Original article

Morphological and morphometric study of collagen and elastic fibers of the gastroduodenal junction of adult and old Wistar rats

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Abstract

The aim of this work was to analyze the elastic fibers and collagen fibers of the gastroduodenal junction in rats at different ages. Through histomorphometric search, data on changes in connective tissue due to aging were obtained in order to understand its anatomical and functional relationships. **Materials and Methods**: We used 34 male rats divided into two groups: adult (03 months) and old group (18 months). To observe the connective tissue fibers of gastroduodenal junction, conventional histological techniques were applied and stained with the Verhoeff's iron hematoxylin and Weigert's resorcin fuchsin; Weigert's resorcin fuchsin after oxidation with 1% aqueous solution of oxone, Picrosirius and hematoxylin-eosin. Slides were analyzed with the program Image Pro-Plus. **Results**: Our results indicate that during aging the linear density, oxytalanic elastic fibers and mature elastic fibers decreased in the old group compared to the adult group, since the elaunin fibers had its linear density increased in the old group. Regarding the linear density of collagen fibers, they did not show statistically significant differences.

Keywords: collagen fibers, elastic fibers, aging, gastroduodenal junction.

1 Introduction

The stomach is divided into three morphologically distinct regions: cardia, fundus and the pyloric region (JUNQUEIRA and CARNEIRO, 2011). According to the current anatomical nomina, the stomach is divided into morphologically cardia, body, fundus and pylorus (BRAZILIAN SOCIETY OF ANATOMY, 2001).

According to Mountcastle (1982), in the pyloric region is located the pyloric sphincter which separates the acidic environment of the stomach from the alkaline environment of the duodenum. This separation is needed for a better functioning of digestive enzymes. The pyloric sphincter has an important functional role, considering that its musculature controls the gastric emptying and prevents gastro duodenal reflux. Thus, any abnormality that interferes with its action may lead to gastroduodenal disorders (DIDIO and MARION, 1968).

In 180 A.D, Galen adopted the term (G.) *stenótis* (narrow) to denote the pyloric canal of the stomach and compared this with a channel (G.) *pylóuros* (= guard temple), and put in Latin, the term pylourus indicated only the distal orifice of the stomach. (FERNANDES, 1999).

The gastroduodenal transition has an important role in gastrointestinal physiology, regulating the flow of chyme from the stomach into the duodenum. Being an anatomical sphincter, features characteristic morphology with circularly arranged muscle fibers. Besides these we have a collagen and elastic frame, accompanying muscle architecture.

The region that delimits the stomach from the duodenum is called the gastroduodenal junction (GDJ) which in turn consists of smooth muscle fibers and connective tissue support. The main connective tissue fibers are: collagen fibers, reticular and elastic that differ according to evolution, acquiring different functions (JUNQUEIRA and CARNEIRO, 2011).

The elastic fibers are formed by elastin (amorphous material) and a small amount of microfibrils. Elastin is a structural glycoprotein and is easily distinguished from collagen fibers. They are thinner and do not present longitudinal striations. Furthermore, elastin branch out and connect to each other, forming an irregular grid. The elastin and microfibrils are highly insoluble structures and form the main components of the extracellular matrix (KIELTY, SHERRATT and SHUTTLEWORTH, 2002; JUNQUEIRA and CARNEIRO, 2011).

Elastic fibers have the function of maintaining the elasticity of the tissue throughout life, and this is being lost during the aging process. This is due to changes in the architecture of these fibers that are highly specific in relation to the tissue where they are present (KIELTY, SHERRATT and SHUTTLEWORTH, 2002; GOSLINE, LILLIE, CARRINGTON et al., 2002).

Elastic fibers are present in various body structures, such as, blood vessels (DAVIS, 1993) and lungs (PIERCE and HOCOTT, 1960). Arrangement of these fibers contribute to the tissue architecture, promoting passive action in the tissue when it is exposed to mechanical stress, and when it returns to its original position (BUCKWALTER,COOPER and MAYNARD,1976).

In a system of elastic fibers, the following types can be distinguished: oxytalanic, elauninic and mature. Each is in a different stage of elastogenesis (PECORA, RODRIGUES, RODRIGUES-JUNIOR et al., 2001). The first component are oxytalanic fibers, characterized by microfibrils that are synthesized and secreted by fibroblasts in the extracellular medium.

