

# Morphometric analysis of muscularis proper and myenteric plexus of the normal human oesophagus. Age related changes

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**Background:** Oesophagus is a muscular tube that transports food and liquids by coordinated contraction of its muscular lining led by stimuli from the nerve plexus. Its muscularis proper layer consists of muscle cells, connective tissue and myenteric plexus. The aim of our histomorphometric study was to reveal detailed characteristics of this layer, cell number, volume, orientation, properties of myenteric plexus as well as changes related to aging.

**Materials and methods:** Oesophagus tissue samples from 17 male cadavers were taken from the cranial and thoracic parts. Samples were divided in 2 groups: younger (ages 21–45) and older (ages 66–78). The tissue was routinely processed, embedded and serially sectioned. Sections were stained with Masson-Goldner and Cresyl-violet dyes. Digital images were analysed with the image analysis software. Statistics were performed with SPSS software.

**Results:** The average thickness of the cranial part of the oesophageal wall and muscularis proper was 2590  $\mu\text{m}$  and 1197  $\mu\text{m}$ , respectively in the younger and 2453  $\mu\text{m}$  and 1144  $\mu\text{m}$  in the older group. Overall volume of the muscle tissue was slightly larger in the thoracic part, and in the younger group compared to the cranial part and the older group. The average number of the striated muscle cells per 100  $\mu\text{m}$  in the cranial part was 771.5 and 749.7 in the younger and the older group, respectively. Striated cells were significantly less present only in the lower thoracic part of the oesophagus. In the older group, smaller striated muscle cells dominated over the larger ones. In the younger group, majority of the striated muscle cells were mid-sized. The thickness of the circular layer of muscularis proper was more affected by aging than the longitudinal one. Ganglion cells number was lower in the older group, but plexus area was unchanged.

**Conclusions:** Aging affects muscularis proper and myenteric plexus of the oesophagus. Major differences can be observed in the striated muscle cells size, volume of the circular layer and number of the ganglionic cells in the myenteric plexus. (Folia Morphol 2013; 72, 3: 223–229)

**Key words:** oesophagus, human, muscularis proper, morphometry, histology, aging

## INTRODUCTION

Oesophagus is a muscular tube that seems to have only one important function — to carry food and liquids from the mouth to the stomach. This role is performed by coordinated contraction of its muscular lining led by stimuli from the nerve plexus situated in its muscularis proper [6, 15, 18]. The histological structure of the muscularis proper of the oesophagus is well known. It has 2 layers, circular and longitudinal, that are responsible for the delicate processes of simultaneous shortening and squeezing of the oesophageal wall. Basic structure of the oesophagus wall is described in the scientific articles and histology textbooks [26]. In the muscularis proper striated and smooth muscle cells are regionally distributed. Cranial part is built from the striated muscle cells only, but along the length of the oesophagus smooth muscle cells are gradually replacing them. On the other hand, deleterious effects of aging on the structure and function of many organs are well recognised [9, 16, 17, 19, 27]. Oesophageal dysfunctions related to aging are clinically well described [4, 8, 10, 14, 23], but histological reports are not numerous and mostly focus on the swallowing reflex structures and the oesophageal sphincter [3, 12, 20, 30]. Interestingly enough, histomorphometric examinations focused on the other parts of the human oesophagus are absent. Therefore, we wanted to fully examine the normal oesophageal muscularis layer and to reveal the specific number, volume, orientation and size of the muscle cells in the oesophageal wall and to examine the number of ganglionic cells in the myenteric plexus in order to identify the changes related to the age of the donor that leads to the oesophageal dysfunction.

