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# Relation between ghrelin level and treatment response in functional constipation

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***Background/aims:*** *Ghrelin stimulates gastrointestinal motility. Although there are some experimental and clinical studies supporting the role of ghrelin for gastrointestinal motility disorders, limited research for constipation has been published. The purpose of this study was to evaluate the possible role of ghrelin in the pathophysiology of functional constipation in childhood.* ***Material and Methods:*** *Forty-three newly diagnosed constipated children aged 1-6 years and 25 healthy age-matched controls were included. Serum ghrelin levels were analyzed initially in both groups. Treatment protocol consisted of dietary modification, lactulose, and administration of pediatric enema. Ghrelin levels of children with functional constipation were reanalyzed after two months of treatment.* ***Results:*** *Initial serum ghrelin levels of constipated patients were found to be lower than those of healthy children ($p<0.001$). Ghrelin levels increased during therapy. The differences between initial and second month serum ghrelin levels of constipated patients were found to be statistically significant ($p<0.05$).* ***Conclusion:*** *Our data supports the potential role of ghrelin in children with functional constipation. Observation of an increase in serum ghrelin levels with nonspecific treatment supports the hypothesis that low serum ghrelin levels might be a result rather than the cause of constipation.*

**Key words:** Ghrelin, motilin, constipation, children

## Fonksiyonel konstipasyonda serum ghrelin seviyesi ve tedavi cevabı arasındaki ilişki

***Giriş ve Amaç:*** *Ghrelin gastrointestinal motiliteyi artırmaktadır. Gastrointestinal motilite bozukluklarında ghrelinin rolü olduğunu destekleyen bazı deneysel ve klinik çalışmalar mevcut olmakla birlikte, konstipasyonla ilgili olarak sınırlı sayıda çalışma yayınlanmıştır. Bu çalışmada, çocukluk döneminde fonksiyonel konstipasyonun patofizyolojisinde ghrelinin olası rolü değerlendirilmiştir.* ***Gereç ve Yöntem:*** *Yaşları 1-6 arasında değişen yeni konstipasyon tanısı almış 43 çocuk ve 25 sağlıklı kontrol grubu çalışmaya dahil edildi. Başlangıç serum ghrelin düzeyleri, her iki grupta da analiz edildi. Tedavi protokolü; diyet, laktuloz ve pediatrik lavman uygulamasından oluşuyordu. Fonksiyonel konstipasyonu olan hastaların ghrelin seviyeleri iki aylık tedavi sonrası tekrar analiz edildi.* ***Bulgular:*** *Konstipasyonu olan hastaların başlangıç serum ghrelin düzeyleri, sağlıklı çocuklardan daha düşük bulunmuştur ($p <0.001$). Ghrelin seviyeleri tedavi sırasında artış göstermiştir. Konstipasyonu olan hastaların ilk ve ikinci ayda ölçülen serum ghrelin düzeyleri arasındaki fark istatistiksel olarak anlamlı bulunmuştur ($p <0.05$).* ***Sonuç:*** *Bu çalışma, ghrelinin fonksiyonel konstipasyonu olan çocuklarda potansiyel rolü olabileceğini desteklemektedir. Nonspesifik tedavi ile serum ghrelin seviyelerinde artış gözlenmesi, düşük ghrelin seviyelerinin konstipasyonun sebebinden çok, sonucu olabileceği hipotezini desteklemektedir.*

**Anahtar kelimeler:** Ghrelin, motilin, konstipasyon, çocuk

## INTRODUCTION

Ghrelin was first discovered during the studies on the hypothalamic regulation of growth hormone secretion by the pituitary gland. Ghrelin, in other words motilin-related peptide, is one of the new members of the gastrointestinal hormones (1-3). It was shown in the stomach as a 28 amino acid peptide for the first time in 1999 (4). In 2000, Date et al. (5) demonstrated that the highest ghrelin expression was detected in the oxyntic mucosa of the stomach and also considerable amounts were de-

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tected in the duodenum, jejunum, ileum, and caecum. In 2002, Wierup et al. (6) reported that ghrelin was mainly found in the pancreas, while an only a few ghrelin cells were located in the stomach during fetal and neonatal period. In 2000, Hosoda et al. (7,8) and later in 2002, Gnanapavan et al. (9) reported the presence of small amounts of ghrelin in the pituitary, kidneys, heart, thyroids, lungs, testicles, liver, pancreas, and hypothalamus.

