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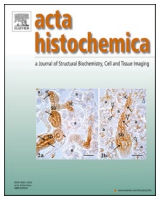

The vulnerability of the human *taenia coli* to alterations in total collagen within the colon of the elderly

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ABSTRACT

The structure of the colonic wall relies on collagen, distributed within the submucosa and the muscularis externa. A recent analysis of total collagen in human ascending colon (AC) suggests that the muscularis externa is more susceptible to age-related increases in collagen among the elderly. However, it is not clear if this change also occurs in the descending colon (DC) or if the circular and longitudinal muscle layers are similarly affected in either region of colon. The aim of this study is to determine the total collagen content in the DC and its distribution between the circular muscle (CM) and *taenia coli* (TC) of the AC and DC of adults and compare the same with tissue from the elderly. Masson's trichrome and Picrosirius red were used to assess total collagen content in the AC and DC; aged 22 – 91 years. Macroscopically normal AC from 22 patients (adults: 22–60 years; 6 male, 6 female; elderly: 70 – 91 years; 6 male, 4 female) and DC from 23 patients (adults: 23–63 years; 6 male, 7 female; elderly: 66 – 88 years; 6 male, 4 female) were obtained following surgery for non-obstructed bowel cancer. The total hydroxyproline content in DC samples was also evaluated. In the DC, tinctorial staining demonstrated an increased occurrence of total collagen fibres in the submucosa of the elderly (159.8 ± 9.6 in elderly vs. 126.9 ± 6.1 in the adults; p 0.05) and in the muscularis externa (respectively 37.4 ± 4.1 vs. 18.8 ± 2.4; p 0.01). In the adult AC and DC, there were no statistically significant differences in the amount of collagen within the CM and TC. In the elderly, the total collagen fibres within the TC was greater in the AC (mean grey intensity: 63.4 ± 3.9% in the elderly vs. 36.6 ± 1.6% in adults; p 0.05) and DC (mean grey intensity: respectively, 59.82 ± 2.4 vs. 40.2 ± 0.9%; p 0.05). In both AC and DC of the elderly samples, several thickened collagen fibrils were microscopically identified within the TC infiltrating to the myenteric plexus. In the TC of the elderly AC, the total collagen fibres were increased by approximately 4% compared to that of the DC. The total collagen concentration in the elderly DC assessed by hydroxyproline assay was increased by approximately 15% compared to the adult. Sex related differences were not found when data combined. We concluded that the total collagen content in the muscularis externa particularly of the TC of human colon increases with age. The subtle change in collagen distribution with age between AC and DC may differentially affect the tensile strength of the colon.

1. Introduction

Chronic constipation, faecal impaction and incontinence are common colonic disorders among the elderly (those over 65 years) (Gallagher and O'Mahony, 2009; Bharucha and Lacy, 2020). In the event that these disorders are poorly treated, the elderly may suffer chronic pain, reduced quality of life and an increased financial burden (Chang et al., 2010; Peery et al., 2019).

Collagen is the most abundant long-lived structural protein in humans and supports the colonic wall to withstand high intraluminal pressures formed during muscle movement (Egorov et al., 2002; Siri et al., 2019). Thomson et al. (1987) found that collagen fibrils in the submucosa of the left or descending colon become smaller and more tightly packed than those in the right or ascending colon with increasing age. Using histochemical and biochemical approaches, we recently reported increases in total collagen content and relative distribution in the

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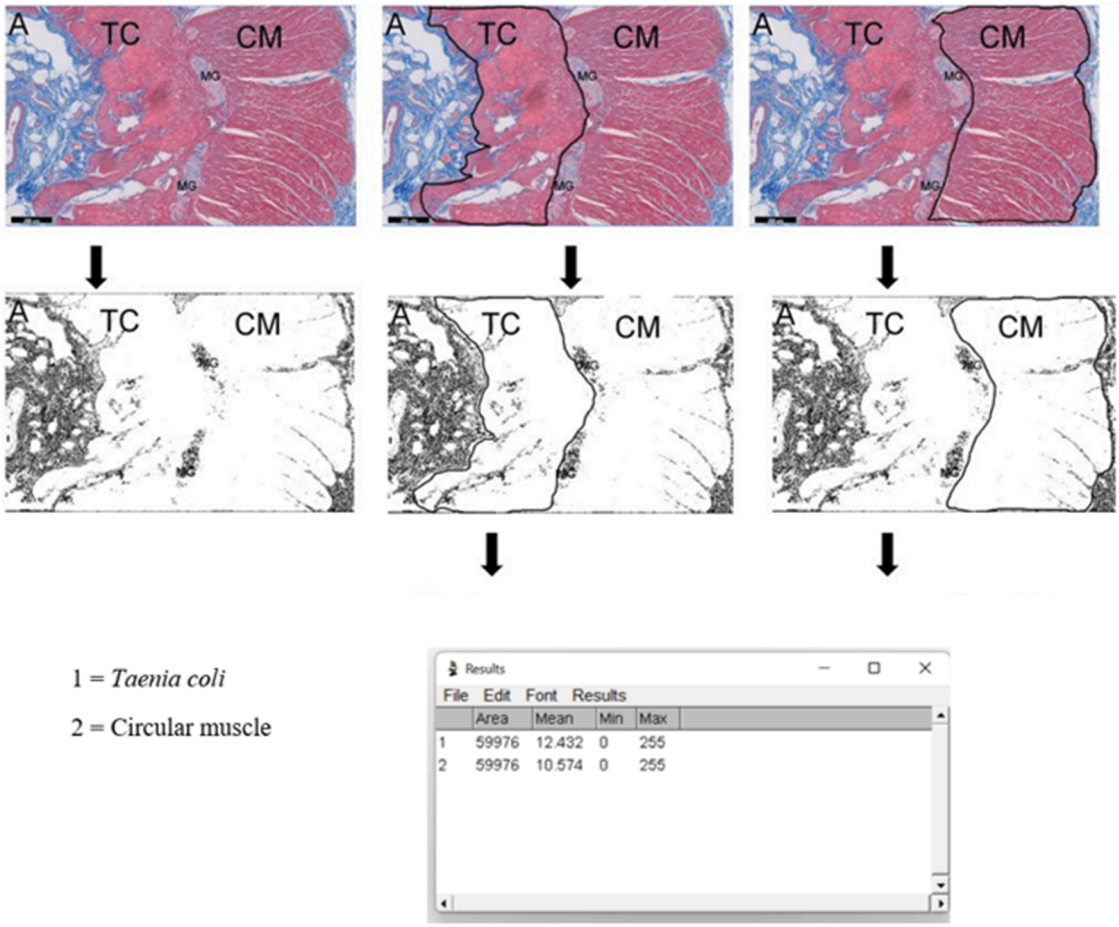


