World Journal of Diabetes

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.4239/wjd.v4.i6.339

World J Diabetes 2013 December 15; 4(6): 339-348
ISSN 1948-9358 (online)

ORIGINAL ARTICLE

Changes of phasic and tonic smooth muscle function of jejunum in type 2 diabetic Goto-Kakizaki rats

Jing-Bo Zhao, Peng-Min Chen, Hans Gregersen

Jing-Bo Zhao, Department of Gastroenterology and Surgery, Aalborg University Hospital, DK 9000 Aalborg, Denmark
Peng-Min Chen, Department of Molecular Biology, Institute of Clinical Medicine, China-Japan Friendship Hospital, Beijing 100029, China
Jing-Bo Zhao, Hans Gregersen, The College of Bioengineering, Chongqing University, Chongqing 400044, China
Hans Gregersen, The GIOME Institute, GIOME FZE, Ras Al-Khaimah 6300, United Arab Emirates
Author contributions: Zhao JB and Chen PM designed the research, performed the experiment, analyzed the data and wrote the manuscript; Gregersen H participated in the study design and revised the manuscript and English grammar.
Supported by Karen Elise Jensens Foundation
Correspondence to: Jing-Bo Zhao, Associate Professor, Mech-Sense, Department of Gastroenterology and Surgery, Aalborg University Hospital, Sdr. Skovvej 15, DK9000 Aalborg, Denmark. jz@rn.dk
Telephone: +45-993-26907 Fax: +45-993-26801
Received: May 1, 2013 Revised: September 15, 2013
Accepted: October 17, 2013
Published online: December 15, 2013

Abstract

AIM: To generate phasic and tonic stress-strain curves for evaluation of intestinal smooth muscle function in type 2 diabetic rats during active and passive conditions.

METHODS: Seven diabetic Goto-Kakizaki (GK) male rats, 32-wk old (GK group), and 9 age-matched normal Wistar rats (Normal group) were included in the study. Jejunal segments were distended up to a pressure of 10 cm H_2O in an organ bath containing 37 ℃ Krebs solution with addition of carbachol (CA). The pressure and outer diameter changes were synchronously recorded. Passive conditions were obtained using calcium-free Krebs solution containing ethylene glycol tetraacetic acid and papaverine. Total phasic, tonic and passive circumferential stress and strain were computed from the diameter and pressure data with reference to the zero-stress state geometry. The active phasic and tonic stresses were defined as the total phasic and tonic stresses minus the passive stress.

RESULTS: Diabetes increased jejunal mucosa and muscle layer thicknesses compared to the Normal group (mucosa, 755.8 ± 63.3 *vs* 633.1 ± 59.1 μm, $P < 0.01$; muscle, 106.3 ± 12.9 *vs* 85.2 ± 11.7 μm, $P < 0.05$). The pressure and stress thresholds were decreased in the GK group after CA application compared to distensions without CA application (pressure, 1.01 ± 0.07 *vs* 1.99 ± 0.19 cmH_2O, $P < 0.01$; stress, 0.11 ± 0.01 *vs* 0.24 ± 0.02 kPa, $P < 0.01$). CA application did not change the pressure and stress threshold in the Normal group (pressure, 2.13 ± 0.32 *vs* 2.34 ± 0.32 cm H_2O, $P > 0.05$; stress, 0.25 ± 0.03 *vs* 0.35 ± 0.06 kPa, $P > 0.05$). The amplitude of total phasic, total tonic, active phasic and active tonic circumferential stresses did not differ for the distensions without CA application between the GK group and the Normal group. However, the total phasic and total tonic stresses increased after CA application in the GK group compared those in the Normal group. When normalized to muscle layer thickness, the amplitude of active stresses before CA application was lowest in the GK group compared with the Normal group. No difference was found during CA application.

CONCLUSION: The stress generated by intestinal muscle normalized to the muscle layer thickness was lowest in GK rats compared to normal rats whereas the response to CA stimulation was preserved.

Key words: Intestine; Diabetes; Muscle function; Stress-strain curves; Carbachol; Rat

Core tip: Length-tension diagrams of smooth muscle strips were obtained in intact segment of intestine *in*

vitro in the present study. We demonstrated that it is a valid tool to evaluate smooth muscle function in intact intestine. Diabetes decreased the force (stress) generated by the smooth muscle normalized to the muscle layer thickness. Since the stress decreased and the muscle layer thickness increased in diabetic rats, it indicates that the intestine, at least in part, remodels in a stress-dependent way. Furthermore, the smooth muscle in Goto-Kakizaki diabetic intestine preserved its response to carbachol stimulation.

Zhao JB, Chen PM, Gregersen H. Changes of phasic and tonic smooth muscle function of jejunum in type 2 diabetic Goto-Kakizaki rats. *World J Diabetes* 2013; 4(6): 339-348 Available from: URL: http://www.wjgnet.com/1948-9358/full/v4/i6/339.htm DOI: http://dx.doi.org/10.4239/wjd.v4.i6.339

INTRODUCTION

Gastrointestinal (GI) disorders are quite common in diabetic patients[1]. Diabetes can affect the entire GI tract including the small intestine. Intestinal motility disorders are common in diabetic patients[1] and diabetic animals[2]. Delayed as well as rapid small intestinal transit was observed in diabetic animal models[3-6]. Small intestinal transit disorders have also been demonstrated in diabetic patients[7-13]. Camilleri and Malagelada[14] reported that intestinal motility was abnormal in about 80% patients with long-standing diabetes with delayed gastric emptying. Both postprandial and fasting intestinal motility disorders were reported in diabetic patients[15,16].

The pathogenesis of intestinal motility disorders in diabetes is complex in nature, multi-factorial (smooth muscle dysfunction, mechano-sensory disorders, autonomic neuropathy, lack of glycemic control, *etc.*) and is not well understood[1]. Our previous studies have shown that the morphological and biomechanical properties of GI tract are altered in type-1 diabetic patients[17] and animals[18-20]. Similar remodeling was also demonstrated in the esophagus[21], stomach[22] and intestine[23] in type-2 diabetic animals. Intestinal morphometric and biomechanical remodeling caused by diabetes may change the smooth muscle function, resulting in altered intestinal motility[20]. However, to the best of our knowledge, contraction thresholds and muscle mechanical properties described in terms of stress and strain in the diabetic intestine have not yet been reported.

It is possible to obtain isometric length-tension diagrams of phasic and tonic smooth muscle contractions *in vitro*[24]. Tools have now been developed for studying the active (phasic and tonic contractions) and passive length-tension behavior in the human gut *in vivo* using impedance planimetric distension[25-27]. From a biomechanical standpoint, muscle mechanical properties must be described in terms of stress and strain, *i.e.*, the force per area and tissue deformation. Computation of the stress depends on the wall thickness which can not be directly measured *in vivo*. However, it is possible to measure the wall thickness *in vitro* and thus to obtain the stress-strain relationship of the intestinal wall with reference to the zero-stress state[28,29]. Hence tonic and phasic stress-strain curves of intestinal contractions can be obtained and by using calcium-free solution containing ethylene glycol tetraacetic acid (EGTA) and papaverine to abolish smooth muscle contractions the passive properties can be obtained as well[28,29].

Carbachol (CA) is a parasympathomimetic drug that directly stimulates cholinergic receptors[30]. It may also act indirectly by promoting release of acetylcholine and by a weak anticholinesterase action[31]. In the present study the intestinal contraction thresholds and the stress-strain curves of smooth muscle contraction were also investigated in response to CA application.

The hypothesis was that the intestinal contraction thresholds and muscle contraction stress-strain curves are altered due to the tissue remodeling induced by diabetes. The aim of this study was to compute stress-strain data for assessment of intestinal smooth muscle function in normal and Goto-Kakizaki (GK) diabetic rats. The contraction threshold and the contraction stress-strain curve in normal and GK diabetic rats during and without CA application are compared.

