



ORIGINAL ARTICLE

# Dose-dependent effect of ghrelin on gastric emptying in rats and the related mechanism of action



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## KEYWORDS

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**Abstract** The aim of this study was to investigate the dose-dependent effect of ghrelin on gastric emptying in rats and the related mechanism of action. Sixty Wistar rats were randomized into control and test groups, which respectively received intraperitoneal injection of normal saline and ghrelin at different doses (0.5 nmol/kg, 1.0 nmol/kg, 1.5 nmol/kg, 2.0 nmol/kg, and 2.5 nmol/kg). After 45 minutes, all rats were gavaged with semisolid paste. The gastric emptying rate was determined 30 minutes later, and the plasma cholecystokinin level was tested by radioimmunoassay. The mean gastric emptying rate in the test groups was significantly higher than in the control group ( $38.24 \pm 7.15\%$  and  $27.18 \pm 2.37\%$ , respectively,  $p < 0.05$ ). Medium and high doses of ghrelin (1.0 nmol/kg, 1.5 nmol/kg, 2.0 nmol/kg, and 2.5 nmol/kg), but not low dose (0.5 nmol/kg), accelerated the gastric emptying. In addition, the plasma cholecystokinin level in the test groups was significantly higher than in the control group ( $p < 0.01$ ). The gastric emptying rate was positively correlated with the plasma cholecystokinin level ( $p < 0.01$ ). Intraperitoneal injection of ghrelin at medium and high doses significantly accelerated gastric emptying in rats.

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## Introduction

Ghrelin, a newly discovered 28 amino acid endogenous peptide, is an endogenous ligand of growth hormone secretagogue receptor (GHSR), which induces extensive biological effects after binding to GHSR. Ghrelin promotes secretion of growth hormone, enhances appetite, lowers fat utilization, increases body weight, maintains a positive energy balance, and regulates energy metabolism [1].

Conflicts of interest: All authors declare no conflicts of interests.

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Ghrelin is produced mainly by gastric endocrine cells and enters the blood circulation. Gastric electrophysiological stimulation can markedly promote gastric endocrine cells to produce ghrelin [2]. Research has shown that ghrelin can accelerate gastric emptying and small intestinal propulsion in *Suncus murinus* and mice [3,4]. High doses of ghrelin can significantly enhance contraction of gastrointestinal smooth muscle [5], and intraventricular or intravenous injection of ghrelin can induce postprandial rapid gastric and duodenal contractions in control rats [6]. However, there are few data on the role of ghrelin in the regulation of gastric emptying. We aimed to observe the effects of intraperitoneally injected ghrelin at different doses on gastric emptying in rats to illuminate the regulatory effect of ghrelin on gastric emptying, and to simultaneously detect plasma cholecystokinin level, so as to explore the mechanism of ghrelin in the regulation of gastric emptying.

## Methods

### Animals and grouping

A total of 60 healthy male Wistar rats weighing 200–250 g were provided by Laboratory Animal Centre of Wenzhou Medical University. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use protocol has been reviewed and approved by the Institutional Animal Care and Use Committee of Wenzhou Medical University.

Rats were maintained under a diurnal cycle at  $22 \pm 3^\circ\text{C}$  with free access to food and water. After 1 week of adaptive raising, the rats were deprived of food for 24 hours and of water for 12 hours, and then randomized into control and test (A–E) groups with 10 rats in each group. The rats in the control and test groups received intraperitoneal injection of normal saline (1 mL) and ghrelin (Sigma, St. Louis, MO, USA) at different doses (A, 0.5 nmol/kg; B, 1.0 nmol/kg; C, 1.5 nmol/kg; D, 2.0 nmol/kg; and E, 2.5 nmol/kg).

### Semisolid paste preparation

Five grams of hydroxymethyl cellulose was dissolved in 125 mL distilled water and the following ingredients were added and mixed to make 150 mL semisolid paste (150 g): 8 g milk powder, 4 g sugar, and 4 g starch [7]. The preparation was stored in the fridge for 12 hours and kept at room temperature for 2 hours before use.

