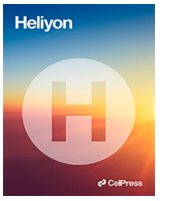
Heliyon 9 (2023) e21007

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



# Effect of Tangshen formula on the remodeling of small intestine and colon in Zucker diabetic fatty rats

Xin Yang [a], Jingbo Zhao [b], Hong Li [a], Lin Pan [a], Jing Guo [a], Jing Li [a], Yuting Zhang [a], Pengmin Chen [a,*], Ping Li [a,**]

[a] *Beijing Key Lab Immune-Mediated Inflammatory Diseases, Institute of Clinical Medicine, China-Japan Friendship Hospital, Beijing (100029), China*
[b] *Anbiping (Chongqing) Pathological Diagnosis Center, Chongqing, China*

ARTICLE INFO

*Keywords:*
Diabetes
ZDF rat
Gut remodeling
Tangshen formula

ABSTRACT

*Background and aim:* Previous study have demonstrated that Tangshen Formula (TSF) could attenuate colonic histomorphological remodeling in the diabetic rat model induced by high fat diet plus low dosage streptozotocin (STZ). However, it is not clear whether TSF has same effect on small intestine and the effect on biomechanical properties of bowel. The aim of this study is to investigate the effect of TSF on histomorphological and biomechanical remodeling of small intestine and colon by using Zucker Diabetic Fatty (ZDF) Rat model.

*Materials and methods:* ZDF rats (obese fa/fa) with blood glucose higher than 11.7 mmol/L were divided into ZDF group (diabetic control group) and ZDF + TSF group (TSF treatment group), the later were intragastrically administered TSF. The ZDF rats (lean fa/+) were served as normal control (ZL) group. The rats in the ZL and ZDF groups were administered with saline. The experimental period covered from 8 weeks to 24 weeks. At the end of experiment, the ileal and colonic segments were studied *in vitro*. The histomorphometry and biomechanical parameters were measured.

*Results:* Compared with ZL group histomorphologically, the wet weight per unit length, wall thickness, wall area and fractions of total and type I and type III collagen in different layers for both ileum and colon increased in ZDF group. Those increasing parameters were partially inhibited in ZDF + TSF group. Compared with ZL group biomechanically, ZDF and ZDF + TSF groups had smaller opening angle and residual strain in ileum, and bigger opening angle and residual strain in colon. Whereas the wall became softer in circumferential direction and stiffer in longitudinal direction for both ileum and colon. However, no difference of biomechanical parameters was found between ZDF and ZDF + TSF groups.

*Conclusion:* The histomorphological and biomechanical remodeling of ileum and colon were happened in ZDF rats ( obese fa/fa ) . TSF could partly attenuate ileal and colonic histomorphological remodeling rather than biomechanical remodeling.

* Corresponding author. Institute of Clinical Medicine, China Japan Friendship Hospital, 2 Yinghua Road , Chaoyang District, Beijing 100029, China

** Corresponding author. Institute of Clinical Medicine, China Japan Friendship Hospital, 2 Yinghua Road , Chaoyang District, Beijing 100029, China

*E-mail addresses:* chenpengmin6@sina.com (P. Chen), lp8675@163.com (P. Li).

https://doi.org/10.1016/j.heliyon.2023.e21007
Received 3 August 2023; Received in revised form 28 September 2023; Accepted 12 October 2023
Available online 16 October 2023

## 1. Introduction

Gastrointestinal (GI) disorders is one of the most common diabetic complications [1]. The main symptoms include abdominal distension, satiety, diarrhea, constipation and fecal incontinence [2]. In order to improve the GI disorder, it is very important to look for the proper medicines.

Patients with diabetic GI disorders are treated conventionally with domperidone, metoclopramide, mosapride citrate, fiber laxatives, lactulose, polyethylene glycol and Cisapride, etc [3–7]. Although these western medications can reduce patients' GI symptoms, however they have certain side effects and are prone to relapse following drug discontinuation [8]. Furthermore, a considerable number of patients are not satisfied with these symptomatic treatments, therefore seeking alternative medicine is important.

It has been demonstrated that Chinese herbs were effective in the treatment of diabetic gastroparesis on clinical practice [8], and had effective in attenuating GI motility disorder in diabetic rats [9,10]. More recently, it has been demonstrated that Chang Run Tong (a formula of Chinese herbs) was better than Forlax to treat constipation, had better follow-up improvement after stopping drugs and no remarkably side effective was found in elderly diabetic patients [11]. Therefore, Chinese herbs could be very good option for the proper alternative medicines to treat diabetic GI disorders [12].

It is well known that diabetes mellitus (DM) induces histomorphological and biomechanical remodeling of small intestine and colon in DM patients [13] and animals [13–15]. Because the close-relationship exists between mechanical behavior and GI function [16,17], it is believed that the function disorders of small intestine and colon in diabetes could be improved if the drug can improve DM-induced GI histomorphological and biomechanical remodeling. Previous studies have demonstrated that some Chinese medicines could improve DM-induced remodeling of small intestine [18–20] and colon [21] in DM rats. Tangshen Formula (TSF) has been used in diabetic patients to treat diabetic kidney diseases and proved to have efficacy [22]. Although no clinical study about the effect of TSF on diabetic gastrointestinal disorders, our group has in previous study demonstrated that TSF could partly attenuate colonic structure remodeling in type 2 diabetic rats [23]. However, it is not clear so far whether TSF has same effect on small intestine and the effect on biomechanical properties of bowel. The present study aims further exploring such effects of TSF.

It is very important to select an appropriate animal model whose symptoms, signs and pathogenesis is close to a human disease for studying effects and possible effective mechanism of medicine. The Zucker diabetic fatty (ZDF) rat (obese fa/fa) shows characteristics such as obesity, insulin resistance and later insulin deficiency, hyperglycemia, hyperlipoidemia, and the mutation in the receptor gene [24]. All of these characteristics are very close to human type 2 diabetes. Therefore, we selected ZDF rats (obese fa/fa) as type 2 diabetic model in the present study. Using this model, the histomorphological and passive biomechanical properties of ileum and colon are studied and whether TSF can attenuate diabetes-induced GI histomorphological and biomechanical remodeling are explored. We hypothesized that the histomorphological and biomechanical remodeling of the ileum and colon can be happen in the ZDF rat (obese fa/fa) model and TSF can have effect on the remodeling of ileum and colon.

## 2. Materials and methods

### 2.1. Reagents

Hank's Balanced Salt Solution (HBSS; cat. G4203), citrate buffer solution (PH6.0; cat. G1202), rabbit polyclonal antibodies against collagen I (cat. GB11022-3) and collagen III (cat. GB111629), Peroxidase Affinipure Goat Anti-Rabbit IgG (cat. GB23303), diaminobenzidine (DAB) kit (cat. G1211), Mayer's hematoxylin (cat. G1004), and bovine serum albumin (BSA; cat. G5001) were purchased from Servicebio (Wuhan, China). Ethylene glycol-bis (β-aminoethyl ether)-N,N,N′,N'-tetraacetic acid (EGTA; cat. 03777) and rat diet (Purina 5008) was purchased from Sigma-Aldrich (Shanghai, China) and Purina Mills, Inc. (Missouri, USA) respectively.

### 2.2. Composition and preparation of Tangshen formula

TSF is comprised of seven natural herbs including *burning bush* (*Euonymus alatus* (*Thunb.*) *Siebold*), *Rehmannia glutinosa Libosch*, *Astragalus Membranaceus*, *Citrus aurantium* L., *cornus fruit* (*Cornus officinalis Sieb. et Zucc.*), *Rheum palmatum* L., and *Panax notoginseng*. The ratios of these raw herbs were 10:5:4:3.4:3:2:1 (W/W) and each gram of the cooked herbs was from 1.77g raw herbs. TSF was processed by Jiangyin Tianjiang Pharmaceutical Company and has been described previously [25]. Briefly, these raw herbs were soaked 30 min and boiled 60 min for two times in distilled water. The decoction was filtered and condensed to 1 g/ml, and then spray dried into fine granules. Authenticating and standardizing of TSF was accomplished through high performance liquid chromatography analysis on marker compounds. The mainly chemical components of TSF are consisted of loganin, calycosin-7-*O*-β- D-glucoside, naringenine-7-rhamnosidoglucoside, naringenin, neohesperidin, and aloe-emodin [25].

### 2.3. Animal experiment

This study was approved by the Ethics Review Committee for Animal Experimentation of the Institute of Clinical Medicine, China-Japan Friendship Hospital, Beijing, and was performed in accordance with the Guiding Principles for the Care and Use of Laboratory Animals (2012- A04).

Twenty male 7-week-old ZDF rats (obese fa/fa) and ten same gender and age ZDF rats (lean fa/+) (ZL) control rats were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). After adaptive feeding standard diet one week, all rats were fed "Purina 5008" diet and allowed free access to food and water under a 12-h light-dark cycle until termination of

experiment. Two rats were housed in one cage. Body weight and fasting blood glucose were first measured before feeding Purina 5008. Then, they were measured every one week and two weeks respectively. After feeding Purina 5008 diet for four weeks, 16 of ZDF rats (obese fa/fa) with blood glucose higher than 11.7 mmol/L were queued based on blood glucose values and then divided into ZDF group (n = 8) and ZDF + TSF group (n = 8) according to serpentine rule. Fasting blood glucose for all ZL control rats were normal and was divided into ZL group (n = 10). After grouping, rats in ZDF + TSF group were intragastrically administered TSF (2.4 g/kg, the dose used was six times higher per unit body weight compared to humans used) which dissolved in saline, and intragastrically administered the same volumetric saline for rats in the ZL and ZDF groups. TSF and saline were given once daily from 12 weeks until termination of experiment.

### 2.4. Sample collection

At the end of experiment, rats were anesthetized by using overdose pentobarbital sodium intraperitoneal injection. A median abdominal incision was made, and blood was draw as much as possible from the abdominal aorta. After euthanized by anesthetized and exsanguinated, 8-cm segment of ileum (5 cm to the end of ileum) and 7-cm segment of colon (2 cm distal to the ileocecal valve) were excised. The lumen content was cleared by gently press using finger and gently rinse using pre-chilled saline. The length and wet weight were measured for each segment. Two-cm segments were obtained from the distal end of the ileum and colon, and then fixed in 10 % phosphate-buffered formalin over 24 h for histological examinations. Remained segments of ileum and colon were put into HBBS solution containing 0.25 % EGTA for biomechanical experiments.

### 2.5. Biomechanical test

#### 2.5.1. No-load and zero-stress experiments

Methods for the experiment of no-load and zero-stress state have been described previously [16,17]. Briefly, three rings about 1.5-mm length were cut from both ileum and colon segments and stored in HBBS solution with EGTA for 5min. A photograph was taken of the cross-section of the rings in the no-load state. Then each ring-shaped segment was cut radially when immersed in the fluid under the microscope. Each ring opened up into a sector. Photographs were taken about 30 min after the radial cutting to allow viscoelastic creep to take place.