Fig. 1. Quantification of total collagen fibres distribution in the *Taenia coli* (TC) and the Circular muscle (CM) in human colon. Collagen fibres were demonstrated using Masson's trichrome staining. **(I)** The blue -stained collagen fibres were separated from the red background using manual threshold of Hue (135 – 195), Saturation (25 – 255) and Brightness (20–255) in ImageJ. **(ii)** Freehand tool was selected to circumscribe an area containing only the *Taenia coli* and Circular muscle without the Myenteric ganglia (MG) on the binarized images. **(iii)** Results for the total collagen content represented by the Mean of the positive pixel in *Taenia coli* (1) and Circular muscle (2) were automatically calculated using Image J software (Version 1.53f51). Scale bars represent 200 μm.

ascending human colon (AC) of the elderly (Baidoo et al., 2022a). In brief, the collagen content of the submucosa and muscularis externa was increased in the elderly (70 – 91 years) compared to younger adults.

It remains unclear if the collagen content of the human descending colon (DC) changes with increasing age and in both regions of colon, if the circular and longitudinal muscle layers are equally affected. These knowledge gaps are important because the circular muscle (CM) and the *taenia coli* (TC) perform distinct functions during movements of the colon (Smith and Robertson, 1998), and the AC and DC regions differ in their physiological primary functions (e.g., respectively, in the absorption of nutrients and water / temporary storage of luminal contents; See Phillips, 1984; Milla, 2009).

The purpose of this study was to assess collagen content in the submucosa and muscularis externa of the DC and analyse the proportion of collagen distribution between the CM and the TC in AC and DC from adult and elderly patients. Then, to understand the mechanism of collagen synthesis in the colonic samples, the amount of hydroxyproline content was assessed. Hydroxyproline is a major constituent of all types of collagen and plays a crucial role in the synthesis and provision of thermodynamic stability of the fibrillar composition (Gorres and Raines, 2010). Changes in the hydroxyproline metabolism have been associated with pathophysiology and pathogenesis of various diseases (Srivastava et al., 2016; Lamandé and Bateman, 2020). In the left colon of old (27 – month-old male) rats, an elevated hydroxyproline content and concentration resulted in a decrease in tensile strength compared to the younger group (Christensen et al., 1992). With increasing age, the hydroxyproline content in human AC increased by 16% (Baidoo et al., 2022a), it is however unclear if similar changes occur in the human DC.

2. Materials and methods

2.1. Subject selection

Macroscopically normal AC from 22 patients (adults: 22–60 years; 6 male, 6 female; elderly: 70 – 91 years; 6 male, 4 female) and DC from 23 patients (adults: 23–63 years; 6 male, 7 female; elderly: 66 – 88 year; 6 male, 4 female) were obtained following surgery for non-obstructed bowel cancer, after informed written consent. The sections of colon were obtained at least 5–10 cm away from the tumour and were prospectively collected. Patient records were examined for current medication and comorbidity (**Table 1.0**). None of the patients underwent surgery had any previous chemoradiotherapy or diagnosis of active inflammatory colonic disease such as diverticular known to affect collagen structure (Wess et al., 1995). This study was approved by the University of Roehampton (LSC 21/339) and by the East London (REC 10/H0703/71) Ethics Committees.

