

## Effect of Tangweian Jianji on the Biomechanical and Morphometric Remodeling of Colon and Rectum in STZ Induced Diabetic Rats

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Received: March 3, 2012

Revised: March 29, 2012

Accepted: April 5, 2012

Published online: July 21, 2012

0.01).

**CONCLUSION:** Although TWAJJ (high dose) treatment could not significantly correct the changes of blood glucose and serum insulin levels in the diabetic rats; it could partly restore the morphometric and biomechanical remodeling of lower GI tract caused by diabetes.

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**Key words:** Diabetes rats; Lower gastrointestinal tract; Tangweian Jianji; Biomechanics; Morphometry; Remodeling

Sha H, Tong XL, Liu GF, Zhen Z, Chen PM, Gregersen H, Zhao J, Effect of Tangweian Jianji on the Biomechanical and Morphometric Remodeling of Colon and Rectum in STZ Induced Diabetic Rats. *Journal of Gastroenterology and Hepatology Research* 2012; 1(6): 86-91 Available from: URL: <http://www.ghrnet.org/index./joghr/>

### ABSTRACT

**AIM:** The aim of the study was to investigate the effect of TWAJJ on the biomechanical and morphometrical remodeling of colon and rectum in streptozotocin (STZ) induced diabetic rats.

**METHODS:** The colonic and rectal segments obtained from diabetic (DM), TWAJJ treated diabetic (TH, high dosage: 10 g/kg; TL, low dosage: 5 g/kg) and normal (CON) rats were used for the study. Blood glucose and serum insulin levels were measured. At the end of experiment, the circumferential length, the wall thickness and the opening angle were measured from the digitized images of those segments and residual strain was computed. Circumferential and longitudinal stresses and strains were computed from the length, diameter and pressure data and from the zero-stress state geometry for the colonic segment.

**RESULTS:** The glucose and insulin levels did not differ among DM, TH and TL groups. Wet weight, wall thickness, cross-sectional wall area, opening angle, and absolute values of residual strain of colonic and rectal segments in DM group were significantly higher than those in CON group ( $P < 0.05$  and  $P < 0.01$ ), whereas those parameters in TH group but not in TL group were significantly lower than those in DM group ( $P < 0.05$ ,  $P < 0.01$ ). Furthermore, the circumferential and longitudinal stiffness of the colonic wall increased in DM group compared those with CON group. TH but not TL treatment could significantly decrease the colonic wall stiffness in both directions ( $P <$

### INTRODUCTION

Diabetic gastrointestinal disorder (DGID) is a common complication of diabetes. Up to 76% of diabetic patients showed significant gastrointestinal (GI) symptoms including dysphagia, early satiety, reflux, constipation, abdominal pain, nausea vomiting and diarrhea<sup>[1,2]</sup>. The motility disturbances of colon and rectum were seen in human diabetes<sup>[3-4]</sup>. Delayed transit of colon was reported in IDDM patients and the symptoms related closely to the region impaired<sup>[5]</sup>. The mean total colonic transit time of the diabetic patients was significantly longer than that in healthy subjects. Furthermore, the diabetic patients with constipation showed longer total, left and recto-sigmoid colonic transit times than those without constipation<sup>[6]</sup>.

The GI tract is functionally subjected to dimensional changes. Hence, biomechanical properties such as the stress-strain relationships are of particularly importance<sup>[7]</sup>. The biomechanical properties are crucial for GI motor function because peristaltic motion that propels the food through the GI tract is a result of interaction of the passive and active tissue forces and the hydrodynamic forces in the food bolus. The remodeling of the mechanical properties reflects the changes in the tissue structure that determine a specific motor dysfunction. Previous study demonstrated that experimental diabetes induces colon morphological and biomechanical remodeling<sup>[8]</sup>. Following the

development of diabetes, the colonic wall becomes thicker and the stiffness of the wall increases in a time-dependent manner. Therefore, the diabetic GI morphological and biomechanical remodeling plays an important role in DGID and has become the new perspective of diabetic GI pathogenesis<sup>[9]</sup>.

The western medicine treatment of DGID mainly focused on symptomatic control with improvement of gastric motility, promoting agents and supportive measures based on blood glucose control<sup>[2]</sup>. However, as its pathogenesis is unclear, the basic paresis factors still exist. Although these therapy methods can partially improve the clinical symptoms, it can not fundamentally reverse the diabetes-induced changes with very high relapse rate. Clinical practice showed that TWAJJ could improve the DGID significantly with low relapse rate, but the mechanism is not fully understood. Therefore, the aim of the present study was to investigate if TWAJJ treatment can improve the morphometric and biomechanical remodeling of colon and rectum in STZ-induced diabetic rats.

