

Tension and stress in the rat and rabbit stomach are location- and direction-dependent

J. ZHAO*, D. LIAO* & H. GREGERSEN*†‡

*Centre of Excellence in Visceral Biomechanics and Pain, Aalborg Hospital, Aalborg, Denmark

†Centre of Sensory-Motor Interaction, Aalborg University, Aalborg, Denmark

‡National Centre of Ultrasound in Gastroenterology, Bergen University and Haukeland Hospital, Bergen, Norway

Abstract Distension studies in the stomach are very common. It is assumed in pressure–volume (barostat) studies of tone and tension in the gastric fundus that the fundus is a sphere, i.e. that the tension in all directions is identical. However, the complex geometry of the stomach indicates a more complex mechanical behaviour. The aim of this study was to determine uniaxial stress–strain properties of gastric strips obtained from rats ($n = 12$) and rabbits ($n = 10$). Furthermore, we aimed to study the gastric zero-stress state since the stomach is one of the remaining parts of the gastrointestinal tract where residual strain studies have not been conducted. Longitudinal strips (in parallel with the lesser curvature) and circumferential strips (perpendicular to the lesser curvature) were cut from the gastric fundus (glandular part) and forestomach (non-glandular part). The residual stress was evaluated as bending angles (unit: degree per unit length and negative when bending outwards). The residual strain was computed from the change in length between the zero-stress state and no-load state. The stress–strain test was performed using a tensile test machine. The thickness and width of each strip were measured from digital images. The strips data were compared with data obtained in the intact stomach in vitro. Most residual stresses and strains were bigger in the glandular part than in the forestomach, and in general the rat stomach had higher values than the rabbit stomach. The glandular strips were stiffer than the forestomach strips and the

longitudinal glandular strips were stiffer than the circumferential glandular strips ($P < 0.05$). The gastric strips were stiffer in rats than in rabbits ($P < 0.01$). The data obtained in the intact rat stomach confirmed the strips data and indicated that those were obtained in the physiological range. In conclusion, the biomechanical properties of the gastric strips from the rat and rabbit are location-dependent, direction-dependent and species-dependent. The assumption in physiological pressure–volume studies that the stomach is a sphere with uniform tension is not valid. Three-dimensional geometric data obtained using imaging technology and mechanical data are needed for evaluation of the stomach function.

Keywords bending angle, gastric strips, strain, stress, uniaxial stretch.

INTRODUCTION

The stomach is an expanded section of the digestive tube between the oesophagus and the small intestine. The major functions of the mammalian stomach are storage, acidification, mixing, and controlled delivery of ingested food to the small intestine.¹ The stomach is to a larger degree than other parts of the gastrointestinal tract functionally subjected to dimensional change.² Hence, the biomechanical properties are of functional importance. However, the stomach has more complex geometry than the intestines. Therefore, from a methodological point of view, it is difficult to study the active and passive biomechanical properties of the stomach.

The stomach has traditionally been studied using pressure–volume and length–tension measures.^{3–5} In a number of more recent studies in animals and humans, the barostat was used to measure pressure–volume relations for evaluation of tone, compliance and tension in the gastric fundus.^{6–8} The main assumptions made in

Address for correspondence

Hans Gregersen, Director and Professor, Center of Excellence in Visceral Biomechanics and Pain, Aalborg Hospital, Hobrovej 42 A, DK-9100 Aalborg, Denmark.

Tel: +45 99322064; fax: +45 98133060;

e-mail: hag@aes.nja.dk

Received: 11 January 2004

Accepted for publication: 18 November 2004

such studies are that the fundus is geometrically a sphere with uniform tension. In other words, it is assumed that the mechanical properties in all directions are the same. However, the geometry of the fundus of the stomach is much more complex than the spheroidal geometry. This is well known from studies using magnetic resonance⁹ and endoscopy. Complex geometry indicates that the mechanical behaviour of the stomach is complex (anisotropic) rather than isotropic.^{10,11} Compared with the compliance parameter, the stress-strain (force per area-deformation) relations are better measures of tissue mechanical properties but the direction and location must be considered. Furthermore, the passive biomechanical properties of the stomach are important for understanding the active biomechanical properties of the stomach muscle.^{11,12} However, only sparse data about these issues have been reported. Egorov and coworkers found in cadaver stomachs that the maximum stress was 0.5 MPa and the destructive strain was 190%.¹³ Gregersen and co-workers recently investigated gastric antral geometry and stress-strain properties by using transabdominal ultrasound scanning during volume-controlled distensions in the human gastric antrum.¹⁴ The stress distribution in the whole stomach is currently being studied using 3D-ultrasound and numerical analysis, and as expected the stress is highly variable (D. Liao *et al.*, unpublished observations). In most other parts of the gastrointestinal tract, it is now known that residual strains exist because tissue rings cut radial open up into sectors representing the zero-stress state.^{11,12,15,16} The physiological applications of the zero-stress state are outlined by Gregersen and coworkers.^{12,13,16} To our best knowledge, the zero-stress state and residual strains in the stomach have not been reported, although these parameters are important in analysing the function and mechanics of the stomach.

The main hypothesis of this study was that the stomach is mechanically anisotropic, i.e. that the stress-strain properties are location- and direction-dependent. The hypothesis is tested by investigating the uniaxial stress-strain relation and zero-stress state of gastric strips in rabbits and rats. The muscle tissue was relaxed pharmacologically and preconditioned in order to obtain stable experimental conditions and to not confound the active and passive tissue properties. Furthermore, the strips data were compared with data obtained in the intact stomach *in vitro*.

MATERIAL AND METHODS

Ten male Wistar rats (3–4 month old, 278 ± 23 g) and 12 male rabbits (3.9 ± 0.1 kg) were used in this study.

Rats and rabbits were chosen because of their small size and since they have quite different eating habits. Five extra rats were used for additional experiments comparing the strips with the intact stomach *in vitro*. Approval of the protocol was obtained from the Danish Committee for Animal Experimentation.

