Age-related alterations in the strength and collagen content of left colon in rats

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Abstract. The biomechanical properties and intestinal wall composition of left colon were studied in 4-month-old, 14-month-old, and 27-month-old male rats. The hydroxyproline content and hydroxyproline concentration in old rats were increased by 36% and 26%, respectively, compared with young rats and by 20% and 17%, respectively, compared with middle-aged rats. In middle-aged rats the maximum load increased by 21%, compared with young rats. In old rats, however, the maximum load decreased by 13%, compared with middle-aged rats. Histological examination showed that the mean crypt height was 9% higher in middle-aged rats and 12% higher in old rats than in young rats. In conclusion, an accumulation of collagenous proteins was found in old rats compared with middle-aged rats and this was accompanied by a decrease in the strength, which may deteriorate the functional integrity of the left colonic wall with age.

Introduction

Increased incidence of diverticular disease with age is related to a decline in the mechanical integrity of the human colonic wall [1, 2], and preoperative observations, radiographic findings and autopsy studies have shown that these changes are found especially in the distal colon probably because of structural changes as a normal concomitant of the ageing process [3].

It is widely accepted that the major load carrying elements of the colon for resisting increases of the intraluminal pressure are the collagens. Most studies on the effect of increasing age on intestinal growth have focused on the mucosa, and decrease in the proliferation rate of intestinal epithelium with age have been demonstrated [4, 5]. In relation to strength the submucosa, however, is the strongest part of the colonic wall and rich with collagens and is responsible for the suture-binding capacity in colorectal surgery [6, 7]. The submucosal layer in humans consists of a collagenous network of fibrils [8] which becomes smaller and more tightly packed in the left colon than those in the right colon with increasing age [9].

Although rats have often been used in ageing research and diverticular have been described in rats [10], the tensile strength of its colon in relation to age has not been described. The aim of the present study was to investigate the influence of increasing age on the biomechanical properties and collagen content of left colon in rats. Moreover, we wanted to study whether age-related alterations in the biomechanical properties of left colon in rats are associated with changes in the composition of the colonic wall.

Materials and methods

Young male rats (4 months old) weighing 470–546 g, middle-aged male rats (14 months old) weighing 524–720 g, and old male rats (27 months old) weighing 464–716 g, all of the same strain (Wistar; Iffa-Credo, Paris, France) were used. The rats were kept at a constant room temperature and humidity and with regulated 12-hour periods of light and darkness and were allowed ad libitum intake of
standard rat pellets (Altromin diet 1324; Chr. Petersen Ltd., Ringsted, Denmark) and water.

On the day of death the rats were anesthetized by an intraperitoneal injection of pentobarbital (50 mg·kg⁻¹ of body weight). After 10 minutes an abdominal midline incision was performed and the rats were killed by exsanguination. The colon was rapidly excised and the internal diameter of the left colon was determined by a caliper. It was then opened along its mesenteric border, gently rinsed of its luminal contents, blotted on moist filter paper, and weighed.

Biomechanical strength. Three 4-mm wide standardized strip specimens were cut circumferentially from each colon proximally to the peritoneal reflection by a multibladed cutting instrument with parallel razor blades. The specimens were mounted between two horizontal clamps in a materials testing machine (Alwetron TCT 5), Lorenzen & Wettre AB, Stockholm, Sweden) with the mucosal surface oriented upwards. The distance between the clamps was 4.0 mm. The specimens were kept moistened by Ringer's solution during the stretching procedure. Each specimen was stretched at a constant deformation rate of 20 mm·min⁻¹ until breaking. The load and deformation values were recorded continuously on a X-Y recorder and the load-deformation curves were read into a computer by a digitizer and transformed to load-strain curves, recording the load value for each strain increment of 1% (Fig. 1). The breaking strength was defined as the load required to break the specimens (maximum load). The strain at maximum load, extensibility, was the deformation at the maximum load divided by the original specimen length. The original specimen length was defined as the sum of the distance between the clamps and the deformation that occurred until a preselected load value of 0.05 Newton was obtained. The energy absorbed during the stretching of a specimen was calculated as the integrated area between the curve and the strain axis from the starting point to the breaking point (relative failure energy). The maximum stiffness of the tissue was measured as the maximum slope of the load-strain curve (\(\tan \beta\)).

