Diabetic gastroparesis (DGP) is characterized by delayed gastric emptying of solid food. Nitrergic neuron-mediated fundus relaxation and intragastric peristalsis are pivotal for gastric emptying and are impaired in DGP. Transient receptor potential vanilloid 1 (TRPV1) ion channels are expressed in gastrointestinal vagal afferent nerves and have a potential role in relevant gastrointestinal disorders. In this study, mice with high-fat diet (HFD)-induced type 2 diabetes mellitus (T2DM), associated with gastroparesis, were used to determine the role of TRPV1 in DGP. After feeding with HFD, mice exhibited obesity, hyperglycemia, insulin resistance, and delayed gastric emptying. Cholinergic- and nitrergic neuron-mediated neuromuscular contractions and relaxation were impaired. The antral tone of the DGP mice was attenuated. Interestingly, activating or suppressing TRPV1 facilitated or inhibited gastric fundus relaxation in normal mice. These effects were neutralized by using a nitric oxide synthase (NOS) inhibitor. Activation or suppression of TRPV1 also increased or reduced NO release. TRPV1 was specifically localized with neuronal NOS in the gastric fundus. These data suggest that TRPV1 activation facilitates gastric fundus relaxation by regulating neuronal NOS and promoting NO release. However, these effects and mechanisms disappeared in mice with DGP induced by HFD diet. TRPV1 expression was only marginally decreased in the fundus of DGP mice. TRPV1 dysfunction may be a potential mechanism underlying the dysfunction of DGP gastric nitrergic neuromuscular relaxation. (Am J Pathol 2023, 193: 548–557; https://doi.org/10.1016/j.ajpath.2023.01.005)
the gastric excitatory vagal motor circuit. The gastric inhibitory vagal motor circuit includes vagal afferent neurons that release inhibitory neurotransmitters [nitric oxide (NO), ATP, and vasoactive intestinal polypeptide] responsible for gastric relaxation and peristalsis. In contrast, the gastric excitatory vagal motor circuit includes preganglionic and post-ganglionic cholinergic neurons that release excitatory transmitters (acetylcholine) in charge of gastric contractions. In previous studies, chronic diabetes–associated hyperglycemia-induced oxidative and inflammatory stress interrupted gastric neuromuscular transmission, which led to loss of relaxation, accommodation, and weak contraction of gastric smooth muscle.

Transient receptor potential vanilloid 1 (TRPV1) is a mammalian TRPV channel, a type of transmembrane nonselective cation channel that is selectively activated by capsaicin (CAP), noxious heat, acidosis, endovanilloids, and calcium ions, leading to neuronal depolarization and action potential. TRPV1 is distributed in the gastrointestinal vagal afferent nerves such as the esophagus, stomach, intestine, colon, and gut. Previous studies showed that numerous TRPV1-immunoreactive nerve fibers were found in rodent and human gastrointestinal smooth muscle layers, myenteric nerve plexus, mucosa, and submucosal layers. TRPV1 has a potential role in relevant gastrointestinal disorders through the vagal afferent nerves. Several studies have shown that activating TRPV1 leads to relaxation in the rat stomach and suppresses TRPV1 reverse sodium hydrosulfide–induced pyloric sphincter muscle relaxation. Moreover, TRPV1 activation also stimulated colonic motility in dogs and protects against endotoxin-induced ileus in mice. These studies indicate that TRPV1 plays a potential role in gastrointestinal motility. However, the specific role and mechanism of TRPV1 in DGP remain unclear.

In the current study, mice with HFD-induced type 2 diabetes mellitus (T2DM) associated with gastroparesis were established to determine their gastric neuromuscular dysfunction profile. In addition, the role of TRPV1 in gastric neuromuscular transmission was investigated in normal mice and DGP mice.

Materials and Methods

All animal experimental protocols were conducted by the Institutional Animal Ethics Committee of Guangzhou University of Chinese Medicine (No. ZYD-2021-170) in compliance with the revised Animals (Scientific Procedures) Act 1986 in the United Kingdom.

Mice and Nutrients

Normal chow (3.5 kcal/g) was purchased from Guangdong Medical Laboratory Animal Center (Foshan, China). Semipur HFD (60% calories as fat, 5.2 kcal/g) was purchased from MediScience Ltd. (Yangzhou, China). Eight-week–old female C57BL/6J mice were purchased from BesTest Biotech Co., Ltd. (Zhuhai, China). Mice were housed under pathogen-free conditions in a temperature-controlled room illuminated for 12 hours every day, and they received humane care in accordance with the study guidelines established by the Guangzhou University of Chinese Medicine Laboratory Animal Holding Care.

