Journal of Biomechanics 91 (2019) 102–108

Contents lists available at ScienceDirect

Journal of Biomechanics

journal homepage: www.elsevier.com/locate/jbiomech
www.JBiomech.com

Mechanical effects of load speed on the human colon

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ARTICLE INFO

Article history:
Accepted 11 May 2019

Keywords:
Colonic mechanical response
Human colon
Biomechanics
Uniaxial solicitation

ABSTRACT

The aim of this study was to examine the mechanical behavior of the colon using tensile tests under different loading speeds.

Specimens were taken from different locations of the colonic frame from refrigerated cadavers. The specimens were submitted to uniaxial tensile tests after preconditioning using a dynamic load (1 m/s), intermediate load (10 cm/s), and quasi-static load (1 cm/s).

A total of 336 specimens taken from 28 colons were tested. The stress-strain analysis for longitudinal specimens indicated a Young's modulus of 3.17 ± 2.05 MPa under dynamic loading (1 m/s), 1.74 ± 1.15 MPa under intermediate loading (10 cm/s), and 1.76 ± 1.21 MPa under quasi-static loading (1 cm/s) with $p < 0.001$. For the circumferential specimen, the stress-strain curves indicated a Young's modulus of 3.15 ± 1.73 MPa under dynamic loading (1 m/s), 2.14 ± 1.3 MPa under intermediate loading (10 cm/s), and 0.63 ± 1.25 MPa under quasi-static loading (1 cm/s) with $p < 0.001$. The curves reveal two types of behaviors of the colon: fast break behavior at high speed traction (1 m/s) and a lower break behavior for lower speeds (10 cm/s and 1 cm/s). The circumferential orientation required greater levels of stress and strain to obtain lesions than the longitudinal orientation. The presence of *taeniae coli* changed the mechanical response during low-speed loading.

Colonic mechanical behavior varies with loading speeds with two different types of mechanical behavior: more fragile behavior under dynamic load and more elastic behavior for quasi-static load.

1. Introduction

Knowledge about the mechanical properties of the digestive tract is essential, and understanding of physiological phenomena can be obtained through quasi-static tests (Egorov et al., 2002). Traumatic phenomena can be investigated with dynamic tests (Crandall et al., 2011). Quasi-static tests can also be used to install materials that allow digestive sutures or surgical simulation. Realistic modeling of soft tissue biomechanics and mechanical interactions between tissues has been shown to be very suitable for simulating soft tissue biomechanics and has been successfully used in a number of image-guidance systems (Johnsen et al., 2015).

The colon is an anisotropic viscoelastic material, and its experimental biomechanical characterization has been limited compared to other abdominal organs (Higa et al., 2007; Carter et al., 2001; Egorov et al., 2002; Watters et al., 1985; Kauer et al., 2002; Yamada, 1970; Fung, 1993; Rubod et al., 2012). The morphology of the colon varies depending on the location. For example, there is a large diameter and thin wall in the ascending colon, but it gradually tapers to a small diameter and thick wall for the sigmoid colon. Like small intestine, the colonic wall is mechanically divided into two layers (from inner to outer): the mucosa, submucosa, inner circular muscular layer, and the outer layer with outer longitudinal muscular layers and serosa (Bourgouin et al., 2012; Massalou et al., 2016).

Only a few tensile tests have been performed on human tissue. For example, Egorov performed such tests under quasi-static load (Egorov et al., 2002), Howes performed high-rate equibiaxial elongation tests (Howes and Hardy, 2012), and our team carried out dynamic tensile tests at 1 m/s (Massalou et al., 2016). Dynamic tests are characterized by loading speeds in the order of m/s, whereas tests are considered static when they are on the order of cm/s or mm/s (Rosen et al., 2008; Rubod et al., 2012; Egorov et al., 2002). No studies have been published concerning the mechanical variability of the human colon subjected to various speeds under uniaxial stress. Our main objective is to determine the mechanical variability of the human colon when subjected to

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https://doi.org/10.1016/j.jbiomech.2019.05.012
0021-9290/

various speeds of uniaxial stress by observing its behavior until complete rupture.