#### 2.5.2. Distension experiment

Methods for the distension experiment have been described previously [15]. Briefly, the distal end of ileum and colon were ligated and proximal were connected with a tube and fixed to a container with HBBS solution (containing 0.25 % EGTA). After two times distension with 10 $cmH_2O$ for ileum and 20 $cmH_2O$ for colon (preconditioning the segments), the segment was inflated with HBBS solution under a gradient pressure of 0, 1, 2, 3, 5, 7.5, 10 $cmH_2O$ for ileum and 0, 2, 4, 6, 10, 15, 20 $cmH_2O$ for colon. Each pressure maintained 1 min and photographs were taken.

### 2.6. Histologic staining

#### 2.6.1. HE and Masson staining

The ileum and colon segments, which were fixed in 10 % phosphate-buffered formalin for over 24 h, were routinely dehydrated, embedded in paraffin, cut for 5-μm thick sections, stained with hematoxylin and eosin (HE) and Masson, made transparent using xylene, and sealed with neutral resin. HE-stained slides were used for general histological observation and measurement of layer thickness, whereas Masson-stained slides were used for total collagen measurement of submucosa and muscle layers. Masson staining is one classic technique for collagen fiber staining. It is mainly suitable for the differential analysis of collagen (green or blue colors) and muscle (red color).

#### 2.6.2. Immunohistochemical staining for type I and type III collagen

Paraffin sections were dewaxed and hydrated using xylene, different concentrations of alcohol and distilled water. Antigen retrieval was accomplished by using 10 mM citrate buffer (pH6.0) which boiled and maintained 18min. For quenching endogenous peroxidase, the sections were incubated with 3 % $H_2O_2$ for 25min at room temperature. Nonspecific staining was blocked by incubated sections with 5 % BSA-PBS buffer at room temperature for 30min. Then the sections were incubated with primary antibody which diluted with 1 % BSA-PBS (1:1000 for against type I collagen antibody and 1:250 for type III collagen) at 4 °C overnight. For negative control, the sections were incubated with normal rabbit serum which pretreated by excessive type I and type III collagen and was diluted with 1 % BSA-PBS (1:1000 for type I collagen and 1:250 for type III collagen). After washing completely using 0.05 % tween-20-PBS, sections were incubated with second antibody marked with HRP for 50min at room temperature. Washing sections completely with 0.05 % tween-20-PBS, the positive area was visualized by incubated sections with DAB solution and then counter staining with Mayer's hematoxylin.

### 2.7. Data analysis

#### 2.7.1. Histomorphological data analyses

The sections with HE, Masson and Collagen immunohistochemical staining for both ileum and colon were scanned by tissue section

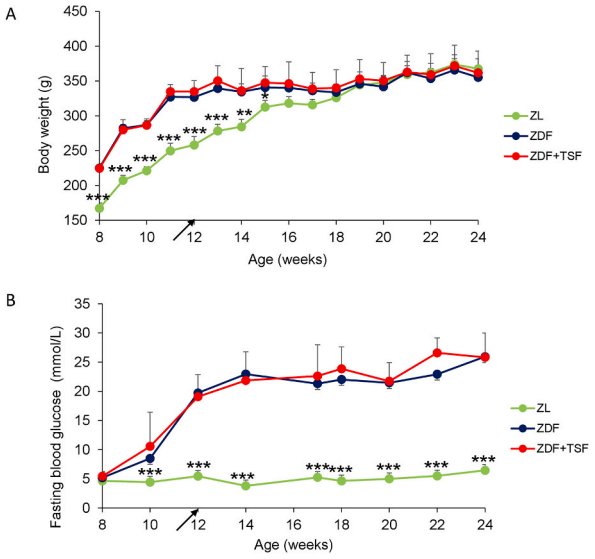



**Fig. 1.** Body weight and fasting blood glucose
Fig. 1 A shows body weight increasing quickly from 8 to 15 weeks for ZL group and from 8 to 12 weeks for ZDF and ZDF + TSF groups. The body weight is significantly lower in ZL group than in ZDF and ZDF + TSF groups from 8 to 15 weeks, where after no difference is found among three groups. Fig. 1 B shows fasting blood glucose of rats in ZDF and ZDF + TSF groups increasing quickly from 8 to 12 weeks, where after increasing slowly until the end of experiment. Whereas the fasting blood glucose level in ZL group remains constant during the entire experiment. From 10 weeks until the end of the experiment, the fasting blood glucose level is significantly higher in ZDF and ZDF + TSF groups than in ZL group, whereas no difference is found between ZDF and ZDF + TSF groups. Arrow (→) indicates the starting time of intragastric with saline (for ZL and ZDF groups) or TSF (for ZDF + TSF group).
Comparing with ZDF and ZDF + TSF groups (Tukey Test): *P < 0.05, **P < 0.01, ***P < 0.001.

digital scanner (Pannoramic MIDI, 3DHISTECH, Budapest, Hungary). The thickness of mucosa (villus and crypt for ileum), submucosa, circumferential and longitudinal muscle were directly measured at the images of HE staining slides by using VENTANA Image Viewer (version 3.2.0, Ventana Medical Systems, Inc.). Furthermore, the thickness of the ileal mucosa (sum of villus height and crypt depth) and the intact wall (sum of all layers) were obtained. Six locations around intestinal sectional ring were randomly selected for measurement.

For analysis of collagen fractions, six locations (almost covering whole circumference of the segment) of each slide through entire wall from ileum and colon at a magnification of 20x were randomly selected and saved as BMP image files by using the VENTANA Image Viewer. Then the different layers were isolated by using Paint software and saved as separated BMP files respectively. For Masson staining, the submucosa and muscle layers for both ileum and colon were selected. For type I and type III collagen staining, the crypt, submucosa and muscle layers for ileum and the mucosa, submucosa and muscle layers for colon were selected. In order to measure the percentage of collagen in different layers, the blue area of collagen in Masson staining and the brown area from collagen immunohistochemistry staining were defined using SigmaScan Pro 4.0 image analysis software (Jandel Scientific, Germany). Finally, the intensity threshold is used to distinguish the color of collagen in the different layers. The area of the collagen and the total area of the different layers were marked with pseudo-color (red), and the area percentage of collagen was calculated by using Matlab program (MATLAB 7.1, The Mathworks Inc. United States).

### *2.7.2. Morphometric data analysis and residual strain computation*

The following data were measured from each specimen for the morphometric analysis: the inner and outer circumference (C), the wall thickness (H), the wall area (A), and the opening angle at zero-stress state [15]. The opening angle 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.

The residual strain was calculated from the morphometric data at no-load and zero stress state as equations [1,2]:

Residual Green strain at the mucosal surface: $E_i=((C_{i\text{-}n}/C_{i\text{-}z})*(C_{i\text{-}n}/C_{i\text{-}z})\text{-}1)/2$ (1)

Residual Green strain at the serosal surface: $E_o=((C_{o\text{-}n}/C_{o\text{-}z})*(C_{o\text{-}n}/C_{o\text{-}z})\text{-}1)/2$ (2)

Where $C$ is the circumference and the subscripts i, o, n and z refer to the inner (mucosal) surface, outer (serosal) surface, no-load state and zero-stress state respectively.

### *2.7.3. Stress and strain analysis*

The stress and strain of the segments in the pressurized state were determined under assumptions that the wall is homogenous and the intestinal shape is cylindrical. The parameters at the pressurized state were calculated from the measured length ($L_p$) and outer

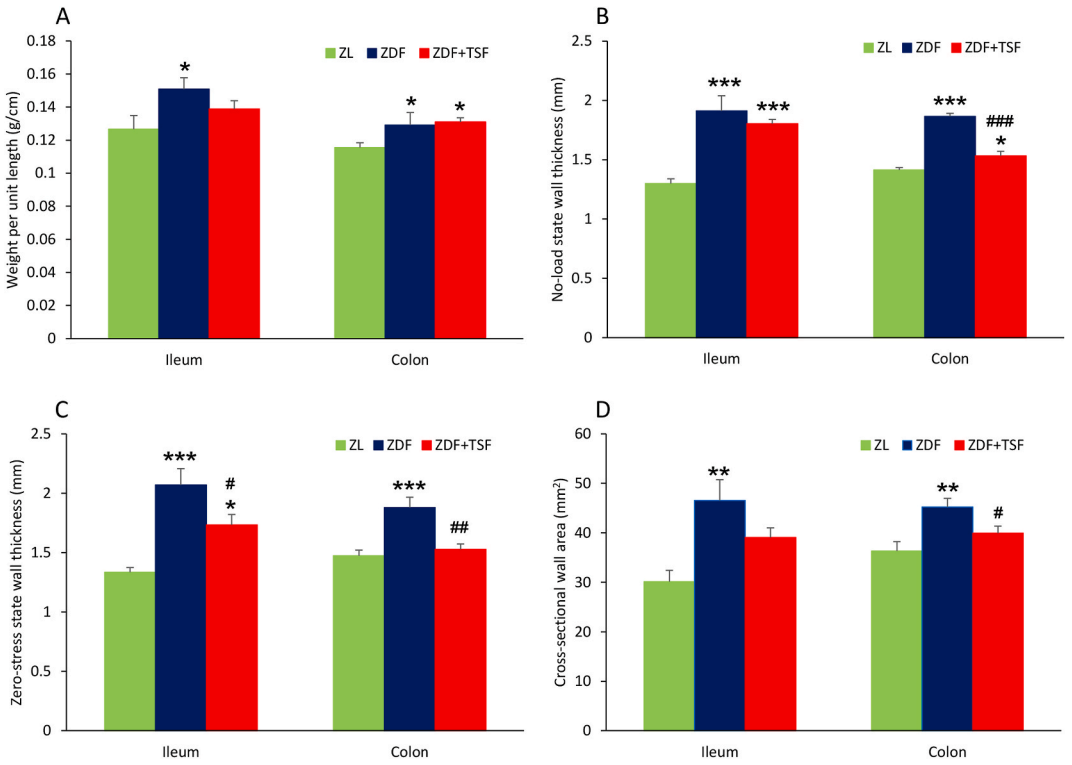



**Fig. 2.** Wet weight, wall thickness, and cross-sectional wall area of ileum and colon
Wet weight per unit length (A) is higher in the ZDF and ZDF + TSF groups than in ZL group, however, for the ileum the extent of increase is lower in the ZDF + TSF group than in the ZDF group. The no-load state wall thickness (B), zero-stress state wall thickness (C) and cross-sectional wall area (D) are bigger in the ZDF and ZDF + TSF groups than in the ZL group, however the extent of increase is lower in the ZDF + TSF group than in the ZDF group.
Comparing with ZL group (Tukey Test): *P < 0.05, **P < 0.01, ***P < 0.001
Comparing with ZDF group (Tukey Test): $^{\#}$P < 0.05, $^{\#\#}$P < 0.01, $^{\#\#\#}$P < 0.001.

diameter ($D_{o\text{-}p}$) of the small intestine and wall area at no-load state ($A_n$) as follows [26]: the longitudinal stretch ratio $\lambda_\varphi = L_p/L_z$; the luminal radius $r_{i\text{-}p}$ = square root (($r_{o\text{-}p}$*$r_{o\text{-}p}$)- $A_n/\pi$); the outer radius $r_{o\text{-}p} = D_{o\text{-}p}/2$; the wall thickness $h_p = r_{o\text{-}p}$- $r_{i\text{-}p}$; the mucosal circumference $C_{i\text{-}p} = 2*\pi*\ r_{i\text{-}p}$; the serosal circumference $C_{o\text{-}p} = 2*\pi*\ r_{o\text{-}p}$; the mid-wall circumference $C_{m\text{-}p}$=($C_{i\text{-}p} + C_{o\text{-}p}$)/2; and the middle-wall circumferential stretch ratio $\lambda_\theta = C_{m\text{-}p}/C_{m\text{-}z}$ (where $C_{m\text{-}z}$=($C_{i\text{-}z} + C_{o\text{-}z}$)/2 is the middle-wall circumference at zero-stress state). The subscripts of i, o, m and p refer to the inner (mucosal) surface, outer (serosal) surface, middle-wall surface, and pressurized state. Then the Kirchhoff 's stress and Green's strain at a given pressure were computed according to the equations of [3–6]:

Circumferential Kirchhoff stress: $S_\theta = \Delta P*r_{i\text{-}p} / (h_p*\lambda_\theta*\lambda_\theta)$ (3)

Longitudinal Kirchhoff's stress: $S_\varphi = \Delta P*(r_{i\text{-}p})*(r_{i\text{-}p}) / (h_p*\lambda_\varphi*\lambda_\varphi*(r_{o\text{-}p} + r_{i\text{-}p}))$ (4)

Circumferential mid-wall Green strain: $E_\theta = (\lambda_\theta*\lambda_\theta - 1)/2$ (5)

Longitudinal Green strain: $E_\varphi = (\lambda_\varphi*\lambda_\varphi - 1)/2$ (6)

where ΔP 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 does not differ between the no-load and zero-stress states [26,27].