Constipation is a common disorder in childhood. It is defined as less than three bowel movements per week or as difficulty in passing stools. The majority of the constipated children have functional constipation (10-12). Organic disease such as congenital megacolon, anorectal malformation, neuronal intestinal dysplasia, metabolic and endocrine disorders or drugs are thought to be responsible for only 10 % of all cases (13). Although painful defecation and stool withholding are generally accepted as major causes, the exact etiology of functional constipation is still not well-known (10,11). Gut regulatory peptides such as motilin and ghrelin might be involved in the pathophysiology of constipation (14). Ghrelin improves digestive motility by accelerating gastric and intestinal motility (14). Although the direct motility effect of ghrelin seems to be restricted to the proximal gastrointestinal tract, there is some evidence for the promoting role of ghrelin on colonic motility. The presence of ghrelin receptor like immunoreactivity was demonstrated in rat colon. On the other hand, it was demonstrated that centrally or peripherally administered ghrelin stimulates colonic motility via hypothalamic NPY1 receptor pathways and stimulation of lumbosacral ghrelin receptors initiates propulsive colonic motility in rats. Although these data are suggestive for the effects of ghrelin on colonic motility, the functional role of colonic ghrelin receptors are still not clear (15-22]. There are many reports about the role of ghrelin and ghrelin receptor agonists in some gastrointestinal motility disorders; however, a study about ghrelin in children with functional constipation has not been encountered so far in the literature. The purpose of this study was to evaluate the possible role of ghrelin in the pathophysiology of functional constipation in childhood.

## MATERIALS and METHODS

Forty-three newly diagnosed constipated children aged between 1 and 6 years old and 25 age- and sex-matched healthy children were included in the study. The diagnosis of constipation was made according to the Rome III criteria which was defined as having at least two of the following characteristics: fewer than three bowel movements weekly; more than one episode of fecal incontinence weekly; large stools in the rectum shown by digital rectal examination or palpable on abdominal examination; occasional passage of large stools; retentive posturing and withholding behavior, and painful defecation.

Serum ghrelin level is known to be affected by circumstances such as obesity, infection, chronic illness and puberty, thus such patients were all excluded in the two groups. Serum ghrelin levels were analyzed initially in both groups. After an overnight fasting, blood samples were taken before 08:00 a.m. Serum samples were stored in -80°C. Serum fasting ghrelin levels were measured using radioimmunoassay method (Ghrelin RIA Kit, KIPMR90). All samples were analyzed in the same run.

Treatment protocol consisted of dietary modification and lactulose. Pediatric enema was administered for the first 2 days to all constipated patients. Lactulose treatment was given with a dose of 1 cc/kg/day. Soft defecation daily or every other day was accepted as therapeutic response. The first interview with constipated patient's parents was performed by telephone at the second week of the treatment. Within the first 2 months, patients' conditions were discussed with families 2-3 times by phone. Lactulose doses were increased step by step according to the information received from the family on the phone. First check-up was performed after 2 months. During follow-up of the patients, lactulose dose has been increased up to 3 cc/kg/day when necessary. Patients who had ongoing complaints despite lactulose given at a dose of 3 cc/kg/day in 6 months were considered as "poor responders". Complete blood count, thyroid function tests, serum calcium levels, celiac markers, barium enema, and colonic manometric studies were performed respectively in poor responders. Serum ghrelin levels were measured again after two months in all constipated patients.

This study was approved by the local Ethics committee in Gazi University School of Medicine. Informed consents of all subjects were taken from parents. This study was granted by the support from Gazi University School of Medicine.

### Statistical Analysis

One-way analysis of variance, post hoc Tukey's test, and chi-square test were performed for variables, where appropriate, to compare patient characteristics. The Kruskal-Wallis test was used for the significance of the difference between the groups in terms of median serum ghrelin values. Wilcoxon signed rank test was used to determine whether there was a statistically significant change between pre- and post-treatment serum ghrelin levels. A p-value <0.05 was considered statistically significant.