2. Methods

2.1. Origin of the tissue

The tested colonic specimens were samples obtained from 28 human subjects (17 females and 11 males, mean age: 85,2 years) stored at 1 °C with an average retention period of 22 days. The removed colon was immediately tested. The use of cadaveric human tissue was part of a protocol approved by the ethics committee of the Medical school of Nice concerning the donation of bodies to science. The study included only adult subjects whose colon showed no signs of pathology (cancer or inflammation). The presence of diverticula was not a reason for exclusion of samples given the high prevalence in the adult human population. However, the colonic specimens did not contain colonic diverticula.

2.2. Tissue sampling

The specimens were colon segments taken from the antimesenteric border after performing a longitudinal opening by following a standardized format using a rectangular punch. Gaur et al. (Gaur et al., 2016) noted that most experimental studies on tensile testing of soft tissues have used aspect ratio between 1.3 and 2 to ensure a shear free deformation and rupture of tissue within the gauge length of the specimen. Hence we chose an aspect ratio of 1.6, with a width of 25 mm and a gauge length of 40 mm. The gripper length being 30 mm, the total length of the specimen was 100 mm.

In longitudinal direction, for each anatomical subject, 2 specimens (one with *taeniae coli* and one without *taeniae coli*) were taken from four colonic segments (ascending, transverse, descending, and sigmoid colon). The specimens with *taeniae coli* were made parallel to the axis of the strip and included the strip. In circumferential direction, for each anatomical subject, only 1 specimen was taken from each colonic segment. The specimens were made perpendicular to the axis of the muscle strip with the strip situated in the middle of the specimen. Therefore, the number of longitudinal specimens is double of circumferential specimen due to the presence or lack of *taeniae coli*. The full description of the specimen preparation protocol has been described in one of our previous articles (Massalou et al., 2016).

2.3. Number of specimens

Specimens were taken from a total of 28 different colons, including 17 from females and 11 from males. The average age was 85.2 ± 10.3 years, and the bodies had been conserved for an average of 22.2 ± 10.1 days. A total of 336 specimens were subjected to tensile tests after preconditioning (Table 1). The tests were conducted with the following conditions:

- 80 longitudinal and 40 circumferential specimens under quasi-static load (1 cm/s),
- 64 longitudinal and 32 circumferential specimens under intermediate load (10 cm/s),
- 80 longitudinal and 40 circumferential specimens under dynamic load (1 m/s).

2.4. Tensile tests

The initial length (L_0) of all the specimens was 40 mm, both longitudinal and circumferential. The experimental characterization of the mechanical behavior of the colon was done using uniaxial tensile tests under dynamic load (1 m/s), intermediate load (10 cm/s), or static load (1 cm/s). The test was performed using a hydraulic test system (MTS 370.10, Landmark®, USA) under controlled displacement. The sample was first hung in the top gripper, and then was clamped in the bottom gripper. It is well known that the determination of the initial state of strain of the soft tissues is very difficult in tensile tests. As previous studies (Gaur et al., 2016) we chose to define the point of zero stress-strain with an arbitrary force value (2 N).

Because the colon is viscoelastic, a pre-conditioning test phase of the specimen was applied with test parameters chosen to avoid the occurrence of lesions. Ten sinusoidal preconditioning cycles were carried out with an amplitude of 6 mm and a speed of 0.5 m/s, as described in a previous study on the small intestine (Bourgouin et al., 2012). The preconditioning phase was immediately followed by a tensile load of 1 m/s, 10 cm/s, or 1 cm/s up to a distance of 10 cm.

2.5. Data acquisition and post-processing of results

The engineering strain δ was calculated from the initial length L_0 (fixed to 40 mm) and the final gauge length L (gripper to gripper strain), using the following equation:

$$\delta(\%) = [(L - L_0)/L_0] * 100.$$

The engineering stress σ was calculated from the initial cross-sectional area S_0 and the load measured by the load cell F, using the following equation:

$$\sigma\ (\text{MPa}) = \text{force (N)} / S_0(\text{mm}^2).$$

The thickness of the specimen was based on literature data (Sandek et al, 2014): 1.2 mm for ascending, 1.2 mm for transverse, 1.4 mm for descending and 1.5 mm for sigmoid.