## MATERIALS AND METHODS

Tissue samples of human oesophagus were collected from the autopsy material of 17 male cadavers aged 21–78 years without previous history of oesophageal diseases. Ethical approval for the research protocol was issued by the Ethics Committee. One sample from the cervical part of the oesophagus (first centimetre) and 3 samples from the thoracic part of the oesophagus were taken from each cadaver: upper (at the height of the ring cartilage of trachea), middle (at the height of the bifurcation of the trachea), and lower (above the oesophago-cardiac junction). Overall, 68 tissue samples of the oesophagus were taken. Approximate length of the samples was 1 cm. Specimens were divided into 2 groups: group one

(7 specimens, age span 21–45 years) and group two (10 specimens, age span 66–78 years). The oesophageal tissue was fixed in 10% buffered paraformaldehyde for 48 h, routinely processed and embedded in paraffin blocks. From each tissue sample 200 serial sections were made at the beginning and at the end of the tissue block, so overall number of sections was 400 for each sample. Celloidin coating of the microscopic slides was performed in order to assure attachment of the tissue section. Two types of staining were applied: Masson-Goldner (for highlighting the muscle cells) and Cresyl-violet for detection of the ganglionic cells in the myenteric plexus. Staining of the serial sections was designed as follow: for each 10 sections, odd ones were stained with Masson-Goldner and even ones were stained with Cresyl-violet. This design was repeated for all the sections. Images of serial sections were captured with digital camera attached to the Olympus BX51 microscope. Measurements, cell classification and image analysis were performed with Image Pro Plus software (Media Cybernetics, USA). Classification of the striated muscle cells according to their diameter was done only on the cells that were sectioned transversely (e.g. ratio of their minimal and maximal diameters was up to 1:3), in order to eliminate the clusters of cells from the image analysis and avoid false measurements. Cells were classified in 3 groups: small (diameter up to 20  $\mu\text{m}$ ), middle (from 20–30  $\mu\text{m}$ ), and large (over 30  $\mu\text{m}$ ). Three-dimensional reconstruction was performed with Amira 4.1.1 software (Mercury Computer System, Germany). Results were presented as mean  $\pm$  standard deviation. Statistical analysis was done using the SPSS software. Estimation of statistical significance between mean values was performed with ANOVA. Level of significance was set at  $p < 0.005$ .

## RESULTS

### Morphometric analysis of the muscle cells in the muscularis proper

Microscopic examination of all the specimens showed no pathological changes in the oesophageal tissue. The average thickness of the cranial part of the oesophageal wall was  $2590 \pm 512 \mu\text{m}$  in group one and  $2470 \pm 482 \mu\text{m}$  in group two. In the thoracic part values were  $2453 \pm 378 \mu\text{m}$  and  $2410 \pm 354 \mu\text{m}$  for group one and two, respectively. Thickness of muscularis proper in the cranial part varied from 913  $\mu\text{m}$  to 1482  $\mu\text{m}$  in group one and from 874  $\mu\text{m}$  to 1414  $\mu\text{m}$

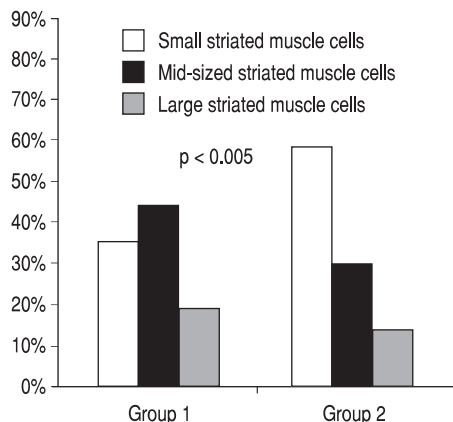
**Table 1.** Measurement results of the oesophageal muscularis proper

