

A biomechanical investigation of the anteromedial and posterolateral bands of the porcine anterior cruciate ligament

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Abstract: The bipartite nature of the porcine anterior cruciate ligament has been documented, but its biomechanics have not been investigated. The need for such knowledge has recently been heightened with xenografting advances such as the introduction of the porcine patellar tendon as a human anterior cruciate ligament graft. The aim of this study is to compare the biomechanical properties of the intact anterior cruciate ligament with that of its two bands. 15 intact porcine anterior cruciate ligament–bone, 15 anteromedial band–bone, and 15 posterolateral band–bone complexes were prepared for tensile testing at 0.33 mm/s. Structural (load, deformation, stiffness, and energy absorbed) and material (stress, strain, modulus, and strain energy density) properties were analysed. Analysis of variance identified significantly higher ultimate load, stiffness, and energy absorbance in the intact porcine ligament when compared with its anteromedial band ($p \leq 0.028$). However, the intact ligament was only significantly higher in ultimate load when compared to its posterolateral band ($p = 0.031$). All ligament–bone complexes failed at similar deformations and strains, suggesting a strain-dependent failure mechanism. The intact porcine anterior cruciate ligament exhibited higher ultimate load, stiffness, and energy absorption than its two bands in isolation. The posterolateral band of the porcine anterior cruciate ligament constitutes a large proportion of the overall restraining function of the entire anterior cruciate ligament.

Keywords: anterior cruciate ligament, anteromedial band, posterolateral band, porcine ligament

1 INTRODUCTION

The anterior cruciate ligament (ACL) is one of the most commonly injured ligaments in humans and its reconstruction is one of the most common orthopaedic surgical procedures [1]. From the current literature, it is evident that up to 25 per cent of all surgical outcomes are suboptimal using a single-bundle technique [2]. This leads to increasing support for double-bundled ACL grafts [1]. ACL

grafting material includes the human patellar tendon and hamstring tendons [3–6] with similar effectiveness in both graft types noted by Woo *et al.* [6] and Aglietti *et al.* [3], while Beynon *et al.* [4] favoured the patellar tendon graft. In a recent study [7], porcine patellar tendon xenografting for human ACL reconstruction showed vastly improved grafting material options for ACL reconstruction.

The porcine femur–anterior cruciate ligament–tibia complex (FATC) is a fibrous (dense regular connective tissue) structure containing a ligament that is structurally [8, 9], functionally, [8, 10] and biomechanically [10] similar to the human ACL. Compared with two other animal models (the goat and the sheep), the porcine ACL is the closest animal

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model of the human both structurally and biomechanically [10]. Both the human and the porcine ACLs can be separated into two separate anatomical bands near their tibial insertion (anteromedial (AMB) and posterolateral (PLB) bands), which arise from a common femoral insertion [8].

Functional similarities between the porcine and human ACLs can be found in their bundle structure and force attenuation. In the human ACL there are fibres that remain at a constant length and tension throughout both flexion and extension, which are termed 'guiding bundles' [11]. The porcine ACL also contains guiding fibres, which are located within the posterolateral band [8]. Load transmission through the porcine ACL is similar to the human ACL [10]. In a comparison of *in-situ* forces in intact ACLs, no statistically significant differences were found between human and porcine specimens tested with anterior tibial loading at 50° and 90° of knee flexion [10]. However, the porcine PLB directed forces more proximally when the AMB was cut. This finding suggested that the human AMB obtained greater loading than the porcine AMB in anterior translation of the tibia.

From the best available evidence, the porcine ACL is the most similar ligament to the human ACL and its bands are structurally and functionally different from each other. Biomechanical investigations of each band of the porcine ACL separately could not be identified in the literature, but differences between the human ACL bands have been found [12–15]. Therefore an investigation of the biomechanics of the porcine ACL bands could improve the understanding of the roles of the human ACL bands and provide a rationale for double-bundled ACL reconstruction using porcine ACL xenograft.

2 METHODS

This study utilized 45 4-month-old porcine knee specimens obtained from a processing plant that follows the Australian regulation for the treatment of livestock for human consumption. The specimens were chilled to 5 °C within 48 h after the animals were culled [16]. Within 4 days the specimens were delivered to the Biomechanics Laboratory, University of South Australia, Australia. All specimens were then individually wrapped in thin plastic coverings to allow easy separation and to minimize water loss. The knee specimens were then frozen for up to 2 months until the day prior to mechanical testing, at which time the specimens were defrosted in a fridge overnight at 4 °C. When fully defrosted, specimens

were removed from the fridge and dissected. Exclusion criteria for this study included porcine knees with observable deformity or degeneration, that were too large to fit in the testing apparatus, and that contained any damage to the ACL.