From strain-stress curves, stress and strain at yield point and ultimate tensile strain and stress were noted. Ultimate strain is the strain value at the point of maximum stress. The elastic modulus was calculated as the slope of a linear curve fit to the

Table 1
Specimens included in this study. F: female; M: male. The number of longitudinal specimens is twice than the circumferential specimens because the longitudinal specimens are taken with and without *taeniae coli*.

Nb of subject	Genre	Nb longitudinal specimens	Nb circumferential specimens	1 cm/s	10 cm/s	1 m/s
6	F	48	24	–	–	72
6	F	48	24	36	36	–
5	F	40	20	40	20	–
4	M	32	16	–	–	48
2	M	16	8	24	–	–
5	M	40	20	20	40	–
28	**17 F/11 M**	**224**	**112**	120	96	120

Bold values are the total of the specimen of the column.

stress–strain region extending from the end of the toe-in region to the yield point, as explained in Gallagher et al. (Gallagher et al. 2012).

Statistical analyses were performed using Statistica software for Windows. The normality condition of the variables was rejected by the Shapiro test (p-value <1%), so we used the nonparametric Kruskal-Wallis test to test whether the independent samples come from the same population. The results were considered statistically significant in cases of $p < 0.05$.

3. Results

For each speed loading (1 cm/s, 10 cm/s and 1 m/s) and each sample orientation (longitudinal and circumferential), the typical responses stress-strain are presented in Fig. 1, and the values of the mechanical parameters are presented in Table 2.

3.1. Influence of speed loading: 1 cm/s versus 10 cm/s versus 1 m/s

The mechanical response of the colon depends on the speed loading. Figs. 1 and 2 reveals two types of behavior of the colon according to the loading speed: few differences were noted in the response between the static and the intermediate speed loads whereas a dynamic tensile load modified the behavior increasing the stiffness of the samples.

The statistical differences of mechanical parameters between the 3 test velocities are presented in Tables 2–4.

The Young modulus and the stress at the second inflexion were statistically correlated with the speed load for both longitudinal and circumferential samples.

The influence of the speed on the strain was observed for the longitudinal samples whereas no significant difference was observed for the circumferential samples.

3.2. Mechanical behavior as a function of the load

3.2.1. Mechanical behavior as a function of the load – Longitudinal specimen

The mechanical response differed according to the speed of the load. There was a significant statistical difference in the modulus (Tables 2, 3 and 5). Stress–strain curves indicated a Young's modulus of 3.17 ± 2.05 MPa in the first quasi-linear phase for each loading speed under dynamic loading (1 m/s), 1.74 ± 1.15 MPa under intermediate loading (10 cm/s), and 1.76 ± 1.21 MPa under quasi-

