STRUCTURE AND AGE CHANGES OF THE CRURAL DIAPHRAGM

A dissertation in partial fulfillment of the requirements for the intercalated Bachelor of Science degree in Anatomy, University of Nairobi

By

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DECLARATION

I hereby confirm that this dissertation is my original work and has not been presented elsewhere for examination.

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Signature _____ Date 13/09/08

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DEDICATION

To the losses, the gains and the constancies:

Uncle Njenga

whose sharp wit and strength of character will be dearly missed

Johari

who has brought joy and beauty into our lives

Mum, Dad and Ng'ang'a

for unwavering faith and support

Kiprono

my best friend and biggest fan

ACKNOWLEDGEMENTS

I am sincerely grateful to my supervisors; Dr. Hassan Saidi, Dr. Paul Odula and Dr. Wycliffe Kaisha, for their constructive criticism and continued support. I am greatly indebted to Dr. Julius Ogeng'o, Dr. Bernard Ndung'u, Dr. Pamela Mandela, Dr. Kirsteen Awori, Prof. Jameela Hassanali, Dr. Moses Obimbo and Dr. S.G. Kiama for their invaluable input. I would also like to thank the other members of teaching staff at the Department of Human Anatomy, University of Nairobi, who may have contributed to this study in one way or another. My thanks to the technical and supporting staff for imparting knowledge necessary for the completion of this study. Finally, I thank my classmates; Njoga Njihia, Charles Masaki and Patrick Gatonga, for being an integral part of a year I will never forget.

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LIST OF ABBREVIATIONS

CoD	Costal diaphragm
CrD	Crural diaphragm
FEL	Fibroelastic lamina(e)
Fig.	Figure
GEJ	Gastro-esophageal junction
GERB	Gastro-esophageal reflux barrier
GERD	Gastro-esophageal reflux disease
HH	Hiatus hernia
ICT	Intramuscular connective tissue
LLC	Limited Liability Company
RC	Right crus
TD	Thoracic diaphragm

SUMMARY

Background: The crural diaphragm is the portion of the thoracic diaphragm arising from the antero-lateral aspects of the upper lumbar vertebrae to form the esophageal hiatus. It is thus a component of the gastro-esophageal reflux barrier, playing a role in the prevention of hiatus hernia and gastro-esophageal reflux disease. The prevalence of these two conditions increases with age and this may be related to an increase in width of the esophageal hiatus. The structural basis for this trend in disease prevalence is unclear. In addition, the pattern of mobilization of the crural diaphragm differs from that of the costal diaphragm, as the former remains tonically contracted around the distal esophagus, while the latter is only mobilized intermittently during respiration. Information on the structural differences between these two components of the thoracic diaphragm is scarce.

Objectives: To describe age-related changes in the structure of the crural diaphragm and determine the structural differences between the crural and costal diaphragms.

Study design: A descriptive cross – sectional study.

Materials and methods: Thirty (30) samples of the crural and costal diaphragms were obtained during autopsy at the Nairobi City and Chiromo mortuaries. Five millimetre segments of the proximal, middle and distal portions of the crural and costal diaphragms were taken. These were then grouped into four age groups (0-19, 20-39, 40-59 and ≥60) and processed for light microscopy. Slides made were observed and photographed using a Zeiss® digital photomicroscope at x40, x100 and x400 magnifications.

Results: The crural diaphragm comprised two components; a proximal tendinous and a distal muscular part. The tendinous part showed an increase in connective tissue content with age while muscle fibres of the muscular part became more regularly oriented. An age-related increase in content, thickness and tortuosity of intramuscular elastic fibres was also observed. The costal diaphragm was homogenously longitudinal throughout its length and showed minimal age-related differences in comparison with the crural diaphragm.

Conclusion: The age-related re-orientation of skeletal muscle fibres and increase in intramuscular connective tissue may contribute to widening of the esophageal hiatus, explaining the increase in prevalence of hiatus hernia and gastro-esophageal reflux disease with age.

INTRODUCTION

The crural diaphragm (CrD) is the part of the thoracic diaphragm (TD) that arises from the anter-lateral aspects of the upper three lumbar vertebrae (Standring, 2005). The other parts of the TD include the costal diaphragm (CoD) and an inconstant sternal diaphragm. These parts of the TD insert into the central tendon. However, some crural fibres, usually of the right crus (Botros *et al.*, 1990), arc around the distal esophagus forming the esophageal hiatus. These arching fibres, known as the extrinsic esophageal sphincter (Mittal and Balaban, 1997; Holloway, 2000), form a component of the gastro-esophageal reflux barrier (GERB) and play a role in the prevention of hiatus hernia (HH) and gastro-esophageal reflux disease (GERD) (Atkinson, 1962; Anderson *et al.*, 1967).

There exists a difference in patterns of mobilization between the CrD and CoD. The CrD remains tonically contracted around the distal esophagus, (Bombeck *et al.*, 1966; Mittal, 1990; Martin *et al.*, 1992) while the CoD is mobilized intermittently during inspiration (Sprung *et al.*, 2006). Mobilization patterns have been shown to affect muscle structure (Jarvinen *et al.*, 2002). However, data on structural differences between the CrD and the CoD are scarce.

The microscopic organization of the CoD has been described (Mayock *et al.*, 1987; Gosselin *et al.*, 1993; Rodrigues and Rodrigues Jũnior, 2000) while that of the CrD has not been elaborated. From gross observations, the CrD is described as being tendinous at its origin and muscular toward the esophageal hiatus (Standring 2005). Generally, skeletal muscle fibres facilitate controlled contraction (Liu *et al.*, 2005) while intramuscular connective tissue (ICT) transmits lateral forces between muscle fibres and maintains elasticity (Bloch and Gonzales-Serratos, 2003; Kjaer *et al.*, 2006).

Age-related changes in content and organization of intramuscular connective tissue have been described in other muscles (Alnaqeeb *et al.*, 1984; Curwin *et al.*, 1994; Gao *et al.*, 2008) including the CoD (Gosselin *et al.*, 1994; Rodrigues and Rodrigues Jūnior, 2000) and associated with mechanical properties. Information on age-related changes in the CrD is however scarce.

Hiatus hernia and gastro-esophageal reflux disease both show an increase in prevalence with age (Dent *et al.*, 2005; El Sherif *et al.*, 2006) and it has been postulated that the mechanism for this is widening of the esophageal hiatus (Ginalski, 1984). The structural basis for this pattern in disease prevalence, particularly with reference to the CrD, has however not been elaborated. The aim of this study is thus to describe the microscopic organization and age changes of the CrD and establish structural differences between the CrD and CoD.

HYPOTHESIS

The microscopic organization of the CrD changes with age.

OBJECTIVES

Broad objective

To describe age-related changes in the the structural organization of the crural diaphragm.

Specific objectives

1. To determine age-related changes in crural diaphragm structure.
2. To determine histomorphologic differences between the crural and costal diaphragm.

STUDY DESIGN

A descriptive cross-sectional study

MATERIALS AND METHODS

Thirty (30) samples of the crural and costal diaphragm were obtained during autopsy from the Nairobi City and Chiromo mortuaries. Ethical approval was sought from the Kenyatta National Hospital Ethics and Research Committee (KNH-ERC) and informed written consent obtained from the next of kin of the deceased. Subjects with observable hiatus hernia or pathology of the upper gastrointestinal tract affecting the structure of the thoracic diaphragm were excluded from the study. Those with a history of gastro-esophageal reflux disease were also excluded. Tissues were obtained within 72 hours after death to avoid *post-mortem* changes. Only segments of the thoracic diaphragm relevant to the study were taken, and these were grouped into four age groups; 0-19 years, 20-39 years, 40-59 years and 60 years and above. The distribution of samples within these age groups is shown in the table below:

Distribution of the samples obtained for the study per age group.

AGE GROUP (YEARS)	NUMBER OF SPECIMENS
0 – 19	6
20 – 39	17
40 – 59	5
≥ 60	2

Exposure of the thoracic diaphragm

A midline skin incision was made over the epigastric region up to the manubriosternal joint. From this point, two other incisions were made extending to the lateral edge of either clavicle (Fig. 1). The skin was reflected to expose the ribcage whose costochondral junctions were severed. The sternum was then removed, exposing the contents of the thoracic cavity. The thoracic diaphragm was then dissected away from its anterior costal attachments and reflected cranially while retracting the abdominal contents caudally. This exposed the CrD along its entire length (Figs. 2a and b).

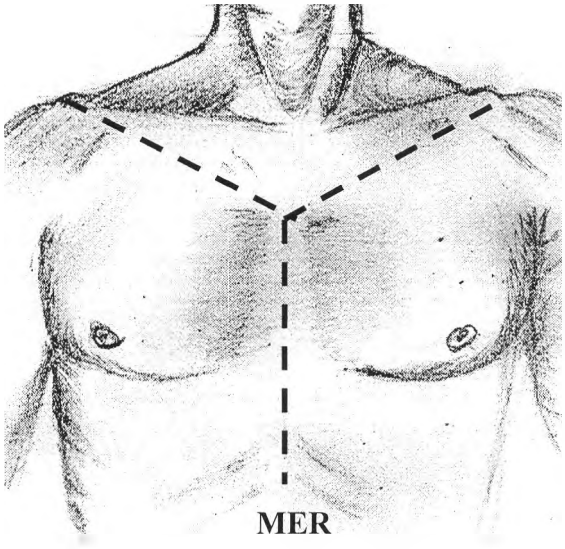
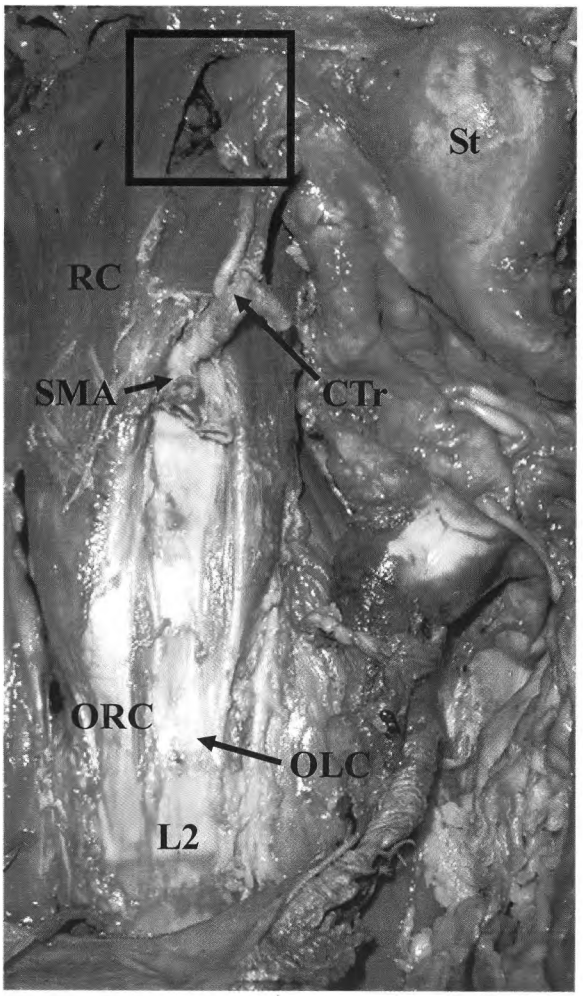


Fig. 1: Image of the anterior thoracic wall, showing the incisions made (dashed lines) for exposure of the thoracic diaphragm. MER – Midline of Epigastric Region.



