Original Communication

REGIONAL VARIATION IN THE MICROSCOPY AND TENSILE STRENGTH OF THE LINEA ALBA IN THE BABOON (*Papio Anubis)*

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RESUMEN

Introducción: La línea alba conecta el rectus abdominis y, por lo tanto, su debilitamiento o el aumento de la tensión intra-abdominal puede resultar en una diastasis rectal. El objetivo de este estudio es investigar la morfología funcional y la resistencia a la tracción de la línea alba en un primate no humano. Materiales y métodos: Utilizando como puntos de referencia el xifoides, el ombligo y el tubérculo púbico, fueron resecados tejidos de la zonas epigástrica, umbilical e hipogástrica de la línea alba de siete babuinos machos. Estos tejidos se procesaron a través del microscopio y tensiometría. Resultados: La línea alba se compone principalmente de fibras de colágeno organizadas en tres láminas, a saber, superficiales, intermedias y profundas, además de algunas fibras elásticas. La lámina intermedia de la línea alba umbilical se caracterizó por estar formada de grupos compactos y gruesos de colágeno alineados longitudinalmente y oblicuamente que se fusionan en el centro y forman una masa. La fuerza máxima para romper la línea alba durante una tracción longitudinal y oblicua fue de 40 N/mm2 y 63.6 N/mm2 con una tensión de 0.35 y 1.19 respectivamente. El módulo de Young de la línea alba mostró que, la línea alba epigástrica y umbilical tuvo el mayor coeficiente de elasticidad media, de 289 N/mm2 y 328 N/mm2, respectivamente, cuando fueron expuestos a una tracción oblicua. Conclusión: La estructura de la línea alba del babuino está diseñada para soportar grandes tensiones o fuerzas multidireccionales.

Palabras claves*: fibras de colágeno; fibras elásticas; diastasis abdominal; hernias ventrales; primate.*

ABSTRACT

Introduction: The linea alba connects the rectus abdominis and thus weakening or increased abdominal pressure may result in diastasis recti. The study aims to investigate the functional morphology and the tensile strength of the linea alba in a non-human primate. Materials and Methods: Using the xiphoid process, the umbilicus, and the pubic tubercle as landmarks, tissues were resected from the epigastric, umbilical and hypogastric parts of the linea alba from seven male baboons. The tissues were processed for microscopy and tensiometry. Results: The linea alba was made up of mainly collagen fibres organized into three laminae namely a superficial, intermediate and deep in addition to a few elastic fibres. The intermediate lamina of the umbilical linea alba was characterized by thick compact bundles of longitudinally and obliquely aligned collagen bundles which fused in the midline to form a mass. The maximal/ ultimate stress needed to tear the linea alba during longitudinal and oblique traction was 40 N/mm^2 and 63.6 N/mm^2 at a strain of 0.35 and 1.19 respectively. The linea alba's Young's modulus showed that on average the epigastric and umbilical linea alba had the highest coefficient elasticity at 289 N/mm^2 and 328 N/mm^2 respectively, when they were exposed to oblique traction. Conclusion: The structure of the baboon linea alba is well organized to withstand strong multidirectional forces.

Key words*: Collagen fibers; elastic fibers; diastasis recti; ventral hernia; primate.*

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Received: 15 July, 2016. **Revised**: 23 July, 2016. **Accepted:** 18 August, 2016.

INTRODUCTION

The linea alba (LA), is a tough midline tendinous structure formed by the interlocking aponeurotic fibers of the three anterolateral abdominal muscles namely external oblique, internal oblique and transversus abdominis muscles crossing from one side to the other (Askar, 1977; Axer et al, 2001a; Standring et al, 2008). The clinical function of the LA is to maintain the abdominal muscles, particularly the rectus abdominis, at a certain proximity to each other (Beer et al, 2009). The split between the two rectus abdominis muscles, often referred to as "rectus abdominis diastasis" (Brauman, 2008; Benjamin et al, 2014), usually results from the stretching and thinning of the LA.

The human abdominal LA has been described, as composed of a highly structured meshwork of obliquely and transversely aligned collagen fibers (Axer et al, 2001a). In another study by Pulei et al (2015), females were found to have more transversely aligned collagen and elastic fibers than males especially in the hypogastric region. The elastic fibers were more abundant in the anterior lamina, in the lateral and in the hypogastric parts of the LA. These regional variations and sex differences were ascribed to reflect differences in function with respect to respiratory and visceral support (Axer et al, 2001a; Pulei et al, 2015). Using a biomechanical study, Grassel et al (2005), proposed that when the LA is exposed to increasing force in a longitudinal, transverse and oblique direction, compliance was highest in the longitudinal direction and least in the transverse direction (Grassel et al, 2005).

