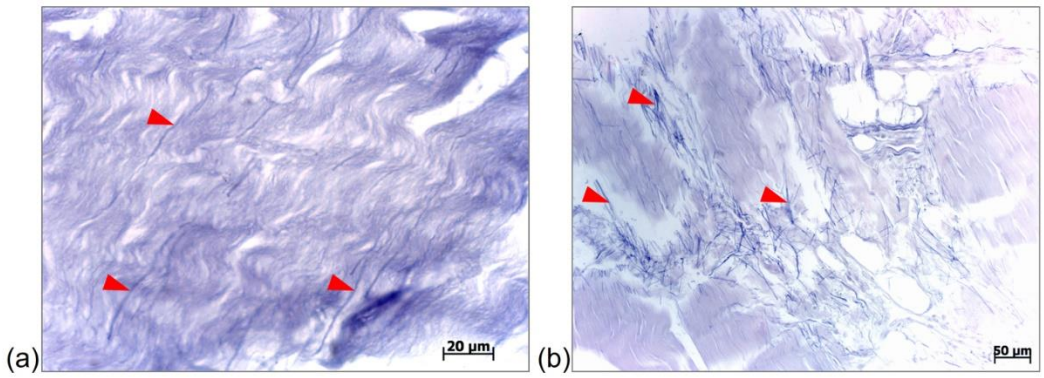

Fig. 6. Image of the fascia lata of the human thigh: (a) clearly visible elastic fibres, stained dark blue; these fibres form more random arrangement and are intermingled between much more numerous collagen fibres; in some areas elastic fibres penetrated collagen bundles resembling “basting stitch” (arrowheads) (Weigert staining); (b) selectively stained elastic fibres (arrowheads), forming a relatively dense, irregular network in the connective tissue (Weigert staining)

In order to precisely analyse the morphological characteristics of the FL and the behaviour of one of its main structural proteins – collagen, under mechanical loading, the material was examined under scanning electron microscopy before and after the uniaxial tensile tests. The SEM images obtained allowed to confirm the layered structure of the studied tissue. (Fig. 7a). Observations from light microscopy, that the individual collagen layers building the FL are mainly formed of parallel bundles of collagen fibres (Fig. 7b), were confirmed.

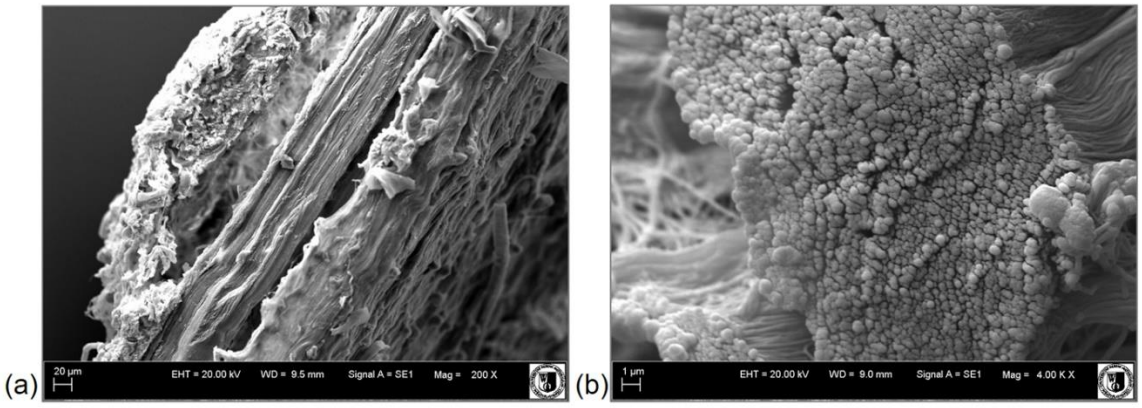

Fig. 7. SEM image of the human fascia lata in cross-section. (a) Clearly visible layered arrangement of collagen fibre bundles in the one of main collagen layers of the fascia (mag. 200x); (b) visible regular parallel arrangement of collagen fibres within a single collagen layer (mag. 4000x)

The collagen fibres were organised in regular bundles running parallel to each other. These bundles, on longitudinal sections, had a characteristic undulating appearance (crimped pattern) (Fig. 8).