### 2.8. Statistical analysis

Data are expressed as mean ± SE unless otherwise indicated. The following parameters among different groups were compared: 1) fasting blood glucose level, body weight and wet weight per unit length of the ileal and colonic segments; 2) morphometry and histological data; 3) opening angles and residual strain; 4) stretch ratio as function of pressures; 5) stress-strain curves; and 6) constants a and b obtained from stress-strain curve fitting by using the exponential function equation $S = (S^{\#} + b)*e^{a*(E-E^{\#})}$. $S^{\#}$ and $E^{\#}$ are the stress and strain at a physiological reference level. One-way ANOVA was used to analyze difference for each parameter among three groups. Two-way-ANOVA were used to analyze body weight and glucose with two factors of groups and times, and stretch ratios with

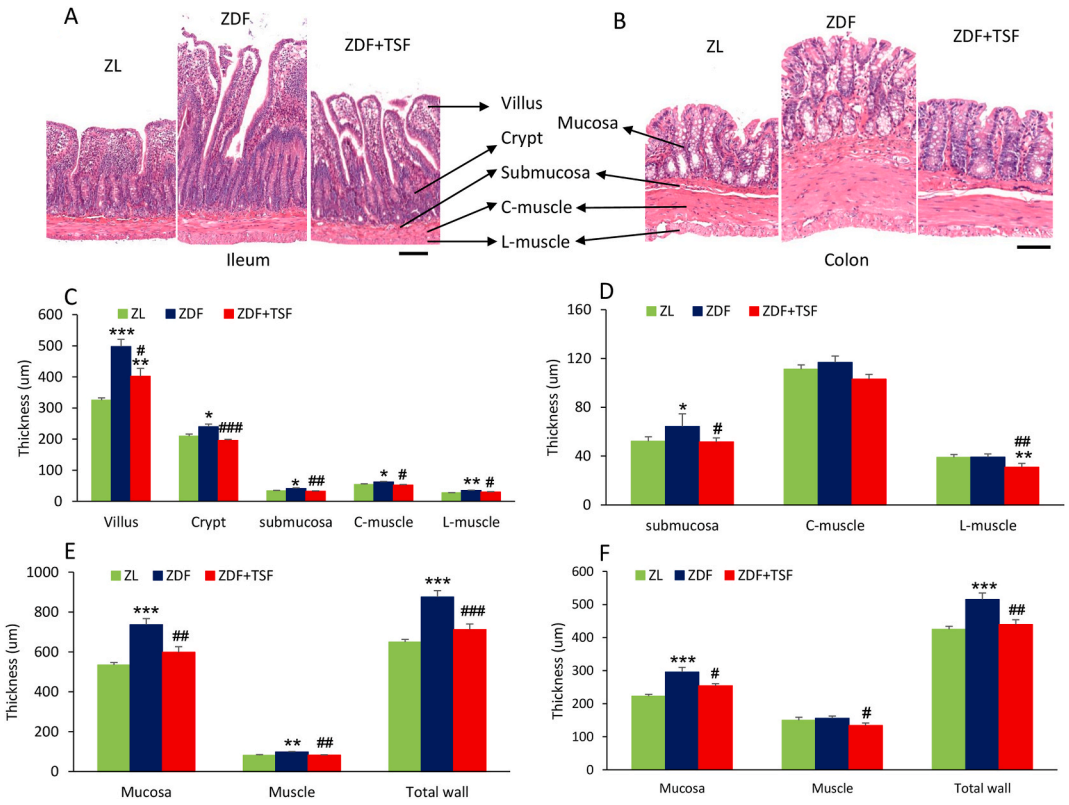



**Fig. 3.** Histological layered wall thickness of ileum and colon

For both ileum (Fig. 3 A, C, E) and colon (Fig. 3 B, D, F), the thickness of different layers was increased in the ZDF group than that in the ZL group and the increasing was partly inhibited by TSF in the ZDF + TSF group. For ZDF group, compared with ZL group, the significant different was found in all layers for ileum (Fig. 3C, E) and in mucosa and total wall thickness for colon (Fig. 3 F). For ZL group, compared with ZDF group, the significant different was found in all layers for ileum (Fig. 3C, E) and in mucosa, muscle and total wall thickness in colon (Fig. 3 F). Interestingly, the longitudinal muscle layer thickness is smaller in ZDF + TSF group than in ZL group (Fig. 3 D).

Comparing with ZL group (Tukey Test): *P < 0.05, **P < 0.01, ***P < 0.001

Comparing with ZDF group (Tukey Test): $^{\#}$P < 0.05, $^{\#\#}$P < 0.01, $^{\#\#\#}$P < 0.001.

two factors of groups and pressures. Multiple tests (Tukey Test) from two-way and one-way analyses were used to analyze each parameter between two different groups among three groups. All statistical analyses were performed by using software of SigmaPlot version 11.0 (Systat Software Inc.). When $P < 0.05$, the results were considered to be significantly different.

## 3. Results

### 3.1. Body weight and fasting blood glucose

Body weight in ZL group increased quickly from 8 to 15 weeks age, where after growing became slower until the end of experiment. Whereas in ZDF and ZDF + TSF groups, the body weight increased quickly from 8 to 12 weeks, then kept relative stable from 13 to 18 weeks, where after increased slowly until the end of experiment (Fig. 1 A). The body weight was significantly lower in ZL group than in ZDF and ZDF + TSF groups from 8 to 15 weeks (Fig. 1 A, two-way ANOVA: $F=112.5$, $P<0.001$), where after no different was found among three groups (Fig. 1 A, $P>0.05$). See detail multiple analysis (Tukey Test) results in Supplementary-Table 1 about the differences between two groups among three groups.

Fasting blood glucose in ZDF and ZDF + TSF groups increased quickly from 8 to 12 weeks of age, where after increased slowly until the end of experiment. No difference was found between ZDF and ZDF + TSF groups (Fig. 1 B, $P>0.05$). Whereas the fasting blood glucose level in ZL group remained constant during the entire experiment. From 10 weeks until the end of the experiment, the fasting blood glucose level was significantly higher in ZDF and ZDF + TSF groups than in ZL group (Fig. 1 B, two-way ANOVA: $F=358.3$, $P<0.001$). See detail multiple analysis (Tukey Test) results in Supplementary-Table 2 about the differences between two groups among three groups.

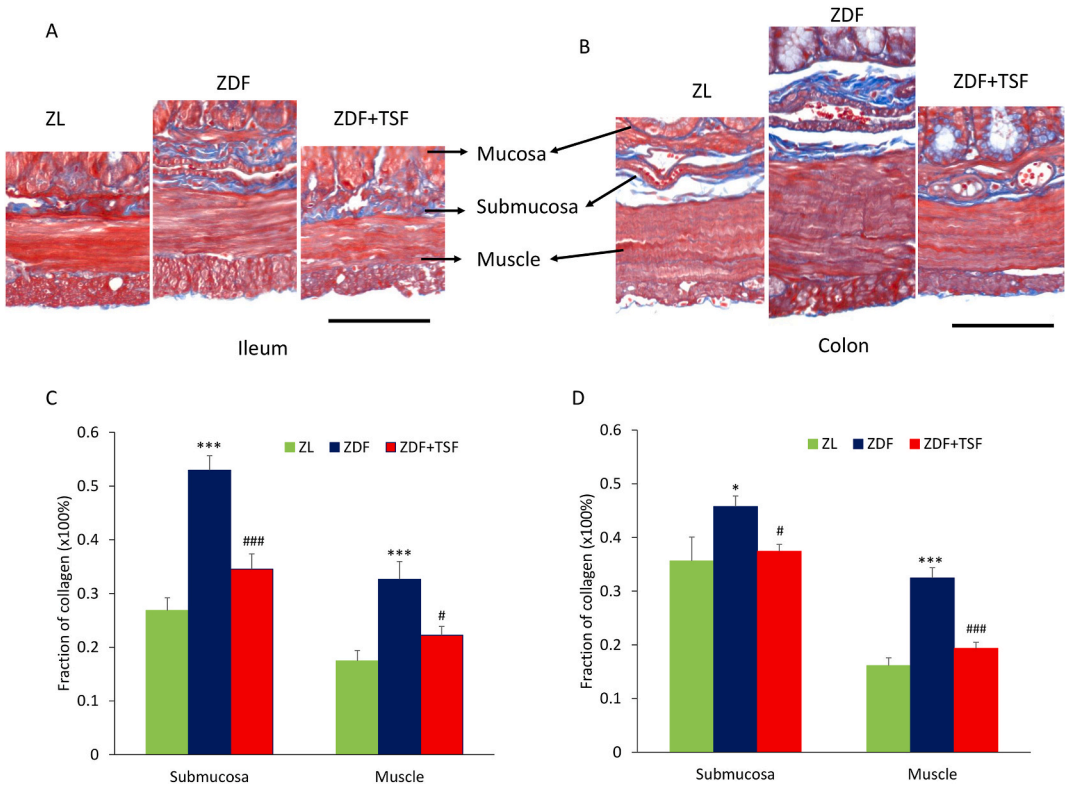



**Fig. 4.** collagen fractions of Masson staining in ileal and colonic wall
For both ileum (Fig. 4 A) and colon (Fig. 4 B), the representative samples show that the blue color intensity of submucosa and muscle layers is weaker in the ZL and ZDF + TSF groups than that in the ZDF group and statistic values (Fig. 4C, D) show the same results.
Comparing with ZL group (Tukey Test): *P < 0.05, ***P < 0.001
Comparing with ZDF group (Tukey Test): $^{\#}$P < 0.05, $^{\#\#\#}$P < 0.001.