MATERIALS AND METHODS

Animals and groups

Seven inherited type 2 diabetic rats (Goto-Kakizaki rats, GK group, 12-wk old, weighing 330 g) were purchased from Taconic Europe DK-8680 Ry, Denmark. Nine age-matched normal rats (same strain as the GK rats) served as controls (Normal group). The rats lived for 32 wk with free access to tap water and food. The body weight was measured on a weekly basis from birth. The fasting glucose level was measured every second weeks from 16 wk after birth and at the day where the experiment was done. Approval of the protocol was obtained from the Danish Committee for Animal Experimentation.

In vitro intestinal preparation

When the scheduled time had arrived, the rats were fasted overnight and then anesthetized with Hypnorm 0.5 mg and Dormicum 0.25 mg per 100 g body weight. The abdominal cavity was opened and a 10-cm-long jejunal segment from 5 cm distal to the ligament of Treitz was harvested. The residual contents in the lumen were gently cleared using physiological saline. One-cm-long specimens were cut from each end of the segment and fixed in 10% formalin for histological examination. Two tissue rings from the proximal end of the segment were cut and used for no-load state and zero-stress state analysis. The remaining segment was inserted into the organ bath containing Krebs solution of the following composition (mmol/L): NaCl, 118; KCl, 4.7; $NaHCO_3$, 25; NaH_2PO_4, 1.0; $MgCl_2$, 1.2; $CaCl_2$-H_2O, 2.5; glucose, 11; ascorbic acid, 0.11 as soon as possible. The Krebs solution was 37 ℃ aerated with a gas mixture (95% O_2 and 5% CO_2,

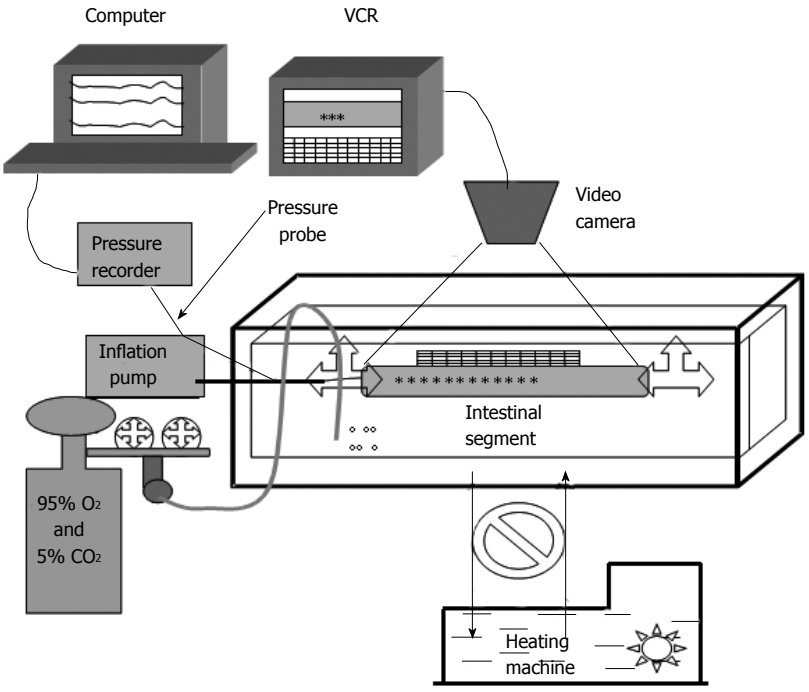

Figure 1 Experimental set-up. The organ bath is composed of an inside chamber and an outside chamber. The Krebs solution contained in the small chamber was maintained constant at 37 ℃ by circulating hot water in the big chamber using a heating machine. The intestinal segment was placed in the small organ bath in the Krebs solution. The intestinal distension was applied by a pump and a pressure probe was used to measure the pressures. The diameter changes of the intestinal segments were videotaped through a stereomicroscope.

pH 7.4). Thirty minutes equilibrating time was needed for recovery of the motility.

Ramp distension experimental set-up

The experimental set-up is shown in Figure 1. The proximal and distal ends of each jejunal segment were tied with silk threads onto two cannulas fixed on two sides inside the organ bath. The resting length of the segment (as measured *in vivo* before excising the segment) was adjusted between two cannulas. The proximal cannula was connected *via* a tube to a fluid container containing the same Krebs solution as mention above. Ramp distension were done using a pump (Genie Programmable Syringe Pump, World Precision Instrument, Stevenage, United Kingdom). After two preconditioning cycles (inflation from 0 to 10 cm H_2O and back again), a ramp distension (0-10 cm H_2O) was done with closed outlet. The intestinal diameters corresponding to pressure recordings in the intestinal segments were videotaped by a CCD camera (Sony, Japan) through a stereomicroscope during the ditensions. The pressure and diameter data acquisition was done at 10 frames per second.

Three minutes after finishing the experiment above, the cholinergic agonist CA (final concentration: 10^{-5} mol) was applied to the organ bath. The same procedure as described above was repeated. Then the Krebs solution was replaced by calcium-free Krebs solution containing 0.4% EGTA and 2 mg papaverine in order to abolish smooth muscle contractions. The same protocol as described above was repeated again. The papaverine causes elevation of cyclic adenosine monophosphate levels; alter mitochondrial respiration and inhibit calcium influx by inhibiting enzyme phosphodiesterase.

Zero-stress state of the intestinal segment

The method for determination of the intestinal zero-stress state has been described in detail previously[19,32]. One-two millimetre wide intestinal rings were transferred to calcium-free Krebs solution containing EGTA and papaverine. A photograph was taken of the cross-section of the rings in the no-load state. Then, each ring was cut radially under the microscope resulting in an open sector geometry. Photographs of the zero-stress state were taken approximately 60 min after the radial cutting to allow viscoelastic creep to occur.

Histological analysis of the small intestine

The intestinal segment was fixed in 10% buffered formalin over 24 h followed by dehydration in a series of graded ethanol (70%, 96% and 99%) and embedding in paraffin. Five-micron sections were cut perpendicular to the mucosa surface and the paraffin was cleared from the slides with coconut oil (over 15 min, 60 ℃). Redehydration was done in 99%, 96% and 70% ethanol followed by staining with hematoxylin and eosin. The layer thickness was measured by the same pathologist in a blinded review.

Mechanical data analysis

Calculation of mechanical parameters was based on the assessment of the no-load state, zero-stress state dimensions and the outer diameters of the specimen at varying pressures[32]. The Kirchhoff's stress and Green's strain in the intestinal wall at a given pressure were computed assuming circular geometry as:

$$\text{Circumferential Kirchhoff's stress: } S_\theta = \frac{\Delta P r_{i,p}}{h_p \lambda_\theta^2} \quad (1)$$

$$\text{Circumferential midwall Green's strain: } E_\theta = \frac{\lambda_\theta^2 - 1}{2} \quad (2)$$

where ΔP is the transmural pressure difference, r is the luminal radius; h is the wall thickness; and λ_θ is the cir-

