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The anisotropic mechanical behaviour of passive skeletal muscle tissue subjected to large tensile strain

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Abstract

The passive mechanical properties of muscle tissue are important for many biomechanics applications. However, significant gaps remain in our understanding of the three-dimensional tensile response of passive skeletal muscle tissue to applied loading. In particular, the nature of the anisotropy remains unclear and the response to loading at intermediate fibre directions and the Poisson's ratios in tension have not been reported. Accordingly, tensile tests were performed along and perpendicular to the muscle fibre direction as well as at 30, 45 and 60 degrees to the muscle fibre direction in samples of *Longissimus dorsi* muscle taken from freshly slaughtered pigs. Strain was measured using an optical non-contact method. The results show the transverse or cross fibre (TT')

direction is broadly linear and is the stiffest (77kPa stress at a stretch of 1.1), but that failure occurs at low stretches (approximately $\lambda = 1.15$). In contrast the longitudinal or fibre direction (L) is nonlinear and much less stiff (10kPa stress at a stretch of 1.1) but failure occurs at higher stretches (approximately $\lambda = 1.65$). An almost sinusoidal variation in stress response was observed at intermediate angles. The following Poisson's ratios were measured: $V_{LT} = V_{LT'} = 0.47$, $V_{TT'} = 0.28$ and $V_{TL} = 0.74$. These observations have not been previously reported and they contribute significantly to our understanding of the three dimensional deformation response of skeletal muscle tissue.

Key words: muscle fibre tension, anisotropy, Poisson's ratio, non-linear

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1. Introduction:

The human body responds to mechanical loading on several timescales (McNamara and Prendergast, 2007, Lissner et al., 1960), but *in vivo* measurements of muscle load, joint reaction force and tissue stress/strain are usually not possible (Erdemir et al., 2007, Davis et al., 2003). In contrast, suitably validated computational models have the potential to predict all of these parameters and thus hold significant promise in advancing health care (Taylor and Humphrey, 2009). In particular they are a powerful means for understanding the musculoskeletal system (Erdemir et al., 2007, Marjoux et al., 1998) and are therefore used in diverse applications from impact biomechanics (Muggenthaler et al., 2008, Ivancic et al., 2007) to rehabilitation engineering (Linder-Ganz et al., 2008, Linder-Ganz et al., 2007), surgical simulation (Lim and De, 2007, Audette et al., 2004) and soft tissue drug transport (Wu and Edelman, 2008). There are also important applications in tissue engineering, as the engineered skeletal muscle tissue needs to have mechanical properties that match those of the replaced native tissue (Hinds et al., 2011, Goldstein et al., 2001).

Finite element models of the human body can be used to predict deformations during transient loading (Raul, Deck et al. 2008). These models require both a description of hard and soft tissue geometries, as well as the determination of their material properties under large deformations. However, the three-dimensional properties of skeletal muscle tissue have not been adequately characterized experimentally and this is an ongoing limitation for computational modelling. Since skeletal muscle tissue accounts for almost half of human body weight (Wang et al., 1997), the constitutive properties of muscle tissue are crucial for many musculoskeletal models.

Skeletal muscle is composed of about 70-80% water, 3% fat and 10% collagen (Vignos and Lefkowitz, 1959). It exhibits a fibre oriented structure to fulfil its force producing function: each muscle is composed of fascicles containing bundles of fibres, which themselves are composed of parallel bundles of myofibrils. Muscle fibres are cells which are very long compared to any other cells in the body (Martini and Nath, 1997). There is a dense network of connective tissues which surrounds groups of fibres, and this too has a hierarchical structure. Epimysium surrounds the whole muscle, perimysium surrounds bundles of muscle fibres and endomysium surrounds each muscle fibre individually. Arising from this structure, and similar to other biological soft tissue, skeletal muscle presents a nonlinear elastic, non-homogeneous, anisotropic and viscoelastic behaviour (Van Looke et al., 2006, Van Looke et al., 2008, Van Looke et al., 2009).