### 3.2. TSF effects on wet weight, wall thickness and wall area of ileum and colon in ZDF rat model

Compared with the ZL group, ileal (One-way ANOVA, *F = 3.958, P=0.037*) and colonic (One-way ANOVA, *F=4.99, P=0.021*) wet weight per unit length increased in the ZDF and ZDF + TSF groups (Fig. 2 A). However, for the ileum the extent of increase was lower in the ZDF + TSF group than in the ZDF group. Significant difference of multiple analysis was found between the ZDF and ZL groups for both ileum (*q=3.965, P=0.029*) and colon (*q=3.597, P=0.050*), and between ZDF + TSF and ZL group for colonic segment (*q=4.011, P=0.03*). See detail multiple analysis (Tukey Test) results in Supplementary-Table 3 about the differences between two groups among three groups.

Compared with the ZL group for the morphometry data, the ZDF group had bigger values for no-load state wall thickness (Fig. 2 B, ileum, *q=7.987, P<0.001; colon, q=16.342, P<0.001*), zero-stress state wall thickness (Fig. 2C, ileum, *q=7.976, P<0.001; colon, q=6.499, P<0.001*) and cross-sectional wall area (Fig. 2 D, ileum, *q=5.644, P=0.002; colon, q=5.141, P=0.005*). Although the values of most morphometry data were bigger in ZDF + TSF group than in the ZL group, the extent of increase was lower in the ZDF + TSF group than in the ZDF group. Compared with ZDF group, the significant differences were found for the colon in no-load state wall thickness (Fig. 2 B, *q=11.670, P<0.001*), zero-stress state wall thickness (Fig. 2C, *q=5.466, P=0.003*) and cross-sectional wall area (Fig. 2 D, $q = 3.964$, $P = 0.049$), and however for the ileum only in the zero-stress state wall thickness (Fig. 2C, $q = 3.820$, $P = 0.049$). See detail one-way ANOVA and multiple analysis (Tukey Test) results in Supplementary-Table 3 about the differences between two groups among three groups.

### 3.3. TSF attenuates layered thickness of ileum and colon in ZDF rat model

The histological measurement data were shown in Fig. 3. Representative images of HE-stained histological slides showed that the thickness of different layers were smaller in ZL and ZDF + TSF groups than in ZDF group for both ileum (Fig. 3 A) and colon (Fig. 3 B). The average data was showed in Fig. 3 C–F. The most parameters were bigger in ZDF group than in ZL group for both ileum (Fig. 3C,E) and colon (Fig. 3 D,F). For the ileum, the significant differences were found in total wall and all layered thickness (*q is from 3.783 to 9.198*; *P is from 0.038 to <0.001*). For the colon, the significant differences were found in mucosa (*q=7.677, P<0.001*), submucosa (*q=5.547, P=0.012*) and total wall thickness (*q=6.250, P<0.001*). Compared with ZDF group, both for ileum (Fig. 3C, E) and colon

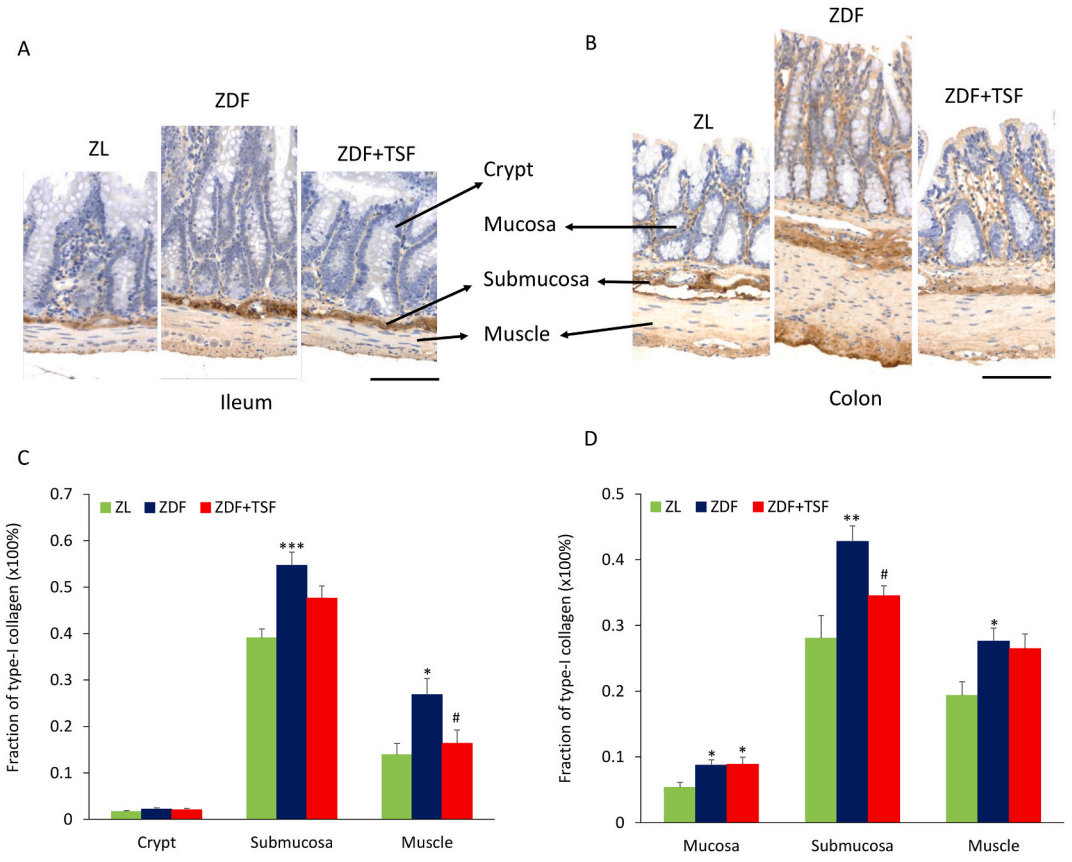



**Fig. 5.** collagen fractions of Type-I collagen immunohistochemistry staining in ileal and colonic wall
For both ileum (A) and colon (B), the representative samples show that the brown color intensity of different layers is weaker in the ZL and ZDF + TSF groups than that in the ZDF group and statistic values (Fig. 5C, D) show the same results. Although higher values for most parameters are found in ZDF + TSF group than that in ZL group, however the less degree is found in ZDF + TSF group than that in ZDF group.
Comparing with ZL group (Tukey Test): *P < 0.05, **P < 0.01, ***P < 0.001
Comparing with ZDF group (Tukey Test): $^{\#}$P < 0.05.

(Fig. 3 D, F), the most parameters were smaller in ZDF + TSF group. For the ileum, the significant differences were found for total wall and all layered thickness (*q is from 3.783 to 6.438*; *P is from 0.038 to <0.001*). For the colon, the differences were found for longitudinal muscle (*q=5.795, P=0.002*), mucosa (*q=4.197, P=0.021*), muscle (*q=3.885, P=0.033*) and total wall thickness (*q=5.047, P=0.006*). See detail one-way ANOVA and multiple analysis (Tukey Test) results in Supplementary-Table 3 about the differences between two groups among three groups.

### *3.4. TSF attenuates collagen content of ileum and colon in ZDF rat model*

Fig. 4 shows the data of collagen analysis of Masson staining. Representative images of Masson-stained histological slides showed that the blue color intensity of collagen in submucosa and muscle layers were weaker in ZL and ZDF + TSF groups than in ZDF group for both ileum (Fig. 4 A) and colon (Fig. 4 B). Compared with ZL group, the average values of collagen fractions were significantly higher in ZDF group for both the ileum (Fig. 4C, submucosa, *q=9.873, P<0.001;* muscle, *q=6.593, P<0.001*) and the colon (Fig. 4 D, submucosa, *q=4.597, P=0.011;* muscle, *q=10.858, P<0.001*). TSF could attenuate collagen fraction increasing in the ileum (Fig. 4C, submucosa, *q=6.762, P<0.001 and muscle, q=4.388, P=0.016*) and colon (Fig. 4 D, submucosa, q = 3.824, P = 0.049 and muscle, *q=8.428, P<0.001*) of ZDF rats (obese fa/fa).

Fig. 5 shows the data of collagen analysis of type I collagen. Representative images of type I collagen immunohistochemistry-stained histological slides showed that the brown color intensity of collagen in different layers were weaker in ZL and ZDF + TSF groups than in ZDF group for both ileum (Fig. 5 A) and colon (Fig. 5 B). Compared with ZL group, the values of collagen fractions were significantly higher in ZDF group for both the ileum (Fig. 5C, submucosa, *q=6.228, P=0.001;* muscle, *q=4.464, P=0.015*) and the colon (Fig. 5 D, mucosa, *q=3.897, P=0.033;* submucosa, *q=5.540, P=0.003;* muscle, *q=3.962, P=0.030*). Compared with ZDF group, the significant smaller values were found in ileal muscle (Fig. 5C, *q=4.193, P=0.049*), and in colonic submucosa (Fig. 5 D, *q=4.476, P=0.020*) in the ZDF + TSF group.

Fig. 6 shows the data of collagen analysis of type III collagen. Representative images of type III collagen immunohistochemistry-

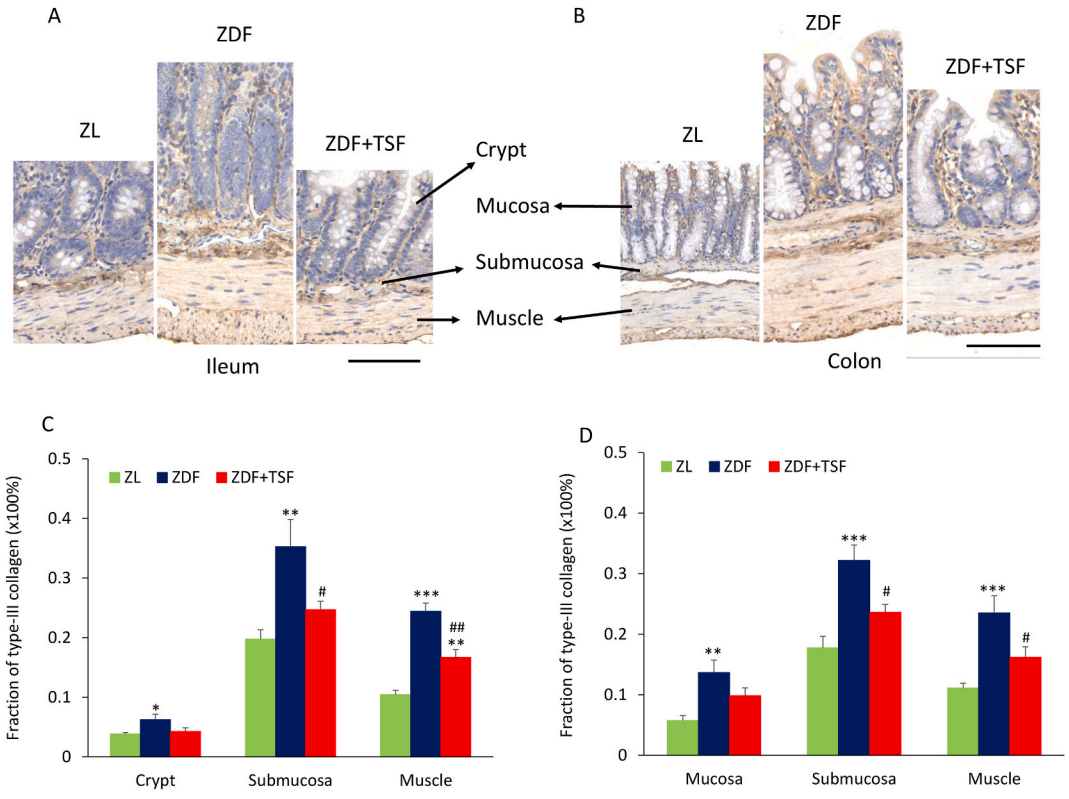



**Fig. 6.** collagen fractions of Type-III collagen immunohistochemistry staining in ileal and colonic wall
For both ileum (A) and colon (B), similarly as Type-I collagen the representative samples show that the brown color intensity of different layers is weaker in the ZL and ZDF + TSF groups than that in the ZDF group and statistic values (Fig. 6C, D) show the same results. Although higher values for most parameters are found in ZDF + TSF group than that in ZL group, however the less degree is found in ZDF + TSF group than that in ZDF group.
Comparing with ZL group (Tukey Test): *P < 0.05, **P < 0.01, ***P < 0.001
Comparing with ZDF group (Tukey Test): $^{\#}$P < 0.05, $^{\#\#}$P < 0.01.