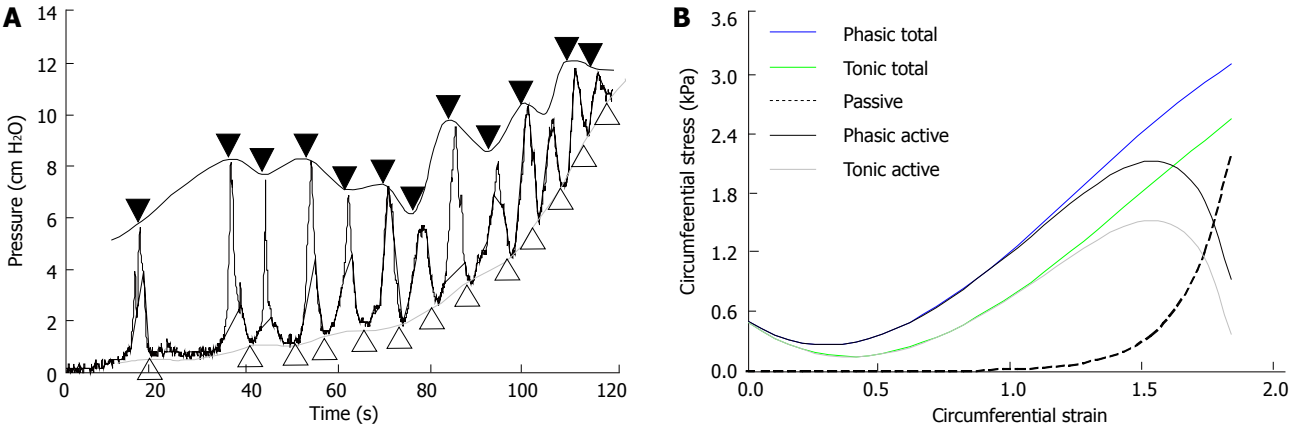

Figure 2 Pressure-diameter curve used for set-up maximum and baseline of contraction, and example of stress-strain curves. A: Maximum amplitudes during contraction and the baseline between the contractions during distension. The closed symbols above the curve mark the phasic part (—). The open symbols under the curve mark the tonic part (—). The pressures from the phasic and tonic parts were used to compute the total phasic and tonic stresses; B: An example of passive, total phasic, total tonic, phasic active and tonic active stresses as function of strains from a normal animal. The passive stress increased exponentially as function of strain, whereas the total phasic and tonic stresses increased in a polynomial way. The phasic and tonic active stresses were obtained by the total phasic and tonic stresses minus the passive stress.

cumferential stretch ratio. Pressure, stress and strain data exactly when the contraction started were used to analyze the contraction thresholds.

The total phasic and the total tonic stresses (both composed of active and passive tissue properties) were extracted from the top points (maximum pressure during the contraction) and the baseline pressure between contractions during the distension (Figure 2A). The passive stress was extracted from the data obtained during distension in the calcium-free Krebs solution with EGTA and papaverine. Since strain was not controlled, it was not possible to directly compare strain between different samples and groups. Therefore, curve fitting was applied and strain points were selected for comparison between samples and groups.

The passive stresses increased in an exponential-like way as function of strain. Consequently the passive stress-strain curves were fitted to the exponential function equation

$$S = (S^* + b)\, e^{a\,(E - E^*)} - b. \quad (3)$$

where S^* and E^* are the stress and strain at a physiological reference level. The total phasic and tonic stress-strain curves increased in a polynomial way as function of strain. Consequently the stress-strain curves were fitted to the polynomial equation

$$S = S_0 + a_1E^3 + a_2E^2 + a_3E^1 \quad (4)$$

where a_1, a_2 and a_3 are constants. Total stresses and active stresses (see below) were calculated both for distensions before and during CA application. The active phasic and tonic stresses were defined as the total phasic and tonic stresses minus the passive stress (Figure 2B).

$$\textit{Active phasic stress} = \textit{total phasic stress} - \textit{passive stress} \quad (5)$$
$$\textit{Active tonic stress} = \textit{total tonic stress} - \textit{passive stress} \quad (6)$$

The active phasic and tonic stresses were normalized to muscle layer thickness as:

$$\textit{Normalized active phasic stress} = \textit{active phasic stress}/\textit{muscle thickness} \quad (7)$$

$$\textit{Normalized active tonic stress} = \textit{active tonic stress}/\textit{muscle thickness} \quad (8)$$

The muscle layer thickness (μm) was obtained from histological measurement.

Statistical analysis

The results were expressed as mean ± SEM unless indicated otherwise. The total phasic, total tonic, active phasic and active tonic stresses were compared between the GK and the Normal groups by analysis of variance and *t* test analyses. The normalized active phasic and tonic stresses as function of strain were also compared between the GK and the Normal groups. The results were regarded as significant when $P < 0.05$.

RESULTS

General data

The body weight and blood glucose level were significantly higher in the GK group than those in the Normal group during the whole experimental period ($P < 0.01$, Table 1). The histological data are presented in Figure 3. Top figure represents examples of muscle layer thickness in the normal (Figure 3A) and GK (Figure 3B) rats. Compared with the normal rats, the muscle layer thickness was bigger in the GK rats. Bottom figure showed different layer thickness of jejunum between Normal and GK groups (Figure 3C). The villous height and circumferential muscle layer thickness of jejunal segments were higher in the GK group compared with the Normal group (villous height, 571.3 ± 34.8 μm *vs* 475.1 ± 44.1 μm, $P < 0.01$; circumferential muscle, 68.9 ± 11.9

Table 1 Body weight and blood glucose level

	Group	Rat age (wk)							
		18	20	22	24	26	28	30	32
Body weight (g)	GK	390.6 ± 20.2[b]	408.1 ± 19.4[b]	421.1 ± 22.1[b]	431.7 ± 23.2[b]	427.5 ± 24.3[b]	436.1 ± 22.5[b]	438.4 ± 21.6	443.5 ± 20.5[b]
	Normal	350.3 ± 16.4	363.3 ± 17.3	371.3 ± 12.5	378.2 ± 15.1	386.8 ± 14.6	391.2 ± 14.3	397.3 ± 13.7	400.6 ± 17.2
Glucose level (mmol/L)	GK	8.89 ± 1.07[a]	8.75 ± 0.32[a]	9.15 ± 1.12[a]	8.97 ± 0.42[a]	8.86 ± 0.36[a]	7.71 ± 0.44[a]	7.92 ± 0.37[a]	8.31 ± 0.38[a]
	Normal	5.91 ± 0.43	6.06 ± 0.29	5.99 ± 0.57	5.93 ± 0.39	6.17 ± 0.54	5.57 ± 0.55	5.55 ± 0.36	5.75 ± 0.48

The body weight and blood glucose level of GK group were significantly higher than those of normal group during the whole experimental period. Compared with normal group, [a]$P < 0.05$, [b]$P < 0.01$. GK: Goto-Kakizaki.

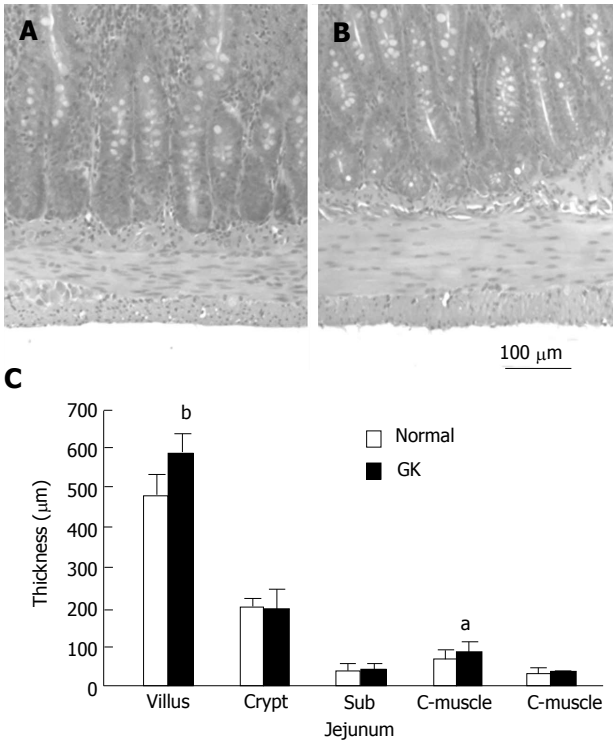

Figure 3 Histological dada. The top figure illustrates the example of jejunal muscle layer in Normal (A) and GK (B) rats. The increasing muscle layer thickness was noted in GK diabetic rat. The bottom figure (C) shows the jejunal layer thickness in two different groups. The villous height and circumferential muscle layer thickness were significantly bigger in GK group (Compared with normal group: [a]$P < 0.05$, [b]$P < 0.01$). GK: Goto-Kakizaki.

um *vs* 57.1 ± 6.4 um, $P < 0.05$). No significant difference was found for other layers ($P > 0.05$).