Rectus abdominis diastasis commonly occurs in child bearing women at different sites along the LA (Bursch, 1987; Hsia and Jones, 2000). The LA of patients with ventral hernias, in epigastric hernias especially, have been reported to consist of exclusively a single midline pattern of decussating collagen fibres compared to the more secure digastric pattern seen in the normal LA structure. Hernias have also been reported in non-human primates (Chaffee and Shehan, 1973; Warren et al, 1979; Carpenter and Riddle, 1980) and other quadrupeds (Vilar et al, 2011). Whereas rectus abdominis diastasis and ventral herniae are common in humans and quadrupeds, there are virtually no reports of their existence in non-human primates (Fitzgibbons et al, 2002; Brengio et al, 2003). The aim of this study is to establish the microscopic structure and tensile strength of the LA of a non-human primate, the baboon (*Papio Anubis*), in order to understand its adaptation to increased abdominal pressure and possibly elucidate further the genesis of the rectus abdominis diastasis as well as ventral herniae.

MATERIALS AND METHODS

The ventral abdominal wall (Fig. 1) was harvested from 7 (seven) healthy male baboons (*Papio Anubis*, weight range 10-13kg), 1 to 2 years old, obtained from the Institute of Primate Research, Kenya. The animals were sedated with ketamine HCL (10mg/kg, vetelar, parnke Davies Co., ponypool Germany) and xylazine (10 mg/kg, Ilium xylazil^{R}, Troy lab Pty limited, Smithfield, Australia) IP/IM and then euthanized with 20% pentobarbitone sodium (80mg/kg body weight).

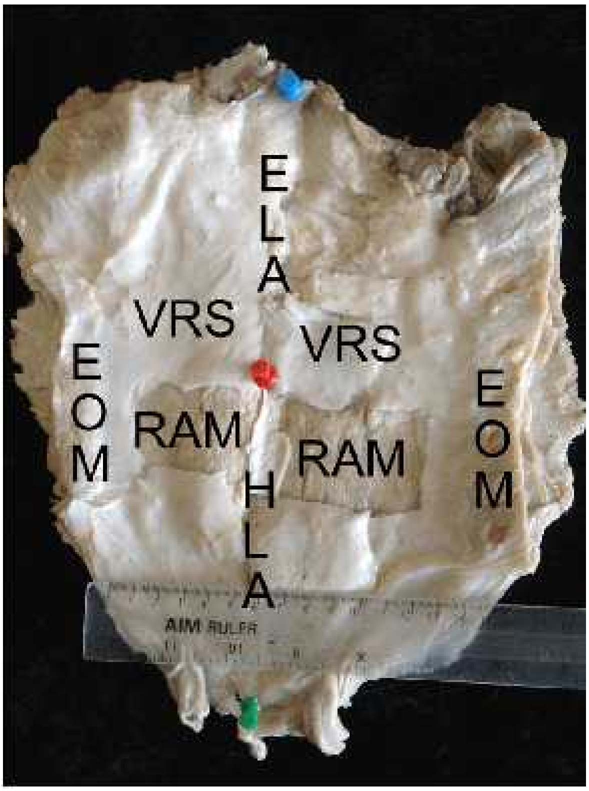


Figure 1- Prosected specimen of the fibrous ventral abdominal wall of the baboon (*Papio Anubis*) illustrating the resection areas from the linea alba, a few muscles and other relevant landmarks. ELA- Epigastric linea alba; HLA- Hypogastric linea alba; ULA/ Red pinhead- Umbilical linea alba; Blue pinhead- the xiphoid process; green pinhead- the pubic symphysis; VRS- Ventral rectus sheath; RAM- Rectus abdominis muscle; EOM- External oblique abdominis muscle.

The skin and the fascia were incised via a midline abdominal incision. This flap was reflected to expose the important landmarks of the LA, namely the xiphoid process, the umbilicus and the pubic symphysis. Using a metal template (40mm x 20mm), pieces of tissue were resected from the mid-epigastric (ELA), the umbilical (ULA) and the mid-hypogastric (HLA) levels of the LA (Fig. 1). They were collected in a longitudinal (L) and oblique direction (O). Specimens cut parallel to the LA were denoted as L (longitudinal), while those cut in a direction parallel to the direction of the oblique muscles were denoted as O (oblique).