These microfibrils are arranged in parallel bundles and are already indicative of the direction and shape of the future elastic fiber. In the next moment, elastin (an amorphous substance) adhered to these microfibrils, which passes to characterize elaunin, the second component of the elastic system. The continuity of this elastin deposition between microfibrils eventually form a thick fiber, which then passes to be called mature fibers. The process of an adequate elastogenesis is recognized by the presence of these three components, and the proportion of these three types varies from tissue to tissue. It seems to depend on the function of organ involved and on the aging process (RODRIGUES-JUNIOR, TOLOSA and CARVALHO, 1990; RODRIGUES, SACHETTI and RODRIGUES-JUNIOR, 1999; AKHTAR, SHERRATT, CRUICKSHANK et al., 2011).

The process of elastogenesis suitable is of great importance, since serious hereditary diseases affecting connective tissue are caused by mutations in components of elastic fibers (ROBINSON and GODFREY, 2000; MILEWICZ, URBAN and BOYD, 2000; LE SAUX, BECK, SACHSINGER et al., 2001).

Collagen is the protein most found in vertebrate tissue, forming something like a quarter of an adult organism. This is a fundamental component of the extracellular matrix. Is a group of proteins with repeated sequences of Gly-XY (Glycine associated with two other amino acids - usually proline and hidroxiprolinas (JENSEN and HOST, 1997). The collagen fibers are responsible for the formation of a framework of support for macromolecules. Are identified at least 28 different types of collagens with a predominance of type I and III. The type I collagen is the most prevalent protein in the human body, followed by collagen type III (KADLER, BALDOCK, BELLA et al., 2007).

The type I collagen fibers are birefringent, have elongated fibers and are grouped in parallel arrangement, being found in bone, dentin, tendons, capsule of various organs and in the dermis. The type III collagen is composed of three chains of alpha-1. They are thinner and shorter fibers. These fibers are synthesized by fibroblasts and reticular cells, and are associated with type I collagen in different ratios. However, are prevalent in tissues with a certain degree of elasticity, such as skin, muscles, ligaments, and fascia. Type III collagen has a distribution similar to the type I, but, while type I is related to support, the type III relates to elasticity itself (NORTON, 1993; CÖR, BARBIC and KRALJ, 2003; JUNQUEIRA and CARNEIRO, 2011). Thus, the elastic and collagen fibers are important for the maintenance of normal tissue resistance.

The objective of this research was to describe and analyze qualitatively and quantitatively the collagen and elastic fibers of the gastroduodenal junction of Wistar rats at different ages.

2 Materials and Methods

For this study we used 34 male Wistar rats (Rattus norvegicus albinus) organized into two groups, old aged 18 months, weighing between 400-700 g, and adult, with 03 months weighing 250-300 g.

The experiment followed the rules and ethical principles of animal experimentation adopted by the Brazilian School of Animal Experimentation (COBEA) and was previously submitted to the Ethics Committee on Using Animals for Experimentation (EAEC) Institute of Biomedical Sciences, University of São Paulo (under No. 2798.) All rats were euthanized by overdose of ketamine (150 mg / kg) and xylazine (10 mg / kg) (CURY, DIAS, SOSTHENES et al., 2013).

After euthanasia of the animals, samples were removed from the gastroduodenal junction, subjected to the usual histological processing.

Cuts were made in the longitudinal series of 5 µm, mounted on slides and stained with conventional iron hematoxylin (Verhoeff) for visualization of elastic fibers, resorcinol fuchsin (Weigert) to highlight elaunin and elastic, resorcinol fuchsin with previous oxidation with 1% aqueous solution of oxone to show oxitalanic, hematoxylin-eosin (HE) to examine the characteristics of collagen fibers and their nuclei, Picrosirius for visualization of collagen fibers (BEHMER, TOLOSA and FREITAS NETO, 1976). Sirius Red staining (picrosirius under polarized light) second Montes and Junqueira (1991), reacts with the collagen molecules and promotes an increase in its ordinary birefringence. This is because dye molecules are aligned along the longitudinal axis of each molecule of collagen. The authors say that the birefringence promoted by the method is, therefore, specific for collagen structures.