Ethical approval for the dissection and testing procedure using scavenged animal tissue was granted through the Institute of Medical and Veterinary Sciences, University of Adelaide, Australia. Dissection removed all soft tissue surrounding the knee joint, sparing only the ACL to produce FATCs. 15 porcine FATC specimens had the posterolateral band of the ACL dissected while another 15 had the anteromedial band dissected. A total of three experimental groups (intact ACL, AMB, and PLB) were produced.

The bone–ACL–bone complex was mounted on to metal brackets on a vertically positioned Hounsfield tensometer (W5063, Tensometer Ltd, UK) using 'Unbreako' reinforced steel rods of 4.8 mm diameter inserted into drill holes in the femur and tibia, 10 cm

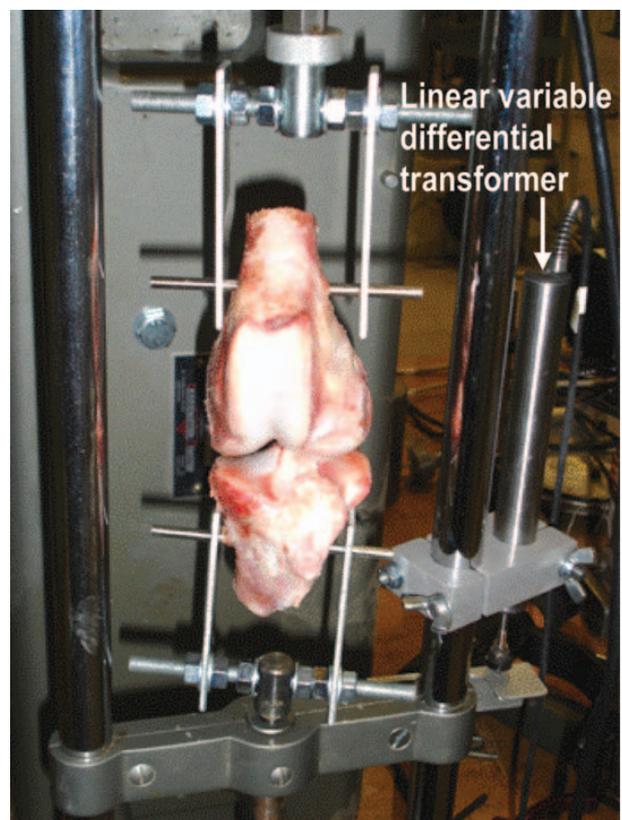


Fig. 1 The porcine knee specimen was mounted on the Hounsfield tensometer using reinforced steel rods and metal brackets. The LVDT, measuring grip-to-grip displacement, was fixed to the tensometer by a clamp and was positioned to the right □

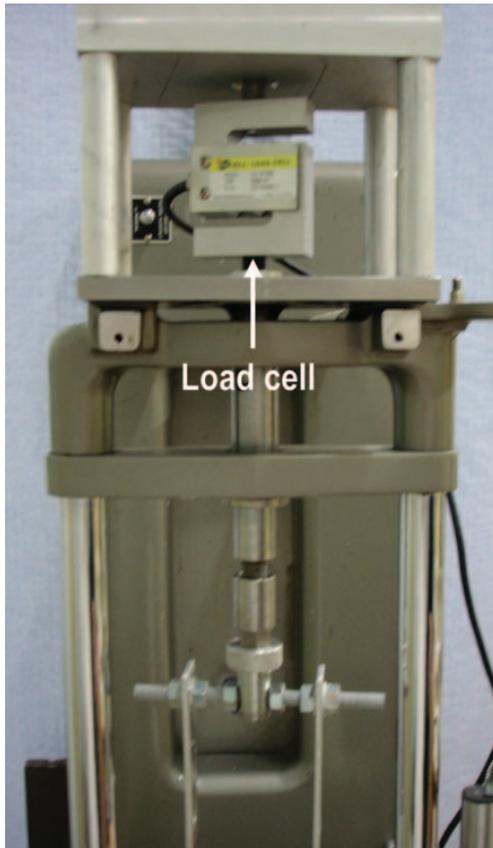


Fig. 2 The superior bracket is attached directly to the load cell, which measures forces transferred through the FATC and input the values into a computer for analysis

apart (Fig. 1). The femoral end was mounted on to the superior (upper) bracket, which was directly attached to a 2.0 kN load cell (DACell UU-K200; 200 kgf range; 0.03 per cent non-linearity; Republic of Korea) (Fig. 2). The tibial end of the complex was mounted on to the inferior (lower) bracket when it had assumed a stationary position after free rotation. A linear variable-differential transformer (LVDT) (Solartron DCR25; ± 25 mm range; 0.3 per cent non-linearity, Leicester) was positioned on the frame of the tensometer to measure the displacement of the inferior bracket. Both the load cell and LVDT were connected to a computer sampling at 10 Hz with the data managed and graphed by Visual Designer software (Intelligent Instrumentation Inc.). Both the superior and the inferior brackets were free to rotate in the transverse and sagittal plane. This allowed the fibres of the ligaments to be naturally aligned to the direction of tensile loading.