Pressure-diameter curves

Figure 4 illustrates ramp distension tracings of the pressure and diameter of jejunal segment in a normal rat with and without CA application. Waves of peristaltic contraction were clearly observed both from pressure and diameter curves (Figure 4A and B). The pressure increased whereas the diameter decreased during each contraction wave. Contractions were stronger after CA application (Figure 4B). Peristaltic contractions were not observed during distensions in calcium-free Krebs solution containing EGTA and papaverine (Figure 4C).

Figure 5 shows jejunal ramp distension pressure-diameter curves during CA application in normal (A) and GK diabetic (B) rats. The peak contraction amplitude is

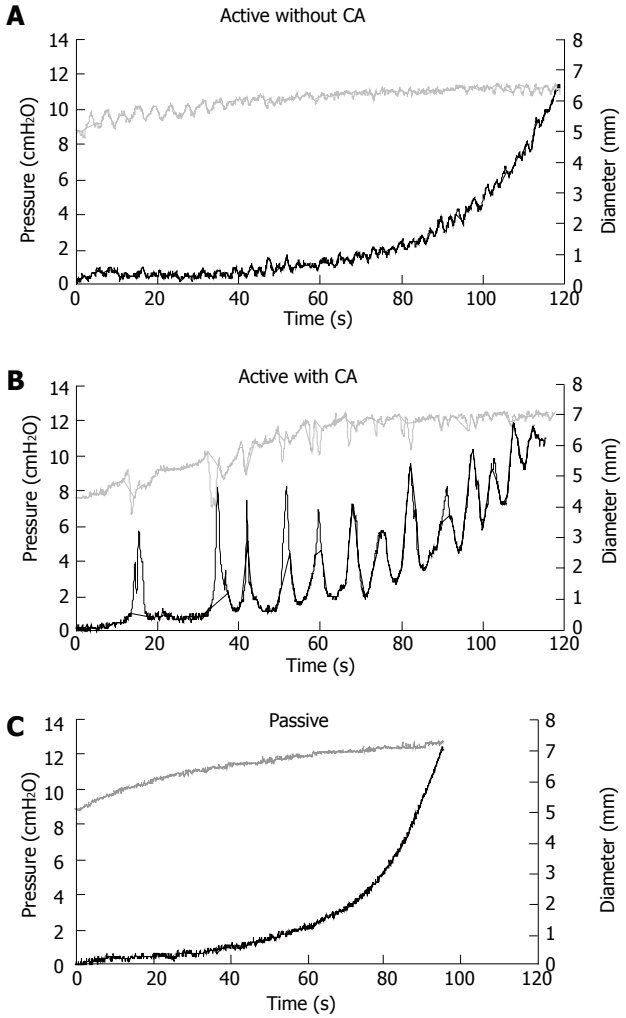

Figure 4 Examples of pressure-diameter curves. Illustration of ramp distension curves of the pressure and diameter of jejunal segment with and without CA application obtained from a Normal rat. Waves of peristaltic contraction were clearly observed (top and middle). The smooth muscle contraction was abolished by papaverine (bottom). The CA application enhanced the peristaltic contraction (middle). CA: Carbachol.

higher in the GK diabetic rat than in the normal rat.

Contraction thresholds

The pressures (A), stresses (B) and strains (C) at the contraction threshold from the GK and Normal groups are shown in Figure 6. During CA application in the GK group, the pressure and stress thresholds were lower compared to the distensions before CA application (pressure threshold,

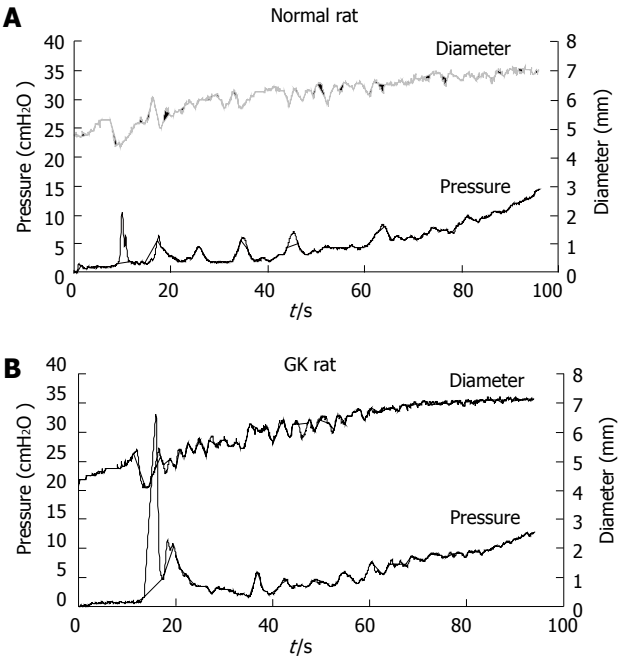

Figure 5 Pressure-diameter curves from normal and goto-Kakizaki groups. Illustration of ramp distension curves of the pressure and diameter of jejunal segment with carbachol application obtained from a Normal (top) and a GK diabetic (bottom) rats. Higher peak of contraction was observed in the GK diabetic rat. CA: Carbachol; GK: Goto-Kakizaki.

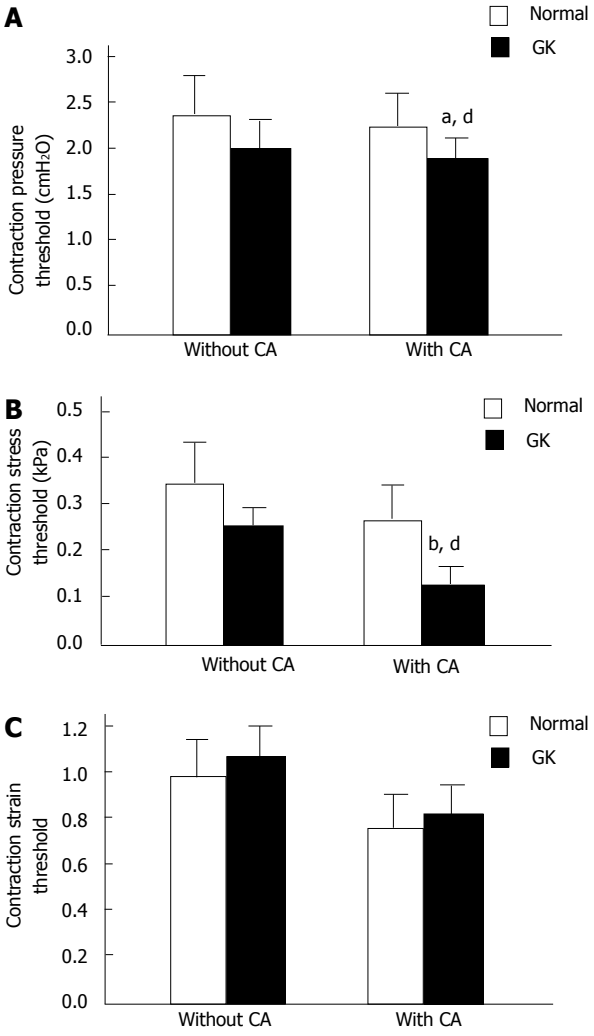

Figure 6 Contraction thresholds. Illustration of the pressures (top), stresses (middle) and strains (bottom) at the contraction threshold in two different groups. The pressure and stress thresholds were significantly decreased in GK group after CA stimulation (compared with Normal group, pressure threshold, $^{a}P < 0.05$; stress threshold, $^{b}P < 0.01$). Furthermore, the pressure and stress thresholds were significantly decreased in GK group with CA application (compared with without CA, $^{d}P < 0.01$) but not in Normal group ($P > 0.05$). The strain at the contraction threshold did not differ between two different groups ($P > 0.05$) and between before and after CA application ($P > 0.05$). CA: Carbachol; GK: Goto-Kakizaki.

