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Full Length Research Paper

Prokinetic effects of ghrelin in streptozotocin-induced diabetic guinea pigs

Wen-Cai Qiu, Cheng-Guang Yang, Jun Yan, Zhi-Gang Wang, Qi Zheng

Department of General Surgery, The Affiliated Sixth Hospital of Medical School, Shanghai Jiaotong University, Shanghai 200233, China.

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The aim of this paper is to investigate the effects of ghrelin on delayed gastrointestinal transit in streptozotocin-induced diabetic guinea pigs. A diabetic guinea pig model was produced by intraperitoneal (i. p.) injection of streptozotocin (STZ, 280 mg/kg). Diabetic guinea pigs were randomized into two main groups: normal guinea pigs and ghrelin-treated diabetic guinea pigs with doses of 0, 10, 20, 50 and 100 µg/kg i.p. Gastric emptying (GE), intestinal transit (IT), and colonic transit (CT) studies were performed in guinea pigs by receiving a phenol red meal following injection of ghrelin. Based on the most effective ghrelin dosage, 1 mg/kg atropine was given 15 min before the ghrelin injection in one group of guinea pigs for each study. The guinea pigs in each group were killed 20 min later, their stomachs, intestines, and colons were harvested immediately, and the amount of phenol red recovered was measured. Percentage of gastric emptying (GE%), intestinal transit (IT%), and colonic transit (CT%) were calculated. We found significantly delayed GE, IT, and CT in the diabetic guinea pigs compared with the control guinea pigs ($P < 0.05$); ghrelin improved both GE and IT, but not CT in the diabetic guinea pigs; the most effective dose of ghrelin was 100 µg/kg, and atropine blocked the prokinetic effects of ghrelin on GE and IT. These results are potentially significant for the clinical treatment of the delayed gastrointestinal transit in diabetes mellitus. In conclusion, ghrelin accelerates delayed GE and IT, but has no effect on CT in the diabetic guinea pigs. The prokinetic effects of ghrelin are probably exerted via the cholinergic pathway in the enteric nervous system. Ghrelin may have a therapeutic potential for diabetic patients with delayed upper gastrointestinal transit.

Key words: Ghrelin, diabetes mellitus, gastric emptying, gastrointestinal transit.

INTRODUCTION

Ghrelin is a peptide synthesized by endocrine cells of the gastric mucosa. The most characteristic functions of this peptide include the stimulation of growth hormone (GH) release (Kojima et al., 1999), the regulation of appetite and nutrient ingestion (Leidy and Campbell, 2011), and the improvement of digestive motility (Asakawa et al., 2001; Dornonville et al., 2004; Masuda et al., 2000). When injected in mice (Asakawa et al., 2001; De Winter et al., 2000), rats (Dornonville et al., 2004; Masuda et al., 2000), or dogs (Trudel et al., 2003), ghrelin accelerates the gastric emptying of a liquid or solid meal. Ghrelin has also been shown to accelerate gastric emptying in postoperative ileus (Trudel et al., 2002), septic ileus (De Winter et al., 2000), and burn-induced slowed gastrointestinal transit (Sallam et al., 2007) in animal models. *In vitro* studies have provided evidence that in addition to known vagus nerve dependent mechanisms, the activity of ghrelin is mediated via the enteric nervous system.

Delayed gastrointestinal transit is a well-known diabetic complication and may lead to discomfort gastrointestinal symptoms, such as frequent vomiting, emaciation, and unpredictable excursion of blood glucose and so on, which all impair the quality of life in diabetic patients (Horowitz et al., 2002; Khoo et al., 2010). Gastrointestinal transit of solid or nutrient liquid meals is abnormally slow in about 50% of diabetic patients (Horowitz et al., 2002).

*Corresponding author. E-mail: sh6_zhengqi@126.com. Tel: +86-21-64369181. Fax: +86-21-64701361.

The delayed gastrointestinal transit may be associated with cardiac autonomic neuropathy, blood glucose concentration, and gastrointestinal symptoms (Horowitz et al., 2002).

In the present study, streptozotocin-induced diabetic guinea pigs were selected as animal model of diabetic gastroparesis, attributed to autonomic neuropathy injury induced by prolonged hyperglycemia (Khalid et al., 2011; Rahbani et al., 2011). It is unknown whether ghrelin can exert a similar prokinetic effect on impaired gastrointestinal motility in streptozotocin-induced diabetic guinea pigs, thus having a clinical role in treating impaired gastrointestinal motility in diabetic patients. We aimed at testing the effect of this newly evolved stomach peptide on delayed gastrointestinal transit in streptozotocin-induced diabetic guinea pigs

MATERIALS AND METHODS

Chemicals

Rat ghrelin was obtained from Tocris Cookson (Bristol, UK). Atropine sulphate, phenol red, and alloxan were obtained from Sigma (St, Louis, MO).