After acclimation for 1 week, all mice were designated into two groups: the normal chow group (n = 15) and the HFD group (n = 15). Following feeding with corresponding diet for 16 weeks, mice were sacrificed by cervical dislocation after anesthesia. Gastric smooth muscle tension studies were performed according to the protocol discussed in the following sections. The rest of the tissues were snap-frozen or fixed in formalin.

Biochemical Assays

Biochemical assays of plasma samples were performed according to our previous research. Blood samples were collected from the retinal vein plexus after the mice were fasted overnight or with feeding. Mice were anesthetized by using isoflurane. Plasma was harvested after centrifugation, and plasma glucose, triglyceride, and total cholesterol levels were determined by using commercial kits from Rsbio (Shanghai, China). Plasma insulin was examined by using ELISA commercial kits from IMD (Hong Kong, China).

Oral Glucose Tolerance Test and Insulin Tolerance Test

An oral glucose tolerance test and insulin tolerance test were performed according to our previous research. Glucose (2 g/kg) was administered intragastrically to mice that had been fasted overnight. Insulin (1 U/kg) was injected intraperitoneally in mice that had been fasted for 6 hours. Insulin sensitivity was evaluated by using the homeostatic model assessment of insulin resistance [fasting blood glucose (mmol/L) × fasting serum insulin (mIU/L)/22.5].

Gastric Emptying Rate

The gastric emptying test of solid food was performed according to earlier research. Each mouse was placed in a separate clean metabolic cage and fasted overnight (providing water) to avoid scatophagy. Dried baked egg yolk (150 mg) was mixed with 150 μL of double distilled water and administered to mice via oral gavage. Mice were fasted for 1 hour without food and water and then sacrificed. The abdominal cavity was opened, pylorus and cardia were clamped, and the stomach was removed. Chyme in the stomach was harvested, dried, and weighed. The rate of gastric emptying was calculated as follows: gastric emptying rate (% in 1 hour) = (1 – gastric content/food intake) × 100.
Histology

Stomachs were harvested, weighed, and cut along the lesser curvature. The fundus, body, and antrum were separated and weighed. Tissue were fixed in formalin, paraffin-embedded, sectioned, and stained with hematoxylin and eosin. Slides were captured by using an IX73 microscope (Olympus, Tokyo, Japan).

Gastric Smooth Muscle Tension Studies (Ex Vivo)

Muscle tension studies were performed as described in earlier research. After euthanasia, stomachs of mice were removed and quickly cut along the lesser curvature. Gastric tissue was transferred to pre-cooled Krebs buffer (NaCl, 118 mmol/L; KCl, 4.75 mmol/L; MgSO₄, 1.18 mmol/L; NaHCO₃, 24.8 mmol/L; KH₂PO₃, 1.18 mmol/L; CaCl₂, 2.5 mmol/L; and C₆H₁₂O₆·H₂O, 10 mmol/L; pH 7.4). Longitudinal smooth muscle strips from the fundus and antrum proximal to the gastric body were obtained from each animal. Each muscle strip was tied with silk and transferred to an organ bath containing Krebs solution maintained at 37°C and continuously gassed with carbogen (95% oxygen + 5% carbon dioxide). Each strip was attached at a fixed hook and a force transducer for measuring isometric tension. Strips were stretched under 0.5 g for 1 hour as equilibration. Changes of force were amplified by a BR4740-ISO510A force transducer (Harvard Apparatus, Holliston, MA). Digital data of tension was displayed and recorded through a force transducer (Harvard Apparatus, Holliston, MA). Dig-}

Gastric Fundus NO Release (Ex Vivo)

Tissue was harvested according to the aforementioned tension protocol. NO release was detected by the probe 3-amino,4-aminomethyl-2',7'-difluorescein, diacetate (DAF-FM DA) (Beyotime Biotecnoogy, Haimen, China) according to the manufacturer’s protocol. After tissue was preloaded with the DAF probe (2 μmol/L), tissue was incubated with CAP or CPZ (5 μmol/L) for 10 minutes in Krebs solution maintained at 37°C and continuously gassed with carbogen (95% oxygen + 5% carbon dioxide) in darkness. The NO release was elicited by 20 Hz EFS. The fluorescent image was captured by using an LSM800 confocal microscope (Zeiss, Oberkochen, German). The integrated fluorescent density was analyzed by using ImageJ version 1.43 software (NIH, Bethesda, MD; http://imagej.nih.gov/ij).