1.01 ± 0.07 *vs* 1.99 ± 0.19 cm H_2O, $F = 14.870$, $P = 0.003$; stress threshold, 0.11 ± 0.01 *vs* 0.24 ± 0.02 kPa, $F = 16.474$, $P = 002$). In the Normal group no difference was found before and during CA application ($P > 0.05$). Compared with the Normal group, the pressure and stress thresholds were lowest in the GK group both for distensions before and during CA application. Significant difference was found during CA stimulation (GK group compared with Normal group, pressure threshold, 1.01 ± 0.07 *vs* 2.13 ± 0.32 cm H_2O, $F = 7.767$, $P = 0.015$; and stress threshold, 0.11 ± 0.01 *vs* 0.25 ± 0.03 kPa, $F = 13.624$, $P = 0.003$). The strain at the contraction threshold did not differ between the GK and Normal groups ($P > 0.05$), nor did strain at the contraction threshold differ before and during CA application in either of the two groups ($P > 0.05$).

Analysis of tonic and phasic stress-strain curves

Total phasic (A), total tonic (B), active phasic (C) and active tonic (D) stresses as function of strains from GK and Normal groups before and during CA application are shown in Figure 7. The amplitude of total phasic, total tonic, active phasic and active tonic circumferential stresses did not differ before CA application. The total phasic and tonic stresses but not active phasic and tonic stresses increased during CA application in the GK group compared with those obtained in the Normal group (total phasic stress, 4.56 ± 0.68 kPa *vs* 3.74 ± 0.47 kPa, $P < 0.05$; total tonic stress, 3.42 ± 0.56 kPa *vs* 2.92 ± 0.34 kPa, $P < 0.05$). Furthermore, the maximum active phasic and active tonic stresses differed during CA application (2.51 ± 0.45 kPa *vs* 1.42 ± 0.27 kPa, $P < 0.01$).

The normalized active phasic (A) and tonic stresses (B) as function of strains are shown in Figure 8. When normalized to muscle layer thickness, the amplitude of active phasic and tonic stresses were significantly lower before CA application in the GK group compared with the Normal group (normalized active phasic stress, 0.0164 ± 0.0018 kPa/μm *vs* 0.0254 ± 0.0013 kPa/μm, $P < 0.05$; normalized active tonic stress, 0.0103 kPa/μm ± 0.0014 *vs* 0.0187 ± 0.0012 kPa/μm, $P < 0.05$) whereas they did not differ during CA application (normalized active phasic stress, 0.0261 ± 0.0047 kPa/μm *vs* 0.0292 ± 0.0036 kPa/μm, $P > 0.05$; normalized active tonic stress, 0.0148 ± 0.0028 kPa *vs* 0.0186 ± 0.0017 kPa, $P > 0.05$).

DISCUSSION

The length-tension diagrams known from physiological and pharmacological studies of smooth muscle strips *in*

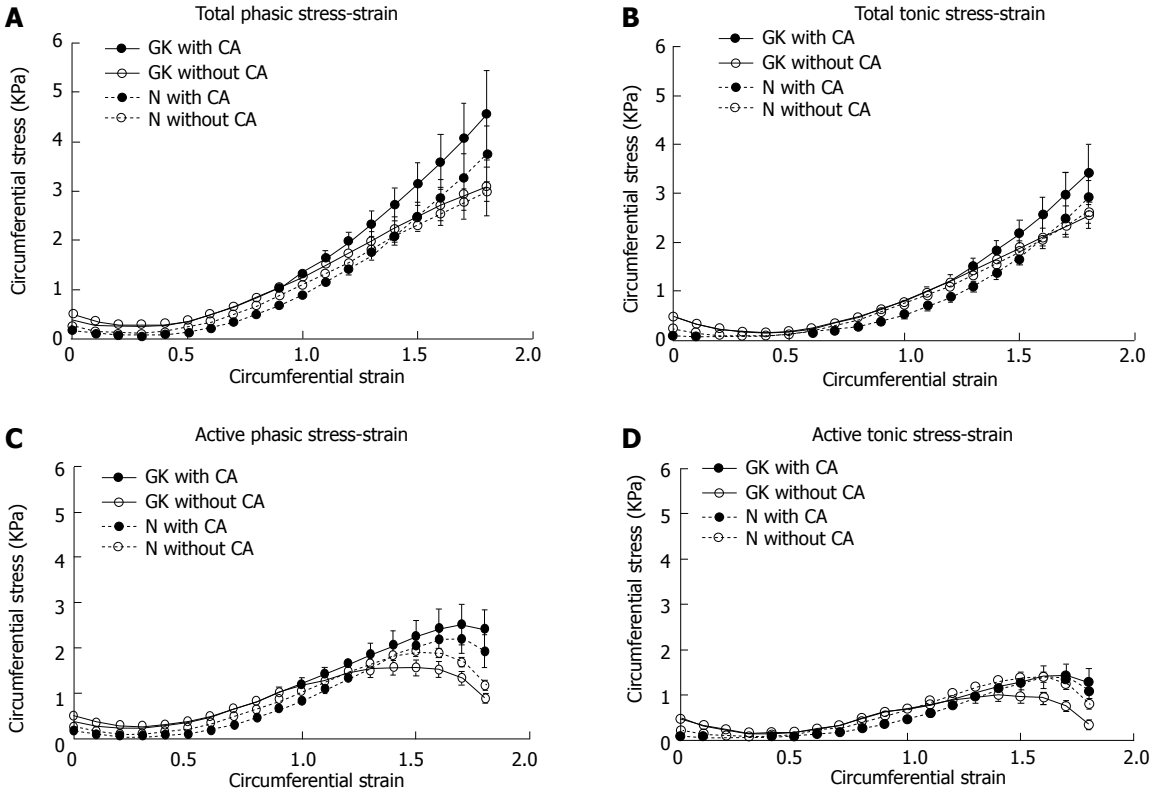

Figure 7 Phasic and tonic stresses as function of strains. The total phasic (A) and tonic stresses (B) as function of strains are presented in the top and the computed active phasic (C) and tonic stresses (D) as function of strain in the bottom. The amplitude of total phasic, total tonic, active phasic and active tonic circumferential stresses did not differ without CA application but significantly increased after CA application in GK group compared those with Normal group ($P < 0.05$). Furthermore, the maximum for the active phasic and active tonic stresses differed ($P < 0.01$). CA: Carbachol; GK: Goto-Kakizaki.