## RESULTS

There were 20 girls/23 boys in the functional constipation group and 13 girls/12 boys in the control group. Median ages of patients with functional constipation and healthy children were 36 (15-66) months and 36 (18-60) months, respectively. There was no difference for age and gender between constipated patients and healthy group. Clinical characteristics are shown in Table 1.

Initial median ghrelin levels in the patients were lower than in the controls: 778 versus 3544 pg/mL (p<0.001) (Figure 1). Within the first 2 months after the diagnosis, phone calls were performed once with 29 patients, twice with 8 patients, and 3 times with 6 patients. Serum ghrelin levels increased after two months of therapy - from 778 pg/mL to 1549 pg/mL (p<0.05) (Figure 1). During the 6-month follow-up period, 8 patients received 1 cc/kg/day, 19 patients received 2 cc/kg/day, and 16 patients received 3 cc/kg/day lactulose treatment. Twelve patients who received 3 cc/kg/day lactulose responded to therapy in that dosage. The remaining four patients who had the highest initial serum ghrelin levels among others did not respond to high-dose lactulose treatment (3 cc/kg/day). These four patients required enema and stimulant drugs and were accepted as poor responders. The clinical characteristics and serum ghrelin levels of poor responders are given in Table 2. There was a positive correlation between initial serum ghrelin levels and lactulose dosage (p <0.05) (R=+0.788) (Figure 2).

## DISCUSSION

This is the first study investigating the relation between serum ghrelin levels and functional constipation. So far, there has been no published study

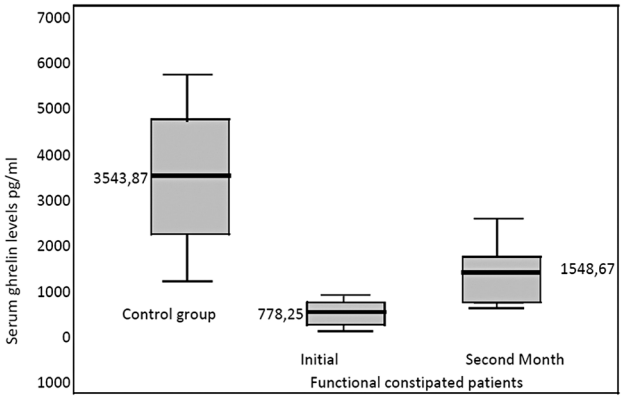


**Figure 1.** Serum ghrelin levels of constipated patients and control group.

**Table 1.** Clinical characteristics of patients with functional constipation and control group

| | Patients with Functional Constipation N=43 (%) | Healthy Children N=25 (%) | *p* |
|---|---|---|---|
| Median age (months) | 36 (15-66) | 36 (18-60) | p>0.05 |
| Female/male | 20 (46.5) / 23 (53.5) | 13 (52) / 12 (48) | p>0.05 |
| Large stools in the rectum | 33 (76.74) | - | |
| Passage of large stools | 38 (88.37) | - | |
| Retentive posturing | 25 (58.13) | - | |
| Withholding behavior | 35 (81.39) | - | |
| Pain during defecation | 36 (83.72) | - | |
| Abdominal distention | 35 (81.39) | - | |
| Poor appetite | 15 (34.88) | - | |
| Urinary tract infection | 11 (11.62) | - | |
| Mild mental retardation | 1 (2.32) | - | |

**Table 2.** Clinical characteristics and serum ghrelin levels of poor responders

| **Patients** | 1 | | 2 | | 3 | | 4 | |
|---|---|---|---|---|---|---|---|---|
| **Age** | 58 months | | 38 months | | 72 months | | 65 months | |
| **Gender** | Girl | | Boy | | Boy | | Boy | |
| **Ghrelin Level (pq/mL)** | Initial | Second month | Initial | Second month | Initial | Second month | Initial | Second month |
| | 942.424 | 987.98 | 988.424 | 995.43 | 969.565 | 995.75 | 976.425 | 1000.98 |
| Large stools in the rectum | + | | - | | + | | - | |
| Passage of large stools | - | | - | | + | | - | |
| Retentive posturing | - | | - | | + | | - | |
| Withholding behavior | - | | - | | + | | + | |
| Pain during defecation | - | | - | | + | | - | |
| Abdominal distention | - | | - | | + | | + | |
| Poor appetite | - | | - | | - | | - | |
| Urinary tract infection | - | | - | | - | | - | |
| Psychological problems | - | | - | | * + | | - | |