The three dimensional, compressive, quasi-static and dynamic mechanical properties of passive skeletal muscle were characterised by Van Looke (Van Looke et al., 2006, Van Looke et al., 2008, Van Looke et al., 2009). The elastic behaviour is nonlinear, and strong viscoelastic effects were observed, as well as significant tissue anisotropy. The direction perpendicular to the muscle fibre direction was found to give the stiffest response when the skeletal muscle was loaded in compression at a quasi-static strain rate of 0.05%/s. This was followed by the fibre direction, and the least stiff response was when the tissue was loaded at 45 degrees to the muscle fibre direction. A Poisson's ratio of 0.5 was observed for the strain in both transverse directions (T and T' in Figure 2) arising from the compressive load applied in the fibre or longitudinal (L) direction, indicating isotropic and incompressible behaviour for this mode of loading. However when compressive load was applied in a transverse direction (T), the resulting expansion in the fibre (L) and the remaining transverse (T') directions was characterized by Poisson's ratios of 0.36 and 0.65 respectively, see Figure 2 (Van Looke et al., 2006), again indicating incompressible but this time also anisotropic behaviour.

The tensile response of passive skeletal muscle is also nonlinear and viscoelastic (Nie et al., 2011, Calvo et al., 2010, Morrow et al., 2010, Martins et al., 1998, Meyers, 1994, Grieve and Armstrong, 1988, Yamada, 1970, Lissner et al., 1960, Martins et al., 2006). Figure 1a shows the fibre direction tensile stress-stretch response observed by different authors, and some of the variability in the data may be a result of viscoelasticity. However, significant variation is evident even for the same applied strain rate: at 0.05% s^{-1} the Morrow et al response profile is stiffer than what was observed by Calvo et al, Martins et al or Yamada.

The stress-strain response is also dependent on the orientation of the fibres in relation to the loading direction, see Figure 1b. In very recent work by Hernandez et al on stomach muscles there was unfortunately no attempt made to measure the fibre direction orientation and their data is therefore not directly comparable (Hernandez et al., 2011). Therefore, to the authors' knowledge, the papers by Morrow et al and Nie et al are the only two which test both the fibre and cross fibre direction, see Figure 1b. However the fibre direction dependant mechanical behaviour of skeletal muscle in tension has not been studied for intermediate angles and this elucidating this relationship is a focus of the present study.

The data of Morrow et al shows the fibre direction to be stiffer than the cross-fibre direction, but the data of Nie et al indicates the opposite, see Figure 1b, though Nie's work was at different strain rates. There appears to be no data on the Poisson's ratios during tensile loading, though these data have been reported for compressive loading (Van Looke et al., 2006). It is evident therefore

that there remain significant gaps in our understanding of the three-dimensional tensile response of passive muscle tissue to applied loading, particularly since the response at intermediate fibre directions and the Poisson's ratios have not been reported. Accordingly, the goal of this paper was to characterise the quasi-static fibre direction dependency of the tensile response of skeletal muscle, including an assessment of the Poisson's ratios.

2. Methods:

2.1 Sample Preparation

Fresh *Longissimus dorsi* skeletal muscle tissue was harvested from 3 month old female pigs. Five animals were used in total. All experiments were approved by the University of Dublin Ethics Committee for the protection of animals used for scientific purposes, according to EU Directive 2010/63/EU. Approximately 10 mm thick and 10 mm wide samples were prepared for quasi-static tensile testing. The sample length requirement according to the tensile testing standard ASTM E8/E8M were taken into account as much as was practically possible. However, it was not feasible to prepare samples with an exact dimensional specification due to the mobility of the freshly harvested skeletal muscle tissue and the limited availability of the tissue in the correct fibre orientation.

	Avg Length (mm)	Std Dev (mm)	Avg Width (mm)	Std Dev (mm)	Avg Thickness (mm)	Std Dev (mm)
Fibre Direction	51.7	3.7	10.8	0.9	10.3	1.3
Cross-Fibre Direction	45.3	2.3	10.1	1.7	10.5	1.2
45 Degrees Direction	49.7	3.1	11.4	0.8	9.9	1.00
60 Degrees Direction	46.7	3.1	11.2	0.3	10.1	0.6
30 Degrees Direction	47.3	3.1	10.7	0.8	10.2	1.7

Table 1: The average muscle tensile sample dimensions and the related standard deviations

The sample length variability was accounted for by adjusting the testing speed in order to maintain a constant strain rate of $0.05\%s^{-1}$. The width and thickness variability was accounted for by using image analysis to determine these two dimensions, see section 2.2 and section 3.3. Six samples were prepared for each of the following muscle fibre orientations: the fibre direction, the cross-fibre (perpendicular to muscle fibres) and 45 degrees to the muscle fibre orientation, see Figure 2. Three samples were prepared with 60 and 30 degrees fibre orientations. The first sample fibre orientation was picked at random, but the same orientation was not repeated until all other muscle fibre orientations had been tested. The test matrix is shown in Table 2. All the experimental tests were carried out within two hours after the death of the animal in order to minimise the effects of rigor mortis on the results (Van Loocke et al., 2006, Kobayashi et al., 1996). All experiments were carried out using a Zwick Z005 tensile testing machine (Zwick GmbH & Co. Ulm, Germany), equipped with a 100 N load-cell. Grated plates were used on the clamps to minimize slippage.