### Gastric emptying test

The rats in the control group and test groups A–E received intraperitoneal injection of normal saline (1 mL) and 0.5 nmol/kg, 1.0 nmol/kg, 1.5 nmol/kg, 2.0 nmol/kg, and 2.5 nmol/kg octanoyl ghrelin, respectively. Each rat was gavaged with 1 mL semisolid paste after 45 minutes. The tail venous blood was collected 30 minutes later under anesthesia with intraperitoneal injection of pentobarbital (the anesthetic dose of phenobarbital was 8 mg/kg). The abdominal cavity was opened, and the pylorus and cardia

were ligated. The stomach was taken out and weighed after being dried with filter paper. It was then cut along the greater curvature, and the gastric content was washed off; the net weight of the stomach was recorded after drying. The difference between the stomach full weight and net weight was defined as the stomach residue weight; the gastric residual rate was the percentage of stomach residue in semisolid paste; the gastric emptying rate was calculated using the following formula:

$$\text{gastric emptying rate} = [1 - (\text{stomach full weight} - \text{net weight})] \times 100\% \quad (1)$$

At the end of the experiments measuring gastric emptying, animals were euthanized by intraperitoneal injection of excess doses of pentobarbital (the anesthetic dosage of phenobarbital was 40 mg/kg, with the concentration as 2%). After intraperitoneal injection of a large dose of phenobarbital, the rats stopped breathing and their hearts stopped beating, so they were judged as dead.

### Detection of cholecystokinin level

The tail venous blood was collected into a tube with 0.3  $\mu\text{L}$  EDTA and 1000 kU aprotinin, followed by cryogenic centrifugation at 3000 g for 10 minutes. The plasma was isolated and the cholecystokinin level was determined using a radioimmunoassay kit (Beijing Haikerui Biotech Center, Beijing, China).

### Statistical analysis

All results are presented as mean  $\pm$  standard error of the mean. The effect of ghrelin on cholecystokinin level in plasma was analyzed by one-way analysis of variance followed by Dunnett's *post hoc* testing. A *p* value  $< 0.05$  was considered statistically significant. Linear regression was used to study the correlation.

## Results

### Comparison of gastric emptying

After intraperitoneal injection of ghrelin, the gastric emptying rate in the test groups ( $38.24 \pm 7.15\%$ ) was significantly higher than that in the control group ( $27.18 \pm 2.37\%$ ;  $p = 4.15 \times 10^{-5}$ ; Table 1).

**Table 1** Comparison of gastric emptying between two groups.

Groups	Cases	Gastric emptying rate (%)
Control	10	$27.03 \pm 2.91$
Test	50	$38.24 \pm 7.15^*$

\* Compared with control group,  $p = 0.0000415$ .

### Dose–effect relationship between ghrelin and gastric emptying

The small dose of ghrelin (0.5 nmol/kg) did not accelerate gastric emptying compared to the control group. Medium and high doses of ghrelin gradually increased gastric emptying rates; the emptying rates were significantly greater than in the control group (Table 2).

### Cholecystokinin level

After intraperitoneal injection of ghrelin at increasing doses (0.5 nmol/kg, 1.0 nmol/kg, 1.5 nmol/kg, 2.0 nmol/kg, and 2.5 nmol/kg), plasma cholecystokinin level was elevated ( $44.17 \pm 5.18$  ng/L,  $48.37 \pm 4.95$  ng/L,  $52.74 \pm 5.3$  ng/L,  $57.86 \pm 4.27$  ng/L, and  $61.47 \pm 5.87$  ng/L, respectively), to levels significantly higher than in the control group (Table 3).

### Correlation analysis

Intraperitoneal injection of ghrelin at different doses accelerated gastric emptying of healthy male Wistar rats. With increasing dose of ghrelin, there is a trend that gastric emptying accelerates. In addition, intraperitoneal injection of ghrelin influenced rat plasma cholecystokinin level. The gastric emptying rate in rats was positively correlated with plasma cholecystokinin level ( $r = 0.708$ ,  $p < 0.01$ ), indicating that cholecystokinin might be involved in the regulation of gastric emptying by ghrelin (Figure 1).

### Discussion

Ghrelin, consisting of 28 amino acids, is a newly discovered endogenous peptide secreted by fundic X/A-like cells, and is the endogenous ligand of GHSR. Increasing electrophysiological stimulation of the rat stomach can lead to the enhancement of ghrelin production by gastric endocrine cells [2], which then binds with GHSR, resulting in extensive biological effects, for example, the promotion of growth hormone release and adjustment of energy metabolism. Ghrelin receptor belongs to the G-protein-coupled family.