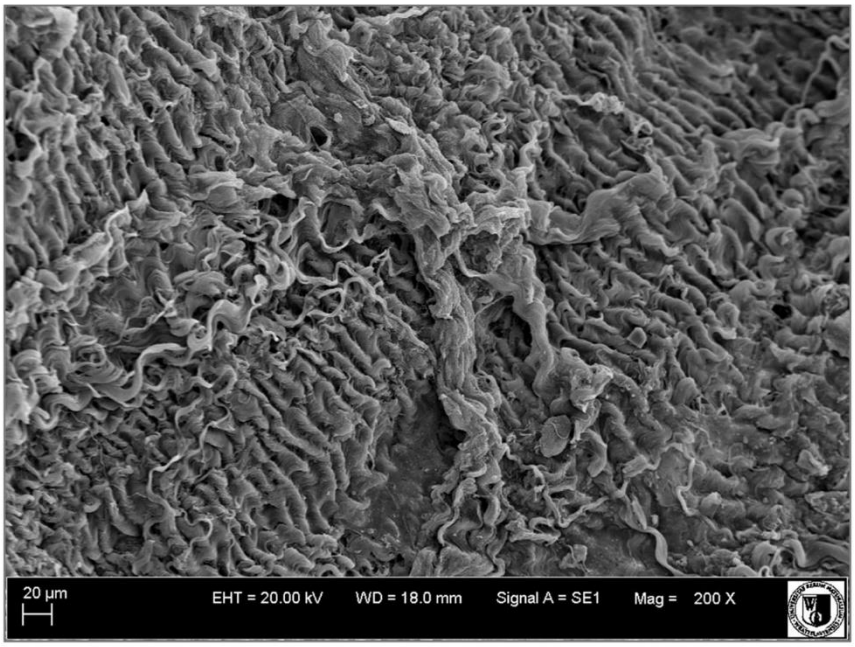

Fig. 8. SEM image of the human fascia lata; collagen bundles exhibiting evident undulation appearance (crimped collagen pattern) (mag. 200x)

When the tissue was subjected to mechanical forces, the regular arrangement of collagen fibres (Fig. 9a) was significantly blurred and disorganised. Apart from the loss of the characteristic undulation, the widening of the spaces between the individual collagen fibre bundles could also be clearly seen (Fig. 9b).

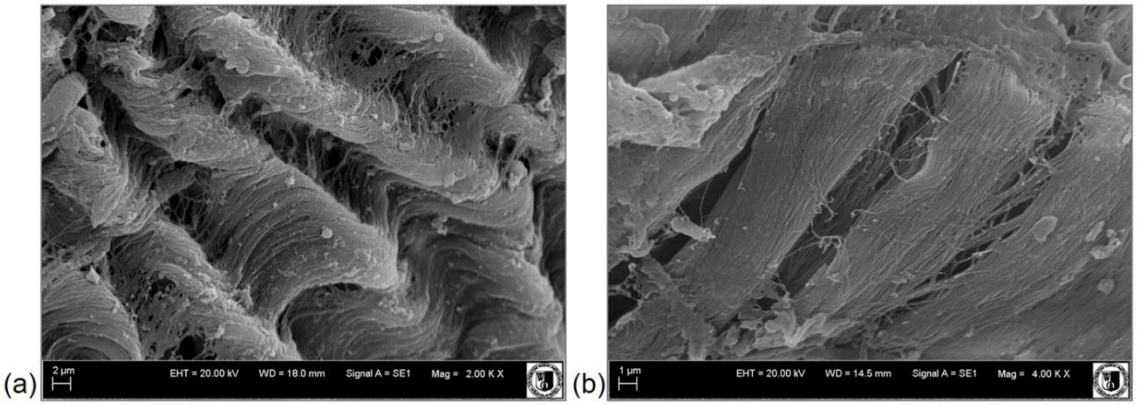

Fig. 9. SEM images of human fascia lata before (a) (mag. 2000x); and after uniaxial tensile test (b) (mag. 4000x)

Individual collagen fibres were fragmented and entangled (Fig. 10).