Load direction	Sample size (N)	nomenclature
Fibre (L)	6	L1, L2, ...L6
Cross Fibre (T)	6	T1, T2, ... T6
45 degrees (45L)	6	45L1, 45L2, ... 45L6
30 degrees (30L)	3	30L1, 30L2, 30L3
60 degrees (60L)	3	60L1, 60L2, 60L3

Table 2: Test matrix summary showing testing direction, sample size and nomenclature employed

2.2 Experiments

A sample of the required geometry was placed between the grated plates, and then the clamps were clamped over the grated plates, see Figure 3. This improved the gripping capabilities of the clamps without over-tightening them, and helped to minimise the unnecessary introduction of stresses around the clamps. For strain evaluation, nine black dots were marked on the sample surface facing the camera (see Figures 3&4). The images were acquired at 1 frame per second using a CCD camera with 800 x 600 pixel resolution. The sample width and thickness was photographed for dimensional analysis. Phosphate buffer saline was continuously sprayed on the sample to avoid drying. Each sample was tested as soon as it was prepared to reduce unnecessary over-exposure to the environment after it had been cut into the required geometry. The testing temperatures ranged from 18-22 degrees Celsius. Samples were loaded at a strain rate of $0.05\%s^{-1}$. A pre-load was determined by assuming that half the sample weight was supported by the top clamp and therefore half the sample mass was used as the preload. After pre loading the muscle was then stretched to a stretch ratio of 1.3, or to failure, whichever came first.

2.3 Data Analysis

A custom Matlab (The Mathworks, Natick Massachusetts, USA) script was used to calculate strains from the camera images, as this eliminated the effects of clamp slippage as well as allowing the local strain variations to be analysed (see Figure 4). For each image, the central coordinates of each dot (determined using the mean of the segmented dot shapes) were recorded and compared to the coordinates of the image at the start of the experimental testing. The difference in coordinates was used to calculate the stretch ratios (Moerman et al., 2010).

Six local Z direction stretch ratios were derived for each sample as follows, see Figure 4:

$$\lambda_{nz} = \frac{ndz}{n0z} \quad \text{Equation 1}$$

where n is a number specifying the specific location (Figure 4a), d signifies the deformed sample length (Figure 4b), 0 is the un-deformed sample length and z is the stretch direction. The local stretch ratios were then used to give the sample average stretch ratio. The Cauchy stress in the Z direction was calculated from the Zwick machine load cell force by assuming incompressibility. For incompressible materials, the volume at the start of a deformation (0) is equal to the volume at the end of a deformation (d):

$$V = V_0 = V_d = A_0 L_0 = A_d L_d \quad \text{Equation 2}$$

Therefore:

$$A_d = \frac{A_0 L_0}{L_d} \quad \text{Equation 3}$$

Applying this to the Cauchy stress (true stress) formula:

$$\sigma = \frac{F}{A_d} = \frac{F}{\frac{A_0 L_0}{L_d}} = \frac{F \lambda}{A_0} \quad \text{Equation 4}$$

Contraction in the Y direction was analysed using the following equation:

$$\lambda_{ny} = \frac{n dy}{n_0 y}, \quad \text{Equation 5}$$

where n is a number specifying the specific location (Figure 4a), d signifies the deformed sample length (Figure 4b), 0 is the un-deformed sample length and Y is the stretching direction.

The average logarithmic strains were then found from:

$$\varepsilon_z = \frac{1}{6} \sum_{n=1}^6 \ln(\lambda_{nz}) \quad \text{and} \quad \varepsilon_y = \frac{1}{6} \sum_{n=1}^6 \ln(\lambda_{ny}) \quad \text{Equation 6}$$

The Poisson's ratios are presented in terms of the longitudinal and transverse directions with respect to the muscle fibres, see Figure 2: for tensile tests aligned with the fibre direction there are two Poisson's ratios: v_{LT} and $v_{LT'}$. for tensile tests aligned perpendicular to the fibre direction there are two Poisson's ratios: v_{TL} and $v_{TT'}$. In each case, the instantaneous Poisson's ratio is minus the ratio of the average induced strain to the average applied strain at each instant during each test. The final Poisson's ratio values presented are the average of the instantaneous values throughout the tests.