2.2. Histochemical and biochemical techniques

Full thickness human colonic tissues were fixed, processed, embedded transversally and serially sectioned as previously described (Baidoo et al., 2022a). In brief, human ascending and descending colon

(~ 10 ×10 mm) were routinely fixed in 10% neutral buffered formalin and processed in xylene before embedding transversely in paraffin wax (to demonstrate mucosal, submucosal, muscularis externa and serosal layers). Carefully consistent serial-sections at 4-μm-thickness on the formalin-fixed, paraffin-embedded samples were generated using a rotary microtome (Leica Biosystems, Buffalo Grove, United States) and mounted on superfrost-plus glass slides. A minimum of fifty serial sections (into a depth of 200 μm) per sample were cut. The first section of each sample was used for haematoxylin and eosin (H&E) staining. For each histochemical staining, a minimum of eight sections at 16 μm separation per patient were used for total collagen content analysis in human AC and DC. Before staining was performed, slides were anonymised to conceal the age and sex of the patients. The sections were deparaffinised, rehydrated and stained for routine H&E, Masson's trichrome (MT) and Picrosirius red (PSR). None of the samples used had any active inflammation, tumour, and structural abnormalities (Feakins and British Society of Gastroenterology, 2013) as assessed with the haematoxylin and eosin staining. For MT staining, sections were loaded on ArtisanLink auto Stainer (Sakura, Tokyo-Japan) and performed according to the manufacturer's instruction. Picrosirus red staining was manually done as described (Junqueira et al., 1979). Briefly, sections were stained in PSR solution (0.1% of Sirius red in saturated aqueous picric acid) for 1 hr. The sections were washed in two changes of acidified water (0.5% acetic acid) for 2 min each, air dried and dehydrated in three changes of 100% ethanol. Stained sections were then cleared in histoclear and mounted with Pertex and coverslipped with glass slide (Sakura, Tokyo-Japan). Results for MT staining yielded; cytoplasm, muscle and erythrocytes stained red and collagen fibres appeared blue. In bright-field microscopy for PSR staining, collagen fibres appeared red on a pale-yellow background.

For analysis of total hydroxyproline content in DC, 35 samples comprising of adults (23–63 years; 6 male, 11 female) and elderly (66 – 88 year; 8 male, 10 female) were used. Analysis of collagen concentration in the colonic samples was performed as previously described (Baidoo et al., 2022a). Hydroxyproline concentration in formalin-fixed paraffin-embedded DC was quantified using a colorimetric assay kit (*QuickZyme* Biosciences, Netherlands). In brief, ten 10 μm sections were transferred to Sarstedt tubes. Next, 150 μl of 6 M HCl was added and the samples hydrolysed for 20 hr at 95°C in a thermoblock. Hydroxyproline standard solutions (6.25 – 300 μgmL^{-1}) and hydrolysated samples were also prepared according to manufacturer's protocol. Both the standard concentration and DC samples were assayed in duplicate and absorbance at 570 nm read on a multiskan ex microplate reader (Thermo Scientific, Singapore) and results averaged. The unknown concentrations of total hydroxyproline in DC hydrolysates were deduced per volume of HCl used, based on the standard calibration curve.

2.3. Analysis of collagen fibres in the muscularis externa

Sequential image acquisition under identical conditions was captured using brightfield microscope equipped with digital camera and collagen fibres were quantified with ImageJ processing (Version 1.53f51; Schneider et al., 2012). Quantitative estimation of collagen fibres within the submucosa and entire muscularis externa for DC was then performed as previously reported (Baidoo et al., 2022a). For the total collagen content within the TC, a tracing tool manually circumscribed an area around the edges of the longitudinal smooth muscle towards the serosa layer and around the myenteric plexus and this was quantified. A similar method was used to obtain an area of only CM (an area around the edges of the smooth muscle towards the submucosa and the myenteric plexus). Collagen in the mucosa and serosa sublayers were not included in this study. Respectively, blue and red colourations indicative of collagen fibres for MT and PSR were separated from background stains in all samples by manual thresholding of Hue (135 – 195; 0–10), Saturation (25 – 255; 20–255) and Brightness (20 – 255; 15–255) in ImageJ. The threshold images were binarized and the proportion of positive pixels within the CM or TC region was determined as the total collagen content (Fig. 1). To estimate the amount of collagen content and relative distribution between the *taenia coli* and the circular muscle, we first calculated the total collagen content in the entire muscularis externa. Percentage differences in total collagen content between the CM and TC was calculated as a percentage of positive pixels value of (either CM or TC) / ∑ positive pixels value of the entire muscularis externa. To avoid normalization of values, the same area size of CM and TC were measured for all samples. Sections with artifacts and irregular staining patterns were not included. Due to variability of the size of CM and TC for AC and DC and between cohorts, all data were considered and collagen content expressed as a mean (± Standard error of mean).

