Does ghrelin explain accelerated gastric emptying in the early stages of diabetes mellitus?

Hajime Ariga, Kenji Imai, Cindy Chen, Christopher Mantyh, Theodore N. Pappas and Toku Takahashi

Department of Surgery, Duke University Medical Center
and Durham Veterans Affairs Medical Center,
Durham, North Carolina

Running head: Accelerated gastric emptying in diabetes

Correspondence:
Toku Takahashi, MD, PhD
Zablocki VA Medical Center
5000 West National Avenue
Milwaukee, WI 53295
Phone: 414-384-2000 (ext 41472)
Fax: 414-382-5374
E-mail: ttakahashi@mcw.edu
ABSTRACT

During the early stages of diabetes, gastric emptying is often accelerated, rather than delayed. The mechanism of accelerated gastric emptying in diabetes has not been fully studied. A recent study showed that plasma ghrelin levels were elevated in diabetes. As postprandial antro-pyloric coordination plays an important role in mediating solid gastric emptying, we hypothesize that the elevated plasma ghrelin levels increase postprandial antro-pyloric coordination to accelerate emptying in the early stages of diabetes. To test this hypothesis, rats were made diabetic by streptozotocin (STZ; 50 mg/kg)-injection and two weeks later pre-and post-prandial plasma ghrelin levels, antro-pyloric coordination and solid gastric emptying were determined. In control rats, plasma ghrelin levels were immediately reduced after feeding. In contrast, plasma ghrelin levels remained within the fasted levels in STZ rats after feeding. In STZ rats, gastric emptying was significantly accelerated (77.4 ± 3.2%, n=6), compared to that of control rats (58.8 ± 2.5%, n=6, p<0.05). Treatments with anti-ghrelin antibodies attenuated accelerated gastric emptying in STZ rats (50.1 ± 3.5 %, n=6, p<0.05), while having little effect in vehicle-control rats. The incidence of postprandial antro-pyloric coordination was significantly increased in STZ rats, compared to that of control rats (p<0.05). Treatments with anti-ghrelin antibodies suppressed this enhanced antro-pyloric coordination in STZ rats. Our study suggests that elevated endogenous ghrelin enhances antro-pyloric coordination, which accelerates gastric emptying in the early stages of diabetes.
INTRODUCTION

It is well recognized that certain populations of diabetic patients have delayed gastric emptying or gastroparesis (5, 36). Diabetic gastroparesis is explained by the autonomic neuropathy associated with diabetes. However, rapid gastric emptying can be seen in subgroups of patients in the early stages of type 2 diabetes (3), non-insulin-dependent diabetes mellitus (NIDDM; type 2 diabetes) (47), and neuropathy-free insulin-dependent diabetes mellitus (IDDM; type 1 diabetes) (28, 45). It has also been reported that gastric emptying is accelerated in the early stages of STZ-induced diabetes in rats (19, 46) and in spontaneously diabetic BB rats (46, 56).

Gastric emptying of solids and liquids is regulated by different mechanisms. The emptying of liquids from the stomach is thought to be primarily a function of the pressure gradient between the stomach and the duodenum (41).

On the other hand, solid gastric emptying is regulated by the coordinated motor activity of the antrum, pylorus and duodenum in humans (4, 41) and dogs (13, 31). Similarly, we have previously demonstrated that the coordinated motor pattern between the antrum and pylorus (antro-pyloric coordination) plays an important role in mediating solid gastric emptying in rats (22, 23, 44) as...
well as dogs (55).

In rats, both the antrum and pylorus randomly contract up to 10 min after feeding and this period seems to reflect the grinding process of the gastric content. Forty min after feeding, contractile patterns become significantly altered to coordinated patterns with low frequency (<3 cycles/min) and high amplitude between the antrum and pylorus. The contraction of the pylorus occurred usually 2-6 sec after the contraction of the antrum. The incidence of antro-pyloric coordination was significantly increased at 40-80 min after the feeding (22, 23, 44). The coordination between the antrum and pylorus is an important factor in the emptying of solid foods (22, 23, 44).

