Vagal Nerve Regulation Is Essential for the Increase in Gastric Motility in Response to Mild Exercise

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It has been shown that mild to moderate exercise can accelerate gastric emptying in humans. However, understanding of the underlying mechanism is hampered by the lack of appropriate animal models. To investigate the effects of mild exercise on gastric motility, we developed an animal model, in which strain gauge transducers were surgically planted on the antral surfaces of female Sprague–Dawley rats. We continuously recorded the contractions of gastric circular muscle in unrestrained conscious rats, divided into four groups: sham-operated exercise, sham-operated sedentary, vagotomized exercise, and vagotomized sedentary. The rats were trained for 3 weeks, and gastric motility was monitored before and after exercise. Although exercise accelerates gastric antral contraction in sham-operated rats, this effect was absent in the vagotomized exercise group, indicating the involvement of the vagal nerve in the exercise-mediated increase in gastric motility. Among the four groups, daily food intake was highest in the sham-operated exercise group. In contrast, the vagotomized exercise group exhibited the smallest body weight gain. Severe gastric retention was observed in vagotomized rats, suggesting a role of the vagal nerve in facilitating food movement and digestion in the stomach. Moreover, at the end of the 3-week exercise, there were no differences in plasma levels of growth hormone, peptide YY, and ghrelin among the four groups. These results indicate that in response to a mild physical exercise challenge, the vagal nerve stimulates gastric motility and enhances the ability of the stomach to process food. Our findings highlight the significance of neuronal control of stomach function.

Keywords: exercise; gastric motility; vagal nerve; rat; gut hormone

as an orexigenic factor (Inui et al. 2004; Schmidt et al. 2007). Ghrelin stimulates short-term food intake, promotes gastric emptying, and regulates body weight. In patients with anorexia or post-bariatric surgery, profound changes in plasma ghrelin levels were found, suggesting that it is an important hormone in the regulation of body weight (Gaddipati et al. 2006; Jürimäe et al. 2007). Studies on the effects of acute or chronic exercise showed that high-intensity exercise could lower plasma ghrelin levels in rats (Ghanbari-Niaki et al. 2008; Wang et al. 2008; Ghanbari-Niaki et al. 2009). Nevertheless, the effects of mild to moderate exercise on plasma ghrelin levels in animals remain unknown.

Growth hormone (GH), a pleiotropic polypeptide hormone secreted by the pituitary gland, plays important physiological roles in regulating body growth and cell proliferation in mammals. Exercise has long been proposed as a potent physiological stimulator for GH secretion. However, its effects on rat GH secretion remain controversial. Using a treadmill-based exercise protocol (24 m/min, 30 min/day, 5 days/week, for 4 weeks), Butkus et al. were unable to find significant changes in plasma GH levels in female rats (Butkus et al. 1995).

Satiety hormone peptide YY (PYY) is another hormone that has been suggested to respond to exercise. There are two main circulating forms of PYY, PYY1-36 and PYY3-36; both have been shown to reduce food intake when administered peripherally. A recent study showed that long-term exercise training in overweight adolescents increases plasma PYY (Jones et al. 2009).

The aim of this study was to determine whether gastric motility would be affected by mild treadmill exercise in vagotomized and sham-operated rats, and to investigate the long-term effects of mild exercise on gastric retention and plasma concentration of ghrelin, GH, and PYY in a rat model.

### Materials and Methods

#### Animals

Twenty-six female Sprague–Dawley rats (CLEA Japan, Inc., Tokyo, Japan), 11-12 weeks old, weighing 210-230 g, which had free access to standard laboratory chow (CE-2; CLEA) and water, were housed individually in plastic cages under conditions of controlled room temperature (22-24°C) and humidity in a 12-h light/12-h dark cycle (0800–2000 h light). The rats were allowed to adapt to the environment for at least 5 days before surgery, and food intake and body weight were monitored during the experiment. All procedures conformed to the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Tohoku University School of Medicine Animal Use and Care Committee.