Immunofluorescence

Gastric fundus tissue was harvested, embedded in optimal cutting temperature compound, and sectioned. After tissue was fixed in 4% paraformaldehyde, it was blocked by Invitrogen blocking solution with 0.1% Triton-X100 (Thermo Fisher Scientific, Waltham, MA). After blocking, sections were incubated overnight at 4°C with rabbit anti-TRPV1 primary antibody (1:200; Abcam, Cambridge, United Kingdom) or anti-neuronal NOS (nNOS) primary antibody (1:200; Cell Signaling Technology, Danvers, MA). An Abberior STAR ORANGE fluorescent dye (Abberior, Göttingen, Germany) and Alexa Flour 488 (Southern Biotechnology (Birmingham, AL) secondary antibody was used to detect the first primary antibody. Hoechst 33342 (4A Biotech, Beijing, China) was used to stain the nuclei. The fluorescent image was captured by using an LSM800 confocal microscope (Zeiss).

Western Blot Analysis

Western blot analysis was performed according to our previous research. Total protein extracts were fractionated by SDS-PAGE and transferred to polyvinylidene difluoride membranes. The membranes were then blocked with 5% non-fat milk in tris-buffered saline with Tween 20 for 2 hours at room temperature and incubated with anti-actin (SAB Signalway Antibody, Greenbelt, MD), anti-nNOS (Cell Signaling Technology), and anti-TRPV1 (Abcam, Waltham, MA) at 4°C overnight, washing three times with tris-buffered saline with Tween 20, and incubated with respective secondary antibodies for 2 hours at room temperature. Protein bands were visualized with SuperSignal West Femto Maximum Sensitivity Substrate (Thermo Fisher Scientific, Rockford, IL) and captured by using an ImageQuant LAS4000 imaging system (Genescipt, Shanghai, China).
Data Analysis

All results are expressed as means ± SEM. Statistical analysis was performed by using StatView 5.0.1 (SAS Institute Inc., Cary, NC). Data from more than two groups were analyzed by using one-way analysis of variance. A t-test was performed to identify differences between two groups. \( P < 0.05 \) was considered significant.

Results

Effects of HFD in Obesity Development

Based on feeding with HFD, calorie intake was significantly higher in the HFD mouse group (Figure 1A). Thus, mice in the HFD group developed obesity (Figure 1B). As expected, the weight of subcutaneous fat and perirenal fat was significantly enhanced after HFD feeding (Figure 1C). In addition, plasma levels of total cholesterol in the HFD group were also higher than those of the normal chow group (Supplemental Figure S1). These data suggest that after feeding mice an HFD for 16 weeks, they developed obesity and hyperlipidemia.

Effects of HFD in T2DM Development

After feeding mice an HFD for 13 weeks, the oral glucose tolerance test and insulin tolerance test were performed. Mice in the HFD group exhibited hyperglycemia as well as impaired glucose and insulin tolerance (Figure 1, D–H). Plasma insulin and homeostatic model assessment of insulin resistance levels were also examined to evaluate insulin resistance and development of T2DM. As expected, hyperinsulinemia and an elevated homeostatic model assessment of insulin resistance index under fasting conditions were observed in mice fed an HFD (Figure 1, I and J). According to these data, mice strongly developed T2DM after HFD feeding. However, plasma glucose levels in the feeding status were similar between normal mice and T2DM mice (Figure 1K). Delayed gastric emptying diminished postprandial glycemia (Figure 2A).

Effects of HFD in DGP Development

After the mice reached a steady T2DM status, a gastric emptying experiment was performed to investigate whether gastroparesis was elicited after 16 weeks of HFD feeding.