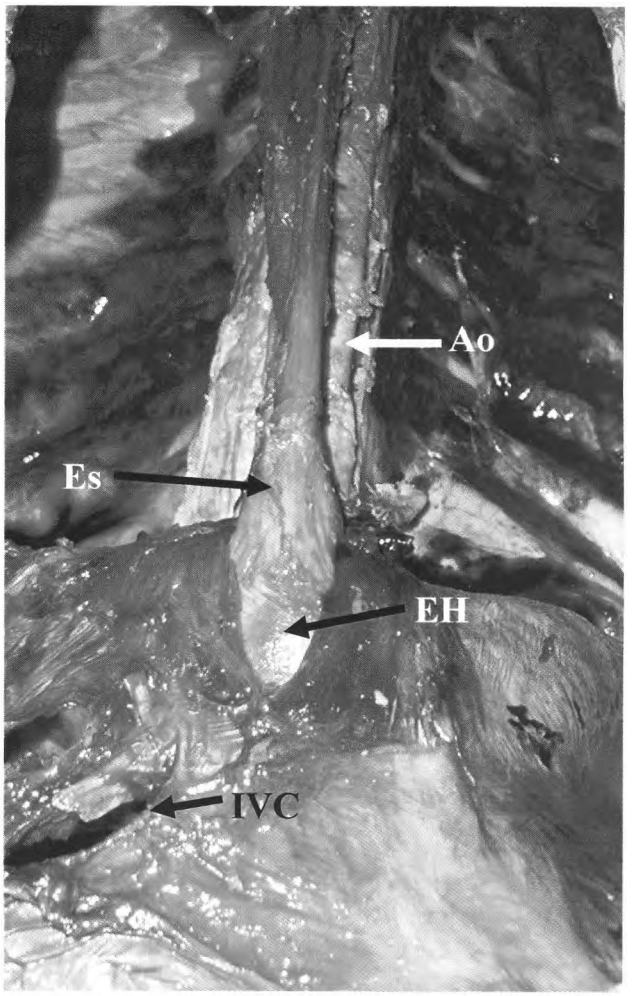


Fig. 2a: Macrograph of the posterior abdominal wall showing the origin of the right **(ORC)** and left crura **(OLC)** from the second lumbar vertebra **(L2). RC** – right crus forming esophageal hiatus **(Rectangle)**; **SMA** – superior mesenteric artery; **CTr** – coeliac trunk; **St** – stomach.

Fig. 2b: Macrograph of the thoracic cavity showing the esophagus **(Es)** in the esophageal hiatus **(EH)** with the aorta **(Ao)** posterior to it. **IVC** – hiatus for inferior vena cava.

Tissue sampling

The crus forming the esophageal hiatus was dissected from its origin on the upper lumbar vertebrae to a point just lateral to the esophageal hiatus. A similar strip of the costal diaphragm was dissected from the same side. These strips were divided into three equal segments and 5 millimetre sections taken from the middle of each segment. These sampling criteria are illustrated in the figures below:

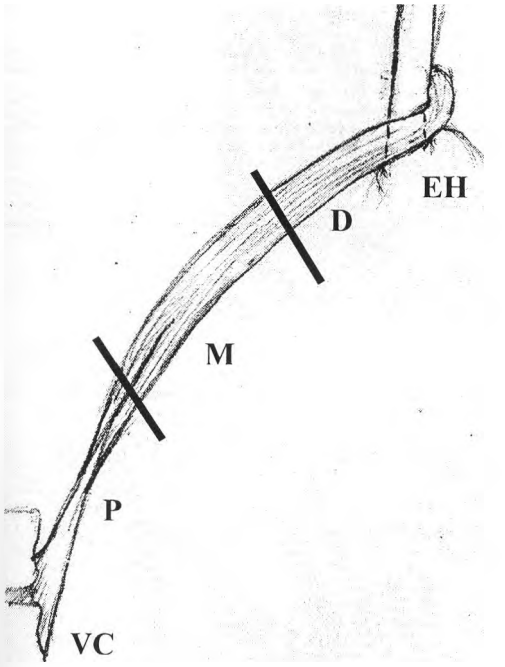


Fig. 3a: Image of the CrD showing segments from which 5mm sections were taken. **P** – proximal; **M** – middle; **D** – distal; **VC** – vertebral column; **EH** – esophageal hiatus.

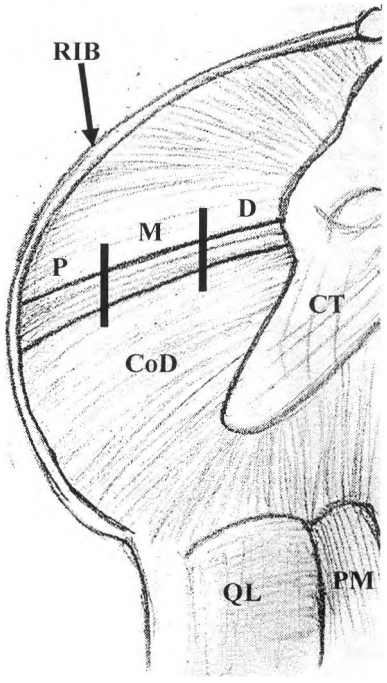


Fig. 3b: Image of the abdominal surface of the thoracic diaphragm showing segments of the CoD from which 5mm sections were taken. **P** – proximal; **M** – middle; **D** – distal; **CT** – central tendon; **QL** – *Quadratus lumborum*; **PM** – *Psoas major.*

Processing for Light Microscopy

After being washed in normal saline, the specimens were fixed by immersion in 10% formal saline for 72 hours. They were then dehydrated in eight graded solutions of ethyl alcohol, from 70% to absolute, for an hour in each solution. The tissues were subsequently cleared in toluene (S.G. 0.866) for 2 hours, followed by wax impregnation in paraffin wax (Paraplast®, McCormick Scientific LLC, USA) in an oven at 58^0C for 12 hours. Embedding was done in paraffin wax set in metal moulds and embedded tissues were cut into 7 micron thick sections with a Leitz Wetzlar® sledge microtome. The cut sections were floated in a water bath at 45^0 C to remove folds and subsequently mounted onto glass slides. These slides were dried in an oven set at 38^0 C for 24 hours.

Two staining protocols were used; Masson's trichrome to study cytoarchitecture and collagen fibre distribution and Weigert's resorcin-fuchsin stain with Van Gieson counterstain to demonstrate elastic fibres (Drury *et al.*, 1967). Slides were mounted with D.P.X mountant® (Unilab Kenya) and observed under a Leica® bright-field microscope (BME Germany) at magnifications x40, x100 and x400. Tissue features were described and recorded in data sheets. Micrographs were then taken using a Zeiss® digital photomicroscope. Micrographs obtained were uploaded onto a personal computer and edited using Macromedia® Fireworks MX® and Microsoft Publisher® (MS Office, 2007).

LEGENDS

Fig. 4a: Micrograph of a transverse section through the proximal segment of the right crus (RC) in a 2½ year old male showing ICT in the endomysium **(En),** perimysium **(P)** and epimysial fibro-elastic lamina. Here the FEL is on both the thoracic **(tFEL)** and abdominal **(aFEL)** surfaces and muscle fibres are oriented longitudinally **(Lo)** (Masson's trichrome stain; magnification x40).

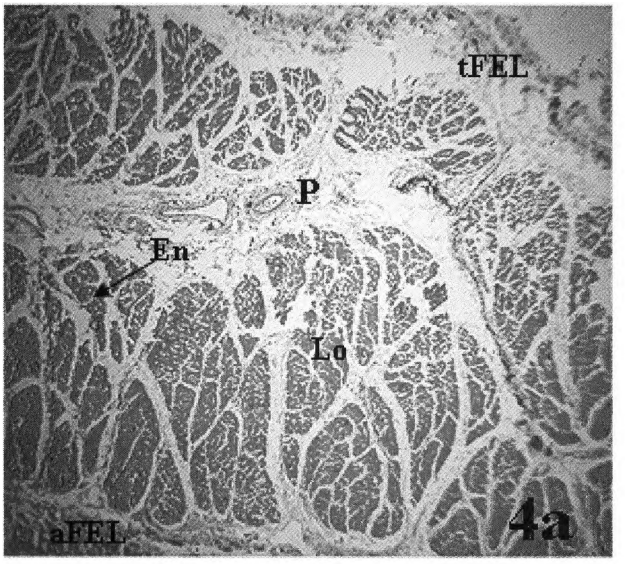
Fig. 4b: Micrograph of a transverse section through the distal segment of the RC in a 2½ year old male showing the muscular component **(white arrowheads)** in ICT **(short black arrows).** The FEL is found only on the thoracic surface **(tFEL). P** – perimysium; **En** - endomysium (Masson's trichrome stain; magnification x100).