#### Surgery

Rats were divided into four groups: the sham-operated exercise group \((n = 8)\); the sham-operated sedentary group \((n = 5)\); the vagotomized exercise group \((n = 8)\); and the vagotomized sedentary group \((n = 5)\). Rats were fasted overnight and anesthetized with intraperitoneal injection of 40 mg/kg sodium pentobarbital (Abbot Laboratories, Chicago, IL). A midline incision was made to provide wide exposure of the upper abdominal organs. After the bilateral subdiaphragmatic trunks of the vagal nerve along the esophagus were exposed, the trunks were dissected from the esophagus. Each branch of the nerve was tied with surgical suture at 2 points separated by approximately 0.5 cm, and then cauterized between the sutures. After subdiaphragmatic vagotomy, to prevent excessive occlusion of the pyloric sphincter, surgical pyloroplasty was performed as follows. An incision was made parallel to the axis of the pylorus, through the pyloric sphincter, and the pylorus wall was reconstructed by silk sutures perpendicular to the pylorus axis. After vagotomy and pyloroplasty, a strain gauge force transducer with a telemetry recording system (Star Medical, Tokyo, Japan) was implanted onto the serosal surface of the antrum or duodenum for recording the circular muscle contractions, and the telemetry wire was fixed in the corner of the peritoneal cavity (Fig. 1A). The stomach was then returned to its normal position. Muscle and skin layers were closed using silk suture. Since pyloroplasty was performed in vagotomized rats, we were unable to record pylorus motility directly. Instead, duodenal motility was recorded.

In the sham operations, the bilateral trunks were only exposed. Pyloroplasty was not performed, and the strain gauge force transducer was implanted onto the serosal surface of the antrum or pylorus. Since pylorus is physically connected to antrum, we wanted to examine whether pylorus movements are regulated cooperatively with the antrum after exercise.

#### Treadmill exercise protocol

Rats were placed in individual cages after surgery. After a 3-week recovery period, rats in the exercise groups were put on a motor-driven treadmill (MK-680, Muromachi, Japan) and walked at a very low speed (8 m/min) 30 min a day for 2 days to adapt to the training environment. Subsequently, the exercise groups were given training according to the following schedule: Three min of warm-up starting at 6 m/min was followed by progressive increases of 3 m/min until 15 m/min, and then run at 15 m/min for 24 min, followed by progressive decreases of 5 m/min until stopped for cool-down. The treadmill slope was always set at 0%.

Mild electrical shock was used at the beginning of each training session and was then turned off to avoid undue stress. Exercise was performed 30 min/day, 5 days/week for 3 weeks. Sedentary groups were exposed to the same environmental conditions during the period that the experimental groups were performing daily exercise sessions.

#### Monitoring of gastric motility

Gastric motility was monitored by calculating the motility index (MI). It is technically impossible to record gastric contractions during exercise because the strong magnetic fields generated by the treadmill motor interfere with the wireless telemetry recording system. During the measurement, the rats were housed in individual cages in unrestrained conditions, and the cage was placed on the receiver (Star Medical). Gastric motility was continuously recorded through wireless communication between the transducers and a personal computer installed with recording and analyzing software (Eight Star version 6; Star Medical) (Fig. 1A).

Quantification of gastric motility was studied by calculating the motility index (MI). MI is equivalent to the area under the curve of the motility recording. MI was calculated from the data immediately before and after treadmill exercise for 15 min each.
Measurement of gastric retention

After the last session of treadmill exercise for 3 weeks, rats were fasted for 24 h.

The rats were anesthetized with intraperitoneal injection of 40 mg/kg sodium pentobarbital, and the stomach was surgically isolated and removed. The gastric content was collected from the stomach, dried, and weighed.

Gastric emptying tests were not performed in the present study. In the pilot study, we found that even after 24-h fasting, a large amount of food remained in the stomachs of vagotomized rats. We therefore decided to test the gastric retention after a 24-h fast instead of performing the gastric emptying test.