Parameter	Age 21–45		Age 46–78	
	Cranial	Thoracic	Cranial	Thoracic
Thickness of the oesophageal wall [ $\mu\text{m}$ ]	2590 $\pm$ 512	2453 $\pm$ 378	2470 $\pm$ 482	2410 $\pm$ 354
Thickness of the muscularis proper [ $\mu\text{m}$ ]	1197 $\pm$ 284	1181 $\pm$ 327	1144 $\pm$ 270	1191 $\pm$ 379
Overall volume of muscle tissue [ $\text{mm}^3/1 \text{mm}^2$ ]	0.4215 $\pm$ 0.085	0.5874 $\pm$ 0.092	0.3844 $\pm$ 0.072	0.5420 $\pm$ 0.073
		0.5752 $\pm$ 0.089		0.5625 $\pm$ 0.064
		0.5996 $\pm$ 0.106		0.5785 $\pm$ 0.102
Volume of striated muscle cells [ $\text{mm}^3/1 \text{mm}^2$ ]	0.4215 $\pm$ 0.085	0.4240 $\pm$ 0.081	0.3844 $\pm$ 0.072	0.3760 $\pm$ 0.57
		0.4020 $\pm$ 0.104		0.3880 $\pm$ 0.061
		0.0052 $\pm$ 0.001		0.0043 $\pm$ 0.001
Volume of smooth muscle cells [ $\text{mm}^3/1 \text{mm}^2$ ]	0	0.1734 $\pm$ 0.82	0	0.1660 $\pm$ 0.092
		0.1732 $\pm$ 0.066		0.1745 $\pm$ 0.068
		0.5844 $\pm$ 0.109		0.5742 $\pm$ 0.64
Number of striated muscle cells per 100 $\mu\text{m}$	771.5 $\pm$ 171.8	586 $\pm$ 112.8	749.7 $\pm$ 107.1	542 $\pm$ 71.6
		432.2 $\pm$ 52.3		404.3 $\pm$ 50.3
		36 $\pm$ 5.5		29 $\pm$ 7.1
Volume ratio of longitudinal/circular oriented cells		1.20 $\pm$ 0.12		1.44 $\pm$ 0.24*
		1.16 $\pm$ 0.14		1.27 $\pm$ 0.21*
		1.10 $\pm$ 0.0.12		1.19 $\pm$ 0.06

\* $p < 0.005$  compared to group one

in group two. For the thoracic part of the oesophagus values were from 854  $\mu\text{m}$  to 1509  $\mu\text{m}$  and from 812  $\mu\text{m}$  to 1570  $\mu\text{m}$ , respectively. Although there were differences in the oesophageal wall thickness among the groups, they were not significant. In the cranial part of the oesophagus muscularis proper was built only from striated muscle cells and in the thoracic part both, smooth and striated muscle cells were present. In the cranial part under the 1  $\text{mm}^2$  of the oesophagus surface in group one mean volume of the striated muscle tissue was 0.4215  $\text{mm}^3$  and in group two — 0.3844  $\text{mm}^3$ . In the upper and middle thoracic part of the oesophagus under the 1  $\text{mm}^2$  of its surface in group one average overall volume of muscle tissue in muscularis proper was 0.5874  $\text{mm}^3$  (striated 0.4130  $\text{mm}^3$  and 0.1744  $\text{mm}^3$  of smooth muscle cells) and in group two — 0.5610  $\text{mm}^3$  (striated 0.3820  $\text{mm}^3$  and 0.1790  $\text{mm}^3$  smooth muscle cells). In the lower part these values were much different because of the dominance of the smooth muscle cells. We also observed that smooth muscle tissue was extended more cranial in the circular muscle layer than in the longitudinal one. Specific values of the muscle cell volume for the upper, middle and lower part of the oesophagus of each group are presented in Table 1. The average