3. Results

Figure 5 shows photographs of a typical test sequence. The first image is taken at the beginning of the tensile testing ($\lambda=1$); the second image is taken at the end of the test (shown here for a fibre directional test at $\lambda=1.35$). The third image shows the marker dot displacements when the last image is superimposed on top of the first image. The coloured markings show the capability of the Matlab code in locating the centre of the dots. The triangles represent the bottom row of dots, the plus symbols represent the middle row and the upper row is shown as circles. The blue colour shows the un-deformed configuration and the red indicates the final deformed configuration.

Typical fibre directions within-sample longitudinal stretch ratio variations at different time points are shown in Figure 6. The results for the six regions shown in Figure 4 are displayed, along with the resulting average stretch and the stretch based on the Zwick machine displacement. Figure 7 shows typical fibre direction within-sample stress variations as a function of applied stretch (derived from image analysis). Figure 8 shows the typical between sample stress-stretch variations for the fibre direction tests. Each individual curve represents one sample for which the average of the six regions shown in Figure 4 is presented. The average across all samples is also shown. Figure 9 shows the between sample stress-stretch variation for the cross-fibre direction tests. Figure 10 shows the stress-stretch response of samples loaded at 45 degrees to the muscle fibre direction. Similar results were found for samples oriented at 30 degrees and 60 degrees to the muscle fibre direction, and all stress-stretch results are shown in Figure 11, with only mean and $\pm$ one standard deviation shown for each test direction for clarity. Finally, the measured Poisson's ratio values as a function of stretch ratio are presented in Figure 12. Linear regressions indicated no influence of stretch ratio on measured Poisson's ratio, and therefore the average values are presented in Table 3.

Fibre Direction	Effective Sample Size	Poisson's Ratio	StDev
$v_{LT} = v_{LT'}$	24	0.47	± 0.02
$v_{T'T}$	24	0.28	± 0.04
v_{TL}	24	0.74	± 0.016

Table 3: Summary of the Poisson's ratios observed during tensile testing of muscle samples

4. Discussion

It is appropriate to consider the testing method, the results obtained and a comparison with existing data before commenting on the significance of the results obtained. These factors are considered sequentially here.

4.1 Testing method

The difference between the image analysis average stretch ratio and the Zwick machine stretch ratio is due to sample slippage. Further tightening the clamps may have addressed this problem, but would have damaged the samples and created regions of high stress concentration. All stress-stretch results are therefore presented with stretches calculated from the more reliable image analysis technique instead. While significantly more laborious, these methods are effective at showing local strain variations and are independent of clamp slippage and reflect a more realistic determination of the applied deformation.

The sample length/width ratio is important in tensile testing to reduce the effect of gripping on stress in the region of interest. For 6 out of 24 of the samples in this study, the length fell short of the recommended ASTM E8 standards, but not by more than 10% (the length is required to be more than five times the width for rectangular specimens). Thinner samples were not feasible as this reduces the number of complete muscle fascicles in the sample. It has been observed that below about 6mm, the sample thickness has a direct influence on the response (Lewis and Purslow, 1990) and although this previous work performed its investigation on cooked meat, there is no evidence to suggest that the microstructure that gives rise to this behavioural response is affected or changed by cooking. No significant difference in response was observed between the samples with a dimensional ratio slightly below the recommended ASTM E8 and those that met the ASTM E8 standard (ASTM International, 2011).

A preload eliminates unavoidable initial compressive forces within samples. The preload should be high enough to straighten muscle fibres in the sample without stretching them. However, if the preload is too high, the typical toe region is partially or totally lost and damage may even occur. The low preload based on half the specimen weight (mean preload was 0.11N) ensured this did not occur in these tests and the results showed that the preload was always less than 1.5% of the maximum force during the testing. The difference between the stretched length after applying the pre-load and the original unloaded length (before preload was applied) was 20% in the fibre direction and 12% in the cross-fibre direction. An approximate comparison to the physiological resting length was possible through an experiment performed on 7 different samples in which muscle tissue dimensions were marked and measured before the tissue was harvested from the pig. The marked tissue was then harvested from its physiological working environment, which resulted in tissue contraction. The difference between the contracted length and the physiological length was found to be an average of 18%. This was found to almost match the stretch achieved using the pre-loads applied in this paper.