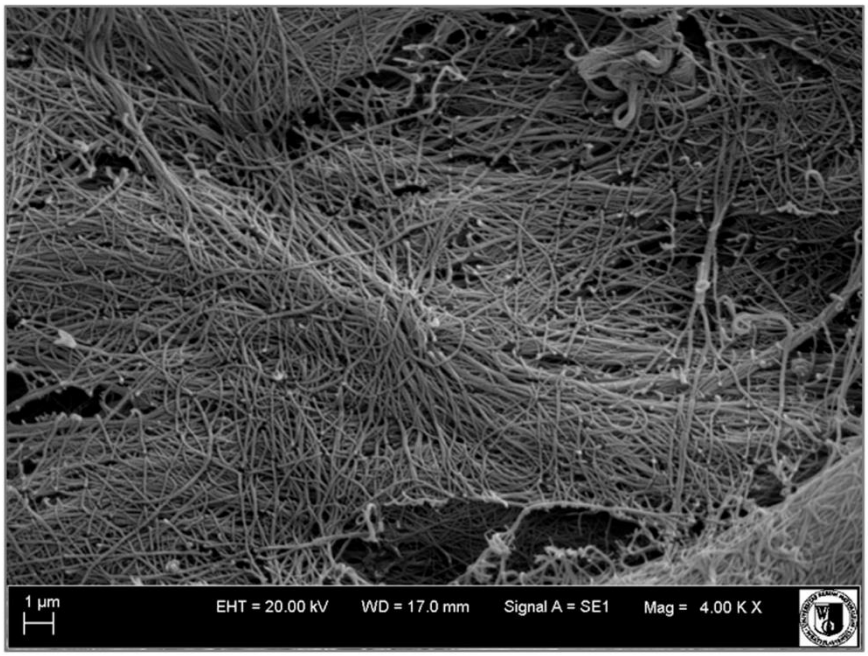

Fig. 10. SEM images of human fascia lata after tensile test; note fragmentation and rupture of individual collagen fibres (mag. 4000x)

In some areas, rupture of individual collagen fibre bundles with subsequent shrinkage and formation of spherical conglomerates could be seen.

4. Discussion

Previously, mechanical studies on fascial tissues were conducted using human fasciae [3][19][21] and in animal models (rabbit, goat, sheep, and dog) [8][28][38]. In most cases, the material was analysed in its native state as fresh samples excised *post mortem* and, less frequently, after fixation by deep freezing or in formalin [21]. From a mechanical perspective, tissue preservation could influence the results of mechanical testing to a great extent [24]. For this reason, in the course of the present study, samples of the human FL were subjected to mechanical testing immediately after collection, as fresh material, without any fixation. Furthermore, it should be emphasised that, when compared to other studies, the number of samples examined by the authors was significantly higher (more than 500 specimens in total). So fat, information on the mechanical properties of the deep fascia was most frequently obtained in uniaxial [3][21][23][] or, less frequently, biaxial [8][22] tensile strength tests, which were generally performed until tissue rupture. Additionally, shear tests were also performed [28]. More recently, attempts have also been made to assess the fascia properties using elastography, but in this case it is difficult to distinguish indisputably, under clinical conditions, between the

deep fascia and other tissues. Numerical models and constitutive modelling of the fascia are also presented [3][22][28]. The authors used uniaxial tensile test; however, in order to better reflect the biomechanical characteristics of the studied tissue, for each specimen, samples were cut out and then stretched in two different directions, focusing on the morphological changes occurring in the structure of the tissue studied under applied loads.

The mean thickness of the FL measured by us was 0.58 ± 0.13 mm, and it was similar to the values obtained by other authors, e.g. Dervin et al. (0.55 ± 0.15 mm) [7], or Otsuka et al. (0.78 ± 0.17 mm) [21], while higher thickness values were obtained by Stecco et al. (0.944 ± 0.156 mm) [32], or Pirri et al. (0.926 ± 0.156 mm) [25]. Differences in the compared thickness values may result from the location from which the study material was collected, as well as from individual characteristics. If the exact thickness of a patient's fascia is known, this can reduce, in a clinical setting, the risk of nerve damage during surgical procedures. Additionally, the analgesic effect of drugs administered as local anaesthetics can be foreseen [25].

After mechanical testing, non-linear stress-strain curves specific for soft tissues were obtained [15][16][36], with two slopes. The source of this two-slope behaviour is thought to arise from the combination of collagen and elastin fibres, where elastin governs deformation at small strains and collagen governs it at larger stresses. Interestingly, when a fascia, a tendon and muscles are compared, it can be seen that the stress-strain curve of the tendon is similar to that of the fascia, with a faster increase in stress values in response to the increase in strain. Fascia, on the other hand, are characterised by greater adaptability than tendons. The tendon reaches non-physiological extension at around 4%, while the deep fascia is able to withstand up to 27% of its elongation. In the case of the muscles, this value is 75% [33].