number of the striated muscle cells under the 100  $\mu\text{m}$  of the oesophageal surface length in the cranial part of the muscularis proper was: in group one — 771.5  $\pm$  171.8, and in group two — 749.7  $\pm$  107.1. The difference was insignificant ( $p > 0.005$ ). In the thoracic part we were unable to determine the exact number of smooth muscle cells, but the number of striated ones depended on the investigated region. Hence, in group one, average value in the upper, middle and lower oesophagus region was 586  $\pm$  112.8, 432.2  $\pm$  52.3 and 36  $\pm$  5.5, respectively, and in group two — 542  $\pm$  71.6, 404.3  $\pm$  50.3 and 29  $\pm$  3.1, also respectively. Microscopic examination of the striated muscle cells orientation in the oesophageal wall (e.g. circular or longitudinal) showed that in the cranial part vast majority of the muscle cells were oriented longitudinally. Classic circular layer of the muscularis proper in this part of the oesophageal wall was almost absent and presented only by a handful of striated muscle cells with the oblique orientation, because this part of the oesophagus was built mostly from the specific muscles involved in swallowing reflex. When orientation of the striated muscle cells in the oesophageal thoracic part was assessed, we observed that in the outer layer of the muscularis proper there were areas of circularly

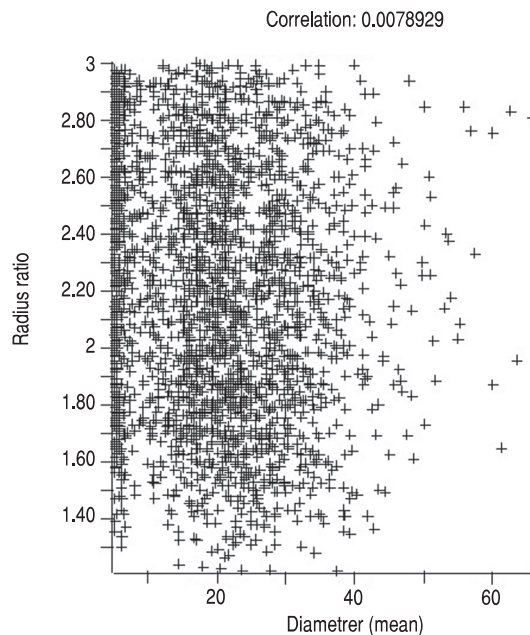


**Figure 1.** Classification of the striated muscle cells according to their mean diameter.

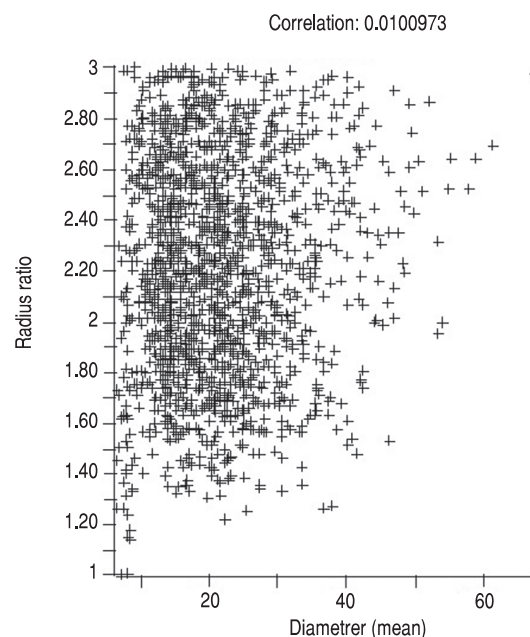
oriented cells. The circular layer was in all segments clearly visible, but in the older group, the volume of the muscle tissue that it was built from was, in the upper and middle part of the oesophagus, significantly smaller (up to 44%,  $p < 0.005$ ) than the volume of the muscle cells that constituted longitudinal layer (Table 1). In the lower oesophageal part this ratio decreased near to 1.19 times. This kind of difference was not observed in the group of younger donors where this ratio was up to 1.20 in the upper part to 1.10 in the lower part of the thoracic oesophagus. Results concerning the classification of the striated muscle cells according to their diameter were quite consistent and almost unrelated to the oesophageal region. In group one, the small cells were present in  $\sim 35 \pm 4\%$ , mid-sized cells comprised  $\sim 45 \pm 5\%$  and large ones around  $20 \pm 4\%$  (Fig. 1). In group two small cells comprised  $\sim 60 \pm 5\%$ , mid-sized  $\sim 30 \pm 4\%$  and large cells around  $10 \pm 3\%$  (Fig. 2). The scattergrams showed "left shift" of the measured cells in older group compared to the younger group and confirm the results mentioned above (Fig. 3). We found that there was significant difference between percentages of small-diameter cells in two groups, being higher in the older one.