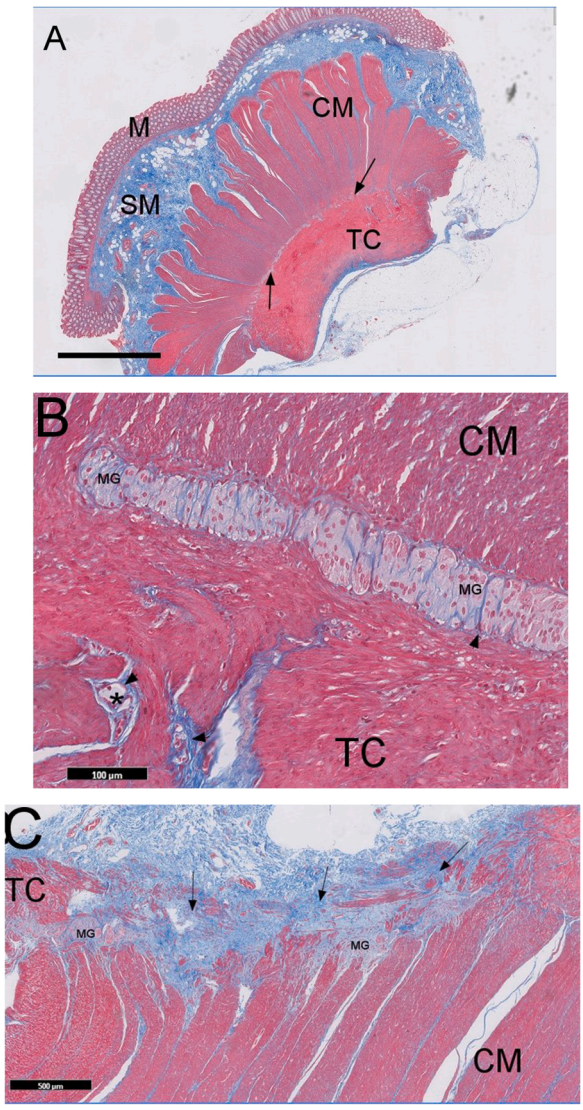


Fig. 2. Representative photomicrograph of human colon stained with Masson's trichrome showing the distribution pattern of collagen fibres in (A) Full thickness adult descending colon; scale bar represent 2 mm; Arrows indicate the myenteric plexus. (B) At higher magnification, collagen bundles (arrowhead) ensheathing the myenteric ganglion and also surrounding a capsule within the taenia coli (*) of adult descending sample; scale bar represent 100 μm.(C) Evidence of *taenia coli* degeneration replaced with bundles of collagen fibres in the elderly descending colon; scale bar represents 500 μm. Mucosa (M), Submucosa (SM), Circular muscle (CM), TC (*Taenia coli*) and Myenteric ganglion (MG).

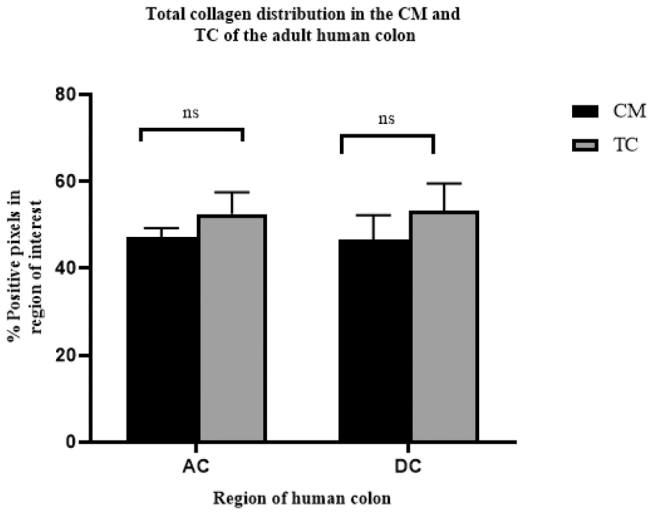


Fig. 3. Total collagen distribution in the circular muscle (CM) and Taenia coli (TC) of adult ascending colon (AC) and descending colon (DC). Human adult (male and female) colon from AC (22–60 years; 6 male, 6 female) and DC (23–63 years; 6 male, 7 female) were stained with Masson's trichrome stain. Blue stained collagen fibres were separated from the background by manual threshold of Hue (135 − 195), Saturation (25 − 255) and Brightness (20−255) and binarized using ImageJ (Version 1.53f51) image processing. Tracing tool was used to circumscribe an area containing only the CM and positive pixel values automatically obtained and expressed in percentages in relationship to the total positive pixel of the entire muscularis externa. Similar approach was used to quantify total collagen for TC. Statistical analysis was performed using two-tailed independent student's t-test and data were represented as mean ± SEM; ns: non significance.

2.4. Statistical analysis

Power analysis was performed prior to sample collection based on a pilot investigation on previously analysed colonic samples for total collagen, determining the minimum number of subjects needed to detect any age-related alterations within the sublayers and between the two regions. This analysis confirmed that power of 95% requires, respectively n = 9 and n = 13 per group for histochemical and biochemical evaluations. Differences in collagen content between adult and the elderly and between AC and DC were compared with a two-tailed independent student's t-test using the Statistical Package for Social Science (IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY) software. GraphPad prism software (Avenida de la Playa La Jolla, USA) was used to plot graphs. $P \leq 0.05$ were chosen for rejection of the null hypothesis. Unless otherwise specified, ***n*** represent the number of patients. All data are expressed as mean ± SEM with (95% confidence interval).