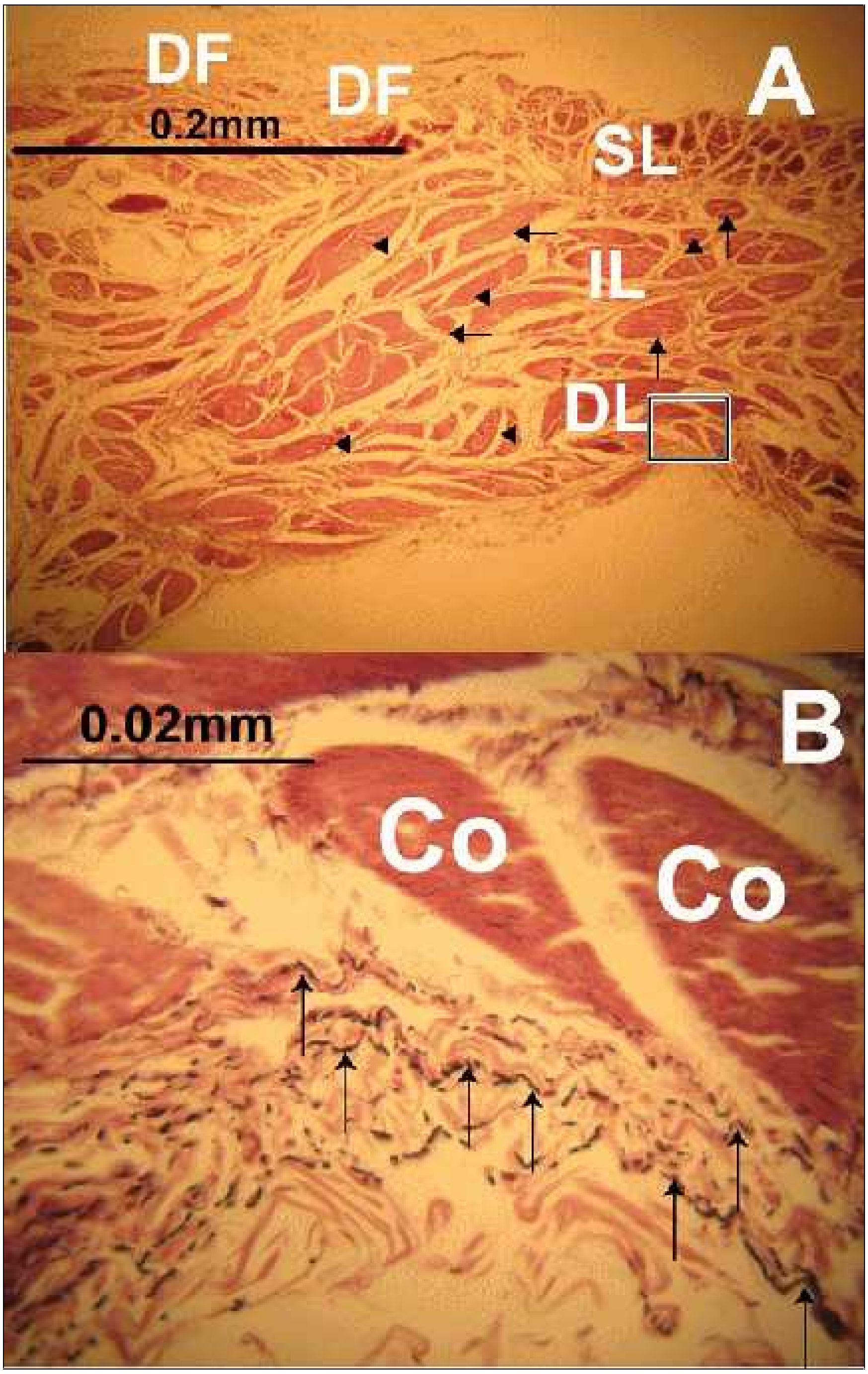


Figure 2A shows the deep fascia (DF) and the three characteristic laminae of the linea alba: superficial (SL), intermediate (IL) and deep (DL) in the ELA. Note the longitudinally aligned collagen fibres (vertical black arrows) and oblique collagen bundles (horizontal black arrows) **Figure 2B** - shows a high magnification power (inset of Fig 2A), illustrating the thin edge of longitudinally elastic fibres (black arrows), found in between the collagen bundles (Co), lining the deep laminae (DL) of the ELA.

