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Biomechanics of Fascia Lata - Uniaxial Tensile Test

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Abstract

Human Fascia Lata (FL) is a connective tissue with a multilayered organization also known as aponeurotic fascia. In each layer (usually two or three) the collagen fibers run in parallel directions, forming an angle between the layers that usually varies between 75° and 80°. Although it is a key structure in several musculoskeletal dysfunctions and in tissue engineering, the investigation of its biomechanics is still challenging as it is difficult to establish a bias-free experimental protocol. This thesis aims to analyze the biomechanical properties of FL ex vivo. The samples were collected from human donors and tested with uniaxial tensile tests in order to investigate the final strength and Young's modulus values between different subjects / layers / directions. The results were analyzed with MATLAB 2021b (The MathWorks, Inc., Natick, Massachusetts, United States). The experimental outcomes show an anisotropic behavior even between different layers. These findings could potentially support surgeons in daily practice (such as graft preparation and placement), engineers in-silico simulation, physiotherapists during musculoskeletal rehabilitation, towards the management of a personalized medicine.

Keywords: Tensile test, Biomechanics, Fascia Lata, Uniaxial, Young Modulus.

Acronym list:

- Young’s Modulus (YMs)
- Fascia Lata (FL)

Introduction

The Fascia Lata (FL) is a deep fascia with a multi-layered structure. The layers are generally two or three. In each layer the collagen's fibers run in parallel directions, between them the collagen's fibers are orientated in different directions forming an inter-layers angle that usually ranges between 75° and 80° (Stecco et al, 2015) [1]. Posteriorly, it is formed by the confluence of the myofascial expansions of the gluteus maximus and medius muscles. Laterally, it is formed by the tensor fasciae latae and, anteriorly, by the expansions of the iliopsoas fascia and the fasciae of the abdominal muscles. The more important longitudinal fibers are on the lateral side and are so thick and resistant that they merit their own name: the iliotibial tract (ITT). The fascia lata forms a firm covering of the limb, strongest on the outer side where the iliotibial tract is present. On the medial surface of the thigh the fascia lata is thin. It creates almost two

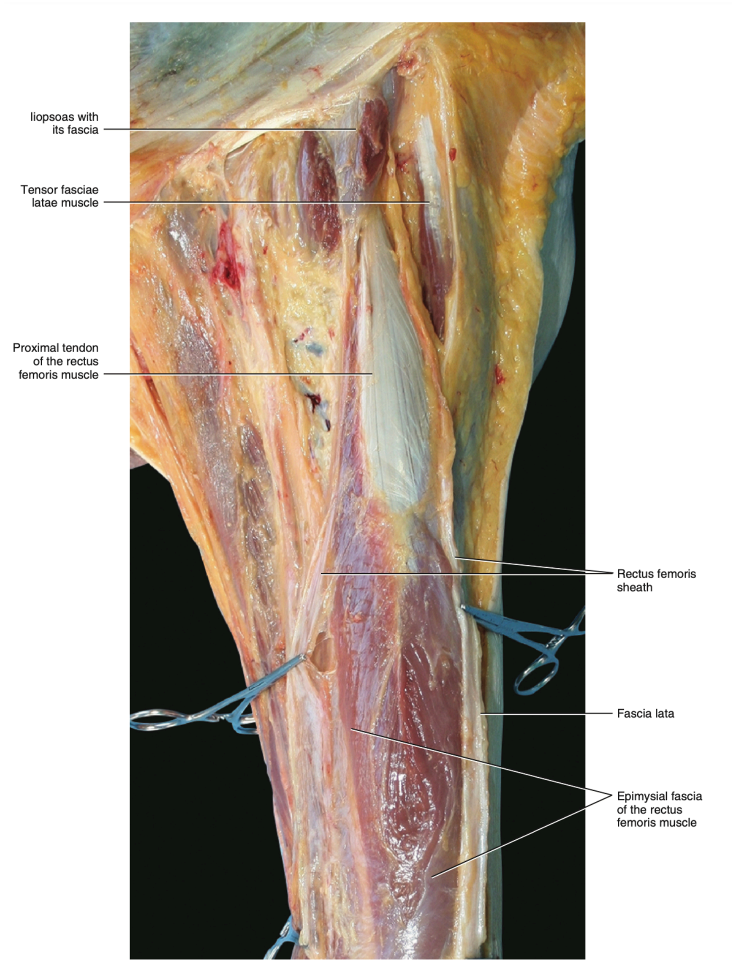


Figure 1: Image from dissection (Stecco et al, 2015) [1].

autonomous sheaths for the sartorius and gracilis muscles. The fascia also forms the adductor canal that is an aponeurotic tunnel in the middle third of the thigh (Stecco et al, 2015) [1]. See *Figure 1*.

Fascia Lata has been demonstrated to be a key structure in clinical practice.

In our daily activities in-vivo forces are transmitted to deep fascia and in case of musculoskeletal dysfunctions this transmission could be altered. For example, according to (Stecco et al, 2014) [11], *"chronic myofascial pain probably causes many alterations of the deep fascia. Past anomalous adhesions of the deep fasciae could have caused pathological lines of force inside the fasciae affecting the deep fascia at a distant location",* concluding that *"clinical experience suggests that a persistent fascial densification/fibrosis will eventually become a compensation along a fascial continuity."*

Anyway, the investigation of the direct link between in-vivo forces and Fascia Lata structures alterations still challenging, for this reason ***ex-vivo*** analysis could be considered as a starting point for its biomechanical characterization.

Moreover, this tissue is often used in tissue engineering as *allograft* [3, 4] or as *autograft* in [5, 6, 7, 8]. Hence, it is necessary to support the use of this structure with experimental evidences in order to improve the clinical approaches, for example supporting surgeons to define the proper graft directions during surgery.

For above mentioned reasons, the structural aspect of Fascia Lata as connective tissue is fundamental in both musculoskeletal dysfunction and tissue engineering, nevertheless from a literature review we evince that is hard to establish a protocol free from *biases* that could not mislead results interpretation.

One example of *bias* is when, during experimental tests, specifically the cutting and testing procedure of the harvested specimen, the collagen fibers directions are not considered *in relation to each layer*. The importance of directionality (fibers orientation VS loading direction) could be expressed with the term anisotropy. "*Anisotropy can be attributed to a material that shows directionally dependent mechanical properties (e.g. stiffness and strength)"* (Stecco et al, 2014) [2]. If considering Fascia Lata, it is defined to be a "*sheet-like structure contains multiple layers of well-organized collagen fibers* *making the tissue highly anisotropic"* (Pancheri et al., 2014) [9]. Since the literature is still poor in experimental protocols that test FL based on the directionality of the ***collagen fibers of each layer***, we have considered this open topic as the main purpose of this thesis, which we have verified through uniaxial tensile tests on FL samples from human donors.

Material & Methods

This thesis was conducted starting from an analysis of the literature in order to understand the missing points that could be reinforced with a further experimental analysis. Therefore, once the open topic of our interest was identified through a gap analysis, we tested it through an experimental protocol.

Literature Review

The review was started with a research on PubMed (search 1) and ScienceDirect (search 2) databases during November 2021. All the articles have been searched, using the following key-words: “Fascia Latae”, “uniaxial tensile test”, “stress-strain”, “mechanical properties”. Other articles (search 3) have been included from other sources.

The total results have been screened according PRISMA approach [10], as first considering Title+Abstract and filtering them in accordance with the inclusion criteria:

- Only English;
- Biomechanical tests;
- Uniaxial test results reported.

Then, the final articles have been analyzed in full text.