Ghrelin, an orexigenic peptide, was discovered as the endogenous ligand for the growth hormone secretagoge receptor (GHS-R) from the rat stomach (30). Ghrelin is produced by X/A-like cells in the stomach (7, 11) and released into circulation after posttranslational processing where n-octanoyl acid becomes attached at serine in position 3 (2), which is essential for its major bioactivity.

It is well established that ghrelin stimulates gastrointestinal motility (16, 29, 38, 51). Ghrelin
accelerates solid gastric emptying in humans (34), rats (17) and mice (8). Gastric emptying of non-nutrient liquid is also accelerated by ghrelin in rats (17, 33, 52) and mice (29).

Circulating levels of ghrelin rise before and decrease after a meal in normal-weight subjects. In contrast, the postprandial reduction of ghrelin is not obvious in obese subjects (32) and the patients with new onset childhood type 1 diabetes (20). In STZ-induced diabetic rats, postprandial ghrelin concentrations are higher than in control rats (24) and return to fasted levels rapidly after feeding (18). Gastric ghrelin-immunoreactive cells are slightly decreased in STZ-induced diabetic rats, while preproghrelin mRNA levels are extremely higher in STZ rats, than in control rats (37). The increased plasma ghrelin levels and the decreased gastric ghrelin cells in the diabetic rats (37) may be due to an increase in ghrelin release from the stomach into the bloodstream.

Based on these evidences, we hypothesize that the alteration of postprandial antro-pyloric coordination induced by elevated plasma ghrelin contributes to rapid gastric emptying in the early stages of diabetes. As it has been shown that diabetic autonomic neuropathy develops 6-8 weeks after the STZ-administration (39, 49), we utilized rats who received STZ-injection 2 weeks prior to
The present study examines whether (1) solid gastric emptying is accelerated, (2) postprandial antro-pyloric coordination is enhanced and (3) if postprandial ghrelin levels are higher in STZ-induced diabetic rats. Anti-ghrelin antibodies were used to determine the role of endogenous ghrelin in mediating accelerated gastric emptying in STZ-induced diabetic rats.

MATERIAL AND METHODS

Animals

Protocols describing the use of rats were approved by the Institutional Animal Care and Use Committee of Duke University and carried out in accordance with the National Institute of Health "Guide for the Care and Use of Laboratory Animals". All efforts were made to minimize animal suffering and to reduce the number of animal in experiments.

Male Sprague-Dawley rats weighing 260-290 g were housed in-group cages with daily illumination from 07:00 to 19:00 (12:12-h light-dark cycle), controlled temperature (22-24 °C) and humidity (30–35%) at least seven days before experiments and given free access to laboratory chow and water ad libitum. Before experiments, rats were fasted for 24 hours but given free access
Induction of diabetes

Rats received intraperitoneal injection of STZ (50 mg/kg) in 0.2 ml of 10 mM citrate buffer (pH 5.5). Control rats received intraperitoneal injection of citrate buffer. All animals were given access to water and suitable rat chow ad libitum. Diabetic rats showed a non fasting glucose concentration of more than 200 mg/dl in tail vein blood. Body weight was monitored daily in both groups.

Measurement of Blood Glucose and Plasma Ghrelin and Insulin before and after feeding

Ten days after STZ injection, intravenous catheter was inserted into the right jugular vein and exteriorized to the back. The catheter was filled with heparinized saline (100 unit/ml).

Four days after surgery, blood sampling was started from 120 min before the feeding. Blood (0.3 ml) was withdrawn from the jugular catheter every 60 min before feeding and every 30 min after feeding (-120, -60, 0, 30, 60, 90, and 120 min). The same volume of saline was injected after each sampling procedure to avoid dehydration. After the measurement of blood glucose level, the remaining blood was collected in tubes containing EDTA and aprotinin (500 kIU/ml) and
immediately centrifuged at 4 °C. As previously reported (21), plasma was aliquoted and added 1.0 N HCl (10% of sample volume) then stored at –80°C. Plasma level of acyl ghrelin was measured by radioimmunoassay (RIA) using a RIA kit (LINCO Research, St Charles, MO). This kit is designed to measure the active form of ghrelin (acyl ghrelin) and does not cross react with des-acyl ghrelin. Intra- and inter-assay coefficient variances were less than 10%. Plasma levels of insulin were also measured by RIA.