Diabetic guinea pigs model

EWG/B guinea pigs of either sex (weighing 200 to 250 g) were obtained from the experimental Animal Center of the Shanghai Academia Sinica, China. All procedures were approved by the Medical Ethics Committee of Shanghai Jiaotong University. Guinea pigs were housed in stainless steel cages at a controlled temperature (22 ± 2°C) and 60 to 65% relative humidity with a normal 12:12 h light/dark cycle. Six guinea pigs were randomly selected as normal controls, and the rest were fed with a high-fat diet. After exposure to the high-fat diet for 3 weeks, the guinea pigs were fasted overnight with free access to water and injected i.p. with streptozotocin (STZ; 280 mg/kg body weight) dissolved in citrate buffer (50 mg/ml); the dose of STZ-induced diabetes in guinea pigs as previously described (Kitazawa et al., 2005). Seventy-two hours later, the fasting blood glucose levels of the guinea pigs were determined using the glucose oxidase method with a Glucose Analyzer (Shanghai Roche Company, China). Guinea pigs with a blood glucose level greater than 11.1 mmol/L were defined as diabetic guinea pigs. Diabetic guinea pigs continued to feed without control of blood glucose for six weeks, and the guinea pigs that were defined to be diabetic guinea pigs with gastroparesis, as confirmed by subsequent tests, were then used for further studies.

Animal grouping for gastrointestinal transit studies

Gastric emptying, intestinal and colonic transit studies were then performed. Within each study, guinea pigs were divided into two groups: a normal (control) group and a diabetic group. Within the diabetic group, guinea pigs were treated with different doses of ghrelin (0, 10, 20, 50 and 100 μg/kg) given in a random order with a total of six guinea pigs in each subgroup. A dose-response curve for ghrelin was obtained in the experiment. Based on the most effective dose of ghrelin, another two groups of 6 guinea pigs were added to each experiment, in which atropine (1 mg/kg) was given 15 min before ghrelin injection.

Gastric emptying

After a 12-h fast, the diabetic guinea pigs were injected with different doses of ghrelin (0, 10, 20, 50 and 100 μg/kg). After ghrelin injection, 2 ml of phenol red semi-liquid test meal (50 mg/100 ml in 0.9% NaCl with 1.5% methylcellulose) was administered intragastrically with an orogastric cannula. 20 min later, the guinea pigs were sacrificed. The stomach was clamped with a string above the lower oesophageal sphincter and a string beneath the pylorus to prevent leakage of phenol red. Gastric emptying was determined spectrophotometrically; the method was adapted from the measurement of gastric emptying in guinea pigs previously described. The stomach of each individual guinea pig was cut just above the lower oesophageal sphincter and the pyloric sphincter. Phenol red remained partly in the lumen of the stomach. The stomach and its contents were put in 5 ml 0.1 mol/L NaOH. The stomach was minced, and then these samples contain the total amount of phenol red present in the stomach. The samples were further diluted to 10 ml with 0.1 mol/L NaOH and left at room temperature for 1 h. 5 ml of the supernatant was then centrifuged at 800 g for 20 min. The absorbance was read at a wavelength of 546 nm with a spectrophotometer (Shanghai Yixian company, China), and then phenol red content that remained in the stomach was calculated. Percentage of gastric emptying of the guinea pigs was calculated as: [(infusion – remained) / infusion] × 100%.

Intestinal and colonic transit

After an overnight fast, guinea pigs were given general anesthesia (2 to 3% isoflurane inhalation) and underwent abdominal surgery. A small polyethylene tube was placed in the duodenum (or colon) via the stomach (or cecum), 0.5 cm distal to the pylorus (or ileocolic junction), fixed with sutures to the gut wall, and then tunneled through the abdominal wall subcutaneously and exited from the skin at the nape of the neck. Medline incision was sutured, and guinea pigs were left to recover in their separate cages. Food and water were abundantly provided. 3 days later, after a 12 h fast, the guinea pigs were administrated intraperitoneally with different doses of ghrelin (0, 10, 20, 50 and 100 μg/kg). After ghrelin injection, 2 ml of phenol red semi-liquid test meal (50 mg/100 ml in 0.9% NaCl with 1.5% methylcellulose) was injected via the implanted polyethylene tube in the duodenum (or colon). After 20 min, the guinea pigs were sacrificed. The distance of phenol red transit and the full length of intestine or colon were calculated. Small intestinal or colonic transit was assessed using the percentage phenol red transit of the full intestinal or colonic length.