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Figure 1  Effects of a high-fat diet (HFD) in obese type 2 diabetes mellitus development. Daily diet consumption (A), body weight changes (B), fat mass (C), plasma glucose kinetics of the oral glucose tolerance test (OGTT) (D), area under the curve (AUC) of OGTT (E), plasma glucose kinetics of the insulin tolerance test (ITT) (F), AUC of ITT (G), glucose changes of ITT (H), plasma insulin (I), homeostatic model assessment of insulin resistance (HOMA-IR) (J), and feeding plasma glucose (Glu) (K) after feeding with an HFD for 16 weeks. Data are expressed as means ± SEM. \( n = 10 \) to 15 per group. *\( P < 0.05 \), **\( P < 0.01 \) versus the normal chow group.
The 1-hour gastric emptying rate was significantly decreased in diabetic mice that were fed an HFD (Figure 2A). Interestingly, the total weight of the stomach, gastric fundus, and body, but not antrum, decreased after feeding with an HFD for 16 weeks (Figure 2, B–F). Gastric smooth muscle atrophy has been observed in nonobese diabetic mice in previous research.20 However, weight loss in the stomach of mice with HFD-induced DGP has not yet been observed. The histomorphologic study indicated a marginal reduction in gastric parietal cells in the stomach of HFD-fed mice (Figure 2G). These cells are responsible for acid secretion and digestion of food. This shows that mice developed T2DM-associated DGP after feeding with an HFD for 16 weeks. Several gastric histomorphologic changes were also observed in DGP mice. Mild atrophy in the forestomach may contribute to the obstruction of food accommodation in DGP. The marginal reduction in gastric parietal cells could be another potential pathogenesis for delayed food digestion and delayed gastric emptying.

Gastric Neuromuscular Dysfunction in DGP Mice

Decreased relaxation, weak contraction of the fundus, loss of tone, and contraction of the antrum are observed in clinical patients with DGP.4 To determine whether these could also be observed in HFD-fed mice, neuromuscular-mediated gastric fundus relaxation, contraction, and antral tone were monitored. The contractile responses induced by CCh were significantly attenuated in the gastric fundus of HFD-fed mice (Figure 3, A and B). EFS-elicited relaxation responses in the gastric fundus were also impaired in HFD-fed mice (Figure 3, C and D). These data suggest that both gastric accommodation and cholinergic-mediated contraction in the fundus were weak in DGP mice. Slow wave–mediated antral tone was also attenuated in the gastric antrum of HFD-fed mice (Figure 3, E–G). Tissue strips from the fundus of both normal and DGP mice showed no significant difference in potassium chloride–induced contraction (Figure 3H, Supplemental Figure S1). These data indicate that these mice had a phenotype similar to that of clinical patients with DGP.

Dysfunction of TRPV1-Mediated Gastric Fundus Relaxation in DGP Mice

To investigate the role of TRPV1 in gastric fundus relaxation in both normal and DGP mice, the selective TRPV1 agonist CAP and antagonist CPZ were used in a strip tension study. CCh-induced contraction of the fundus strips was attenuated by CAP treatment (Figure 4, A and B). However, CAP treatment did not neutralize CCh-induced contraction in the fundus strips of HFD-fed DGP mice (Figure 4, A and C). Conversely, EFS-elicited relaxation of the gastric fundus was significantly neutralized by CPZ pretreatment in mice fed normal chow (Figure 4, D and E). However, this effect almost disappeared in DGP mice that were fed an HFD and was only observed at 5 Hz EFS (Figure 4, D and F). These data indicate that TRPV1 plays a potential role in gastric fundus relaxation. However, this effect was not observed in mice with HFD-induced DGP.
Dysfunction of TRPV1 Mediated NO Release in Diabetic Mice

The mechanism of action of TRPV1 on fundus relaxation was investigated using the nNOS inhibitor L-NAME in normal chow and HFD-fed mice. After pretreatment with L-NAME, CAP-induced relaxation disappeared in the fundus strips of normal chow-fed mice (Figure 5, A and B). This result indicated that TRPV1-mediated fundus relaxation is associated with NO. EFS elicited fundus relaxation in normal chow mice, which shrunk after L-NAME pretreatment (Figure 5, D and E). This corroborated previous research showing that nitrergic neurons play a dominant role in fundus relaxation.\(^3\) In addition, EFS elicited NO release facilitated by CAP pretreatment and was suppressed by CPZ pretreatment (Figure 5, G and H). These data indicate that TRPV1

Figure 3  Gastric neuromuscular dysfunction in diabetic gastroparesis mice. Representative tracings showing contraction (A) and summary of fundus contraction (B) induced by carbachol (CCh). Summary of fundus relaxation (C) and representative tracing showing relaxation responses (D) induced by electrical field stimulation (EFS). Summary of amplitude (E), frequency (F), and representative tracings showing tone of antral contraction (G). Summary of fundus contraction induced by potassium chloride (KCL) (H). Data are expressed as means ± SEM. \(n = 6\) to 8 per group (A–D, H); \(n = 3\) to 5 per group (E–G). *\(P < 0.05\), **\(P < 0.01\) versus the normal chow group. HFD, high-fat diet.

