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Regional Variation in the Microscopy and Tensile Strength of the Linea Alba in the Male Goat *(Capra Hircus)*

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ABSTRACT

The linea alba (LA), a midline tendinous structure formed by the interlocking anterior abdominal wall aponeurotic fibers, acts as a passive tensile band that prevents sagging of the lower abdomen. The microstructure and the strength of these fibres would thus be expected to reflect on the forces that act on the linea alba. This study was undertaken to establish the histomorphology, morphometry and tensiometric characteristics of the linea alba of the goat so as to elucidate on the elements that are responsible for maintaining the integrity of the wall. Tissues resected from the linea alba in six male goats and prepared for routine light microscopy and stained with Weigert resorcin–fuchsin stain revealed three distinct laminae namely a superficial, intermediate and deep. The superficial lamina of goat epigastric linea alba, which was closely apposed to the overlying elastic tunica flava abdominis (deep fascia), was made up of predominantly obliquely aligned collagen bundles, an intermediate lamina comprising longitudinally aligned collagen bundles and a deep lamina consisting of transversely oriented collagen fibres. When exposed to longitudinal traction, the epigastric LA on average was the stiffest at 35 N/mm^2 with an elastic coefficient 350 N/mm^2 while the hypogastric LA was the weakest at 26.5 N/mm^2 with an elastic coefficient 217 N/mm^2 respectively. In conclusion, the epigastric LA was well suited for longitudinal load strength to support the compound stomach during grazing while the relatively elastic hypogastric LA was most suited for allowing a degree of midline sagging during browzing. The variations noted in tensiometry suggest that the hypogastric linea alba is more compliant to stretch but more predisposed to tearing than the epigastric line alba especially when exposed to sudden violent force or trauma.

Key Words: linea alba, histomorphology, tensile strength, collagen fibers, elastic fibers.

INTRODUCTION

The linea alba (LA), a midline structure, together with the fibrous ventral abdominal wall plays a key role in the stabilization of the abdominal wall (Axer et al 2001a). This tough tendinous structure is formed by the interlocking aponeurotic fibers of three ventrolateral abdominal muscles: external oblique, internal oblique and transversus abdominis muscle passing from one side to the other (Askar 1977; Standring 2008).

The function of the linea alba is to maintain the abdominal muscles, particularly the rectus abdominis muscles, at a certain proximity to each other (Beer et al, 2009). The linea alba in man has been described as composed of a highly structured meshwork of oblique and transverse collagen fibers (Axer et al 2001a, Pulei et al 2015). Elastic fibres have also been found in the superficial lamina and in the lateral and the caudal parts of the LA (Pulei et al, 2015). These authors also noted that females had more transverse collagen and elastic fibres than males

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especially in the infraumbilical region. Very few studies have been done on animals (Acostasantamaria 2015).

In a study done on quadrupeds, Smit (2002) proposed that the linea alba acts as a passive tensile band which assists in conserving energy by bearing the weight of the intra-abdominal organs and hence prevent sagging of the abdominal wall. Other studies have shown that the tensile strength of the LA is directly proportional to its thickness and density (Korenkov et al, 2001). Using a biomechanical study, Grässel et al (2005) proposed that when the linea alba is exposed to increasing force in the longitudinal, transverse and oblique direction, compliance was highest in the longitudinal direction and least in the transverse direction. However, the structure or tensile strength of the linea alba of the goat is largely unknown.

The aim of this study is to describe the histomorphology of the LA and evaluate its tensile strength in the goat. This may explain how the LA of the goat, as a grazer and browser, is adapted to its function.

MATERIALS AND METHODS

The fibrous ventral abdominal wall was harvested from 1- 2year old 6 (six) healthy male goats (*Capra hircus*) weight range 10-13kg. The goats were procured from a local butchery immediately after slaughter. The skin and fat were incised along a midline abdominal incision to form skin flaps that exposed the fibrous ventral abdominal wall. The xiphoid process, the umbilicus, and the pubic tubercle were used as landmarks. Using a metal template (40mm x 20mm), pieces of linea alba were resected from the mid-epigastric (ELA), the umbilical (ULA) and hypogastric (HLA) (Fig. 1).