The stained slides were observed and digitized microscope NIKON Eclipse E600/NIS-Elements AR for documentation and further qualitative and quantitative.

Statistical analysis was performed by comparing the linear density of collagen fibers and muscle of adult and old groups. The results were subjected to statistical analysis of variance (ANOVA), followed by Tukey's "t" test, which was adopted significance level "p" equal to or less than 0.05.

3 Results

The images obtained through analysis by light microscopy of sections stained with hematoxylin-eosin in old and adults animals demonstrated the smooth muscle fibers distributed in the muscular region of the mucosa and submucosa. Nucleus are shown centralized, a typical characteristic of this type of tissue (Figures 1a, b).

Using picrosirius staining and analyzing with polarized light microscope to highlight the collagen type I, that have shades of orange and red, and type III with green color, there is a prevalence of type III collagen in the adult group and type I in the old (Figures 1c, d).

Histological sections of the gastroduodenal region using Weigert staining with prior oxidation made possible the

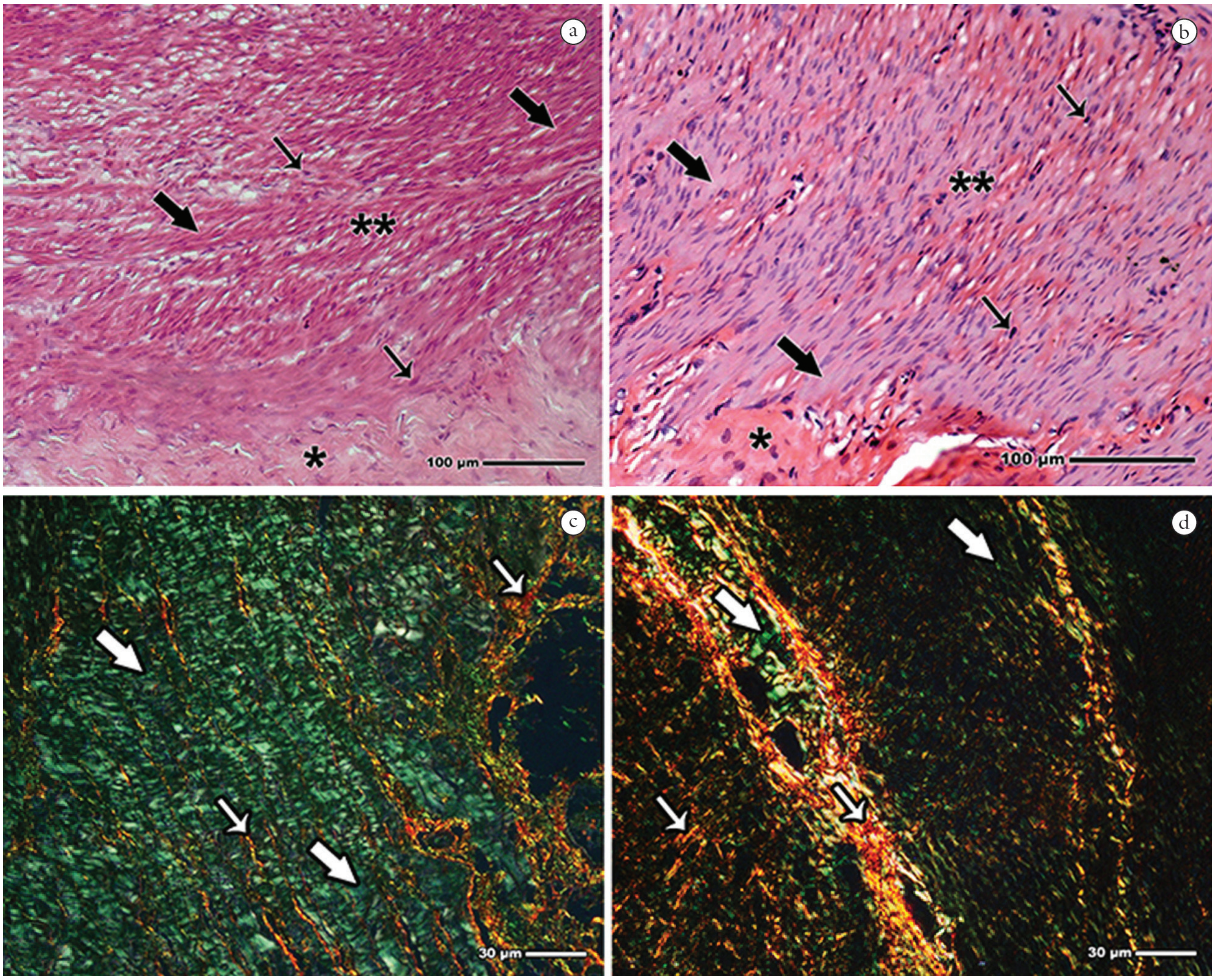

Figure 1. Light microscopy of the gastroduodenal junction of adult (Figures ac) and old Wistar rats (Figures bd). (ab) Cell nucleus (thin arrows) and smooth muscle fibers (thick arrows), the region of the submucosa (*), muscularis mucosa region (**). Magnification: 200x, bar: 100 µm . Staining: hematoxylin-eosin. (cd) show the collagen type I (thin arrows) and type III(thick arrows). Magnification: 400x, bar: 30 µm. Staining method: picrosirius, image under polarized light.

observation of oxytalanic fibers. In the adult group note that these are distributed parallel between them and have a thin thickness. In the old group note that these are thicker and not straight as in the adult (Figures 2a, b).

Using only Weigert staining, it is possible to highlight the elaunin fibers, in which there is a lot in the adult group with straight, narrow and parallel features. In the old group note a lesser amount with tortuous and thick shape compared to the group adult (Figures 2c, d).