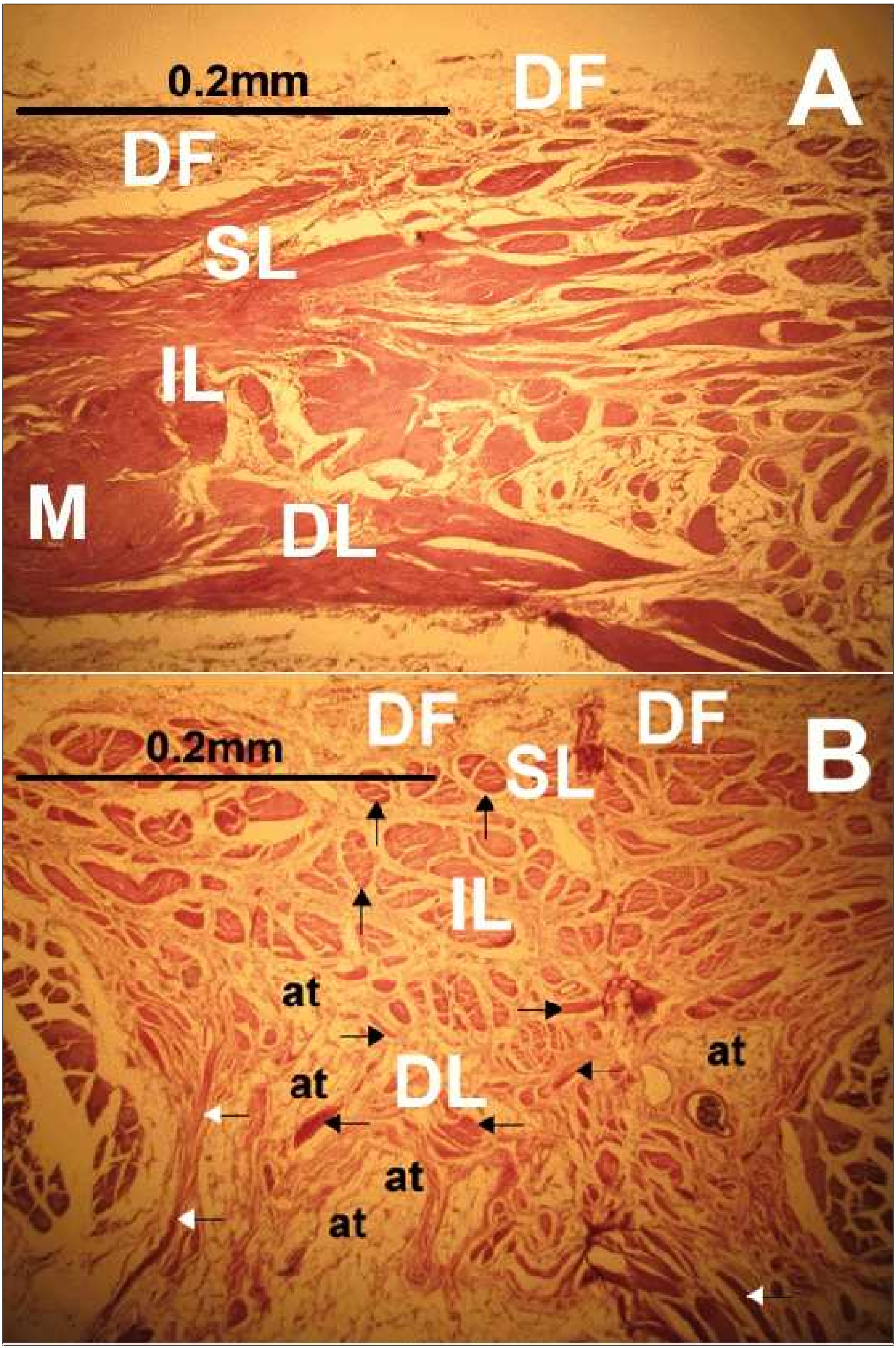


Figure 3A shows the collagen bundles arising from the superficial (SL), intermediate (IL) and deep (DL) laminae fusing to form a midline mass (M) in ULA. **Figure 3B** illustrates the longitudinally aligned collagen fibres (vertical black arrows), oblique collagen bundles (horizontal white arrowheads) and transversely aligned collagen bundles (horizontal black arrows) found in the superficial (SL), intermediate (IL) and deep (DL) laminae of the HLA. Note the adipose tissue (at) interspersed between the collagen bundles in the deep lamina (DL).