3. Results

3.1. Histochemical quantification of total collagen content in muscularis externa of adult human

In the assessment of haematoxylin and eosin-stained slides, none of the samples from both adult and elderly group had any active inflammation or pathologies. Adult colonic samples revealed well-defined MT blue-stained (compared with background; Fig. 2A) and PSR red-stained collagen fibres in the CM and TC. At a higher magnification, collagen fibres were noted to ensheathe the myenteric ganglion. In both the muscle layer of the adult sections, closely packed, blue-stained collagen fibrils were noted and in some sections, occasional sighting of collagen bundles surrounding a capsule in the TC were also present (Fig. 2B). When total collagen content in the CM and TC of the adult samples were compared, there were no statistically significant differences in either the

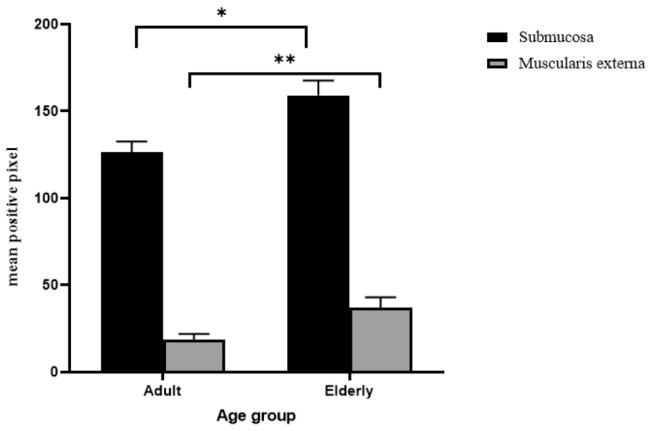


Fig. 4. The distribution of total collagen fibres in the submucosa and overall muscularis externa of human descending colon (DC) in adult and elderly populations. Full thickness adult and elderly DC (adults: 23–63 years; 6 male, 7 female; elderly: 66 – 88 year; 6 male, 4 female) were stained with Masson's trichrome stain. Blue stained collagen fibres were separated from the background by manual threshold of Hue (135 − 195), Saturation (25 − 255) and Brightness (20−255) and binarized using ImageJ (Version 1.53f51) image processing. Tracing tool was used to circumscribe an area containing the submucosa and the overall muscularis externa (both the circular and longitudinal muscle layer) and positive pixel values automatically obtained. All data were obtained from a mean of each ROI. Total collagen content between adult and the elderly per each ROI were compared by a two-tailed independent student's t-test. Statistical significance is: * $P < 0.05$; * * $P < 0.01$.

AC (22–60 years; 6 male, 6 female) or DC (23–63 years; 6 male, 7 female) (Fig. 3.) We did not detect any statistically significant differences in the measurement of total collagen content between MT and PSR methods ($Z = -1.791$, $P = 0.066$). Hereafter, data from MT staining were used for the analysis of total collagen content in the investigative sublayers of human colon.

3.2. Total collagen content in the submucosa and muscularis externa of descending colon of the elderly

In both the adult and elderly human descending colon, histological sections revealed blue stained fibres occupying most of the submucosa. Evidence of excess collagen fibres in the *taenia coli* of the elderly descending colon (Fig. 2C), were visually noted in 4 out of 10 samples. When the blue-stained collagen fibres in the human descending colon were separated from the red background and measured, the total collagen content in the submucosa and the overall *muscularis externa* was greater in the elderly samples compared to the adult (Fig. 4).

3.3. Total collagen content in the CM and TC of elderly human colon

To investigate the distribution of total collagen fibres within the CM and TC of the elderly, human AC from 22 patients (adults: 22–60 years; 6 male, 6 female; elderly: 70 – 91 years; 6 male, 4 female) and DC from 23 patients (adults: 23–63 years; 6 male, 7 female; elderly: 66 – 88 year; 6 male, 4 female) were stained with MT and PSR. Microscopically, the muscularis externa of the elderly sections, in contrast to the adult, greater occurrence of collagenous fibres was visibly noted for both staining types (Fig. 5 A - D). Tightly packed collagen fibres were seen in the TC as well as in the region occupying the myenteric plexus. In some samples from the elderly (about 45–50%), consistent bundles of striking thickened collagen fibres deposition were seen within the outer areas of TC towards the serosal layer. These thickened bundles appear to displace the TC occurring in both regions of the elderly but were more pronounced in the AC samples compared to the DC. MT-stained slides were used for the assessment of total collagen distribution in the CM and TC for both regions of the colon. Greater collagen content existed in the TC