Light microscopy

A transverse section of each region (20mm x 10mm) was fixed in 10% formaldehyde solution. Thereafter, they were trimmed and dehydrated through increasing concentrations of alcohol from 50% to 100%, cleared with cedar wood oil and then embedded in paraffin wax. Seven micrometer (7um) sections were cut from paraffin blocks, collected on slides, stained with the Weigert resorcin–fuchsin stain and counterstained with Van Gieson stain for demonstration of elastic fibres (Drury 1967). Fifty-six sections from each region were selected for histomorphometry. They were examined with light microscope at various magnifications. Photomicrographs were taken using digital photomicroscope and images analyzed with ImageJ software (Schneider, 2012). The different layers of the LA thickness were measured in millimeters and the ratios plotted on a graph.

Tensiometry

The present study used a protocol employed by Seifert et al,(2012) using a Hounsfield tensometer (80035; Pesola, Baar, AG Switzerland). Fresh strips of 30mm x 20mm were excised from the epigastric, umbilical and hypogastric parts of the linea alba from 6 different goats. The strips were kept in phosphate buffered saline before mechanical testing.

During mechanical testing, the strips were placed firmly in metal screw clamps with rubber pieces covering the clamp ends of the tensometer, to be stretched automatically 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 the clamps) were measured using a vernier caliper before loading was started. Force (N) and displacement (mm) were measured on a xy plotter, and these points subsequently recorded as stress (force per cross sectional area) and strain (fractional change in length) and replotted in Microsoft Excel (Microsoft Office Professional 2013). Longitudinal load was applied to the strips and the ultimate tensile strength at failure strain were recorded as the yield point. For each tissue specimen, stiffness (Young's modulus) or coefficient of elasticity was calculated by determining the slope of a line that fit the most linear portion of the stress-strain curve.

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 then plotted in a graph to compare the three regions examined. The tissues were kept wet throughout the tests. No prior preconditioning was done on the tissues.

RESULTS

Microscopy

The LA of the three regions contained collagen and elastic fibers which were organized into three laminae namely, superficial, intermediate and deep one in relation to the subcutaneous tissue. In all the three regions, the intermediate lamina consisted of rows of longitudinally aligned collagen fibers while the deep lamina, consisted of rows of transversely aligned thick collagen bundles (Figure 2 A - C). Laterally the linea alba had a prominent zone of elastic fibres lying next to the rectus abdominis muscle (Figure 2D).

The superficial lamina of all three regions were made up of a few rows of obliquely arranged collagen fibres which were closely apposed to a ventral zone of longitudinally aligned elastic fibres found in the deep fascia (Figure 2). In the hypogastric region, the collagen bundles in the superficial and intermediate lamina became more distinct, while the transversely aligned collagen bundles in the deep lamina reduced markedly in size and thickness (Figure 2 A - C). In addition, the zone of longitudinally aligned elastic fibers seen in the deep fascia overlying the superficial zone of the epigastric linea alba became more prominent and thicker in size in the hypogastric region (Figure 2 A - C).

In terms of size, the deep lamina reduced drastically from 0.179mm to 0.065mm craniocaudally compared to the elastic deep fascia which increased in size from about 10% of the wall section in the ELA (p-0.002) to more than 20% in the HLA (Figure 3).

Tensiometry

During longitudinal mechanical loading, the linea alba displayed the typical stress-strain curves for destructive tensile testing of soft tissues before breaking at its waist with a characteristic plane. Collagen fibril failure, which is characteristic of the plastic region (yield or failure region), was quite prominent in all the curves (Figure 4).