## MATERIALS AND METHODS

### Animal model and groups

Forty male Sprague Dawley (SD) rats weighing 220-250g were included in this study. Thirty rats were made diabetic by a single tail vein injection of 40 mg/kg streptozotocin (STZ, Sigma-Aldrich, China). This dose of STZ resulted in a random blood glucose level  $\geq 16.7$  mmol/L in 90% of rats after 7 d of injection. The remaining 10% of rats were excluded from this study. Twenty-seven STZ induced diabetic rats were subdivided into three groups ( $n=9$  in each group), i.e., diabetic control group (DM); high dose of TWAJJ group (TH) and low dose of TWAJJ group (TL). Another 10 rats of similar age and body weight from the same vendor were used as non-diabetic control group (CON). The rats in DM, TH, TL and CON groups were fed with the same isocaloric diet.

### Drugs and administration methods

TWAJJ is composed of *Atractylodes macrocephala*, *Citrus aurantium*, *Wine rhubarb*, *Pinellia* and *Ginseng* provided by Guang'anmen Hospital, China Academy of Chinese Sciences. The medicine was directly injected into stomach lumen by gastric lavage once daily from the beginning of experiment. The dosage was 10 g/kg for TH and 5 g/kg for TL respectively. The rats of DM and CON groups were only poured the physiological saline.

### Experimental procedures

Body weight and blood glucose levels were measured at 2-weeks intervals after initiating the experiment. The insulin level was measured at the end of the experiment.

The experimental period was 60 d. At the ending of the experiment, the rats were fasting overnight and anesthetized with 4% Chloral hydrate (10 mL/kg, ip). Following laparotomy, the middle part of colon and whole rectum were harvested. After gently cleaning the lumen of the segments with saline, the length and the wet weight were measured. The colonic segment were divided into two parts, the proximal 1 cm long segment were used for zero-stress state experiment. The remaining part was used for distension test. The rectal segment was only used for the zero-stress state experiment.

### Zero-stress state experiment

For obtaining data on the zero-stress state, three 1-2 mm wide colonic and rectal rings were cut and placed in the Krebs solution with EGTA (100 mg/L) at room temperature. The composition of Krebs solution (mmol/L) is: NaCl, 118; KCl, 4.7;  $\text{NaHCO}_3$ , 25;  $\text{NaH}_2\text{PO}_4$ , 1.0; MgCl,

1.2; and ascorbic acid, 0.11. A photograph was taken of the cross-section of the rings by camera (Canon, Japan) and it is presented as the no-load state. Then each ring-shaped segment was cut radially from the opposite mesentery site and the photographs were taken about 60 min after the radial cutting to allow viscoelastic creep to take place. This is presented as the zero-stress state.

### Distension test

The distal end of the remaining colonic segment was tied with a suture and the proximal end was cannulated with a tube for the distension experiment. After preconditioning of the segment two times from 0 to 20 cmH<sub>2</sub>O, it was inflated with Krebs solution using a step-wise distension protocol up to 20 cmH<sub>2</sub>O (0, 1, 2, 3, 5, 10, 15 and 20 cm H<sub>2</sub>O). The segment conformed to a cylindrical geometry during the distensions. Each pressure lasted for 2 m and then the outer diameter and length of the segment was photographed by the camera (Canon, Japan).

### Mechanical data analysis

The morphometric data were obtained from digitized images of the segments in the zero-stress, no-load and pressurized states. Measurements were undertaken using image analysis software (Sigmascan ver. 4.0, Sigma Corp., San Rafael, CA, USA). The following data were measured from each specimen: the circumferential length (C), the wall thickness (h), the wall area (A), and the opening angle at zero-stress state ( $\alpha$ ). The subscripts i, o, n, z and p refer to the inner (mucosal) surface, outer (serosal) surface, no-load state, zero-stress state and pressurized condition. The opening angle  $\alpha$  was defined as the angle subtended by two radii drawn from the midpoint of the inner wall to the inner tips of two ends of the specimen. Furthermore, the outer diameter (D) and the length (L<sub>p</sub>) were measured from the images of the pressurized segments.