Experimental procedures

The rats and rabbits were anaesthetized with Hypnorm 0.5 mg and Dormicum 0.25 mg per 100 g body weight (Hypnorm : Dormicum : sterile water = 1 : 1 : 2; subcutaneous injection). Following laparotomy, the whole stomach with the distal part of the oesophagus and the proximal part of the duodenum was excised. The rats were killed by CO₂ inhalation overdose after the stomach was taken out of the abdominal cavity, in accordance with guidelines from the Danish Committee for Animal Experimentation. The stomach was immersed in calcium-free Krebs solution with EGTA (0.26 mmol L⁻¹) aerated with a gas mixture of 95% O₂ and 5% CO₂ at pH 7.4. Muscle activity was not observed visually or recorded in the test machine after immersion in this solution. The composition of the Krebs solution (g L⁻¹) was NaCl, 6.89; KCl, 0.35; NaHCO₃, 2.1; NaH₂PO₄, 0.16; MgCl, 0.24; glucose, 2.1. The stomach was opened paramedian to the greater curvature and gently washed using saline. Two strips (about 15 mm long and 3 mm wide) of the stomach wall were cut both in longitudinal direction (parallel with the lesser curvature) and circumferential direction (perpendicular to the lesser curvature) from the gastric fundus (glandular part) and the forestomach (non-glandular part) (Fig. 1). One of the two strips was used for zero-stress state measurement and the other strip was used for the stress-strain experiment. The length of the strips was measured before the stress-strain experiment.

Mechanical stress-strain experiments

The test was conducted in a tensile test machine consisting of organ bath, motion table, force transducer and electronics. The gastric strip was bathed in an oxygenized physiological Krebs solution. The two ends of each strip were tied with silk threads to cannulas and the length between the threads was measured in the organ bath. The cannulas were connected to rods that could be moved at controlled velocities by a motor. One of the rods was attached to a force transducer. The distance between the rods was adjusted manually to the *in vitro* length of the strips. The force (*F*) was recorded in real time by the force transducer. Three cycles of loadings and unloadings

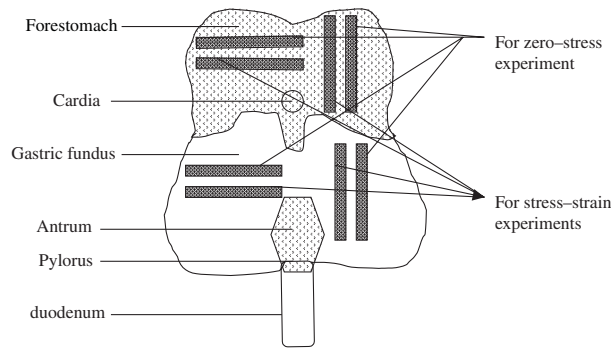


Figure 1 Sketch of rat or rabbit stomach opened paramedian to the greater curvature. Two strips of the stomach wall both in longitudinal (parallel with the lesser curvature) and circumferential (perpendicular to the lesser curvature) directions were cut from the gastric fundus (glandular part) and fore-stomach (non-glandular part). One strip was used for the zero-stress state analysis, the other strip was used for the stretch analysis.

were done up to 1.5 stretch ratio (length divided by reference length). The first two cycles were for preconditioning the strip mechanically.¹² The preconditioning behaviour is believed to be due to strain softening (a non-viscoelastic behaviour observed during preconditioning) in the tissue in response to the loading.¹² The third cycle was used for the stress-strain analysis. The strain rate was 0.5 mm s^{-1} . The whole segment was photographed using a video camera (Sony CCD Camera, DXC-151A, Tokyo, Japan) for later analysis of length and width.

Mechanical data analysis

The morphometric data were obtained from digitized images and from the strips in the no-load state, the zero-stress state and stretched states. Measurements were done using image analysis software (Sigmascan 4.0; Sigma Corp., San Rafael, CA, USA). The following data were measured from each specimen: the inner and outer length at the no-load state and zero-stress state, the thickness and width at the no-load state. L , h and w refer to the length, thickness and width, respectively. The wall thickness and the width were averaged from 10 measurement point along the whole length of the strip. The subscripts i, o, n, z, ns and s refer to the inner (mucosal surface), outer (serosal) surface, no-load state, zero-stress state, no-stretch and stretch condition. The bending angle (φ) was defined as the angle of bending when a gastric strip was cut.¹² In the case of bending angle $>360^\circ$, the strip was cut to two or three shorter segments. If a strip bends outwards the bending angle will be negative.

The measured data was used for computation of biomechanical parameters defined as

The bending angle per unit length :

$$\frac{\varphi - 180}{(L_{n-i} + L_{n-o})/2} \quad (1)$$

Residual Green's strain at the mucosal surface :

$$E_i = \frac{\left(\frac{L_{i-n}}{L_{i-z}}\right)^2 - 1}{2} \quad (2)$$

Residual Green's strain at the serosal surface :

$$E_o = \frac{\left(\frac{L_{o-n}}{L_{o-z}}\right)^2 - 1}{2} \quad (3)$$

Residual strain difference :

$$E_{\text{difference}} = E_o - E_i \quad (4)$$

$E_{\text{difference}}$ normalized to the wall thickness :

$$E_{\text{difference}}/h \quad (5)$$

The stress and strain of the stomach strips in the loaded state were determined as average values, independent of the layered structure of the wall. The stress and stretch ratio in a gastric wall strip were computed as

$$\text{Stress : } T_s = \frac{F}{A_0} \quad (6)$$

where F is the force recorded, and A_0 is the cross-sectional area at zero-stress state (computed from wall thickness h and width w as $A_0 = wh$)

$$\text{Stretch ratio : } \lambda = \frac{L_s}{L_{ns}} \quad (7)$$

The tissue constants a and b were obtained with curve fitting using the equation.¹²

$$T = b * \{\exp[a * (\lambda - 1)] - 1\} \quad (8)$$

Young's modulus (a stiffness constant) is defined as

$$Y(T) = \frac{dT}{d\lambda} = a(T + b) \quad (9)$$

Additional experiments on the intact stomach *in vitro*

Five male Wistar rats weighting 300–350 g were used for these experiments. After obtaining smooth muscle relaxation, the whole stomach with the distal part of the oesophagus and the proximal part of the duodenum

was excised. The stomach was rinsed with saline solution introduced into the lumen through the distal oesophagus.

