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Experimental Evaluation of Fiber Orientation Based Material Properties of Skeletal Muscle in Tension

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Abstract: Biomechanical researches are essential to develop new techniques to improve the clinical relevance. Skeletal muscle generates the force which results in the motion of human body, so it is essential to study the mechanical and structural properties of skeletal muscle. Many researchers have carried out mechanical study of skeletal muscle with in-vivo testing. This work aims to examine anisotropic mechanical behavior of skeletal muscle with in vitro test (tensile test).
It is important to understand the mechanical and structural behavior of skeletal muscle when it is subjected to external loading; the research aims to determine the structural properties of skeletal muscle by tensile testing. Tensile testing is performed on 5 samples of skeletal muscle of a goat at the rate of 1mm/min with fiber orientation along the length and 45˚ inclined to the length. It is found that muscle is stiffer in the direction parallel to the muscle fiber than at 45˚ to the muscle fibers. The tensile strength of the skeletal muscle along the fiber direction is 0.44 MPa at maximum load of 110 N and for direction 45˚ inclined to the muscle fibers, the strength is 0.234 MPa at max load 43 N. The displacement of Muscle sample against the maximum load is small along the length of the muscle fiber i.e. under longitudinal elongation [15.257 mm] as compared to 45˚ inclined to the length of skeletal muscle [17.775 mm] and under cross fiber elongation [19.7291mm by FEA]. The testing is not performed for 90˚ fiber orientation due to unavailability of soft tissue in cross fiber direction of the required specification, but finite element analysis is done on the skeletal muscle for the cross fiber orientation. As the fiber orientation within skeletal muscle differs with respect to the length of the muscle, the stiffness of skeletal muscle is also changing effectively. Hence skeletal muscle exhibits the anisotropic mechanical behavior.

Keywords: In-vitro, tensile test, fiber orientation, stiffer, skeletal muscle, anisotropic, FEA (Finite element analysis).

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1 Introduction

Basic and major content of skeletal muscle include about 70 – 80 % water, 10-12 % collagen and 3% fat [1]. Each skeletal muscle fiber is a single cylindrical muscle cell. An individual skeletal muscle may be made up of hundreds, or even thousands, of muscle fibers bundled together and wrapped in a connective tissue covering. Each muscle is surrounded by a connective tissue sheath called the epimysium. Fascia, connective tissue outside the epimysium, surrounds and separates the muscles. Portions of the epimysium project inward to divide the muscle into compartments. Each compartment contains a bundle of muscle fibers, see fig. 1. Each bundle of muscle fiber is called a fasciculus and is surrounded by a layer of connective tissue called the perimysium. Within the fasciculus, each individual muscle cell, called a muscle fiber, is surrounded by connective tissue called the endomysium [2]. It shows that the muscles are composed of oriented fibers to perform its basic function of force generation. As the biological soft tissue exhibits the non linear, viscoelastic and anisotropic behavior, with the same perspective fiber oriented muscle also exhibit the same material behavior [3-6].

Mechanical characterization of soft tissue by in vitro method is very difficult because of its hyperelastic, viscoelastic and highly nonlinear behavior [7,8]. Skeletal muscle is highly nonlinear and hyperelastic in behavior with a perspective of development of Bioengineering and tissue engineering, it is essential to measure the mechanical characteristics of skeletal muscle, which could improve the understanding of how various conditions affects the performance of muscle [9-11]. It could also help to develop the bio-mimetic materials and advances the clinical evaluation of muscle. Measurement of muscle force and tissue load-displacement is not possible. In contrast, advancement in computational models of skeletal muscle was made from the basic Hill-type muscle model to three dimensional constitutive muscle models [12]. Finite element models of the human body were developed to study the deformations for static and transient loading [13]. To analyze the behavior of these models, it requires a well defined material properties of soft tissue and hard tissue under large deformations. Although hyperelastic and anisotropic properties of skeletal muscle tissue have not been well defined experimentally, this puts the limitation for computational models of skeletal muscle. Skeletal muscle tissue contributes half of the body weight; hence well defined properties of skeletal muscle tissue are required for many constitutive models of skeletal muscle and for understanding the musculoskeletal system in diverse applications [14].