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

$$\text{Residual Green's strain at the mucosal surface: } E_i = \frac{\left(\frac{C_{i-n}}{C_{i-z}}\right)^2 - 1}{2} \quad [1]$$

$$\text{Residual Green's strain at the serosal surface: } E_o = \frac{\left(\frac{C_{o-n}}{C_{o-z}}\right)^2 - 1}{2} \quad [2]$$

The stress and strain of the colonic segment in the pressurized state were determined under assumptions that the wall was homogenous and the organ shape was cylindrical. Calculation was done from knowing the no-load state dimensions, the outer diameters and lengths of the specimen at varying pressures, and assuming incompressibility of the wall. The longitudinal stretch ratio,  $\lambda_\phi = \frac{L_p}{L_z}$ ; the luminal radius,  $r_{i-p} = \sqrt{r_{o-p}^2 - \frac{A_n}{\pi\lambda_\phi}}$ ; the wall thickness,  $h_p = r_{o-p} - r_{i-p}$ ; the mucosal circumferential length,  $C_{i-p} = 2 \times \pi \times r_{i-p}$ ; the serosal circumferential length,  $C_{o-p} = 2 \times \pi \times r_{o-p}$ ; the mid-wall circumferential length,  $C_{m-p} = \frac{C_{i-p} + C_{o-p}}{2}$ ; the circumferential stretch ratio,  $\lambda_\phi = \frac{C_{m-p}}{C_{m-z}}$  (where the middle-wall circumferential length at zero-stress state,  $C_{m-z} = \frac{C_{i-z} + C_{o-z}}{2}$ ) were computed. Then the Kirchhoff's stress and Green's strain in a wall at a given pressure were computed according to the following equations:

$$\text{Circumferential Kirchhoff's stress: } S_\theta = \frac{\Delta P r_{i-p}}{h_p \lambda_\phi^2} \quad [3]$$

$$\text{Longitudinal Kirchhoff's stress: } S_\phi = \frac{\Delta P r_{i-p}^2}{h_p \lambda_\phi^2 (r_{o-p} + r_{i-p})} \quad [4]$$

$$\text{Circumferential mid-wall Green's strain: } E_\theta = \frac{\lambda_\phi^2 - 1}{2} \quad [5]$$

$$\text{Longitudinal Green's strain: } E_{\phi} = \frac{\lambda_{\phi}^2 - 1}{2} \quad [6]$$

$\Delta P$  is the transmural pressure difference. The longitudinal mid-wall stretch ratio was referenced to the no-load state because tissue strips could not be cut for obtaining the zero-stress state in longitudinal direction. However, the longitudinal mid-wall length in rat intestine does not differ between the no-load and zero-stress states<sup>[10]</sup>.

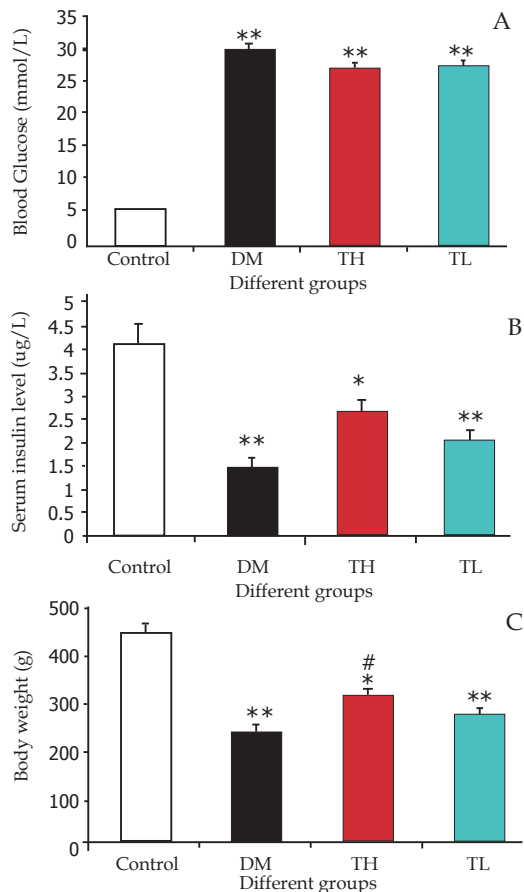
**Statistical analysis**

The data were representative of a normal distribution and accordingly the results were expressed as means±SEM. The stress-strain curve for each direction was fitted using the exponential function equation  $S = (S^* + b)e^{a(E-E^*)} - b$ .  $S^*$  and  $E^*$  are the stress and strain at a physiological reference level<sup>[7]</sup>. The constants  $a$  and  $b$  from the above exponential function were used for the statistical evaluation of the stress-strain data. Analysis of variance was used to detect the differences of different parameters in different groups (Sigmatat 2.0TM). The results were regarded as significant when  $p < 0.05$ .