Following the guideline proposed by (Gordon et al, 2017) [11], the full articles have been systematically stored in a table (see *Appendix A*). This table included the following sections:

I. Specimen
    I. Human
    II. Animal
    III. Age
    IV. M/F (%)
II. Tissue Histology, Morphometry and structural Configuration
    I. Tissue Layers
        I. Number

II. Thickness

III. Specimen Preparation

I. Tissue Storage

I. Fresh <48h

II. Freezed

III. Fixed

II. Mount of the Specimen

I. Soft tissue grip

II. Cryoclamps

III. Sophisticated Clamps

III. Cut of the Specimen

I. Region

II. Dimension/Shape

IV. Preconditioning

I. Number of Cycles

II. Strain Rate

V. Uniaxial Test

I. Strain Rate

II. Stress/Strain at break

III. Young’s Modulus

This review has been fundamental for the definition of the experimental testing protocol.

Experimental test

We have analyzed 4 subjects Fascia Lata patches (fresh-frozen, stored at -80°C) harvested from human donors according to *Body Donation Program* of the Institute of Anatomy of the University of Padua [12]. The subjects were homogeneously distributed in term of gender (50% males, 50% females), of a mean age 70 years old (between 54-89 yo), without history of FL alterations during lifetime. The patches have been cut (as composite) into rectangular strips in four different configurations according to collagen directions for each layer (see *Table 1*).

Layer	Direction 1	Direction 2
1	Principal	Transverse
2	Principal	Transverse

Table 1: Specimens different configurations cut from patches.

The strips have been mounted into mechanical tester (Model Mach-1, ©Biomomentum Inc.) grips and tested with uniaxial tensile test after preconditioning step. Preconditioning is done in order to generate a stable viscoelastic response within the tissue during testing. Typically, this involves applying a small subfailure tensile load over several sinusoidal cycles at specific extension rate. "The number of preconditioning cycles required to reach a stable or repeatable response" (e.g., w10 cycles) (Gordon et al, 2017) [11]. In our specific case, the number of preconditioning cycles was 10, the strain rates were of $1\%s^{-1}$ and $0.5\%s^{-1}$ respectively for preconditioning and failure tests. Strips dimensions (thickness, width, length) have been acquired with a manual caliper in multiple points to assess operator's reliability. For each configuration and subject, the sample size was decided on the basis of the availability of native tissue. Sample size for each configurations and dimensionality are reported in *Appendix B - Table 1-2*. Then, data collected have been analyzed with Matlab 2021b [13].

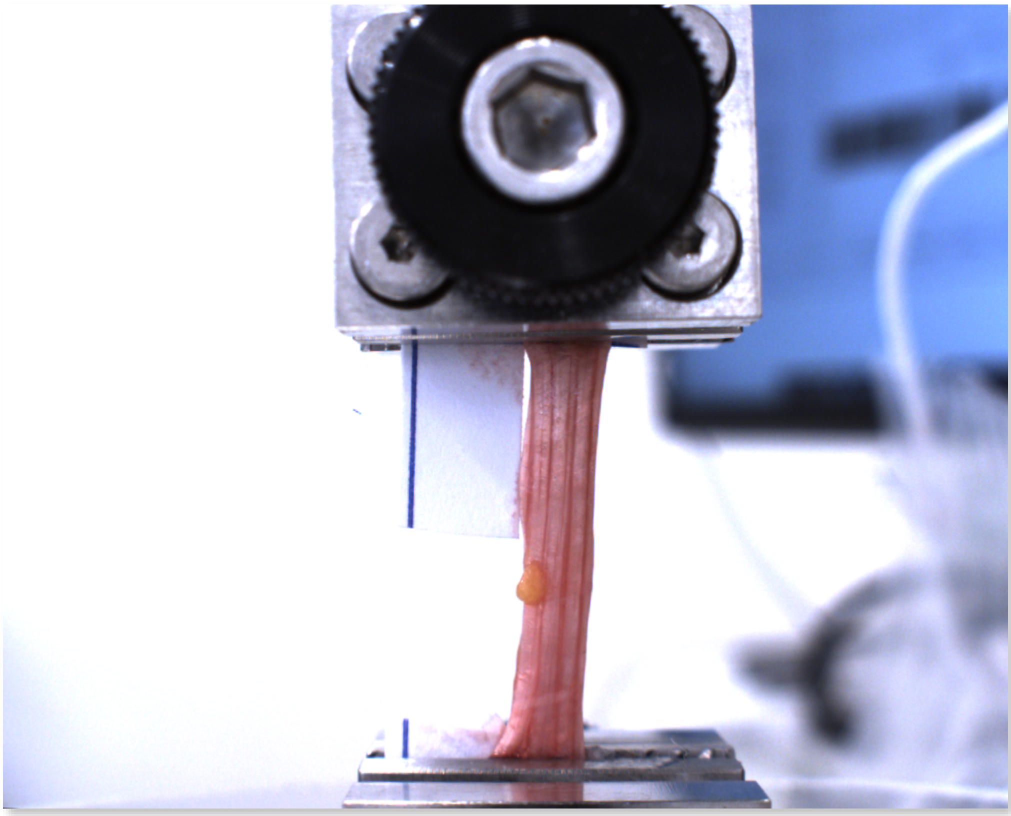


Figure 2: A Fascia Lata specimen during uniaxial tensile test

Figure 3: A Fascia lata specimen after the uniaxial tensile test

Specifically, failure protocols outcomes have been elaborated to estimate the slope of the following regions (see *Figure 4*) and the stress-strain values at break:

- TOE region;
- TRANSITION region;
- LINEAR (Young's Modulus) region;
- UP TO FAILURE region.

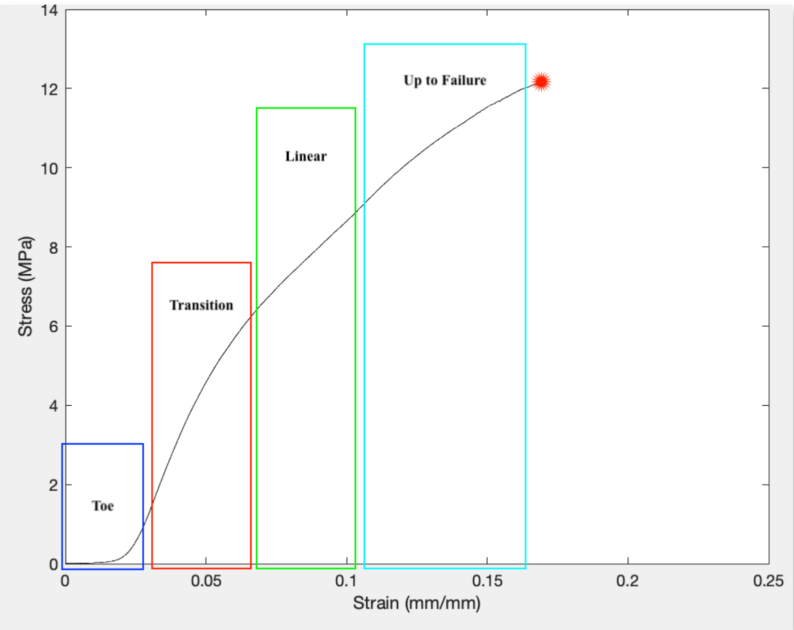


Figure 4: Example of different slope regions from failure test.

Algorithm	*Slopes calculation and Stress-strain at break*
INPUT: 4 selected points chosen by the user related to the beginning of each region of slope (Toe, transitioning, linear, up to failure), Delta strain values (length of each interval). OUTPUT: Slope ALGORITHM: - The user selects the 4 points; - Linear Fitting through *Curve-fitting* function; - Slope detection; - Printing the previous values (***Line slopes***) in a chart; - Printing also ***Stress-Strain at break*** chart.	

Table 2: Semi interactive algorithm to extract the slope values for each region and output stress-strain at break.

An ad hoc semi-interactive algorithm has been implemented (see *Table 2*), where the slope value for each region was found using a linear fit function from *built-in* Matlab app *(Curve fitting)*.

Results

In the current section we reported the results from literature review (see also *Appendix A - Table with literature results*) and experimental tests.