The skeletal muscle structure reveals that it can behave as transversely isotropic [10]. Some investigators characterize muscle in the fiber direction, where as transversely isotropic behavior may be characterized by testing the soft tissue under fiber direction (Longitudinal extension) and cross fiber direction (Transverse extension)

[6,15,16]. While some of the investigators measured the stress-strain characteristics of muscle across the entire musculotendinous unit, results could not isolate the properties of muscle tissue itself [17-19].

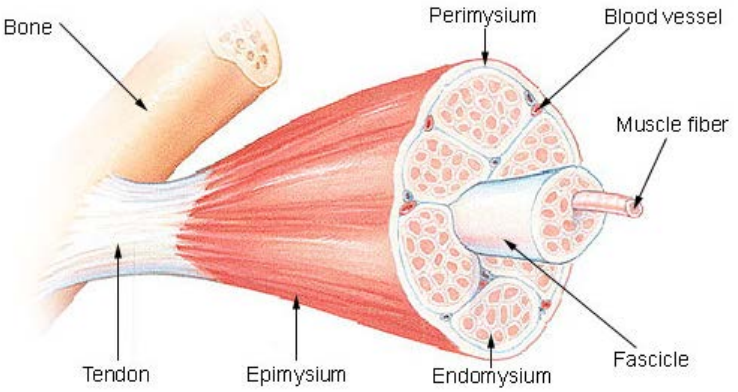


Figure 1: Structure of skeletal muscle

Transversely isotropic material properties of skeletal muscle have been determined experimentally under tension; results reported that muscle is stiffer in the fiber direction as compared to transverse direction [3,4,10,14,20]. Studies of the muscle under compression reported that skeletal muscle tissue is much stiffer in the transverse direction than in the fiber direction [5,20]. In the per view of this conflict, some authors could provide the experimental data for tensile loading that muscle is stiffer in transverse direction than in the fiber direction during the experiment for tensile loading [6,20,21].

The mechanical behavior of skeletal muscle depends on the fiber direction in tension has been studied at intermediate angles by Michael Takaza et al. The result shows the muscle could be stiffer in the transverse or cross fiber direction than the fiber direction, but the results from Van Fe et al., Morrow et al., Linder-Ganz, Blemker and Delp have indicated the opposite, see Fig.2. The difference in material properties of the skeletal muscle tissue was significant see table 1. Therefore, it is concluded that there is disparity in understanding of the tensile response of passive muscle to load. The aim of this paper is to characterize the passive muscle in tension and to assess of fiber direction dependency of the tensile response of skeletal muscle. As a conflict arises from the previous studies on the tensile response of passive muscle, muscle response with respect to the fiber direction is a focus of the present paper.

This research aims to 1] determine the fiber oriented passive behavior of the skeletal muscle, 2] compute the stiffness of skeletal muscle along the different fiber orientation of the muscle fibers by in vitro test and finite element analysis and 3] compare the in vitro test results with the finite element analysis. Overall the null hypothesis of the present study is, the skeletal muscle is stiffer in the fiber direction than cross fiber direction at significance level of 5 % (H_0: $E_P > E_C$ at $t_{0.05}$). The alternate hypothesis of the study is the skeletal muscle is stiffer in cross fiber direction than the fiber direction (H_1: $E_P < E_C$ at $t_{0.05}$).

E_C= Mean modulus of elasticity of muscle in cross fiber direction.

E_P= Mean modulus of elasticity of muscle in fiber direction.

Table 1: Tensile response of passive skeletal muscle.