Statistical analysis

Statistical analysis of the obtained data was executed using one-way ANOVA for multiple comparisons. Data are expressed as mean ± SE. $P < 0.05$ was considered to be statistically significant.

RESULTS

Gastric emptying and intestinal and colonic transit in diabetic guinea pigs

We found significantly delayed gastric emptying, intestinal and colonic transits in diabetic guinea pigs. Percentage of gastric emptying was significantly decreased in diabetic guinea pigs vs. normal guinea pigs ($P < 0.05$, Figure 1A). The intestinal transit was decreased

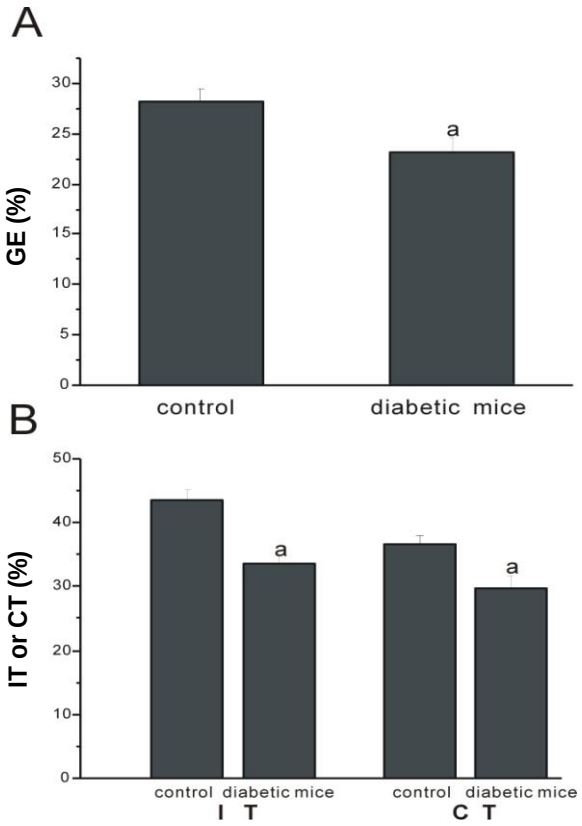

Figure 1. Delayed gastric emptying and intestinal and colonic transits in diabetic guinea pigs. A) Percentage of gastric emptying was significantly decreased in diabetic guinea pigs, [a]P < 0.05 versus control. B) Percentage of intestinal and colonic transits were significantly decreased in diabetic guinea pigs, [a]P < 0.05 vs. control.

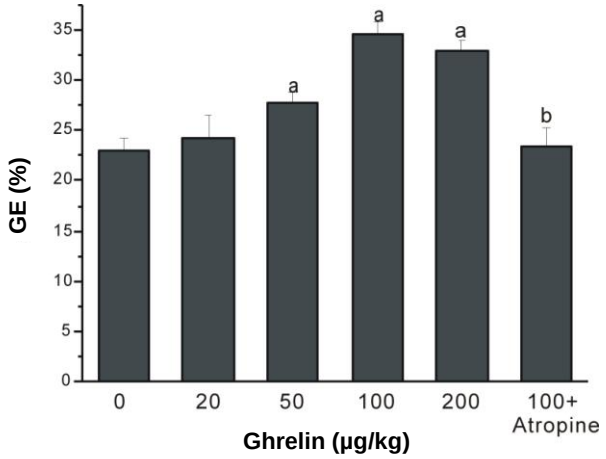

Figure 2. Ghrelin significantly increased delayed gastric emptying in the diabetic guinea pigs, [a]P < 0.05 vs. control. Atropine (1 mg/kg) blocked the 100 μg/kg ghrelin dose effect on the gastric emptying percentage in the diabetic guinea pigs, [b]P < 0.05 vs. 100 μg/kg ghrelin dose.