The stomach was suspended with the greater curvature down in an organ bath by fixing the distal oesophagus and the proximal duodenum in vertical orientation to a bar above the organ bath. The bar was then lowered to completely submerge the stomach into the organ bath. The organ bath was filled with Krebs solution with EGTA (100 mg L^{-1}) aerated with a gas mixture of 95% O_2 and 5% CO_2 at pH 7.4. The proximal duodenum end was closed after pressure equilibration. The distal oesophagus was connected via a tube to a fluid container containing Krebs solution. The container was used for applying static gastric luminal pressures ranging from 0 to 8 cmH_2O . Three minutes were allowed at each pressure level for equilibration.

The stomach was scanned at each pressure level using a 20 MHz, 360° ultrasonic probe (UM-3R; Olympus, Tokyo, Japan) positioned in water. The catheter was fixed on a Z-shape frame and guided by a micromanipulator along an axis parallel to the gastric longitudinal direction. Cross-sectional images along the stomach with interval distance of 1 mm were captured and recorded by a super VHS videotape recorder.

The images were digitized from the VHS videotape and saved as BMP files with image resolution from 0.10 to 0.23 mm per pixel. The images were imported into a MATLAB program. The inner and outer wall was then identified by grayscale thresholding and semi-automated edge detection. This procedure was done for approximately 30–45 cross-sections in each stomach. Hence, the 3-D gastric surface was obtained from the transverse cross-sectional images along the straight longitudinal axis of the stomach.

The circumferential strain was calculated based on the average circumference in approximately the same four slices near the interface in both the glandular and non-glandular part. The longitudinal strain along the greater curvatures was calculated in the glandular, the non-glandular part and the whole stomach. The strain was expressed as the stretch ratio with the geometry at pressure of 0 cmH_2O as reference.

$$\lambda = L/L_0 \quad (10)$$

where λ is the stretch ratio, L and L_0 are the length at the pressurised and the reference state. Calculation of the bending angle was based on three points at each of three nearby locations on the surface of the stomach. A Matlab program computed the bending angle. The bending angle per unit length is comparable between the strips and intact stomach experiments.

Statistical analysis

The data were representative of a normal distribution and accordingly the results were expressed as mean values \pm SEM. The constants a and b obtained from the curve fitting procedure were used for statistical comparison of the stress-strain data. Two-way analysis of variance was used to detect the differences between locations and species (Sigmastat 2.0TM, Sigma Corp. San Rafael, CA, USA). In case of significance, the data were evaluated in pairs by a multiple comparison procedure (Student–Newman–Keuls method). Linear regression analysis was used to demonstrate possible association between the elastic modulus and stress parameters. The results were regarded as significant when $P < 0.05$.

RESULTS

Morphometry data

The wall thickness was significantly thicker in the glandular stomach (rat $0.9 \pm 0.1 \text{ mm}$; rabbit $2.1 \pm 0.2 \text{ mm}$) than in the forestomach (rat $0.5 \pm 0.1 \text{ mm}$; rabbit $1.1 \pm 0.1 \text{ mm}$). The wall thickness was larger in the rabbit stomach than in the rat stomach ($P < 0.001$). The wall thickness did not vary along the length of the strips in either the glandular stomach and the forestomach in both species.

Mechanical experimental data

The bending angle per unit length, residual strains, residual strain difference and residual strain difference normalized to the wall thickness of the gastric strips are shown in Fig. 2. The bending angle per unit length (Fig. 2A) was significantly larger in the glandular strips than in the forestomach strips both in the rats and rabbits ($F > 37.6$, $P < 0.001$). The bending angle was significantly larger in the rat than in the rabbit both in the glandular part and the forestomach ($F > 75.4$, $P < 0.001$). The bending angles did not differ between the two directions at the same location. The same accounted for the inner and outer residual strain (Fig. 2B, inner residual strain, $F > 127.3$, $P < 0.001$ for locations and $F > 5.3$, $P < 0.05$ for the species; outer residual strain, $F > 142.8$, $P < 0.001$ for locations and $F > 5.1$, $P < 0.05$ for the species), residual strain difference (Fig. 2C, $F > 37.6$, $P < 0.001$ for locations and $F > 4.8$, $P < 0.05$ for the species) and residual strain difference normalized to the wall thickness (Fig. 2D, $F > 42.9$, $P < 0.001$ for locations and $F > 13.5$, $P < 0.01$ for the species). The absolute value of the residual strain and residual

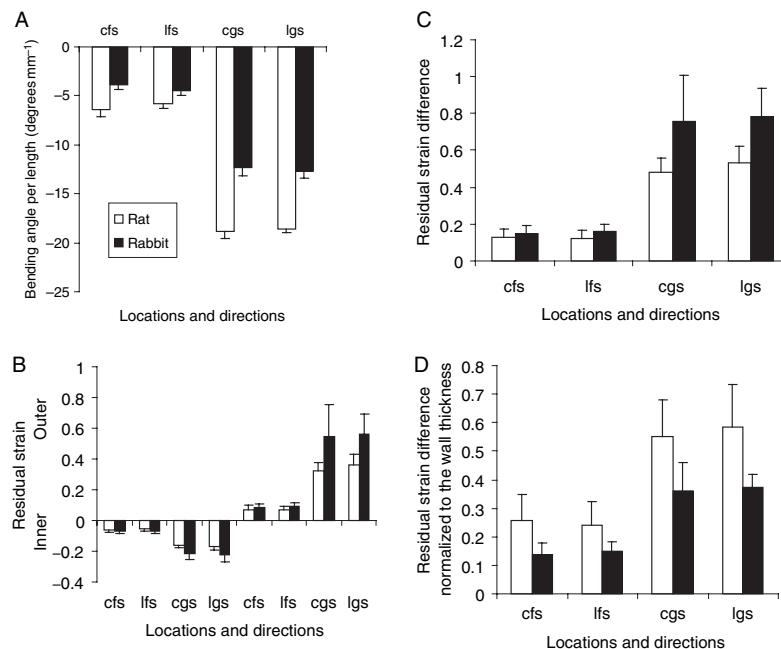


Figure 2 The bending angle per unit length (2A), residual strain (2B), residual strain difference (2C) and residual strain difference normalized to the wall thickness (2D) of the gastric strips are shown (mean \pm SEM). See the text for statistics. c, circumferential; l, longitudinal; g, glandular; f, forestomach; s, strips.