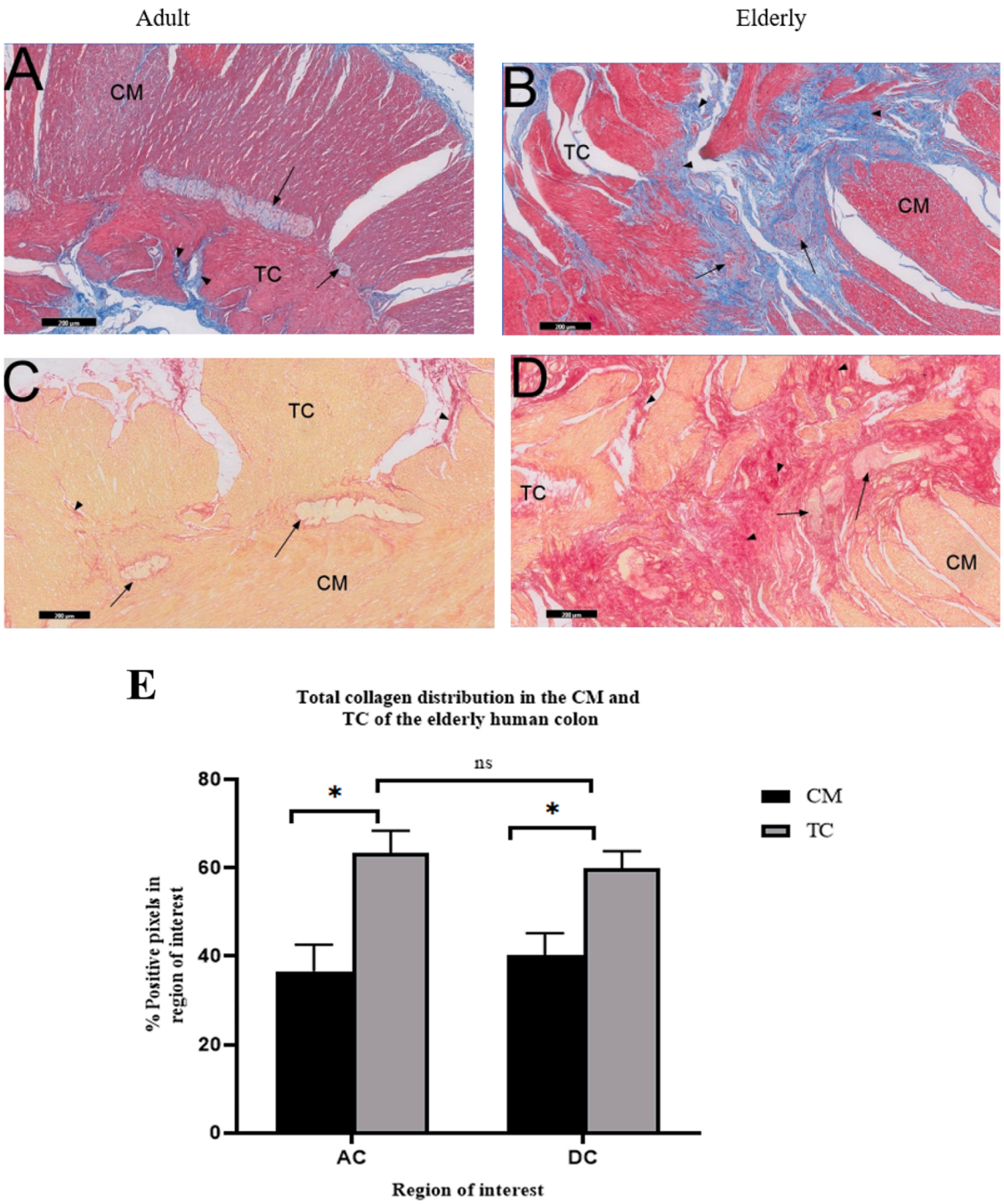


Fig. 5. Influence of ageing on total collagen distribution in the circular muscle (CM) and *Taenia coli* (TC) of human ascending colon (AC) and descending colon (DC). Representative photomicrograph of muscularis externa of the adult (< 65 years) and elderly (≥ 65 years) colonic tissues were stained with MT (**A** and **B**) and PSR (**C** and **D**) staining. Respectively, blue and red colourations indicate collagen fibres for MT and PSR (arrowhead). Collagen fibres were seen surrounding the myenteric ganglion (arrow) located between the CM and TC. In (**E**), Collagen content was quantified as the mean positive pixel value within the CM and TC of the elderly AC and DC samples with ImageJ (Version 1.53f51) processing. Statistical analysis showed that increasing age results in greater occurrence of collagen fibres in the TC than the CM in the elderly of both regions. However, when the TC of the elderly AC was compared to the same region of the elderly DC, there was no changes in the amount of collagen fibres distribution (Scale bar 200 μm, * $P \leq 0.05$; ns: non-significant).

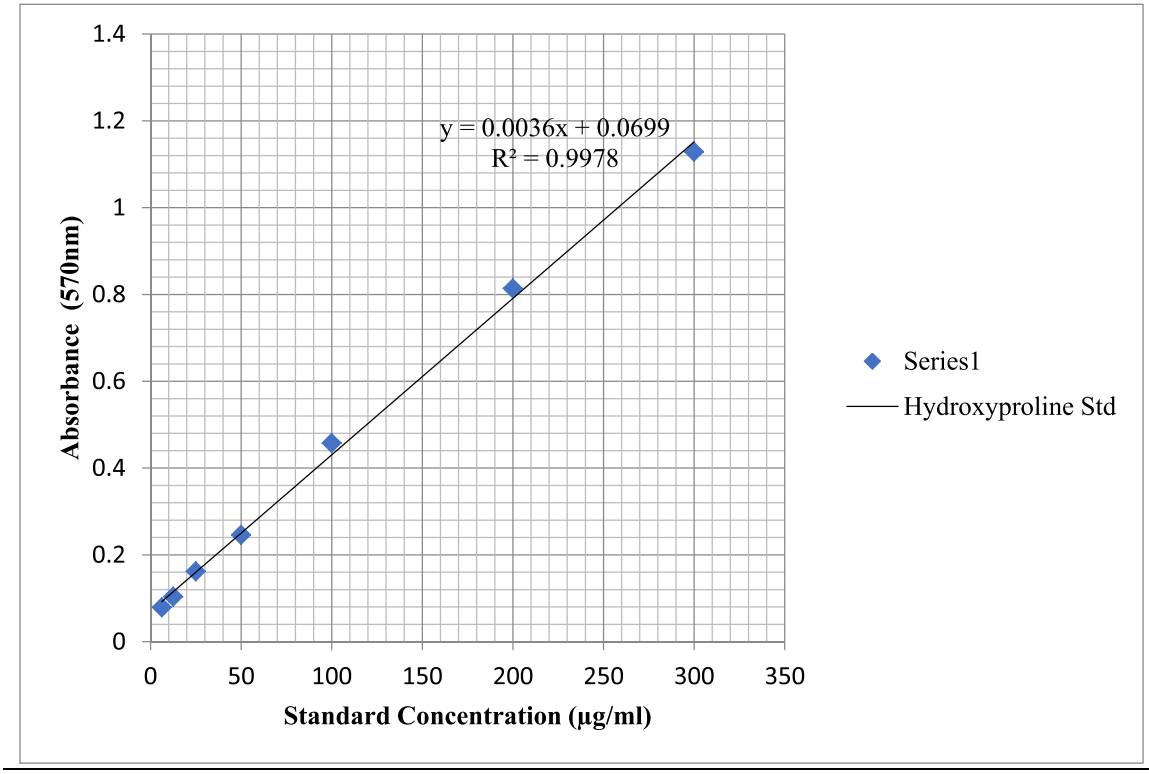
of the elderly (male and female) samples in both regions compared with the CM. However, when the total collagen content in the CM of the elderly AC (male and female) was compared with the same group of the DC, there was no significance difference in the distribution of total collagen fibres between the two regions. Similar changes were found when the collagen content in the TC were compared between the elderly samples in both regions (Fig. 5). However, a small increase of 4% collagen content was detected in the elderly AC sample compared to the same group of DC.