Literature Review

On the basis of the approaches presented in Materials and Methods for the analysis of the articles, the following is a summary of the significant information that emerged from the comparisons among some of the most relevant studies analyzed (5 articles selected/reported).

- I. Specimen:
  - I. Human [14] [15] [16]
    - I. Age: 26 [14], 75-95 [16],
    - II. M/F (%): 78 (14M-4F) [14], 50 [16]
  - II. Animal: Porcine [17], Goats [9],
- II. Tissue Histology, Morphometry and structural Configuration
  - I. Tissue Layers
    - I. Number: n/a
    - II. Thickness: 15,6mm [14], 355,6 µm [15], lateral site 0.8+/-0.2 other sites 0.2-0.3 [16], longitudinal 246.47 ± 42.8µm transversal 61.37 ± 8.7µm [9]
- III. Specimen Preparation
  - I. Tissue Storage
    - I. Fresh <48h [15], [9]
    - II. Freezed [14], [17]
    - III. Fixed: [16]
  - II. Mount of the Specimen
    - I. Soft tissue grip: Fixed to clamps with multiple small teeth [17], [15]
    - II. Cryoclamps: n/a
    - III. Sophisticated Clamps [14] [16] [9]

III. Cut of the Specimen

I. Region: *not specified [14] [17] [15] [9]*, specific-site location (anterior-lateral-medial-posterior) [16]

II. Dimension: 46.7x15.7mm [14], 10x2cm [17], 119.38x15.24mm [15], 40x40mm [16]

IV. Preconditioning

I. Number of Cycles: 1 [16], 5 [9]

II. Strain Rate: 25mm/min [16]

V. Uniaxial Test

I. Strain Rate: 25mm/min [16], 0.0015%/s^{-1} [9]

II. Stress-strain at break: 78.7 ± 4.6 MPa [14], five different measurement 24.34 - 34 MPa [17], transversal 38.4 ± 10.3MPa longitudinal 8.6 ± 2.9MPa [9]

VI. Results

I. Young modulus: 397,5 ± 17,1 MPa [14], higher in longitudinal (275.9 ± 83.2 MPa lateral, 71.6 ± 3 1.7 MPa medial, 107.8 ± 63.5 MPa posterior) than the transverse (lateral 3.2 ± 2.3 MPa, others 31.9–41.9 MPa) [16], *Preconditioning*

In our case we have conducted 10 cycles of preconditioning in order to prepare the sample before tensional test. In the table below is shown that it was possible to stop it before reaching the tenth cycle because the sample reached a stable response (95% of the loss) in less cycles, mean value after 5th cycle (see *Table 3, Figure 5).*

S1-S2	**S3**	**S4**
4	5	6
7	6	6
5	5	4
3	4	6
3	4	5
5	5	7
3	6	6
4	5	5
5	6	6
3	4	8
3	6	outlier
5	7	4
9		
4		
4		
6		
4,6	5,3	5,7
5,2		

Table 3: Cycles of Preconditioning.

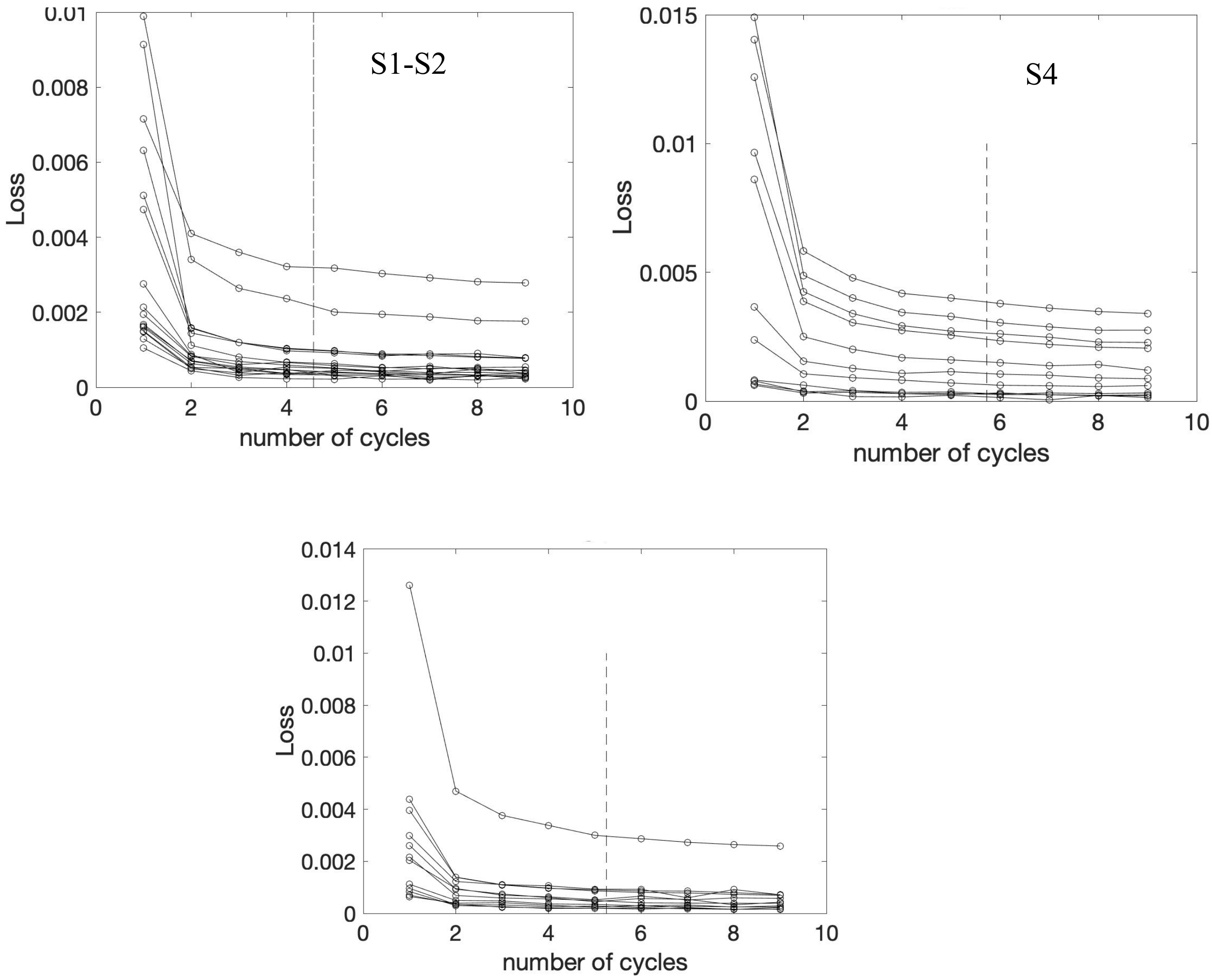


Figure 5: Repeatable response after a certain number of cycles for each subject.

Uniaxial tensile test (up to failure)

In *Figure 6* an example of uniaxial tensile test.