Figure 4  Dysfunction of transient receptor potential vanilloid 1–mediated gastric fundus relaxation in diabetic gastroparesis mice. Representative tracings showing precontraction induced by carbachol (CCh) followed by relaxation due to capsaicin (CAP) treatment (A) and summary of relaxation changes of fundus after CAP treatment from the normal chow group (B) and the high-fat diet (HFD) group (C). Representative tracings showing precontraction induced by CCh followed by relaxation due to increasing frequencies of electrical field stimulation (EFS) (D) and summary of relaxation changes of fundus after EFS from the normal chow group (E) and the HFD group (F). Data are expressed as means ± SEM. \(n = 8\) to 8 per group. *\(P < 0.05\), **\(P < 0.01\) versus the CAP or EFS group.
mediates fundus relaxation by regulating NO release. However, L-NAME pretreatment had no effect on CAP-induced fundus relaxation (Figure 5, A and C), and EFS elicited fundus relaxation in DGP mice fed an HFD (Figure 5, D and F). CAP-induced fundus relaxation was weak in DGP mice. The effects of CAP/CPZ on NO release were observed using direct DAF staining, and were attenuated in the fundus of the DGP mice (Figure 5, G and H). This indicated that TRPV1-mediated gastric fundus nitrergic neuromuscular relaxation was dysfunctional in mice with HFD-induced DGP. NO-mediated relaxation was weak in the fundus of DGP mice (Figures 3C and 5D), and NO release was lower than that in normal chow-fed mice (Figure 5, G and H).

Expression of TRPV1 and nNOS in Gastric Fundus

TRPV1 and nNOS immunoreactivity were detected in both the normal chow and the DGP mouse fundus. Most TRPV1 staining was specifically co-localized with nNOS (Figure 6A). This result indicates that TRPV1 may interact with nNOS and regulate NO release. However, the expression of TRPV1 was only marginally decreased in the fundus of HFD-induced DGP mice (Figure 6, B and C). This may be indicative of a functional disorder instead of a change in the expression of TRPV1 in the fundus of DGP mice. Interestingly, nNOS protein expression in DGP mice was similar to that in normal mice (Supplemental Figure S1). While loss of antral nitrergic neurons in DGP has been reported, a different study reported that dysfunction of gastric motility in DGP is associated with a damaged neuromuscular network, but no loss of nitrergic neuron density. Therefore, dysfunction of fundus relaxation in HFD-induced DGP may be associated with a damaged TRPV1/ nNOS network.

Discussion

Dysfunction of the gastric cholinergic—mediated gastric excitatory vagal motor circuit and the nitrergic-mediated gastric inhibitory vagal motor circuit are pivotal mechanisms of DGP, leading to poor fundus relaxation, contractions, and antral contraction. Many researchers have investigated DGP in mice with streptozocin-induced type 1 DM or in transgenic mice. However, most patients with diabetes in the clinical setting have T2DM. Although ample literature has shown that men are more susceptible to insulin resistance and T2DM, more women than men are affected by DGP. Therefore, most studies on DGP have focused on female animals instead of male animals.
Hence, HFD-induced female DGP mice were used in this study. Decreased relaxation, weak contraction of the fundus, loss of tone, and contraction of the antrum are observed in clinical patients with DGP.4 The current study investigated whether the DGP animal model had a phenotype similar to that of a patient with DGP. Based on current results, CCh-induced fundus contraction responses were attenuated in the DGP mice, indicating impaired cholinergic-mediated fundus contraction. Conversely, nitrergic neuron-mediated fundus relaxation almost disappeared in these DGP mice. These data indicate that accommodation, peristalsis, and pulverization, but not cholinergic/nitrergic neuron-mediated fundus contraction and relaxation, are impaired in DGP mice.

Slow wave-mediated antral tone and contraction also play critical roles in gastric emptying. In a previous study, abnormal slow waves and antral tone contributed to delayed gastric emptying.29 Therefore, the antral tone in normal and DGP mice was monitored here. As expected, the antral tone in the DGP mice was significantly reduced. In addition to gastric motility, these DGP mice exhibited stomach weight loss and marginal parietal cell reduction. A review showed that gastric atrophy can be observed in patients with long-standing diabetes.30 Taken together, these data suggest that mice with HFD-induced DGP exhibit symptoms similar to those of clinical patients with DGP.