Tensile Response of passive skeletal muscle				
Authors	Strain rate ($\%s^{-1}$)	Modulus of Elasticity (KPa)		Remarks
		Fiber direction	Cross fiber direction	
Morrow et al., 2008 (Rabbit) [3]	0.05	767	81	Fiber direction stiffer
Morrow et al., 2010 (Rabbit) [4]	0.05	447	22.4	Fiber direction stiffer
Nie et al.,2011 (Pig) [21]	5.00	100	59	Fiber direction stiffer
Calvo et al.,2010 (Rat) [22]	0.025	46		Fiber direction stiffer
Martin et al., 2006 (Pig) [7]		35		Fiber direction stiffer
Blemker and Delp, 2005 [10]		2700	50	Fiber direction stiffer
Mathur et al., 2001 [23]		100-700		Fiber direction stiffer
Michael Takaza et al. 2013 [6]	0.05	10	77	Cross fiber direction stiffer

2 Experimental Method

2.1 Testing Specimen

Fresh skeletal muscle tissues are extracted from 2 year old male goat. Samples of skeletal muscle for tensile test are prepared for it as per the specification of ASTM E8M for tensile test method. Sample size is approximately 10mm x 10mm in cross section and 60 mm in length. It is difficult to prepare the sample of the exact dimension as per the standard specification because of insufficient muscle tissues extracted from the goat with correct fiber orientation. Samples are prepared and categorized into two categories as per fiber orientation with respect to the length. Sample size for the present study is calculated with 5% significance level and statistical power of 95% to follow the hypothesis. The hypothesis of this study will

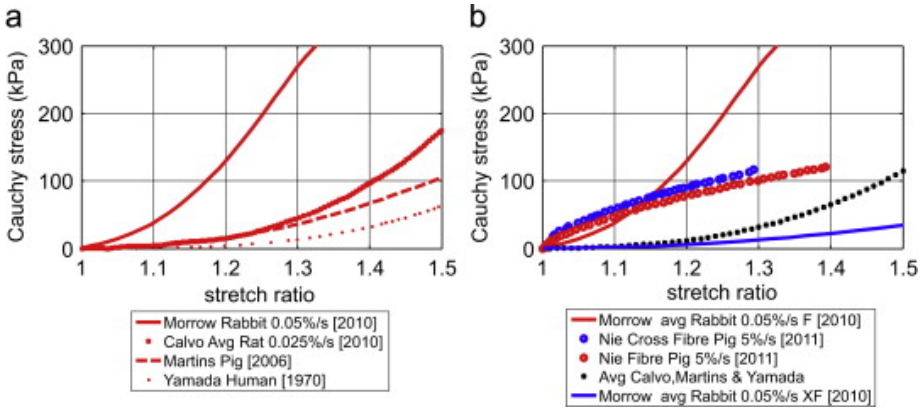


Figure 2: Tensile response of skeletal muscle from literature [6]: a. Fiber direction, b. Cross fiber direction.

report that skeletal muscle is stiffer in the fiber direction than cross fiber direction. Survey of existing studies resulted into 19% margin of error [3,4] and standard deviation in modulus of elasticity in cross fiber direction is 22KPa [3,4,6,10,21]. Sample size for this research is calculated using following equation (1) [24,25].

$$n = \frac{1.96^2 \sigma^2}{E^2} \tag{1}$$

$n =$ Sample size

$\sigma =$ standard deviation (22 Kpa from the survey of existing studies)

$E =$ margin of error (19% from the survey of existing studies)

$$n = \frac{1.96^2 * 22^2}{19^2} = 5.150 \approx 5$$

Statistically calculated sample size of the present research is 5 specimens in each set.

A set of 5 samples with fiber orientation in the direction of the length while the other set is about the same sample size with fiber orientation 45˚ inclined to length. Samples with fiber orientation 90˚ inclined to length are difficult to extract due to limitation on availability of the required tissue. Tensile test is performed on only two sample sets using Instron 3345 biomaterials tensile test machine with 5 KN load cell as shown in figure 3. The test is performed well within few hours after the animal's death to optimize the effect of Mortis on results [6,16]. All the samples are preserved in formalin solution. Bio grips with serrated faces are used to clamp

the sample which gives better grip and minimize the effect of slippage and initial stress on the results.