stained histological slides showed that the brown color intensity of collagen in different layers were weaker in ZL and ZDF + TSF groups than in ZDF group for both ileum (Fig. 6 A) and colon (Fig. 6 B). Compared with ZL group, the values of collagen fractions were significantly higher in ZDF group for both the ileum (Fig. 6C, crypt, *q=4.330, P=0.020;* submucosa, *q=6.519, P=0.001;* muscle, *q=12.386, P<0.001*) and the colon (Fig. 6 D, mucosa, *q=5.763, P=0.003;* submucosa, *q=7.436, P<0.001;* muscle, *q=6.948, P<0.001*). Compared with ZDF group, the significant smaller values were found for ileal submucosa (Fig. 6C, *q=4.218, P=0.024*) and muscle (Fig. 6C, *q=6.497, P=0.001*), and in colonic submucosa (Fig. 6 D, *q=4.266, P=0.022*) and muscle (Fig. 6 D, *q=3.976, P=0.033*) in the ZDF + TSF group.

See detail one-way ANOVA and multiple analysis (Tukey Test) results in Supplementary-Table 3 about the differences between two groups among three groups.

### 3.5. *TSF likely has no effect on biomechanical remodeling of ileum and colon in ZDF rat model*

The opening angle and residual strains data were shown in Fig. 7. Generally, the absolute values of opening angle (Fig. 7 A) and residual strains (Fig. 7 B, C) were smaller in ileum and bigger in colon in ZDF group than in ZL group. However, the significant difference was only found for inner residual strain in colon (Fig. 7 B, *q=3.908, P=0.032*) and outer residual strain in ileum (Fig. 7C, *q=3.716, P=0.042*). The changes of opening angle and residual strain were slighter in ZDF + TSF group than in ZDF group, however no significant difference was found (*P>0.05*). See detail one-way ANOVA and multiple analysis (Tukey Test) results in Supplementary-Table 3 about the differences between two groups among three groups.

Circumferential (A, B) and longitudinal (C, D) stretch ratio of ileum (A, C) and colon (B, D) were shown in Fig. 8. Two-way ANOVA analysis indicated the significant differences among three groups for both circumferential stretch ratio (ileum, Fig. 8 A, F = 55.099, P < 0.001; colon, Fig. 8 B, F = 37.175, P < 0.001) and longitudinal stretch ratio (ileum, Fig. 8C, two-way ANOVA, F = 7.346, P < 0.001; colon, Fig. 8 D, two-way ANOVA, F = 3.003, P < 0.05). Multiple comparison (Tukey test) indicated that the circumferential stretch ratio was significantly bigger in ZDF (ileum: *q* from 4.110 to 5.019, *P* from 0.010 to <0.001; colon: *q* from 3.757 to 4.669, *P* from 0.024 to 0.004) and ZDF + TSF groups (ileum: *q* from 4.276 to 5.463, *P* from 0.007 to <0.001; colon: *q* from 2.534 to 3.637, *P* from 0.177 to 0.031) than in ZL group, however, no difference was found between ZDF and ZDF + TSF groups (P > 0.05). For the longitudinal stretch

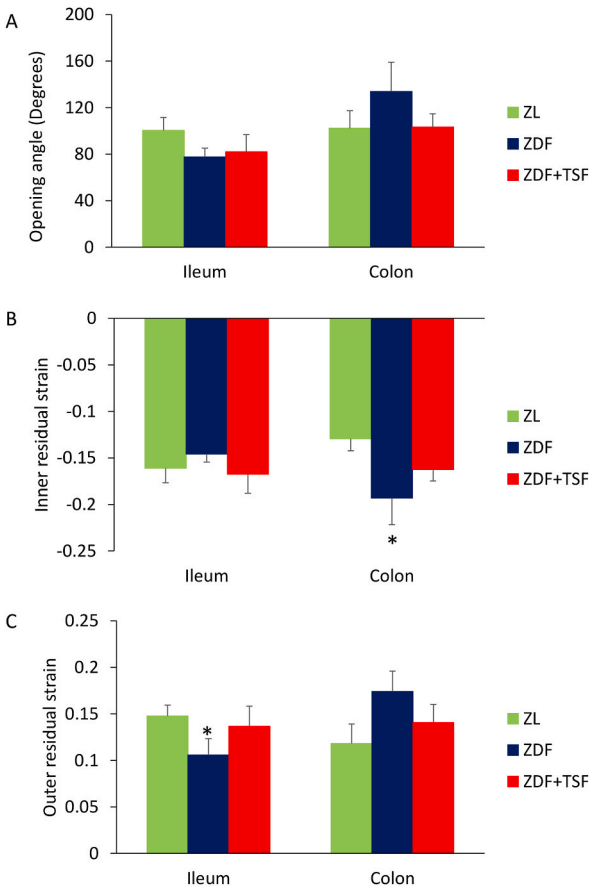



**Fig. 7.** Opening angle and residual strain
Although the absolute values of opening angle (A) and residual strains (inner, B; outer, C) tend to be smaller in ileum and bigger in colon in ZDF group than in ZL group. However, the significant difference is only found for inner residual strain in colon (B) and outer residual strain in ileum (C). No significant difference is found between ZDF + TSF and ZDF groups.
Comparing with ZL group (Tukey Test): *P < 0.05.

ratio, only at 10 cm$H_2O$ pressure point for ileum (Fig. 8C), ZDF group was significantly smaller than ZL group ($q=3.832$, $P=0.018$). In addition, no difference was found between ZDF and ZDF + TSF groups ($P > 0.05$). See detail multiple analysis (Tukey Test) results in Supplementary-Table 4 about the differences among three groups.

Circumferential and longitudinal stress-strain curves of ileum (A, C) and colon (B, D) were shown in Fig. 9. Compared with ZL group, the circumferential stress-strain curves (Fig. 9 A, B) shifted to the right indicating softer. However, one-way ANOVA analysis of constant a and b showed no significant difference among different groups for both ileum (Fig. 10 A, $F < 1.4$, $P > 0.2$) and colon (Fig. 10 B, constant a, $F < 2.5$, $P > 0.1$). Whereas, the longitudinal stress-strain curves (Fig. 9C, D) shifted to the left indicating stiffening. However, one-way ANOVA analysis also showed no significant difference among different groups for both ileum (Fig. 10C, $F < 0.7$, $P > 0.4$) and colon (Fig. 10 D, $F < 1.9$, $P > 0.2$).

## 4. Discussions

Our previous study has demonstrated that Tangshen Formula could partly attenuate colonic structure remodeling in type 2 diabetic rat model [23]. However, that study did not analyze the biomechanical properties and did not include small intestinal segments. In the present study, we confirmed colonic histomorphological remodeling in ZDF rats (obese fa/fa) and the TSF could partly attenuate the remodeling. At same time, we also found that the ileal histomorphologically remodeled in ZDF rats (obese fa/fa) and TSF could partly attenuate the remodeling as well. Furthermore, it was demonstrated that both ileal and colonic segments somehow remodeled biomechanically, TSF had no significantly effect on biomechanical remodeling in ZDF rats (obese fa/fa). Therefore, TSF could attenuate ileal and colonic histomorphological remodeling rather than biomechanical remodeling in ZDF rats (obese fa/fa).

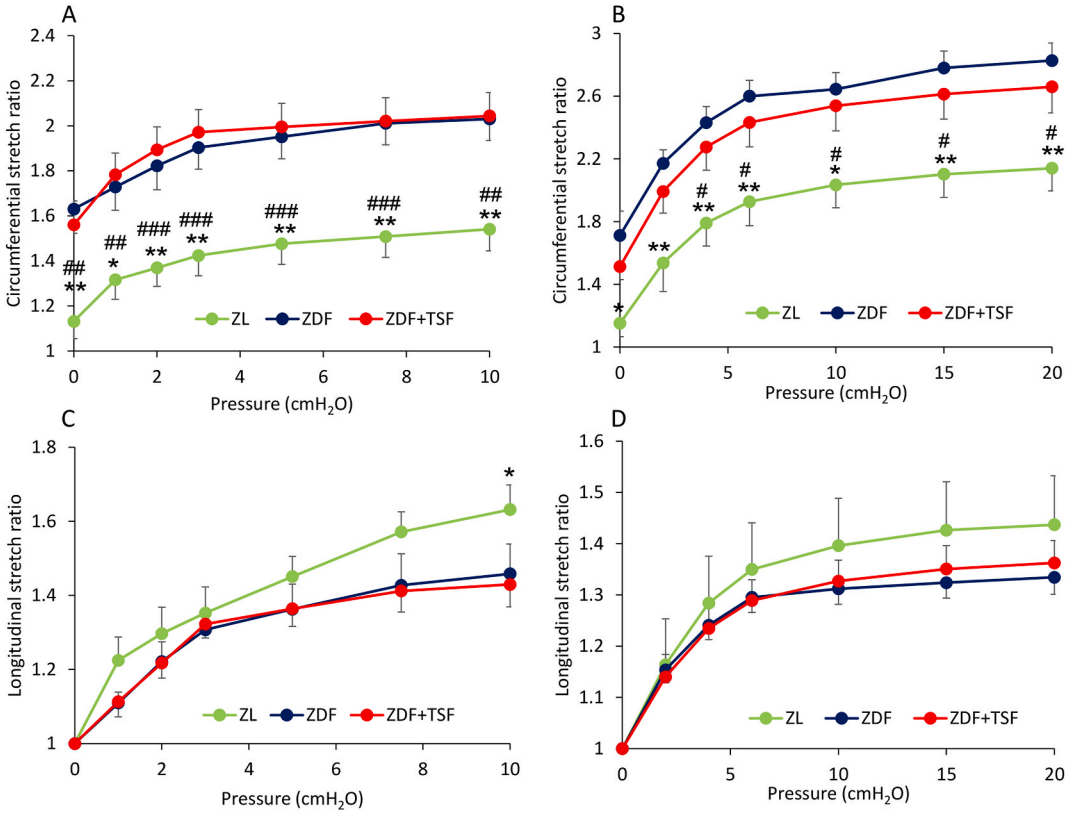



**Fig. 8.** Stretch ratio as function of the pressures
The circumferential stretch ratios are bigger in ZDF and ZDF + TSF groups than that in ZL group for both ileum (A) and colon (B). However, no difference is found between ZDF and ZDF + TSF groups. Whereas the longitudinal stretch ratios are smaller in ZDF and ZDF + TSF groups than that in ZL group for both ileum (C) and colon (D). Similarly, no difference is found between ZDF and ZDF + TSF groups.
Comparing with ZDF group (Tukey Test): *P < 0.05, **P < 0.01
Comparing with ZDF + TSF group (Tukey Test): $^{\#}$P < 0.05, $^{\#\#}$P < 0.01, $^{\#\#\#}$P < 0.001.