*: a slight diminution in IQ

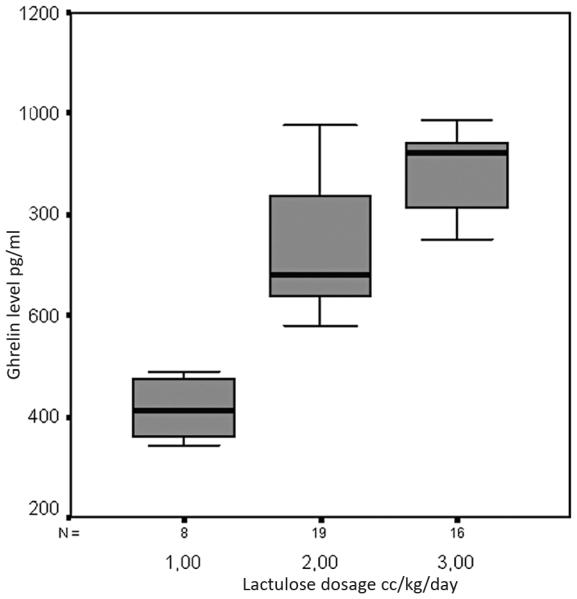


**Figure 2.** The association between initial serum ghrelin levels and lactulose dosage.

investigating this issue in either adults or children. We showed that serum ghrelin levels are lower in children with functional constipation compared to healthy children which improved with treatment.

The studies for the past 10 years were about the effects of ghrelin in gut-brain axis and were identifying different forms of ghrelin in blood. In the last few years, both the animal and human studies focused on ghrelin levels in situations such as glucose homeostasis, obesity, irritable bowel syndrome, gastritis, and motility disorders.

El Salhy et al. (23) simultaneously analyzed serum ghrelin levels and ghrelin cell density in oxyntic stomach mucosa of patients with irritable bowel syndrome. As a result, no significant difference was found between irritable bowel syndrome patients and control group in their study. Similarly, Sjölund et al. (24) reported that serum ghrelin and motilin levels of patients with irritable bowel syndrome and control group showed no significant difference. However, Takamori et al. (25) reported that gastric emptying time was longer in functional dyspepsia patients than in the control group, and total serum ghrelin levels were lower in these patients compared with the control group. According to Takamori et al's study (25), it might be claimed that low total ghrelin levels may extend gastric emptying time and may cause dyspeptic symptoms.

Falken et al. (17) reported the first and single study about ghrelin in healthy volunteers. According to this study, administration of i.v. ghrelin increased gastric emptying rate, while it has no effect on orocecal or colonic transit. Although there was no evidence for a direct effect of ghrelin in the colon, it was shown that ghrelin receptor agonists reduced the first bowel movement time after surgery (26). This postsurgical recovery of the gastrointestinal motility was explained by activation of gastrocolic reflex and shortened overall transit ti-

me by stimulating gastric emptying time and small intestinal transit time with ghrelin receptor agonist. Gut regulatory peptides such as motilin and ghrelin might be involved in the pathophysiology of constipation (14). Based on the results of the studies mentioned above, we decided to investigate the serum ghrelin levels of children with constipation.

Animal studies about ghrelin's effect on colonic motility are quite interesting. Ghrelin accelerates gastric emptying and enhances intestinal transit time in conscious rats and mice (19,27,28). During gastric motility tests, it was shown that ghrelin augmented phase III-like contractions and these contractions disappeared with ghrelin receptor antagonists and atropine. In rats, gastric phase II-I-like contractions and increase in gastric emptying with ghrelin vanished by vagotomy or treatment with capsaicin, showing that vago-vagal reflex pathway participates in stimulating action of ghrelin. Besides a vago-vagal reflex pathway, the functional role of peripheral enteric ghrelin receptor has been demonstrated in isolated gastrointestinal strips of rats and mice (18,19,21,29). However, ghrelin did not stimulate canine gastrointestinal motility in vivo (20). As a result, based on the above-mentioned literatures, there is a gut-brain axis for regulation of ghrelin levels. The transfer of information about ghrelin to the brain through the vagus might also contribute the prokinetic effect of ghrelin.