**RESULTS**

**The blood glucose, serum insulin levels and body weight**

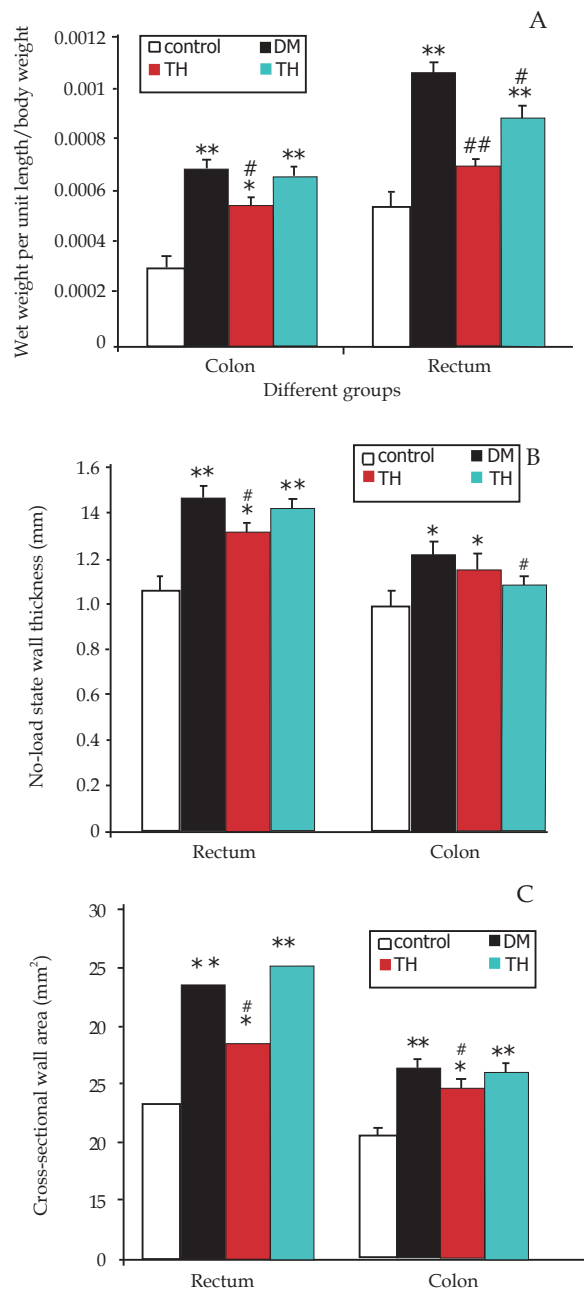
The blood glucose, serum insulin levels and body weight at the ending of experiment were shown in Figure 1. The blood glucose level was about 4-fold higher in DM group compared with CON group (Figure 1A,  $p < 0.01$ ). Whereas the serum insulin level was significantly lower in DM group compared with CON group (Figure 1B,  $p < 0.01$ ). The body weight in DM group decreased nearly 50% compared with that in CON group (Figure 1C). Compared with DM group, the blood glucose level did not significantly decrease (Figure 1A,  $p > 0.05$ ) and serum insulin level did not significantly increase (Figure 1B,  $p > 0.05$ ) in TH and TL groups. It indicated that both the low and high dosage of TWAJJ did not obviously affect the blood glucose and serum insulin level. However, after treatment with high dosage of TWAJJ could partially increased the body weight compared with that of DM group (Figure 1C,  $p < 0.05$ )



**Figure 1** The blood glucose levels (A), the serum insulin level (B) and body weight (C) in different groups. Compared with Control group: \* $p < 0.05$ , \*\* $p < 0.01$ ; Compared with DM group: # $p < 0.05$

**The weight/cm to body weight ratio, wall thickness and wall area**

The wet weight per unit length to body weight ratio (Figure 2A), no-load wall thickness (Figure 2B) and cross-section wall area (Figure 2C) of colonic and rectal segments were significantly increased in DM group compared with CON group ( $p < 0.01$ ). After treatment with TH, those parameters significantly decreased in both of two segments (Figure 2,  $p < 0.05$  and  $p < 0.01$ ); however those parameters did not significantly change in TL group ( $p > 0.05$ ) with exception of wall thickness of colon (Figure 2B,  $p < 0.05$ ).