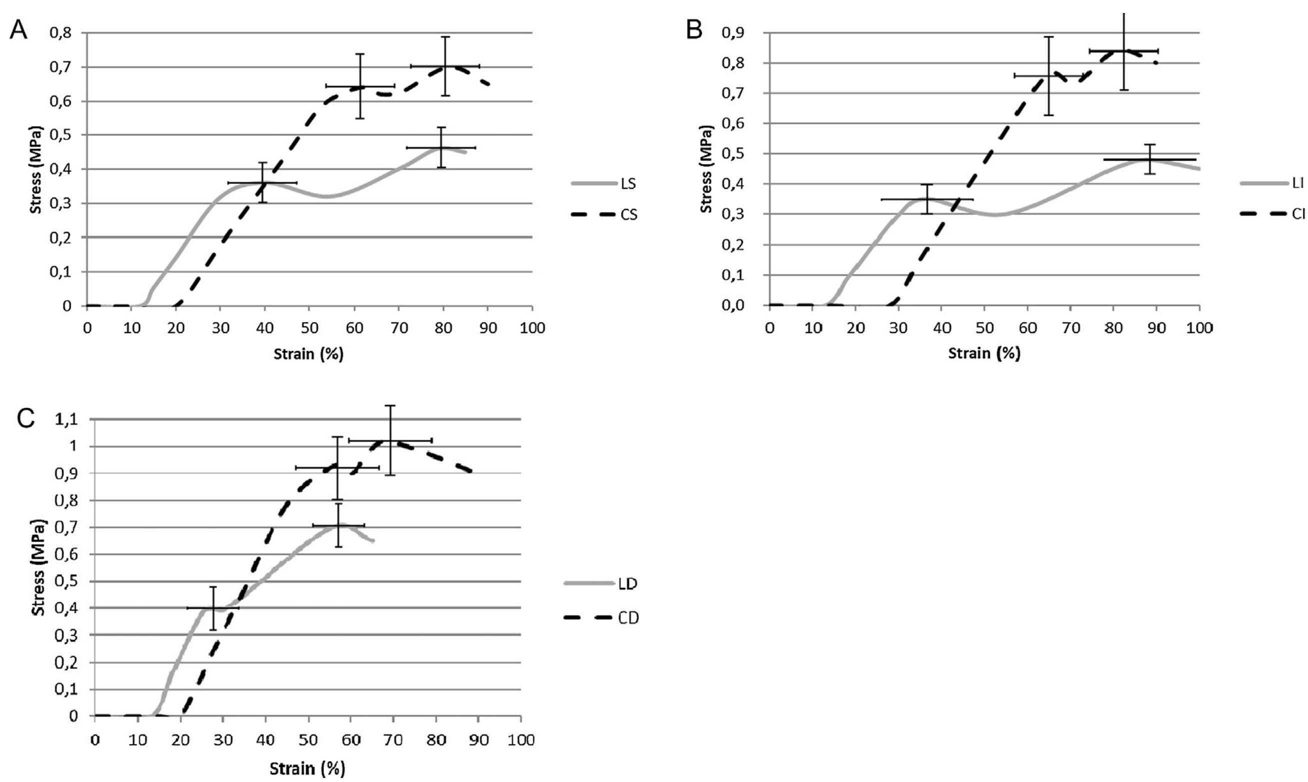

Fig. 1. Stress-strain curves for uniaxial test. A. 1 cm/s. LS: longitudinal static; CS: circumferential static. B. 10 cm/s. LI: longitudinal intermediate; CI: circumferential intermediate. C. 1 m/s. LD: longitudinal dynamic; CD: circumferential dynamic. The standard deviations are represented on these curves for the rupture points during the tensile tests.

Table 2
mechanical parameters values for longitudinal and circumferential samples at different loading speed.

	1 cm/s		10 cm/s		1 m/s	
	Longitudinal	Circumferential	Longitudinal	Circumferential	Longitudinal	Circumferential
Modulus of the elastic phase (MPa)	1.76 ± 1.21	0.63 ± 1.25	1.74 ± 1.15	2.14 ± 1.3	3.17 ± 2.05	3.15 ± 1.73
Strain at 1st inflexion (%)	40.45 ± 25.49	61.31 ± 21.96	36.72 ± 19.84	64.63 ± 24.23	27.61 ± 14.44	56.06 ± 15.04
Stress at 1st inflexion (MPa)	0.36 ± 0.21	0.64 ± 0.38	0.35 ± 0.21	0.76 ± 0.38	0.42 ± 0.29	0.93 ± 0.52
Strain at 2nd inflexion (%)	78.75 ± 45.15	81.24 ± 37.54	87.76 ± 51.63	80.76 ± 33.43	55.41 ± 31.66	68.6 ± 21.72
Stress at 2nd inflexion (MPa)	0.46 ± 0.22	0.7 ± 0.35	0.48 ± 0.22	0.84 ± 0.39	0.7 ± 0.34	1.02 ± 0.5