Measurement of solid gastric emptying

Two weeks after STZ-injection, rats were fasted for 24 hrs and received 1.5 g of solid caloric meal (PMI Feeds, Richmond, IN; 3.04 kcal/g, protein 23.4%, fat 4.5%, fiber, 5.3%), as previously described (23). Thirty minutes before the solid meal ingestion, a specific anti-ghrelin antibody (rabbit anti-Ghrelin (rats/mice) IgG, designed for acyl ghrelin, 10 µg/kg; 0.5 ml) or non-immuno specific antibody (0.5 ml) as an isotype control were injected intraperitoneally. It has been shown that anti-ghrelin antibody (4 µg/rat) significantly inhibited food intake (50), suggesting that endogenous orexigenic action of ghrelin is blocked by ghrelin antibody (4 µg/rat). Ninety minutes after feeding, rats were euthanized by intraperitoneal injection of pentobarbital sodium (250
mg/kg). During the experiment of gastric emptying for 90 min, water was not given.

The stomach was surgically isolated and removed. The gastric content was recovered from the stomach, dried, and weighed. Solid gastric emptying was calculated according to the following formula, as previously described (23).

\[
\text{Gastric emptying (\%) = } [1 - \frac{\text{dried weight of food recovered from stomach}}{\text{weight of food intake}}] \times 100.
\]

Recording of postprandial antro-pyloric coordination

Different rats were used to investigate antro-pyloric coordination. One week after STZ or vehicle-injection, rats were anesthetized with pentobarbital sodium (45 mg/kg, IP). Through a midline laparotomy, two strain gauge transducers were implanted on the serosal surface of the gastric antrum and the pylorus to monitor the contractions of circular muscle, as previously described (22, 23, 44).

The wires from transducers were exteriorized through abdominal wall, placed under the skin and ran up towards the back. Exposed wires were shielded in a protective jacket (Star Medical, Tokyo, Japan). After the surgery, rats were housed individually with standard diets and tap water.
Rats were allowed to recover for one week before motility recording studies.

After a 24 hr-fasting, the wires from transducers were connected to a recording system (Power-Lab model 8SP, ADI instruments, Colorado Springs, CO) and contractions of the antrum and pylorus were monitored for 120 min in conscious and freely moving rats. Thirty minutes after administration of a specific anti-ghrelin antibody (10 µg/kg; 0.5 ml, IP) or non-immuno specific antibody (10 µg/kg, 0.5 ml, IP), rats were given a preweighed solid meal (1.5 g). Postprandial gastric motility was recorded continuously for 120 min.

*Evaluation of antro-pyloric coordination*

As previously described (22, 23, 44), antro-pyloric coordination was defined as a single contraction at the antrum that propagated aborally in the pylorus within 10 sec and was followed by quiescence period (more than 20 sec). Individual contractions were defined as > 2 g change of > 1 sec duration.

To evaluate the change of antro-pyloric coordination in each group, the number of incidences of antro-pyloric coordination was calculated every 20 min after the feeding for up to 120 min, as previously reported (22, 23).
Materials

STZ was purchased from Sigma-Aldrich (St Louis, MO). Anti-ghrelin antibody was purchased from Phoenix Pharmaceuticals (Belmont, CA). Non-immune isotype control antibody was purchased from Phoenix Pharmaceuticals (Belmont, CA). RIA kit was purchased from LINCO Research (St Charles, MO).

Statistical Analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Tukey test or by Mann-Whitney U test, depending on the dataset concern, by using StatMate III (ATMS Co. Ltd., Tokyo, Japan). P values <0.05 were considered as significant. All results were expressed as means ± SE.