Blood collection and hormone assays

Blood samples were drawn from the anesthetized rats via a cardiac puncture. The blood was collected in tubes containing EDTA and aprotinin (500 kIU/ml). After centrifugation, plasma was aliquoted, and for ghrelin, 1.0 N HCl (10% of sample volume) was added. The plasma fraction was stored at −80°C until assayed.

The plasma level of active ghrelin was measured using an ELISA kit (Mitsubishi Chemical Medience Corporation, Tokyo, Japan). The plasma level of GH was measured using another ELISA kit (Millipore, St. Charles, MO), and the plasma level of PYY was measured using an EIA kit (Yanaihira, Japan). For each hormone, all samples were assayed in the same batch to avoid inter-assay variability. The intra-assay variations were <10%.

Data analysis

The results are presented as means ± SEM. Data were analyzed using SPSS 15.0 J software (SPSS Inc). Comparisons of MI before and after treadmill exercise were tested by repeated measures of analysis of variance. Other comparisons in the 4 groups were initially tested by analysis of variance; significant differences among the 4 groups were then compared using Tukey’s test with a significance level of \( p < 0.05 \).

Results

Effects of mild treadmill exercise on gastroduodenal motility

After adaptation to the treadmill exercise for 1 week, we recorded gastroduodenal motility in the 2 exercise groups before and after treadmill exercise for both fasting and fed state rats between 5:00 pm and 8:00 pm on three different days.

Rats exhibited two different gastric motility patterns in both fasting and fed states. Cyclic occurrence of intense contractions was observed in the fasting state, whereas regular phasic contractions were observed in the fed state.

In the sham-operated group, gastric antral motility enhanced after treadmill exercise regardless of feeding state. Such enhancement normally lasted about 30 min to 1 h after the treadmill was completely stopped (Fig. 2A). No obvious change was observed in pyloric motility (Fig. 2B). In the fed state, antral MI significantly increased from 20.6 ±
7.3 g·min (before exercise) to 42.3 ± 12.5 g·min (after exercise) ($p < 0.05$, Fig. 4). However, no significant difference was observed in pyloric MI (26.43 ± 1.9 g·min vs. 27.6 ± 2.2 g·min, Fig. 4).

In the vagotomized group, in both fasting and fed states, gastric antral and duodenal motilities remained unchanged after treadmill exercise (Fig. 3). In the fed state, antral MI was 24.1 ± 4.4 g·min before exercise, and to 25.0 ± 4.7 g·min after exercise (Fig. 4). In the same state, duodenal MI was 10.1 ± 1.6 g·min before exercise and 9.8 ± 1.4 g·min after exercise (Fig. 4).

**Effects of mild treadmill exercise on body weight and daily food intake**

Significantly lower body weight gain was observed in the vagotomized exercise group during the training period (11.0 ± 3.6 g) compared to the sham-operated exercise (32.9 ± 3.6 g), sham-operated sedentary (24.1 ± 4.6 g), or vagotomized sedentary groups (24.8 ± 4.1 g) ($p < 0.05$ for each comparison, Fig. 5A). In the final week of the experiment, daily food intake was significantly higher in the sham-operated exercise group (18 ± 0.9 g) compared to the sham-operated sedentary (14.8 ± 1.1 g), vagotomized exercise (13.6 ± 1.0 g), or vagotomized sedentary groups (14.1 ± 1.2 g) ($p < 0.05$ for each comparison, Fig. 5B).

**Effects of mild treadmill exercise on gastric retention**

After 3 weeks of mild treadmill exercise, severe gastric retention was observed in the vagotomized exercise group (Fig. 5C, Fig. 6). The dry weight of gastric content after 24-h fasting was 0.65 ± 0.1 g in the vagotomized exercise group, which was significantly higher than that of the vagotomized sedentary (0.29 ± 0.1 g), sham-operated exercise (0.15 ± 0.1 g), and sham-operated sedentary groups (0.04 ± 0.1 g) ($p < 0.05$ for each comparison, Fig. 5C).