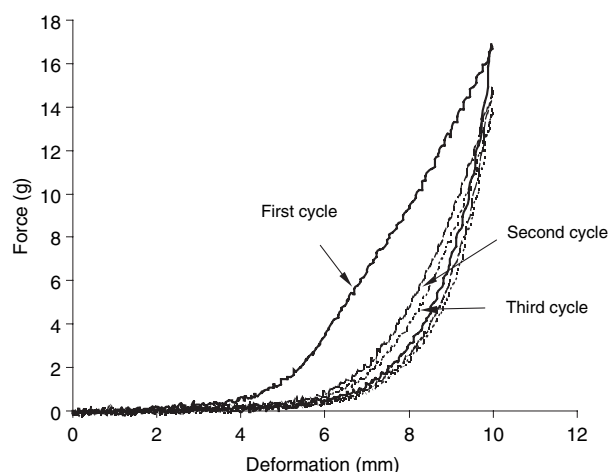


Figure 3 Original recording of force–deformation relationship during the preconditioning process. The hysteresis loop decreases with succeeding cycles until the third cycle. The preconditioning behaviour is believed to be because of strain softening in the tissue in response to the loading.¹³

strain difference was bigger in the rabbit stomach than in the rat stomach.

When a strip is tested in a tensile testing machine by imposing a cyclically varying strain, the stress response will show a hysteresis loop for each cycle. Figure 3 shows a recording of the force–deformation relationship from a glandular strip of the rabbit stomach. The area of the hysteresis loop decreases with succeeding cycles and the curves shift to the right,

rapidly at first, then tending to a steady state at the third cycle. This mechanical phenomenon observed in soft tissues and rubber materials is called preconditioning¹². For the stomach three preconditioning cycles are necessary to obtain repeatable results. The stress–strain data provided below are from the last (preconditioned) loading curve.

Uniaxial stress–strain relationships of the gastric strips are shown in Fig. 4. The stress–strain curves differed between the strips obtained at different locations and directions. Both for the rat and rabbit, the stress–strain curve for the longitudinal glandular strip was located to the left, indicating that those strips were the stiffest. The curve for the longitudinal forestomach strip was located to the right, indicating it was softest. The rat gastric strips were stiffer than the rabbit gastric strips (Fig. 4). Statistical analysis of the *a* and *b* constants confirmed that the stress–strain properties are location-, direction-, and species-dependent (Fig. 5 and Table 1).

A strong linear association exists between the stress and young's modulus in the glandular part (Fig. 6). R^2 in the glandular strips ranged from 0.9425 to 0.9669 whereas it ranged from 0.5407 to 0.5694 in the forestomach strips.

Comparison between the isotropic model and the anisotropic model

Isotropic and anisotropic tension models were compared as outlined in the Appendix. The two models

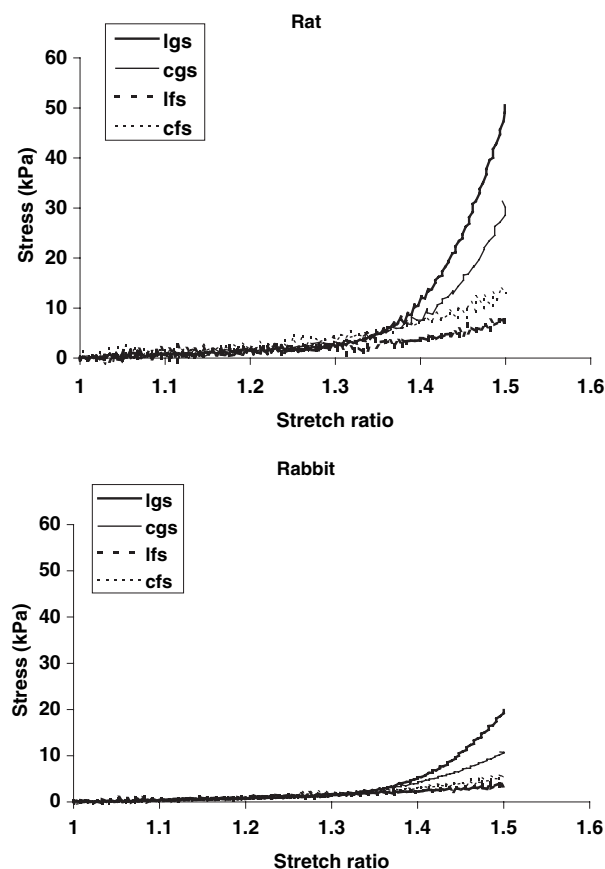


Figure 4 The uniaxial stress-strain relationship of the gastric strips obtained at different locations and directions. The stress-strain curves in order from left to right were lgs, cgs, cfs and lfs. where: c, circumferential; l, longitudinal; g, glandular; f, forestomach; s, strips. The data indicate that the longitudinal glandular strip was the stiffest and the longitudinal forestomach strip was the softest. The strips from the rat stomach were stiffer than that from the rabbit stomach.

were deformed to the same degree and **Young's modulus and Poisson ratio for the anisotropic model were taken from the experimental data obtained in the rats, i.e. $E_1 = 111$ kPa, $E_2 = 54$ kPa and $\nu_1 = \nu_2 = 0.45$. Young's modulus of the isotropic model was averaged from the anisotropic data, i.e. $E = (E_1 + E_2)/2 = 83$ kPa, $\nu = 0.45$.** The relative tension error between the two models was thus found to be 25.2% in the x direction and 53.7% in the y direction.