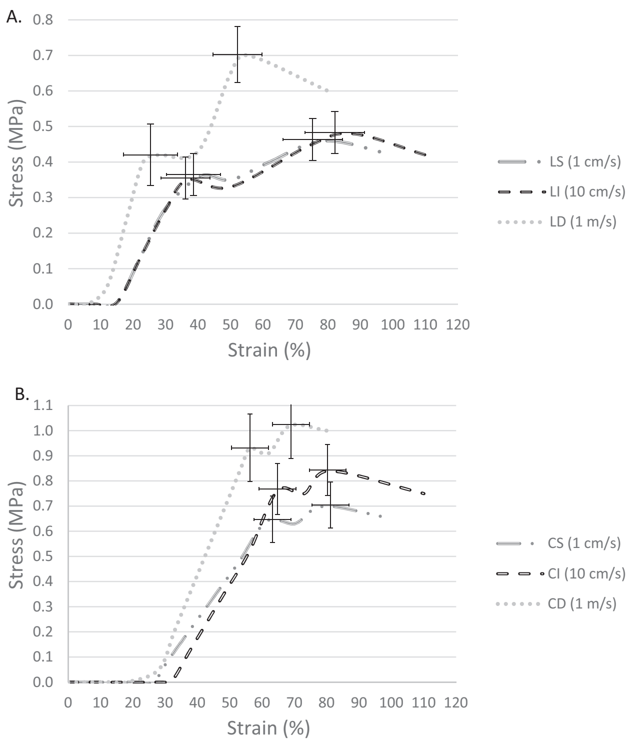

Fig. 2. Stress-strain curves for the 3 protocols under longitudinal and circumferential solicitation. L: longitudinal and C: circumferential. S: static (1 cm/s); I: intermediate (10 cm/s); D: dynamic (1 m/s). The standard deviations are represented on these curves for the rupture points during the tensile tests.

Table 3
Statistical analysis of the speed influence on the mechanical behavior for both longitudinal and circumferential samples. Bold p-values are statistically significant.

	p-value	
	Longitudinal	Circumferential
	1 cm/s vs 10 cm/s vs 1 m/s	**1 cm/s vs 10 cm/s vs 1 m/s**
Modulus of the elastic phase (MPa)	**<0.001**	**<0.001**
Strain at 1st inflexion (%)	**<0.001**	0.45
Stress at 1st inflexion (MPa)	0.31	**0.005**
Strain at 2nd inflexion (%)	**<0.001**	0.18
Stress at 2nd inflexion (MPa)	**<0.001**	**0.001**

static loading (1 cm/s) with $p < 0.001$ (Fig. 2.A). The curves reveal two types of behavior of the colon according to the loading speed: fast break behavior at high-speed traction (dynamic protocol at 1 m/s) and a different type of behavior for lower speeds (intermediate protocols at 10 cm/s and quasi-static at 1 cm/s). Changes in loading speed resulted in different profile curves of the colon, changed the Young's modulus but did not modify the stress necessary for the first point of rupture.

3.2.2. Mechanical behavior as a function of the load – Circumferential specimen

The mechanical response differed according to the speed of the load. There was a significant statistical difference in the Young's modulus (Tables 3, 4 and 6). The stress–strain curves indicated a Young's modulus of 3.15 ± 1.73 MPa in the first quasi-linear phase for each loading speed under dynamic loading (1 m/s), 2.14 ± 1.3 MPa under intermediate loading (10 cm/s), and 0.63 ± 1.25 MPa under quasi-static loading (1 cm/s) with $p < 0.001$ (Fig. 2.B). Dynamic loading results in more fragile mechanical behavior than slower loads.

Table 4
Statistical analysis of the sample orientation influence on the mechanical behavior at different loading speed. Bold p-values are statistically significant.

	p-value Longitudinal versus circumferential		
	1 cm/s	10 cm/s	1 m/s
Modulus of the elastic phase (MPa)	0.93	0.1	0.88
Strain at 1st inflexion (%)	**<0.001**	**<0.001**	**<0.001**
Stress at 1st inflexion (MPa)	**<0.001**	**<0.001**	**<0.001**
Strain at 2nd inflexion (%)	0.35	0.74	**<0.001**
Stress at 2nd inflexion (MPa)	**<0.001**	**<0.001**	**<0.001**

3.3. Mechanical behavior as a function of the taeniae coli – Longitudinal specimen

For longitudinal specimens, we performed tests with or without the *taeniae coli* for the three different speed loadings. The mechanical response with or without *taeniae coli* differed depending on the speed of the load: there was no effect for dynamic load, but the *taeniae coli* modified the mechanical behavior of the specimens for lower-speed load (Table 5, Fig. 3).