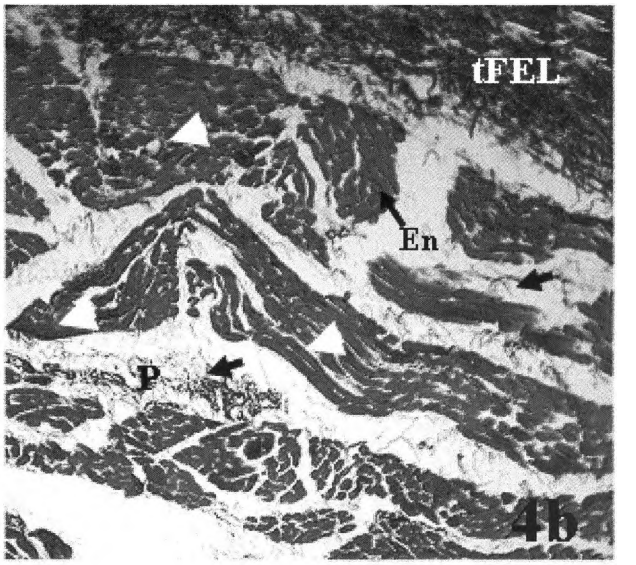
Fig. 4c: Micrograph of a transverse section through the proximal segment of the RC in a 20 year old male showing the tendinous component of the CrD. Collagen fibres are arranged in thick irregular laminae **(arrowheads)** (Masson's trichrome stain; magnification x40).

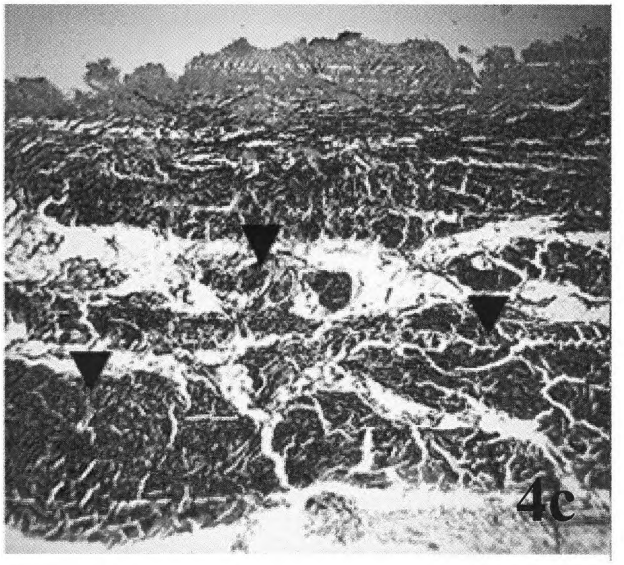
Fig. 4d: Micrograph of a transverse section through the distal segment of the RC in a 20 year old male showing the muscular component of the CrD, comprising longitudinal muscle fibres **(Lo)** in ICT **(arrowheads). tFEL** – thoracic fibroelastic lamina. **P** – perimysium; **En** - endomysium (Masson's trichrome stain; magnification x40).

Fig. 4e: Micrograph of a longitudinal section through the proximal segment of the RC in a 50 year old male, showing the tendinous component of the CrD with collagen fibres arranged in thick longitudinal bundles **(arrowheads)** (Masson's trichrome stain; magnification x40).

Fig. 4f: Micrograph of a longitudinal section through the distal segment of the RC in a 50 year old male showing the muscular component of the CrD with discontinuous muscle fibres **(arrowheads)** (Masson's trichrome stain; magnification x100).


tFEL
P
En
Lo
aFEL
4a


tFEL
En
P
4b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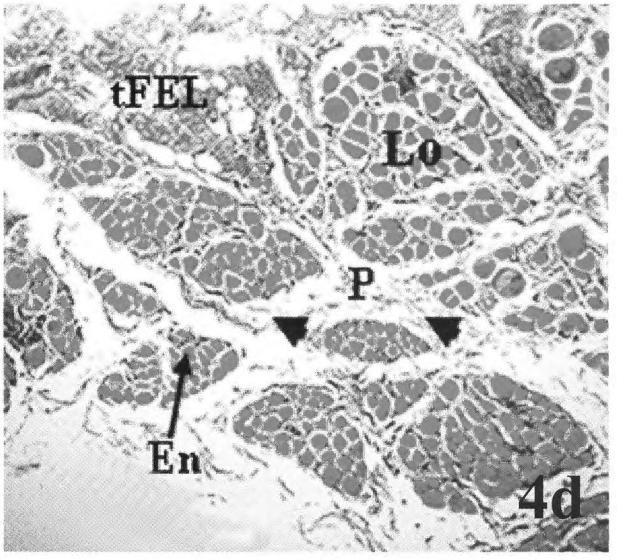
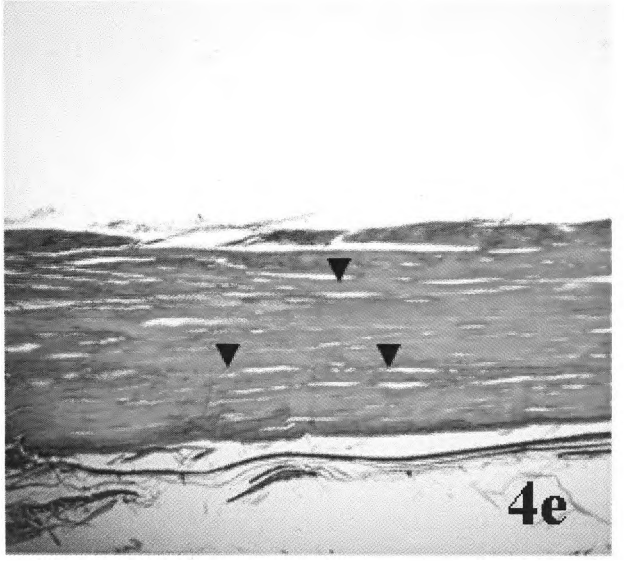

tFEL
Lo
P
En
4d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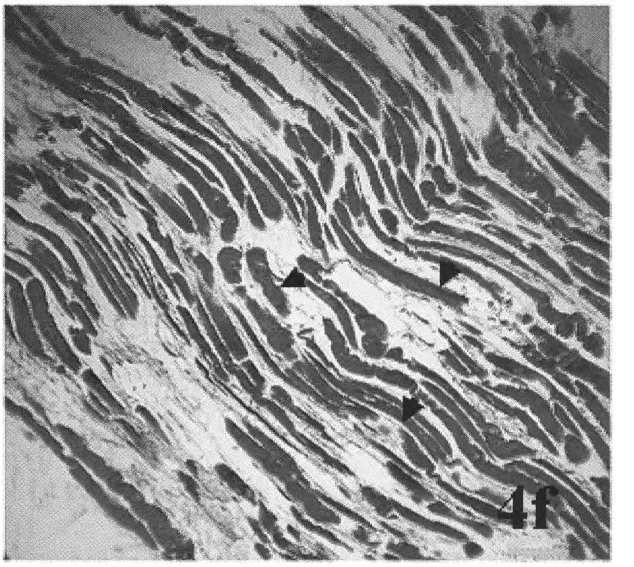

4f

Differences in muscle fibre orientation among age groups

In the distal segment in the 0-19 age group, muscle fibre orientation was heterogenous with transverse, oblique and longitudinal fibres present. In the 2½ year old male, transverse, longitudinal and oblique fibres were present toward the thoracic surface while only transverse and longitudinal ones were observed toward the abdominal surface (Fig. 5a). In the 16 year old female, thoracic oblique and abdominal transverse fibres were observed (Fig. 5b). In the older age groups, muscle fibres were homogenously longitudinal throughout the length of the CrD (Figs. 4f and 5c).

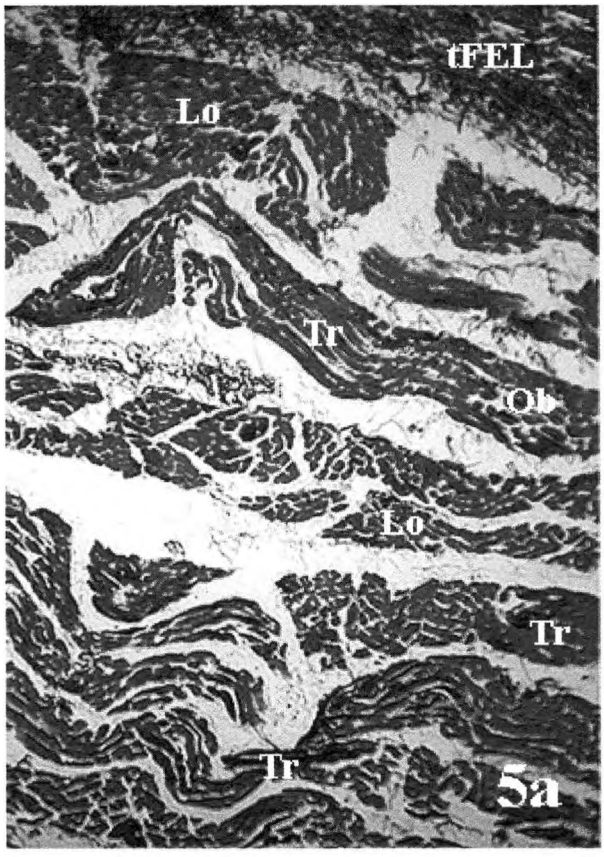
LEGENDS

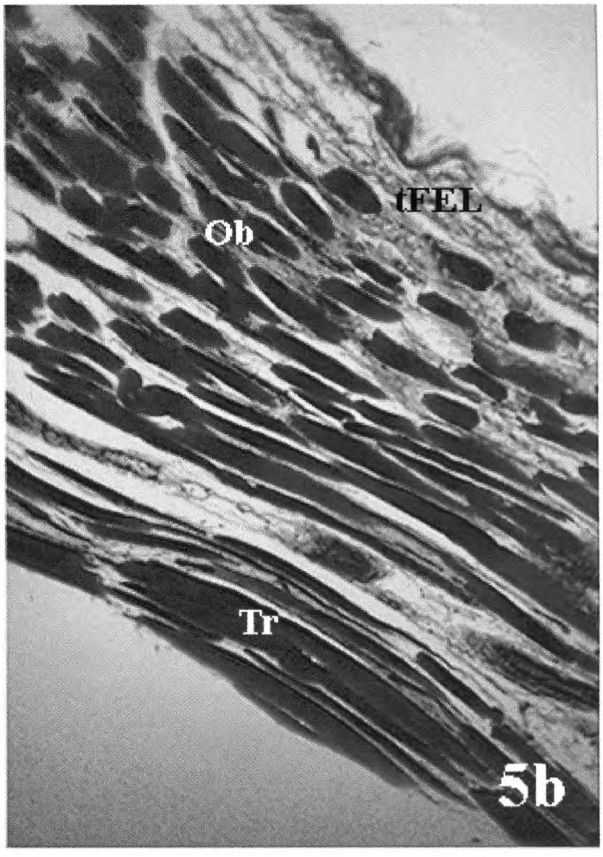
Fig. 5a: Micrograph of a transverse section through the distal segment of the RC in a 2½ year old male showing heterogenous muscle fibre orientation. Fibres toward the thoracic surface are longitudinal **(Lo)**, oblique **(Ob)** and transverse **(Tr)**; those toward the abdominal surface are longitudinal and transverse. **tFEL** – thoracic fibro-elastic lamina (Masson's trichrome stain; magnification x100).