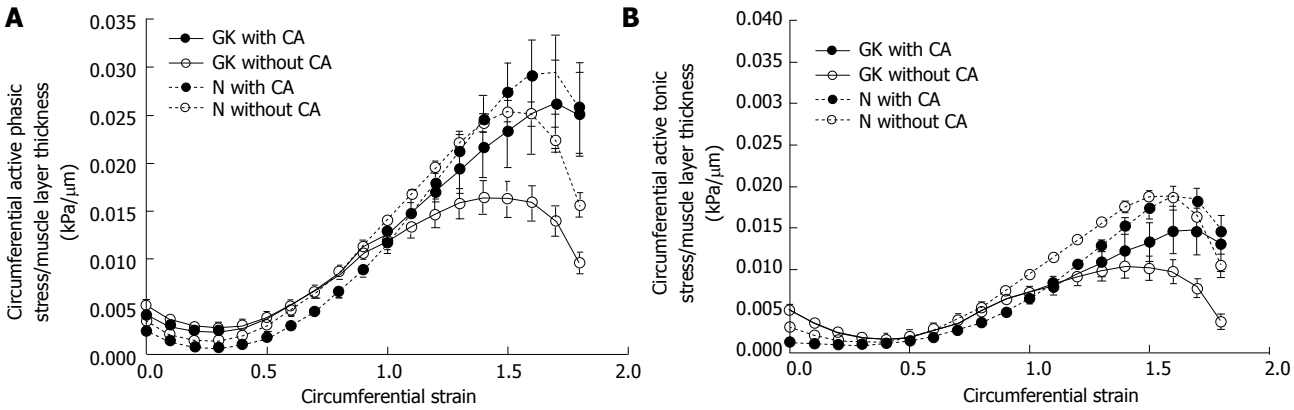

Figure 8 Normalized active phasic and tonic stresses as function of strains. The normalized active phasic (top) and tonic stresses (bottom) illustrated as function of strains. When normalized to muscle layer thickness, the amplitude of active stresses significantly decreased without CA application ($P < 0.05$) and did not differ after CA application ($P > 0.05$). CA: Carbachol.

vitro[25,33] can be reproduced in intact segment of intestine *in vitro* as shown in the present and previous studies[28,29]. The main findings of the current study were that the amplitudes of total phasic, total tonic, active phasic and active tonic circumferential stresses did not differ before CA application but the total phasic and total tonic stresses increased during CA application in GK diabetic rats. However, when normalized to muscle layer thickness, the active stress amplitudes decreased before CA application but did not differ during CA application in the GK diabetic rats. Furthermore, the pressure and stress thresholds were significantly lower in the GK group compared with the Normal group during CA application.

The mechanical properties of the small intestine can be divided into passive properties arising from connective tissue element, tonic active properties reflecting baseline muscle activity, and phasic active property reflecting the contractile forces of distension-induced neuromuscular function. The passive and the active stress-

strain curves depend on the wall structure, the wall mechanical properties and the smooth muscle contractile properties. Recent studies demonstrated that histological and biomechanical remodeling of the small intestine, such as residual strain changes and intestinal wall stiffening, occurred in diabetic patients[17] and animals[19,20,23,34]. Such diabetes-induced remodeling may affect intestinal smooth muscle contractility.

Many human[1,35] and animal[19,20,35-37] studies have shown that diabetes causes structural changes of intestinal smooth muscle. Furthermore, several studies have demonstrated that function of intestinal smooth muscle also changed due to diabetes[36,38-42]. There are many ways to study smooth muscle contractility[43]. However from biomechanical point of view, muscle mechanical properties are best described in terms of stress and strain, *i.e.*, the force per area and tissue deformation[28,29]. Therefore, stress-strain data are important for understanding the mechanical function of remodeled smooth muscle in the diabetic intestine. Length-tension diagrams have been derived from the human gastric antrum and duodenum[32,44], although butylscopolamine may not have abolished all phasic activity when analyzing the passive properties. The present and previous[28,29] *in vitro* studies produced tonic and phasic stress-strain curves referenced to the passive stress-strain curve because papaverine completely abolished all smooth muscle activity. Computation of the stress depends on the wall thickness which cannot easily be measured *in vivo*. However it can be measured *in vitro*.

In the present study the thicknesses of intestinal mucosa layer and muscle layer increased in diabetic rats. The increase in the muscle layer thickness is mainly due to circumferential muscle layer thickening and like caused by the increased circumferential stress. Before CA application, stress-strain data analysis of the contraction showed that the amplitude of total phasic, total tonic, active phasic and active tonic circumferential stresses as function of strains did not differ between diabetic and normal rats. However when normalized to muscle layer thickness, the amplitude of active phasic and tonic stresses significantly decreased. It indicates that the remodeled smooth muscle cells may be somehow altered due to long-term diabetes. The information regarding the direct effects of diabetes on ultrastructural changes of intestinal smooth muscle cell is very limited. However, one report indicated that streptozotocin-induced diabetes caused ultra-structural changes of venous and arterial smooth muscle cells which may account for the specific vascular complications[45]. Such changes may also occur in the diabetic intestinal smooth muscle and account for decreasing active contraction force per unit. This is needed to further study. However, the decreasing active contraction force per unit is likely to be compensated by increasing muscle layer thickness which is regulated in stress-dependent way. Furthermore, it is worthwhile to notice that the maximum of active phasic and active tonic stresses differ. This probably means that they are regulated in different ways.

It is interesting at the present study to notice that after CA application, the amplitude of total phasic and total tonic stresses increased in the GK diabetic rats and the decreased amplitude of active stresses normalized to muscle layer thickness in the GK diabetic rats was corrected. Furthermore, the pressure and stress thresholds were significantly smaller in GK group compared with Normal group after CA application. All these together indicate that the intestinal muscle in the diabetic GK rats preserved their responses to the CA stimulation or even have higher sensitivity to CA application. It is well known that CA can directly stimulate muscarinic and nicotinic cholinergic receptors[30]. Therefore the receptor density likely has important impact on the GK diabetic intestinal smooth muscle cells. Although no direct evident was obtained at present study, it was in one previous study demonstrated that the detrusor supersensitivity was observed after only 1 wk of untreated STZ-induced diabetes in the rat. The overactivity was associated with an enhanced sensitivity to carbachol, which could be partly explained by an increase in receptor density[46]. It maybe the same case in the GK diabetic intestine and need to identify in the future. But the controversial results were also reported on diabetic colon[47] and gastric antrum[48]. The former study has been shown that the contractility of the proximal colon in response to CA was weaker in the diabetic rats[47]. The later study demonstrated that gastric antral smooth muscle cells from streptozotocin-induced rats and db/db spontaneously diabetic mice impair their contractile response to CA[48]. These controversial results may indicate the different regions of gastrointestinal tract in the diabetes have different reaction to the CA stimulation.

In summary, the force (stress) generated by the smooth muscle per unit was decreased in GK diabetic rats and it is likely to be compensated by smooth muscle proliferation. It is indicating that the intestine remodels in a stress-dependent way. Furthermore, the smooth muscle in GK diabetic intestine preserves its response to the stimulation of CA same as the smooth muscle in the normal small intestine.

ACKNOWLEDGMENTS

The technicians Ole Sørensen, Torben Madsen and Jens Sørensen are thanked for handling the animals.

COMMENTS

Background

The intestinal motility disorders have been found both in the diabetic patients and animals. However, the pathogenesis of intestinal motility disorders in diabetes mellitus is complex and is not well understood. The morphological and biomechanical remodeling of gastrointestinal tract occurred in the diabetes which may change the smooth muscle function, and then affect the intestinal motility.

Research frontiers

There are many ways to study smooth muscle contractility, however from biomechanical point of view; muscle mechanical properties are better to be

described in terms of stress and strain, *i.e.*, the force per area and tissue deformation. Therefore, stress-strain data are important for understanding the mechanical function of remodeled smooth muscle in the diabetic intestine.

Innovations and breakthroughs

The present *in vitro* study produced tonic and phasic stress-strain curves referenced to the passive stress-strain curve because papaverine completely abolished all smooth muscle activity. Computation of the stress depends on the wall thickness which cannot be directly measured *in vivo*. However it can be measured *in vitro*. Furthermore, the authors use carbachol as stimulator which is a parasympathomimetic drug that directly stimulates cholinergic receptors.