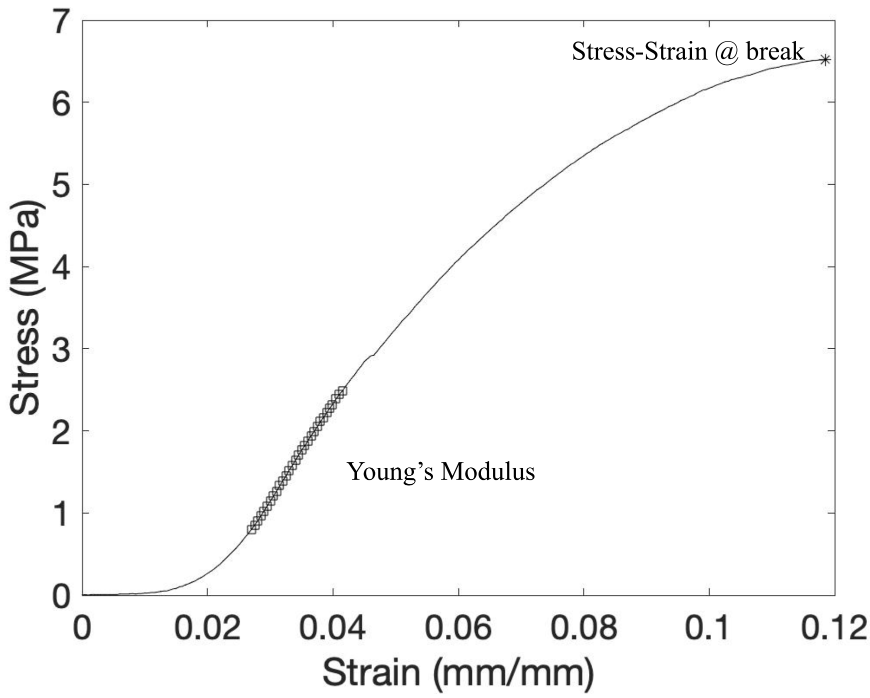


Figure 6: Stress-Strain @ break and Young's Modulus from uniaxial tensile test, as example.

In *Table 4-5* the values of strain-stress at break have been reported (mean values) excluding outliers. Raw data have been reported in *Appendix B - Table 3-4-5-6.*

	S1				**S2**			
	MEAN VALUE	DEV.ST	MEAN VALUE	DEV.ST	MEAN VALUE	DEV.ST	MEAN VALUE	DEV.ST
	Strain @ break		**Stress @ break**		**Strain @ break**		**Stress @ break**	
L1 P	0,132	0,060	5,410	2,689	0,121	0,043	2,089	0,744
L1 T	N/A	N/A	N/A	N/A	0,084	0,084	0,966	0,377
L2 P	0,222	0,125	0,759	0,361	0,135	0,032	1,063	0,184
L2 T	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Table 4: mean value and dev.st of strain@break and stress@break of S1-S2

	S3				S4			
	MEAN VALUE	DEV.ST	MEAN VALUE	DEV.ST	MEAN VALUE	DEV.ST	MEAN VALUE	DEV.ST
	Strain @ break		**Stress @ break**		**Strain @ break**		**Stress @ break**	
L1 P	0,132	0,053	5,243	4,779	0,180	0,067	1,510	1,183
L1 T	0,232	0,191	0,475	0,014	0,182	0,111	9,011	2,993
L2 P	0,200	0,088	1,979	0,735	0,163	0,038	12,040	1,047
L2 T	0,198	0,014	4,102	1,083	0,149	0,015	1,385	0,214

Table 5: mean value and dev.st of strain@break and stress@break of S3-S4

In *Table 6-7-8-9* has been reported the slope of each region and the relative strain (mean values). In Figure 7 the Young's Modulus graphical rendering. Raw data have been reported in Appendix B - Table 3-4-5-6. The relative strain has been computed as follow: $Relativestrain = strainstart/strainatbreak$

LAYER	DIRECTION	REGION	SLOPE		RELATIVE STRAIN	
			MEAN VALUE	STANDARD DEVIATION	MEAN VALUE	STANDARD DEVIATION
S1						
I	Principal	Toe	18,189	14,726	0,090	0,062
		Transition	54,841	26,435	0,200	0,127
		Linear	67,137	24,853	0,403	0,184
		Up to failure	13,394	10,509	0,871	0,058
II	Principal	Toe	2,433	0,606	0,049	0,011
		Transition	4,131	2,223	0,127	0,064
		Linear	5,946	1,811	0,303	0,017
		Up to failure	2,247	1,110	0,851	0,059

Table 6: mean value and standard deviation of the slope of the S1

I	Principal	Toe	7,285	3,665	0,084	0,042
		Transition	42,319	21,034	0,216	0,083
		Linear	29,165	11,587	0,406	0,072
		Up to failure	9,069	6,547	0,833	0,023
	Transverse	Toe	4,299	2,457	0,077	0,008
		Transition	10,941	2,870	0,286	0,021
		Linear	14,411	2,696	0,554	0,025
		Up to failure	7,885	3,844	0,895	0,012
II	Principal	Toe	4,572	1,248	0,066	0,015
		Transition	8,107	0,535	0,209	0,011
		Linear	10,279	1,647	0,458	0,034
		Up to failure	2,680	0,873	0,887	0,023

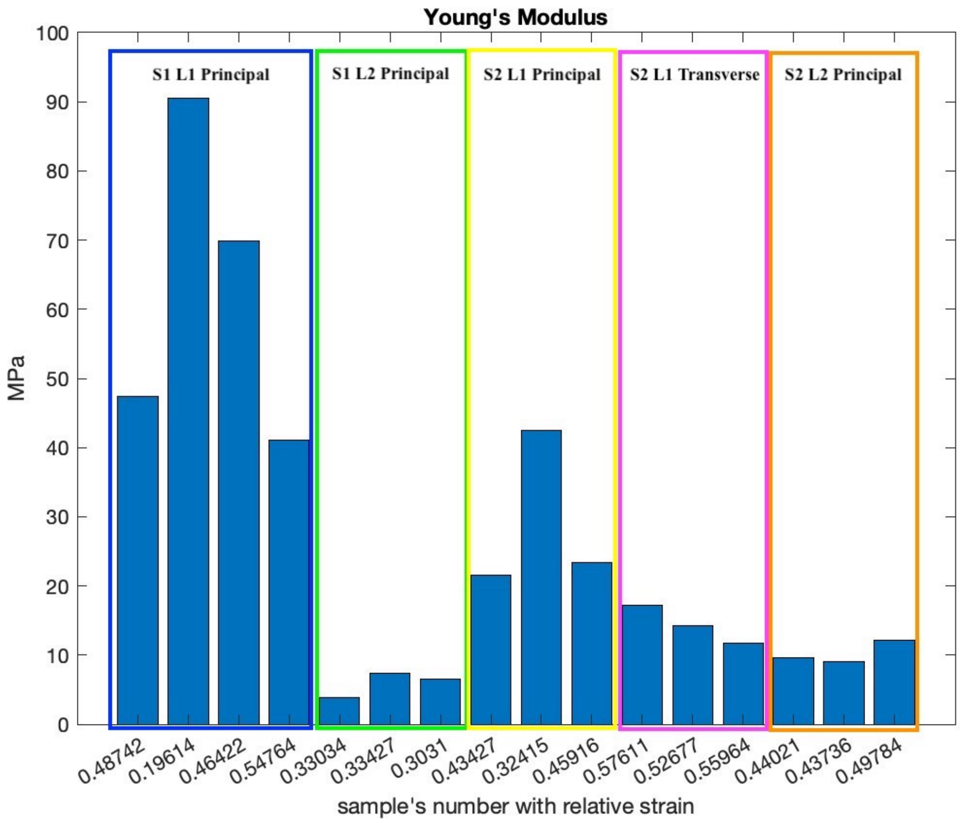
Table 7: mean value and standard deviation of the slope of the S2