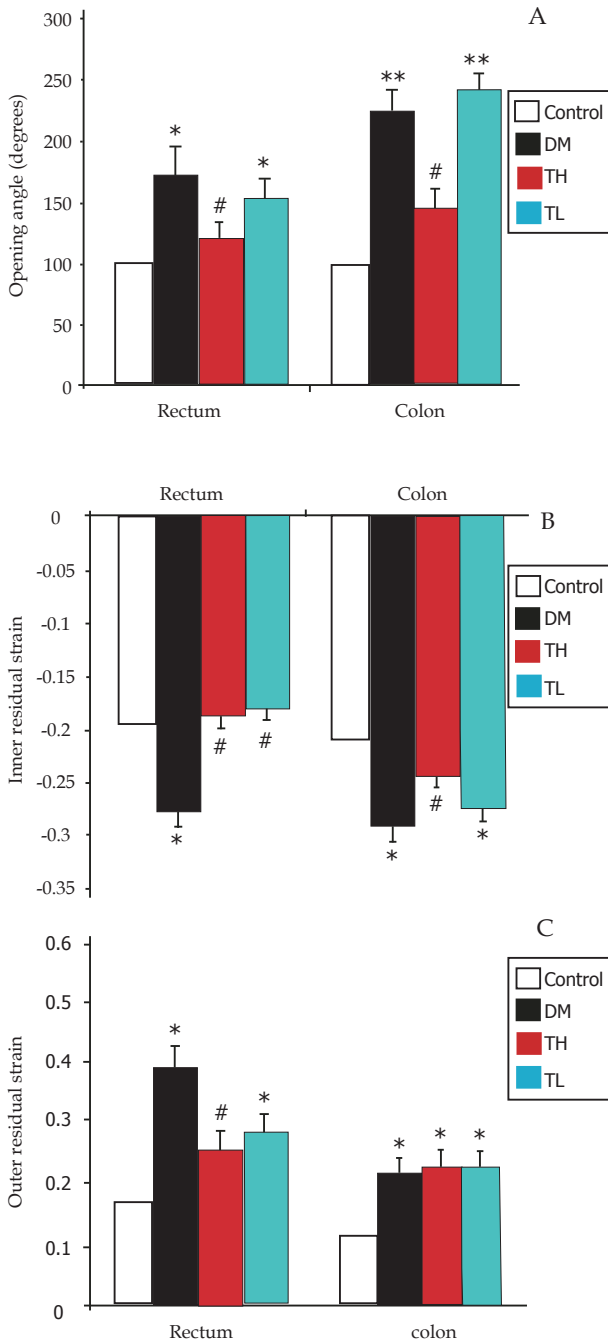


**Figure 2** Wet weight per unit length to body weight ration (A), wall thickness (B) and wall area (C) in different segments of different groups. Compared with Control group: \* $p < 0.05$ , \*\* $p < 0.01$ ; Compared with DM group: # $p < 0.05$ , ## $p < 0.01$ .

**Opening angle and residual strain**

At the end of the experiment, the opening angle of both colonic and rectal segments was significantly increased in DM group compared with CON group (Figure 3A,  $p < 0.05$ ). Treatment with high dosage of TWAJJ significantly decreased the opening angle (Figure 3A,  $p < 0.05$ ); whereas the opening angle did not change in TL group ( $p > 0.05$ ).

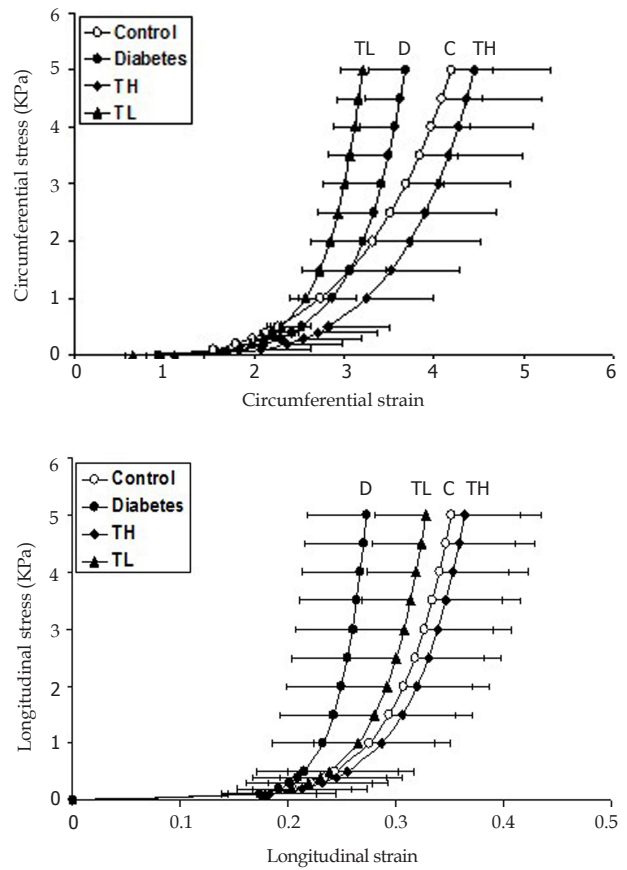
The similar pattern as the opening angle was found for the inner residual strain in both segments and outer residual strain for rectal segment (Figure 3B), i.e., the absolute value of residual strain of both colonic and rectal segments was significantly increased in DM group compared with CON group (Figure 3B and 3C,  $p < 0.05$ ). Treatment with high dosage of TWAJJ (TH group) could partially recover the changes of residual strain (Figure 3B and 3C,  $p < 0.05$ ), but not in TL group.