(a) (b)

Figure 3: Test specimen: (a) Specimen clamped in the grips, (b) Specimen during a tensile test.

2.2 Experimentation

Samples of skeletal muscle tissue are divided into two sets, labelled as Muscle-P [fiber orientation along the length] and Muscle-I [fiber orientation 45˚ inclined to length]. The sample is clamped between the crossheads on test machine using granular rubber grips with 250 N grip forces to avoid damage of the sample at grips. Before the start of the test, samples are taken out of formalin solution and cut into required geometry appropriate to the ASTM E8M standard specification. All the samples are tested with the use of the automated tensile test machine so load – displacement [stress-strain] data is recorded automatically by software which is connected to the machine. The test temperature is maintained between 18˚ to 30˚C and humidity 60 %. All the samples are loaded at a strain rate of $0.15s^{-1}$. For the validation of experimental results, finite element method a mathematical tool is used.

3 Finite Element analysis

Skeletal Muscle tissue is almost incompressible. Incompressibility is a constraint to the model for finite element analysis (FEA) of skeletal muscle. To satisfy the incompressibility condition in FEA, Poisson's ratio is assumed as 0.499. Mixed displacement-pressure formulation was developed and that is implemented in an ANSYS element description of hyperelastic almost incompressible materials. In this formulation, the pressure is introduced as a new degree of freedom at the element level. The strain energy density must in addition balance the new degree of freedom and the pressure calculated by the volume changes. In the present study, experimental results are simulated using FEA. Finite element analysis is performed on the model of skeletal muscle of the dimensions similar to that of experimental specimen. The hyperelastic behavior of skeletal muscle tissue is simulated using finite element method using Ansys version 14.5 (Ansys, Inc.). FEA is performed with the two distinct material parameters as per their fiber direction; one is having fiber direction along the length and other with cross fiber direction to length. Finite element analysis of all the models is explained in following section.

3.1 Geometry

Model of skeletal muscle tissue is as per the dimensions of the specimen used in the experiment (10 mm $\times$ 10 mm $\times$ 60 mm). The same model is used for different fiber orientations. The fiber orientations as along the length (longitudinal fiber direction), 45° inclined to the length and perpendicular to the length (cross fiber direction) are simulated using different hyperelastic material properties for each.

3.2 Material

The material behavior is considered as hyperelastic for finite element analysis of skeletal muscle tissue. Mooney Rivlin 5 parameter model is used for specifying the hyperelasticity of skeletal muscle tissue for each case. To incorporate the incompressibility condition, poisson's ratio is considered as 0.499 and compressibility parameter is taken as 0. For all the fiber direction, this study used different hyperelastic material parameters see table 2.

3.3 Meshing

For the hyperelastic behavior of skeletal muscle tissue with incompressibility and distinct fiber orientations, the element used for meshing is a SOLID 185 in Ansys software. SOLID185 is used for 3-D modelling of solid structures. It is defined by eight nodes having three degrees of freedom at each node: translations in the nodal x, y, and z directions. The element has plasticity, hyperelasticity, stress stiffening,

Table 2: Material Parameters for skeletal Muscle [4].

Material Parameters.	For Longitudinal fiber direction [Fiber orientation 0˚]	For Cross fiber direction [Fiber orientation 90˚]
Poisson's ratio	0.499	0.499
Density [Kg/ mm^3]	1.073e-6	1.073e-6
C_{10} [MPa]	0.74639	0.078742
C_{01} [MPa]	-0.749	-0.082404
C_{20} [MPa]	-0.25071	-0.02500
C_{11} [MPa]	0.77279	0.09465
C_{02} [MPa]	-0.84041	-0.082404
d	0	0
Where, C_{10}, C_{01}, C_{20}, C_{11} and C_{01} are the Mooney–Rivlin hyperelastic material parameters. d-Compressibility parameter		

creep, large deflection, and large strain capabilities. It also has the mixed formulation capability for simulating deformations of nearly incompressible elastoplastic materials, and fully incompressible hyperelastic materials. The model is discretized into small elements using Solid 8 node brick element, i.e. SOLID 185 using volume, mapped meshing see figure 4.