RESULTS

Body weight changes in control and STZ rats

Figure 1 shows the changes in body weight, 2 weeks after STZ or vehicle injection. Diabetic rats
gradually gained weight during the 2 weeks after STZ (50 mg/kg) injection. The body weight increment in STZ-injected rats was significantly less than the weight gain in control rats over the same time period (Fig. 1).

**Blood glucose levels before and after feeding in control and STZ rats**

Blood glucose levels were measured before and after feeding in control and STZ rats. The blood glucose levels in STZ rats at 60 min before feeding were 123.4 ± 19.2 mg/dl (n=5), which was significantly higher than the levels in control rats (72.4 ± 2.6 mg/dl, p<0.05, n=5) (Fig. 2A).

Blood glucose levels increased immediately after feeding in STZ rats. Blood glucose levels at 30 min after feeding in STZ rats were 235.6 ± 23.3 mg/dl.

In STZ rats, blood glucose levels reached a maximum at 60 min after feeding (257.0 ± 30.8 mg/dl), which was significantly higher than glucose levels in control rats (118.2 ± 5.9 mg/dl, p<0.01) (Fig. 2A).

**Plasma Insulin levels before and after feeding in control and STZ rats**

Before feeding, plasma insulin levels were 0.45 ± 0.04 pg/ml (n=6) in control rats. Sixty min
after feeding, plasma insulin levels significantly increased to $1.43 \pm 0.15$ pg/ml ($n=6$) in control rats.

Two weeks after STZ injection, pre-prandial insulin levels were $0.31 \pm 0.02$ pg/ml, which were not significantly different from the levels in control rats. However, post-prandial plasma insulin levels in STZ rats ($0.38 \pm 0.03$ pg/ml, $n=6$) were significantly decreased from the levels in control rats ($P<0.01$).

**Plasma ghrelin levels before and after feeding in control and STZ rats**

In a fasting state, plasma ghrelin levels were not significantly different between control and STZ rats (Fig. 2B).

In control rats, plasma ghrelin levels were significantly reduced from $220.7 \pm 42.1$ pg/ml to $99.0 \pm 21.6$ pg/ml ($n=5$) 30 min after feeding. Plasma ghrelin levels were further reduced to $54.1 \pm 11.2$ pg/ml ($n=5$) 90 min after feeding.

In contrast, plasma ghrelin level remained within the levels of fasted animals even after feeding STZ rats. Postprandial plasma ghrelin levels were $268.4 \pm 68.3$ pg/ml at 30 min after
feeding and 207.9 ± 37.3 pg/ml at 120 after feeding. Both were significantly higher than the levels in control rats (n=5, p<0.05) (Fig. 2B).

_Solid gastric emptying in control and STZ rats_

All rats consumed the entire 1.5g of solid meal within 8-10 min (8.7 ± 0.5 min, n=24). In control rats, solid gastric emptying at 90 minutes after feeding was 58.8 ± 2.5% (n=6). Gastric emptying was significantly accelerated to 77.4 ± 3.2%, p<0.05, n=6) in STZ rats, compared to that in control rats (Fig. 3).

The treatment with the anti-ghrelin antibody did not affect gastric emptying in control rats. In contrast, accelerated gastric emptying was not observed in STZ rats that received anti-ghrelin antibody (n=6, p<0.01) (Fig. 3).

_Antro-pyloric coordination in control and STZ rats_

Antro-pyloric coordination was not obvious within 40 min after feeding in control rats. The number of antro-pyloric coordination events was 1.25 ± 0.25 times/20 min within 40 minutes after feeding in control rats (Fig. 4A and Fig. 5).
Forty min after feeding, antro-pyloric coordination was frequently observed and reached to its peak (8.25 ± 0.85 times/20 min) 60-80 min after feeding in control rats. Eighty min after feeding, the number of antro-pyloric coordination events gradually decreased (Fig. 5).

In contrast, antro-pyloric coordination events were frequently observed even 0-20 min after feeding in STZ rats (4.25 ± 0.63 times/20 min, p<0.05 vs control rats (Fig. 4B and Fig. 5). The number of antro-pyloric coordination events reached a peak at 20-40 min after feeding (11.50 ± 3.52 times/20 min). The number of antro-pyloric coordination events in STZ rats was significantly higher than in control rats at 20-40, 80-100 and 10-120 min after feeding (Fig. 5).