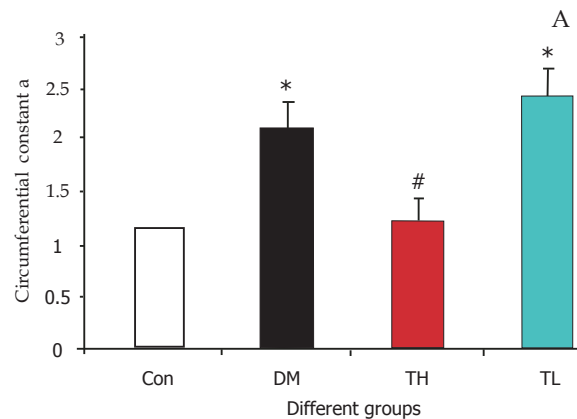
**Figure 3** Opening angle (A), inner residual strain (B) and outer residual strain (C) in different segments of different groups. Compared with Control group: \* $p < 0.05$ , \*\* $p < 0.01$ ; Compared with DM group: # $p < 0.05$ .

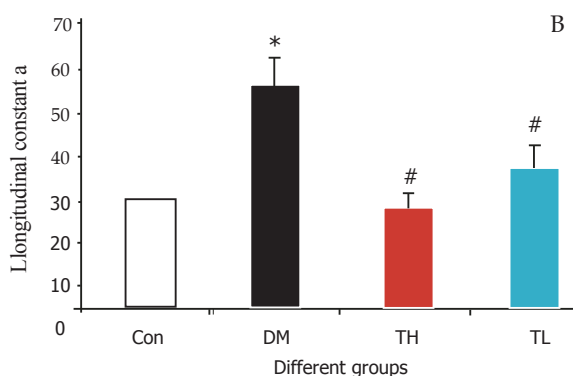
**Stress-strain distribution**

At the end of the experiment, the stress-strain analysis showed that both the circumferential and longitudinal stress-strain curves of colonic segment (Figure 4A and 4B) in DM group shifted to the left compared with those in CON group. It indicated that the colonic wall became stiffer due to diabetes. Computation of constant  $a$  showed the significant difference between DM group and CON group (Figure 5A and 5B,  $p < 0.05$ ). High dosage of TWAJJ (TH) treatment significantly decreases the stiffness of colonic wall in both circumferential (Figure 4A, 5A,  $p < 0.05$ ) and longitudinal (Figure 4B, 5B,  $p < 0.05$ ) direction. Low dosage of TWAJJ treatment (TL) did not show the improvement of the stiffening of colonic wall caused by diabetes (Figure 4 and 5,  $p > 0.05$ ).



**Figure 4** Circumferential and longitudinal stress-strain curves of esophageal and jejunal segments among different groups.





**Figure 5** Circumferential (A) and longitudinal (B) constant a of colonic segments in different groups. Compared with Control group: \* $p < 0.05$ ; Compared with DM group: # $p < 0.05$ .

## DISCUSSION

According to WHO report; it is expected to reach 370 million DM patients by 2030. The serious impact caused by diabetes and its complications are not only on the patients themselves but also the heavy financial burden to the state and families<sup>[11]</sup>. DGID is a common complication of diabetes<sup>[2]</sup>. The ano-rectal symptoms in the diabetic patients correlated better with the manometric abnormalities<sup>[4]</sup>. The manometric abnormalities may relate to the changes of biomechanical properties in the diabetes<sup>[9]</sup>. Previous study showed that prominent proliferation and biomechanical remodeling of colonic wall was occurred in experimental diabetes<sup>[8]</sup>. The main findings were found at the present study that the morphometric and biomechanical remodeling occurred in STZ-induced diabetic rats. This confirmed the findings of previous study<sup>[8]</sup>. Furthermore, it was demonstrated that TWAJJ (high dose) treatment could partly restore the morphometric and biomechanical remodeling of colonic and rectal segments caused by diabetes.

In the view of traditional Chinese medicine, DGID is in the category of fullness, epigastric pain, vomiting, diarrhea, and constipation concurrent with diabetes. The major syndrome of DGID is spleen deficiency and qi stagnation. The principle treatment is to strengthen spleen and move stagnation. TWAJJ is a clinic practical Chinese medicine compound on the diabetic gastrointestinal dysfunction<sup>[12-14]</sup> composed of several herbs, such as *Atractylodes macrocephala*, *Citrus aurantium*, *Pinellia downbear* and *Ginseng*. Each of these herbs has some specific effects<sup>[15-20]</sup>. However, the TWAJJ seems mainly to effect on up-GI tract, such as gastroparesis. We also in another study (unpublished data) demonstrated that the treatment with high dosage of TWAJJ could partially improve the morphometric and biomechanical remodeling of Up-GI tract. This indicated the mechanism of TWAJJ effect on DGID is at least partially through this pathway. Therefore, it is also interesting to investigate if TWAJJ can through the same pathway to affect the lower GI tract in the diabetes.