In our study, initial serum ghrelin levels were lower in patients than in controls and they increased during lactulose therapy gradually. The question that arises in this point is whether high serum ghrelin levels via unknown mechanisms may have a role in the treatment of constipation or treatment of constipation leads to an increase in serum ghrelin levels. Unfortunately, our study was not designed to answer this question.

We hypothesized that treatment of functional constipation may lead to increase in serum ghrelin levels either by reversing central dysregulation ghrelin pathways or by increasing ghrelin receptors in the gastrointestinal tract. In addition, normalization of defecation with treatment, resolution of gut distention, changes in gut content, or other unknown mechanisms may promote ghrelin secretion in the gastrointestinal tract.

According to our study, 8 patients with functional constipation received lactulose treatment of 1 cc/kg/day and 19 patients received lactulose treatment of 2 cc/kg/day. In our study, initial serum ghrelin levels of constipated patients were low and among them, patients with the lower serum ghrelin levels did not require additional dosage for recovery. In other words, 16 of 43 constipated patients were receiving 3 cc/kg/day lactulose treatment and initial serum ghrelin levels were higher. 4 of 16 constipated patients had the highest serum ghrelin levels and did not respond to treatment even though in high lactulose dosages. There was no difference between initial and second month serum ghrelin levels of these 4 poor responder patients who are still under treatment and we did not observe an increase with therapy.

An interesting result of our study is the strong positive correlation between serum ghrelin levels and oral lactulose treatment dosage ($p<0.05$) ($R=+0,788$). Children with functional constipation with low serum ghrelin levels required low dosage of oral lactulose treatment. However, higher ghrelin levels required higher doses of oral lactulose for treatment. We took oral lactulose dosage as an indirect measure of treatment response in our study. There were 4 poor responders despite of high dose of lactulose treatment. We speculate that these outliers may be a subgroup with a different mechanism involved in functional constipation or there may be other factors/disease that we could not diagnose despite detailed history and investigation.

The limitation of our study was that we considered the clinical situations that may affect serum ghrelin levels; however, although effects of appetite and feeding on ghrelin were known, the dietary regimens were not same in all groups. Under 2 years of age, Z scores would not be suitable; therefore, percentile values were used for inclusion criteria, and children with normal development pattern were included in the study. There was no significant difference between the two groups in terms of nutritional status. In our study, in order to clarify the relationship between the etiology of constipation and serum ghrelin levels, serum ghrelin levels have to be checked more than twice during the follow-up period. However, in our study, ghrelin levels were measured only twice.

In conclusion, studies concerning the effects of ghrelin on gastrointestinal disease still continue in the literature. Besides animal experiments, clinical trials in humans are very limited especially for constipation. In our study, we observed low se-

rum ghrelin levels in constipated patients and ghrelin levels increased with treatment. The constipated patients with the lowest initial ghrelin levels showed the best response to treatment. We need larger groups of patients in order to claim that there is a relationship between ghrelin, colonic motility and functional constipation. Moreover, we need additional studies to clarify that serum ghrelin levels may be an indicator for response to therapy.