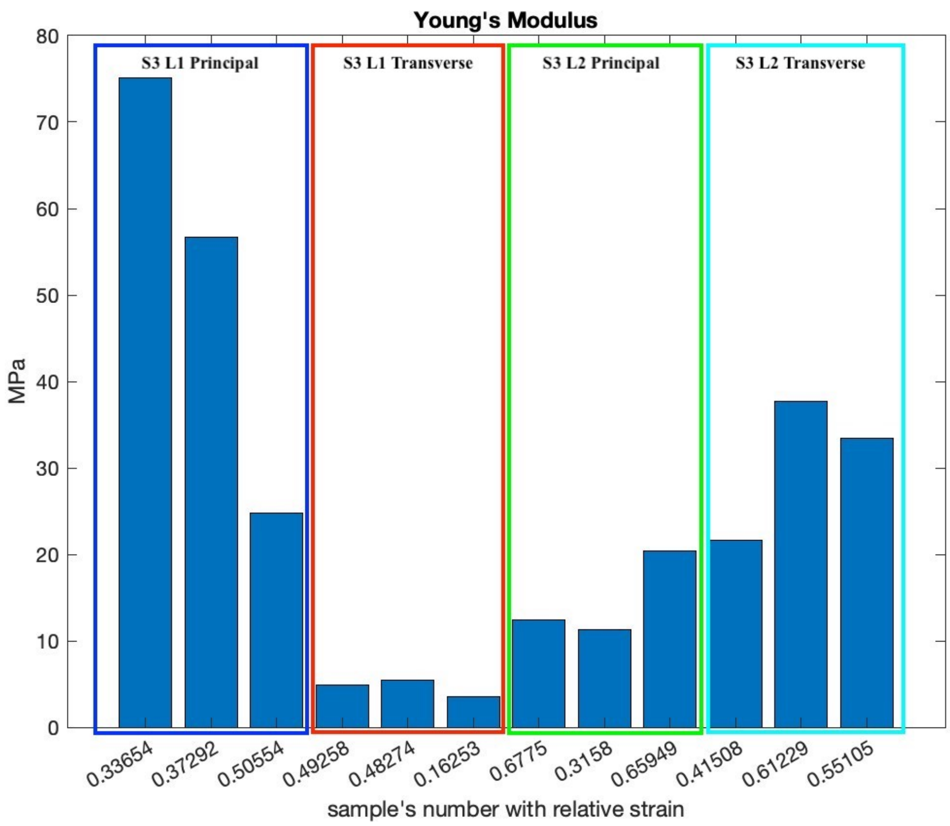
LAYER	DIRECTION	REGION	SLOPE		RELATIVE STRAIN	
			MEAN VALUE	STANDARD DEVIATION	MEAN VALUE	STANDARD DEVIATION
I	Principal	Toe	17,403	14,490	0,070	0,026
		Transition	78,111	48,478	0,210	0,072
		Linear	52,161	25,477	0,405	0,089
		Up to failure	7,442	6,873	0,824	0,052
	Transverse	Toe	2,631	0,441	0,046	0,023
		Transition	5,040	0,857	0,172	0,102
		Linear	4,636	0,961	0,379	0,188
		Up to failure	1,536	1,032	0,857	0,070
II	Principal	Toe	5,017	1,239	0,060	0,033
		Transition	10,826	3,590	0,226	0,105
		Linear	14,710	4,946	0,551	0,204
		Up to failure	11,373	9,039	0,910	0,015
	Transverse	Toe	6,948	2,387	0,054	0,008
		Transition	18,209	3,255	0,259	0,052
		Linear	30,892	8,356	0,526	0,101
		Up to failure	18,634	5,908	0,871	0,003

Table 8: mean value and standard deviation of the slope of the S3

LAYER	DIRECTION	REGION	SLOPE		RELATIVE STRAIN	
			MEAN VALUE	STANDARD DEVIATION	MEAN VALUE	STANDARD DEVIATION
I	Principal	Toe	5,215	3,906	0,077	0,038
		Transition	12,578	12,432	0,245	0,104
		Linear	15,305	14,058	0,424	0,135
		Up to failure	8,978	10,027	0,917	0,048
	Transverse	Toe	39,478	9,931	0,095	0,060
		Transition	137,071	59,877	0,233	0,096
		Linear	107,918	23,052	0,367	0,135
		Up to failure	24,772	21,449	0,858	0,078
II	Principal	Toe	94,854	66,481	0,104	0,042
		Transition	233,084	43,130	0,194	0,059
		Linear	124,665	31,325	0,280	0,083
		Up to failure	28,129	23,410	0,874	0,078
	Transverse	Toe	6,206	1,036	0,110	0,072
		Transition	12,224	1,447	0,292	0,122
		Linear	13,084	2,663	0,520	0,197
		Up to failure	7,101	4,720	0,870	0,097

Table 9: mean value and standard deviation of the slope of the S4


Young's Modulus
MPa
S1 L1 Principal
S1 L2 Principal
S2 L1 Principal
S2 L1 Transverse
S2 L2 Principal
0.48742
0.19614
0.46422
0.54764
0.33034
0.33427
0.3031
0.43427
0.32415
0.45916
0.57611
0.52677
0.55964
0.44021
0.43736
0.49784
sample's number with relative strain


Young's Modulus
MPa
S3 L1 Principal
S3 L1 Transverse
S3 L2 Principal
S3 L2 Transverse
0.33654
0.37292
0.50554
0.49258
0.48274
0.16253
0.6775
0.3158
0.65949
0.41508
0.61229
0.55105
sample's number with relative strain

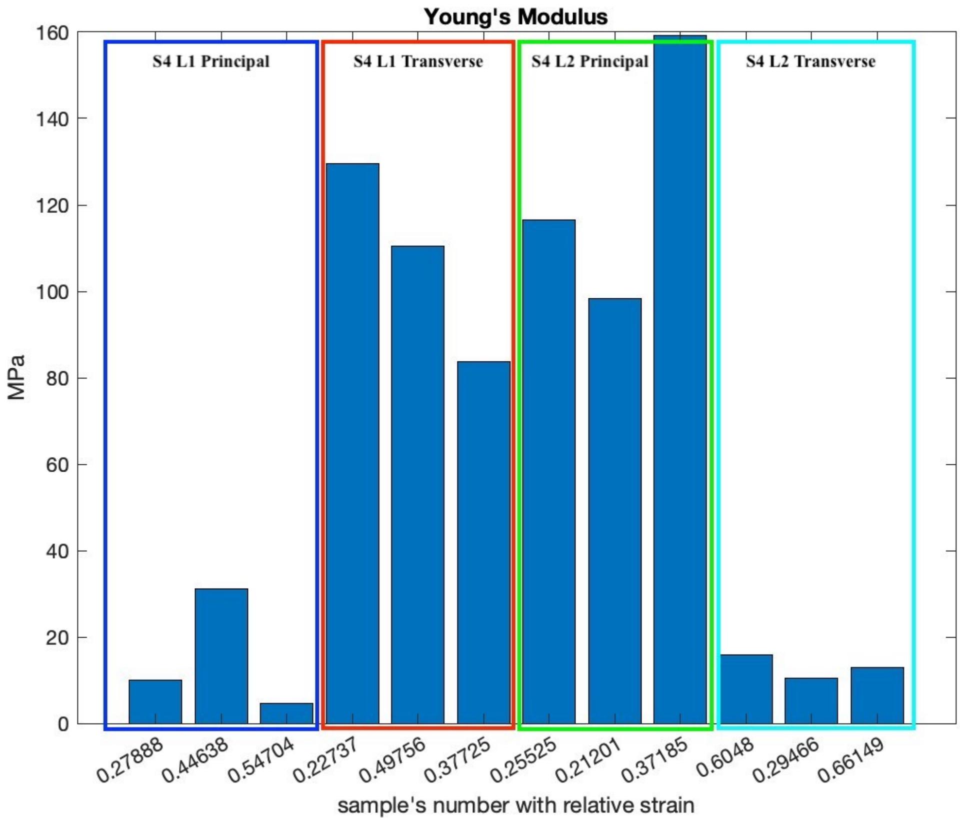


Figure 7: Young's Modulus graphical rendering of S1-S2-S3-S4

Discussion

From our analysis, experimental evidences on Fascia Lata are *mainly* focused in testing *animals* (like goats and porcine) [9, 17] or *humans* donors [14, 15, 16], in all these articles *uniaxial tensile tests* have been performed [14, 15, 16, 17, 9]. The specimen was generally stored with 3 different methods as fresh [9, 15], freezed [14, 17] or fixed with formalin [16]. Specimen fixation could alter biomechanical properties [16], but freezing it has been reported not to "… deep-freezing had no effect on the length and strength of fascia latae" (Kayurapan et al, 2010) [17]. Specimens were mounted mostly on sophisticated clamps [9, 14, 16] or on soft tissue grips [15, 17] e.g. fixed to clamps with multiple small teeth [17]. Gripping force should be monitored as trade off between avoiding slippage and tissue alterations.