Using a preload of 2 N, the cross-sectional area of the midsubstance of the ligament was estimated by multiple measurements using two Vernier callipers

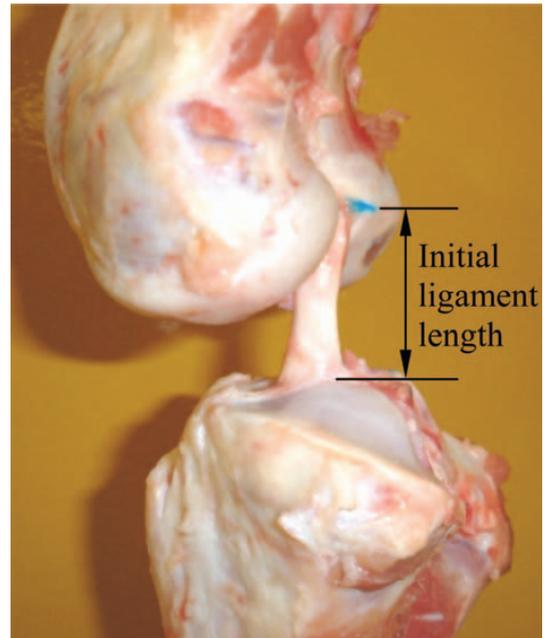


Fig. 3 The initial ligament length of a porcine specimen was estimated using the initial vertical length from the superior aspect of the femoral insertion to the posterior aspect of the tibial insertion with of a preload of 2 N

(± 0.05 mm) positioned at 90° to each other. This initial preloading (2 N) was used to allow some method of standardization (calibration) of the different specimens before each testing session began. The use of the two Vernier callipers assumed a rectangular area. Since ligaments are primarily circular in cross-section, this rectangle would be an overestimation of the area and would represent an underestimation of Young's modulus. It is important to identify that this cross-sectional area of the ligament was only taken at the beginning of the test and therefore the stress and strain measurements would not be true engineering measurements. The initial length of the ligament was measured as the vertical distance between the most superior point on the femoral attachment to the most posterior point on the tibial attachment using the same Vernier calliper (Fig. 3).

Tensile testing was divided into three groups (intact ACL, AMB, and PLB bands) with each individual porcine specimen being displaced at a rate of 0.33 mm/s to failure point. The literature suggests that a higher strain rate significantly alters the stress-strain curve [17–19]. Testing was carried out by displacement of the inferior bracket at a rate of 0.33 mm/s, while the superior bracket was held stationary. This displacement rate was comparable with those previously described in the literature [17,