One of the mechanical parameters assessed by the authors of this study was the value of the maximum failure force (F_{max}). Today, however, this parameter is analysed less frequently, and generally replaced by the maximum failure stress (σ_{max}) [4]. In addition to maximum failure stress (σ_{max}), parameters typically analysed when assessing the biomechanical properties of soft tissues are the stiffness coefficient (k) and Young's modulus (E).

The stiffness values obtained by us were comparable to the results obtained by other authors testing fresh fascia samples (3.3 ± 0.58 N/mm) [37], while lower than those obtained in the studies of authors evaluating formalin-fixed fascia specimens (24.1 ± 9.1N/mm) [21]. The above difference can be explained by the effect of formalin on the level of soft tissue stiffness, as it was demonstrated that formalin fixation increased stiffness of tissues; namely, formalin stiffens samples due to the formation of irreversible cross-links between the amino groups in

collagen [34]. Furthermore, we observed less stiffness in the transverse direction (when compared to the longitudinal direction), which may be related to the fascia ability to adapt to the effects of muscle fibre contraction. It can be suspected that as the specimen is elongated in the main direction in line with the orientation of the collagen fibres, the stiffness of the tissue changes when a certain strain value is exceeded. The fibres are then stretched and arranged in the direction of loading. However, they do not contribute to the stiffness of the tissue when stretched in the transverse direction [31]. For the longitudinal specimens tested, lower values were obtained for the measured mechanical parameters, including stiffness, in age group I than in the group of older donors. It is suspected that this situation results from the fact that, with age, the production of collagen fibres decreases, and the connective tissue structures become more rigid. One of possible reasons explaining higher values of the stiffness coefficient as we age is the fact that the fibroblast activity decreases, and therefore the amount of collagen they produce also falls. Simultaneously, ageing is associated with qualitative changes in collagen fibres; type I collagen predominates, collagen fibres become chaotically distributed. Similar processes occur in elastic fibres [10][12]. Additionally, as shown recently, postmenopausal fasciae contain more type I collagen and less type III collagen, and this explains why, in women, fasciae become stiffer with age [20].

Another biomechanical parameter analysed by us was the maximum failure stress (σ_{max}). This parameter was also assessed by other authors [3][12][19][38][39]; however, due to differences in protocols regarding the test procedure (e.g.: method of sample preservation and storage, method of hydration, sample shape, elongation speed), it is often difficult to directly compare the results obtained. Additionally, information on specific parameters is not always available. In the FL samples examined by us, in general, the stiffness value was lower compared to the work of other authors. For example, Zwirner et al. recorded in fresh-frozen samples σ_{max} of 29.0 ± 17.0 MPa [39], Manon et al. in the studied frozen material determined the σ_{max} value of 96.8 ± 3.2 MPa [19], while Balsly et al. showed the σ_{max} of 93.4 ± 28.2 MPa [1], when examining frozen dried sections. Our results mostly resemble those obtained by Bonaldi et al. with a result of 6.3 ± 4.1 MPa for fresh-frozen tissue [3]. This large variety of results for σ_{max} obtained by the various authors may also be explained by higher test speed until rupture, which could lead to slight stiffening of the material. Due to the viscoelastic behaviour that characterises biological tissues, the selected strain rate usually plays a significant role in the final test result, with a significant increase in both σ_{max} and E and, in consequence, a decrease in maximum strain with the increasing strain rate [3].

In the case of the conventional Young's modulus (*E*), values obtained by us ranged between ca. 63–83MPa for longitudinal specimens, and between ca. 8–20 MPa for transverse specimens. The values obtained by individual authors for *E* were even more varied than the values of *k* and σ_{max}. They ranged from 1 to 500MPa, with a few exceptions, particularly for Manon et al., who obtained the value of ca. 1500MPa [4][19]. Our results were most similar to those obtained by Bonaldi et al. (longitudinal:102.2 ± 60.6MPa; transverse 8.0 ± 3.6MPa) [3]. Values of the conventional Young's modulus in the FL samples studied by us were higher for older individuals. As with stiffness, this difference may be related to changes in the fascial tissue structure occurring with age (increase in the quantitative proportion of type I collagen, decrease in the total number of collagen fibres, decrease in amount of elastin and elastin cross-links) [10][12].