**Table 2** Dose–effect relationship between ghrelin and gastric emptying in rats.<sup>a</sup>

Groups	Ghrelin dose (nmol/kg)	Cases	Gastric emptying rate (%)
Control		10	$27.03 \pm 2.91$
A	0.5	10	$29.78 \pm 4.68$
B	1.0	10	$34.59 \pm 4.07^{\#}$
C	1.5	10	$38.33 \pm 4.41^{\#}$
D	2.0	10	$41.88 \pm 3.94^{\#}$
E	2.5	10	$46.64 \pm 4.14^{\#}$

<sup>#</sup> Compared with the control group, the difference was statistically significant.

<sup>a</sup> Compared with control group, (A)  $p = 0.066$ , (B)  $p = 7.6 \times 10^{-5}$ , (C)  $p = 1.2 \times 10^{-6}$ , (D)  $p = 1.6 \times 10^{-9}$ , and (E)  $p = 1.8 \times 10^{-10}$ .

**Table 3** Effects of ghrelin on plasma cholecystokinin level in rats.

Groups	Ghrelin dose (nmol/kg)	Cases	Cholecystokinin (ng/L)	$p^*$
Control		10	$44.17 \pm 5.18$	
A	0.5	10	$48.37 \pm 4.95$	0.04
B	1.0	10	$52.74 \pm 5.3$	0.0009
C	1.5	10	$57.86 \pm 4.27$	$2.3 \times 10^{-6}$
D	2.0	10	$61.47 \pm 5.87$	$7.97 \times 10^{-7}$
E	2.5	10	$66.73 \pm 4.74$	$3.5 \times 10^{-9}$

\* Compared with control group.

The structural similarity of the peptide has stimulated interest in the relationship between ghrelin and gastric emptying or intestinal motility.

In our research, ghrelin was found to accelerate gastric emptying in rats, and to some degree, it was a dose–effect relationship. Gastric emptying increased after intraperitoneal injection of low-dose ghrelin compared with the control group but the difference was not significant. However, intermediate and large doses of ghrelin promoted gradual gastric emptying. By contrast, intraperitoneally injected ghrelin at all doses increased cholecystokinin level in rat plasma, which correlated to some extent with the gastric emptying rate.

It has been proven in rat and mouse studies that ghrelin and ghrelin agonist (growth hormone releasing peptide-6) accelerated gastric emptying [8,9], and that ghrelin also promoted gastric emptying in a rat model with postoperative obstruction [10]. In human studies, ghrelin was found to accelerate gastric emptying in healthy controls [4] and in patients with idiopathic or diabetic gastroparesis [11,12]. Endogenous ghrelin can modulate the Phase III-like contraction that appears in the rat or mouse stomach [6], induce the earlier Phase III of Migrating Motor Complex (MMC) of the stomach and the duodenum, and enhance contraction in Phase III of MMC. Continuous intravenous infusion of ghrelin to patients following open colectomy could increase the speed of gastric emptying during infusion, improve the recovery of intestinal tract function, shorten the anal exhaust time, and affect the release of certain gastrointestinal hormones, thereby indicating that the effect of ghrelin on gastrointestinal motility works through humoral factors [13]. The ghrelin agonist TZIP-101 significantly alleviates nausea and vomiting in patient with gastroparesis [14]. Recent studies have indicated that a daily dose of 10–40 mg TZIP-102 improved the symptoms of diabetic gastroparesis. However, there was no correlation between symptom improvement and gastric emptying change in diabetic gastroparesis [15]. Oral or intraperitoneal injection of levodopa significantly inhibits the gastric emptying of healthy rats, while ghrelin clearly reverses such inhibition [16]. Our research was consistent with these results, indicating that ghrelin could accelerate gastric emptying. However, some studies have yielded different results: one study indicated that intravenous injected ghrelin had no significant effect on gastrointestinal movement in dogs in fasting and postprandial conditions, while large doses of intravenous injected ghrelin significantly reduced the