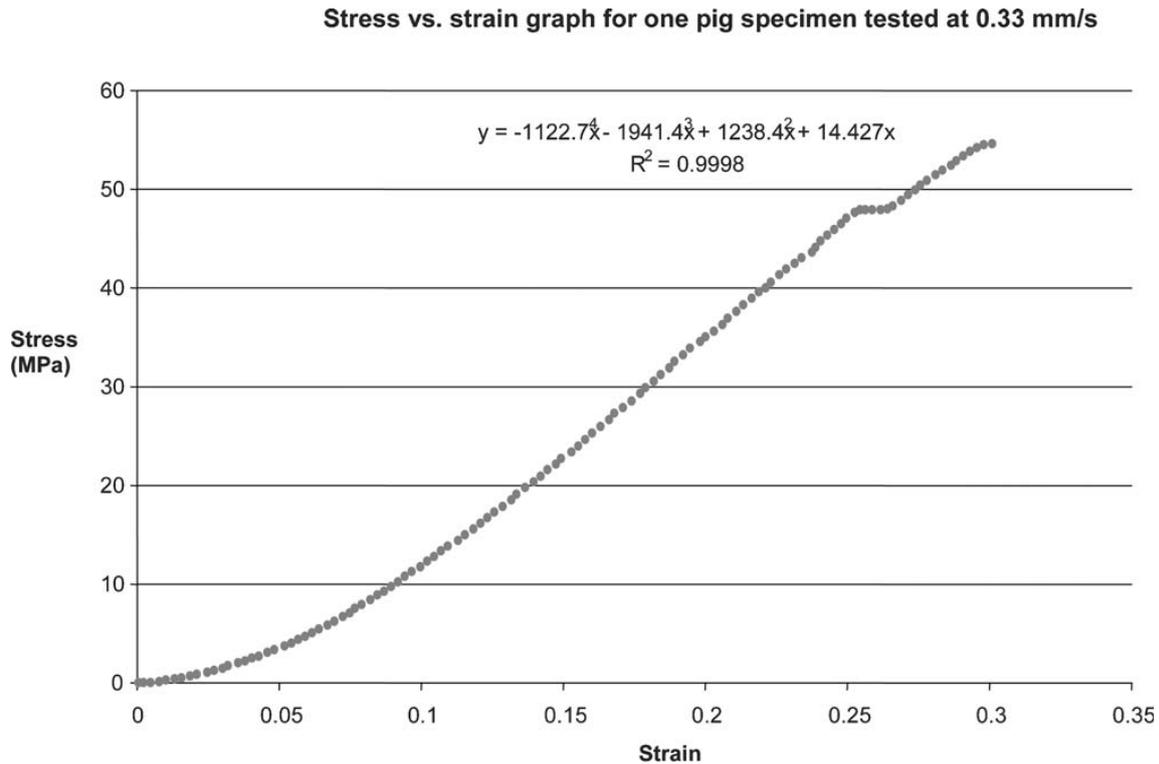


Fig. 4 The stress–strain curve obtained from one porcine specimen showing also the mathematical modelling of the curve and the R^2 (coefficient of determination) value.

19, 20] and was calculated by Visual Designer software as the change in grip-to-grip displacement divided by each sampling period of 0.1 s. A taring load of 2 N was used prior to each test to allow for standardization of specimens before test. Testing did not include any cyclic loading or preconditioning of the ligaments primarily because the work was interested in determining biomechanical structural and material properties *in vitro* rather than physiological properties usually experienced *in vivo*. Continual rehydration of the specimens with saline solution was not possible throughout the testing procedure. Data collection was divided into individual 1 h sessions to limit ligament dehydration. This was because the experimental apparatus was not specifically designed for testing animal ligaments (it was modified so that it best suited the specimens under test) and spraying the device with a saline solution would not have been a possibility in this open engineering laboratory environment. However, the specimens were wrapped in a saline cloth prior to and directly before being mounted in the testing apparatus. In addition, the complete testing of one specimen once mounted in the experimental device took no longer than 15 min.

Raw data collected in the form of load (N) and deformation (mm) were converted into stress

(MPa) and strain (ratio) respectively to allow for comparison of biomechanical properties between specimens of different sizes. The load and stress of each specimen were plotted against the deformation and strain respectively to obtain load–deformation and stress–strain graphs (Fig. 4). Failure point values for load and deformation were defined in the load–deformation curve as the values with the highest load and these were extracted for statistical analysis. Stress was calculated as the ratio of load to midsubstance cross-sectional area. Strain was calculated as the ratio of grip-to-grip displacement to the initial length of the ligament. Energy absorption (N mm) and strain energy density (MPa) at failure were determined by the area under the load–deformation and stress–strain curves up to the failure point respectively. Stiffness (N/mm) and elastic modulus (MPa) were calculated from the slopes of the linear regions of the load–deformation and stress–strain curves respectively. The calculations for stiffness and modulus were carried out at the inflection points of the load–displacement and stress–strain curves. These points were obtained by first fitting a fourth-order polynomial to the data and then using the second differential of this equation to find the inflection point of the curves.

Table 1 Means and standard deviations (SDs) for all variables of each experimental group

Group	Statistic	Load (N)	Deformation (mm)	Stiffness (N/mm)	Energy absorbed (N mm)	Stress (MPa)	Strain (ratio)	Modulus (MPa)	Strain energy density (MPa)
Intact ACL	Mean	853.53	8.67	147.02	3715.36	32.22	0.32	147.76	5.40
	SD	252.32	1.70	29.79	1991.53	15.63	0.08	61.75	4.08
AMB	Mean	453.47	8.72	79.17	1739.19	19.65	0.27	111.12	2.36
	SD	127.61	1.75	15.73	533.02	6.91	0.06	30.09	0.99
PLB	Mean	588.81	7.08	130.27	2057.88	23.27	0.30	123.32	3.14
	SD	144.55	2.70	27.47	1110.51	6.66	0.10	45.55	1.13

The data were analysed using the analysis of variance (ANOVA) technique from the Statistical Package for Social Sciences (SPSS) version 14.0. The ANOVA assessed differences in the means of each variable (load, deformation, stiffness, energy absorbed, stress, strain, modulus, and strain energy density) between each experimental group (intact ACL, AMB, and PLB). The Levene test of equality of error variances and the Shapiro–Wilk test of normality were conducted on all groups of data to test for equality of variance and normality of distribution respectively. All statistical testing was conducted with $\alpha = 0.05$.

3 RESULTS

Testing was performed on 44 knee specimens as one of the porcine femur–PLB–tibia complex specimens was damaged during dissection (15 intact, 15 AMB, and 14 PLB). Specimens that showed bony avulsion prior to ultimate failure was also excluded, which resulted in the inclusion of a total of 30 knee specimens being included for statistical analysis (11 intact, 11 AMB, and eight PLB).