The typical stress-strain curve seen during longitudinal loading of the linea alba did not seem to vary significantly among the 3 regions.The maximal/ultimate stress needed (load to failure) to fracture the linea alba during longitudinal traction was 35N/mm^2 at a strain of 0.15 (Figure 4). The epigastric linea alba had a signficantly higher load to failure of 35 N/mm^2 compared to the hypogastric linea alba (26.5 N/mm^2) respectively during longitudinal traction. The most strain the LA encountered before breaking at its ultimate breaking point was seen in the hypogastrium at a strain of 0.21. (Figure 4).

The linea alba's Youngs modulus was calculated over linear portions of the stress strain curves. These values show that on average, the epigastric linea alba had a higher elastic coefficient of 350 N/mm^2 compared to the umbilical and the hypogastric which had 227 N/mm^2 and 217 N/mm^2 respectively (Figure 5).

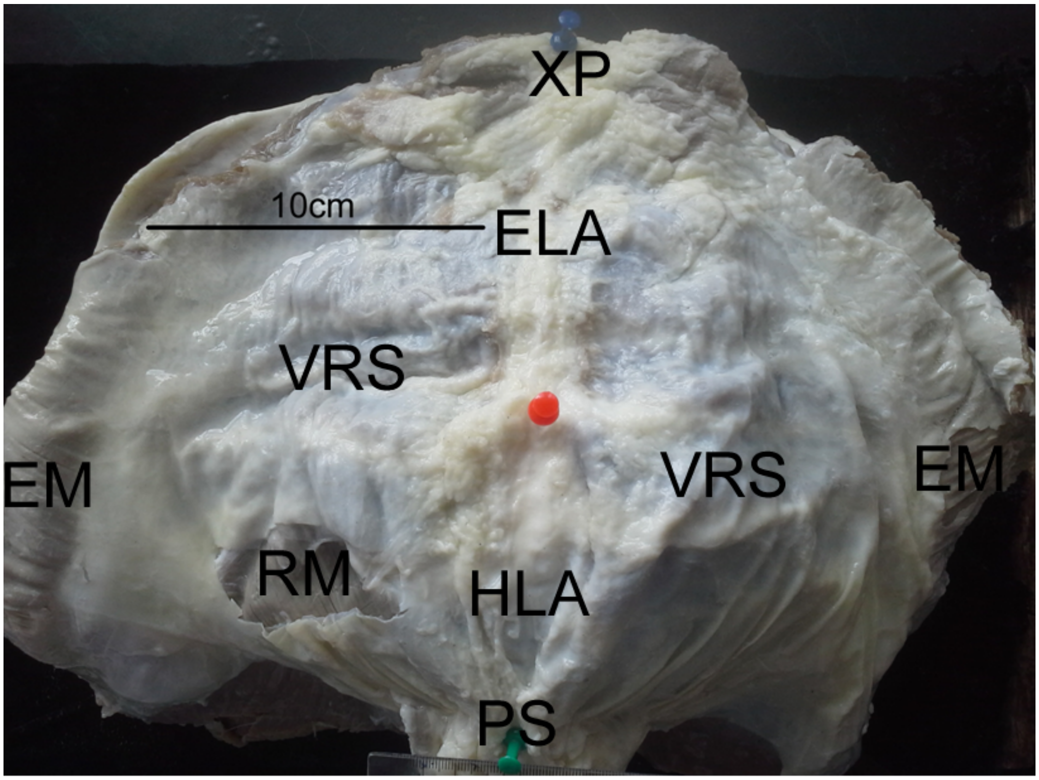

Figure 1: A picture of the ventral abdominal wall of a 2-year old male goat. Legend: - Shows the resection areas from the linea alba, and other relevant landmarks; Ventral rectus sheath (VRS), External oblique muscle (EM), Rectus abdominis muscle (RM), Epigastric linea alba (ELA); Umbilical linea alba (red pin); Hypogastric linea alba (HLA), the pubic symphysis (PS/ green pin), the xiphoid process (XP/ blue pin).