A transverse strip (20mm x 10mm), excised off each of the tissues were then fixed in 10% formol-saline. Thereafter, they were trimmed and then dehydrated through increasing concentr-ations of alcohol from 50% to 100%, cleared with cedar wood oil before they were embedded in paraffin wax. Seven micrometer (7um) sections were cut from the paraffin blocks using a Leitz Wetzlar sledge microtome (Leica^R Model SM2400, Leica Microsystems, Nussloch GmbH, Germany), collected on slides, stained with Weigert resorcin-fuchsin stain and counterstained with Van Gieson stain (Drury et al, 1967). Fifty six sections from each region were selected for histomorphometry. They were examined under a Leica light microscope (BME model, Germany) at magnification of x40 and x400. Photomicrographs were taken using Zeiss^{TM} digital photomicroscope (Carl Zeiss AG, Oborkochen, Germany) and images analyzed with Image J software (Schneider et al, 2012). The thicknesses of the different layers of the LA were measured in millimeters and the ratios were then plotted on a graph.
Using a protocol employed by Seifert et al (2012), thirty (30) tissue strips, which were fresh from five (5) different baboons, were kept in a phosphate buffered saline before being exposed to mechanical testing. The strips were placed firmly in metal screw clamps with rubber pieces covering the clamp ends of the Hounsfield tensometer (80035; Pesola, Baar, AG Switzerland), to be stretched at a rate of 20mm/min. Each test took on average 2 to 4 minutes to complete. Tissue width (mm) and thickness (mm) at the waist (around the middle third of the tissue between clamps) were measured using a Vernier caliper before loading was started. The distance between each clamp was standardized at 12mm. Force (N) and displacement (mm) were measured on XY plotter, and these points were subsequently recorded as stress (force per cross sectional area) and strain (fractional change in length) and replotted in Microsoft Excel (Microsoft Office Professional 2013). Tensile strength and failure strain were also recorded.
When a sample tore or ruptured outside the measured area (outside the waist), the result was excluded from the analysis. The mean stress-strain curves were the plotted in a graph to compare the three regions examined. No prior preconditioning was done to the tissues.

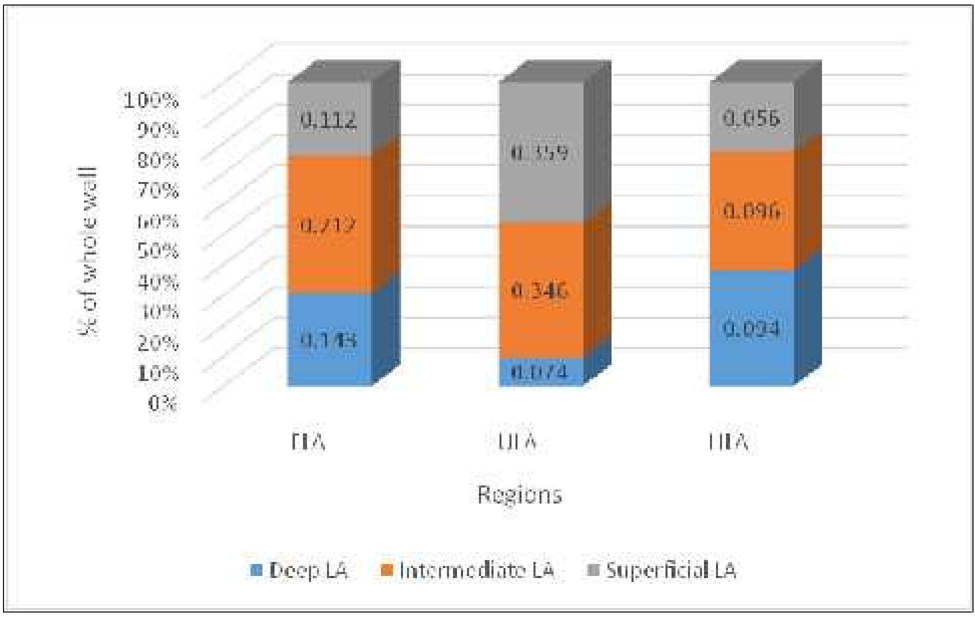
Baboon LA	Epigastric	SD	Umbilical	SD	Hypogastric	SD
Thickness (mm)	**0.467**	**0.073**	**0.779**	**0.064**	**0.246**	**0.052**
UTS (N/mm^2) for longitudinal traction	**32.4**	**0.5**	**18.8**	**1.1**	**40**	**0.3**
UTS (N/mm^2) for oblique traction	**40**	**3.4**	**23.6**	**2.3**	**63.6**	**4.1**

Table 1 - Comparison of mean thickness and mean ultimate tensile strength (UTS) of the LA. Note how the umbilical LA was the thickest, while the hypogastric LA had the highest UTS of 63.6 N/mm^2 when exposed to oblique traction. SD: Standard deviation.