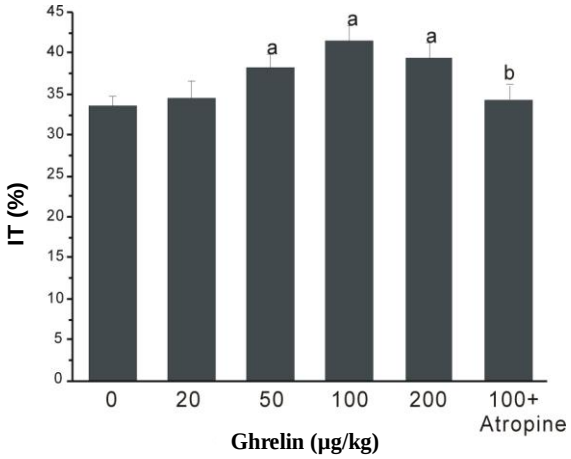

Figure 3. Ghrelin significantly increased delayed intestinal transit in the diabetic guinea pigs, [a]P < 0.05 vs. control. Atropine (1 mg/kg) blocked the 100 μg/kg ghrelin dose effect on the intestinal transit percentage in the diabetic guinea pigs, [b]P < 0.05 vs. 100 μg/kg ghrelin dose.

significantly in diabetic guinea pigs vs. normal guinea pigs (P < 0.05, Figure 1B). The colonic transit was decreased significantly in diabetic guinea pigs vs. normal guinea pigs (P < 0.05, Figure 1B).

Effect of ghrelin on delayed gastric emptying in diabetic mice

Ghrelin significantly accelerated delayed gastric emptying in diabetic guinea pigs. All of these doses except 10 μg/kg normalized delayed gastric emptying in diabetic mice (P < 0.05, Figure 2). We considered the dosage of 100 μg/kg most effective in increasing gastric emptying.

Effect of ghrelin on delayed small intestinal transit in diabetic mice

Ghrelin significantly accelerated delayed intestinal transit in diabetic guinea pigs. All of these doses except 10 μg/kg normalized delayed intestinal transit (P < 0.05, Figure 3). Accordingly, the 100 μg/kg ghrelin dose was considered as most effective in accelerating intestinal transit.

Effect of ghrelin on delayed colonic transit in diabetic mice

Ghrelin had no effects on delayed colonic transit. All of these doses were unable to accelerate delayed intestinal transit (P < 0.05, Figure 4).

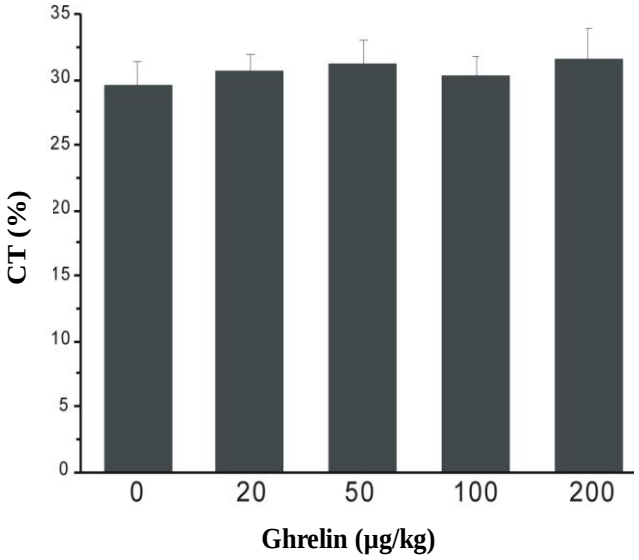

Figure 4. Ghrelin had no effect on delayed colonic transit in the diabetic guinea pigs, $p > 0.05$ vs. control.

Effect of atropine on delayed gastric emptying and intestinal transit

Atropine blocked the prokinetic effect of 100 μg/kg ghrelin dose on gastric emptying and intestinal transit ($P < 0.05$; Figures 2 and 3).

DISCUSSION

In this study, significantly delayed gastric emptying, intestinal and colonic transit were found in the streptozotocin-induced diabetic guinea pigs, indicated that streptozotocin-induced diabetic guinea pigs could be used as an animal model of diabetic gastroparesis. Ghrelin at the effective dose was able to accelerate delayed gastric emptying and intestinal transit but had no effect on colonic transit in the diabetic guinea pigs. The most effective dose of ghrelin to accelerate upper gastrointestinal transit was 100 μg/kg, while atropine blocked the ghrelin effect on gastric emptying and intestinal transit. Ghrelin had no effect on colon transit. This is similar to the results seen in the animal models of postoperative ileus (Trudel et al., 2002) and in burn-induced gastrointestinal delayed transit (Sallam et al., 2007). We believe that this might be related to the distribution of ghrelin receptors along the gut, which decreases distally (Date et al., 2001; Kojima and Kangawa, 2005).