The colon and rectum are tubular organs. One important function is transportation of the content by the wall movement; therefore the biomechanical properties of the wall are important for the function<sup>[21]</sup>. The biomechanical properties of the colon and rectum depend on its structure and can be evaluated by opening angle, residual stress and strain, and stress-strain relationship<sup>[8]</sup>. The previous studies demonstrated the colonic wall remodeled during the development of the diabetes. The present study confirmed the previous findings that the morphometric and biomechanical remodeling of colonic wall occurred in STZ-induced

diabetic rats. Furthermore, it was also showed the remodeling was occurred in the rectal segment. Although the treatment with TWAJJ did not significantly changes the blood glucose and serum insulin levels, high dosage of TWAJJ does partially improve the morphometric and biomechanical remodeling caused by diabetes. Improvement of the morphometric remodeling expresses as reducing the wall thickness and area. Improvement of biomechanical remodeling expresses as reducing the opening angle, the absolute value of residual strain and decreasing the wall stiffness. The present study clearly indicated that the high dosage of TWAJJ could partially improve the biomechanical remodeling of lower GI wall in the STZ-induced diabetic rats.

The morphological changes, such as increasing wall thickness caused by diabetes will change the relative location of mechanosensitive afferents in the GI tract<sup>[22-23]</sup>. The alterations of residual stress and wall stiffness in the diabetes will change the tension and stress distribution in the location of mechanosensitive afferents. Therefore the morphometric and biomechanical remodeling of GI wall in the diabetes in this way will affect the GI sensory and motor function. High dosage of TWAJJ could partially correct the disorders of morphometric and biomechanical remodeling caused by diabetes; therefore can improve the motor and sensory dysfunction of diabetic GI tract through this mechanism.

In this study, we adopted STZ-induced diabetic rat model. In order to get high glucose level and obvious remodeling of GI tract, we did not treat with insulin for the diabetic rats. However, it is well known that in the nowadays very common settings where patients are insulin- treated for diabetes type 1, or non-insulin dependent diabetes type 2. Therefore, we will consider including the group of type-1 diabetic rats treated with insulin and adapt the type-2 diabetic animal model in the future studies. Furthermore, in order to overcome the possible variations among the different groups and to investigate the effect of TWAJJ on different time point during the development of diabetes, the data in different study groups of rats at baseline and at certain time intervals after initiating the experiment should be taken into account in the future studies.

In conclusion, High dosage of TWAJJ treatment could partly improve the biomechanical and morphometric remodeling of colonic and rectal wall in the diabetes. This indicated that the TWAJJ not only improve the up-GI disorders in the diabetes, but also may improve the low GI disorders in the diabetes. Therefore, it is possible to explore the new application of TWAJJ to the diabetic patients in the clinics. Furthermore, it is important to develop some Chinese herbs, such as TWAJJ, to improve the morphometric and biomechanical remodeling, and then further improves GI dysfunction caused by diabetes.

## ACKNOWLEDGEMENTS

The study was financially supported by National Key Basic Research Program of China (973), No.2010CB530600. Mingyi Yuan and Jia-Cheng Zhang are thanked for help to do animal experiments.

## REFERENCES

- 1 Icks A, Haastert B, Rathmann W, Wareham N. Prevalence of gastrointestinal symptoms in patients with type 2 diabetes: a population-based study. *Arch Intern Med* 2002; **162**: 1067-109; author reply 1069
- 2 Shakil A, Church RJ, Rao SS. Gastrointestinal complications of diabetes. *Am Fam Physician* 2008; **77**: 1697-1702
- 3 Chandrasekharan B, Anitha M, Blatt R, Shahnavaz N, Kooby D, Staley C, Mwangi S, Jones DP, Sitaraman SV, Srinivasan S. Colonic motor dysfunction in human diabetes is associated