RESULTS

When examined under light microscopy, the ELA, ULA and HLA were found to contain mainly collagen fibers with a few scattered elastic fibers. The collagen fibers were organized into three laminae namely: superficial, intermediate and deep one, in relation to the subcutaneous tissue (Fig. 2 and 3).
The superficial lamina of the ELA was made up of predominantly longitudinally arranged collagen bundles. The intermediate lamina consisted of an admixture of longitudinal and oblique collagen fibers. The deep lamina consisted of mainly thick, obliquely aligned collagen bundles (Fig. 2A). These thick collagen bundles on the dorsal surface were lined with fine longitudinally aligned elastic fibers (Fig. 2B).
Caudally at ULA, all the laminae showed increased number of collagen bundles. These became thicker towards the center of ULA to fuse into a thick mass (Fig. 3A).
The three laminae of HLA consisted of predominantly longitudinally aligned collagen fibers. The superficial and intermediate laminae had thicker collagen bundles while the deep lamina had thinner fibers. Fine longitudinally arranged elastic fibers were noted in the deep fascia lying on the ventral surface of the superficial lamina (Fig. 3B).
Using the Image J analyzer, on average, the ULA was noted to be thickest (0.779mm) while the HLA was the thinnest at 0.246mm (Table 1). The superficial lamina increased from less the 24% and 23% of the wall section in ELA and HLA respectively to 46% in ULA (p-0.003). Proportion-

ately, the deep lamina was the thinnest (9.5%) relative to the total wall thickness at the level of the ULA (Graphics 1).
During longitudinal and oblique loading, the LA displayed typical stress-strain curves for destructive tensile testing soft tissues before breaking at its waist with a characteristic plane. Collagen fibril failure, which is characteristic of the plastic region (region yield or failure) was quite prominent in all curves. Likewise, collagen fibril straightening at the elastic region (toe and linear region) was more easily demonstrable during longitudinal traction compared to oblique traction. The maximal/ ultimate tensile strength needed to tear the LA during longitudinal traction and oblique traction was 40N/mm^2 and 63.6 N/mm^2 at a strain of 0.35 and 1.19 respectively (Graphics 2).



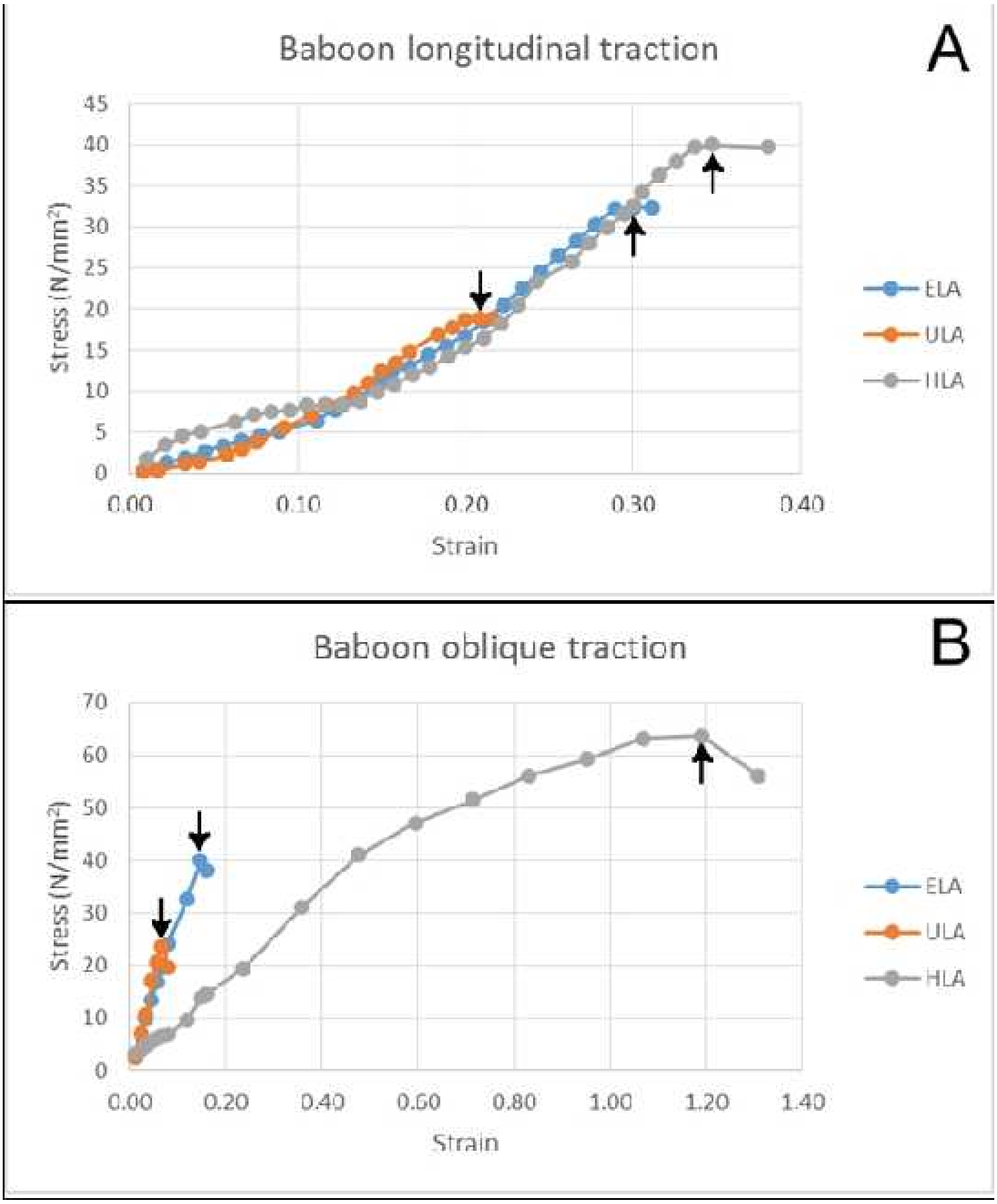
Graphic 1: A bar chart showing percentage proportions mean measurements of the different laminae of the baboon LA at different regions. Note that unlike the superficial and intermediate lamina (p-0.002), the deep lamina gradually reduced in size proportionately towards the umbilical LA (ULA) (p- 0.018).