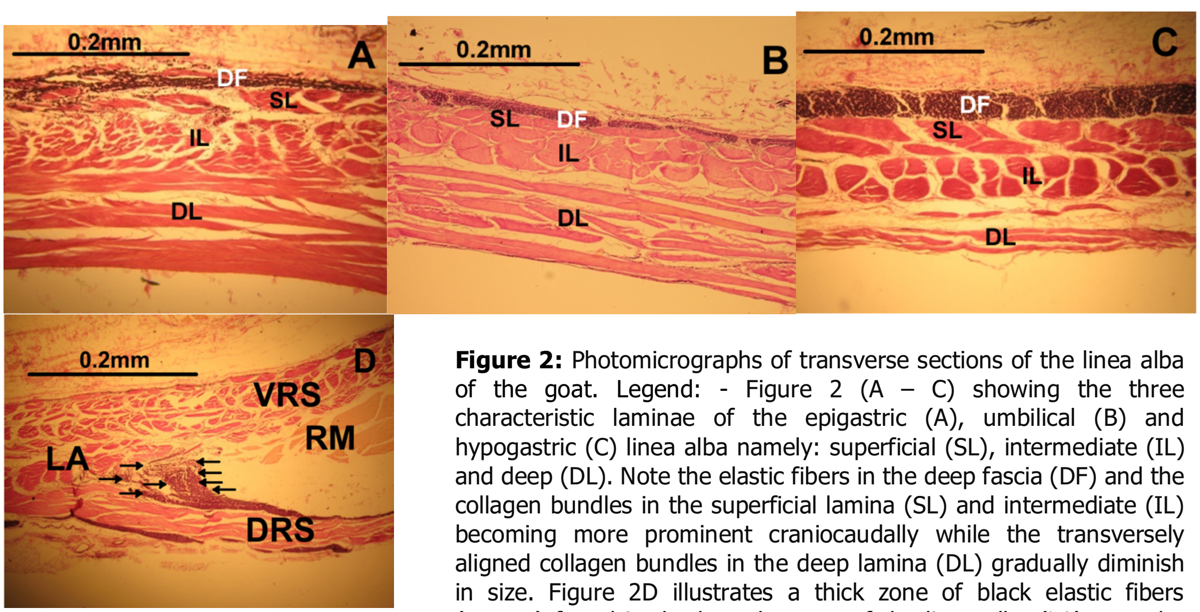

Figure 2: Photomicrographs of transverse sections of the linea alba of the goat. Legend: - Figure 2 (A – C) showing the three characteristic laminae of the epigastric (A), umbilical (B) and hypogastric (C) linea alba namely: superficial (SL), intermediate (IL) and deep (DL). Note the elastic fibers in the deep fascia (DF) and the collagen bundles in the superficial lamina (SL) and intermediate (IL) becoming more prominent craniocaudally while the transversely aligned collagen bundles in the deep lamina (DL) gradually diminish in size. Figure 2D illustrates a thick zone of black elastic fibers (arrows) found in the lateral aspect of the linea alba (LA) near the rectus abdominis muscle (RM). These elastic fibres extend towards the dorsal rectus sheath (DRS).