Means, standard deviations, and coefficients of variance were calculated for all eight variables, after mathematical calculation of stiffness, modulus, energy absorbed, and strain energy density (Table 1). The Levene test of equality of error variances demonstrated that the error variances of the majority of parameters were not equal; hence post-hoc testing was performed with the Tamhane test. Box plots were utilized to exclude outliers, as defined by SPSS 14.0, from the final ANOVA.

Graphical representations of only the statistically significant mean differences have been presented as box plots containing error bars at the 95 per cent confidence level (Figs 5 to 7). Numerous significant differences in the dependent variables were found between the intact porcine FATC and both the anteromedial and the posterolateral band–bone complexes. The ultimate load values were significantly higher for the intact porcine FATC group than

for the AMB and PLB specimens ($p \leq 0.031$). Stiffness of the PLB was similar to that of the intact FATC, but both the intact FATC and the PLB specimens had significantly higher stiffnesses than the AMB specimens ($p \leq 0.001$). Energy absorption by the intact porcine FATC group was significantly higher than that of the AMB group ($p = 0.028$).

The mean energy absorbed by the intact porcine FATC was approximately equal to the sum of the energy absorbed by both the AMB and the PLB. Deformation and strain were not statistically significantly different for all three structures; hence it could be expected that the summation of the two bands (AMB and PLB to form the entire ACL) would absorb the total of energy absorbed by both bands.

No material properties (stress, strain, modulus, and strain energy density) of the intact porcine, AMB, and PLB sample groups were statistically significantly different. As the material properties of a ligament–bone complex reflect the biomechanical properties of the ligament midsubstance, the similarities in the material properties of the three struc-

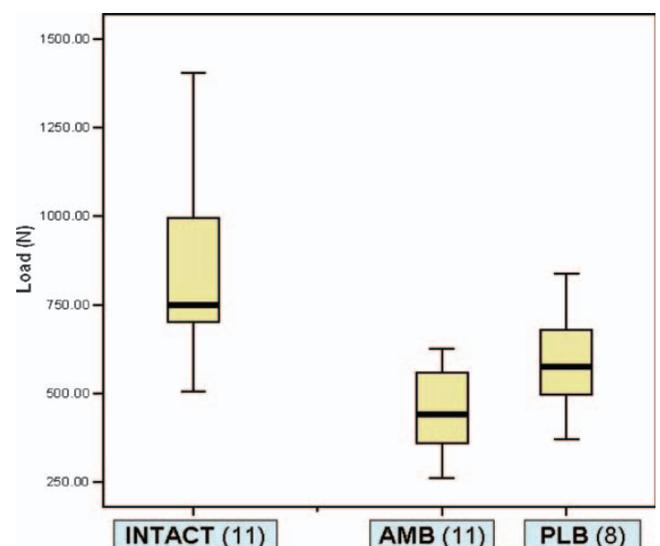


Fig. 5 Box plot showing the mean, range, and error bars for the loads of all groups tested at the 95 per cent confidence level

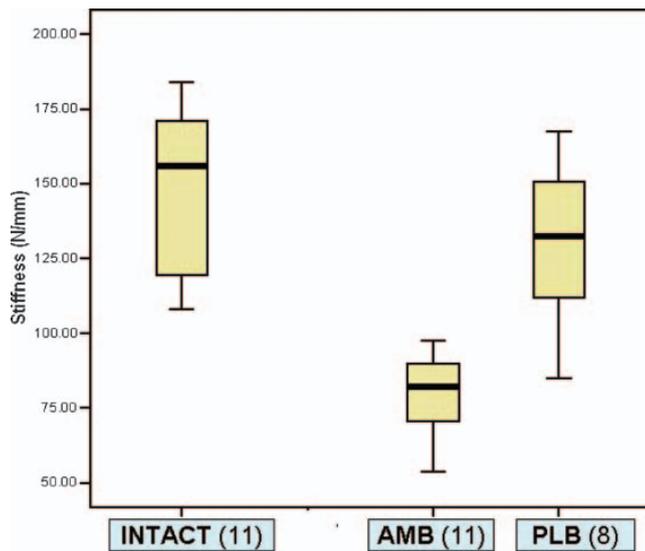


Fig. 6 Box plot showing the mean, range, and error bars for the stiffnesses of all groups tested at the 95 per cent confidence level

tures suggest a more or less homogeneous tissue composition throughout the porcine FATC.

4 DISCUSSION

The attachments of both bands on the lateral femoral condyle were observed to be similar in size and location, but considerable dissimilarity exists in the tibial insertion, which was the likely source of any biomechanical disparities between the AMB and PLB. The AMB was observed to insert over a larger area and at more acute angles than the PLB did. The similarities between the stiffnesses of the intact porcine FATC and PLB complex suggest that the PLB constitutes a considerable proportion of the load-bearing properties of the entire porcine ACL. Therefore, the porcine PLB resembles more of the entire ACL than the AMB.