The main shape used for the specimens was rectangular [14-17], and has been performed according various dimensions. Fascia Lata has been reported to have various thickness according to site location (Site-dependence of facia lata could be important also to define how the collagen fibers are oriented). Anyway, no information in term of layers differences have been specified. As example, Authors reported its bi-layered structures without proving the differences between layers with mechanical tests [14, 9]. Uniaxial test was conducted in all specimens [9, 14, 15, 16, 17] with various strain rate and maximum stress. Anyway, some difficulties emerged from literature in comparing uniaxial test outcomes from different works because, in some of them, key information regarding protocols details and results analysis such as preconditioning [14, 15, 17] and Young Modulus [9, 15, 17] respectively, still missing. For all the above mentioned reasons we decided to test with uniaxial load, human specimens of fresh frozen FL ex-vivo; cutting them into rectangular shape and testing them according to collagen fibers direction for each layers; monitoring gripping force to not alter tissue properties during experiments; performing a specific preconditioning protocol before failure cycle and specifying testing conditions (temperature monitoring) and parameters such as stain rate. Outcomes have been evaluated also in term of *toe/transition/YMs/up to failure* slope regions and stress-strain values at break. Toe region is defined by a crimping state between collagen fibers in which loading the specimen results in stretching the fibers before reaching the linear region. Different structures in the human body have a different toe region that depends on the orientation of the fibers: *"The more regular and parallel the collagenous fiber organization, the shorter the toe segment"* (Threlkeld et al, 1992) [18]. Transition region is the interface between toe region and the linear one. Linear region is where the tissue perform at his peak and the stress strain curve follows a linear trend. Collagen fibers un-crimping allows the layers to glide more freely between each other, resulting in ideal physiological working range. When the load is exceeding the maximum stress allowed, the fibers tend to break as long as the whole section, resulting in the rupture and damage of the specimen (failure).

In *Table 10-11* we reported the slope of the toe, transition, linear and up to failure regions as well as the relative strain values as outcomes of our experimental tests. Anyway, from the analyzed literature works we have not specific reference values in term of toe/transition/up to failure regions slopes.

SLOPE						
LAYER		MEAN VALUE	REGION	MEAN VALUE	LAYER	
I	Principal	5,215	Toe	6,206	Transverse	II
		12,578	Transition	12,224		
		15,305	Linear	13,084		
		8,978	Up to failure	7,101		
	Transverse	39,478	Toe	94,854	Principal	
		137,071	Transition	233,084		
		107,918	Linear	124,665		
		24,772	Up to failure	28,129		

Table 10: S4 slope comparison between region of two layers

RELATIVE STRAIN						
LAYER		MEAN VALUE	REGION	MEAN VALUE	LAYER	
I	Principal	0,077	Toe	0,110	Transverse	II
		0,245	Transition	0,292		
		0,424	Linear	0,520		
		0,917	Up to failure	0,870		
	Transverse	0,095	Toe	0,104	Principal	
		0,233	Transition	0,194		
		0,367	Linear	0,280		
		0,858	Up to failure	0,874		

Table 11: S4 relative strain of the two layers compared

Taking into consideration mostly the aspect regarding the YMs and the stess-strain values, our results confirmed that the FL has a different biomechanical behavior between subjects, confirming tissue anisotropy according to layers/directions (see *Table 12*).

Young's Modulus (MPa)									
Subject		S1		S2		S3		S4	
Layer	Direction	Mean	SD	Mean	SD	Mean	SD	Mean	SD
I	P	67,137	24,853	29,165	11,587	52,161	25,477	15,305	14,058
	T	-		14,411	2,696	4,636	0,961	137,071	59,877
II	P	5,946	1,811	10,279	1,647	14,710	4,946	124,665	31,325
	T	-		-		30,892	8,356	13,084	2,663

Table 12: Young's Modulus (mean values).

Moreover, e.g, in the fourth subject the YM (see *Table 10*) of the transverse cut of the first layer and the principal cut of the second layer, have almost the same values. This evidence suggests that also the inter-layer angle has a potential influence on the mechanical behavior of the composite tissue. In fact the principal direction of one layersalmost "collapses" on the transverse direction of the other layer and that is due to the fact that the interlayer angle, in the fourth specimen, is almost 90º (see *Table 13*).

	S1	S2	S3	S4
Inter-Layers Angle (°)	61	81	77	86

Table 13: Inter-layer Angle

	S1				S2			
	MEAN VALUE	DEV.ST	MEAN VALUE	DEV.ST	MEAN VALUE	DEV.ST	MEAN VALUE	DEV.ST
	Strain @ break		**Stress @ break**		**Strain @ break**		**Stress @ break**	
L1 P	0,132	0,060	5,410	2,689	0,121	0,043	2,089	0,744
L1 T	N/A	N/A	N/A	N/A	0,084	0,084	0,966	0,377
L2 P	0,222	0,125	0,759	0,361	0,135	0,032	1,063	0,184
L2 T	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	S3				**S4**			
	MEAN VALUE	DEV.ST	MEAN VALUE	DEV.ST	MEAN VALUE	DEV.ST	MEAN VALUE	DEV.ST
	Strain @ break		**Stress @ break**		**Strain @ break**		**Stress @ break**	
L1 P	0,132	0,053	5,243	4,779	0,180	0,067	1,510	1,183
L1 T	0,232	0,191	0,475	0,014	0,182	0,111	9,011	2,993
L2 P	0,200	0,088	1,979	0,735	0,163	0,038	12,040	1,047
L2 T	0,198	0,014	4,102	1,083	0,149	0,015	1,385	0,214

Table 14: mean value and dev.st of strain@break and stress@break

Conclusion

From the literature review we found out that FL has a multilayer structure, but literature still lacks of evidences that prove tissue anisotropy between layers. During this thesis project we tried to define an experimental protocol to prove this anisotropic aspect of the tissue though uniaxial tensile tests. Our data give evidences in term of slopes of toe, transition, linear and up to failure regions as well as stress-strain values at break. Outcomes highlight that the biomechanical behavior changes according to subjects/ layers/directions, confirming tissue anisotropy. In general, considering stress strain values we evince that where strain increases the relative stress decreases. Moreover, taking into consideration the Young's Modulus we can define that each layer has a dominant direction (principal or transverse) that changes if we analyze the other layer. The more the interlayer angle come close to 90º the more the dominant direction of a layer collapses on the dominant direction of the other one.

Therefore we conclude that the bi-layer structure of the FL influences the mechanical properties of the tissue (anisotropic behavior).

In conclusion we reached our purpose of trying to explain the importance of considering layers anisotropy to give more information to surgeons for the cut of the grafts, to engineers to perform in-silico simulation, to physiotherapists during musculoskeletal rehabilitation, all towards the management of a personalized medicine.