Previous studies have shown that oxidative and inflammatory stress is the primary pathogenesis of gastric neuromuscular dysfunction.5,6,22,23 However, the mechanism underlying gastric neuromuscular dysfunction in HFD-induced DGP mice remains unclear. The membrane-bound G protein–coupled bile acid receptor TGR5 is expressed in gastrointestinal vagal nitrergic nerves.31 Previous research has shown that bile acids activate TGR5 and play a role in ascending contraction, descending relaxation, and colonic peristalsis.32 Both effects are required for defecation in mice. Delayed gut transit was observed in tgr5-ko mice, indicating that neuronal membrane–binding proteins were associated with gastrointestinal motility. TRPV1 ion channels are also expressed in the gastrointestinal vagal afferent neuronal membranes (smooth muscle, myenteric nerve plexus, mucosa, and submucosal layer), which play critical roles in gastric motility.9–11 In previous studies, the TRPV1 agonist CAP promoted gastric relaxation of the fundus and body.13,33,34 Moreover, activating TRPV1 also ameliorates endotoxin-induced ileus through afferent neurons.15 In contrast, a reduced response of gastric vagal tension receptors to stretch was observed in trpv1-ko mice.35 Clinical research also revealed that homozygosity in TRPV1 315C influences susceptibility to functional dyspepsia.36 Collectively, these studies indicate that TRPV1 has potential roles in gastric mobility. Therefore, the effects of activating and suppressing TRPV1 on HFD-induced DGP mice were investigated.

While the activation and suppression of TRPV1 had opposite effects on gastric relaxation of the fundus, these profiles were not observed in the HFD-induced DGP mice. Several studies have shown that TRPV1 is involved in nNOS activity. Activation of TRPV1 involves the up-regulation of NOS and the death of retinal cells.37 Another study revealed that nNOS in hippocampal hyperexcitability is involved in cannabinoid/TRPV1 signaling.38
Moreover, nNOS was shown to regulate load-induced skeletal muscle hypertrophy by activating TRPV1.39

Based on the role of nitrergic neurons in gastric fundus relaxation, we hypothesized that TRPV1-induced fundus relaxation involves the release of nNOS and NO. In tissue strip tension studies, TRPV1-induced fundus relaxation was neutralized by pretreatment with the NOS inhibitor L-NAME. In addition to the tension studies, DAF staining was performed to investigate NO release in the gastric fundus. In this experiment, EFS elicited NO release from nitrergic neurons. This NO release was obviously enhanced after TRPV1 agonist treatment and suppressed by TRPV1 antagonist pretreatment. We therefore hypothesized that TRPV1 promotes gastric fundus relaxation through nNOS activity and facilitates NO release. Finally, TRPV1 and nNOS immunoreactivity and localization were also detected. TRPV1 staining was specifically localized within nNOS in the same cells. These data also suggest that TRPV1 may have potential effects on nNOS.

In contrast, activating TRPV1-induced gastric fundus relaxation disappeared in HFD-induced DGP mice. Nitrergic vagal neurons play a dominant role in fundus relaxation and dysfunction in nonobese DGP in mice.40,41 This study showed that relaxation of the gastric fundus in the DGP group was weak. The NOS inhibitor L-NAME had little effect on fundus relaxation in the DGP mice. These results indicated that nitrergic vagal neuron-mediated fundus relaxation was attenuated in HFD-fed DGP mice. Although TRPV1 was shown to be involved in NO release, TRPV1 activation failed to promote NO release in the gastric fundus of these DGP mice. However, the protein expression of nNOS was still robust and primarily detected in the gastric fundus. The expression of TRPV1 was only marginally reduced in DGP mice. Based on the almost total loss of function of TRPV1 in DGP mice, these marginal decreases in TRPV1 expression were insufficient to explain this phenotype. This led to the possibility of it being a functional disorder instead of a change in the expression of TRPV1 in the fundus of DGP mice. This dysfunction could be a potential mechanism for impaired nitrergic neuron transmission in the gastric fundus.

In conclusion, TRPV1 activation has a positive effect on nNOS activity and NO release for gastric fundus relaxation in normal mice. However, these effects were not observed in HFD-induced T2DM mice with DGP. This dysfunction may be a potential mechanism for the dysfunction of gastric nitrergic neuromuscular relaxation in patients with DGP.

Author Contributions
S.X., H.C., and S.G. designed the research; S.X., S.L., and Y.P. performed experiments; S.X., R.W., Y.Z., and S.L. analyzed data; S.X., B.H., and Y.X. wrote the manuscript; and H.L., J.L., B.T., and H.C. revised the manuscript. All authors read and approved the final study.

Supplemental Data
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