The anatomical and functional analysis of the porcine ACL by Fuss [8] yielded similar findings, where it was noted that the posterolateral band of the porcine ACL contained all the guiding bundles of the ACL and this is therefore functionally analogous to the entire human ACL. Both Butler *et al.* [14] and Xerogeanes *et al.* [10] noticed higher load attenuation by the AMB, which seemingly conflicts with the results of the current study. Butler *et al.* [14] divided the human ACL into anteromedial, anterolateral, and posterior bands and noticed significantly greater stresses within the anteromedial and anterolateral bands. Results from that study were obtained from a different anatomical structure (the human FATC)

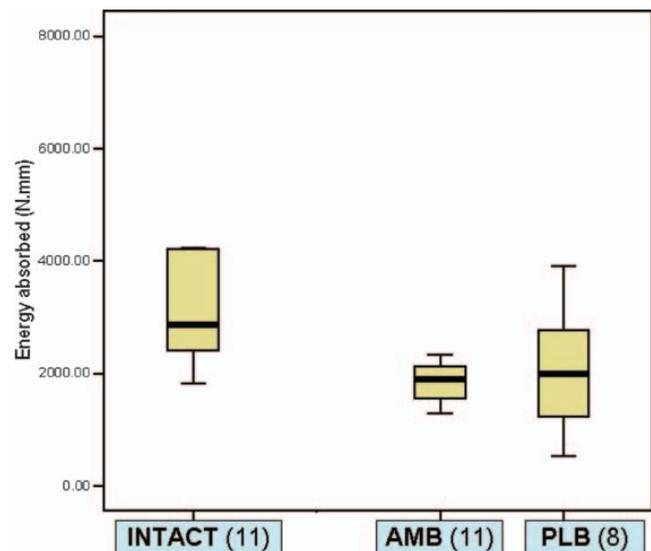


Fig. 7 Box plot showing the mean, range, and error bars for energy absorbed of all groups tested at the 95 per cent confidence level

and different band division. Therefore the 'anteromedial bands' of the two studies are not likely to contain the same anatomical fibre groups. Xerogeanes *et al.* [10] placed an anterior translatory tibial load on the porcine ACL as opposed to the inferior displacement of the tibia conducted in the current study. The higher *in-situ* forces within the AMB are consistent with the fact that the AMB has a greater function in restraining anterior translation of tibia on the femur [11, 12, 21].

Deformation and strain at failure point were not different for the intact porcine FATC, PLB, and AMB complexes in the current study. Butler *et al.* [14] noticed a similar trend of strain dependence in ligamentous failure in bands of human FATC and speculated on the relationship of strain on failure. They concluded that the human FATC failed when a certain strain was achieved and this was not limited by any maximal load or stress. As the results of the current study ascertained that intact porcine FATC, AMB, and PLB complexes failed at similar deformations and strains, the strain dependence of the ligament-bone complex failure, if it does exist, is not affected by insertion size, angulation, or cross-sectional area.

The mean energy absorbed by the intact porcine FATC was approximately equal to the sum of the energy absorbed by both the AMB and the PLB. The deformations and strains are statistically similar for all three structures as previously reported, and so it could be expected that the summation of the energy absorbed by the two bands (AMB and PLB to form

the entire ACL) individually would absorb the total of energy absorbed by both bands together. However, the energy absorbed by the intact ACL was not significantly higher than the energy absorbed by the PLB, further suggesting that the PLB contributes to a major proportion of the biomechanical properties of the entire ACL.

No material property of the intact porcine, AMB, and PLB specimen groups was significantly different. As the material properties of a ligament–bone complex reflect the biomechanical properties of the ligament midsubstance, the similarities in the material properties of the three structures suggest a more or less homogeneous tissue composition throughout the porcine FATC. However, the material properties reported in this study incorporated characteristics of the ligament–bone interfaces. It was observed that the attachments of the AMB lay at a more acute angle with the tibial articular surface than the PLB insertion. The location and angle of ligamentous attachments have been shown to be significant in altering the tensile properties of the ligament–bone complexes [22–24]. When a ligament–bone complex is loaded in the longitudinal direction of its fibres, force uptake is maximized owing to the simultaneous tension of the majority of load-bearing fibres. Therefore the PLB should be expected to experience higher stiffness and modulus than the AMB does. Stiffness in the PLB was indeed found to be significantly greater than that of the AMB, but the moduli were not. The probable reason for the similarity in the modulus is that the ligament midsubstance has a much greater contribution to the material properties of the ligament–bone complex than the ligament–bone interface has. Hence any disparities caused as a result of the distinct attachment structure between the AMB and PLB would be less influenced than the similarities in the midsubstance tissue composition.