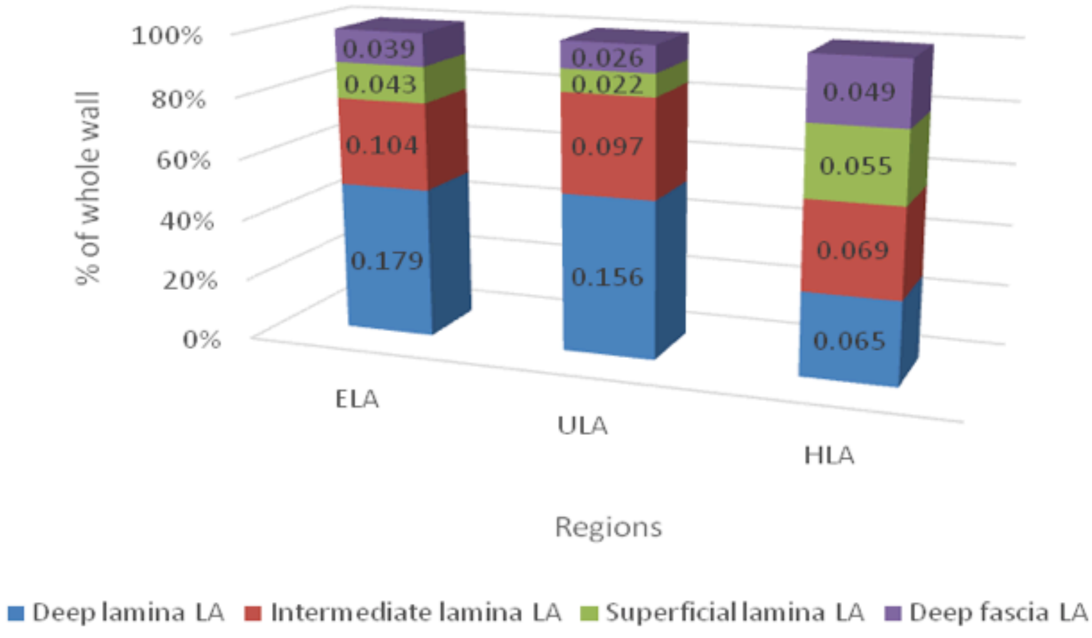

Figure 3: A bar chart showing percentage proportions mean measurements of the goat linea alba at different regions. Legend: - Note how the intermediate and deep lamina gradually reduced in size proportionately craniocaudally, compared to the deep fascia and superficial lamina which increased in size relatively from the epigastric linea alba (ELA) to the hypogastric linea alba (HLA). (N.B the measurements inserted within the bars are in millimeters.)

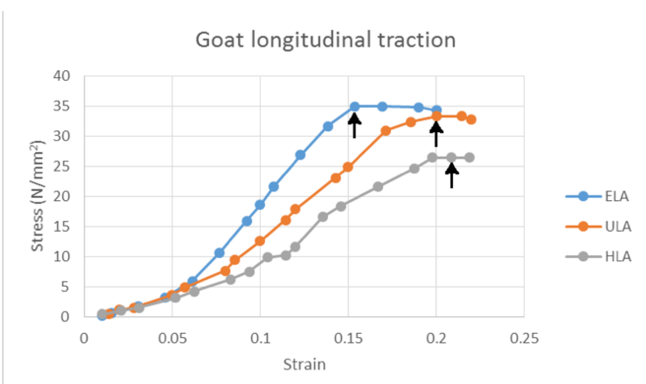

Figure 4: A graph showing the longitudinal stress/ strain curves on the various parts of the linea alba. Legend: - The arrows are pointing to the yield point of each region of the linea alba i.e epigastric linea alba (ELA); umbilical linea alba (ULA) and hypogastric linea alba (HLA).

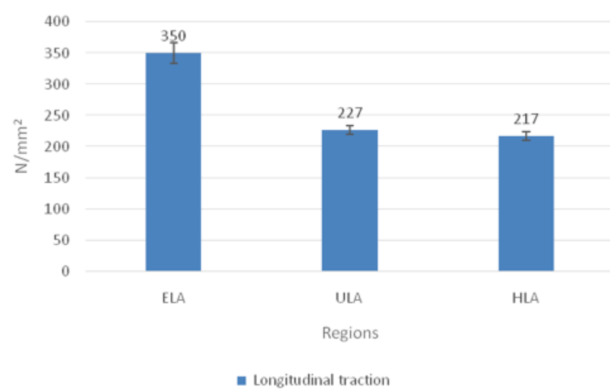

Figure 5: A bar chart showing Young's modulus of the different regions of the goat's linea alba when it was exposed to longitudinal traction. Legend: - Note how the epigastric linea alba (ELA) recorded the greatest elastic coefficient at 350 N/mm^2 compared to the umbilical linea alba (ULA) and hypogastric linea alba (HLA).