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Appendix A - Table with literature results

	Specimen	
	Age	%M/F
Effects of structure and strain maesurement thechnique on the material properties of young human tendons and fascia [14]	26	78% (14-4)
Effect of gamma sterilisation and deepfreezing on length and strength of fascia lata [17]	the speciments were taken form porcines	
Tensile strength and elasticity tests on human fascia lata [15]	N/A	
Site specificity of mechanical and structural properties [16]	75-95	50%
A constitutive description of the anisotropic response of the fascia lata [9]	the speciments were taken form goats	

	Tissue Hystology, Morphometry and structural configuration	
	Tissue Layers	
	Thickness	Number of layers
Effects of structure and strain maesurement thechnique on the material properties of young human tendons and [14]	15,6 mm	N/A
Effect of gamma sterilisation and deepfreezing on length and strength of fascia latae [17]	N/A	N/A
Tensile strength and elasticity tests on human fascia lata [15]	355,6 µm	N/A
Site specificity of mechanical and structural properties [16]	lateral site 0.8 +/- 0.2 other sites 0.2-0.3	N/A
A constitutive description of the anisotropic response of the fascia lata [9]	longitudinal 246.47 +/- 42.8 µm transversal 61.37 +/- 8.7 µm	N/A

	Specimen Preparation		
	Tissue Storage		
	Fresh <48h	Freezed	Fixed
Effects of structure and strain maesurement thechnique on the material properties of young human tendons and fascia [14]		X	
Effect of gamma sterilisation and deepfreezing on length and strength of fascia latae [17]		X	
Tensile strength and elasticity tests on human fascia lata [15]	X		
Site specificity of mechanical and structural properties [16]			Formalin
A constitutive description of the anisotropic response of the fascia lata [9]	X		

	Specimen Preparation		
	Mount of the Speciment		
	Soft Tissue Grips	Cryoclamps	Sofisticated Clamps
Effects of structure and strain maesurement thechnique on the material properties of young human tendons and fascia [14]	N/A		
Effect of gamma sterilisation and deepfreezing on length and strength of fascia latae [17]	fixed to clamps with multiple small teeth		
Tensile strength and elasticity tests on human fascia lata [15]	X		
Site specificity of mechanical and structural properties [16]			X
A constitutive description of the anisotropic response of the fascia lata [9]			X

	Specimen Preparation		
	Cut of the Speciment		Cut of the Speciment
	Region	Dimension	Region
Effects of structure and strain maesurement thechnique on the material properties of young human tendons and fascia [14]	fascia lata	46,7x15,7mm	fascia lata
Effect of gamma sterilisation and deepfreezing on length and strength of fascia lata [17]	Fascia lata	10x2 cm	Fascia lata
Tensile strength and elasticity tests on human fascia lata [15]	fascia lata	119,38x15,24 mm	fascia lata
Site specificity of mechanical and structural properties [16]	anterior, lateral, medial, posterior	40x40 mm	anterior, lateral, medial, posterior
A constitutive description of the anisotropic response of the fascia lata [9]	Facia lata	N/A	Facia lata

	Preconditioning	
	Number of Cycles	Strain Rate
Effects of structure and strain maesurement thechnique on the material properties of young human tendons and fascia [14]	N/A	N/A
Effect of gamma sterilisation and deepfreezing on length and strength of fascia lata [17]	N/A	N/A
Tensile strength and elasticity tests on human fascia lata [15]	N/A	N/A
Site specificity of mechanical and structural properties [16]	1	25mm/min
A constitutive description of the anisotropic response of the fascia lata [9]	one pair of preconditioning 5	N/A

	Unilaxial Test	
	Strain Rate	Maximum Stress
Effects of structure and strain maesurement thechnique on the material properties of young human tendons and fascia [14]	N/A	78,7 +/- 4,6 Mpa
Effect of gamma sterilisation and deepfreezing on length and strength of fascia lata [17]	N/A	MPa 1 24,34±9.3 2 27.63±8.1 3 24.24±7.9 4 26.09±3.0 5 34.00±6.8
Tensile strength and elasticity tests on human fascia lata [15]	N/A	N/A
Site specificity of mechanical and structural properties [16]	25mm/min	N/A
A constitutive description of the anisotropic response of the fascia lata [9]	0.0015^(-1)s	(stress cauchy failure) transversal 38.4 +/- 10.3 Mpa longitudinal 8.6 +/- 2.9 Mpa

	Results	
	Young Modulus	Conclusions
Effects of structure and strain maesurement thechnique on the material properties of young human tendons and fascia [14]	397,5 +/- 17,1 Mpa	Significant differences were measured between the material properties of tendons and fascia lata preparations taken from young human donors (partially caused by tissue structure). results have implications in the selection of biological substitutes as replacements for torn ligaments, in the design of synthetic ligament implants, and in mathematical modeling of both soft tissues and the knee.
Effect of gamma sterilisation and deepfreezing on length and strength of fascia lata [17]	N/A	The maximum tensile strength was not significantly different among groups 1 to 4, but was significantly higher in group 5. (Group 1 control, 2-3-4 freezed and gamma irradiated, 5 fixed on a wooden board)
Tensile strength and elasticity tests on human fascia lata [15]	N/A	The sutures should be made longitudinally in the direction of the fibers, since the fascia lata has very little strength in the transverse direction
Site specificity of mechanical and structural properties [16]	2-90 higher in longitudinal (275.9 ± 83.2 MPa lateral, 71.6 ± 3 1.7 MPa medial, 107.8 ± 63.5 MPa posterior) than the transverse (lateral 3.2 ± 2.3 Mpa, others 31.9–41.9 MP)	The present study shows that the fascia lata possesses sites and gender dependence of the morphological characteristics and elastic properties
A constitutive description of the anisotropic response of the fascia lata [9]	N/A	a suitable strain-energy function to describe the anisotropic response of the fascia

Appendix B - Tables with experimental results

Number of specimens FL - Failure	Layer 1		Layer 2	
Subject/Direction	Principal	Transverse	Principal	Transverse
S2	4	-	3	-
S3	3	3	3	-
S4	3	3	3	3
S5	3	3	3	3

Table 1

Failure FL							
Subject	Layer	Direction	Dimensions	Initial length (mm)	Width (mm)	Thickness (mm)	Temperature (°C)
S1	L1	P	1 (OUTLIER - double curves due to slippage)	30	3	0,7	19,5
			2	30	3	0.7	19,3
			3	30	3	0,65	19,3
			4	30	3	0,6	19,3
	L2	P	1	30	3	0,6	19,9
			2	30	3	0,7	20,5
			3	30	3	0,8	20,5
S2	L1	P	1 (NO OUTLIER - uncertain slippage)	30	3	0,95	21,5
			2	30	3	0,7	22
			3	30	3	0,6	22
		T	1	30	3	0,4	21,5
			2	30	3	0,5	21,7
			3	30	3	0,6	21,8
			1	30	3	0,6	21,7

	L2	P	2	30	3	0,6	21,9
			3	30	3	0,65	20,9
S3	L1	P	1	30	6,3	0,4	19,4
			2 (OUTLIER - slippage)	30	6	0,6	19,5
			3	30	4,7	0,8	19,8
		T	1	30	6,4	0,6	19,7
			2	30	4,6	0,5	19,8
			3	30	4,3	0,5	19,9
	L2	P	1	30	4,4	1	19,5
			2	30	2,8	0,6	19,6
			3	30	3	0,7	20
		T	1	30	3,5	0,5	17,5
			2	30	4,2	0,5	17,9
			3 (NO OUTLIER- Twisted)	30	2,4	0,6	18
S4	L1	P	1	30	3,1	1	20,4
			2	30	2,9	0,9	22
			3	30	3,4	0,9	21
		T	1	30	3,9	0,8	18,4
			2	30	3,2	0,8	22,3
			3	30	3,5	0,7	20,5
	L2	P	1	30	3,5	0,7	21,3
			2	30	3,6	0,7	21,9
			3	30	3,1	0,6	21,5
		T	1	30	4,4	0,6	20,5
			2	30	4	0,6	22,7
			3	30	3,6	0,5	21,3