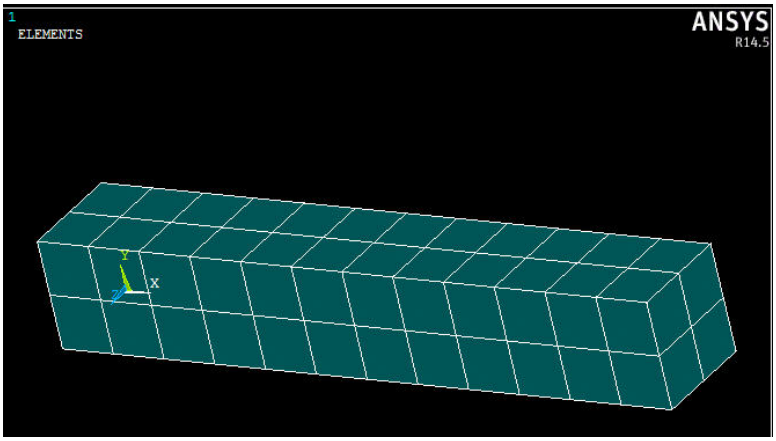


Figure 4: Volume Mapped Meshing Using SOLID 185 (8 Node Brick Element).

3.4 Boundary conditions and convergence conditions

In order to simulate the hyperelastic material behavior of skeletal muscle, boundary conditions (constraints) are applied to get the solution. All DOF at one end are set to zero while the opposite end is subjected to tensile load of 110 N see figure 5. For non linear analysis large deformation is active and Elastic modulus is kept zero for convergence. For nonlinear material behavior, some solution control is provided. To get the solution, the numbers of substeps are set to 100, maximum numbers of substeps are 1000 and minimum substeps are 10 in order to increase the load in steps. Once all the controls are set, the problem is solved to get the deformation and stress-strain on the skeletal tissue for the given load.

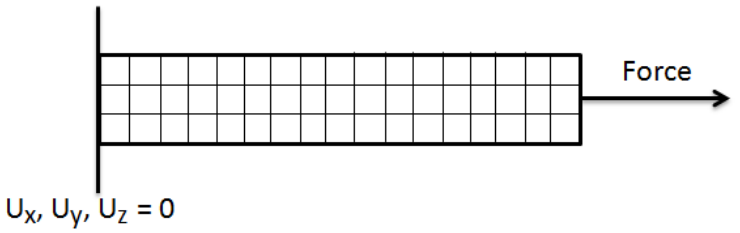


Figure 5: Boundary Condition for Finite Element Analysis

4 Results and Discussion

Experimental results are reported as the deformation of 17.775 mm at the maximum load of 43 N under the elongation of fiber orientation 45˚ to the length while under longitudinal elongation it is 15.257 mm at the load of 110N see table 3. The experimental results are validated using finite element analysis with the same experimental constraints, but material data is used from the literature. The deformations resulted from finite element analysis for both the fiber orientations are 17.7981 mm and 15.1908 mm see figure 6. The results held good agreement between experimental and finite element analysis. The failure was observed as a separation of muscle fibers within muscle tissue. The tensile strength is higher in longitudinal elongation (0.43 MPa) compared to that of in cross fiber elongation (0.23 MPa). The resulting linear model from longitudinal and cross fiber elongation tests (1.53MPa and 0.43MPa) are higher than reported by Morrow et al see table 3 [3,4]. The results of the present study show that the ultimate stress in skeletal muscle tissue under longitudinal elongation is higher than that of cross fiber elongation or elongation under 45˚ fiber orientation see figure 7. In converse of this, failure strain is sufficiently higher under cross fiber or 45˚ fiber orientation elongation than under longitudinal

elongation.