No evidence of torsion was observed during testing, possibly due to the micro-structural behavioural response of skeletal muscles to external passive loading. The perimysium collagen fibres are arranged in a cross-ply manner, with an angle of 55° to the muscle fibre direction muscle is un-stretched, and have been observed to change to 20° with respect to the main muscle fibre direction when stretched, at which stage it is thought the thicker stronger perimysium collagen fibres bear almost all the load (Passerieux et al., 2007, Scheip et al., 2006, Monti et al., 1999, Trotter and Purslow, 1992, Street, 1983). However, although a somewhat regular fibre angle has been observed for the perimysium, the endomysium surrounding individual muscle fibres has a more random fibre orientation (Purslow, 2010). Accordingly, it is perhaps not surprising that no obvious torsional mode was observed for any of the different muscle fibre orientations in this study.

4.2 Interpretation of results

The typical fibre direction within-sample stretch ratio variation (Figure 6) shows a range of the local variations in stretch within a sample, similar to findings observed by Palmer et al for tensile testing of rat *soleus* muscle (Palmer et al., 2011). The corresponding within-sample stress variations (difference between maximum and minimum) as a function of applied stretch (Figure 7) are of lower magnitude and are relatively constant as stretch increases: 12% of mean stress at $\lambda=1.15$, 11% of mean stress at $\lambda=1.2$ and 11% of mean stress at $\lambda=1.3$ for the sample shown. These approximately constant stress variations are assumed to be due to the muscle fibre's ability to transmit force to the surrounding muscle fibres. This seems to corroborate the previous findings that skeletal muscle fibres can laterally transmit 80% of their loads (Monti et al., 1999, Huijing, 1999, Street, 1983). Figures 8, 9 and 10 show the between-sample stress-stretch variations for the fibre, cross-fibre and 45 degrees to the fibre direction tests respectively. For the fibre direction (Figure 8), the typical nonlinear increase in stiffness with stretch is observed. The between sample variation is high as a percentage of the mean stress at low strains, but this decreases with stretch: 54% at $\lambda=1.1$, 31% at $\lambda=1.2$ and 26% at $\lambda=1.3$. For the cross fibre direction (Figure 9), a stiff and largely linear response was observed, with failure occurring at stretches beyond 1.15. The between sample stress stretch variation is highest at low strains and again decreases relative to the mean stress at high strains: 59% at $\lambda=1.04$, 40% at $\lambda=1.08$ and 24% at $\lambda=1.12$. Since for the current study samples were harvested from 5 different animals, it is likely that most of the observed variation can be attributed to intra-animal variation. Figure 10 shows the stress-stretch response of samples loaded at 45 degrees to the muscle fibre direction. Again, a mildly non-linear relationship is observed. The between sample variation as a percentage of mean is high at low strains, but this decreases at higher stretches: 83% at $\lambda=1.04$, 54% at $\lambda=1.08$ and 42% at $\lambda=1.12$. All stress-stretch results are shown in Figure 11, with only mean and $\pm$ one standard deviation shown for each test direction for clarity. There is a clear influence of fibre direction on the stress-stretch relationships observed, with the

cross fibre direction significantly stiffer than the fibre direction. To observe the change in stiffness with fibre angle, Figure 13 shows two views of a three-dimensional landscape plot, illustrating how stress increases with stretch and also with angle relative to the main muscle fibre direction. Furthermore, it can be seen that the relationship with angle is not linear, but rather follows a more sinusoidal shape. The fibre direction response was observed to be the most compliant, and this seems intuitive since it is the normal physiological working direction for muscle.