Additional experiments on the intact stomach *in vitro*

The distensions experiments demonstrated that the volume distensibility of the forestomach was higher than that of the glandular stomach ($P < 0.01$) (Fig. 7). This was confirmed when expressing the stretch ratio

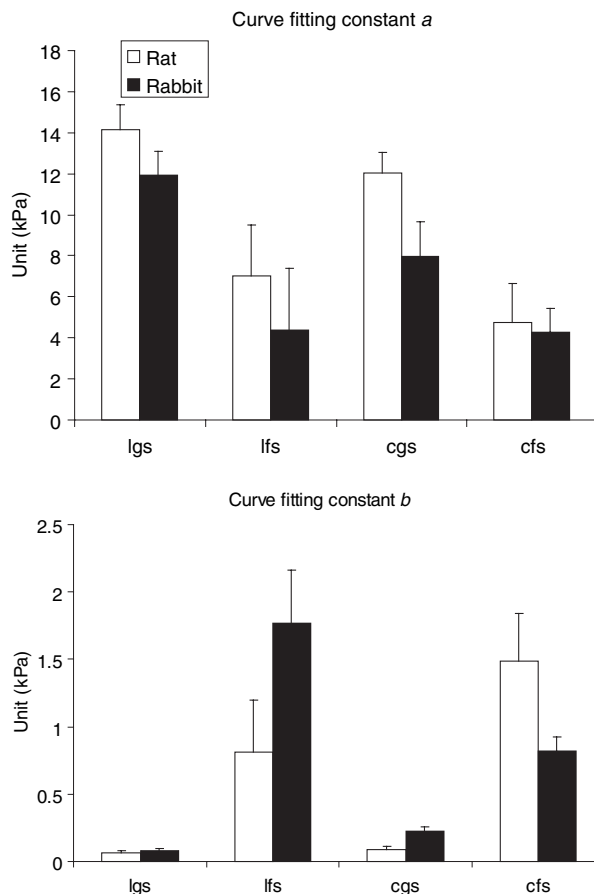


Figure 5 The curve fitting constants a and b are shown (mean \pm SEM). Both a and b differed significantly between the different locations and directions, and between the species. Detailed statistical results are given in Table 1. c, Circumferential; l, longitudinal; g, glandular; f, forestomach; s, strips.

as function of the distension pressure or as function of the volume. The stretch ratios in circular direction were higher than in longitudinal direction. The stretch ratios obtained at the applied pressures up to 8 cmH₂O were higher than those applied in the strips experiments. The bending angle per unit length decreased as function of the distending pressure and the volume ($P < 0.01$) but did not show much difference between the direction and segment of the stomach. The bending angle per unit length was positive (Fig. 7) in contrast to the bending angles measured in the zero-stress state (bending outwards, Fig. 2).

DISCUSSION

The stomach has four basic functions¹: (1) storage reservoir; (2) substantial chemical and enzymatic digestion, particularly of proteins; (3) vigorous contractions of gastric smooth muscle mix and grind food-

Table 1 One-way ANOVA *post hoc* analysis results of constant *a* and *b*

	Different locations and different directions			
	<i>a</i>		<i>b</i>	
	Rat	Rabbit	Rat	Rabbit
GL–GC				
<i>F</i> -value	37.5	20.98	15.18	0.942
<i>P</i> -value	<0.001	<0.001	<0.001	0.342
FL–FC				
<i>F</i> -value	0.01	5.73	1.21	1.54
<i>P</i> -value	0.925	<0.05	0.286	0.229
GL–FL				
<i>F</i> -value	54.39	77.60	11.06	14.56
<i>P</i> -value	<0.001	<0.001	<0.01	<0.001
GC–FC				
<i>F</i> -value	33.09	149.89	31.187	15.84
<i>P</i> -value	<0.001	<0.001	<0.001	<0.001
GL–FC				
<i>F</i> -value	219.57	198.84	52.19	16.41
<i>P</i> -value	<0.001	<0.001	<0.001	0.001
GC–FL				
<i>F</i> -value	10.83	40.51	3.63	3.445
<i>P</i> -value	<0.01	<0.001	0.073	0.078
Different species				
	<i>a</i>		<i>b</i>	
	Rat vs rabbit		Rat vs rabbit	
GL–GL				
<i>F</i> -value	18.54		1.036	
<i>P</i> -value	<0.001		0.321	
GC–GC				
<i>F</i> -value	47.79		12.63	
<i>P</i> -value	<0.001		<0.01	
FL–FL				
<i>F</i> -value	4.82		4.17	
<i>P</i> -value	<0.05		<0.05	
FC–FC				
<i>F</i> -value	0.46		2.79	
<i>P</i> -value	0.513		0.111	

G, glandular part; F, forestomach; C, circumferential; L, longitudinal.

stuffs with gastric secretions; and (4) after the food has mixed with the stomach secretions, it is slowly released into the small intestine for further digestion. The mechanical function is important for these basic functions. To fully understand the physiology of stomach function we need to know the basic mechanical characteristics of the tissue, starting with the geometry of the zero-stress state and the passive stress-strain properties.

The major findings in this study were that the residual stresses and strains and the stress-strain

properties in the stomach were species-, location-, and direction-dependent. The strongest correlation between the stress and modulus was found in the glandular gastric strips.

Structure–mechanics relations

The rabbit has a very large stomach with a relatively narrow diameter small intestine. The stomach functions largely as a food reservoir and is usually never empty.¹⁷ The rabbit stomach consists of the fundic region and the pyloric region. The rat stomach is composed of a forestomach and glandular stomach.¹⁸ The structure differs between the glandular part and the forestomach.^{17,18} The wall is thickest in the glandular part and the layered structure also differs between the two locations. The biomechanical properties depend on the tissue components and structure. Therefore, the anisotropic mechanical behaviour of the stomach is not surprising due to the complex geometry and the fact that muscle layers have different directions. In order to obtain stable experimental conditions, we relaxed the smooth muscle. Thus, the data represent the passive mechanical properties that from other tissues are known to behave in an exponential way. The zero-stress state and the passive properties are determined by non-contractile tissue components such as the collagen and other matrix components and considered to be the parallel element in Hill's well known model.¹⁹ The passive elements must be determined before we can fully understand the active muscle properties of living tissues since the function of the muscle depends on the parallel element.^{10,12}

The zero-stress state and residual strains

The zero-stress state and residual strains have been studied in most of the gastrointestinal tract but not in the stomach before now. Axial variation of opening angle and residual strain distribution exist in the normal oesophagus,^{16,20} intestine²¹ and colon.¹⁵ Changes in tissue structure (non-homogeneous remodelling) due to disease alter the distribution of residual strain in the tubular parts of the gastrointestinal tract.^{22–24} All gastrointestinal studies done so far show that the rings open into sectors when cut radially. The gastric strips deformed towards the serosal side after the dissection. This implies that the mucosa is under compression in the no-load state whereas the muscle layers are in tension. Although the pressure in the proximal stomach under physiological conditions rarely reaches high pressures, the gastric volume changes considerably