Mature elastic fibers were observed in the adult group looking straight and thick, confronting the old group. Note that the same fibers exhibit fewer, slender and sinuous features (Figures 2e, f).

The average area of collagen type I in the adult group was 0.114 ± 0.046 µm^2, with the median value of 0.099 µm^2. The old group showed an average area of 0.164 µm^2 ± 0.061, median value of 0.166 µm^2. Statistical results show a larger area of type I collagen in the old group, however, these results are not significant ($p = 0.055$) and are shown in the Figure 3a.

The average area of type III collagen in adult rats was 0.146 ± 0.047 µm^2, with a median value of 0.149 µm^2. In the old the average area was 0.156 ± 0.038 µm^2 and the median value of 0.150 µm^2. There were no statistically significant differences between the two groups ($p = 0.612$), the data are graphed in Figure 3b.

Analysis of the average linear density of oxytalanic fibers in the adult group was 0.0036 ± 0.0007, with median value of 0.0037. The old group showed linear average value 0.0027 ± 0.0005, median value of 0.0027. In this case, the adult group has a higher elaunin density, being statistically significant ($p = 0.001$). This is expressed in the Figure 3c.

The average linear density of elaunin fibers in the adult group was 0.0027 ± 0.0004, with median value of 0.0028. In the old group the average linear density was 0.0037 ± 0.0006, with a median value of 0.0038. These results show a higher density of oxytalanic fibers in the old group compared to adults, with significant statistical difference ($p = 0.001$). Data presented in the graph in Figure 3d.

Data of average linear density of mature elastic fibers in the adult group were 0.0055 ± 0.0007, with median value of 0.0054. In the old group, average data were of 0.0044 ± 0.0016, with median value of 0.0047. Presented data indicates a higher density of these fibers in the adult group, but not statistically significant ($p = 0.055$). Data exposed in Figure 3e.

4 Discussion

Our study aimed to analyze the fibers from elastic system and the collagen from gastroduodenal junction (GDJ), facing the aging process. The importance of this work is given by the lack of data in this type of tissue in the literature.