### *4.1. Histomorphological and biomechanical remodeling of small intestine and colon in ZDF rat model*

ZDF rats (obese fa/fa) as spontaneous diabetic animal model exhibit the symptoms and signs observed in human Type 2 DM patients both in pre-diabetes and in late stage [28]. ZDF rats (obese fa/fa) start to develop Type 2 DM as early as 10 weeks of age, reaching 100 % incidence at around 20 weeks of age [29]. Therefore, ZDF rat (obese fa/fa) model is widely used in the research for type 2 DM and related complications all over the world [30–32]. Hence, we adopted ZDF male rats (obese fa/fa) as type 2 DM model in our study. At present study, the body weight and fasting blood glucose levels in ZDF male rats (obese fa/fa) were significantly higher than that in ZDF rats (lean fa/+) (Fig. 1). The highest fasting blood glucose level was up to about 26 mmol/L during the period of the experiment.

Nowadays it is well known that the patients with both type 1 and type 2 diabetes often have gastrointestinal problems, named diabetic gastrointestinal complication [33–35]. It has been demonstrated that the histomorphological and biomechanical remodeling of gastrointestinal tract including small intestine and colon occurred during the development of diabetes in several diabetic animals [2]. In STZ-induced diabetic rat models, both small intestine [15,18–20,36] and colon [14,20,21,36] expressed as increasing in wet weight per unit length, total wall and layered wall thickness, and wall area. Collagen increasing in colonic mucosa has been reported [37]. Biomechanically, both small intestine and colon expressed as changing in opening angle and residual strain and increasing in wall stiffness for both circumferential and longitudinal directions in STZ-induced diabetic rats [14,15,18–21]. In GK diabetic rat (another spontaneous type 2 diabetes model), intestinal histological [38–40] and biomechanical [39,40] remodeling has been reported, and similar data were obtained as STZ-induced diabetic rat model. Recently, our group has demonstrated the histological remodeling of colon such as wet weight and layer thicknesses increasing in high fat feeding plus low dose STZ-induced type 2 diabetic rat model [23]. However, to the best of our knowledge, no study in relation to histological and biomechanical remodeling of GI tract has been reported in world-widely used ZDF type 2 diabetic model so far. At present study, we for the first time demonstrated the small intestinal and colonic remodeling in ZDF rats (obese fa/fa). The similar histomorphological remodeling was happened in ileum and colon in ZDF rats (obese fa/fa) comparable with STZ induced diabetic rats, i.e., wet weight, wall thickness, wall area, layered wall thickness and collagen contents in the wall increased. Surprisingly, the biomechanical remodeling is somehow different compared with the data discovered previously in STZ-induced diabetic rats. ZDF rats (obese fa/fa) had smaller opening angle and residual strain in ileum, and bigger

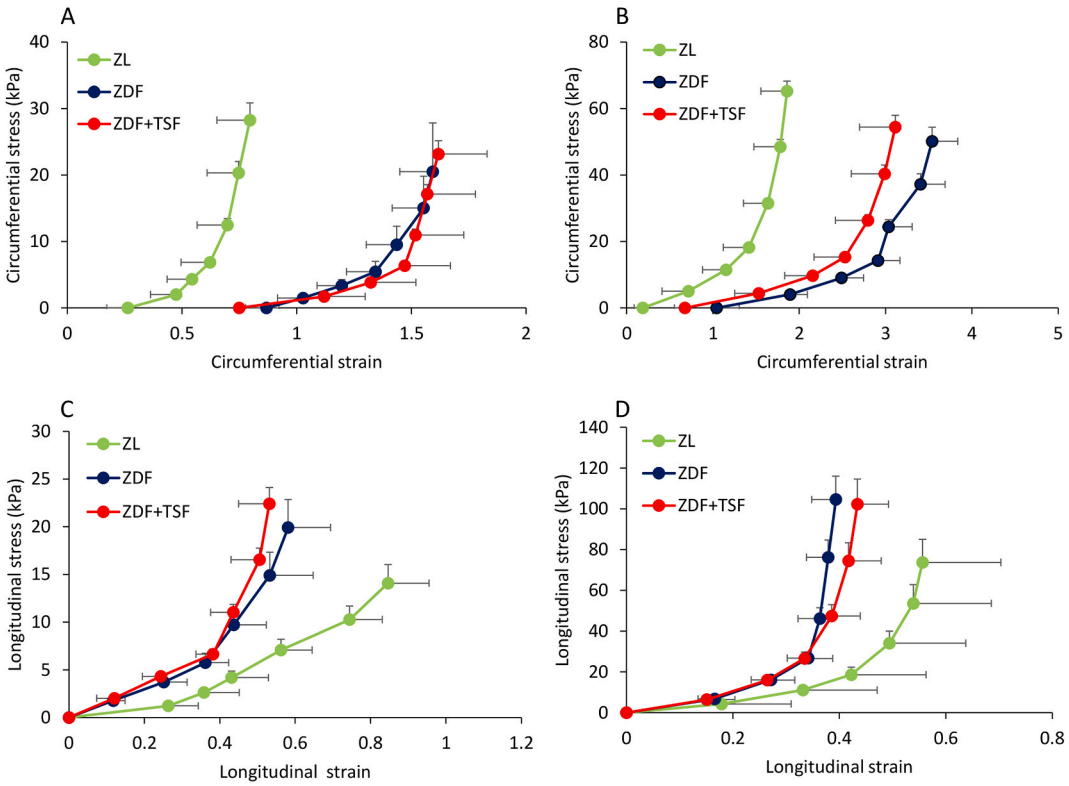



**Fig. 9.** Stress-strain relationships
Comparing with ZL group, the circumferential stress-strain curves in ZDF and ZDF + TSF groups for both ileum (A) and colon (B) shift to the right indicating softening. Whereas, the longitudinal stress-strain curves in ZDF and ZDF + TSF groups for both ileum (C) and colon (D) shift to the left indicating stiffening.

opening angle and residual strain in colon. Similar with STZ-induced diabetic rats, the wall became stiffer in longitudinal direction for both ileum and colon; opposite with STZ-induced diabetic rats, however, the wall became softer in circumferential direction for both ileum and colon. It is well known that ZDF rat (obese fa/fa) model has deficiencies in its leptin receptors and therefore researchers often use it for obesity and type 2 diabetes studies [28–32]. ZDF rat (obese fa/fa) model is different in some aspects with other diabetic models [41]. For example, the body weight curve in the ZDF rats (Fig. 1 in the present paper) differs significantly from that in the STZ rats (Fig. 1 in reference 23). The different performances in the body weight change between the two diabetic rat models might imply the existence of varied mechanisms during the model generation. However, we don't know the cause-relationship exactly about the differences in the present study. We need to further explore the mechanisms such as the detail tissue structure, orientation of collagen and muscle fibers, molecular pathways as well as the effect of obesity on GI biomechanical properties in future studies.

### *4.2. Effect of TSF on diabetes-induced intestinal and colonic remodeling*

Traditional Chinese Medicine (TCM) including herb medicine have been widely used to treat diabetes and proved to have efficacy in improving diabetic symptoms and complications [42]. It has been demonstrated that several Chinese herb medicines could improve diabetes-induced small intestinal and colon remodeling in diabetic animal models [11,19,20,23]. TSF is a traditional Chinese herbal compound and showed effective for diabetic kidney diseases in both diabetic patients [22] and diabetic animals [43]. Recently our group demonstrated that TSF could partly improve colonic structure remodeling in high fat diet feeding plus low dose STZ-induced type 2 diabetic rat model [23]. At present study in ZDF rat (obese fa/fa) model, we confirmed the improvement of TSF not only on colonic histomorphological remodeling but also on ileal histomorphological remodeling (Figs. 2–4) including decreasing wet weight, wall thickness, wall area, layered wall thickness and collagen fractions in different layers. However, against our hypothesis TSF did not show the improvement on passive biomechanical remodeling for both ileum and colon in the ZDF rat (obese fa/fa) models (Figs. 5–7). In future studies, we need to confirm this data and further investigate whether TSF have effect on active mechanical properties such as muscle contractions in ZDF rat (obese fa/fa) model.

Same as our previous study [23], TSF did not have effect on the blood glucose level in ZDF rats (obese fa/fa) (Fig. 1). Therefore, it seems that the effect of TSF on small intestinal and colonic remodeling in ZDF rats (obese fa/fa) not through improving hyperglycemia. Although we did not investigate the mechanisms about the effect of TSF on small intestinal and colonic remodeling at present study, several studies of mechanisms about TSF effect on diabetic kidney diseases [43–48] and diabetic-colonic histologic remodeling [23]

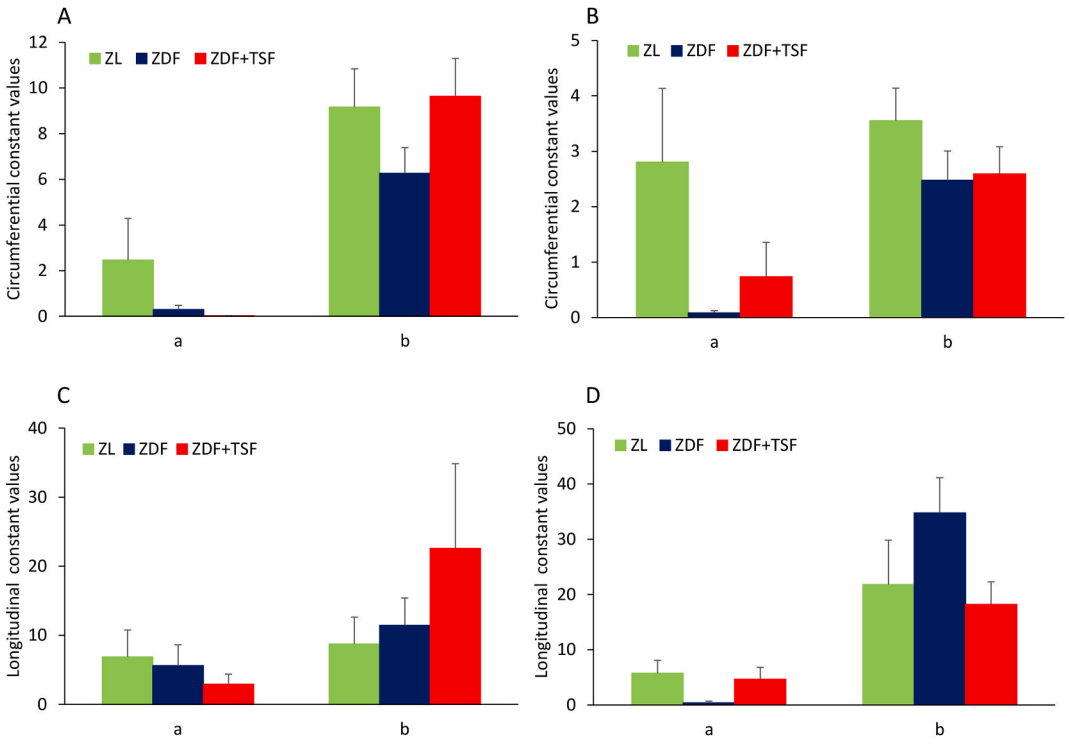



**Fig. 10.** Constant a and b obtained from stress-strain curves fitting
One-way ANOVA and multiple comparison (Tukey Test) in showed that both circumferential (A, B) and longitudinal (C, D) constant a and b show no significant difference among different groups for both ileum (A, C) and colon (B, D).