The stiffest direction is the cross-fibre response, but this has a low failure stretch ($\lambda=1.15$). The fibre direction response observed was less stiff than the responses observed by both Morrow et al and Nie et al (Nie et al., 2011, Morrow et al., 2010), but close to the findings of Calvo et al (Calvo et al., 2010), see Figure 14 and Table 4. Calvo et al. used a slightly lower strain rate of 0.025%/s, see Figure 4 and Table 4, and this may partially account for their lower stiffness results, as it is known that skeletal muscle is viscoelastic even at very low strain rates (Van Looche et al., 2008). Morrow et al. observed stiffer fibre directional responses than was observed in this paper (Morrow et al., 2010), and this may be largely attributed to the different experimental protocol used. More importantly, Morrow et al., 2010 froze their samples within an hour of the animal sacrifice, but no effort was made to discuss the thawing process they used. Although it has been found that a freeze thaw cycle does not affect the post rigor response of muscle tissue (Van Ee et al., 2000), the thawing process remains an important part of their experiments as it is likely to have allowed rigor mortis to set in and make the tissue much stiffer (the rigor mortis characterisation investigation performed on freshly harvested porcine tissue (Van Looche, et al., 2006, showed that the testing window is two hours after death before rigor mortis starts influencing the passive response of porcine skeletal muscles). The thawing process is likely to have allowed rigor mortis to set in and make the tissue much stiffer (rigor mortis characterisation investigation performed on freshly harvested porcine tissue (Van Looche et al., 2006) showed that the testing window is two hours after death before stiffening commences). The 1 hour window left after freezing makes it unlikely that Morrow et al. was able to thaw and test the tissue in its fresh state. Therefore it is our view that Morrow's experimental tests were probably performed on aged tissue (post rigor mortis). Furthermore, Morrow et al. performed their uniaxial passive testing on complete muscles which may have given rise to complicated geometric shapes, therefore resulting in incorrect stress calculations. It is therefore imperative to test skeletal muscle within two hours after death to avoid rigor mortis effects and to retain a hydrated environment (Van Looche et al., 2006, Kobayashi et al., 1996) and to test samples with as uniform a cross section as possible. Although Morrow et al may have less reliable strain estimates since they did not use image analysis for strain estimation, this should mean they would underestimate stress. It is more likely that their higher stiffness values result mainly from the use of aged tissue and possibly also from differences in the amount of perimysium in the skeletal muscle, which can vary between 0.4% - 4.8% of dry weight (Purslow, 2010, KJÆR, 2004, Purslow, 1999.). The epimysium would also influence the measured stiffness, but it appears from the Morrow paper that epimysium was not present. Differences in the amount of perimysium may also explain why lower stretches to failure were observed in the present work, while Hernandez et al observed stretch ratios up to 2.5 using samples harvested from rabbit external oblique (Hernandez et al., 2011). The porcine *Longissimus dorsi* muscles used in our tests may not be exposed to large stretches in the fibre direction and to only very small stretches in the cross-fibre during physiological activities. The Nie et al fibre response profile is unusual in that the typical toe region and increasing stiffness were not observed (Nie et al., 2011), and it is unclear why this is, though the use of different sample dimensional ratios can also contribute to the differences in Figure 14. The cross-fibre response observed in our work was in broad agreement with the Nie et al cross-fibre response up to a stretch ratio of 1.07, and their results also showed that the cross-fibre response is stiffer than the fibre response. This observation contradicts the findings of Morrow et al,

and this might be attributed mainly to the different experimental protocol employed. The typical toe region in soft tissue stretch due to the crimped collagenous fibres straightening out (Monti et al., 1999, Street, 1983, Trotter and Purslow, 1992) is absent from the Nie et al data (Nie et al., 2011) possibly due to a high preload, but those authors do not discuss the magnitude of the preload they used.

Author	Strain rate (%/s)	Fibre Direction Stress (kPa) at $\lambda=1.3$	Cross-Fibre Direction Stress (kPa) at $\lambda=1.1$	45° Direction. Stress (kPa) at $\lambda=1.1$
Morrow et al., 2010 Rabbit	0.05	269	2	
Nie et al., 2011 Pig	5.00	100	59	
Takaza et al Pig	0.05	100	77	50
Calvo et al., 2010 Rat	0.025	46		
Martins et al., 2006 Pig	?	35		
Yamada , 1970 Human	?	14		

Table 4: Showing varying tensile responses observed by different authors (No strain rates for the last two were given).