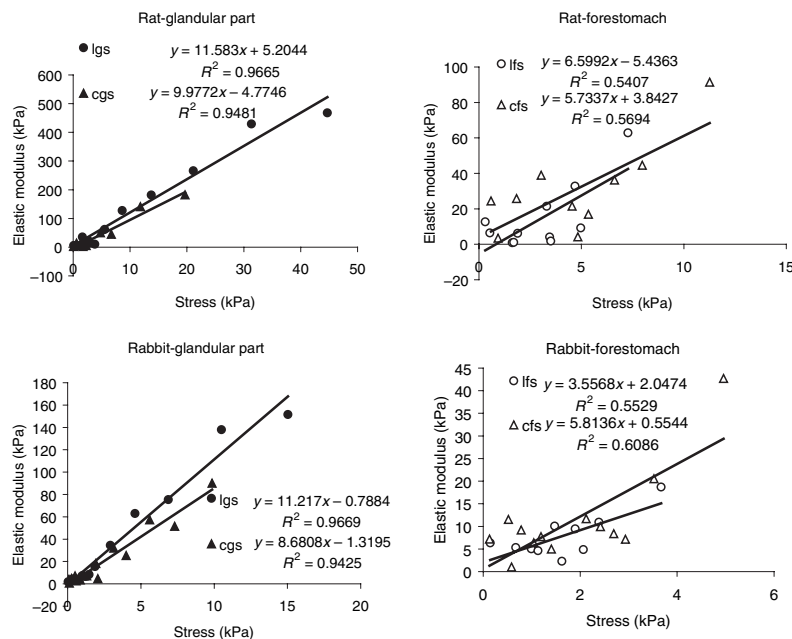


Figure 6 Association between the stress and the elastic modulus. The strongest linear correlation exists in the glandular gastric strips. c, Circumferential; l, longitudinal; g, glandular; f, forestomach; s, strips.

between the empty and filled state. Thus, the compressed mucosa may be better protected against the large dimensional changes in the stomach.^{11,12} Our results demonstrated that the residual strain was more negative in the rabbit stomach mucosa than in the rat stomach mucosa. The rabbit stomach functions largely as a food reservoir and is usually never empty. Therefore, the mucosa of the rabbit stomach is more stretched than that of the rat stomach under physiological conditions and may require larger residual strains.

Tension considerations

Tonic contraction of the proximal stomach (fundus) plays an important role in liquid emptying.^{25,26} The function of the fundus is traditionally studied by length-tension studies in muscle strips³⁻⁵ and pressure-volume (barostat) studies of tone and tension.^{6-8,27,28} The length-tension studies have shown differences in passive mechanical behaviour as well as in properties of the various gastric muscle layers. The tension in the pressure-volume studies is computed by applying Laplace's law ($T = P \cdot r/2$). The major assumptions for Laplace's law as used in those studies are that the organ is spherical, very thin-walled, and in static force equilibrium, i.e. the inertial forces are zero. Furthermore, it is assumed that the tension is constant everywhere. Distrutti and co-workers computed the error if the stomach geometry is ellipsoidal rather than spherical²⁹ based on a model applicable to cylindrical

organ.¹¹ However, the gastric fundus has a much more complex geometry than the spherical and the ellipsoidal geometry. Complex geometry indicates that the tension distribution of the stomach is complex. The current study showed that the material properties of the fundus were anisotropic. The comparison of the two models shows that the tension error is very large both in longitudinal (>50%) and circumferential (>25%) direction. Since we only consider two orthogonal directions, the error in other directions may be larger than we have shown here. Therefore, investigations of gastric tone must take into account the complex geometry and the anisotropic biomechanical properties of the gastric wall.

Physiological implications

The analysis of the intact stomach demonstrates that the stretch ratios obtained in the strips experiments were in the low pressure range. Furthermore, the intact stomach data confirmed the strips findings, i.e. that the properties are location- and direction-dependent. As expected the bending angle per unit length decreased when the mechanical load was increased and it was positive in contrast to the bending angles measured at the zero-stress state.

A residual strain gradient existed from the mucosa to the serosa of the gastric wall. This may have implications for the function of the mechanoreceptors (nerve endings of the sensory afferent nerves) in the gastric wall. It is well known that the receptors are located