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## REFERENCES

1. Kojima M, Kangawa K. Ghrelin: structure and function. Physiol Rev 2005; 85:495-522.
2. Peeters TL. Ghrelin: a new player in the control of gastrointestinal functions. Gut 2005; 54:1638-49.
3. Van der Lely AJ, Tschöp M, Heiman ML, et al. Biological, physiological, pathophysiological, and pharmacological aspects of ghrelin. Endocr Rev 2004; 25:426-57.
4. Kojima M, Hosoda H, Date Y, et al. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 1999; 402:656-60.
5. Date Y, Kojima M, Hosoda H, et al. Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. Endocrinology 2000; 141:4255-61.
6. Wierup N, Svensson H, Mulder H, et al. The ghrelin cell: a novel developmentally regulated islet cell in the human pancreas. Regul Pept 2002; 107:63-9.
7. Hosoda H, Kojima M, Matsuo H, et al. Ghrelin and des-acyl ghrelin: two major forms of rat ghrelin peptide in gastrointestinal tissue. Biochem Biophys Res Commun 2000; 279:909-13.
8. Hosoda H, Kojima M, Matsuo H, et al. Purification and characterization of rat des-Gln14-Ghrelin, a second endogenous ligand for the growth hormone secretagogue receptor. J Biol Chem 2000; 275:21995-22000.
9. Gnanapavan S, Kola B, Bustin SA, et al. The tissue distribution of the mRNA of ghrelin and subtypes of its receptor, GHS-R, in humans. J Clin Endocrinol Metab 2002; 87:2988
10. Benninga MA, Voskuijl WP, Taminiau JA. Childhood constipation: is there new light in the tunnel? J Pediatr Gastroenterol Nutr 2004; 39:448-64.
11. Hyman PE, Milla PJ, Benninga MA, et al. Childhood functional gastrointestinal disorders: neonate/toddler. Gastroenterology 2006; 130:1519-26.
12. Tabbers MM, Boluyt N, Berger MY, et al. Clinical practice: diagnosis and treatment of functional constipation. Eur J Pediatr 2011; 170:955-63.
13. Rasquin A, Di Lorenzo C, Forbes D, et al. Childhood functional gastrointestinal disorders: child/adolescent. Gastroenterology 2006; 130:1527-37.
14. De Smet B, Mitselos A, Depoortere I. Motilin and ghrelin as prokinetic drug targets. Pharmacol Ther 2009; 123:207-23.
15. Cowley MA, Grove KL. Ghrelin. Satisfying a hunger for the mechanism. Endocrinology 2004; 145:2604-6.
16. Date Y, Marakami N, Toshinai K, et al. The role of the gastric afferent vagal nerve in ghrelin-induced feeding and growth hormone secretion in rats. Gastroenterology 2002; 123:1120-8.
17. Falkén Y, Hellström PM, Sanger GJ, et al. Actions of prolonged ghrelin infusion on gastrointestinal transit and glucose homeostasis in humans. Neurogastroenterol Motil 2010; 22:e192-200.
18. Fujino K, Inui A, Asakawa A, et al. Ghrelin induces fasted motor activity of the gastrointestinal tract in conscious fed rats. J Physiol 2003; 550:227-40.
19. Kitazawa T, De Smet B, Verbeke K, et al. Gastric motor effects of peptide and non-peptide ghrelin agonists in mice in vivo and in vitro. Gut 2005; 54:1078-84.
20. Ohno T, Kamiyama Y, Aihara R, et al. Ghrelin does not stimulate gastrointestinal motility and gastric emptying: an experimental study of conscious dogs. Neurogastroenterol Motil 2006; 18:129-35.
21. Tack J, Depoortere I, Bisschops R, et al. Influence of ghrelin on interdigestive gastrointestinal motility in humans. Gut 2006; 55:327-33.
22. Zheng J, Ariga H, Taniguchi H, et al. Ghrelin regulates gastric phase III-like contractions in freely moving conscious mice. Neurogastroenterol Motil 2009; 21:78-84.
23. El-Salhy M, Lillebø E, Reinemo A, et al. Ghrelin in patients with irritable bowel syndrome. Int J Mol Med 2009; 23:703-7.
24. Sjölund K, Ekman R, Wierup N. Covariation of plasma ghrelin and motilin in irritable bowel syndrome. Peptides 2010; 31:1109-12.
25. Takamori K, Mizuta Y, Takeshima F, et al. Relation among plasma ghrelin level, gastric emptying, and psychologic condition in patients with functional dyspepsia. J Clin Gastroenterol 2007; 41:477-83.
26. Venkova K, Mann W, Nelson R, et al. Efficacy of ipamorelin, a novel ghrelin mimetic, in a rodent model of postoperative ileus. J Pharmacol Exp Ther 2009; 329:1110-6.
27. Dornonville de la Cour C, Lindström E, Norlén P, et al. Ghrelin stimulates gastric emptying but is without effect on acid secretion and gastric endocrine cells. Regul Pept 2004; 120:23-32.
28. Trudel L, Tomasetto C, Rio MC, et al. Ghrelin/motilin-related peptide is a potent prokinetic to reverse gastric postoperative ileus in rat. Am J Physiol Gastrointest Liver Physiol 2002; 282:G948-52.
29. Masuda Y, Tanaka T, Inomata N, et al. Ghrelin stimulates gastric acid secretion and motility in rats. Biochem Biophys Res Commun 2000; 276:905-8.