Treatment with anti-ghrelin antibody did not affect the number of antro-pyloric coordination events in control rats (data not shown). However, the early manifestation of antro-pyloric coordination within 40 min was not observed after treatment with anti-ghrelin antibodies in STZ rats (Fig. 4C).

Anti-ghrelin antibody treatment also significantly reduced the number of antro-pyloric coordination events at 40-60, 80-100 and 10-120 min after feeding in STZ rats (Fig. 5).
DISCUSSION

It is widely accepted that gastric emptying is delayed in the late stages of diabetes (5, 36). However, rapid gastric emptying has been reported in other subgroups of patients (3, 28, 45, 47) and diabetic rats (19, 46, 56). Although a great deal of attention has been paid to diabetic gastroparesis, the mechanism of accelerated gastric emptying in the early stages of diabetes has not been fully studied.

In rats (53) and healthy subjects (6, 54), circulating plasma ghrelin levels decrease immediately after ingestion of a meal. In contrast, postprandial reduction of plasma ghrelin levels is impaired in obese subjects (14, 32) and patients with new onset of type 1 diabetes (20). It has been shown that the postprandial ghrelin levels remain high (24) or quickly return to the fasting levels (18) in STZ-induced diabetic rats.

Our present study demonstrates that in control rats plasma ghrelin levels decrease immediately from 221 to 99 pg/ml within 30 min after feeding and plateau within 60 min at a slightly lower level of 68 pg/ml. In contrast, postprandial reductions in plasma ghrelin levels were significantly impaired in STZ rats. The postprandial ghrelin levels remained high even at 120
min after feeding (208 pg/ml) in STZ rats. These results are consistent with the previous reports in diabetic patients (43).

Solid gastric emptying is enhanced by exogenously applied ghrelin in humans (34), rats (17) and mice (8). Ghrelin also can accelerate gastric emptying of non-nutrient liquids in rats (17, 33, 52) and mice (12).

We have recently demonstrated that enhanced manifestation of antro-pyloric coordination plays an important role in ghrelin-induced acceleration of solid gastric emptying (1). Intraperitoneal-administration of ghrelin (4-8 µg/kg) accelerated gastric emptying. The number of antro-pyloric coordination events, which was not obvious within 20–40 min after feeding in saline-injected rats, were significantly increased in ghrelin-injected rats (1).

The number of antro-pyloric coordination events was not increased within 40 min after feeding in control rats. In STZ rats, however, the incidence of antro-pyloric coordination was frequently observed within 20 min after feeding and reached a peak at 20 to 40 min after feeding. To verify that elevated ghrelin is involved in mediating accelerated gastric emptying and enhanced
antro-pyloric coordination, STZ rats received anti-ghrelin antibodies 30 min before feeding.

Since available ghrelin receptor antagonists are limited and (D-lys3)GHRP-6 (a ghrelin receptor antagonist) has been shown to interact with a 5-HT$_{2B}$ receptor (9), we used anti-ghrelin antibody in our current study. We showed that administration of anti-ghrelin antibodies significantly reduced the number of antro-pyloric coordination events and accelerated gastric emptying in STZ rats. This strongly suggests that elevated plasma ghrelin promotes antro-pyloric coordination and accelerates solid gastric emptying in STZ-induced diabetic rats. In contrast, anti-ghrelin antibodies did not affect solid gastric emptying in normal rats. This suggests that endogenous ghrelin levels may have a minor role in mediating solid gastric emptying under normal conditions.

As shown in Fig. 1, body weight in STZ rats was significantly less than in control rats. This may raise the possibility that the observed differences in gastric emptying and antro-pyloric coordinations between controls and STZ rats are secondary to different body weight.

However, our preliminary study showed that there were no significant differences of gastric emptying between the rats of 250 g (59.6±6.7%, n=6) and 300 g (61.3±6.5%, n=6).
As it has been demonstrated that both fat weight and plasma leptin levels were reduced in STZ rats (24), we cannot exclude the possibility that reduced levels of leptin may affect plasma ghrelin levels.