In comparison with the existing studies on skeletal muscle under compression by Van Loocke et al and under tension by Takaza et al reported that skeletal muscle tissue is a biomodular material [6,16]. The result of the present study suggested that cross fiber direction modulus is lower than that of in fiber direction see table 3. Mechanical properties of skeletal muscle were also examined experimentally under tension, results reported that muscle is stiffer in the fiber direction as compared to cross fiber direction, this held good agreement between numerical and experimental studies in the literature [3,4,10,14,20].

The results of experimental study of the present research are validated using finite element analysis. Experimental results of skeletal muscle along the fiber direction and 45° inclined fiber orientation to the length are validated using finite element method. Once the correctness of the finite element method is established between the results of experiment and finite element analysis, properties in the cross fiber direction were computed using finite element analysis. Overall, both finite element analysis and experimental results prove the research hypothesis that muscle is stiffer in the fiber direction compared to that in cross fiber direction. In this study, the testing is performed using 5KN load cell because of testing device limitations. Soft tissue may have to test using a small load cell in order to achieve lower testing rate. On the higher load cell this test is performed with a lower test rate so as to achieve the predicted results. This study is conducted on limited specimen due to unavailability of muscle tissue of required specification in cross fiber direction. Therefore, results in cross fiber direction are from its simulation using finite element analysis. It shows good agreement with the results from existing studies [3,4,10,21]. The viscoelastic behavior of the skeletal muscle is not considered in the simulation of finite element analysis; this is another limitation of the present study.

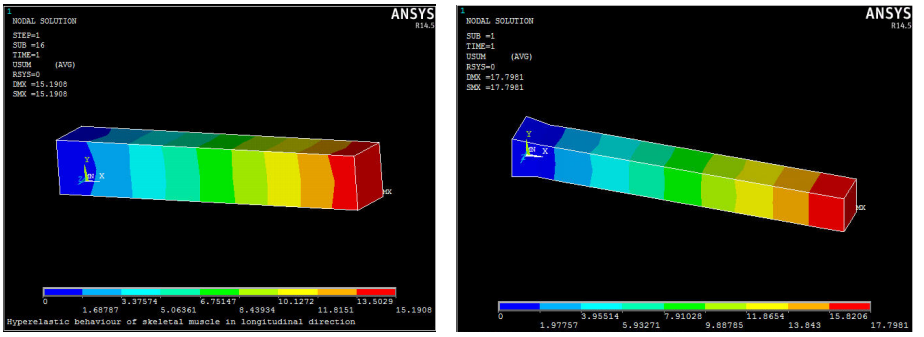
4.1 Statistical hypothesis test

Statistical analysis of the hypothesis of the present research is done using a paired t-test method with significance level of 5 %. The null hypothesis is tested by using the results of modulus of elasticity of the skeletal muscle in the fiber direction (E_P) and cross fiber direction (E_C). Sets of the specimen are having the same sample size, therefore increase in modulus of elasticity (d) in the fiber direction compared to cross fiber direction is used to test the null hypothesis of the study see table 4.

Sample size (n) = 5

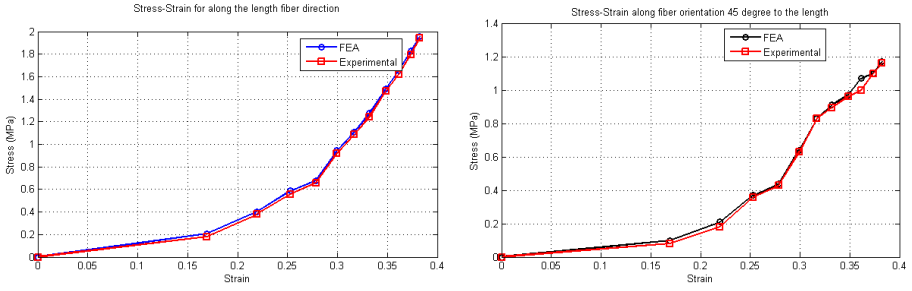
Degree of freedom (df) = n-1 = 5-1 = 4

Null hypothesis H_0: The skeletal muscle is stiffer in fiber direction than cross fiber



(a) (b)

Figure 6: Deformation with different orientation of the muscle fibers within the muscle tissue: (a) Fiber orientation along the length (Longitudinal Elongation), (b) 45° fiber orientation to the length



(a) (b)

Figure 7: Stress-Strain behavior of skeletal muscle: (a) Along the length fiber direction, (b) fiber orientation 45° inclined to the length.