There is no doubt that the mechanical properties of the connective tissue are to a large extent determined by its structure. In the case of the deep fascia of the thigh, being a cell poor, collagen–dominated structure, this is particularly evident. Structural studies conducted to date show that fascia lata is formed by several layers of regular fibrous connective tissue [4][32], divided by thin layers of loose connective tissue. Nevertheless, there are discrepancies as to the exact number of collagen layers in this fascia. Currently, the deep fascia is described as a structure consisting of 2 to 3 layers of the connective tissue with different orientation and density of collagen fibres (mainly of type I and III collagen). Within individual layers, the collagen fibres are arranged parallel to each other, but when adjacent layers are compared, it is found that the fibres are arranged differently, forming an angle of 67–80º or 70–85º depending on the author of the study [2][22]. On the basis of their studies, the authors believe that the human FL consists of three collagen layers, with one of them (the middle) being the thickest. These layers, as observed by other authors, are separated by loose connective tissue containing vascular structures, adipose tissue and cellular elements [27][32].

To date, the morphological structure of the human fascia (particularly the deep fascia) has only been assessed prior to mechanical testing. In the course of the present study, the authors carried out a structural assessment of the fascial tissue not only before but also after the uniaxial tensile tests. Thus, it was possible to observe the behaviour of the collagen fibres under the applied loads. Collagen is a protein of great tensile strength. Due to the fact that collagen fibres are able to move and glide within the amorphous ground substance they can cope with compression and tension, still they are inelastic. Nevertheless deep fasciae, like other dense connective tissues, are characterized by a unique morphological pattern in a form of intense waviness of collagen bundles. This pattern is defined as crimp pattern and is an innate property

of type I collagen fibres. This unique crimp arrangement is crucial for biomechanics, as it largely determines the nonlinear (strain-dependent) biomechanical behaviour of the tissues. Collagen fibre bundles elongate under tension to their physiological length and recoil when tension is released. That is why thanks to crimp pattern fascia lata despite being collagen dominated tissue exhibit also elastic properties [13].

The conducted analysis demonstrated that the collagen fibres in the unloaded samples were arranged parallel to each other in each layer, however, in different layers the fibres intersected in different directions, in line with the observations of [32].

With the use of scanning electron microscopy (SEM), it was possible to observe the behaviour of collagen fibre bundles before and after mechanical testing in an even more precise way. SEM images confirmed a clear loss of order in collagen fibre bundles after loading; additionally, severe fragmentation of collagen fibres could be seen. This may explain why, after subjecting the deep fascia to supra-physiological forces *in vivo*, its regeneration and return to full functional capacity is not always possible. When considering the structure of fascial tissues, an element that still needs to be clarified is the content and spatial distribution of elastic fibres in the deep fascia, as they are much less studied than collagen fibres. At the same time, one of the most controversial issues is whether fascia is elastic structure. Although the presence of cells with contractile properties – myofibroblasts – within fascial structures was demonstrated [30], yet, firstly, they were not numerous, and secondly, significantly more myofibroblasts were found in pathologically altered fasciae (e.g. palmar fibromatosis, hypertrophic scars, chronic low back pain) [27]. Therefore, it is not known whether they are present in the fascia under physiological conditions, or whether they are formed from fibroblasts only in consequence of an injury of inflammation. It can therefore be suspected that elastic fibres are nevertheless mainly responsible for the elastic properties of the fascia, although it was demonstrated that the mean amount of elastic fibres in the deep fascia is only about 1% [27]. In this respect, deep fasciae closely resemble tendons, as these structures are also characterised by very low content of elastic fibres and a clear dominance of collagen. In the examined fascia, elastic fibres were much less abundant compared to collagen fibres. The most characteristic feature of these fibres is their susceptibility to stretching and returning to their original length once the tensile force is removed, which is undoubtedly crucial for the biomechanical properties of the fascia. In routine histological staining, elastic elements were difficult to visualise, not only because of the significant quantitative predominance of collagen fibres, but also because of the close packing of collagen fibres. Therefore, histochemical special – Weigert's stain was used to visualise the elastic fibres [14]. This resulted in more efficient detection of elastic fibre structures in the