Applications

The mechanical properties of the small intestine can be divided into passive property, tonic active property and phasic active property. By understanding how the phasic and tonic properties of intestinal smooth muscle are changed in the diabetes, this study may represent a future strategy for studying the intestinal smooth function in different diseases.

Terminology

Stress is the force per area and strain is the tissue deformation. Stress-strain data are important for understanding the mechanical function of smooth muscle. Carbachol is a parasympathomimetic drug that directly stimulates muscarinic and nicotinic cholinergic receptors. The receptor density has important impact on the function of smooth muscle cells.

Peer review

The main goal of this paper was to examine whether the function of jejunal smooth muscle was altered during diabetes. To do this, the authors proposed to generate phasic and tonic stress-strains curves in normal Wistar and diabetic rats before and after parasympathetic stimulation with carbachol. The experimental plan seems to be sound and follows a logical progression.

REFERENCES

1 **Horowitz M,** Samsom M. Gastrointestinal function in diabetes mellitus. Chichester, England: John Wiley & Sons, Ltd, 2004: 1-349
2 **Yamamoto T**, Watabe K, Nakahara M, Ogiyama H, Kiyohara T, Tsutsui S, Tamura S, Shinomura Y, Hayashi N. Disturbed gastrointestinal motility and decreased interstitial cells of Cajal in diabetic db/db mice. *J Gastroenterol Hepatol* 2008; **23**: 660-667 [PMID: 18341539 DOI: 10.1111/j.1440-1746.2008.05326.x]
3 **Chang FY**, Lee SD, Yeh GH, Wang PS. Hyperglycaemia is responsible for the inhibited gastrointestinal transit in the early diabetic rat. *Acta Physiol Scand* 1995; **155**: 457-462 [PMID: 8719265 DOI: 10.1111/j.1748-1716.1995.tb09995.x]
4 **Kumar MS**, Prashanth KV. alpha-Lipoic acid ameliorates altered colonic contractility and intestinal transit in STZ-diabetic rats. *Indian J Exp Biol* 2004; **42**: 279-282 [PMID: 15233297]
5 **El-Salhy M**. Gastrointestinal transit in nonobese diabetic mouse: an animal model of human diabetes type 1. *J Diabetes Complications* 2001; **15**: 277-284 [PMID: 11561557 DOI: 10.1016/S1056-8727(01)00158-1]
6 **El-Salhy M**. Gastrointestinal transit in an animal model of human diabetes type 2: relationship to gut neuroendocrine peptide contents. *Ups J Med Sci* 2002; **107**: 101-110 [PMID: 12602782 DOI: 10.3109/2000-1967-133]
7 **Scarpello JH**, Greaves M, Sladen GE. Small intestinal transit in diabetics. *Br Med J* 1976; **2**: 1225-1226 [PMID: 990859 DOI: 10.1136/bmj.2.6046.1225]
8 **de Boer SY**, Masclee AA, lam WF, Schipper J, Jansen JB, Lamers CB. Hyperglycemia modulates gallbladder motility and small intestinal transit time in man. *Dig Dis Sci* 1993; **38**: 2228-2235 [PMID: 8261826 DOI: 10.1007/BF01299901]
9 **Iida M**, Ikeda M, Kishimoto M, Tsujino T, Kaneto H, Matsuhisa M, Kajimoto Y, Watarai T, Yamasaki Y, Hori M. Evaluation of gut motility in type II diabetes by the radiopaque marker method. *J Gastroenterol Hepatol* 2000; **15**: 381-385 [PMID: 10824881 DOI: 10.1046/j.1440-1746.2000.02076.x]
10 **Kawagishi T**, Nishizawa Y, Okuno Y, Sekiya K, Morii H. Segmental gut transit in diabetes mellitus: effect of cisapride. *Diabetes Res Clin Pract* 1992; **17**: 137-144 [PMID: 1425148 DOI: 10.1016/0168-8227(92)90159-O]
11 **Folwaczny C**, Hundegger K, Volger C, Sorodoc J, Kühn M, Tatsch K, Landgraf R, Karbach U. Measurement of transit disorders in different gastrointestinal segments of patients with diabetes mellitus in relation to duration and severity of the disease by use of the metal-detector test. *Z Gastroenterol* 1995; **33**: 517-526 [PMID: 8525655]
12 **Keshavarzian A**, Iber FL, Dangleis MD, Cornish R. Intestinal-transit and lactose intolerance in chronic alcoholics. *Am J Clin Nutr* 1986; **44**: 70-76 [PMID: 3728351]
13 **Nguyen HN**, Silny J, Wüller S, Marschall HU, Rau G, Matern S. Abnormal postprandial duodenal chyme transport in patients with long standing insulin dependent diabetes mellitus. *Gut* 1997; **41**: 624-631 [PMID: 9414968 DOI: 10.1136/gut.41.5.624]
14 **Camilleri M**, Malagelada JR. Abnormal intestinal motility in diabetics with the gastroparesis syndrome. *Eur J Clin Invest* 1984; **14**: 420-427 [PMID: 6441717 DOI: 10.1111/j.1365-2362.1984.tb01206.x]
15 **Dooley CP**, el Newihi HM, Zeidler A, Valenzuela JE. Abnormalities of the migrating motor complex in diabetics with autonomic neuropathy and diarrhea. *Scand J Gastroenterol* 1988; **23**: 217-223 [PMID: 3363294 DOI: 10.3109/00365528809103971]
16 **Samsom M**, Jebbink RJ, Akkermans LM, van Berge-Henegouwen GP, Smout AJ. Abnormalities of antroduodenal motility in type I diabetes. *Diabetes Care* 1996; **19**: 21-27 [PMID: 8720528 DOI: 10.2337/diacare.19.1.21]
17 **Frokjaer JB**, Andersen SD, Ejskjaer N, Funch-Jensen P, Drewes AM, Gregersen H. Impaired contractility and remodeling of the upper gastrointestinal tract in diabetes mellitus type-1. *World J Gastroenterol* 2007; **13**: 4881-4890 [PMID: 17828820]
18 **Yang J**, Zhao J, Zeng Y, Gregersen H. Biomechanical properties of the rat oesophagus in experimental type-1 diabetes. *Neurogastroenterol Motil* 2004; **16**: 195-203 [PMID: 15086873 DOI: 10.1111/j.1365-2982.2004.00495.x]
19 **Zhao J**, Yang J, Gregersen H. Biomechanical and morphometric intestinal remodelling during experimental diabetes in rats. *Diabetologia* 2003; **46**: 1688-1697 [PMID: 14593459 DOI: 10.1007/s00125-003-1233-2]
20 **Zhao J**, Frøkjaer JB, Drewes AM, Ejskjaer N. Upper gastrointestinal sensory-motor dysfunction in diabetes mellitus. *World J Gastroenterol* 2006; **12**: 2846-2857 [PMID: 16718808]
21 **Zhao J**, Liao D, Gregersen H. Biomechanical and histomorphometric esophageal remodeling in type 2 diabetic GK rats. *J Diabetes Complications* 2007; **21**: 34-40 [PMID: 17189872 DOI: 10.1016/j.jdiacomp.2005.12.001]
22 **Liao D**, Zhao J, Gregersen H. Three-dimensional geometry analysis of the stomach in type II diabetic GK rats. *Diabetes Res Clin Pract* 2006; **71**: 1-13 [PMID: 16054265 DOI: 10.1016/j.diabres.2005.05.016]
23 **Zhao J**, Chen P, Gregersen H. Morpho-mechanical intestinal remodeling in type 2 diabetic GK rats--is it related to advanced glycation end product formation? *J Biomech* 2013; **46**: 1128-1134 [PMID: 23403079 DOI: 10.1016/j.jbiomech.2013.01.010]
24 **Longhurst PA**, Kang JS, Wein AJ, Levin RM. Comparative length-tension relationship of urinary bladder strips from hamsters, rats, guinea-pigs, rabbits and cats. *Comp Biochem Physiol A Comp Physiol* 1990; **96**: 221-225 [PMID: 1975540 DOI: 10.1016/0300-9629(90)90069-5]
25 **Drewes AM**, Schipper KP, Dimcevski G, Petersen P, Andersen OK, Gregersen H, Arendt-Nielsen L. Multi-modal induction and assessment of allodynia and hyperalgesia in the human oesophagus. *Eur J Pain* 2003; **7**: 539-549 [PMID: 14575667 DOI: 10.1016/S1090-3801(03)00053-3]
26 **Gao C**, Arendt-Nielsen L, Liu W, Petersen P, Drewes AM,