The mechanism of elevated ghrelin levels in diabetes remains to be investigated. Ghrelin increases adiposity and mediates energy balance (53). Negative energy balance in diabetic rats might induce a compensatory signal to up-regulate ghrelin mRNA expression and to increase ghrelin generation and secretion in the stomach (37).

Glucose and/or insulin have been suggested as important elements in the mechanisms regulating plasma ghrelin levels. Hyperinsulinemic-euglycemic clamp studies demonstrated that insulin suppresses endogenous ghrelin release in humans (15, 48) and rats (40). This suggests that insulin, rather than glucose, is essential for the suppression of ghrelin levels.

Postprandial ghrelin remains increased in type 1 diabetes when the patients don’t receive insulin therapy (43). Ex-vivo studies, using isolated perfused rat stomach, showed that ghrelin secretion from the stomach was suppressed by insulin infusion (27, 35), indicating that insulin regulates circulating ghrelin levels. STZ destroys beta cells of the pancreas and reduces insulin
secretion (25). Plasma insulin concentration is significantly reduced 2 weeks after STZ injection, compared to that of vehicle–injected rats (24). Ghrelin is also known to inhibit glucose-induced insulin release (10). It is conceivable that the hyper-ghrelinemia observed in STZ rats may further reduce insulin secretion.

It is unclear whether high plasma levels of ghrelin and accelerated gastric emptying observed in the early stages of diabetes are due to hypoinsulinemia. Nowak et al. previously showed that delayed gastric emptying in STZ-induced diabetic rats was improved by insulin treatments (46). Further studies are required to determine if insulin replacement in STZ rats is sufficient to reverse accelerated gastric emptying, increase antropyloric activity and elevate plasma ghrelin levels.

It has been shown that fasting and postprandial blood glucose levels were 178 mg/dl and 314 mg/dl, respectively, when 65 mg/kg of STZ was administered in rats (42). Similarly, others demonstrated that fasting blood glucose levels were 210 mg/dl after STZ (65 mg/kg) injection (26). In our study, we used STZ at 50 mg/kg in order to induce a mild form of diabetes. As shown in Fig. 2, the fasting and postprandial blood glucose levels were 112 mg/dl and 257 mg/dl, respectively, in rats treated with STZ (50 mg/kg). Body weight was not significantly increased 2 weeks after STZ (65 mg/kg)
injection (24, 26). In contrast, when STZ was applied at a dosage of 50 mg/kg, rats could gain their body weight (40 g) 2 weeks after STZ injection in our experiment (Fig 1).

As far as we know, this is the first study to demonstrate that accelerated gastric emptying occurs in an early stage of diabetes in rats. Use of this animal model may contribute to a better understanding of the mechanism by which diabetic hyperphagia, which is frequently observed in patients in the early stages of diabetes, contributes to accelerated gastric emptying.

In the early stages of diabetes, accelerated solid gastric emptying could make it easier to overeat, which is well recognized for exacerbating diabetes. If we could successfully control the hyperphagia and accelerated gastric emptying in the early stages of diabetes, this could possibly prevent and/or delay the development of gastroparesis and/or neuropathy, two complications that develop later in diabetes.

**Acknowledgement:**

This study was supported in part by VA Merit Review (T.T.)
References


10. Dezaki K, Hosoda H, Kakei M, Hashiguchi S, Watanabe M, Kangawa K, and Yada T. Endogenous ghrelin in pancreatic islets restricts insulin release by attenuating Ca2+...


39. Mattingly GE and Fischer VW. Peripheral neuropathy following prolonged exposure to
40. McCowen KC, Maykel JA, Bistrian BR, and Ling PR. Circulating ghrelin concentrations are lowered by intravenous glucose or hyperinsulinemic euglycemic conditions in rodents. *J Endocrinol* 175: R7-11, 2002.