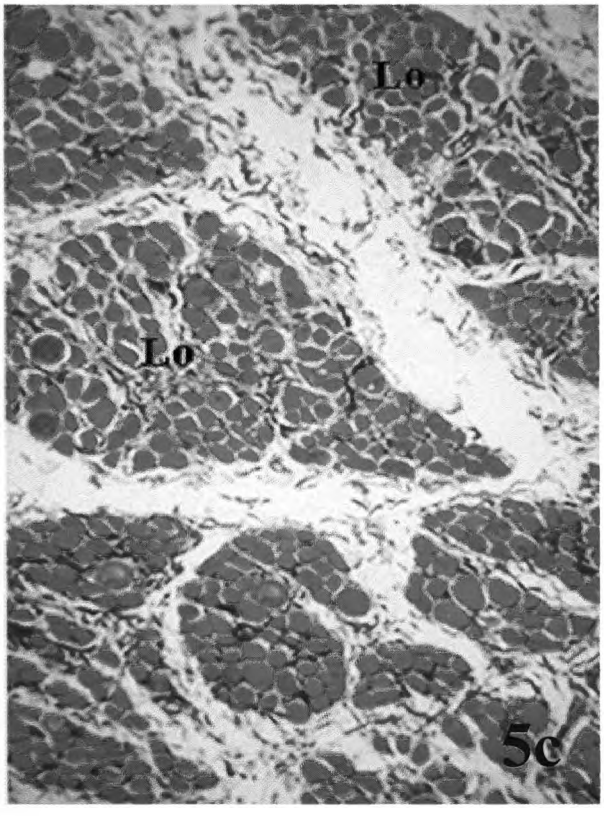
Fig. 5b: Micrograph of a transverse section through the distal segment of the RC in a 16 year old female showing muscle fibre orientation. Muscle fibres toward the thoracic surface are oblique **(Ob)**; those toward the abdominal surface are transverse **(Tr)**. **tFEL** – thoracic fibro-elastic lamina (Masson's trichrome stain; magnification x100).

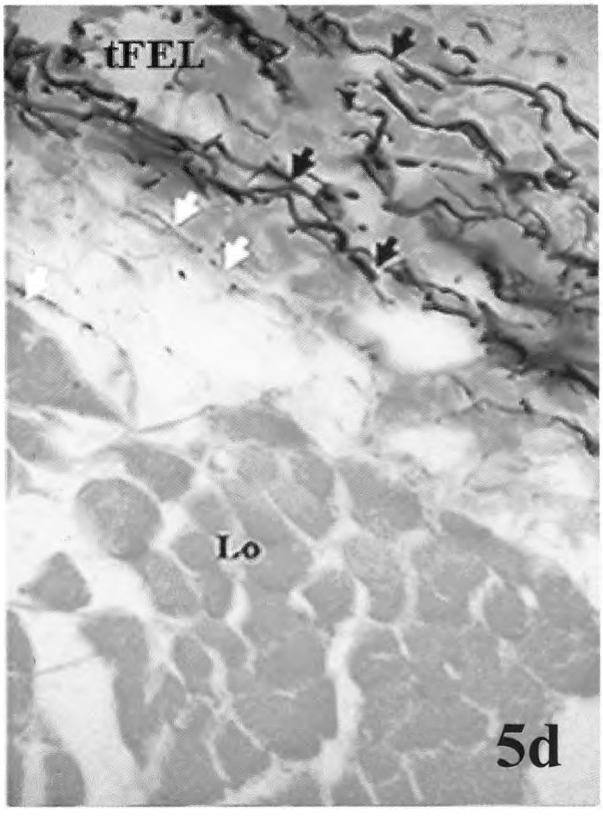
Fig. 5c: Micrograph of a transverse section through the distal segment of the RC in a 20 year old male showing homogenously longitudinal skeletal muscle bundles **(Lo)** (Masson's trichrome stain; magnification x100).

Fig. 5d: Micrograph of a transverse section through the proximal segment of the RC in a 2½ year old male showing the elastic fibre orientation in the thoracic fibro-elastic lamina **(tFEL)**. Superficial fibres are thick, tortuous and oriented transversely **(black arrows)**; deep fibres are fine and oriented longitudinally **(white arrows)**. **Lo** – longitudinal skeletal muscle bundles (Weigert's resorcin-fuchsin stain with Van Gieson counterstain; magnification x100).


tFEL
Lo
Tr
Ob
Lo
Tr
Tr
5a


tFEL
Ob
Tr
5b


Lo
Lo
5c


tFEL
Lo
5d

Differences in connective tissue among age groups

In all age groups, elastic fibres were present in the endomysium, perimysium and epimysial FEL. Epimysial elastic fibres were organised in two layers; a deep layer consisting of fine longitudinal fibres and a superficial layer comprising thick, tortuous and transverse fibres. This orientation was maintained throughout the length of the CrD (Fig. 5d). In the younger age groups, perimysial fibres were thick and tortuous at the CrD origin (Fig. 6a) and decreased in content toward the esophageal hiatus (Fig. 6b). Endomysial elastic fibres were fine and oriented longitudinally throughout the CrD length (Figs. 6a and b). In the older age groups, there was an increase in thickness, content and tortuosity of elastic fibres toward the esophageal hiatus. This change was particularly marked in the endomysium (Figs. 6d and f). Elastic fibre content at the CrD origin in the older age groups was limited to fine fibres within tendinous bundles (Figs. 6c and e).

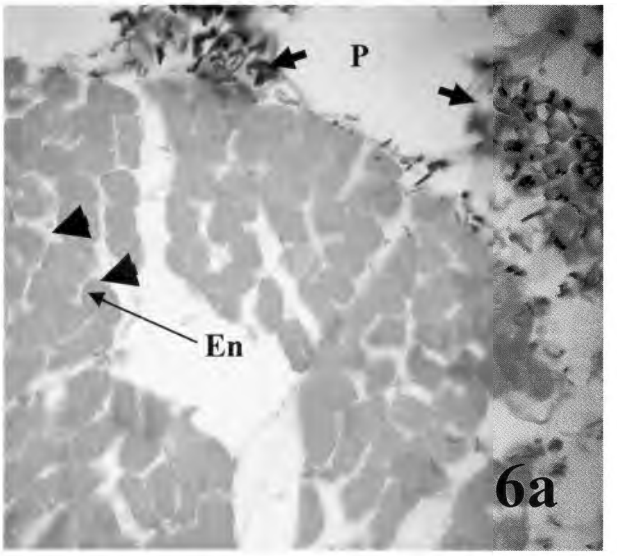
LEGENDS

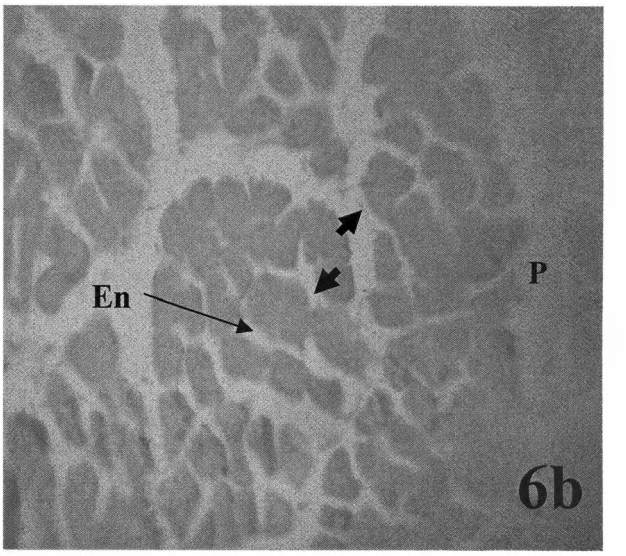
Fig. 6a: Micrograph of a transverse section through the proximal segment of the RC in a 2½ year old male showing intramuscular elastic fibre distribution. Perimysial fibres **(P)** are thick, tortuous and irregular **(short arrows)** Endomysial fibres **(En)** are fine and oriented longitudinally **(arrowheads)** (Weigert's resorcin-fuchsin stain with Van Gieson counterstain; magnification x400).

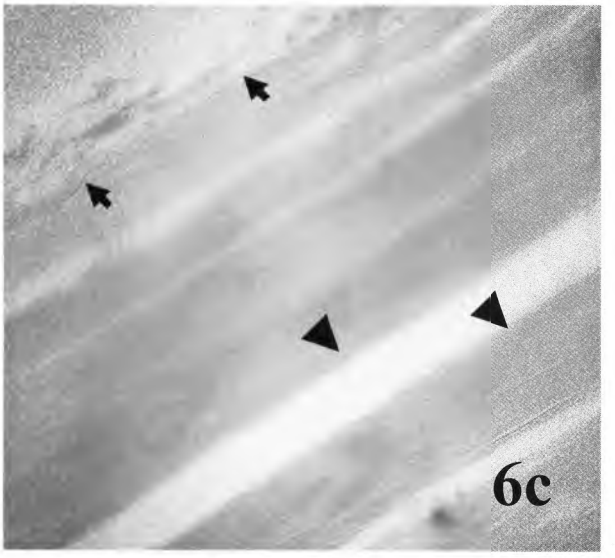
Fig. 6b: Micrograph of a transverse section through the distal segment of the RC in a 2½ year old male showing fine longitudinal elastic fibres in both perimysial **(P)** and endomysial **(En)** spaces. (Weigert's resorcin-fuchsin stain with Van Gieson counterstain; magnification x400).