have been reported. Zhao et al. reported that TSF could attenuate diabetic renal injuries by upregulating autophagy through inhibiting PLZF expression, which in turn resulted in an increase in autophagic degradation of collagen III [44]. Zhao et al. also showed that TSF significantly inhibited diabetic renal injury through attenuating renal inflammation by modulating gut microbiota and decreasing levels of lipopolysaccharide and indoxyl sulfate [46]. Zhang et al. demonstrated that TSF could reduce glomerulosclerotic index and renal tubular interstitial fibrosis in type 1 and type 2 diabetic rats through inhibiting overexpression of transforming growth factor-$\beta$1 (TGF-$\beta$1) [48]. Zhao et al. discovered that TSF could inhibit expressions of nuclear factor kappa B (NF-$\kappa$B) and proinflammatory cytokines, such as tumor necrosis factor-$\alpha$ (TNF-$\alpha$), interleukin-1 (IL-1), and monocyte chemoattractant protein-1 in the kidney of diabetic rats [43]. Recently our group has demonstrated in a type 2 diabetic rat model that TSF down regulated expressions of NF-$\kappa$B, interferon-$\gamma$ (IFN-$\gamma$), interleukin-6 (IL-6), TGF-$\beta$1, and Smad2/3 in the colon wall [23]. The level of expression of NF-$\kappa$B was associated with those of TGF-$\beta$1 and Smad2/3, whereas the expression of TGF-$\beta$1 was associated with the most colonic histomorphometric parameters [24]. More recently, our group found that the TSF had protective effects on ICC through repair of the epithelial junctions, which attenuates inflammation and inflammation-initiated apoptosis in colon of DM rats [49]. Based on the above mentioned, we have reasons to believe that similar mechanisms may be account for the effect of TSF on small intestinal and colonic remodeling in ZDF rats (obese fa/fa). However, we need to confirm and explore direct cause-result relationships about the effect of TSF on gastrointestinal remodeling in ZDF rats (obese fa/fa) in future studies.

### *4.3. Limitations of the study*

At present study, we have several weak points that are needed to address in our future studies. Firstly, the detail structural analyses of gastrointestinal wall including the orientations of muscle and collagen fibers are needed to be done. Secondly, the active biomechanical properties of gastrointestinal wall such as contraction properties and muscle mechanics are needed to be investigated. Thirdly, the molecular pathways in relation to the effect of TSF on gastrointestinal remodeling in ZDF rats (obese fa/fa) are needed to study in detail as well. Finally, the dose of TSF used in this study was six times higher per unit body weight than that used in humans. Although this is a widely accepted equivalent dose between human and rat, it cannot be completely equal due to the large species differences between human and rat. The results obtained from this study may have some difference with the effect of TSF on the human gastrointestinal tract with the dose of human used.

## 5. In conclusion

The small intestine and colon histomorphologically and biomechanically remodeled during the development of DM in the ZDF rat (obese fa/fa) models as we demonstrated in other diabetic rat models. However, in contrast with previous findings related to wall stiffening in both circumferential and longitudinal directions, at present study, the small intestinal and colonic wall became stiffer in longitudinal direction and softer in circumferential direction in ZDF rats (obese fa/fa). Application of TSF could partly improve small intestinal and colonic histomorphological remodeling in ZDF rats (obese fa/fa), but had no role on biomechanical remodeling. The effect of TSF on diabetes-induced small intestinal and colonic remodeling may indicate the potential role of TSF on the treatment of gastrointestinal disorders in diabetic patients.

## Declarations of ethics

This study was approved by the Ethics Review Committee for Animal Experimentation of the Institute of Clinical Medicine, China-Japan Friendship Hospital, Beijing, and was performed in accordance with the Guiding Principles for the Care and Use of Laboratory Animals (2012- A04). The approval number is zryhyy21-21-04-02.

## CRediT authorship contribution statement

**Xin Yang:** Conceptualization, Data curation, Methodology, Writing – original draft. **Jingbo Zhao:** Conceptualization, Formal analysis, Methodology, Software, Writing – original draft, Validation. **Hong Li:** Project administration, Resources. **Lin Pan:** Data curation, Methodology. **Jing Guo:** Methodology, Resources. **Jing Li:** Methodology, Resources. **Yuting Zhang:** Data curation, Methodology, Resources. **Pengmin Chen:** Funding acquisition, Investigation, Methodology, Supervision, Validation, Writing – review & editing. **Ping Li:** Supervision, Validation, Visualization, Writing – review & editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements and funding

This study is supported by National Natural Science Foundation of China (No. 81873135). Zhang Hao-Jun, Zhao Ting-Ting, Zhao Hai-Ling, Yan Mei-Hua provided different helps for in this study.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e21007.

## References

[1] R. Mare, I. Sporea, Gastrointestinal and liver complications in patients with diabetes mellitus-A review of the literature, J. Clin. Med. 11 (17) (2022) 5223, https://doi.org/10.3390/jcm11175223. PMID: 36079153.
[2] M. Zhao, D. Liao, J. Zhao, Diabetes-induced mechanophysiological changes in the small intestine and colon, World J. Diabetes 8 (6) (2017) 249–269, https://doi.org/10.4239/wjd.v8.i6.249. PMID: 28694926.
[3] Y.T. Du, C.K. Rayner, K.L. Jones, N.J. Talley, M. Horowitz, Gastrointestinal symptoms in diabetes: prevalence, assessment, pathogenesis, and management, Diabetes Care 41 (3) (2018) 627–637, https://doi.org/10.2337/dc17-1536. PMID: 29463666.
[4] G. Barbara, M.R. Barbaro, G. Marasco, C. Cremon, Chronic constipation: from pathophysiology to management, Minerva Gastroenterol. 69 (2) (2023) 277–290, https://doi.org/10.23736/S2724-5985.22.03335-6. Epub 2023 Feb 2. PMID: 36727654.
[5] V.G. Prasad, P. Abraham, Management of chronic constipation in patients with diabetes mellitus, Indian J. Gastroenterol. 36 (1) (2017) 11–22, https://doi.org/10.1007/s12664-016-0724-2. Epub 2016 Dec 17. PMID: 27987136.
[6] M. Mínguez, A. López Higueras, J. Júdez, Use of polyethylene glycol in functional constipation and fecal impaction, Rev. Esp. Enferm. Dig. 108 (12) (2016) 790–806, https://doi.org/10.17235/reed.2016.4571/2016. PMID: 27871178.
[7] P. Paré, R.N. Fedorak, Systematic review of stimulant and nonstimulant laxatives for the treatment of functional constipation, Chin. J. Gastroenterol. Hepatol. 28 (10) (2014) 549–557, https://doi.org/10.1155/2014/631740. PMID: 25390617.
[8] Y.X. Zhang, Y.J. Zhang, R.Y. Miao, X.Y. Fang, J.H. Wei, Y. Wei, J.R. Lin, J.X. Tian, Effectiveness and safety of traditional Chinese medicine decoction for diabetic gastroparesis: a network meta-analysis, World J. Diabetes 14 (3) (2023) 313–342, https://doi.org/10.4239/wjd.v14.i3.313. PMID: 37035221.
[9] Y. Nam, J.M. Lee, Y. Wang, H.S. Ha, U.D. Sohn, The effect of Flos Lonicerae Japonicae extract on gastro-intestinal motility function, J. Ethnopharmacol. 179 (2016) 280–290, https://doi.org/10.1016/j.jep.2015.12.056. PMID: 26743226.
[10] X. Xiong, W. Peng, L. Chen, H. Liu, W. Huang, B. Yang, Y. Wang, Z. Xing, P. Gan, K. Nie, Traditional Chinese medicine Zhiqiao-Houpu herb-pair induce bidirectional effects on gastric motility in rats, J. Ethnopharmacol. 175 (2015) 444–450, https://doi.org/10.1016/j.jep.2015.10.001. PMID: 26456365.
[11] D. Zhao, J.B. Zhao, Comparison of Chang Run Tong and forlaxin treatment of constipation in elderly diabetic patients, J Altern Complement Med 24 (5) (2018) 472–480, https://doi.org/10.1089/acm.2018.0052. PMID: 29698053.
[12] H. Liu, B. Yu, M. Zhang, K. Liu, F.C. Wang, X.Y. Gao, Treatment of diabetic gastroparesis by complementary and alternative medicines, Medicines (Basel) 2 (3) (2015) 212–219, https://doi.org/10.3390/medicines2030212. PMID: 28930208.

[13] J. Zhao, J.B. Frøkjaer, A.M. Drewes, N. Ejskjaer, Upper gastrointestinal sensory-motor dysfunction in diabetes mellitus, World J. Gastroenterol. 12 (18) (2006) 2846–2857, https://doi.org/10.3748/wjg.v12.i18.2846. PMID: 16718808.

[14] J. Zhao, T. Nakaguchi, H. Gregersen, Biomechanical and histomorphometric colon remodelling in STZ-induced diabetic rats, Dig. Dis. Sci. 54 (8) (2009) 1636–1642, https://doi.org/10.1007/s10620-008-0540-3. PMID: 18989775.

[15] J. Zhao, J. Yang, H. Gregersen, Biomechanical and morphometric intestinal remodelling during experimental diabetes in rats, Diabetologia 46 (12) (2003) 1688–1697, https://doi.org/10.1007/s00125-003-1233-2. PMID: 14593459.

[16] B. Patel, A. Gizzi, J. Hashemi, Y. Awakeem, H. Gregersen, G. Kassab, Biomechanical constitutive modeling of the gastrointestinal tissues: a systematic review, Mater. Des. 217 (2022), 110576, https://doi.org/10.1016/j.matdes.2022.110576. PMID: 35935127.

[17] J.B. Kola, T. Docsa, K. Uray, Mechanosensing in the physiology and pathology of the gastrointestinal tract, Int. J. Mol. Sci. 24 (1) (2022) 177, https://doi.org/10.3390/ijms24010177. PMID: 36613619.

[18] H. Sha, J.B. Zhao, Z.Y. Zhang, S.P. Zhou, X.L. Tong, F.Y. Zhuang, H. Gregersen, Effect of Kaiyu Qingwei Jianji on the morphometry and residual strain distribution of small intestine in experimental diabetic rats, World J. Gastroenterol. 12 (44) (2006) 7149–7154, https://doi.org/10.3748/wjg.v12.i44.7149. PMID: 17131477.