For an intermediate speed, the taeniae leads to a significant increase of the modulus and a decrease of the strain at first inflexion. For a static speed, the taeniae leads to a significant decrease of the strain and stress at first inflexion and an increase of strain at second inflexion.

3.4. Mechanical behavior as a function of location

The mechanical response differs slightly depending on the colonic segment location: ascending, transverse, descending or sigmoid colon. Only Young's modulus and stress at 1st point of inflexion are modified by the location of the specimens for some speed solicitation:

- for longitudinal and dynamical solicitation (1 m/s): Young's modulus $p = 0.03$ and stress at 1st point of inflexion $p = 0.08$;
- for circumferential and intermediate solicitation (10 cm/s): Young's modulus is $p = 0.06$ and stress at 1st point of inflexion $p = 0.04$;
- for circumferential and static solicitation (1 cm/s): Young's modulus is $p = 0.06$ and stress at 1st point of inflexion $p = 0.01$.

4. Discussion

This study on the human colon completes the earlier work of Yamada (1970), Fung (1993), Egorov et al. (2002), and our laboratory Massalou et al. (2016). From a mechanical point of view, the colon is composed of viscoelastic tissue and described as contractile and anisotropic (Rubod et al., 2012; Egorov et al., 2002; Fung, 1993). Knowledge of the passive properties of the colon is crucial for understanding colonic functioning (Fung, 1991). Gregersen and Kassab (1996) demonstrated a better reflection of passive mechanical behavior with circular segments of a hollow organ rather than uniaxial tensile samples. However, the digestive tract is an anisotropic material (Rubod et al., 2012; Yamada, 1970; Fan et al., 2004; Gao and Gregersen, 2000; Liao et al., 2004), and the use of longitudinal samples allows a more precise characterization of the longitudinal fibers (Gao and Gregersen, 2000).

The objective of this study was to describe the differences in mechanical behavior of refrigerated human colon subjected to different rates of uniaxial tensile stress. The colon behaved as a viscoelastic material with different mechanical responses that depend on the speed of loading. The dynamic solicitation leads to lower rupture strain. The typical curves in the quasi-static and intermediate tests are very similar to the curves published by

Table 5
Mechanical parameters values for longitudinal samples with and without taeniae coli at different loading speed.

	Longitudinal					
	1 cm/s		10 cm/s		1 m/s	
	Taeniae	No taeniae	Taeniae	No taeniae	Taeniae	No taeniae
Modulus of the elastic phase (MPa)	1.93 ± 1.21	1.60 ± 1.20	2.01 ± 1.15	1.46 ± 1.09	3.41 ± 2.3	2.92 ± 1.75
Strain at 1st inflexion (%)	35.40 ± 28.75	45.38 ± 21.06	28.09 ± 12.94	45.62 ± 21.88	26.59 ± 15.10	28.65 ± 13.86
Stress at 1st inflexion (MPa)	0.31 ± 0.13	0.42 ± 0.25	0.31 ± 0.18	0.39 ± 0.23	0.40 ± 0.30	0.43 ± 0.28
Strain at 2nd inflexion (%)	91.71 ± 52.85	66.10 ± 31.99	91.96 ± 62.09	83.43 ± 38.56	54.30 ± 35.67	56.56 ± 27.37
Stress at 2nd inflexion (MPa)	0.44 ± 0.39	0.48 ± 0.25	0.46 ± 0.21	0.50 ± 0.22	0.68 ± 0.20	0.72 ± 0.28

Table 6
Statistical analysis of the taeniae coli influence on the mechanical behavior for the longitudinal specimen at different speed loading. Bold p-values are statistically significant.