Gastrointestinal motility disturbances including esophageal motor dysfunction, gastroparesis, constipation and diarrhea, are common in patients with diabetes mellitus. Gastrointestinal transit was significantly slower in the diabetic animal model of human diabetes (Kojima and Kangawa 2005; Anjaneyulu and Ramarao, 2002; El-Salhy, 2002). It is also known that inhibition of the gastrointestinal motility has been reported in humans with diabetes mellitus (Triantafyllou et al., 2002; Jung et al., 2003). The mechanisms of slowed gastrointestinal transit in diabetes mellitus are not very clear, although several mechanisms have been proposed (Horowitz et al., 2002). Among them, autonomic neuropathy, which is a complication of long-standing diabetes mellitus, has been widely accepted as the culprit. It may lead to an absence of a postprandial gastrointestinal response, a reflex that should be present in healthy people (Triantafyllou et al., 2002). Recently, several studies showed that an acute change in the blood glucose concentration also had a major effect on the gastrointestinal motor function in healthy subjects. In particular, acute hyperglycemia inhibited both the gastrointestinal and ascending component of the peristaltic reflex. Poor glycemic control has the potential to cause delayed gastrointestinal transit in diabetic patients (Jung et al., 2003).

To the best of our knowledge, this is the first time to report the effect of ghrelin on gastrointestinal dysmotility in the diabetic guinea pigs. Ghrelin is known to possess prokinetic characteristics. Ghrelin increased gastric emptying (Kitazawa et al., 2005; Konturek et al., 2005) of healthy mice. Ghrelin also improved gastric emptying (Levin et al., 2005; Fukuda et al., 2004), increased the frequency of migrating motor complex, and the intestinal transit (Edholm et al., 2004) of healthy rats. In healthy dogs, ghrelin stimulated antral contractility and antroduodenal coordination, hence its accountability to increase gastric emptying (Ohno et al., 2006). In healthy volunteers (Levin et al., 2005), normal weights (Tack et al., 2006), and gastroparetic (Murray et al., 2005) human subjects, ghrelin also increased gastric emptying. The mode of action of ghrelin seems to rely on neural mechanisms. Isolated strips of muscle *in vitro* failed to contract significantly when exposed to ghrelin. *In vivo*, the gastrokinetic effect of ghrelin in rats was abolished by atropine as well as by vagotomy. Diabetic gastroparesis is classically attributed to autonomic neuropathy induced by prolonged hyperglycemia; this condition did not preclude the gastrokinetic effect of ghrelin in our investigation. Our data in the diabetic guinea pigs, with slowed gastrointestinal transit strongly suggests that ghrelin can exert its prokinetic action on the upper alimentary tract via the enteric nervous mechanisms. Ghrelin as well as ghrelin receptors have been identified in the enteric nervous system, and we can postulate that these structures are probably of functional significance. In our study, we also showed that atropine blocked the 100 μg/kg ghrelin dose effect on gastric emptying and intestinal transit, suggesting that the prokinetic effect of ghrelin was perhaps mediated via the cholinergic pathway in the enteric nervous system. Other mechanisms involve tachykininergic pathways, as

demonstrated in the electrical field stimulation studies in isolated rat stomach (Kojima and Kangawa, 2005). Based on this study, ghrelin yields a strong potential for treatment of diabetic patents with delayed gastrointestinal transit. Clinically, improvement of gastrointestinal transit will facilitate enteral resuscitation, correcting patients' blood glucose concentrations and reducing patients' discomfort gastrointestinal symptoms.

In conclusion, ghrelin accelerates delayed gastric emptying and intestinal transit in the diabetic guinea pigs, an action perhaps mediated via the cholinergic pathway in the enteric nerve system. It is reasonable to assume that ghrelin can be used as a potential drug for the treatment of diabetic patents with delayed gastrointestinal transit; its pharmacotherapeutic potential deserves to be explored further in diabetic patients suffering of delayed upper gastrointestinal transit.

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REFERENCES

Anjaneyulu M, Ramarao P(2002).Studies on gastrointestinal tract functional changes in diabetic animals. Methods Find Exp. Clin. Pharm., 24: 71-75.