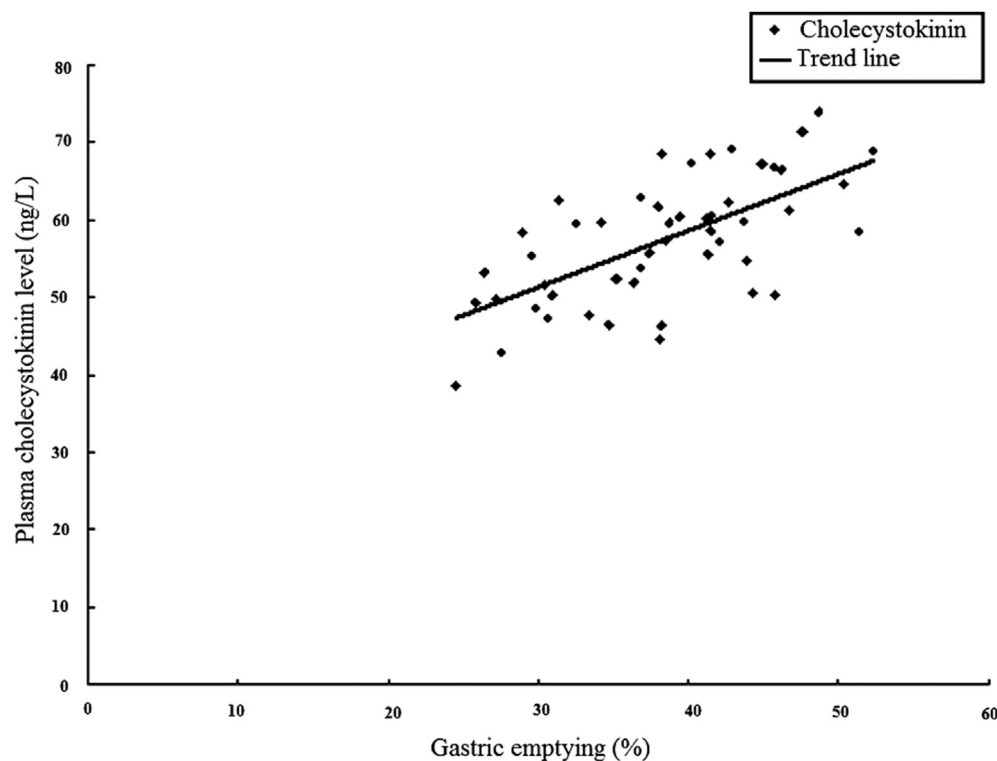


Figure 1. Correlation analysis of gastric emptying rats with plasma cholecystokinin level.

motility index of the gastric body with no enhancement to gastric emptying [17]. These differences may reflect the different clinical implications between intravenous and intraperitoneal injection. Different molecular forms of ghrelin have demonstrated different effects on gastric emptying. Symplectic acylated ghrelin enhanced rat gastric emptying, while deacylated ghrelin applied intracerebroventricularly inhibited rat gastric emptying following intake of a semifluid diet [18]. In another study, it was demonstrated that the effect of ghrelin on gastric emptying could also be affected by the form and composition of gastric content. Peripheral intravenous ghrelin administration could promote gastric emptying of non-nutritive liquid material, but not of nutritive liquid material.

Cholecystokinin, which exists extensively in many parts of the human body and is one of the most studied gastrointestinal hormones, is a kind of brain-gut peptide with wide ranging biological activities which not only stimulate gallbladder contraction, but also play an important role in the regulation of overall gastrointestinal movement. Cholecystokinin can inhibit the gastric emptying of solids and liquids by means of relaxing the proximal stomach, increasing the pyloric sphincter tension, and altering the gastric emptying pattern [19,20]. Although many studies support the view that cholecystokinin inhibits gastric emptying physiologically, due to the complexity of gastric emptying regulation and gastric motility, the exact role of cholecystokinin within this process is still uncertain. Ghrelin can promote the release of gastrointestinal hormones, such as cholecystokinin, glucagon-like peptide 1 (GLP-1), and nitric oxide (NO) [21]. Some researchers have indicated that gastric emptying of high-fat diet-induced obese rats was positively correlated with plasma ghrelin level, and

negatively associated with plasma cholecystokinin and leptin level [4]. However, in our research, the rat plasma cholecystokinin concentration and the gastric emptying speed increased after intraperitoneal injection of ghrelin, and the plasma cholecystokinin concentration was positively correlated with the gastric emptying rate, which is opposite to the above-mentioned results. In our study, the increasing cholecystokinin level after ghrelin injection was confirmed, and the plasma cholecystokinin concentration was positively correlated with the gastric emptying rate. We supposed that the high level of cholecystokinin could be increased the effect of gastric emptying through diastolic proximal stomach and improve the tension of pyloric sphincter. Furthermore, the high level cholecystokinin could increase the excitatory effect on the function of the small and large intestines, causing small intestine and colon movement. Whether cholecystokinin plays a role in the gastric emptying regulation of ghrelin is still to be confirmed.

In conclusion, intraperitoneal ghrelin injection can accelerate gastric emptying. However, the pathophysiological mechanism by which ghrelin affects gastric emptying requires further investigation.

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