[19] G.F. Liu, J.B. Zhao, Z. Zhen, H. Sha, P.M. Chen, M. Li, J.C. Zhang, M.Z. Yuan, W. Gao, H. Gregersen, X.L. Tong, Effect of tangweian jianji on upper gastrointestinal remodeling in streptozotocin-induced diabetic rats, World J. Gastroenterol. 18 (35) (2012) 4875–4884, https://doi.org/10.3748/wjg.v18.i35.4875. PMID: 23002359.

[20] J. Tian, M. Li, J. Zhao, J. Li, G. Liu, Z. Zhen, Y. Cao, H. Gregersen, X. Tong, Research on the traditional Chinese medicine treating gastrointestinal motility in diabetic rats by improving biomechanical remodeling and neuroendocrine regulation, Am J Transl Res 9 (5) (2017) 2219–2230. PMID: 28559973.

[21] H. Sha, D. Zhao, X. Tong, H. Gregersen, J. Zhao, Mechanism investigation of the improvement of Chang Run Tong on the colonic remodeling in streptozotocin-induced diabetic rats, J. Diabetes Res. 2016 (2016), 1826281, https://doi.org/10.1155/2016/1826281. PMID: 26839890.

[22] P. Li, Y. Chen, J. Liu, J. Hong, Y. Deng, F. Yang, X. Jin, J. Gao, J. Li, H. Fang, G. Liu, L. Shi, J. Du, Y. Li, M. Yan, Y. Wen, W. Yang, Efficacy and safety of tangshen formula on patients with type 2 diabetic kidney disease: a multicenter double-blinded randomized placebo-controlled trial, PLoS One 10 (5) (2015), e0126027, https://doi.org/10.1371/journal.pone.0126027. PMID: 25938778.

[23] P. Chen, J. Zhao, H. Zhang, X. Yang, T. Zhao, H. Zhang, M. Yan, L. Pan, X. Li, Y. Zhang, P. Li, Tangshen formula attenuates colonic structure remodeling in type 2 diabetic rats, Evid Based Complement Alternat Med 2017 (2017), 4064156, https://doi.org/10.1155/2017/4064156. PMID: 28303157.

[24] B. Wang, P.C. Chandrasekera, J.J. Pippin, Leptin- and leptin receptor-deficient rodent models: relevance for human type 2 diabetes, Curr. Diabetes Rev. 10 (2) (2014) 131–145, https://doi.org/10.2174/1573399810666140508121012. PMID: 24809394.

[25] Q. Kong, H. Zhang, T. Zhao, W. Zhang, M. Yan, X. Dong, P. Li, Tangshen formula attenuates hepatic steatosis by inhibiting hepatic lipogenesis and augmenting fatty acid oxidation in db/db mice, Int. J. Mol. Med. 38 (6) (2016) 1715–1726, https://doi.org/10.3892/ijmm.2016.2799. PMID: 27840945.

[26] J. Zhao, J. Yang, L. Vinter-Jensen, F. Zhuang, H. Gregersen, The morphometry and biomechanical properties of the rat small intestine after systemic treatment with epidermal growth factor, Biorheology 39 (6) (2002) 719–733. PMID: 12454438.

[27] Y. Dou, Y. Fan, J. Zhao, H. Gregersen, Longitudinal residual strain and stress-strain relationship in rat small intestine, Biomed. Eng. Online 5 (2006) 37, https://doi.org/10.1186/1475-925X-5-37. PMID: 16759387.

[28] K.M. Wasan, V. Risovic, V.G. Yuen, J.H. McNeill, Differences in plasma homocysteine levels between Zucker fatty and Zucker diabetic fatty rats following 3 weeks oral administration of organic vanadium compounds, J. Trace Elem. Med. Biol. 19 (4) (2006) 251–258, https://doi.org/10.1016/j.jtemb.2005.10.001. PMID: 16443173.

[29] N. Yokoi, M. Hoshino, S. Hidaka, E. Yoshida, M. Beppu, R. Hoshikawa, K. Sudo, A. Kawada, S. Takagi, S. Seino, A novel rat model of type 2 diabetes: the zucker fatty diabetes mellitus ZFDM rat, J. Diabetes Res. 2013 (2013), 103731, https://doi.org/10.1155/2013/103731. PMID: 23671847.

[30] M. Shiota, R.L. Printz, Diabetes in Zucker diabetic fatty rat, Methods Mol. Biol. 933 (2012) 103–123, https://doi.org/10.1007/978-1-62703-068-7_8. PMID: 22893404.

[31] Y. Katsuda, T. Ohta, K. Miyajima, Y. Kemmochi, T. Sasase, B. Tong, M. Shinohara, T. Yamada, Diabetic complications in obese type 2 diabetic rat models, Exp. Anim. 63 (2) (2014) 121–132, https://doi.org/10.1538/expanim.63.121. PMID: 24770637.

[32] S. Pandey, M.C. Dvorakova, Future perspective of diabetic animal models, Endocr., Metab. Immune Disord.: Drug Targets 20 (1) (2020) 25–38, https://doi.org/10.2174/1871530319666190626143832. PMID: 31241444.

[33] A.E. Zawada, M. Moszak, D. Skrzypczak, M. Grzymisławski, Gastrointestinal complications in patients with diabetes mellitus, Adv. Clin. Exp. Med. 27 (4) (2018) 567–572, https://doi.org/10.17219/acem/67961. PMID: 29533548.

[34] A.H. Kurniawan, B.H. Suwandi, U. Kholili, Diabetic gastroenteropathy: a complication of diabetes mellitus, Acta Med. Indones. 51 (3) (2019) 263–271. PMID: 31699951.

[35] M.J. Concepción Zavaleta, J.G. Gonzáles Yovera, D.M. Moreno Marreros, L.D.P. Rafael Robles, K.R. Palomino Taype, K.N. Soto Gálvez, L.F. Arriola Torres, J. C. Coronado Arroyo, L.A. Concepción Urteaga, Diabetic gastroenteropathy: an underdiagnosed complication, World J. Diabetes 12 (6) (2021) 794–809, https://doi.org/10.4239/wjd.v12.i6.794. PMID: 34168729.

[36] M. Esteves-Monteiro, D. Menezes-Pinto, M. Ferreira-Duarte, P. Dias-Pereira, M. Morato, M. Duarte-Araújo, Histomorphometry changes and decreased reactivity to angiotensin II in the ileum and colon of streptozotocin-induced diabetic rats, Int. J. Mol. Sci. 23 (21) (2022), 13233, https://doi.org/10.3390/ijms232113233. PMID: 36362021.

[37] M.C. D'Arpino, A.G. Fuchs, S.S. Sánchez, S.M. Honoré, Extracellular matrix remodeling and TGF-β1/Smad signaling in diabetic colon mucosa, Cell Biol. Int. 42 (4) (2018) 443–456, https://doi.org/10.1002/cbin.10916. PMID: 29227005.

[38] J.N.B. Pereira, G.M. Murata, F.T. Sato, A.R. Marosti, C.R.O. Carvalho, R. Curi, Small intestine remodeling in male Goto-Kakizaki rats, Physiol Rep 9 (3) (2021), e14755, https://doi.org/10.14814/phy2.14755. PMID: 33580916.

[39] J. Zhao, J. Yang, D. Liao, H. Gregersen, Interdependency between mechanical parameters and afferent nerve discharge in remodeled diabetic Goto-Kakizaki rat intestine, Clin. Exp. Gastroenterol. 10 (2017) 303–314, https://doi.org/10.2147/CEG.S145016. PMID: 29238211.

[40] J. Zhao, P. Chen, H. Gregersen, Morpho-mechanical intestinal remodeling in type 2 diabetic GK rats–is it related to advanced glycation end product formation? J. Biomech. 46 (6) (2013) 1128–1134, https://doi.org/10.1016/j.jbiomech.2013.01.010. PMID: 23403079.

[41] A. Al-Awar, K. Kupai, M. Veszelka, G. Szűcs, Z. Attieh, Z. Murlasits, S. Török, A. Pósa, C. Varga, Experimental diabetes mellitus in different animal models, J. Diabetes Res. 2016 (2016), 9051426, https://doi.org/10.1155/2016/9051426. PMID: 27595114.

[42] B. Pang, Q. Zhou, T.Y. Zhao, L.S. He, J. Guo, H.D. Chen, L.H. Zhao, X.L. Tong, Innovative thoughts on treating diabetes from the perspective of traditional Chinese medicine, Evid Based Complement Alternat Med 2015 (2015), 905432, https://doi.org/10.1155/2015/905432. PMID: 26504482.

[43] T. Zhao, S. Sun, H. Zhang, X. Huang, M. Yan, X. Dong, Y. Wen, H. Wang, H.Y. Lan, P. Li, Therapeutic effects of tangshen formula on diabetic nephropathy in rats, PLoS One 11 (1) (2016), e0147693, https://doi.org/10.1371/journal.pone.0147693. PMID: 26807792.

[44] H. Zhao, X. Li, T. Zhao, H. Zhang, M. Yan, X. Dong, P. Chen, L. Ma, P. Li, Tangshen formula attenuates diabetic renal injuries by upregulating autophagy via inhibition of PLZF expression, PLoS One 12 (2) (2017), e0171475, https://doi.org/10.1371/journal.pone.0171475. PMID: 28182710.

[45] P. Liu, L. Peng, H. Zhang, P.M. Tang, T. Zhao, M. Yan, H. Zhao, X. Huang, H. Lan, P. Li, Tangshen formula attenuates diabetic nephropathy by promoting ABCA1-mediated renal cholesterol efflux in db/db mice, Front. Physiol. 9 (2018) 343, https://doi.org/10.3389/fphys.2018.00343. PMID: 29681863.

[46] T. Zhao, H. Zhang, X. Yin, H. Zhao, L. Ma, M. Yan, L. Peng, Q. Wang, X. Dong, P. Li, Tangshen formula modulates gut Microbiota and reduces gut-derived toxins in diabetic nephropathy rats, Biomed. Pharmacother. 129 (2020), 110325, https://doi.org/10.1016/j.biopha.2020.110325. PMID: 32535383.

[47] N. Li, T. Zhao, Y. Cao, H. Zhang, L. Peng, Y. Wang, X. Zhou, Q. Wang, J. Li, M. Yan, X. Dong, H. Zhao, P. Li, Tangshen formula attenuates diabetic kidney injury by imparting anti-pyroptotic effects via the TXNIP-NLRP3-GSDMD Axis, Front. Pharmacol. 11 (2021), 623489, https://doi.org/10.3389/fphar.2020.623489. PMID: 33584307.

[48] H. Zhang, P. Li, F.J. Burczynski, Y. Gong, P. Choy, H. Sha, J. Li, Attenuation of diabetic nephropathy in otsuka long-evans tokushima fatty (OLETF) rats with a combination of Chinese herbs (tangshen formula), Evid Based Complement Alternat Med 2011 (2011), 613737, https://doi.org/10.1155/2011/613737. PMID: 21274280.

[49] P.M. Chen, J.B. Zhao, X. Yang, J. Li, H.J. Zhang, H.C. Zhang, Y.T. Zhang, P. Li, Protective effect of tangshen formula (糖肾方) on interstitial cells of cajal in colon of diabetic rats, Chin. J. Integr. Med. 28 (1) (2022) 43–51, https://doi.org/10.1007/s11655-021-3297-6. PMID: 34581938.