Table 3: Mechanical Characteristics of Skeletal muscle.

Sample	Break Load (N)	Tensile Strength (MPa) Mean ± S.D.	Modulus (MPa) Mean ± S.D.	Deformation Experimental	Deformation From FEA (mm)
Fiber along the length	110 N	0.44 ± 0.22	E_P: 1.59 ± 0.33	15.257 mm	15.1908
45° Fiber orientation	43 N	0.23 ± 0.14	E_I: 0.621 ± 0.13	17.775 mm	17.7981
Cross fiber orientation	43 N	____	E_C: (By FEA) 0.43 ± 0.13	____	19.7291
E_P= Mean modulus of elasticity of muscle in fiber direction E_I= Mean modulus of elasticity of muscle in inclined fiber direction E_C= Mean modulus of elasticity of muscle in cross fiber direction					

Table 4: Increase in modulus of elasticity of the skeletal muscle.

Sample	E_P (MPa)	E_C(MPa)	(d) = E_P - E_C	d^2
1	2.775	0.292	2.483	6.165
2	1.587	0.315	1.272	1.617
3	0.494	0.917	-0.423	0.178
4	1.359	0.210	1.149	1.320
5	1.745	0.436	1.309	1.713
n=5			$\Sigma d = 5.79$	$\Sigma d^2 = 10.993$

direction.

$H_0 : E_p > E_c$

Alternative hypothesis H_1: The skeletal muscle is stiffer in cross fiber direction than fiber direction

$H_1 : E_p < E_c$

As fiber direction and cross fiber direction muscle samples are having the same sample size, the mean value of the increase in modulus of elasticity (d) in the fiber direction compared to cross fiber direction is given by equation (2) [26].

$$\bar{d} = \frac{\Sigma d}{n} = 5.79/5 = 1.158 \tag{2}$$

The standard deviation for a paired t-test method is given by equation (3) [26].

$$\begin{aligned} S^2 &= \frac{1}{n-1}\left[\sum d^2 - \frac{(\sum d)^2}{n}\right] \\ &= 1/4(10.993 - -6.704) \\ &= 1.057 \end{aligned} \tag{3}$$

$S = 1.028$

Test Statistics t is given by equation (4) [26].

$$t = \bar{d}/\left(s/\sqrt{n}\right) = 1.158/(1.028/2.236) \tag{4}$$

$t = 2.518$

Citical value from the table of t test for df = 4 at 5 % significance level ($t_{0.05}$) is 2.776.

Since the calculated value of t from the equation (4) is less than the critical value of t at 5 % significance level for 4 deaf ($2.518 < 2.776$), accept the null hypothesis ($E_P > E_C$). Therefore, muscle is stiffer in the fiber direction than cross fiber direction.

Conclusion

The results of the present study hold good agreement with the majority of studies in the recent past under tensile test. The results of experimental and finite element analysis are closely matching and prove the anisotropic behavior of skeletal muscle under tension. The present study suggests that as the fiber orientation moves from 0° to 90° with respect to the length; modulus of elasticity decreases and failure strain increases. This study provides the data which is sufficient to characterize skeletal muscle tissue as anisotropic and hyperelastic. Providing a reliable and quantitative tool to estimate muscle mechanical properties could also be useful for rehabilitation and performance training. Whether all these clinical applications could benefit from these measurements is still subject to debate and would need a large amount of clinical trials to be established. The work undertaken in current study might pave the way toward such investigations. Neuromuscular diseases directly affect proteins; some of them may greatly change the mechanical properties of muscle tissue as are sometimes described by clinicians [27,28]. So if the change in the mechanical material properties of the muscle is observed it will indicate the need of diagnosis of the disease.

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Conflict of Interest

No conflict of interest between authors and supporters.

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