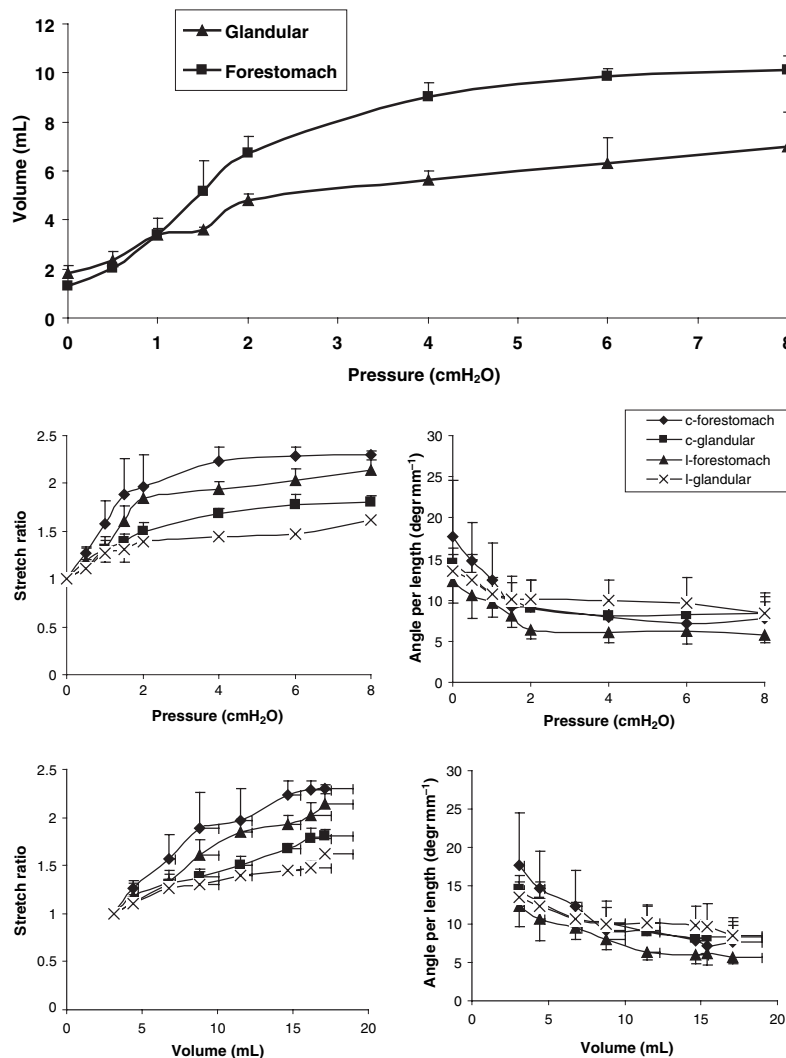


Figure 7 Data from the experiments on the intact stomach *in vitro*. The volume (top figure) and stretch ratio data (left graphs) show that the forestomach is most elastic and that the stretch ratios are direction- and location-dependent. The bending angle per unit length shown in the right graphs decrease as function of the distending load.

both in the submucosa and in the muscle layers. This suggests that the populations of receptors from different locations in the wall may have different zero-settings and respond differently to the stimuli. The mechanoreceptors in the stomach are intra-ganglionic lamina endings (IGLEs) that may function as tension-sensitive endings within the smooth muscle-lined regions of the stomach.^{30–32} Both distension and contraction may activate the mechanoreceptors. Gastric distension triggers vago-vagal reflexes influencing gastric accommodation, activation of antral peristalsis and inhibition of food intake. In addition, gastric distension is also an important trigger for evoking transient relaxations of the lower oesophageal sphincter. The present study (Figs 2 & 4–6) and the classic data by Yamada³³ clearly demonstrate the complex biomechanical behaviour of the stomach. Therefore, during distension the tension varies between locations and

directions in the stomach because of the anisotropic material properties of the stomach.

Pain arising from the stomach is an important clinical problem.³⁴ A large number of studies have focused on gastric tension and sensory responses during distension in animals and humans. Gastric pain is likely mediated by afferent fibres in sympathetic nerves.³⁴ In accordance with studies in other organs the receptors will more likely be directly stimulated by local wall deformation or stress. It is, therefore, likely that the largest contribution to the sensory responses come from regions of the stomach where the stress is high. Hence, determination of the peak stresses and their location are important for understanding the mechanical environment at the receptor sites and the afferent signalling. The stress distributions are related to the geometry at the zero-stress and loaded states, the mechanical load as well as to the material properties.¹² The tension in this study for

the anisotropic model and the isotropic model has an error more than 50% in longitudinal direction and more than 25% in circumferential direction. Therefore, the material properties at different locations and in different direction must be taken into account in studies analysing stress and tension in the stomach and mechanoreceptor-mediated afferent responses. This study is an attempt to provide some of the most basic data for further analysis in 2D and 3D mechanical models.

In conclusion, the biomechanical properties of the gastric strips from the rat and rabbit are location-dependent, direction-dependent and species-dependent. The assumption that the stomach is a sphere with uniform tension is not valid, i.e. a single tension cannot be derived from pressure–volume measurements. Three-dimensional geometric data obtained using medical imaging technology and mechanical data are needed for more detained evaluation of the stomach function.

ACKNOWLEDGMENT

This study was supported by the Karen Elise Jensens Foundation and the Danish Technical Research Council. The technicians Ole Sørensen, Torben Madsen and Jens Sørensen are thanked for handling the animals.

APPENDIX

Comparison between an isotropic model and the anisotropic model

Consider a rectangular plate of sizes L and W , and small thickness h with bi-axial tension. For the isotropic model, Young's modulus and Poisson ratio are E and ν . For the anisotropic model, Young's modulus are E_1 and E_2 and Poisson ratios are ν_1 and ν_2 in the x direction and the y directions. We assume that the deformation in the two models is the same and the length and width in the deformed state are l and w . Hence according to the mechanical theory outlined by Fung,¹⁹ the strains in the x and y directions, ϵ_x, ϵ_y of the two models are identical. Then the tensions in the two models are:

For the isotropic model,

$$T_{x-\text{iso}} = \frac{Eh}{1 - \nu^2} (\epsilon_x + \nu \epsilon_y) \quad (\text{A1})$$

$$T_{y-\text{iso}} = \frac{Eh}{1 - \nu^2} (\epsilon_y + \nu \epsilon_x)$$

For the anisotropic model

$$T_{x-\text{aniso}} = \frac{E_1 h}{1 - \nu_1 \nu_2} (\epsilon_x + \nu_1 \epsilon_y) \quad (\text{A2})$$

$$T_{y-\text{aniso}} = \frac{E_2 h}{1 - \nu_1 \nu_2} (\epsilon_y + \nu_2 \epsilon_x)$$

where the subscript x, y means the x and y direction. iso and aniso mean the isotropic and the anisotropic model. h is the wall thickness.

The relative tension difference between the two models is

$$R_x = \frac{|T_{x-\text{aniso}} - T_{x-\text{iso}}|}{T_{x-\text{aniso}}} \times 100\% \quad (\text{A3})$$

$$R_y = \frac{|T_{y-\text{aniso}} - T_{y-\text{iso}}|}{T_{y-\text{aniso}}} \times 100\%$$