The greatest strain encountered in the LA was in the HLA at 0.35 and 1.19 when it was exposed to longitudinal and oblique traction respectively to reach its ultimate breaking point. Regionally, the HLA also exhibited the largest toe region compared to the other regions (Graphics 2).
The LA's Young's modulus was calculated over linear stress-strain curves. These values showed that, the ULA and the ELA had the highest coefficient of elasticity with about the same value when exposed to oblique traction. However, when exposed to longitudinal traction, all these three regions exhibited the same coefficient of elasticity (Graphics 3).

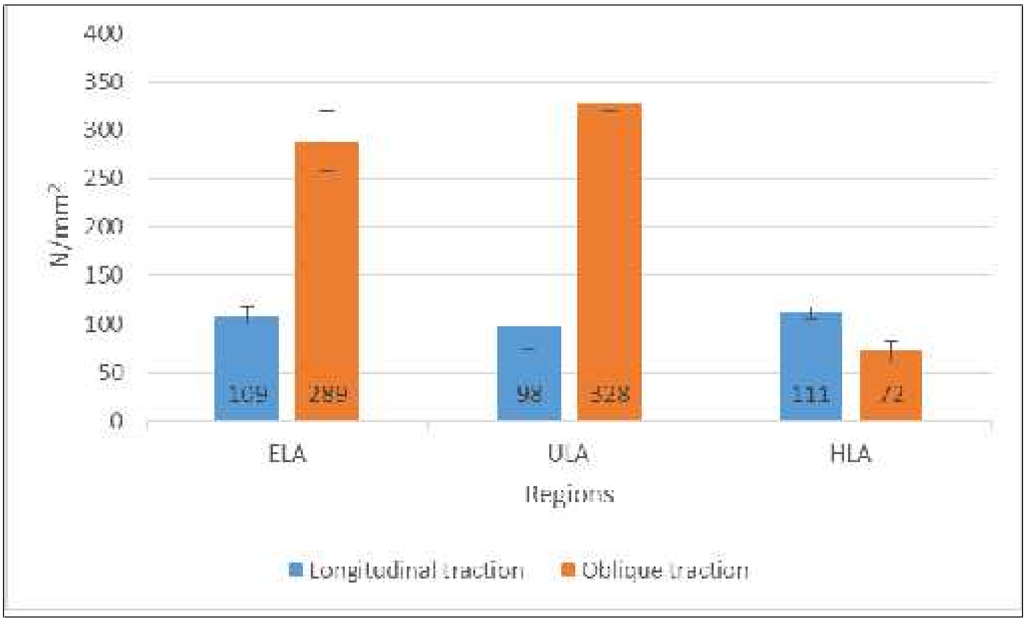
DISCUSSION

The regional variations in structure of the equine LA and associated tensiometric properties have been shown to be well designed to withstand the biomechanical stress generated by intra-abdominal pressure (Trostle et al, 1994). Kirker-head et al (1989) reported that the epigastric and hypogastric equine LA is well structured to accommodate the rapid forces associated with strenuous truncal movements (Kirker-head et al, 1989). In contrast, our study in the baboon has shown higher concentrations of oblique and transverse collagen in the ELA and the ULA. This would enable these parts to withstand a greater deal of stress during vigorous truncal movements compared to the HLA. The preponderance of the longitudinal collagen fibres seen in the HLA is similar to the findings seen in the male human LA (Grassel et al, 2005; Pulei et al, 2015).
In our study, the LA's calculated coefficient of elasticity showed that on the average, the ELA and the ULA had a higher coefficient of elasticity when exposed to traction compared to the HLA. This is in contradiction to biomechanical studies

done by others (Rath et al, 1999), who reported that the human HLA exhibited larger coefficient elasticity than the other regions.
In our study, the greatest strain encountered was seen in the HLA at 0.35 and 1.19 when it was exposed to longitudinal and oblique traction respectively at its ultimate tensile strength breaking point. Thick collagen fibrils resist greater tensile strength as opposed to thin ones (Grassel et al, 2005). It is proposed that while the intermediate and deep laminae confer strength to the ELA and ULA, the longitudinally aligned collagen bundles are responsible for the low coefficient of biomechanical elasticity seen in the HLA. These properties would enable the LA to uniformly distribute the forces generated by the muscles (Ushiki, 2002). Hence, with its low coefficient of elasticity and high strain, the HLA would be predisposed to diastasis recti when compared to other regions.



Graphic 2: Shows the longitudinal and oblique stress- strain curves of the tissue strips from the various regions of the baboon LA. Note the arrows pointing to the ultimate tensile strength of each part of the LA when exposed to longitudinal (A) and oblique (B) traction.