The aging of connective system fibers have been studied in previous studies by other authors in different

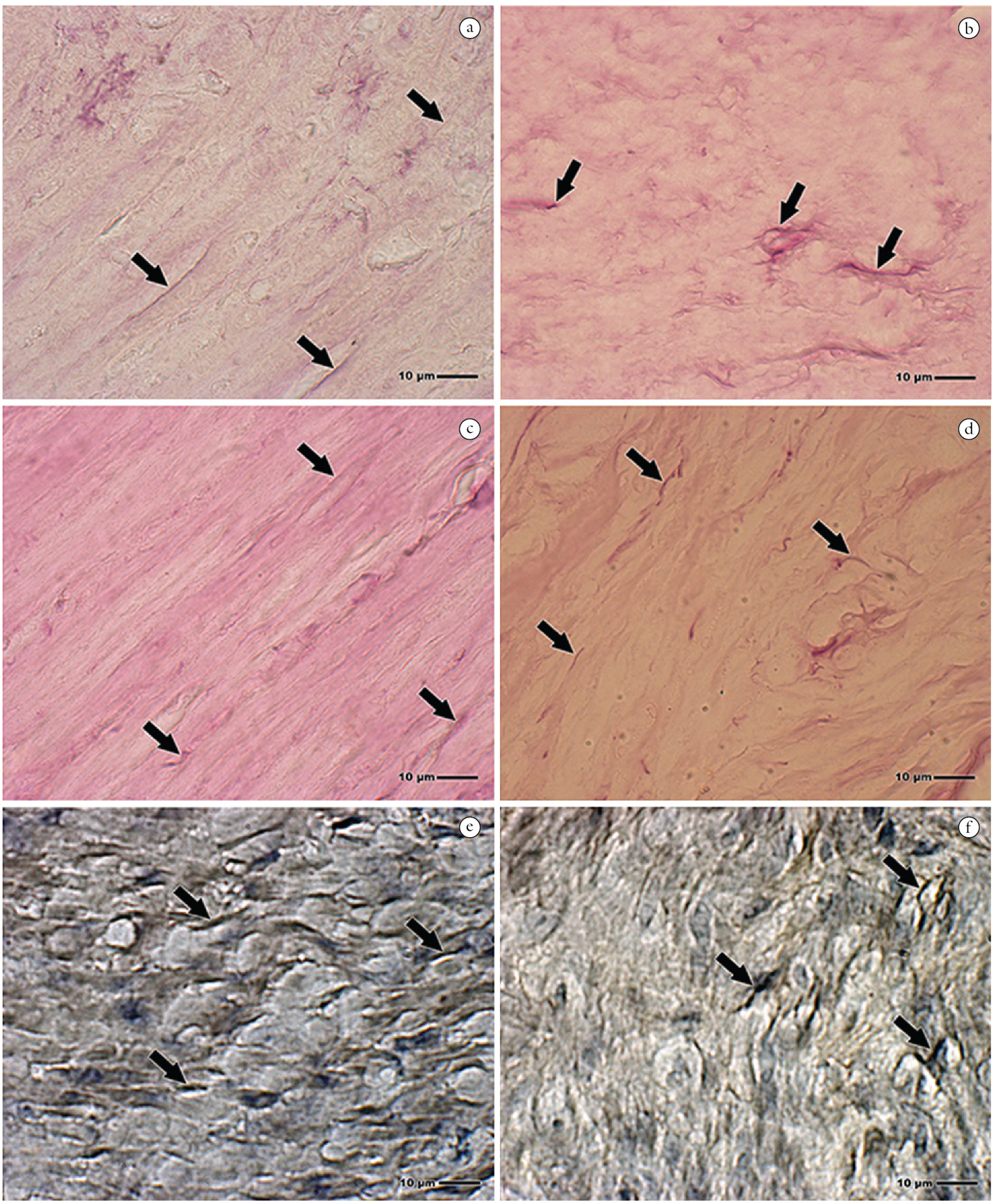

Figure 2. Light microscopy of the gastroduodenal junction of adult (Figures ace) and old Wistar rats (Figures bdf). (a-b) show the oxytalanic fibers (arrows). Magnification: 1000x, bar: 10 µm. Staining Method: Weigert with prior oxidation. (c-d) show the elaunin fibers (arrows). Magnification: 1000x, bar: 10 µm. Staining method: Weigert. (ef) show the mature elastic fibers (arrows). Magnification: 1000x, bar: 10 µm.Staining method: Verhoeff.