studied tissue, and visualisation of even the finest fibres. Similarly to other authors, in our study we showed that elastic fibres were more abundant in the two thinner outer layers of the fascia lata and less abundant in the thicker middle layer [26]. Furthermore, the elastic fibres in the middle layer were not only parallel to the main direction of the collagen bundles, but, as other authors have also shown, pierced these compact fibrous bundles (as in a basting stitch), to, presumably, improve the elastic response of the fascia under loading. We also agree that elastic fibres in thinner fibrous layers exhibit an organisation interdependent of collagen bundle arrangement, and that they are much more numerous within loose connective tissue layers separating the 3 main fibrous layer.

5. Conclusions

Summing up, the mechanical characteristics obtained for the studied tissue confirmed its anisotropic behaviour. An undoubted value of the study carried out was visualisation of morphological changes occurring in the FL under the influence of applied loads, which was shown both at the histological and, using SEM, at the ultrastructural levels. This is crucial for understanding the biomechanics of the fascia lata *in vivo* and its many applications in the medical field. This knowledge can contribute to improvement in clinicians' understanding of the musculo-fascial system and the role that deep fascia can play in musculoskeletal dysfunctions. This opens up new avenues for research into the wider use of the fascia in plastic and reconstructive surgery and helps to determine the fascia suitability as graft material, depending on the direction of the forces at the target location in the patient. When considering the structure of fascial tissues, an element that still needs to be clarified is the content and spatial distribution of elastic fibres in the deep fascia, as they are much less studied than collagen fibres. In order to reduce discrepancies in the results obtained, it is advisable to develop a reproducible methodology for collecting and fixing study material, as well as to standardise methods for mechanical tests.

Conflict of interest

The author declares that there is no conflict of interest regarding the presented study.

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References

[1] Balsly C.R., Cotter A.T., Williams L.A., Gaskins B.D., Moore M.A., Wolfinbarger L. Jr., Effect of low dose and moderate dose gamma irradiation on the mechanical properties of bone and soft tissue allografts, Cell Tissue Bank., 2008, 9(4), DOI:10.1007/s10561-008-9069-0.

[2] Benetazzo L., Bizzego A., De Caro R., Frigo G., Guidolin D., Stecco C., 3D reconstruction of the crural and thoracolumbar fasciae, Surg Radiol Anat., 2011, 33(10), DOI:10.1007/s00276-010-0757-7.

[3] Bonaldi L., Berardo A., Pirri C., Stecco C., Carniel E.L., Fontanella C.G., Mechanical characterization of human fascia lata: uniaxial tensile tests from fresh-frozen cadaver samples and constitutive modelling, Bioengineering (Basel), 2023, 10(2), DOI:10.3390/bioengineering10020226.

[4] Bonaldi L., Fontanella C.G., Stecco C., Berardo A., Design, implementation and effectiveness of human fascia lata biomechanics for tissue engineering, J Biomech., 2024, DOI:10.1016/j.jbiomech.2024.112369.

[5] Dawidowicz J., Szotek S., Matysiak N., Mielańczyk Ł., Maksymowicz K., Electron microscopy of human fascia lata: focus on telocytes., J Cell Mol Med., 2015,19(10), DOI:10.1111/jcmm.12665.

[6] Dawidowicz J., Matysiak N., Szotek S., Maksymowicz K., Telocytes of Fascial Structures, Adv Exp Med Biol., 2016, 913, DOI:10.1007/978-981-10-1061-3_26.

[7] Derwin K.A., Baker A.R., Spragg R.K., Leigh D.R., Farhat W., Iannotti J.P., Regional variability, processing methods, and biophysical properties of human fascia lata extracellular matrix, J Biomed Mater Res A, 2008, 84(2), DOI: 10.1002/jbm.a.31455.

[8] Eng C.M., Pancheri F.Q., Lieberman D.E., Biewener A.A., Dorfmann L,. Directional differences in the biaxial material properties of fascia lata and the implications for fascia function, Ann Biomed Eng., 2014, 42(6), DOI:10.1007/s10439-014-0999-3.

[9] Fede C., Pirri C., Fan C., Petrelli L., Guidolin D., De Caro R., Stecco C. A., Closer look at the cellular and molecular components of the deep/muscular fasciae, Int J Mol Sci., 2021, 22(3), DOI: 10.3390/ijms22031411.