REFERENCES

- 1 Guyton AC and Hall JE. *Textbook of Medical Physiology*, 10th edition. Philadelphia, USA: W.B. Saunders Company, 2000: 718–63.
- 2 Schulze-Delrieu K, Herman RJ, Shirazi SS, Brown BP. Contractions move contents by changing the configuration of the isolated cat stomach. *Am J Physiol* 1998; **274**: G359–69.
- 3 Haffner JFW, Stadaas J. Pressure responses to cholinergic and adrenergic agents in the fundus, corpus, and antrum of isolated rabbit stomachs. *Acta Chir Scand* 1972; **138**: 713–9.
- 4 Milenov K, Golenhofen K. Contractile responses of longitudinal and circular smooth muscle of the canine stomach to prostaglandin E and $F_{2\alpha}$. *Prostaglandins Leukotrienes Med* 1982; **8**: 287–300.
- 5 Schulze-Delrieu K, Shirazi SS. Pressure and length adaptations in isolated cat stomach. *Am J Physiol* 1987; **252**: G92–9.
- 6 Azpiroz F, Malagelada JR. Physiologic variations in canine gastric tone measured by an electronic barostat. *Am J Physiol* 1985; **248**: G229–37.
- 7 Azpiroz F, Malagelada JR. Gastric tone measured by an electronic barostat in health and postsurgical gastroparesis. *Gastroenterology* 1987; **92**: 934–43.
- 8 Whitehead WE, Delvaux M. Working Team. Standardization of barostat procedures for testing smooth tone and sensory thresholds in the gastrointestinal tract. *Dig Dis Sci* 1997; **42**: 223–41.
- 9 Schwizer W, Fraser R, Borovicka J, Asal K, Crelier G, Kunz P, Boesiger P, Fried M. Measurement of proximal and distal gastric motility with magnetic resonance image. *Am J Physiol* 1996; **271**: G217–22.
- 10 Gregersen H, Christensen J. Gastrointestinal tone. *Neurogastroenterol Motil* 2000; **12**: 501–8.
- 11 Gregersen H, Kassab GS. Biomechanics of the gastrointestinal tract. *Neurogastroenterol Motil* 1996; **8**: 277–97.

- 12 Gregersen H. *Biomechanics of the Gastrointestinal Tract. New Perspectives in Motility Research and Diagnostics*. London: Springer-Verlag, 2002: 137–255.
- 13 Egorov VI, Schastlivtsev IV, Prut EV, Baranov AO, Turusov RA. Mechanical properties of the human gastrointestinal tract. *J Biomech* 2002; **35**: 1417–25.
- 14 Gregersen H, Gilja OH, Hausken T, Heimdal A, Gao C, Metre K, Odegaard S, Berstad A. Mechanical properties in the human gastric antrum using B-mode ultrasonography and antral distension. *Am J Physiol* 2002; **283**: G368–75.
- 15 Gao C, Gregersen H. Biomechanical and morphological properties in rat large intestine. *J Biomech* 2000; **33**: 1089–97.
- 16 Gregersen H, Kassab G, Pallencaoe E, Lee C, Chien S, Skalak R, Fung YC. Morphometry and strain distribution in guinea pig duodenum with reference to the zero-stress state. *Am J Physiol* 1997; **273**: G865–74.
- 17 McLaughlin C, Chiasson RB. *Laboratory Anatomy of the Rabbit. Textbook*, 3rd ed. New York: McGraw-Hill Professional Publishing, 1990: **124**.
- 18 Chiasson RB. *Laboratory Anatomy of the White Rat. Textbook*, 5th edn. New York: McGraw-Hill Professional Publishing, 1987: **144**.
- 19 Fung YC. *Biomechanics. Properties of Living Tissues*. Berlin: Springer-Verlag, 1993: 38–40; 399–411.
- 20 Lu X, Gregersen H. Regional distribution of axial strain and circumferential residual strain in the layered rabbit oesophagus. *J Biomech* 2001; **34**: 225–33.
- 21 Zhao JB, Sha H, Zhuang FY, Gregersen H. Morphological properties and residual strain along the small intestine in rats. *World J Gastroenterol* 2002; **8**: 312–7.
- 22 Dou Y, Lu X, Zhao J, Gregersen H. Morphometric and biomechanical remodelling in the intestine after small bowel resection in the rat. *Neurogastroenterol Motil* 2002; **14**: 43–53.
- 23 Gregersen H, Weis SM, McCulloch AD. Oesophageal morphometry and residual strain in a mouse model of osteogenesis imperfecta. *Neurogastroenterol Motil* 2001; **13**: 457–64.
- 24 Yang J, Zhao J, Zeng Y, Vinter-Jensen L, Gregersen H. Morphological properties of the zero-stress state in rat large intestine during systemic EGF treatment. *Dig Dis Sci* 2003; **48**: 442–8.
- 25 Kelly KA. Gastric emptying of liquids and solids: roles of the proximal and distal stomach. *Am J Physiol* 1978; **235**: E552–5.
- 26 Paterson CA, Anvari M, Tougas G, Huizinga JD. Nitrogenic and cholinergic vagal pathways involved in the regulation of canine proximal gastric tone: an in vivo study. *Neurogastroenterol Motil* 2000; **12**: 301–6.
- 27 Kuiken SD, Vergeer M, Heisterkamp SH, Tytgat GN, Boeckxstaens GE. Role of nitric oxide in gastric motor and sensory functions in healthy subjects. *Gut* 2002; **51**: 212–8.
- 28 Penning C, Vu MK, Delemarre JB, Masclee AA. Proximal gastric motor and sensory function in slow transit constipation. *Scand J Gastroenterol* 2001; **36**: 1267–73.
- 29 Distrutti E, Azpiroz F, Soldevilla A, Malagelada JR. Gastric wall tension determines perception of gastric distension. *Gastroenterology* 1999; **116**: 1035–42.
- 30 Noriyuki O, Sengupta JN, Gebhart GF. Mechanosensitive properties of gastric vagal afferent fibers in the rat. *J Neurophysiol* 1999; **82**: 2210–20.
- 31 Page AJ, Martin CM, Blackshaw LA. Vagal mechanoreceptors and chemoreceptors in mouse stomach and esophagus. *J Neurophysiol* 2002; **87**: 2095–103.
- 32 Zagoronyuk VP, Chen BN, Brookes SJH. Intraganglionic laminar endings are mechano-transduction sites of vagal tension receptors in the guinea-pig stomach. *J Physiol* 2001; **534**: 255–69.
- 33 Yamada H, Evans FG. In: Evans FG, ed. *Strength of Biological Materials*. Baltimore, MD: The Williams & Wilkins Co., pp. 155–204.
- 34 Cervero F. Sensory innervation of the viscera: peripheral basis of visceral pain. *Physiol Rev* 1994; **74**: 95–138.