5 LIMITATIONS

There are a number of limitations that should be acknowledged in this study. First, specimen acquisition is limited in the detail of information regarding each specimen. The specific age, weight, and history of each animal from which the specimens were obtained is unknown, but an approximation was provided by the meat-processing plant. The approximate mean ages of the pigs from which the specimens were obtained were 4 months old. Skeletal immaturity is suspected within the porcine specimens and the suspicions were supported by the

high proportion of avulsion failures during tensile testing. Owing to data loss from pre-failure bony avulsions, a number of sample groups were diminished in size, reducing the statistical power.

Random variations in bony alignment as a result of the drilling process were unavoidable, but similar variations would arise from alternative methods of bony fixation using clamps or bone–cement cups. Possible ligament dehydration could have occurred despite the short testing sessions.

Estimations of the ligament midsubstance cross-sectional area produced using Vernier callipers could be overestimates of the actual area for the ligaments. Calculations of strain were conducted from assumptions that the initial vertical distance between the superior aspect of the ACL femoral insertion and the posterior aspect of the tibial insertion represented the ligament fibre length. The actual initial ligament lengths were underrated using the described method as it is known that the ACL travels anteriorly from the femur and fibre lengths within the ACL are not homogeneous. Finally, the displacement measurement of the tensometer is not the true ligament displacement. Without having strain gauges embedded in the ligament or laser or video measurement techniques this is a very difficult measure to obtain accurately. At failure loads of 850 N it could be argued that there would potentially be a small amount of movement in the bone pins. However, the bone pin locations were examined after the testing had been completed and there was no damaged or deformity present in any of the specimens. The pins that were fitted into the bones were as ‘tight’ a fit at the end as they were at the beginning of the process.

6 CONCLUSION

This study showed that the PLB of the porcine ACL is significantly stiffer than the AMB. As expected, the intact porcine ACL contains higher load-bearing and energy-absorbing properties than both separate bands. All three ligament–bone complexes failed at similar strains, which implies that the stretching of a ligament induces its rupture. As ligament deformation is related to the forces applied, the clinical correlation between the force of injury loading and ligamentous damage is expected. With current advances in xenografting technology [7], porcine ACL grafts for human ACL reconstructions could be a future possibility. However, it is important to understand both the mechanical and the structural properties of the porcine ACL. Further research is therefore needed to compare the biomechanics of

more porcine and human ACLs, as well as a review of the current xenografting protocols, before any application to surgery can be made with scientific confidence.

CONFLICT OF INTEREST STATEMENT

To the best of our knowledge, there is no conflict of interest with regard to this research.