[10] Fede C., Fan C., Pirri C., Petrelli L., Biz C., Porzionato A., Macchi V., De Caro R., Stecco C., The effects of aging on the intramuscular connective tissue, Int J Mol Sci., 2022 23(19), DOI: 10.3390/ijms231911061.

[11] Gerwin R.D., Classification, epidemiology, and natural history of myofascial pain syndrome, Curr Pain Headache Rep., 2001, 5(5), DOI: 10.1007/s11916-001-0052-8.

[12] Hammer N., Lingslebe U., Aust G., Milani T.L., Hädrich C., Steinke H., Ultimate stress and age-dependent deformation characteristics of the iliotibial tract, J Mech Behav Biomed Mater., 2012, 16, DOI:10.1016/j.jmbbm.2012.04.025.

[13] Jan N.J., Brazile B.L., Hu D., Grube G., Wallace J., Gogola A., Sigal I.A., Crimp around the globe; patterns of collagen crimp across the corneoscleral shell, Exp Eye Res., 2018 172, DOI: 10.1016/j.exer.2018.04.003.

[14] Kobielarz M., Szotek S., Głowacki M., Dawidowicz J., Pezowicz C., Qualitative and quantitative assessment of collagen and elastin in annulus fibrosus of the physiologic and scoliotic intervertebral discs, J Mech Behav Biomed Mater., 2016, 62, DOI: 10.1016/j.jmbbm.2016.04.033.

[15] Kobielarz M., Effect of collagen fibres and elastic lamellae content on the mechanical behaviour of abdominal aortic aneurysms, Acta of Bioengineering and Biomechanics, 2020, 22(3), DOI:10.37190/ABB-01580-2020-02.

[16] Kozuń M., Chwiłkowska A., Pezowicz C., Kobielarz M., Influence of atherosclerosis on anisotropy and incompressibility of the human thoracic aortic wall, Biocybernetics and Biomedical Engineering, 2021, 41(1), DOI:10.1016/j.bbe.2020.11.004.

[17] Kuchera M.L., Kuchera W., Osteopathic Principles in Practice, Greyden Press USA, 1994.

[18] Langevin H.M., Fascia mobility, proprioception, and myofascial pain, Life (Basel), 2021,11(7), DOI:10.3390/life11070668.

[19] Manon, J., Evrard, R., Maistriaux, L., Fiev´e, L., Heller, U., Magnin, D., Boisson, J., Kadlub, N., Schubert, T., Lengel´e, B., Behets, C., Cornu, O., Periosteum and fascia lata: Are they so different? Front. Bioeng. Biotechnol., 2022, 10, DOI: 10.3389/fbioe.2022.944828.

[20] Mika A., Oleksy Ł., Fede C., Pirri C., Stecco C., Editorial: Fascia as a multi-purpose structure of connective tissue – dysfunction, diagnostics and treatment, Front Med (Lausanne), 2024, 11, DOI:10.3389/fmed.2024.1472116.

[21] Otsuka S., Yakura T., Ohmichi Y., Ohmichi M., Naito M., Nakano T., Kawakami Y., Site specificity of mechanical and structural properties of human fascia lata and their gender differences: A cadaveric study, J Biomech, 2018, 77, DOI: 10.1016/j.jbiomech.2018.06.018.

[22] Pancheri F.Q., Eng C.M., Lieberman D.E., Biewener A.A., Dorfmann L., A constitutive description of the anisotropic response of the fascia lata, J Mech Behav Biomed Mater., 2014, 30, DOI: 10.1016/j.jmbbm.2013.12.002.

[23] Pavan P.G., Stecco A., Stern R., Stecco C., Painful connections: densification versus fibrosis of fascia, Curr Pain Headache Rep., 2014, 18(8), DOI:10.1007/s11916-014-0441-4.

[24] Pillet B., Badel P., Pierrat B., Effects of cryo-preservation on skeletal muscle tissues mechanical behavior under tensile and peeling tests until rupture, J Mech Behav Biomed Mater., 2022,132, DOI:10.1016/j.jmbbm.2022.105273.