**Quantification study of the myenteric plexus**

The elements of the myenteric plexus were situated in the connective tissue between the layers of the muscularis proper in a web-like manner. In our study, only neurons of the myenteric plexus were counted and the results of the number of these cells and surface area of the neural structure in both groups measured per area (recalculated to  $1 \text{ cm}^2$  of the oesophageal surface) are presented in Table 2. The results showed



**Figure 2.** Distribution of striated muscle cells according to their mean diameter in group one.



**Figure 3.** Distribution of striated muscle cells according to their mean diameter in group two.

significant ( $p < 0.005$ ) loss of the ganglion cells in group two. The average number of neurons in group one was  $61.58 \pm 12.85$  and in group two —  $47.62 \pm 10.42$ . Expressed in percentage loss was  $\sim 23\%$ . In the same time we were not able to detect significant changes of the surface area of the myenteric plexus

**Table 2.** Age-related changes of oesophageal myenteric plexus

Parameters	Age 21–45	Age 46–78
Surface area of the myenteric plexus [mm <sup>2</sup> /cm <sup>2</sup> ]	0.0106 ± 0.00142	0.0111 ± 0.00112
Number of ganglions [cells/cm <sup>2</sup> ]	61.58 ± 12.85	47.62 ± 10.42*
Number of ganglions per 1 mm <sup>3</sup> of plexus volume	8.6 ± 1.7	7.1 ± 1.6

\*p &lt; 0.005

compared to the younger group (Table 2). Serial sectioning of the oesophagus wall enabled 3-dimensional reconstruction of the myenteric plexus, as well as data related to the number of ganglionic cells per unit volume (1 mm<sup>3</sup>) of the plexus itself. We verified that in group one there were 8.6 ± 1.7 cells and in group two — 7.2 ± 1.6 cells per 1 mm<sup>3</sup> of myenteric plexus. Loss of ganglion cells verified with this method was ~19%. This, in our opinion more precise approach, confirmed similar loss of ganglionic cells with age.

## DISCUSSION

Human oesophagus is a muscular tube that transports food and liquids to the stomach. Although epithelial lining, glands and connective tissue elements are important for the proper functioning of the oesophagus, one can understand that muscular layer and myenteric plexus are the most responsible for the normal propulsion of the swallowed material [6, 26]. On the other side, aging, as a normal biological process with the deleterious effect on the function of numerous organs, in the gastrointestinal system can cause various disorders [7, 9–11]. Many reports on the oesophagus function and motility disorders, as well as clinical effects of aging on the oesophagus exist [8, 14, 19], but comparative and quantitative analysis studies concerning the effect of aging on cells in the muscularis proper are absent. Scientists frequently pointed out systemic diseases as a primary reason for age-related dysfunction of the oesophagus [22, 29] as well as changes of myenteric plexus related to aging [11, 19, 31]. Our study proved that muscular layer also undergoes changes related to aging. Precise measurement showed that thickness of the oesophageal wall, as well as thickness of the muscularis proper decrease with age. Our findings concerning the thickness of the oesophageal wall are, in part, in correlation to the results of Xia et al. [32]. They used computed tomography to measure the thickness of the oesophageal wall in dilatation and reported the values from 1.87 mm to 2.7 mm with cranial part being thicker than the thoracic one. We can agree on