**Effects of mild treadmill exercise on plasma ghrelin, GH, and PYY after 24-h fasting**

The plasma active ghrelin level was 29.5 ± 8.1 fmol/ml in the sham-operated exercise group and 20.9 ± 9.8 fmol/ml in the vagotomized exercise group, no significant differences were observed among the 4 groups ($p = 0.822$) (Fig. 7A). The plasma GH level was 24.5 ± 9.2 ng/ml in the sham-operated and 17.4 ± 8.1 ng/ml in the vagotomized exercise groups, no significant differences were observed among the 4 groups ($p = 0.503$) (Fig. 7B).

No significant differences were observed in plasma PYY levels among the 4 groups ($p = 0.243$) (Fig. 7C).

**Discussion**

In the present study, we successfully developed a novel
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A rodent model that enabled us to monitor gastric motility in real time under unrestrained, conscious conditions. Specifically, a wireless strain gauge force transducer was surgically implanted on the serosal surfaces of the antrum, pylorus, and duodenum. Gastric motility was continuously recorded using a telemetry recording system. We also used this system for testing gastric motility changes in the sham-operated and vagotomized rats throughout the 3-week training program.

The present study revealed that mild exercise can significantly enhance antral motility in sham-operated rats, which may explain accelerated gastric emptying and increased daily food intake observed among these rats. In the vagotomized exercise group, enhancement of gastric antral motility by mild treadmill exercise was absent, suggesting that mild treadmill exercise accelerates gastric motility through the vagal nerve. Formerly, increases in gastric emptying during mild intensity treadmill exercise was assumed to be related to increases in intragastric pressure brought about by contractile activity of the abdominal muscles (Neufer et al. 1989).

Vagotomy has been used extensively as a tool to ascertain the role of the vagal nerve in the control of gastric motility. In the days following vagotomy, a period of hypomotility is followed by periods of increased activity. In considering the mechanism of changes in gastric motility occurring after vagotomy, the role of adaptive changes in the enteric nervous system and the gastric smooth muscle has been given due consideration (Andrews and Bingham 1990). In the present study, we found that vagotomy affected antral motility, and that the sham-operated rats showed a characteristic pattern of regular phasic contractions in the fed state. We also found that 3-6 weeks after vagotomy, antral MI in the vagotomized rats was significantly lower compared to that in the sham-operated rats in the fasted state, which indicates that total recovery of gastric antral contractions does not occur in vagotomized rats. On the other hand, since rats are nocturnal animals, changes in eating behavior as well as delayed gastric emptying after vagotomy may have made MI in vagotomized rats have no significant difference to that in sham-operated rats in the test period.

Pumping action of the antrum is weakened after vagal denervation (Cowley et al. 1971; Medina 1982), and animals subjected to a total vagotomy show gastric distension and retention (Kraly et al. 1986). In the present study, a greater amount of gastric content and greater gastric distension were observed in the vagotomized exercise group than in the vagotomized sedentary group after 24-h fasting. The reason may be that when the counteracting action of the vagal nerve is abolished, exercise-mediated neurological responses such as an increase in sympathetic tone might further decrease the blood flow of the stomach during exercise (Brouns and Beckers 1993), resulting in loosening of the stomach wall and gastric distension.