DISCUSSION

The microscopic structure of the linea alba has been reported by several workers in humans (Askar, 1977; Axer et al, 2001a, b; Pulei et al 2007; Pulei et al 2015) and pigs (Acostasantamaria et al, 2015). Our study has for the first time reported the microscopic structure of the linea alba the goat. Pulei et al, (2009) reported the linea alba appeared to be arranged in 3 laminae namely; anterior/ superficial, middle/ intermediate and posterior/ deep. They further reported that the elastic fibres which were longitudinally and obliquely disposed in the entire length of the LA while collagen fibres were transversely arranged in the middle lamina but inconsistent in the other 2 laminae. The elastic fibres were more abundant in the superficial lamina, in the lateral and caudal parts of the LA.

Our study showed that the linea alba of the goat, like in man, was made up of three distinct laminae namely a superficial, intermediate and deep lamina. In addition, the study also showed that the lateral, ventral and caudal aspects of the goat linea alba had a high amount of elastic fibres. This is similar to the findings by Pulei et al, (2015). In our study, we noted however, that this zone of elastic fibres were actually part of the deep fascia of the LA intimately adherent to the underlying superficial lamina and not part of the superficial lamina of the linea alba.

Our study also noted that the goat LA comprised of an intermediate and deep lamina made up of longitudinally aligned collagen bundles and transversely oriented collagen fibers respectively. The latter were more prominent cranially towards the epigastric linea alba. The transversely aligned collagen bundles seen in deep lamina of the male goat LA are similar to what was seen in the human female (Pulei et al, 2015) and in the porcine (Acostasantamaria et al, 2015).

This thus extends the argument that about the role of the admixture of oblique and transversely aligned collagen fibers observed in the goat line alba may constitute a "strong aponeurosis of variable tension" which would serve various functions (Pulei et al, 2015). The thicker deep lamina, which contains transversely aligned collagen fibers and represents the aponeurotic fibers from the transversus abdominis muscle, seems to explain the main reason for the great compliance (350 N/mm^2) noted in the goat epigastric linea alba compared to the hypogastric linea alba.

This study further illustrated that goat epigastric linea alba was the thickest (0.365mm) while the hypogastric was the thinnest (0.238mm) respectively. On average, the epigastric linea alba had also a higher elastic coefficient of 350 N/mm^2 compared to the umbilical and the hypogastric which had 227 N/mm^2 and 217 N/mm^2 respectively. This would be in agreement with Korenkov's proposal which states that 'The tensile strength of the line alba is directly proportional to its thickness and density (Korenkov et al, 2011).

Considering that most of the weight of the compound stomach will be loaded on the epigastrium and hence the linea alba, the crisscrossing multidirectional collagen bundles and in particular the transverse fibres of the deep lamina would allow for changes in the shape of the abdominal wall, while at the same time strengthen the epigastric linea alba during browsing or grazing. The substantial amount of elastic fibers seen in the deep fascia of the linea alba of the goat, not comparable with the findings in man (Pulei et al, 2015), make it more compliant to stretch during grazing.

In conclusion, the alignment of the collagen and elastic fibers noted may confer reversible stretchability to the LA as shown by tensiometry. The ELA was well suited for longitudinal load strength to support the compound stomach during grazing while the relatively elastic HLA was most suited for allowing of midline sagging during browzing especially when the abdomen is extremely distended. The variations noted in tensiometry suggest that the hypogastric linea alba is more compliant to stretch but more predisposed to tearing than the epigastric line alba especially when exposed to sudden violent force or trauma.

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Conflict of Interest

All the authors declare that there is no conflict of interest with reference to the publication of this article. This material has not been published and is not under current consideration elsewhere

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