Anjaneyulu M, Ramarao P(2002).Studies on gastrointestinal tract functional changes in diabetic animals. Methods Find Exp. Clin. Pharm., 24: 71-75.

Asakawa A, Inui A, Kaga T, Yuzuriha H, Nagata T, Ueno N, Makino S, Fujimiya M, Niijima A, Fujino MA, Kasuga M(2001). Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin. Gastroenterol., 120: 337-345.

Asakawa A, Inui A, Kaga T, Yuzuriha H, Nagata T, Ueno N, Makino S, Fujimiya M, Niijima A, Fujino MA, Kasuga M(2001). Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin. Gastroenterol., 120: 337-345.

Date Y, Nakazato M, Murakami N, Kojima M, Kangawa K, Matsukura S(2001). Ghrelin acts in the central nervous system to stimulate gastric acid secretion. Biochem. Biophys. Res. Commun., 280: 904-907.

Date Y, Nakazato M, Murakami N, Kojima M, Kangawa K, Matsukura S(2001). Ghrelin acts in the central nervous system to stimulate gastric acid secretion. Biochem. Biophys. Res. Commun., 280: 904–907.

De Winter BY, De Man JG, Seerden TC, Depoortere I, Herman AG, Peeters TL, Pelckmans PA (2000). Effect of ghrelin and growth hormone-releasing peptide 6 on septic ileus in mice. Neurogastroenterol Motil. 16: 439-446.

De Winter BY, De Man JG, Seerden TC, Depoortere I, Herman AG, Peeters TL, Pelckmans PA (2000). Effect of ghrelin and growth hormone-releasing peptide 6 on septic ileus in mice. Neurogastroenterol Motil. 16: 439-446.

Dornonville C, Lindstrom E, Norlen P, Hakanson R (2004).Ghrelin stimulates gastric emptying but is without effect on acid secretion and gastric endocrine cells. Regul. Pept., 120: 23-32.

Dornonville C, Lindstrom E, Norlen P, Hakanson R (2004).Ghrelin stimulates gastric emptying but is without effect on acid secretion and gastric endocrine cells. Regul. Pept., 120: 23-32.

Edholm T, Levin F, Hellstrom PM, Schmidt PT (2004). Ghrelin stimulates motility in the small intestine of rats through intrinsic cholinergic neurons. Regul. Pept., 121: 25-30.

Edholm T, Levin F, Hellstrom PM, Schmidt PT (2004). Ghrelin stimulates motility in the small intestine of rats through intrinsic cholinergic neurons. Regul. Pept., 121: 25-30.

El-Salhy M (2002).Gastrointestinal Transit in Relation to Gut Endocrine Cells in Animal Models of Human Diabetes. Ups J. Med. Sci., 107: 23-33.

El-Salhy M (2002).Gastrointestinal Transit in Relation to Gut Endocrine Cells in Animal Models of Human Diabetes. Ups J. Med. Sci., 107: 23-33.

El-Salhy M(2002). Gastrointestinal Transit in an Animal Model of Human Diabetes Type 2: Relationship to gut neuroendocrine peptide contents. Ups J. Med. Sci., 107: 101-110.

El-Salhy M(2002). Gastrointestinal Transit in an Animal Model of Human Diabetes Type 2: Relationship to gut neuroendocrine peptide contents. Ups J. Med. Sci., 107: 101-110.

Fukuda H, Mizuta Y, Isomoto H, Takeshima F, Ohnita K, Ohba K, Omagari K, Taniyama K, Kohno S(2004). Ghrelin enhances gastric motility through direct stimulation of intrinsic neural pathways and capsaicinsensitive afferent neurones in rats. Scand J. Gastroenterol., 12: 1209-1214.

Fukuda H, Mizuta Y, Isomoto H, Takeshima F, Ohnita K, Ohba K, Omagari K, Taniyama K, Kohno S(2004). Ghrelin enhances gastric motility through direct stimulation of intrinsic neural pathways and capsaicinsensitive afferent neurones in rats. Scand J. Gastroenterol., 12: 1209-1214.

Horowitz M, O'Donovan D, Jones KL, Feinle C, Rayner CK, Samson M(2002). Gastric emptying in diabetes: Clinical significance and treatment. Diabetic Med., 19: 177- 194.

Horowitz M, O'Donovan D, Jones KL, Feinle C, Rayner CK, Samson M(2002). Gastric emptying in diabetes: Clinical significance and treatment. Diabetic Med., 19: 177- 194.