Table 2

Layer	Direction	Toe	Relative strain if compared to strain at break (strain/ strain @ break) - Toe	Transition	Relative strain if compared to strain at break (strain/ strain @ break) - Transition	YM	Relative strain if compared to strain at break (strain/ strain @ break) - Linear	Up to failure	Relative strain if compared to strain at break (strain/ strain @ break) - Up to failure
I	P	12,143	0,154	50,767	0,308	47,472	0,487	25,622	0,891
	P	33,113	0,036	71,721	0,092	90,512	0,196	17,827	0,814
	P	17,784	0,076	68,426	0,169	69,869	0,464	20,960	0,869
	P	3,670	0,158	24,376	0,340	41,031	0,548	1,395	0,931
II	P	2,928	0,061	3,496	0,120	3,910	0,330	0,967	0,783
	P	2,614	0,049	6,602	0,135	7,378	0,334	2,926	0,876
	P	1,757	0,038	2,294	0,126	6,549	0,303	2,849	0,893
I	P	7,183	0,127	29,094	0,308	21,610	0,434	15,093	0,847
	P	6,849	0,044	31,290	0,148	42,506	0,324	2,101	0,845
	P	7,824	0,082	66,574	0,190	23,379	0,459	10,013	0,807
	T	7,136	0,071	14,035	0,278	17,159	0,576	12,284	0,909
	T	2,895	0,073	10,421	0,310	14,306	0,527	6,203	0,888
	T	2,865	0,086	8,367	0,270	11,770	0,560	5,169	0,888
II	P	4,694	0,077	10,238	0,203	9,572	0,440	2,117	0,895
	P	3,268	0,049	10,892	0,203	9,104	0,437	2,238	0,860
	P	5,755	0,071	11,300	0,222	12,162	0,498	3,686	0,905

Table 3: Slope S1-S2

Layer	Direction	Toe	Relative strain if compared to strain at break (strain/ strain @ break) - Toe	Transition	Relative strain if compared to strain at break (strain/ strain @ break) - Transition	YM	Relative strain if compared to strain at break (strain/ strain @ break) - Linear	Up to failure	Relative strain if compared to strain at break (strain/ strain @ break) - Up to failure
I	P	33,658	0,053	133,262	0,151	75,086	0,337	15,273	0,770
	P	12,709	0,057	58,836	0,191	56,664	0,373	2,409	0,828
	P	5,842	0,100	42,235	0,290	24,732	0,506	4,644	0,874
	T	2,142	0,062	5,777	0,220	4,882	0,493	2,426	0,923
	T	2,998	0,057	5,245	0,242	5,450	0,483	1,777	0,864
	T	2,752	0,020	4,099	0,055	3,577	0,163	0,404	0,783
II	P	3,769	0,096	6,853	0,337	12,467	0,677	8,214	0,912
	P	5,037	0,032	11,790	0,128	11,282	0,316	4,338	0,894
	P	6,247	0,051	13,836	0,212	20,380	0,659	21,568	0,924
	T	7,423	0,048	14,746	0,208	21,576	0,415	17,859	0,870
	T	4,359	0,052	18,676	0,311	37,724	0,612	24,891	0,869
	T	9,062	0,063	21,206	0,259	33,375	0,551	13,152	0,875

Table 4: Slope S3

Layer	Direction	Toe	Relative strain if compared to strain at break (strain/ strain @ break) - Toe	Transition	Relative strain if compared to strain at break (strain/ strain @ break) - Transition	YM	Relative strain if compared to strain at break (strain/ strain @ break) - Linear	Up to failure	Relative strain if compared to strain at break (strain/ strain @ break) - Up to failure
I	**P**	3,630	0,043	6,966	0,164	10,064	0,279	3,191	0,955
	P	9,664	0,069	26,827	0,209	31,231	0,446	20,556	0,863
	P	2,349	0,118	3,940	0,363	4,621	0,547	3,187	0,934
	T	28,542	0,033	130,279	0,125	129,61	0,227	1,452	0,789
	T	47,933	0,153	200,054	0,311	110,41	0,498	43,657	0,842
	T	41,960	0,099	80,879	0,262	83,719	0,377	29,208	0,942
II	**P**	34,412	0,082	183,443	0,188	116,46	0,255	46,310	0,798
	P	84,090	0,077	254,443	0,138	98,253	0,212	36,363	0,870
	P	166,06	0,153	261,367	0,256	159,27	0,372	1,715	0,955
	T	5,258	0,190	11,958	0,422	15,867	0,605	4,961	0,932
	T	7,312	0,050	13,786	0,181	10,561	0,295	3,830	0,758
	T	6,047	0,090	10,929	0,272	12,824	0,661	12,512	0,920

Table 5: Slope S4

Layer	Direction	Strain @ break	Stress @ break
		S1-S2	
I S1	P	0,078	2,512
	P	0,197	7,366
	P	0,119	6,520
	P	0,080	2,343
II S1	P	0,150	0,342
	P	0,226	0,972
	P	0,291	0,963
I S2	P	0,072	1,238
	P	0,139	2,413
	P	0,153	2,616
	T	0,099	1,394
	T	0,076	0,820
	T	0,076	0,683
II S2	P	0,163	1,271
	P	0,143	0,922
	P	0,100	0,996
		MEAN VALUE	
		0,130	**1,929**

Layer	Direction	Strain @ break	Stress @ break	Strain @ break	Stress @ break
		S3		S4	
I	P	0,170	8,622	0,257	1,203
	P	0,195	6,668	0,139	2,815
	P	0,095	1,864	0,144	0,510
	T	0,130	0,490	0,304	12,467
	T	0,114	0,470	0,086	7,304
	T	0,453	0,463	0,157	7,263
II	P	0,141	1,157	0,171	12,189
	P	0,301	2,211	0,196	10,926
	P	0,159	2,571	0,121	13,004
	T	0,188	2,958	0,132	1,253
	T	0,214	5,112	0,158	1,270
	T	0,192	4,236	0,157	1,632
		MEAN VALUE		**MEAN VALUE**	
		0,196	**2,741**	**0,155**	**5,526**

Table 6: Strain-stress at break S1-S2, S3, S4

LAYER	DIRECTION	REGION	SLOPE		RELATIVE STRAIN	
			MEAN VALUE	STANDARD DEVIATION	MEAN VALUE	STANDARD DEVIATION
S1						
I	Principal	Toe	18,189	14,726	0,090	0,062
		Transition	54,841	26,435	0,200	0,127
		Linear	67,137	24,853	0,403	0,184
		Up to failure	13,394	10,509	0,871	0,058
II	Principal	Toe	2,433	0,606	0,049	0,011
		Transition	4,131	2,223	0,127	0,064
		Linear	5,946	1,811	0,303	0,017
		Up to failure	2,247	1,110	0,851	0,059
S2						
I	Principal	Toe	7,285	3,665	0,084	0,042
		Transition	42,319	21,034	0,216	0,083
		Linear	29,165	11,587	0,406	0,072
		Up to failure	9,069	6,547	0,833	0,023
	Transverse	Toe	4,299	2,457	0,077	0,008
		Transition	10,941	2,870	0,286	0,021
		Linear	14,411	2,696	0,554	0,025
		Up to failure	7,885	3,844	0,895	0,012
II	Principal	Toe	4,572	1,248	0,066	0,015
		Transition	8,107	0,535	0,209	0,011
		Linear	10,279	1,647	0,458	0,034
		Up to failure	2,680	0,873	0,887	0,023

Table 7: Slope and relative strain of S1-S2

SLOPE						
LAYER		MEAN VALUE	REGION	MEAN VALUE	LAYER	
I	Principal	17,403	Toe	6,948	Transverse	II
		78,111	Transition	18,209		
		52,161	Linear	30,892		
		7,442	Up to failure	18,634		
	Transverse	2,631	Toe	5,017	Principal	
		5,040	Transition	10,826		
		4,636	Linear	14,710		
		1,536	Up to failure	11,373		

Table 8: Slope of S3

RELATIVE STRAIN						
LAYER		MEAN VALUE	REGION	MEAN VALUE	LAYER	
I	Principal	0,070	Toe	0,054	Transverse	II
		0,210	Transition	0,259		
		0,405	Linear	0,526		
		0,824	Up to failure	0,871		
	Transverse	0,046	Toe	0,060	Principal	
		0,172	Transition	0,226		
		0,379	Linear	0,551		
		0,857	Up to failure	0,910		

Table 9: Relative strain of S3