[25] Pirri C., Fede C., Petrelli L., Guidolin D., Fan C., De Caro R., Stecco C., An anatomical comparison of the fasciae of the thigh: A macroscopic, microscopic and ultrasound imaging study, J Anat., 2021; 238(4), DOI: 10.1111/joa.13360.

[26] Pirri C., Fede C., Petrelli L., Guidolin D., Fan C., De Caro R., Stecco C., Elastic Fibres in the subcutaneous tissue: Is there a difference between superficial and muscular fascia? A cadaver study, Skin Res Technol., 2022, 28(1), DOI: 10.1111/srt.13084.

[27] Pirri C., Caroccia B., Angelini A., Piazza M., Petrelli L., Caputo I., Montemurro C., Ruggieri P., De Caro R., Stecco C., A new player in the mechanobiology of deep fascia: yes-associated protein (YAP), Int J Mol Sci., 2023, 24(20), DOI: 10.3390/ijms242015389.

[28] Ruiz-Alejos D., Peña J.A., Perez M.M., Peña E., Experiments and Constitutive Model for Deep and Superficial Fascia. Digital Image Correlation and Finite Element Validation, Strain, 2016, 52, DOI: 10.1111/str.12198.

[29] Schleip R., Jäger H., Klingler W., What is 'fascia'? A review of different nomenclatures, J Bodyw Mov Ther., 2012, 16(4), DOI:10.1016/j.jbmt.2012.08.001.

[30] Schleip R., Klingler W., Active contractile properties of fascia., Clin Anat., 2019, 32(7), DOI:10.1002/ca.23391.

[31] Stecco C., Pavan P.G., Porzionato A., Macchi V., Lancerotto L., Carniel E.L., Natali A.N., De Caro R., Mechanics of crural fascia: from anatomy to constitutive modelling, Surg Radiol Anat., 2009, 31(7), DOI: 10.1007/s00276-009-0474-2.

[32] Stecco C., Functional Altas of the Human Fascial System, Churchill Livingstone: London, UK, 2014.

[33] Stecco C., Pirri C., Fede C., Yucesoy C., De Caro R., Stecco A., Fascial or Muscle Stretching? A Narrative Review., Appl. Sci., 2021, 11, 307, DOI: DOI: 10.3390/app11010307.

[34] Steinke H., Lingslebe U., Böhme J., Slowik V., Shim V., Hädrich C., Hammer N., Deformation behavior of the iliotibial tract under different states of fixation., Med Eng Phys., 2012, 34(9), DOI: 10.1016/j.medengphy.2011.12.009.

[35] Szotek S., Dawidowicz J., Eyden B., Matysiak N., Czogalla A., Dudzik G., Leśniewicz A., Maksymowicz K., Morphological features of fascia lata in relation to fascia diseases. Ultrastruct Pathol., 2016, 40(6), DOI: 10.1080/01913123.2016.1239665.

[36] Szotek S., Dawidowicz J., Geniusz M., Kozak M., Łukomski R., Czogalla A., The biomechanical characteristics of spinal dura mater in the context of its basic morphology, Acta of Bioengineering and Biomechanics, 2021, 23(4), 149-159.

[37] Thomas O.L., Morrison C., Howard L., Oni O.O.A., The biomechanical properties of fascia lata grafts: a preliminary study, Injury, 1998, 29(3), DOI: 10.1016/s0020-1383(97)00192-7.

[38] Wang H.Q., Wei Y.Y., Wu Z.X., Luo Z.J., Impact of leg lengthening on viscoelastic properties of the deep fascia, BMC Musculoskelet Disord., 2009,10, DOI:10.1186/1471-2474-10-105.

[39] Zwirner J., Babian C., Ondruschka B., Schleifenbaum S., Scholze M., Waddell N.J., Hammer N., Tensile properties of the human iliotibial tract depend on height and weight, Med Eng Phys., 2019, 69, DOI: 10.1016/j.medengphy.2019.05.001.

[40] Żak M., Pezowicz C., Analysis of the impact of the course of hydration on the mechanical properties of the annulus fibrosus of the intervertebral disc, Eur Spine J, 2016, 25(9), DOI: 10.1007/s00586-016-4704-0.

[41] Żak M., Pezowicz C., Effect of overload on changes in mechanical and structural properties of the annulus fibrosus of the intervertebral disc, Biomech Model Mechanobiol, 2021, 20(6), DOI:10.1007/s10237-021-01505-w.