Jung HK, Kim DY, Moon IH, Hong YS (2003). Colonic transit time in diabetic patients-comparison with healthy subjects and the effect of autonomic neuropathy.Yonsei Med. J., 44: 265-272.

Jung HK, Kim DY, Moon IH, Hong YS (2003). Colonic transit time in diabetic patients-comparison with healthy subjects and the effect of autonomic neuropathy.Yonsei Med. J., 44: 265-272.

Khalid S, Al-Numair, Govindasamy C, Mohammed A, Alsai (2011). Effect of camel milk on collagen abnormalities in streptozotocin-diabetic rats. Afr. J. Pharm. Pharm., 5: 238-243.

Khalid S, Al-Numair, Govindasamy C, Mohammed A, Alsai (2011). Effect of camel milk on collagen abnormalities in streptozotocin-diabetic rats. Afr. J. Pharm. Pharm., 5: 238-243.

Khoo J, Rayner CK, Feinle-Bisset C, Jones KL, Horowitz M(2010). Gastrointestinal hormonal dysfunction in gastroparesis and functional dyspepsia. Neurogastroenterol Motil., 22(12): 8-1270.

Khoo J, Rayner CK, Feinle-Bisset C, Jones KL, Horowitz M(2010). Gastrointestinal hormonal dysfunction in gastroparesis and functional dyspepsia. Neurogastroenterol Motil., 22(12): 8-1270.

Kitazawa T, De Smet B, Verbeke K, Depoortere I, Peeters TL(2005). Gastric motor effects of peptide and non-peptide ghrelin agonists in mice in vivo and in vitro. Gut., 54: 1078-1084.

Kitazawa T, De Smet B, Verbeke K, Depoortere I, Peeters TL(2005). Gastric motor effects of peptide and non-peptide ghrelin agonists in mice in vivo and in vitro. Gut., 54: 1078-1084.

Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K (1999). Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature. 402: 656-660.

Kojima M, Kangawa K(2005). Ghrelin: structure and function. Phys. Rev., 85: 495–522.

Kojima M, Kangawa K(2005). Ghrelin: structure and function. Phys. Rev., 85: 495–522.

Konturek PC, Brzozowski T, Pajdo R, Nikiforuk A, Kwiecien S, Harsch I, Drozdowicz D, Hahn EG, Konturek SJ (2005). Ghrelin-a new gastroprotective factor in gastric mucosa. J. Phys. Pharm., 55: 325-336.

Konturek PC, Brzozowski T, Pajdo R, Nikiforuk A, Kwiecien S, Harsch I, Drozdowicz D, Hahn EG, Konturek SJ (2005). Ghrelin-a new gastroprotective factor in gastric mucosa. J. Phys. Pharm., 55: 325-336.

Leidy HJ, Campbell WW (2011). The effect of eating frequency on appetite control and food intake: brief synopsis of controlled feeding