Determination of dry weight (DW), defatted dry weight (DDW), fat concentration, and hydroxyproline content. Colonic samples of 10 mm, collected 12 mm proximally to the peritoneal reflection, were freeze dried, weighed (DW) and defatted in acetone for 72 hours with one change of the acetone after 48 hours, freeze dried and weighed again (DDW). The fat concentration was determined as the difference between the dry weight and the defatted dry weight and expressed in percentage of the dry weight. The hydroxyproline content, as a measurement of collagens, was then determined after acid hydrolysis, according to Woessner [12]. The hydroxyproline concentration was expressed in percentage of the DDW.

Histology. Segments of 10 mm for light microscopy were collected 22 mm proximally to the peritoneal reflection and immediately fixed in a 4% formaldehyde solution buffered by a 0.15 M phosphate buffer (pH = 7.4) for 24 hours. The segments were then embedded in paraffin capsules and three 5 μm thick sections from each colon segment were cut at right angle to the longitudinal axis of the colon and stained with hematoxylin and eosin. The microscopic images (Olympus BH-2, Tokyo, Japan) were projected on a table with a mirror at a magnification of × 60 and histological measurements were performed by means of a micrometer. The lengths of 9 crypts were measured from each specimen. A whole crypt was defined as a crypt in which the entire length of the crypt, from the muscularis mucosa to the mucosal surface, could be visualised. The measurements were made in a blinded fashion.

Statistical methods. Data were expressed as mean values ± SEM for the groups. Differences between groups were analyzed by the Kruskal-Wallis test, followed by the Mann-Whitney two-sample test. Probability values < 0.05 were considered statistically significant.

Results

Body weight. The body weights of the middle-aged and old rats were higher than the body weight of the young rats (Table 1).

Colonic diameter, wet weight, dry weight (DW), defatted dry weight (DDW) and fat concentration (Table 1). There was no difference in the diameter of left colon among the groups. The wet weight of the entire colon in the old rats (6538 ± 410 mg; mean ± SEM) was 38% higher than in the young rats (4726 ± 183 mg; p < 0.005) and 27% higher than in the middle-aged rats (5137 ± 120 mg; p < 0.005).

The DW of left colon in the old rats was 30% higher than in the young rats (p < 0.05). There was no differences between the groups after defatting of the tissue (DDW). The fat concentration of the left colon in young rats was 8.9 ± 4.6% of the dry weight, in middle-aged rats 10.7 ± 2.2.6%, and in old rats 20.9 ± 6.6% of the dry

Table 1. Body weight, diameter and weight parameters of left colon in young, middle-aged and old rats. Values are mean ± SEM

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>4</th>
<th>14</th>
<th>27</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of rats</td>
<td>15</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>515 ± 8</td>
<td>597 ± 14 b</td>
<td>608 ± 21 b</td>
</tr>
<tr>
<td>Colonic diameter (mm)</td>
<td>5.1 ± 0.2</td>
<td>5.1 ± 0.2</td>
<td>4.7 ± 0.3</td>
</tr>
<tr>
<td>Dry weight (DW; mg·cm⁻¹)</td>
<td>20.9 ± 1.2</td>
<td>21.9 ± 0.8</td>
<td>27.3 ± 2.5 a</td>
</tr>
<tr>
<td>Defatted dry weight (DDW; mg·cm⁻¹)</td>
<td>19.1 ± 1.0</td>
<td>19.4 ± 0.7</td>
<td>20.2 ± 1.3</td>
</tr>
<tr>
<td>Fat concentration (% of DDW)</td>
<td>8.9 ± 1.0</td>
<td>10.7 ± 2.6</td>
<td>20.9 ± 6.6</td>
</tr>
</tbody>
</table>

\( a p < 0.05, b p < 0.005, \text{ vs 4-month-old} \)