Graphic 3: A bar chart showing Young's modulus of the different regions of the baboon's LA when it is exposed to various traction forces. Note how the epigastric LA (ELA) and umbilical LA (ULA) recorded the highest elastic coefficient when exposed to oblique traction.

The admixture of longitudinal and oblique collagen fibres observed in the ELA and the ULA of the baboon may constitute a" strong aponeurosis of the variable tension" (Axer et al, 2001b; Fitzgibbons et al, 2002). The crisscrossing thick collagen fibers seen in ULA would withstand the changes in the shape of the abdominal wall without the resultant tearing of fibres or stretching of the spaces between the fibres and hence reduce the risk of diastasis recti or ventral hernia formation.

Our findings in the baboon LA are consistent with findings of Kirilova et al (2009), who studied the viscoelasticity properties of the human abdominal fascia. They noted that samples cut parallel to the direction of the collagen exhibited 3 times the elastic modulus and had nearly twice the ultimate tensile strength compared to those cut perpendicular to the fibres (Kirilova et al, 2009).

In conclusion, the findings of the study suggest that the baboon ELA and ULA are well organized for withstanding great forces. However, weakening of the LA or excessive abdominal pressure may result in diastasis recti or ventral hernia particularly in the hypogastric LA.

Conflict of interest

None.

Funding

Deans Committee Research Grant of the University of Nairobi, Kenya.

Justification for animal invasive procedure

The tissues were obtained from animals sacrificed at the end of experiments at the Institute of Primate Research (IPR). These researchers at IPR used the baboon as a model, to investigate the effect of preclinical drugs on various parasites harmful to humans. The study protocols followed by the researchers at IPR were approved by the Institutional Scientific Evaluation and Review Committee (ISERC) and the Animal Care and Use Committee of the Institute of Primate Research, Nairobi, Kenya.

Contributions

Conceived and designed the study: - P.O.O and S.G.K. Performed the test and gathered data: - P.O.O. Analyzed data: - All. Wrote paper: - All.

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ACKNOWLEDGEMENTS

We would like to thank Mr. J. Mugweru of the department of Veterinary Anatomy and Physiology. Mr. John Kahiro and David Macharia for assisting during the material testing at the Department of Mechanical Engineering, University of Nairobi. We would like to express gratitude to Dr. M. Ngotho and the staff of Institute of Primate Research, National Museums of Kenya for allowing us to collect tissues. We would also like to appreciate the efforts of Sarah Mungania and Acleus Murunga for assistance with animal care and materials procurement as well as Margaret Irungu for help with the histological processing of the tissues. Last but not least to Dr. Charles Kamotho and Luis Franceschi, for agreeing to translate the abstract.