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REFERENCES

- 1 **Steckel, H., Starman, J. S., Baums, M. H., Klinger, H. M., Schultz, W., and Fu, F. H.** The double-bundle technique for anterior cruciate ligament reconstruction: a systematic overview. *Scand. J. Med. Sci. Sports*, 2007, **17**, 99–108.
- 2 **Zelle, B. A., Brucker, P. U., Feng, M. T., and Fu, F. H.** Anatomical double-bundle anterior cruciate ligament reconstruction. *Sports Med.*, 2006, **36**, 99–108.
- 3 **Aglietti, P., Giron, F., Buzzzi, R., Biddau, F., and Sasso, F.** Anterior cruciate ligament reconstruction: bone-patellar tendon-bone compared with double semitendinosus and gracilis tendon grafts. A prospective, randomized clinical trial. *J. Bone Jt Surg. Am.*, 2004, **86**, 2143–2155.
- 4 **Beynon, B. D., Johnson, R. J., Fleming, B. C., Kannus, P., Kaplan, M., Samani, J., and Renström, P.** Anterior cruciate ligament replacement: comparison of bone-patellar tendon-bone grafts with two-strand hamstring grafts: a prospective, randomized study. *J. Bone Jt Surg. Am.*, 2002, **84**, 1503–1513.
- 5 **Williams, R. J., Hyman, J., Petrigliano, F., Rozentel, T., and Wickiewicz, T. L.** Anterior cruciate ligament reconstruction with a four-strand hamstring tendon autograft. *J. Bone Jt Surg. Am.*, 2004, **86**, 225–232.
- 6 **Woo, S. L., Kanamori, A., Zeminski, J., Yagi, M., Papageorgiou, C., and Fu, F. H.** The effectiveness of reconstruction of the anterior cruciate ligament with hamstrings and patellar tendon: a cadaveric study comparing anterior tibial and rotational loads. *J. Bone Jt Surg. Am.*, 2002, **84**, 907–914.
- 7 **Stone, K. R., Abdel-Motal, U. M., Walgenbach, A. W., Turek, T. J., and Galili, U.** Replacement of human anterior cruciate ligaments with pig ligaments: a model for anti-non-gal antibody response in long-term xenotransplantation. *Transplantation*, 2007, **83**, 211–219.
- 8 **Fuss, F. K.** Anatomy and function of the cruciate ligaments of the domestic pig (*Sus scrofa domestica*): a comparison with human cruciates. *J. Anat.*, 1991, **178**, 11–20.
- 9 **Kääb, M. J., Gwynn, I. A., and Nötzli, H. P.** Collagen fibre arrangement in the tibial plateau articular cartilage of man and other mammalian species. *J. Anat.*, 1998, **193**, 23–34.
- 10 **Xerogeanes, J. W., Fox, R. J., Takeda, Y., Kim, H., Ishibashi, Y., Carlin, G. J., and Woo, S. L.** A functional comparison of animal anterior cruciate ligament models to the human anterior cruciate ligament. *Ann. Biomed. Engng*, 1998, **26**, 345–352.
- 11 **Fuss, F. K.** Anatomy of the cruciate ligaments and their function in extension and flexion of the human knee joint. *Am. J. Anat.*, 1989, **184**, 165–176.
- 12 **Amis, A. A. and Dawkins, G. P.** Functional anatomy of the anterior cruciate ligament. Fibre bundle actions related to ligament replacement and injuries. *J. Bone Jt Surg. Br.*, 1991, **73**, 260–267.
- 13 **Bach, J. M., Hull, M. L., and Patterson, H. A.** Direct measurement of strain in the posterolateral bundle of the anterior cruciate ligament. *J. Biomech.*, 1997, **30**, 281–283.
- 14 **Butler, D. L., Guan, Y., Kay, M. D., Cummings, J. F., Feder, S. M., and Levy, M. S.** Location-dependent variations in the material properties of the anterior cruciate ligament. *J. Biomech.*, 1992, **25**, 511–518.
- 15 **Gabriel, M. T., Wong, E. K., Woo, S. L., Yagi, M., and Debski, R. E.** Distribution of in situ forces in the anterior cruciate ligament in response to rotatory loads. *J. Orthop. Res.*, 2004, **22**, 85–89.
- 16 **Edge, M. K., Maguire, T., Dorian, J., and Barnett, J. L.** National Animal welfare standards at livestock processing establishments preparing meat for human consumption. 1. The Standards, 2005 (Australian Meat Industry Council, Crows Nest, New South Wales).
- 17 **Noyes, F. R., Delucas, J. L., and Torvik, P. J.** Biomechanics of anterior cruciate ligament failure: an analysis of strain-rate sensitivity and mechanisms of failure in primate. *J. Bone Jt Surg. Am.*, 1974, **56**, 236–253.
- 18 **Lydon, C., Crisco, J. J., Panjabi, M., and Galloway, M.** Effect of elongation rate on the failure properties of the rabbit anterior cruciate ligament. *Clin. Biomech.*, 1995, **10**(8), 428–433.
- 19 **Pioletti, D. P., Rakotomanana, L. R., and Leyvraz, P. F.** Strain rate effect on the mechanical behavior of the anterior cruciate ligament-bone complex. *Med. Engng Phys.*, 1999, **21**, 95–100.
- 20 **Woo, S. L., Newton, P. O., MacKenna, D. A., and Lyon, R. M.** A comparative evaluation of the mechanical properties of the rabbit medial collateral and anterior cruciate ligaments. *J. Biomech.*, 1992, **25**, 377–386.
- 21 **Chhabra, A., Starman, J. S., Ferretti, M., Vidal, A. F., Zantop, T., and Fu, F. H.** Anatomic, radiographic, biomechanical, and kinematic evaluation of the anterior cruciate ligament and its two functional bundles. *J. Bone Jt Surg. Am.*, 2006, **88**, 2–10.
- 22 **Mommersteeg, T. J., Kooloos, J. G., Blankevoort, L., Kauer, J. M., Huikes, R., and Roeling, F. Q.** The fibre bundle anatomy of human anterior cruciate ligaments. *J. Anat.*, 1995, **187**, 461–471.

- 23 Woo, S. L., Hollis, J. M., Roux, R. D., Gomez, M. A., Inoue, M., Kleiner, J. B., and Akeson, W. H. Effects of knee flexion on the structural properties of the rabbit femur–anterior cruciate ligament–tibia complex (FATC). *J. Biomech.*, 1987, **20**, 557–563.
- 24 Woo, S. L., Kanamori, A., Zeminski, J., Yagi, M., Papageorgiou, C., and Fu, F. H. The effectiveness of reconstruction of the anterior cruciate ligament with hamstrings and patellar tendon: a cadaveric study comparing anterior tibial and rotational loads. *J. Bone Jt Surg. Am.*, 1991, **84**, 907–914.