tissues: skin (COTA-PEREIRA, RODRIGO and BITTENCOURT-SAMPAIO, 1976; NISHIMORI, EDWARDS, PEARSE et al., 2001; LANGTON, SHERRATT, GRIFFITHS et al., 2010), skin and gums (GOGLY, GODEAU and GILBERT, 1997), intestine (ORBERG, KLEIN and HILTNER, 1982), periodontal ligament (SAWADA, SUGAWARA, ASAI et al., 2006), arteries (HOSODA, KAWANO and YAMASAWA et al., 1984; BRUEL and OXLUND, 1996; CATTELL, ANDERSON and HASLETON, 1996; CHOPARD,

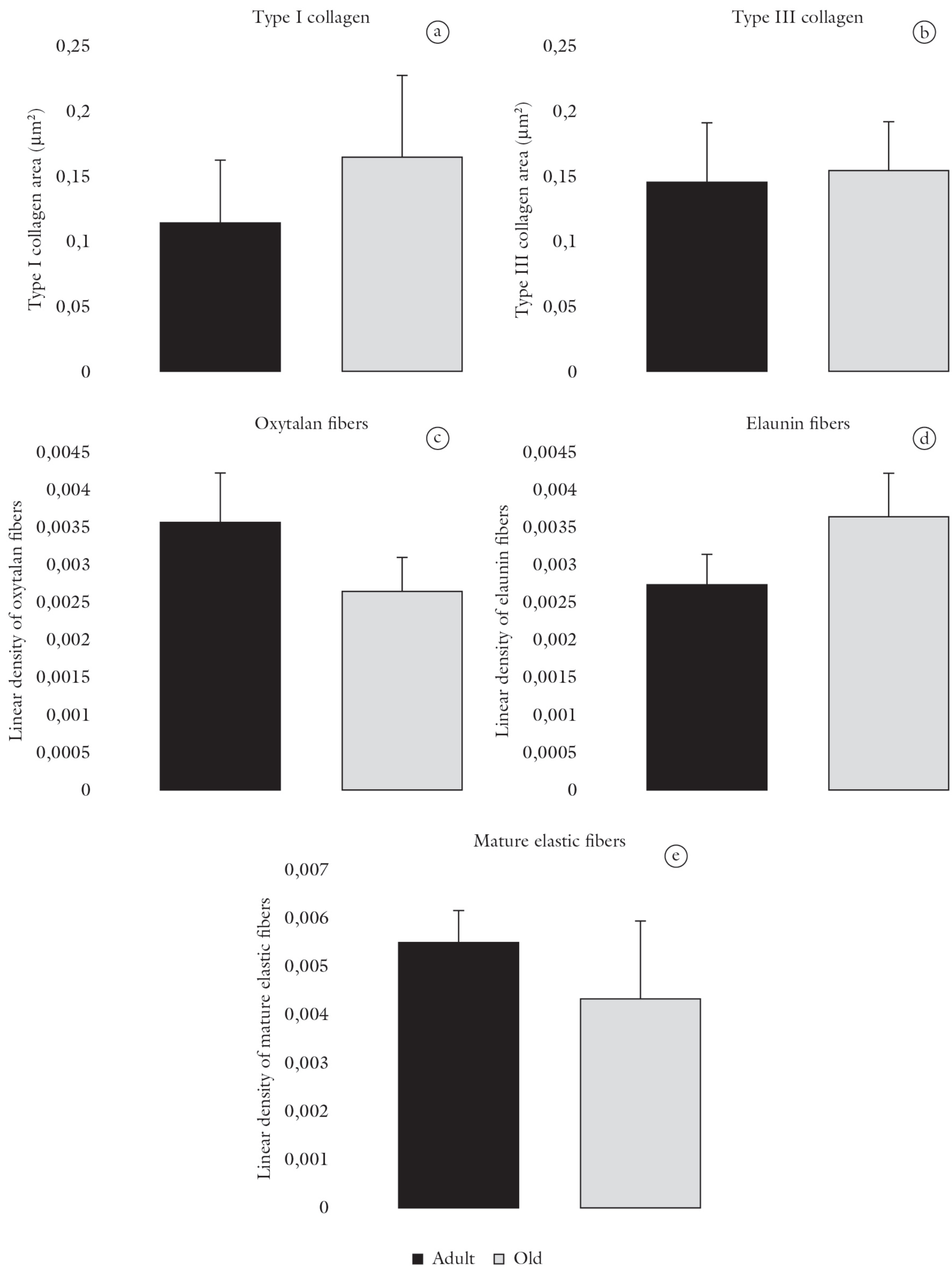

Figure 3 Graphs of quantitative analysis of the elastic fibers of rats and old adults. (a) shows the area of collagen type I fibers. (b) Area of collagen type III fibers. (c) Linear density of oxytalanic fibers. (d) Linear density of elaunin fibers. (e) Linear density of mature elastic fibers.