50. Solomon A, De Fanti BA, and Martinez JA. Peripheral ghrelin participates in the glucostatic signaling mediated by the ventromedial and lateral hypothalamus neurons.


56. Young AA, Gedulin B, Vine W, Percy A, and Rink TJ. Gastric emptying is accelerated
Figure Legend

Figure 1 Daily body weight in control and STZ rats. Control rats increased their body weight from 265.0 ± 1.8 to 342.5 ± 2.8 g (n=5) 2 weeks after vehicle-injection. STZ rats gained their weight from 265.8 ± 7.0 to 305.8 ± 7.2 g (n=5). The increment was significantly lower in STZ rats, compared to that of control rats (* p<0.05 vs control rats). An arrow indicates STZ or vehicle injection.

Figure 2 Plasma glucose (A) and ghrelin (B) levels and before and after feeding in control and STZ rats. Blood glucose level 60 min before feeding was 123.4 ± 19.2 mg/dl (n=5) in STZ rats, which was significantly higher than that of control rats (72.4 ± 2.6 mg/dl, p<0.05, n=5). The blood glucose level increased immediately after feeding in STZ rats. Blood glucose level at 30 min after feeding was 235.6 ± 23.3 mg/dl in STZ rats.

Plasma ghrelin level just before feeding was not significantly different between control and STZ rats (220.7 ± 42.1 and 282.1 ± 32.1 pg/ml, respectively). Plasma ghrelin level decreased immediately after feeding in control rats. However, there was no significant decrease of plasma ghrelin levels observed after feeding in STZ rats (* p<0.05, ** p<0.01 vs control rats, † p<0.05, †† p<0.01 vs before feeding). Arrows indicate feeding.

Figure 3 Solid gastric emptying in control and STZ rats. Gastric emptying was significantly accelerated in STZ rats (77.4 ± 3.2 %, n=6), compared to that of control rats (58.8 ± 2.5 %,
n=6, p<0.05). The accelerated gastric emptying was abolished by anti-ghrelin antibody (Ghr-Ab) administration (* p<0.05, ** p<0.01).

**Figure 4** Postprandial contractions of the antrum and pylorus in control rats (A), vehicle-treated STZ rats (B) and ghrelin antibody-treated STZ rats (C). Phasic contractions were observed immediately after feeding in the antrum and pylorus, which were not synchronized to each other. Forty min after feeding, antro-pyloric coordination was frequently observed and reached to its peak 60-80 min after feeding in control rats. Eighty min after feeding, the incidence of antro-pyloric coordination was gradually reduced (A).

The initial “un-synchronized” motor pattern was observed in a very short period after feeding in STZ rats. Antro-pyloric coordination was frequently observed even 0-20 min after feeding in STZ rats (B). In contrast, the early manifestation of antro-pyloric coordination within 40 min was no more observed after the treatment with an anti-ghrelin antibody in STZ rats (C). A magnified view demonstrated the incidence of antro-pyloric coordination (showed by asterisks). An arrow indicates feeding.

**Figure 5** The number of incidence of antro-pyloric coordination in control rats, vehicle-treated STZ rats and anti-ghrelin antibody-treated STZ rats. In control rats, the incidence of antro-pyloric coordination was increased 40 minutes after feeding and reached to its peak at 60 to 80 minutes (8.25 ± 0.85 times/20 min).

In STZ rats, the incidence of antro-pyloric coordination was frequently observed within 20 minutes after feeding (4.25 ± 0.63 times/20 min) and reached to its peak at 20 to 40
minutes (11.50 ± 3.52 times/20 min). In STZ rats who received anti-ghrelin antibody, the early manifestation of antro-pyloric coordination within 40 minutes was significantly reduced. The anti-ghrelin antibody also significantly reduced the incidence of antro-pyloric coordination 40-80 min after feeding in STZ rats (* p<0.05 vs control rats, † p<0.05 vs STZ + vehicle rats).
Figure 2

(A) Glucose (mg/dl) levels over time for Control and STZ groups.

(B) Ghrelin (pg/ml) levels over time for Control and STZ groups.
Figure 3
Figure 4