Gregersen H. Sensory and biomechanical responses to ramp-controlled distension of the human duodenum. *Am J Physiol Gastrointest Liver Physiol* 2003; **284**: G461-G471 [PMID: 12431908]
27 **Gregersen H**, Gilja OH, Hausken T, Heimdal A, Gao C, Matre K, Ødegaard S, Berstad A. Mechanical properties in the human gastric antrum using B-mode ultrasonography and antral distension. *Am J Physiol Gastrointest Liver Physiol* 2002; **283**: G368-G375 [PMID: 12121884]
28 **Zhao J**, Liao D, Gregersen H. Phasic and tonic stress-strain data obtained in intact intestinal segment in vitro. *Dig Dis Sci* 2008; **53**: 3145-3151 [PMID: 18461453 DOI: 10.1007/s10620-008-0277-z]
29 **Zhao J**, Liao D, Yang J, Gregersen H. Phasic and tonic smooth muscle function of the partially obstructed guinea pig intestine. *J Biomed Biotechnol* 2011; **2011**: 489720 [PMID: 22162636 DOI: 10.1155/2011/489720]
30 **van Zwieten PA**, Doods HN. Muscarinic receptors and drugs in cardiovascular medicine. *Cardiovasc Drugs Ther* 1995; **9**: 159-167 [PMID: 7786837 DOI: 10.1007/BF00877757]
31 **Marshall IG**. Actions of acetylcholine and carbachol on the chick biventer cervicis muscle. *Br J Pharmacol* 1971; **42**: 462-472 [PMID: 5560904 DOI: 10.1111/j.1476-5381.1971.tb07131.x]
32 **Gregersen H.** Biomechanics of the Gastrointestinal Tract. London : Springer-Verlag, 2002: 1-268
33 **Pedersen J**, Drewes AM, Gregersen H. New analysis for the study of the muscle function in the human oesophagus. *Neurogastroenterol Motil* 2005; **17**: 767-772 [PMID: 16185317 DOI: 10.1111/j.1365-2982.2005.00652.x]
34 **Jørgensen CS**, Ahrensberg JM, Gregersen H, Flyvberg A. Tension-strain relations and morphometry of rat small intestine in experimental diabetes. *Dig Dis Sci* 2001; **46**: 960-967 [PMID: 11341665 DOI: 10.1023/A:1010737323153]
35 **Folwaczny C**, Riepl R, Tschöp M, Landgraf R. Gastrointestinal involvement in patients with diabetes mellitus: Part I (first of two parts). Epidemiology, pathophysiology, clinical findings. *Z Gastroenterol* 1999; **37**: 803-815 [PMID: 10522367]
36 **Nowak TV**, Harrington B, Weisbruch JP, Kalbfleisch JH. Structural and functional characteristics of muscle from diabetic rodent small intestine. *Am J Physiol* 1990; **258**: G690-G698 [PMID: 2185667]
37 **Verne GN**, Sninsky CA. Diabetes and the gastrointestinal tract. *Gastroenterol Clin North Am* 1998; **27**: 861-874, vi-vii [PMID: 9890116 DOI: 10.1016/S0889-8553(05)70035-2]
38 **Nowak TV**, Harrington B, Kalbfleisch JH, Amatruda JM. Evidence for abnormal cholinergic neuromuscular transmission in diabetic rat small intestine. *Gastroenterology* 1986; **91**: 124-132 [PMID: 3011579]
39 **Nowak TV**, Harrington B, Kalbfleisch J. Adaptation of cholinergic enteric neuromuscular transmission in diabetic rat small intestine. *Diabetes* 1990; **39**: 891-897 [PMID: 1973670 DOI: 10.2337/diabetes.39.8.891]
40 **Nobe K**, Momose K, Sakai Y. Effects of Kampo medicine, keishi-ka shakuyaku-to (TJ-60) on alteration of diacylglycerol metabolism in gastrointestinal smooth muscle of diabetic rats. *Acta Pharmacol Sin* 2002; **23**: 1173-1180 [PMID: 12466057]
41 **Ordög T**, Hayashi Y, Gibbons SJ. Cellular pathogenesis of diabetic gastroenteropathy. *Minerva Gastroenterol Dietol* 2009; **55**: 315-343 [PMID: 19829287]
42 **Hu W**, Feng P. Myosin light chain kinase is involved in the mechanism of gastrointestinal dysfunction in diabetic rats. *Dig Dis Sci* 2012; **57**: 1197-1202 [PMID: 22302242 DOI: 10.1007/s10620-012-2041-7]
43 **Gregersen H**, Liao D, Pedersen J, Drewes AM. A new method for evaluation of intestinal muscle contraction properties: studies in normal subjects and in patients with systemic sclerosis. *Neurogastroenterol Motil* 2007; **19**: 11-19 [PMID: 17187584 DOI: 10.1111/j.1365-2982.2006.00837.x]
44 **Pedersen J**, Gao C, Egekvist H, Bjerring P, Arendt-Nielsen L, Gregersen H, Drewes AM. Pain and biomechanical responses to distention of the duodenum in patients with systemic sclerosis. *Gastroenterology* 2003; **124**: 1230-1239 [PMID: 12730864 DOI: 10.1016/S0016-5085(03)00265-8]
45 **Mompeo B**, Popov D, Sima A, Constantinescu E, Simionescu M. Diabetes-induced structural changes of venous and arterial endothelium and smooth muscle cells. *J Submicrosc Cytol Pathol* 1998; **30**: 475-484 [PMID: 9851055]
46 **Stevens LA**, Sellers DJ, McKay NG, Chapple CR, Chess-Williams R. Muscarinic receptor function, density and G-protein coupling in the overactive diabetic rat bladder. *Auton Autacoid Pharmacol* 2006; **26**: 303-309 [PMID: 16879496 DOI: 10.1111/j.1474-8673.2006.00371.x]
47 **Kim SJ**, Park JH, Song DK, Park KS, Lee JE, Kim ES, Cho KB, Jang BK, Chung WJ, Hwang JS, Kwon JG, Kim TW. Alterations of colonic contractility in long-term diabetic rat model. *J Neurogastroenterol Motil* 2011; **17**: 372-380 [PMID: 22148106 DOI: 10.5056/jnm.2011.17.4.372]
48 **Soulié ML**, Cros G, Serrano JJ, Bali JP. Impairment of contractile response to carbachol and muscarinic receptor coupling in gastric antral smooth muscle cells isolated from diabetic streptozotocin-treated rats and db/db mice. *Mol Cell Biochem* 1992; **109**: 185-188 [PMID: 1385642 DOI: 10.1007/BF00229775]

P- Reviewer: Navedo M **S- Editor:** Zhai HH **L- Editor:** A
E- Editor: Wu HL

百世登
Baishideng®

Published by **Baishideng Publishing Group Co., Limited**
Flat C, 23/F., Lucky Plaza,
315-321 Lockhart Road, Wan Chai, Hong Kong, China
Fax: +852-65557188
Telephone: +852-31779906
E-mail: bpgoffice@wjgnet.com
http://www.wjgnet.com