Fig. 6c and e: Micrographs of longitudinal sections through the proximal segment of the RC in a 29 year old female and a 90 year old female respectively, showing longitudinal collagen bundles (arrowheads) interspersed with fine elastic fibres **(short arrows)** (Weigert's resorcin-fuchsin stain with Van Gieson counterstain; magnification x400).

Figs. 6d and f: Micrographs of transverse sections through the distal segments of the RC in 29 and 90 year old females respectively, showing thick, tortuous and irregular elastic fibres in the endomysium (En). (Weigert's resorcin-fuchsin stain with Van Gieson counterstain; magnification x400).


P
En
6a


En
P
6b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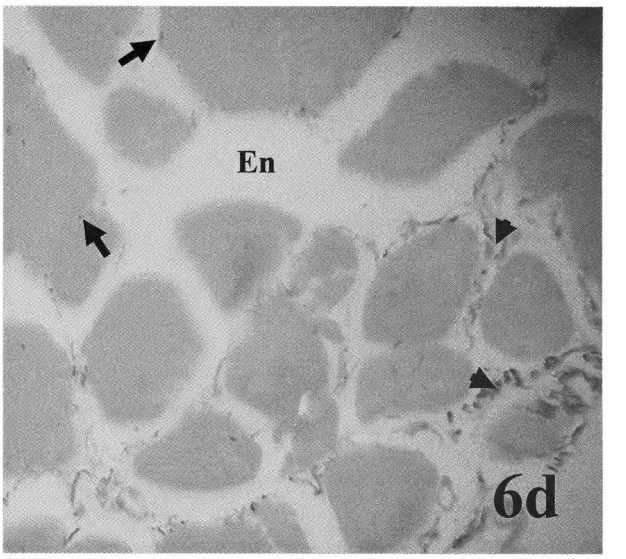
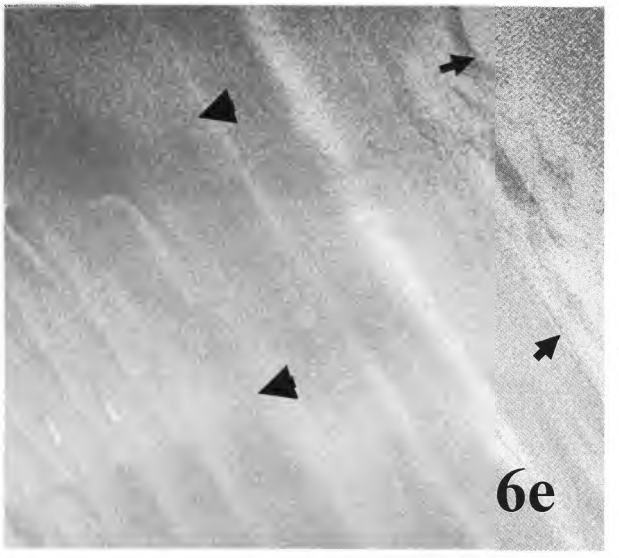

En
6d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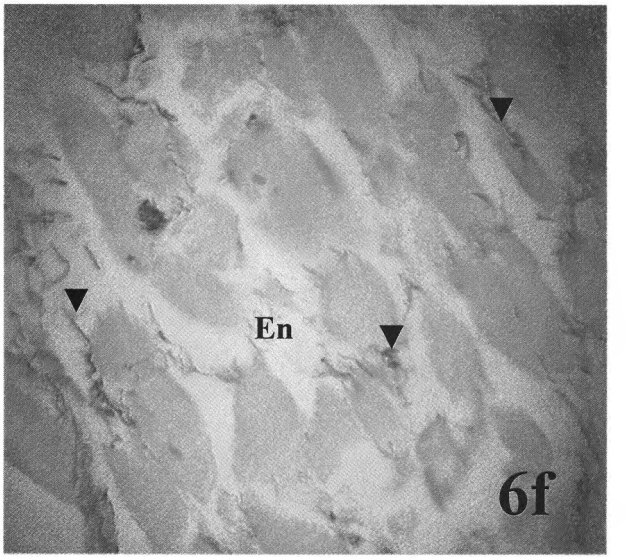

En
6f

In all age groups, intramuscular collagen fibre content increased toward the CrD origin. In the younger age groups, this manifested as an increase in the endomysial, perimysial and FEL collagen content (Fig. 7a) while in the older age groups, tendinous fibres were present (Figs. 7c and e). These collagen fibres were irregular in orientation (Figs. 7a, b, d and f) except in the tendinous portion in the older age groups (Fig. 7e).

LEGENDS

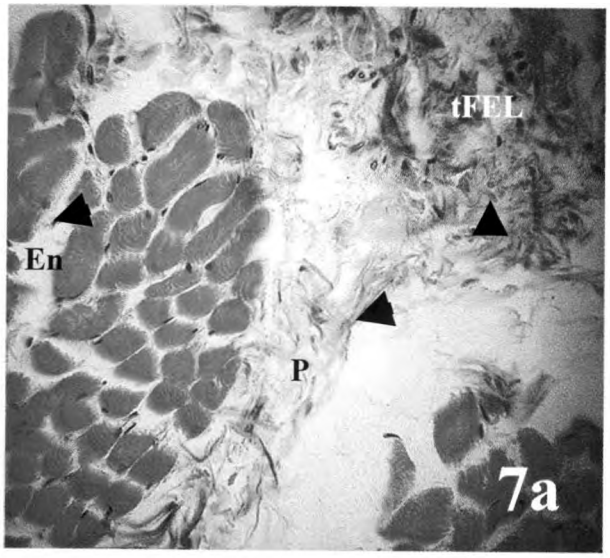
Fig. 7a: Micrograph of a transverse section through the proximal segment of the RC in a 2½ year old male showing irregular collagen fibres **(arrowheads)** in the endomysium **(En),** perimysium **(P)**, and thoracic fibroelastic lamina **(tFEL)**. (Masson's trichrome stain; magnification x400).

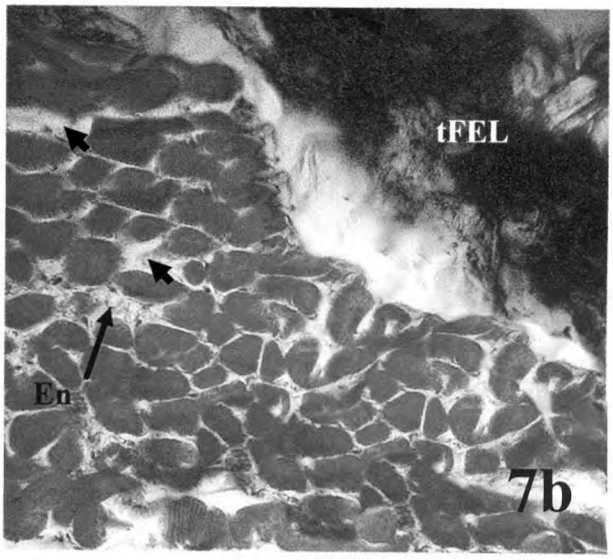
Fig. 7b: Micrograph of a transverse section through the distal segment of the RC in a 2½ year old male showing thick and irregular collagen fibres in the thoracic fibro-elastic lamina **(tFEL)** and fine ones in the endomysium **(En; short arrows)** (Masson's trichrome stain; magnification x400).

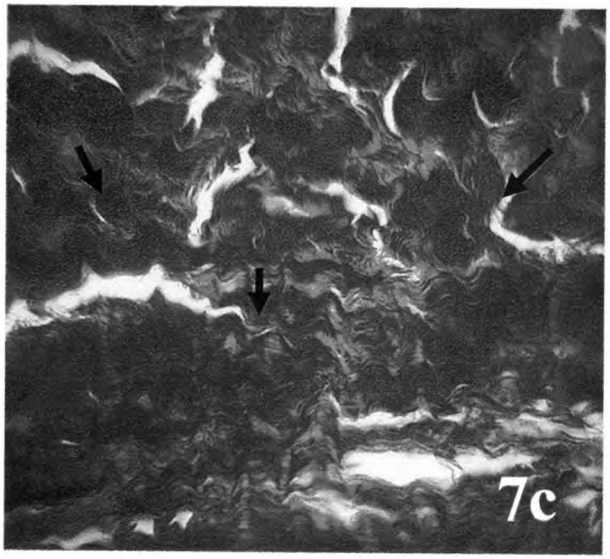
Fig. 7c: Micrograph of a transverse section through the proximal segment of the RC in a 20 year old male showing tendinous collagen fibres arranged in irregular laminae **(short arrows)** (Masson's trichrome stain; magnification x400).

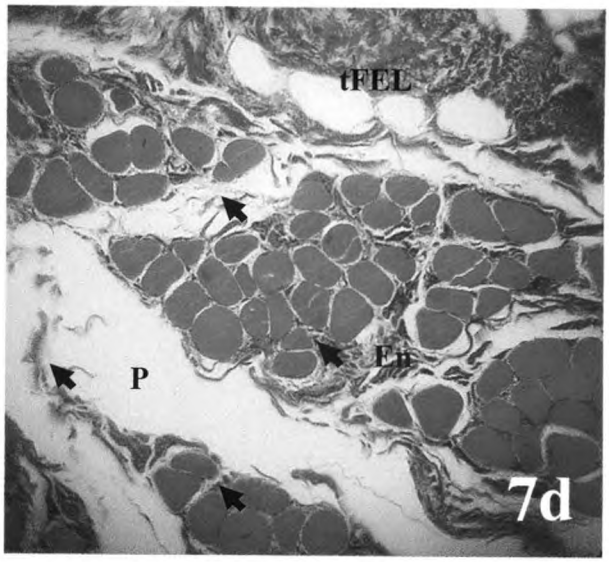
Fig. 7d and f: Micrographs of transverse sections through the distal segment of the RC in a 20 year old male and 90 year old female respectively, showing irregular collagen fibres **(short arrows)** in the endomysium **(En)**, perimysium **(P)** and thoracic fibroelastic lamina **(tFEL)** (Masson's trichrome stain; magnification x400).