3.4. Hydroxyproline content in human descending colon

The result for the hydroxyproline standard curve used to determine concentration of hydroxyproline in the hydrolysate descending samples is presented in **supplementary sheet 1.0**.

supplementary sheet 1.0.

Hydroxyproline standard (μg/ml)	Average absorbance reading (570 nm)
300	1.1285
200	0.8135
100	0.4575
50	0.246
25	0.162
12.5	0.1035
6.25	0.079
0	0.0011



Shows a graph of standard curve of mean absorbance (A_{570} – blank) against standard known collagen concentration. The standard curve was used to convert the A_{570} values of the test samples to converted-hydroxyproline concentration of collagen in the hydrolysed human descending colonic.

$$Y = 0{\cdot}0036X + 0.0699$$

$$R^2 = 0.9978$$

$$X = \frac{Y - 0.0699}{0.0036}$$

R = Correlation Coefficient.
X = Hydroxyproline concentration in unknown samples.
Y = Absorbance (570 nm).

The total hydroxyproline content was higher in DC from the elderly (66 – 88 year; 8 male, 10 female) compared to the adult (23–63 years; 6 male, 11 female) group (respectively 48.91 ± 1.5 vs. 36.5 ± 1.3 μg/ml; $P < 0.05$), indicating an increased in collagenous protein by approximately 15%.

3.5. *Sex-related differences in the distribution of collagen content in colonic sublayers*

The specimen numbers were too small to detect a robust significance of any sex-related differences in the distribution of total collagen within the investigating sublayers and between regions. However, when all data were considered and compared, we did not detect any change in the amount of total collagen content distributed between the elderly male and female in both the CM and TC of the AC (70 – 91 year; 6 male, 4 female; CM: respectively 14.6 ± 0.6 vs. 15.2 ± 1.3; TC: 25.4 ± 1.5 vs 29.8 ± 1.8; $P > 0.05$) and DC (66 – 88 year; 6 male, 4 female; CM: 16.9 ± 0.7 vs. 19.7 ± 1.5; TC: 25.9 ± 1.8 vs 29.3 ± 0.3; $P > 0.05$).

4. Discussion

We have previously demonstrated an increased collagen content within the muscularis externa and the submucosa of AC from the elderly (Baidoo et al., 2022a). The present study extends these findings for the DC and in addition, for the first time, show that the increase was greater in the TC, compared with the CM.

Morphologically, collagen in the submucosa of the DC appeared tightly packed with fine and some loose fibrils network. These observations may account for a lower grey intensity value recorded in the elderly DC, representing the amount of collagen content in the tissue when data were compared to what has been previously reported for AC (Baidoo et al., 2022a). In part, the data are consistent with Thomson et al. (1987), who found that collagen fibrils in the descending colon become smaller and more tightly packed than those in the ascending colon with increasing age.

The present study found no differences in the amount of collagen fibres within CM and TC for adult samples in both AC and DC. However, an age-related increase in total collagen distribution occurred in the TC of AC and DC, but not in the CM. There were no clear sex related differences in the amount of collagen distribution in the CM and TC for

both AC and DC, although a greater number of samples are needed before a definitive conclusion can be reached.

In this study a small increase (4%) in collagen content was detected in the TC of the elderly AC compared to elderly DC. Whether this subtle increase selectively affects function, merits further investigation. Interestingly, this finding is consistent with what has been reported in the TC of 2-year-old guinea pigs compared with younger animals (Gabella, 2001). For the human colon, a striking observation of blue-stained fibres dominance within the TC of the elderly AC samples compared to that of the DC, could account for the apparent difference. In further analysis, ageing of the human DC reported elevated hydroxyproline concentration (by 15%), an observation also reported in human AC (Baidoo et al., 2022a) and in old rats (27-months-old male), accompanied by a maximum load decrease compared to young rats (Christensen et al., 1992). Total collagen in the mucosa and the serosa were not studied in the present investigation as they do not provide any biomechanical integrity to the colonic wall (Egorov et al., 2002; Siri et al., 2019).