The Poisson's ratio results presented in Figure 12 and Table 3 show an important component of the deformation response in tension, and this is the first time these data have been reported. A Poisson's ratio of 0.47 ± 0.02 was observed for transverse contraction when samples were loaded in the fibre direction (longitudinal). Despite the fact that a small degree of fluid loss was observed, the passive response of skeletal muscle to tensile loading in the fibre (L) direction is therefore almost a volume preserving event, with equal contraction in the two transverse directions (T and T'). Due to the transversely isotropic and almost incompressible nature of muscle tissue, this was expected, and is in agreement with assumed Poisson's ratios used by Meier, Vannah and Beldie (Beldie et al., 2010, Meier and Blickhan, 2000, Vannah and Childress, 1993). Furthermore, it also agrees well with the reported compressive Poisson's ratio of 0.5 ± 0.04 for fibre direction loading (Van Looke et al., 2006). The cross-fibre (T) tensile loading is more complex and gives rise to two different Poisson's ratios. A Poisson's ratio of 0.28 was observed when the contracting side considered was the remaining orthogonal cross-fibre direction (T'). However, a Poisson's ratio of 0.74 was observed when the contracting side considered was the fibre (L) direction. Thus when tensile load is applied in the transverse (T) direction, an almost volume preserving contraction response is observed in both the fibre direction (L) and in the other cross-fibre (T') direction, but the magnitude of the fibre direction contraction was much greater than the cross-fibre contraction, indicating that the cross-fibre (T') direction requires more internal force to contract than the less stiff fibre direction (L). The sum of the two Poisson's ratios is close to 1 in both loading cases, which corroborates the assumption that skeletal muscle tissue is incompressible. These figures are different to the equivalent compressive Poisson's ratios observed by Van Looke et al (0.64 and 0.36 respectively), see Table 5, which shows a complete Poisson's ratio characterisation of quasi-static skeletal muscle tissue in both tension and compression. The Poisson's ratio of 0.47 for tension (rather than 0.5 for compression) may be due to biological fluid losses observed when the specimen was subjected to longitudinal tensile loading. For tensile loading in the cross-fibre (T) direction, the specimen was observed to be more compliant in the longitudinal (L) contraction than in the other cross-fibre (T')

contraction. This is the opposite of what has been observed in compression (Van Loocke et al., 2006). While a non-linear stress-strain response was observed by Van Loocke for the cross-fibre (T') compression response, an almost linear response was observed for the cross-fibre (T') tension response in the present work. The magnitude of stress responses observed for all directions for the tensile tests presented here is much higher than the observed compressive responses from the literature.

	Takaza et al. (Tension)	Van Loocke et al. (Compression)
$V_{LT} = V_{LT'}$	0.47	0.5
$V_{TT'}$	0.28	0.65
V_{TL}	0.74	0.36

Table 5: Tensile Poisson's ratios observed in the present work, and compressive data observed by Van Loocke et al (Van Loocke et al., 2006).

In summary, the experiments performed in this study were performed on freshly harvested samples that were cut into a well-defined geometric shape in order to facilitate stress calculations and tested within two hours after death. In contrast, Morrow et al (Morrow et al., 2010) most likely performed their experiments inside the rigor mortis window, which would explain the very stiff response they observed. In addition, non-defined geometric shapes were used by Morrow et al., which complicated their stress calculations. Hernandez et al (Hernandez et al., 2011) did not establish different fibre orientations, and therefore their work is not directly comparable with ours. The only other tensile experimental work using different fibre orientation was by Nie et al (Nie et al., 2011), who found unusual stress-strain curve shapes, but their tests were performed at higher strain rates, and therefore direct comparison to the present work is not possible. The present paper is the only one to have used quasi-static tensile load to experimentally characterize freshly harvested skeletal muscle tissue, with the muscle fibre angle being oriented sequentially from zero degrees to 90 degrees to the loading direction. The tensile Poisson's ratios presented in this paper have not been reported before. The preload applied and discussed in this paper is a good practical way of introducing a pre-load that is repeatable and appears to be quite close to the physiological working length. No other authors have discussed their pre-load or its relationship with the muscle's physiological resting length.

1. Conclusions

The three dimensional stress-stretch behaviour of samples from freshly slaughtered porcine *Longissimus dorsi* muscle has been reported. Tests were performed along and perpendicular to the muscle fibre direction, as well as at 30, 45 and 60 degrees to the muscle fibre direction. The results show the cross-fibre direction is broadly linear and is the stiffest (77kPa stress at a stretch of 1.1), but failure occurs at low stretches ($\lambda = 1.15$). In contrast the fibre direction is nonlinear and much less stiff (10kPa stress at a stretch of 1.1) but failure occurs at higher stretches ($\lambda = 1.65$). An approximately sinusoidal variation in stiffness was observed at intermediate angles. The following Poisson's ratios were measured: $V_{LT} = V_{LT'} = 0.47$, $V_{TT'} = 0.28$ and $V_{TL} = 0.74$. These observations have not been previously reported, and they contribute significantly to our understanding of the three dimensional deformation response of skeletal muscle tissue.