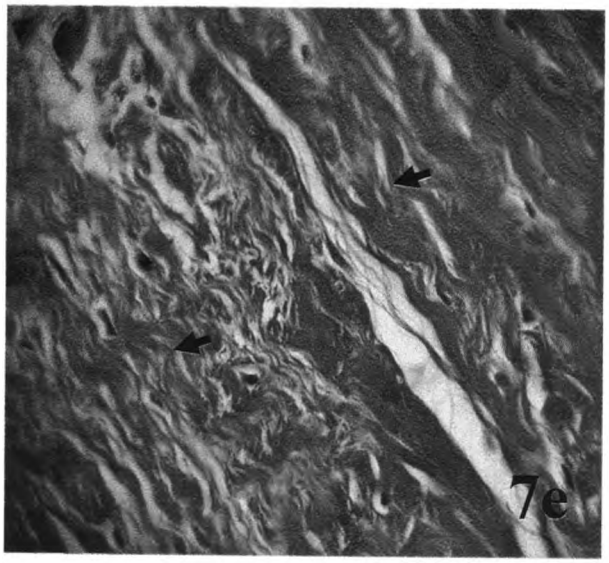
Fig. 7e: Micrograph of a longitudinal section of the proximal segment of the RC in a 90 year old female showing longitudinal collagen bundles **(short arrows)** (Masson's trichrome stain; magnification x400).

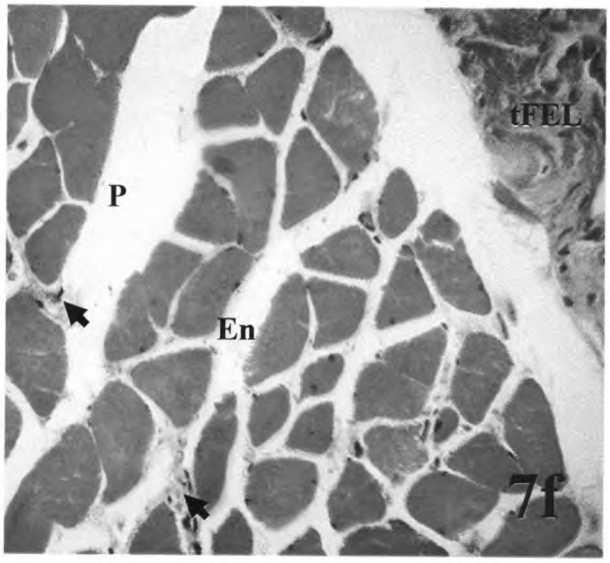

tFEL
En
P
7a


tFEL
En
7b


7c


tFEL
P
En
7d


7e


tFEL
P
En
7f

Comparison between the crural and costal diaphragms

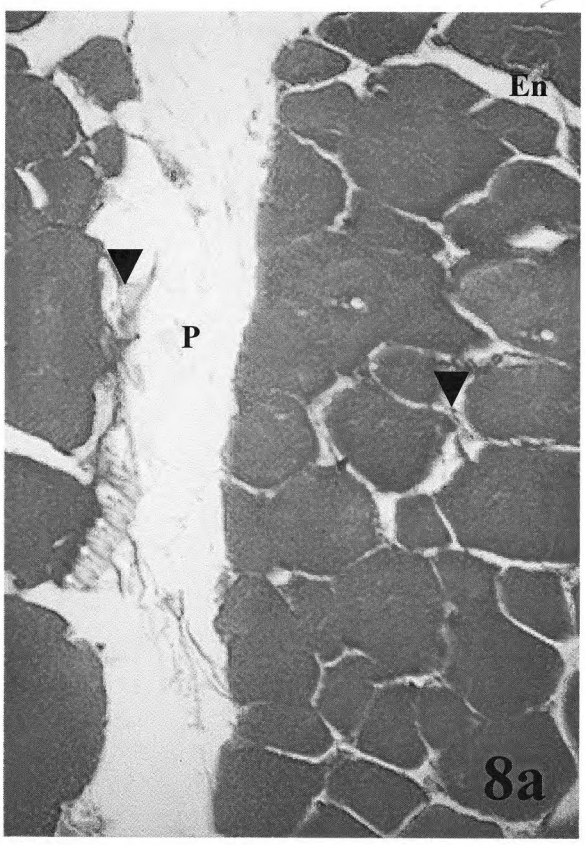
The structure of the CoD was consistent throughout its length (Figs 8a and b). Skeletal muscle fibres were homogenously longitudinal and ICT content was constantly minimal. No notable age-related differences in connective tissue content, distribution, orientation or structure were present (Figs. 9a and b).

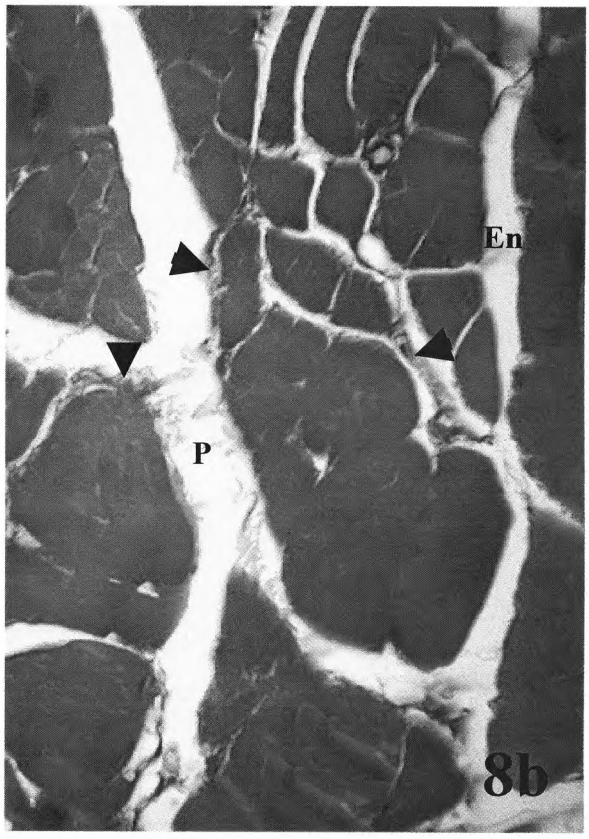
LEGENDS

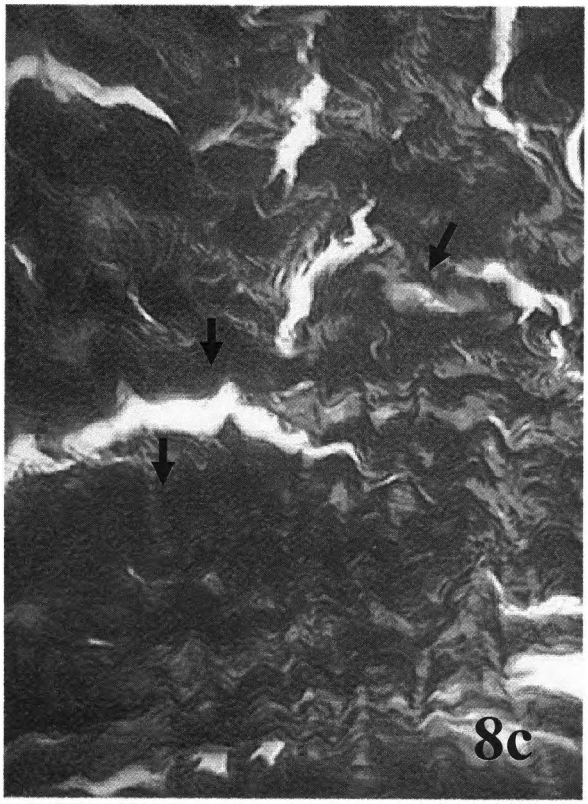
Figs. 8a and b: Micrographs of transverse sections through the proximal and distal segments of the CoD in a 20 year old male, comparing skeletal muscle fibre orientation and collagen fibre content. In both sections, skeletal muscle fibres are longitudinal **(Lo)** with minimal collagen fibre content **(arrowheads)** in the endomysium **(En) and perimysium (P)** (Masson's trichrome stain; magnification x400).

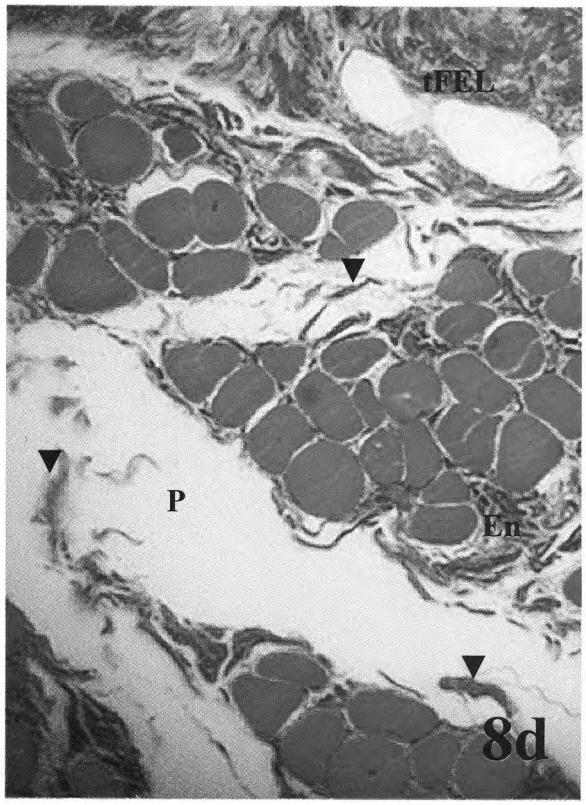
Figs. 8c and d: Micrographs of transverse sections through the proximal and distal segments of the right crus in a 20 year old male, comparing tissue composition. The proximal segment is composed of irregular laminae of tendinous collagen fibres **(short arrows)** while the distal segment is muscular with collagen fibres **(arrowheads)** in the endomysium **(En)**, perimysium **(P)** and thoracic fibroelastic lamina **(tFEL)** (Masson's trichrome stain; magnification x400).

Nuclei?