Although not investigated in this study, we speculated that higher collagen content in the colon of the elderly may be an indicative of a progressive accumulation of advanced glycation end-products, reported to reduce tissue viscoelasticity by limiting fibre-fibre and fibril-fibril sliding (Gautieri et al., 2017). It would seem plausible that high degree of cross-linkage in the elderly samples will have far-reaching consequences in disrupting many different aspects of homeostasis within the submucosa and the muscularis externa.

This study reported a higher collagen content with age in the regions that coincide with the location of the submucosal and myenteric plexus, neural plexuses that, respectively, largely control the secretory and motility functions of the intestine. There has also been a report of age-related collagen modifications involving carbamylation and fragmentation contributing to stiffness within tissue (Birch, 2018), indicating that functional roles of the enteric and extrinsic nervous system could be impacted as a result. In this regard, the overall tensile strength and biomechanics of the AC would be more affected compared to DC, thus likely to explain the observed significant stool retention in human AC compared to DC during ageing (Gau et al., 2022). Regardless, higher collagen content in incontinent patients (mean age, 51.5 years), have been implicated in the inability of the anal canal and sphincters to maintain pressure and achieve maximum squeeze pressure (Speakman et al., 1995).

There are limitations in this present study. First, the samples were obtained from patients undergoing cancer resection and tissues were removed 5 – 10 cm way from the tumour, considered as 'macroscopical normal'. This procedure has often been employed in studying the functions of the human gastrointestinal tract (Sanger et al., 2013), as it is impossible to obtain sections of colon from healthy patients for research. All samples used in this study were initially assessed for tumour or active inflammation with haematoxylin and eosin staining prior to collagen analysis. None had other co-morbidities affecting the bowel, therefore observed changes reported herein argue for an effect of ageing. Second, it is to be acknowledged that there are over 28 collagen types (Ricard-Blum, 2011), however, our methods account for the absolute collagen rather than the currently known subtypes of which may be singularly affected with age in different ways. Third, second-harmonic generation microscopy has emerged as a powerful technique for imaging collagen fibres more robustly than the conventional methods (Mao et al., 2016), but this is expensive and not routinely used in diagnostic settings. Regardless, our work analysed up to at least 200 μm deep into the colonic tissue which is an appreciable thickness to produce a significant outcome. Additionally, the sample size for age-related sex differences in collagen content distribution was small, making it difficult to draw a firm conclusion; a larger sample size is required. Nevertheless, the present study has provided detailed analysis on the distribution of total collagen content in the sublayers of human AC and DC using systematic and reproducible methods. Finally, the current study used tissues from women of menopausal age, an inevitable factor in ageing studies. A decline in oestrogen level has been associated with a changed content and quality of collagen within the skin of menopausal women (see Stevenson and Thornton, 2007; Rzepecki et al., 2019). If similar changes occur in human colon, this might be expected to increase the variability of data obtained within and between our 'adult' male and female population. In the present study the amounts of collagen – and their variability - within 'adult' males and females were similar. However, larger, more complex temporal studies, ideally using non-invasive measures of collagen, are needed to address the possibility of menopause-related changes within the human colon. Accordingly, our experiments extend previous studies on the distribution of total collagen fibres within the muscularis externa in human ascending colon and highlight a novel greater collagen content pertaining to the *taenia coli* in the elderly.

It is possible that the structural changes reported in this study have an effect on the tensile strength of the colon but not necessarily a direct influence on motility (e.g., the maximum motility ability of circular muscle from the AC and DC were similar in both adult and elderly populations; Broad et al., 2019). Although evidence of decline in density of S100 immunoreactive EGCs within the myenteric ganglion and circular muscle layer per unit area of ageing human descending colon has recently been reported (Baidoo et al., 2022b); the new findings warrant a parallel physiological studies involving the structural interplay between colonic collagen and the enteric (neurons and enteric glia) and extrinsic nervous system merit further investigation. Additionally, compared to circular muscle, the TC appears more vulnerable to an age-related collagen increase, prompting a need to look for changes in functions of the TC among the elderly.

In conclusion, by assessing the collagen distribution in individual sublayers, the present study indicates that the TC of the human colon particularly the AC maybe more vulnerable to age-related increase in collagen deposition which in turn could influence the mechanical function of the *muscularis externa*, contributing to functional disturbances commonly seen in the elderly.

Ethics approval

Approved by the University of Roehampton ethics committee (LSC 21/339) and the East London ethics committee (REC 10/H0703/71).

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Contributors

NB critically reviewed, designed and conducted the experiments, analysed the data. and cowrote the manuscript with GJS, AB co-designed the experiments, analysed the data and supervised the overall project, GJS facilitated the identification, collection and governance. of human tissue collection for this study, all authors participated in its construction and refinement.

Patient consent

All patients provided written informed consent for the donation of tissue (REC 10/H0703/71; East London ethics committee).

Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Data Availability

Data will be made available on request.

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Competing interests

Not applicable.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.acthis.2022.151958.

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