Interestingly, we did observe significantly lower weight gain in the vagotomized exercise group compared to the vagotomized sedentary group, even though no significant differences in daily food intake were observed between the 2 groups. Exercise leads to higher metabolic rate, increased energy expenditure, and increased appetite. However, under vagotomized conditions, food digestion might be compromised by gastric distension and severe gastric retention. Thus, decreased nutrition intake and higher energy expenditure in rats in the vagotomized exercise group might explain their reduced weight gain.
Mild exercise might also affect the whole body and not just gastrointestinal motility. We also examined the plasma concentrations of ghrelin, GH, and PYY after the training to determine whether mild exercise and vagotomy had affected the secretion of these hormones, which might exert a secondary effect on gastric motility, body weight, and appetite of the rats. Previous studies of other groups have reported either decreased plasma ghrelin levels or little effect on plasma GH levels in female rats following intensive exercise (Butkus et al. 1995; Ghanbari-Niaki et al. 2009). In contrast, data in the present study showed that mild exercise may have little effect on the GH level but no effect on PYY and ghrelin levels. Difference in exercise protocols and intensities might explain these discrepancies. Nevertheless, these changes remained after vagotomy, suggesting that secretion of ghrelin and PYY might be partially mediated or not mediated by vagal nerves (Inui et al. 2004; Hosoda and Kangawa 2008; Yakabi et al. 2008).

Exercise intensity used in this study (treadmill exercise for 30 min at 15 m/min after running habituation) was categorized as mild exercise because it was sufficient to generate minimal running stress in rats but was still below the lactate threshold (LT). Elevated energy expenditure through mild exercise will promote glucose intake and its usage through glycolysis. Indeed, it was shown previously that rats running at this speed tend to have lower plasma glucose levels. In contrast, elevations of plasma glucose levels have been observed in rats running at supra-LT speed (Soya et al.)
Fig. 6. Gastric distension and gastric content after 24-h fasting. After moderate treadmill exercise for 3 weeks, greater gastric distension and gastric content were observed in the vagotomized exercise group.

Fig. 7. Plasma concentrations of active-ghrelin, GH, and PYY after 24-h fasting.
(A) No significant differences were observed in plasma active-ghrelin concentrations among the 4 groups.
(B) No significant differences were observed in plasma GH concentrations among the 4 groups.
(C) No significant differences were observed in plasma PYY concentrations among the 4 groups.
Both human and animal studies have shown that changes in plasma glucose levels can act as modulators of gastric motility (Chang et al. 2006). During or after mild exercise, decreased glucose levels might also stimulate vagal efferent firing and accelerate antral contractions.

What are the stimulating factors responsible for the exercise-mediated acceleration of gastric motility? As we have mentioned above, both blood glucose levels and ghrelin may be important. Previous studies showed that in response to insulin-induced hypoglycemina, gastric motility was accelerated (McCann and Stricker 1986). It remains to be determined whether lower blood glucose resulting from mild exercise has a similar effect. Ghrelin has been demonstrated to regulate interdigestive contractions in rats (Taniguchi et al. 2008). Such effect of ghrelin can be blocked by vagotomy in vivo. Interestingly, ghrelin receptors are present on both vagal afferents and nodose ganglion (Peeters 2003). Both vagal afferent and efferent pathways might play important roles in regulating gastric motility. The efferent pathway might exert direct stimuli, whereas gut hormones can regulate gastric motility through a vago-vagal reflex. Thus, ghrelin might stimulate vagal afferents directly, which activate vagal efferent cholinergic pathways subsequently.

It is believed that during exercise, sympathetic nerves are activated while parasympathetic nerve activities are suppressed, leading to faster heartbeat; these effects are reversed in recovery phase. In the present study, we were only able to record gastric contractions after exercise because the strong magnetic fields generated by the treadmill motor would interfere with the wireless telemetry recording system. Since there is a 3-minute cool-down period in each exercise session, the acceleration of gastric motility observed in our study after mild exercise could result from enhanced activities of parasympathetic nerves.

In conclusion, our results demonstrated that in response to mild physical exercise challenges, the vagal nerve stimulates gastric motility and enhances the capability of the gastrointestinal tract to process food and absorb nutrients. Our findings highlight the significance of such neuronal control of gastric function. The unrestrained, real-time gastric motility detection model developed in the present study will serve as a powerful tool to further understand the complex hormonal/neurological regulations of gastric motility.

References
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