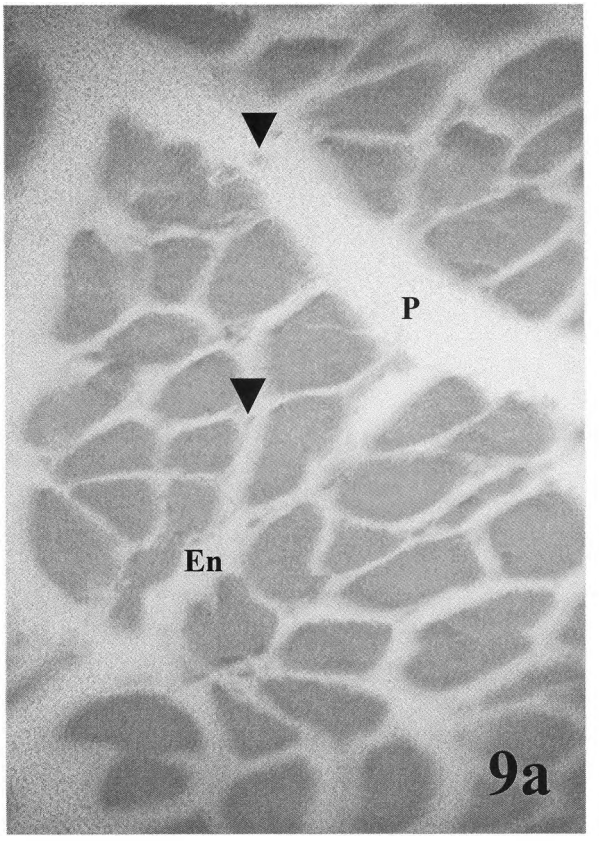


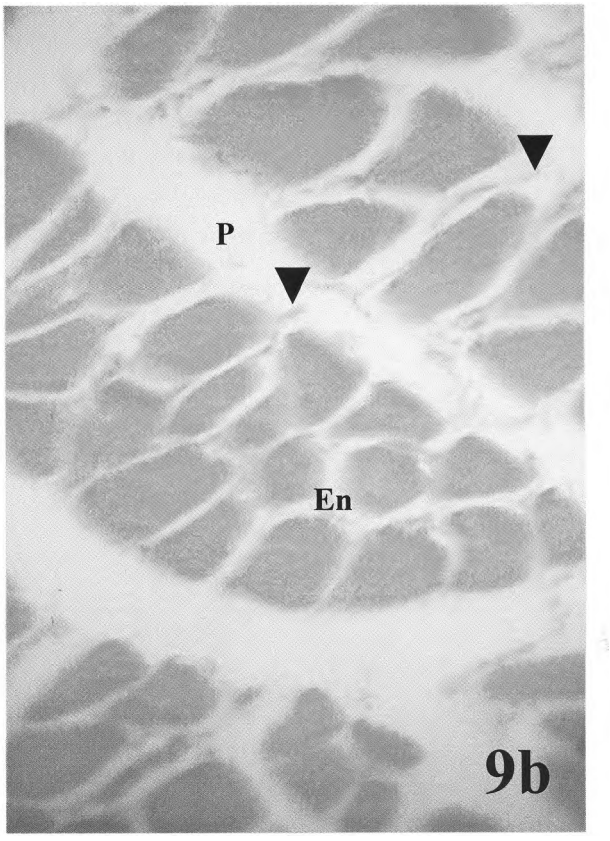


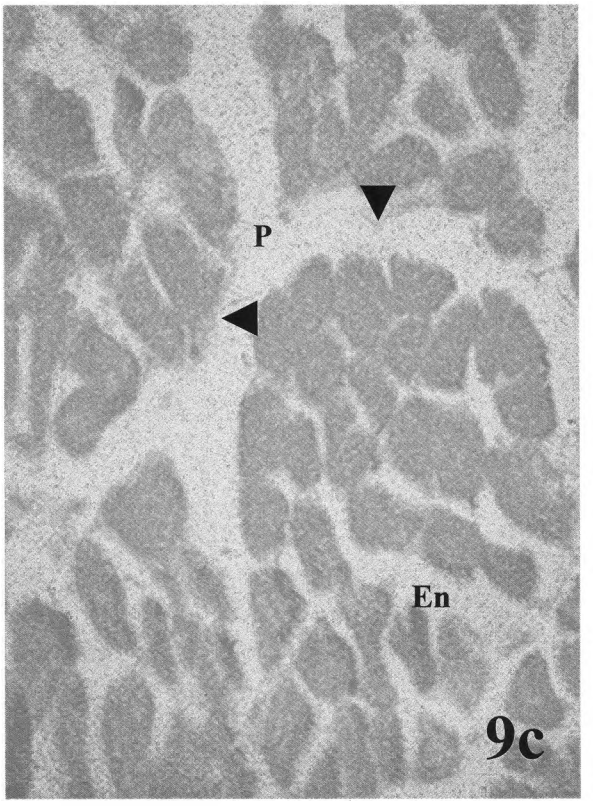
LEGENDS

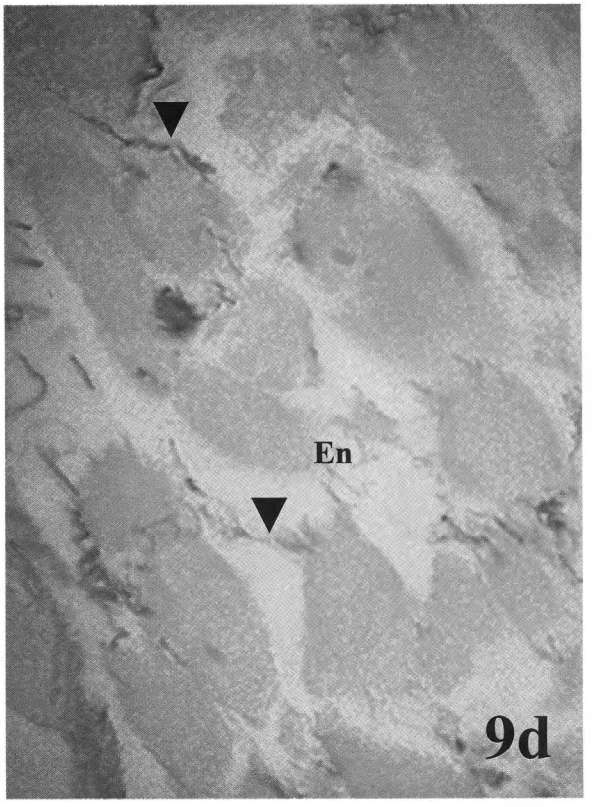
Figs. 9a and b: Micrographs of transverse sections through the distal segments of the CoD in a 2½ year old male and 90 year old female respectively, showing minimal difference in elastic fibre content **(arrowheads)** between the two. **P** – perimysium; **En** – endomysium (Weigert's resorcin-fuchsin stain with Van Gieson counterstain; magnification x400).

Figs. 9c and d: Micrographs of transverse sections through the distal segments of the RC in a 2½ year old male and a 90 year old female respectively, showing differences in elastic fibre content, orientation and structure. The fibres in the 90 year old female are thicker, tortuous and more irregular in orientation than those in the 2½ year old male **(arrowheads) P –** perimysium; **En** – endomysium (Weigert's resorcin-fuchsin stain with Van Gieson counterstain; magnification x400).









Can hardly see elastic fiber!
Look like pavement

DISCUSSION

The crural diaphragm in the present study was found to be a musculo-tendinous structure, with a tendinous origin at the vertebral column and a muscular portion that fans out from this tendon. This structure has been described in previous reports from gross observations (Standring, 2005). Two variations were observed in relation to this structure; a regional difference across the thoracic diaphragm where no such origin was observed in the costal diaphragm, and an age-related change where this origin comprised muscle in the younger age groups. Tissue structure is an adaptation to function, and in this regard, the crural diaphragm functions mainly in respiration (Sprung, *et al.*, 2006) and maintenance of gastro-esophageal junction integrity (Mittal *et al.*, 1987; Mittal, 1990; Pandolfino *et al.*, 2007). In addition, the crural diaphragm maintains spinal column stability (Hodges *et al.*, 2003; Shirley *et al.*, 2003) by increasing intra-abdominal pressure, particularly during trunk and limb movements (Hodges and Gandevia, 2000; DePalo *et al.*, 2004). It has been shown that the vertebral stiffness which occurs as a result of this action is highest between the 2nd and 4th lumbar vertebrae, suggesting that this area is subjected to most force (Hodges *et al.*, 2005). As this is where the origin of the crural diaphragm lies, its tendinous origin may be adaptive to forces it is subjected to during movement (Shadwick, 1990; Curwin *et al.*, 1994). This would explain the lack of a tendinous origin at the costal diaphragm. The increase in prominence and thickness of tendons with age has also been observed in other areas (Shadwick, 1990, Curwin *et al.*, 1994). With regard to the crural diaphragm, this may be related to acquisition of limb movements and lumbar lordosis with age.

Skeletal muscle fibre orientation

The muscular component of the crural diaphragm, by controlled and sustained contraction around the esophageal hiatus, plays a role in the prevention of hiatus hernia and gastro-

esophageal reflux disease (Holloway, 2000; Pandolfino *et al.*, 2007). In this study, muscle fibres around the esophageal hiatus were heterogenous in orientation in the younger age groups and homogenously longitudinal in older age groups. This change in orientation may be related to growth-related changes in the width of the esophagus (Biancani *et al.*, 1977), with fibres re-orienting to accommodate a wider esophagus. In addition, models of the thoracic diaphragm have shown that with alteration of orientation, muscle fibres are susceptible to distortion and compromise of function (Boriek and Rodarte, 1994; Angelillo *et al.*, 1997). Muscle fibre orientation in the thoracic diaphragm must thus be restricted, and at the crural diaphragm, this optimum orientation is theorized to be a combination of transverse and longitudinal fibres (Angellilo *et al.*, 1997). A predominantly longitudinal muscle orientation at the esophageal hiatus may thus contribute to widening of the esophageal hiatus, predisposing to hiatus hernia and gastro-esophageal reflux disease.

Skeletal muscle fibres were also found to be discontinuous along the length of the crural diaphragm. This finding corroborates a study on the thoracic diaphragm which demonstrated numerous neuromuscular junctions along its length (Boriek *et al.*, 1998). As each fibre has one neuromuscular junction, these findings show that skeletal muscle fibres of the thoracic diaphragm are arranged serially from origin to insertion. The importance of this arrangement is unclear, but may be related to the fact that diaphragmatic muscle fibres are subjected to forces both along and across their gradient (Hwang *et al.*, 2005) and may thus need a more complex arrangement to cope with these forces.