	LS Taeniae versus no taeniae	LI Taeniae versus no taeniae	LD Taeniae versus no taeniae
Modulus of the elastic phase (MPa)	0.07	**0.02**	0.26
Strain at 1st inflexion (%)	**<0.001**	**<0.001**	0.19
Stress at 1st inflexion (MPa)	**0.04**	0.21	0.75
Strain at 2nd inflexion (%)	**0.04**	0.95	0.78
Stress at 2nd inflexion (MPa)	0.78	0.5	0.14

Egorov et al. (2002) during transverse tests of static traction on transverse colon samples. They demonstrated a modification of the colonic mechanical response by *taeniae coli* (longitudinal muscle strips of the colon). Under static and intermediate stress, our study also showed a mechanical impact of the *taeniae coli*. However, in dynamic traction, we did not observe an effect of *taeniae coli*, even though the levels of deformation seem identical between the study by Egorov et al. and ours. We did not study the impact of *taeniae coli* separately.

Other anisotropic and viscoelastic materials appear to behave in the same way. For striated muscular fibers, an increase of the speed of stress decreases the force necessary for rupture in both static and dynamic situations (Roberts, 2016; Rosario et al., 2016). As in our study, there is fragile behavior under dynamic stress, while for slower stresses, the results are then superimposable and more elastic. Many structures within the muscles intervene in this reaction: actomyosin bypasses, actin and myosin filaments, titin, and the scaffolding of the connective tissue of the extracellular matrix (Fallqvist and Kroon, 2013; Fallqvist et al., 2014; Kroon, 2011; Roberts, 2016). Collagen is likely the main determinant for the intestinal wall stiffness since collagen in most tissues is the stress-bearing structure (Fung, 1993). It was found that the collagen distribution was axial preferred in both layers and the mucosa contained more collagen. The collagen distributes more axially for both the muscle and mucosal layers; the collagen content is higher for the mucosa than for the muscle. The load-bearing collagen content increases and the collagen fibers rotate towards to the axial direction with the increase of the axial stretch (Yang et al., 2006). This collagen axial-orientation could explain the need for more stress for longitudinal tensile tests to obtain similar damages than circumferential tensile tests. The predominant collagen content in the mucosa could also explain the more visco-elastic behavior of the internal layer of the colon.

The anisotropic material of the colon has different mechanical properties between longitudinal and circumferential uniaxial