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Editable Equation

$$\lambda_{nz} = \frac{ndz}{n0z} \quad \text{Equation 1}$$

$$V = V_0 = V_d = A_0 L_0 = A_d L_d \quad \text{Equation 2}$$

$$A_d = \frac{A_0 L_0}{L_d} \quad \text{Equation 3}$$

$$\sigma = \frac{F}{A_d} = \frac{F}{\frac{A_0 L_0}{L_d}} = \frac{F L_d}{A_0 L_0} \quad \text{Equation 4}$$

$$\lambda_{ny} = \frac{ndy}{n0y}, \quad \text{Equation 5}$$

$$\varepsilon_z = \frac{1}{6} \sum_{n=1}^6 \ln(\lambda_{nz}) \quad \text{and} \quad \varepsilon_y = \frac{1}{6} \sum_{n=1}^6 \ln(\lambda_{ny}) \quad \text{Equation 6}$$

Editable Tables

Fibre Direction	Effective Sample Size	Poisson's Ratio	StDev
$V_{LT} = V_{LT'}$	24	0.47	± 0.02
$V_{TT'}$	24	0.28	± 0.04
V_{TL}	24	0.74	± 0.016

Table 3: Summary of the Poisson's ratios observed during tensile testing of muscle samples

	Takaza et al. (Tension)	Van Loocke et al. (Compression)
$V_{LT} = V_{LT'}$	0.47	0.5
$V_{TT'}$	0.28	0.65
V_{TL}	0.74	0.36

Table 5: Tensile Poisson's ratios observed in the present work, and compressive data observed by Van Loocke et al (Van Loocke et al., 2006).

Figure 1: Literature findings for tensile stress - stretch response for skeletal muscle: (a) fibre direction responses, (b) comparison between fibre and cross - fibre direction responses.

Figure 2: Schematic illustration of fibre orientation in different samples. Definition of muscle fibre longitudinal (L) and transverse directions (T and T') and observed Poisson's ratios from previous compression testing (Van Loocke et al., 2006). Key: $V_{LT} = V_{LT'}$: Transverse expansion resulting from longitudinal compression; $V_{TT'}$: transverse (T') expansion due to transverse compression (T); V_{TL} : longitudinal (L) expansion due to transverse compression (T).

Figure 3: Typical cross-fibre direction sample shown towards end of testing: the depth (in the fibre direction) has contracted significantly when compared to the cross-fibre direction (where markers are marked). The nine black dots visible on the sample surface were used for image analysis to calculate displacements. Washers were used to counter balance the screws' centre of gravity.

Figure 4: Schematic illustration of the sample numbering before (a) and after (b) deformation and the labelled arrows inside show the different distances analysed. The different arrow regions are shown labelled using ordinary numbers for simplicity. The stretch and Poisson's ratio calculation regions are numbered in the two images on the right.

Figure 5: Typical fibre direction test sequence showing sequentially the un-deformed (initial, $\lambda=1$) image, the final deformed ($\lambda=1.35$) image and a superposition of the two (final plus initial).

Figure 6: Typical fibre direction within-sample longitudinal stretch ratio variation at different time points (Sample 5 shown here). The individual region results (see Figure 4), the average and the stretch based on the Zwick machine displacement are shown.

Figure 7: Typical within-sample stress variation (shown here is fibre direction sample L5).

Figure 8: Between-sample stress versus stretch variation for fibre direction tests.

Figure 9: Between-sample stress versus stretch variation for the cross-fibre direction tests.

Figure 10: Between-sample stress versus stretch variation for the 45 degrees tests.

Figure 11: Fibre direction responses (mean and ± 1 standard deviation) shown for all tests performed: fibre, cross fibre and at 30, 45 and 60 degrees to the fibre direction.

Figure 12: The Poisson's ratio versus stretch ratio responses observed. Linear regression fits are also shown.

Figure 13: The experimental Cauchy stress and stretch curves in 3D in relation to the muscle fibre angle. For visualisation a periodic cubic surface fit is shown (shaded towards stress magnitude), and this is shown with a rotation of the muscle fibre orientation with respect to the loading axis. In addition surface plots (transparent grey) are shown for the anisotropy landscape (± 1 standard deviation).

Figure 14: Comparison of current data with existing literature findings (Nie et al., 2011, Calvo et al., 2010, Morrow et al., 2010).