Intramuscular connective tissue fibres

An age-related increase in elastic fibre content, thickness and tortuosity in the crural diaphragm was observed in this study. Similar findings have been made in human skin (Tsuji and Hamada,

1981; Imayama and Braverman, 1989), transversalis fascia (Rodrigues Jũnior *et al.*, 1990), the interfoveolar ligament (Quintas *et al.*, 2000) and the rat costal diaphragm (Rodrigues and Rodrigues Jũnior, 2000). One reason postulated for these alterations in elastic fibres is a decrease in the rate of fibre turnover with subsequent accumulation and cross-linking (Tsuji and Hamada, 1981). This suggests changes in elastogenesis with age and has subsequently been confirmed in studies of the elastic fibre system (Rodrigues Jũnior *et al.*, 1990; Quintas *et al.*, 2000; Rodrigues and Rodrigues Jũnior, 2000). These studies showed an age-related decrease in immature oxytalan fibres coupled with an increase in mature and elaunin fibres. The staining protocol used in the present study stains mature and elaunin fibres (Rodrigues and Rodrigues Jũnior, 2000). Functionally, mature and elaunin fibres confer tissue elasticity (Ross, 1973) thus an increase in their content would be expected to increase elasticity. However, this increase is coupled with several other factors that decrease the functional capacity of these fibres. First, oxytalan fibres confer tissue resistance, thus their concomitant decrease would allow the tissue to become compliant without recoil (Ross, 1973). Secondly, collagen fibres also tend to increase with age and may exert forces on the elastic fibres which would distort them (Fukuda *et al.*, 1989). In addition, elastic fibres also undergo degenerative changes with age, such as loss of their fibrillar structure (de Carvalho Filho *et al.*, 1996) and increase in foreign deposits and inclusions (Stadler and Orfanos, 1978). These structural changes alter the overall functional capacity of intramuscular elastic fibres, possibly increasing compliance at the esophageal hiatus and predisposing to hiatus hernia and gastro-esophageal reflux disease.

No significant age-related changes in intramuscular collagen fibres were noted in the present study. Skeletal muscle fibres have however been shown to exhibit increases in intramuscular collagen with age (Alnaqeeb *et al.*, 1984; Gosselin *et al.*, 1994; Rodrigues *et al.*, 1996). The

possible reason for this disparity in findings is a difference in methodology, as these earlier studies employed electron microscopy while the present was a light microscopic study. Further, the present study noted a fibroelastic lamina present mostly on the thoracic surface. A similar structure has been described in previous studies (Griffiths *et al.*, 1992) and postulated to function in supporting diaphragmatic passive tension. The thoracic diaphragm is under constant tension on its thoracic surface due to negative intra-pleural pressure, thus an elastic component on this surface may aid in regulating changes in lung volume and prevent damage to the diaphragm (Griffiths and Berger, 1996).

Comparison between the crural and costal diaphragm

In the present study, the age-related changes in the crural diaphragm were not noted in the costal diaphragm. Rodrigues and Rodrigues Jũnior (2000) have however demonstrated such changes in the rat costal diaphragm. These workers employed electron microscopy thus may have been able to visualize these fibres better than a light microscopic study would. The same study, however, focused only on the costal diaphragm. This suggests that the lack of observable age-related changes in the costal diaphragm in the present study was not a complete lack of change, rather a less overt alteration than that in the crural diaphragm. The synchrony of the action of the crural and the costal diaphragms in inspiration is dependent on their uniform insertion into the central tendon (Angellilo *et al.*, 1997). However, the crural fibres that form the esophageal hiatus do not insert into the central tendon and act autonomously in their contribution to the gastro-esophageal reflux barrier (Mittal *et al.*, 1987; Mittal and Balaban, 1997; Holloway, 2000). The action of the crural diaphragm in the formation of the gastro-esophageal reflux barrier is partly dependent on the integrity of its intramuscular connective tissue, as this facilitates lateral force transmission and maintenance of muscle elastic properties (Bloch and Gonzales-Serratos, 2003; Kjaer *et al.*,

2006). Skeletal muscle structure is affected by differences in muscle mobilization patterns, with studies showing that with immobilization, intramuscular connective tissue fibres become thicker, irregular and increase in number (Jarvinen *et al.*, 2002). Such changes were noted with age in the crural diaphragm in the present study suggesting that there is a reduction in its mobilization with age. These findings tally with previous reports of decreases in mechanical work by the diaphragm with age (Sprung *et al.*, 2006) due to a decrease in muscle elastic properties (Gosselin *et al.*, 1994). The difference in the age-related changes in the costal and crural diaphragm suggests that the mechanical work required of the crural diaphragm is more than that which its elastic properties at a particular age can accommodate. This would result in a more rapid age – related weakening of the crural diaphragm compared to the costal diaphragm.

The findings of the present study could serve to explain observations of increase in hiatus width with age. With regard to aging changes related to the crural diaphragm, studies have shown an age-related increase in hiatus width with age (Ginalski, 1984) and subsequently associated these increases in width with an increase in prevalence of hiatus hernia and gastro-esophageal reflux disease (Dent *et al.*, 2005; El Sherif *et al.*, 2006).

CONCLUSION

The crural diaphragm exhibits age-related changes in skeletal muscle fibre orientation and intramuscular connective tissue content and structure. Such changes are less marked in the costal diaphragm and this probably relates to their differences in function and patterns of mobilization. The present study might have been enhanced by employing histochemical and electron microscopic methods, and a more uniform sample distribution to age groups. The latter was however limited by availability at autopsy. Nonetheless, the findings of this study may contribute to understanding on the etiology of hiatus hernia and gastro-esophageal reflux disease.

SUGGESTIONS FOR FURTHER STUDIES

- Employment of stereological, electron microscopic and histochemical methods in comparing crural and costal diaphragms in order to provide a more accurate comparison between the two.
- Experimental assessment of structural differences between an intact and compromised (by hiatus hernia or gastroesophageal disease) crural diaphragm so as to infer functional implications.

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APPENDIX I: DATA SHEET

Case number: ____________________

Sex: M: ☐ F: ☐

Age group (specifying age): 0-19 ☐ : 20-39 ☐ : 40-59 ☐ : >60 ☐ :

Segment of muscle strip:

☐ Proximal

☐ Middle

☐ Distal

Description:

a. **Skeletal muscle fibres** -

- -

- -

b. **Connective tissue fibres** -

- -

- -

APPENDIX II: CONSENT FORM (English)

Study number _____

Aim of the study

The crural diaphragm is a muscle that slings round the upper part of the stomach, stopping stomach acid from being forced back into the esophagus (the part of the digestive system between the stomach and the throat). Several pathologies have been attributed to this muscle though its precise structure remains unknown. This study attempts to describe the microscopic structure of this muscle which may be useful in identifying risk factors for these pathologies.

Benefits

This study may be useful in understanding the cause of illnesses affecting this region.

Humble request

In order to carry out this study, we will need specimens during autopsy. If you agree for your next of kin to participate, measurements will be taken with the organs intact and only small blocks will be extracted for histology. There will be no mutilation or whole organ extraction. The tissue blocks will then be buried at Lang'ata cemetery.

Confidentiality

The name of the deceased will remain confidential and no information concerning him/ her will appear on either the data sheets or the final thesis.

I the undersigned have been explained to and understood the above and willingly accept to let the deceased participate in the study.

Signature/ Thumbprint _____

Date: _____

I the investigator, having explained in detail the purpose of this study, hereby submit that privacy of the data collected will be maintained and only details relevant to the study revealed.

Signature: _____

Date: _____

APPENDIX III: KIBALI CHA RUHUSA (Kiswahili)

Nambari ya uchunguzi _____

Lengo la uchunguzi

Kiunguzo cha tumbo huzuiliwa kuingia koromeo na misuli inayofunga funuo la tumbo. Misuli hii yaweza kuhusika katika magonjwa kadhaa ambayo yanaweza kusababishwa na maumbile yake.

Manufaa

Kuelewa maumbile ya misuli hii kunaweza kusaidia kutambua na kupunguza magonjwa ya sehemu hii ya mwili.

Maombi

Ukikubali marehemu asaidie katika uchunguzi huu, vipimo vitachukuliwa bila kudhuru viungo na vipande vidogo kuchukuliwa ili kufanya uchunguzi na darubini.

Maadili na Usiri

Hakuna viungo vyovyote vitakavyotolewa.Vipande vitakavyobaki vitazikwa kwenye makaburi ya Lang'ata.

Jina la marehemu halitatumika mahali popote katika uchunguzi huu wala katika matokeo yatakayochapishwa.

Mimi, baada ya kuelezewa lengo na manufaa ya uchunguzi huu vilivyo, nimekubali marehemu asaidie katika uchunguzi huu.

Sahihi/ Kidole cha gumba _____

Mimi mchunguzi nimewaelezea jamaa wa marehemu kuhusu uchunguzi huu ipasavyo na naapa kutimiza usiri wa matokeo yoyote yasiyohusika na uchunguzi.

Sahihi ya mtafiti _____

Tarehe _____

APPENDIX IV: ETHICAL APPROVAL LETTER

KENYATTA NATIONAL HOSPITAL
Hospital Rd. along, Ngong Rd.
P.O. Box 20723, Nairobi
Tel: 2726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi
Email: knhadmin@knh.or.ke

Ref: KNH-ERC/ 01/ 319

9th April, 2008

Wanjiku Njongo
Dept. of Human Anatomy
UNIVERSITY OF NAIROBI

Dear Wanjiku

RESEARCH PROPOSAL: "STRUCTURE AND AGE CHANGES OF THE CRURAL DIAPHRAGM" (UP38/3/2008)

Your above revised research proposal refers.

This is to inform you that permission has been granted by the KNH-Ethics & Research Committee to carry out research on "Structure and Age Changes of the Crural Diaphragm".

By a copy of this letter, I am requesting the relevant persons to accord you the professional support and other materials that may be useful to your research.

Yours sincerely

PROF A N GUANTAI
SECRETARY, KNH-ERC

c.c. Prof. K.M. Bhatt, Chairperson, KNH-ERC
The Deputy Director CS, KNH
The Dean, School of Medicine, UoN
The Chairman, Dept. of Human Anatomy, UoN
Supervisors: Dr. Hassan Saidi, Dept. of Human Anatomy, UoN
Dr. Paul O. Odula, Dept. of Human Anatomy, UoN
Dr. Wycliffe Kaisha, Dept. of Human Anatomy, UoN