- with enteric neuronal loss and increased oxidative stress. *Neurogastroenterol Motil* 2011; **23**: 131-118, e26
- 4 Rosztóczy A, Róka R, Várkonyi TT, Lengyel C, Izbéki F, Lovonics J, Wittmann T. Regional differences in the manifestation of gastrointestinal motor disorders in type 1 diabetic patients with autonomic neuropathy. *Z Gastroenterol* 2004; **42**: 1295-1300
  - 5 Iber FL, Parveen S, Vandrunen M, Sood KB, Reza F, Serlovsky R, Reddy S. Relation of symptoms to impaired stomach, small bowel, and colon motility in long-standing diabetes. *Dig Dis Sci* 1993; **38**: 45-50
  - 6 Jung HK, Kim DY, Moon IH, Hong YS. Colonic transit time in diabetic patients--comparison with healthy subjects and the effect of autonomic neuropathy. *Yonsei Med J* 2003; **44**: 265-272
  - 7 Gregersen H. *Biomechanics of the Gastrointestinal Tract*. London : Springer-Verlag, 2002
  - 8 Zhao J, Nakaguchi T, Gregersen H. Biomechanical and histomorphometric colon remodelling in STZ-induced diabetic rats. *Dig Dis Sci* 2009; **54**: 1636-1642
  - 9 Zhao J, Liao D, Yang J, Gregersen H. Biomechanical Remodelling of the Diabetic Gastrointestinal Tract. In: Levy JH. eds: *Biomechanics: Principles, Trends and Applications*. USA: Nova Publishers; 2010: 137-162
  - 10 Dou Y, Fan Y, Zhao J, Gregersen H. Longitudinal residual strain and stress-strain relationship in rat small intestine. *Biomed Eng Online* 2006; **5**: 37
  - 11 Yang W, Lu J, Weng J, Jia W, Ji L, Xiao J, Shan Z, Liu J, Tian H, Ji Q, Zhu D, Ge J, Lin L, Chen L, Guo X, Zhao Z, Li Q, Zhou Z, Shan G, He J. Prevalence of diabetes among men and women in China. *N Engl J Med* 2010; **362**: 1090-1101
  - 12 Liu W, Tong X. Examples of experiences in the treatment of diabetic gastroparesis. *Drug Evaluation (Chinese)* 2009; **12**: 495-498
  - 13 Tong X, Dong L. Treatment with Chinese medicine on diabetes induced gastrointestinal function disorders. *New World of Diabetes (Chinese)* 2007; **1**: 16-17
  - 14 Wang J, Tong X. Diagnosis and treatment with Chinese medicine on diabetic gastroparesis. 《Proceedings of ninth National Academic Conference of Chinese medicine and diabetes》 2006
  - 15 Wang F. Research progression of *Atractylodes*' pharmacological and clinical applications in treatment of gastrointestinal diseases. *Medical Journal of Chinese People's Health* 2008; **20**: 1646
  - 16 Huang G, Sheng S, Zhang Q. Zhishi on small intestinal smooth muscle function. *Journal of China Medical University* 1993; **1**: 49-52
  - 17 Liu Y, Ye F, Wang R, Qiu GQ, Cai Y, Sun Y. Effects of Zhishi (*Fructus Aurantii Immaturus*) and its medicinal serum on extracorporeal colonic muscle strips in rats with slow transit constipation. *Journal of Beijing University of Traditional Chinese Medicine* 2010; **33**: 402-405
  - 18 Wu H, Cai BC, Rong GX, Ye DJ. The effect of *Pinellia* processed by ginger juice on gastric and intestinal function of animals. *China Journal of Chinese Materia Medica* 1994; **19**: 535-537, 574
  - 19 Cho WC, Chung WS, Lee SK, Leung AW, Cheng CH, Yue KK. Ginsenoside Re of *Panax ginseng* possesses significant antioxidant and antihyperlipidemic efficacies in streptozotocin-induced diabetic rats. *Eur J Pharmacol* 2006; **550**: 173-179
  - 20 Zhang Z, Li X, Lv W, Yang Y, Gao H, Yang J, Shen Y, Ning G. Ginsenoside Re reduces insulin resistance through inhibition of c-Jun NH2-terminal kinase and nuclear factor-kappaB. *Mol Endocrinol* 2008; **22**: 186-195
  - 21 Zhao J, Liao D, Gregersen H. Biomechanics of the Gastrointestinal Tract in Health and Disease. In: Levy JH. eds: *Biomechanics: Principles, Trends and Applications*. USA: Nova Publishers; 2010; 163-206
  - 22 Raab M, Neuhuber WL. Number and distribution of intraganglionic laminar endings in the mouse esophagus as demonstrated with two different immunohistochemical markers. *J Histochem Cytochem* 2005; **53**: 1023-1031
  - 23 Swithers SE, Baronowsky E, Powley TL. Vagal intraganglionic laminar endings and intramuscular arrays mature at different rates in pre-weanling rat stomach. *Auton Neurosci* 2002; **102**: 13-19
- Peer reviewers:** I. Michael Leitman, MD, Chief Of General Surgery, Albert Einstein College Of Medicine-Beth Israel Medical Center, 10 Union Square East, 2M, New York, USA; Claudio Chiesa, MD, Institute of Translational Pharmacology, National Research Council Via del Fosso del Cavaliere, 100, 00133-Rome, Italy.