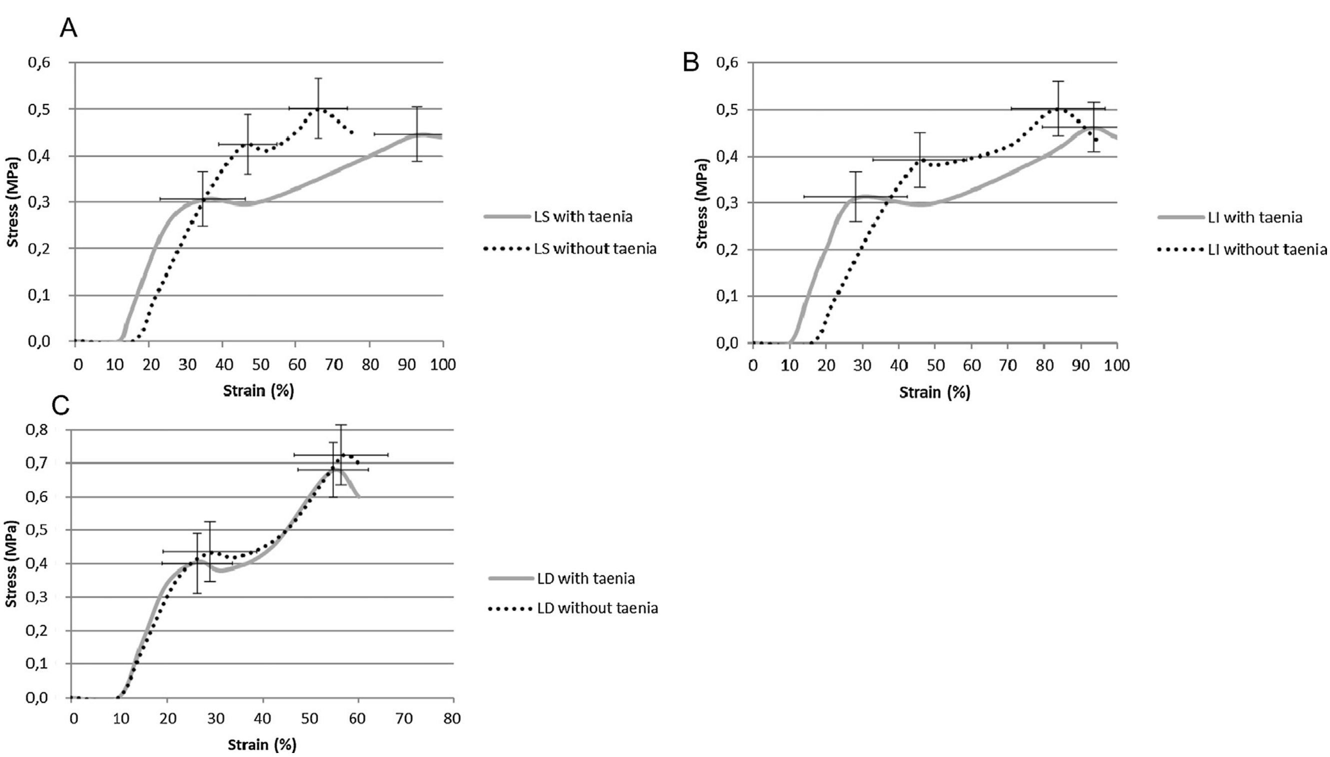

Fig. 3. Stress-strain curves with or without *taeniae coli* for uniaxial test. A. 1 cm/s. LS: longitudinal static. B. 10 cm/s. LI: longitudinal intermediate. C. 1 m/s. LD: longitudinal dynamic. The standard deviations are represented on these curves for the breaking points during the tensile tests.

stress tests (Massalou et al., 2016; Merlo and Cohen, 1988). Howes and Hardy (Howes and Hardy, 2012) also highlighted this property in uniaxial and bi-axial dynamic tests. We confirmed this property for dynamic speeds as well as slower loads. Indeed, the mechanical response of the colon subjected to circumferential traction is more elastic, requiring higher levels of stress and strain to obtain lesions in the specimens.

Other factors may also modify the experimental results with respect to the behavior of the colon in vivo:

- Active properties of muscle cells responsible for the propulsion of the digestive contents (Kroon, 2010).
- Modification of digestive tonicity: intestinal mechanoreceptors are sensitive to the stress stimulus and a linear association between stress relaxation and afferent discharge adaptation has been found (Liao et al., 2012).
- Modification of the composition of the inter or intracellular fluid: the presence of certain neuropeptides or the concentration of calcium will modify the recorded mechanical response (La et al., 2005; Merlo and Cohen, 1988; Middleton et al., 1993; Washabau and Sammarco, 1996).
- Pathological phenomena: inflammatory bowel diseases or the deletion of certain genes can lead to a modification of the mechanical behavior of the colon (Onori et al., 2005; Sung et al., 2015; Yang et al., 2009).

It is therefore difficult to determine the mechanical behavior of the human colon in a physiological situation, as well as in our tests. The completion of bi-axial tests would describe the behavior of the colon more completely. Since the tissue has a muscular layer, the synchronous realization of contraction tests would also approach the behavior of the human colon in vivo (Murtada et al., 2017). This experimental study has made it possible to obtain reference values for the colon when subjected to different stresses. These values could be used for finite element models of virtual trauma and quasi-static simulation, as in the case of surgical simulation or the improvement of colonic stent deployments.

5. Conclusion

Tensile tests were performed on human colic samples at 1 cm/s, 10 cm/s and 1 m/s. There is variability in the mechanical behavior of the colon as a function of the loading speed. The colonic tissue behaves in the same way under static and intermediate stress, and then its behavior becomes more fragile under dynamic stress. In the case of quasi-static stress, *taeniae coli* modify the mechanical response of the colon. Regardless of the loading speed, the circumferential stress requires higher levels of stress and strain to obtain lesions. This study could be useful to improve the biofidelity of colon numerical models.

Funding

None.

Declaration of Competing Interest

None.

Acknowledgements

English editing by American Manuscript Editors©.

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