LUCAS, GERHARD et al.,1998), heart (CARVALHO-FILHO, CARVALHO and SOUZA, 1996), lungs (JANSSENS, PACHE and NICOD, 1999; LAI-FOOK and HYATT, 2000), in the diaphragm and rectus abdominis muscles (RODRIGUES and RODRIGUES, 2000) and in the interspinous ligament (BARROS, RODRIGUES, RODRIGUES et al., 2002).

Our results showed no alterations of collagen fibers in young and old group. However evaluations made in the gut of rats by Orberg, Klein and Hiltner (1982), demonstrated that increased fiber length during maturation, but remains constant during aging, while their diameters do not seem to change with age. However, in the skin and gums, as described by Gogly, Godeau and Gilbert (1997), there is an increase in the diameter of collagen fibers with age, contrary to the results observed by Orberg, Klein and Hiltner (1982). However, our results are similar to those of (AÏT-BELKACEM, GUILBERT, ROCHE et al., 2012) who studied tail tendons from rats. The authors point out that networks of fibrils became thicker and scarce and that the length of the collagen fibers increased significantly with advancing age.

However our results are consistent, in parts, with the study of Takubo, Hirai, Muro et al. (1999), regarding the effect of aging in the extracellular matrix of lungs and age-associated changes in the levels of collagen and elastin in rats at different ages. The authors state that there were no significant changes in the content of elastin, collagen type I and III and in the extracellular matrix with aging.

Many studies, over the years, describe elastic system changes with respect to aging tissues. According to our results, with respect to the elastic system in the GDJ, mature elastic and oxytalanic fibers showed lower linear density in the old group compared to the adult group,. However, the linear density of elaunin fibers increased in the old group compared to the adult. Similar results were described by Reisdoerfer, Pereira and Chopard (2009), however, at the vesico-urethral junction, mature elastic and oxytalanic fibers had decreased its length and the elauninic had increased its length with aging. Our results also agree with the Cota-Pereira, Rodrigo and Ferreira (1976). According to these authors, the linear density of elaunin fibers increased proportionally in the aging process.

Results of this study are similar to other authors who worked with different tissues Barros, Rodrigues, Rodrigues et al. (2002), analyzed the interspinals ligaments. In addition to structural changes that occur in elastic fibers, aging leads to the disappearance of oxytalan fibers, which are responsible for tissue resistance leading to the flaccid aspect. In the transversalis fascia occurs a decrease in oxytalan and an increasing of amorphous substance (RODRIGUES, TOLOSA and CARVALHO, 1990). Therefore, the reduction of this type of fiber, depending on age, can be responsible for changes in resistance of the transversalis fascia. Other authors observed that in advanced ages, there is a progressive increase in linear density of mature elastic and elauninic fibers and reduction of oxytalan fibers in diaphragm and rectus abdominis muscles (RODRIGUES and RODRIGUES, 2000). Their results are partial consistent with our result.

According to the tissue in which the elastic fiber system is present, it can play different functions, such as determining stress resistance, absorbing tension, giving support and elasticity (CARVALHO-FILHO, CARVALHO and SOUZA, 1996). Sephel and Davidson (1986), and Uitto, Fazio and Olsen (1988) explain that with advanced ages, rates of biosynthesis of elastin decreases. Collagen, in turn, plays a dominant role in maintaining the structure of various tissues and also has many other important functions, for example, adhesion, differentiation and tissue remodeling (MYLLYHARJU and KIVIRIKKO, 2004).

5 Conclusion

The present results show that in aged animals the linear density of elastic fibers and mature had oxytalan decreased. However, the elaunin fibers revealed increased linear density compared to the adult group. collagen fibers, did not present any changes in both groups. Therefore, it is possible to conclude that pylorus integrity was conserved in all animals and ages studied.

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