the wall thickness, but in our study thoracic part was thicker than cranial one and the difference between 2 values was around 3%. Overall volume of the muscle tissue of the muscularis proper in the group of younger donors is larger than in the older group, but, again, the difference is not significant (less than 10%). This is in correlation with the findings of manometric studies that showed the lowering of the oesophageal peristaltic wave pressure with age [13, 25]. The level of peristaltic wave reduction in their study is similar to the loss of overall muscle tissue volume that we found in the older group. Along the oesophagus tube striated muscle cells are being replaced with the smooth ones. This transition is not simple abrupt replacement related to the muscle bundles, but also the addition of muscle tissue in order to increase the muscle volume as shown in our study. We verified that in the thoracic oesophagus overall muscle tissue volume is increasing along the oesophagus in both groups, but highest values in the lower oesophagus were observed in the younger group. Unfortunately, we found no data on human material to compare this finding. Serial sectioning performed in our investigation proved that smooth muscle cells are extended more cranial in the circular layer of muscularis proper, which is in correlation with the previously published results [20]. The examination of the average number of the striated muscle cells in all 3 segments of the oesophagus shows that in the younger group this value is slightly higher than in older one, but the difference is less than 5%. As expected, their number is decreasing throughout the length of the oesophagus, but crucial change is observed in the lower part, while its mid-portion still possesses significant number of the striated muscle cells. The examination of the orientation of the muscle cells and the volume of the circular and longitudinal layer of the muscularis proper in the oesophagus shows that in the older group circular layer is much more affected by the aging process than the longitudinal layer, especially in the upper and mid-portion. This observation was never published before and the reduction of the muscle volume was

up to 44%. From the reports on the general effect of aging on gastrointestinal system [1, 21] we expected equal effect of aging on all muscle structures of the oesophagus but, as shown in our study, it is not true. We cannot offer good explanation for this finding, but potential reasons could be the eating habits of the elderly (more liquid food) and diminished need for the longitudinal contraction of the oesophageal wall. From the results of classification of the muscle cells according to their mean diameter, it is obvious that cells with smaller diameter comprise majority (around 60%) of the muscle cells in the older group. On the other hand these cells are significantly less present (around 35%) in the oesophageal wall of the younger group. This difference can be observed from 2 scattergrams. Hence, aging affects oesophageal striated muscle cells and, as a result, has reduction of the mean cell diameter, while the overall number of cells, as well as the thickness of the muscle layer remains practically unchanged. In the available literature, again, we found no data to compare our results. However, we can conclude that during aging, the connective tissue is filling the space once occupied by muscle cells increasing the rigidity of the oesophagus, which is in correlation with the previously published results [14]. When myenteric plexus was examined in our study, some well-established facts were confirmed. Our results show the decrease of neuron number in the older group, but without the reduction of the surface area of plexus itself. This finding is verified in the reports of Philips et al. [24] and Wu et al. [31], but some authors reported the opposite results in the other parts of the gut [2, 5]. They argued that, with age, increased number and size of the ganglions occur. In order to resolve this disagreement we examined ganglionic cell number to myenteric plexus volume ratio and confirmed the very similar percentage of cell loss, without significant reduction of plexus itself during aging. In the previously published clinical reports [11, 19, 28, 29] authors suggested that motility disorders in the gastrointestinal system are related to the aging and they can be caused by some systemic diseases. We confirmed that aging has a significant deleterious effect on the neuronal cell population in the whole oesophagus, not only in the sphincters region. Unfortunately, the staining method that was used in our study prevented us from further characterising the myenteric neuron types and the effect of aging on ganglion classes, but it would be interesting to find out which classes on neurons are most affected by aging.

## CONCLUSIONS

To summarise, aging affects oesophageal muscularis proper. Major changes occur in striated muscle cell diameter, thickness of the circular muscle layer and number of the ganglionic cells. On the other hand, muscle cell number and overall volume and thickness of the oesophageal wall remain practically unchanged. The consequences of these changes are oesophageal dysfunction, increased rigidity and reduction of the peristaltic wave's amplitude.

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