studies. J Nutr., 141(1): 7-154.
Leidy HJ, Campbell WW (2011). The effect of eating frequency on appetite control and food intake: brief synopsis of controlled feeding studies. J Nutr., 141(1): 7-154.
Levin F, Edholm T, Ehrstrom M, Wallin B, Schmidt PT, Kirchgessner AM, Hilsted LM, Hellstrom PM, Naslund E (2005). Effect of peripherally administered ghrelin on gastric emptying and acid secretion in the rat. Regul. Pept., 131: 171-174.
Levin F, Edholm T, Ehrstrom M, Wallin B, Schmidt PT, Kirchgessner AM, Hilsted LM, Hellstrom PM, Naslund E (2005). Effect of peripherally administered ghrelin on gastric emptying and acid secretion in the rat. Regul. Pept., 131: 171-174.
Masuda Y, Tanaka T, Inomata N, Ohnuma N, Tanaka S, Itoh Z, Hosoda H, Kojima M, Kangawa K(2000).Ghrelin stimulates gastric acid secretion and motility in rats. Biochem. Biophys. Res. Commun., 276: 905-908.
Masuda Y, Tanaka T, Inomata N, Ohnuma N, Tanaka S, Itoh Z, Hosoda H, Kojima M, Kangawa K(2000).Ghrelin stimulates gastric acid secretion and motility in rats. Biochem. Biophys. Res. Commun., 276: 905-908.
Murray CD, Martin NM, Patterson M, Taylor SA, Ghatei MA, KammMA,Johnston C, Bloom SR, Emmanuel AV(2005). Ghrelin enhances gastric emptying in diabetic gastroparesis: a double blind, placebo controlled, crossover study. Gut., 54: 1693-1698. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K (1999). Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature. 402: 656-660.
Murray CD, Martin NM, Patterson M, Taylor SA, Ghatei MA, KammMA,Johnston C, Bloom SR, Emmanuel AV(2005). Ghrelin enhances gastric emptying in diabetic gastroparesis: a double blind, placebo controlled, crossover study. Gut., 54: 1693-1698.
Ohno T, Kamiyama Y, Aihara R, Nakabayashi T, Mochiki E, Asao T, Kuwano H (2006). Ghrelin does not stimulate gastrointestinal motility and gastric emptying: an experimental study of conscious dogs. Neurogastroenterol. Motil., 18: 129-135.
Ohno T, Kamiyama Y, Aihara R, Nakabayashi T, Mochiki E, Asao T, Kuwano H (2006). Ghrelin does not stimulate gastrointestinal motility and gastric emptying: an experimental study of conscious dogs. Neurogastroenterol. Motil., 18: 129-135.
Rahbani M, Mohajeri D, Rezaie A, Doustar Y, Nazeri Mehrdad (2011). Attenuation of oxidative stress of hepatic tissue by ethanolic extract of saffron (dried stigmas of Crocus sativus L.) in streptozotocin (STZ)-induced diabetic rats. Afr. J. Pharm. Pharm., 5: 2166 - 2173.
Rahbani M, Mohajeri D, Rezaie A, Doustar Y, Nazeri Mehrdad (2011). Attenuation of oxidative stress of hepatic tissue by ethanolic extract of saffron (dried stigmas of Crocus sativus L.) in streptozotocin (STZ)-induced diabetic rats. Afr. J. Pharm. Pharm., 5: 2166 - 2173.
Sallam HS, Oliveira HM, Gan HT, Herndon DN, Chen JD (2007).Ghrelin improvesburn-induced delayed gastrointestinal transit in rats. Am. J. Phys. Regul. Int. Comp. Phys., 292: R253-257.
Sallam HS, Oliveira HM, Gan HT, Herndon DN, Chen JD (2007).Ghrelin improvesburn-induced delayed gastrointestinal transit in rats. Am. J. Phys. Regul. Int. Comp. Phys., 292: R253-257.
Tack J, Depoortere I, Bisschops R, Delporte C, Coulie B, Meulemans A, Janssens J, Peeters T (2006). Influence of ghrelin on interdigestive gastrointestinal motility in humans. Gut. 55: 327-333.
Tack J, Depoortere I, Bisschops R, Delporte C, Coulie B, Meulemans A, Janssens J, Peeters T (2006). Influence of ghrelin on interdigestive gastrointestinal motility in humans. Gut. 55: 327-333.
Triantafyllou K, Kalantzis C, Papadopoulos AA, Apostolopoulos P, Rokkas T, Kalantzis N, Ladas SD(2002).Video-capsule endoscopy gastric and small bowel transit time and completeness of the examination in patients with diabetes mellitus. Dig. Liver Dis. 39: 575-580.
Triantafyllou K, Kalantzis C, Papadopoulos AA, Apostolopoulos P, Rokkas T, Kalantzis N, Ladas SD(2002).Video-capsule endoscopy gastric and small bowel transit time and completeness of the examination in patients with diabetes mellitus. Dig. Liver Dis. 39: 575-580.
Trudel L, Bouin M, Tomasetto C, Eberling P, St-Pierre S, Bannon P,L'Heureux MC, Poitras P(2003). Two new peptides to improve gastric ileus in dog. Peptides. 24: 531-534.
Trudel L, Bouin M, Tomasetto C, Eberling P, St-Pierre S, Bannon P,L'Heureux MC, Poitras P(2003). Two new peptides to improve gastric ileus in dog. Peptides. 24: 531-534.
Trudel L, Tomasetto C, Rio MC, Bouin M, Plourde V, Eberling P, Poitras P(2002).Ghrelin/motilin-related peptide is a potent prokinetic to reverse gastric postoperative ileus in rat. Am. J. Phys. Gastrointest. Liver Physiol., 282: G948-952.
Trudel L, Tomasetto C, Rio MC, Bouin M, Plourde V, Eberling P, Poitras P(2002).Ghrelin/motilin-related peptide is a potent prokinetic to reverse gastric postoperative ileus in rat. Am. J. Phys. Gastrointest. Liver Physiol., 282: G948-952.