

Effect of Acute Gastric Electrical Stimulation on the Systemic Release of Hormones and Plasma Glucose in Dogs

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Abstract This study evaluated the effect of gastric electrical stimulation (GES) with various parameters on plasma concentrations of satiety-related peptides and glucose. GES was performed in nine healthy dogs via electrodes implanted in the middle of the lesser curvature. Four sessions were performed in each animal: control, stimulation with IGS (implantable gastric stimulation for obesity, 0.3 m sec), modified IGS (2 msec), and long pulses (300 msec). Blood samples were collected at 15 and 0 min before the meal and at 15, 30, and 60 min after the meal. GES was initiated 30 min before the first blood sample and maintained throughout collection. Plasma ghrelin, leptin, insulin and glucose were measured. The total AUCs of plasma ghrelin and leptin were not significantly affected by GES. The total AUC of plasma insulin was significantly lower with IGS and long pulse parameters ($P < 0.05$). The total AUC for plasma glucose was significantly lower in sessions with long pulses and modified IGS parameters ($P < 0.05$). We conclude that acute GES is able to change the release of some satiety-related peptides.

Whether this is associated with the changed eating behavior and weight loss in obese patients needs further investigation.

Keywords Gastric electrical stimulation · Ghrelin · LeptinI · Insulin · Glucose

Background

Recently, gastric electrical stimulation (GES) has been introduced as a therapeutic option for patients with morbid obesity. This therapy is achieved by delivering currents of short pulse trains via electrodes implanted at the distal antrum along the lesser curvature to gastric smooth muscle. Animal studies have shown that electrical stimulation at the lesser curvature can affect gastric motility [1–3], reduce food intake, and consequently lead to weight loss [4]. Clinical trials have been conducted, and the preliminary results have also shown that implantable gastric stimulation (IGS) can induce satiety and significant weight loss in morbidly obese patients [5–8].

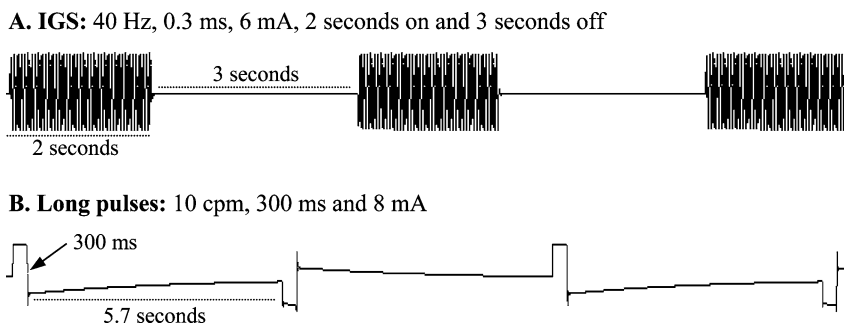
The mechanisms by which IGS benefits obese patients, however, are not fully understood. It has been postulated that IGS may facilitate weight loss by enhancing neuroendocrine satiety mechanisms. Ghrelin is one of those that can induce weight gain and adiposity by stimulating food intake and decreasing fat use or energy expenditure [9–11]. On the other hand, leptin and insulin have been considered as satiety peptides, as they tend to inhibit food intake and promote energy expenditure [12, 13]. Ghrelin and leptin are both expressed abundantly in stomach and are secreted into the gastric lumen and the circulatory system once stimulated [10, 11, 14–17]. Endocrine secretion of insulin from pancreas is under the influence of various neural pathways [18–20]. All

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Fig. 1 Stimulation parameters applied included the following. (A) IGS: 40 Hz, 0.3 msec, 6 mA, 2 sec on and 3 sec off. (B) Long pulses: 10 cpm, 300 msec, and 8 mA. Modified IGS parameters are similar to those for IGS, except that the pulse width was increased from 0.3 to 2 msec



these peptides are released during eating and may function in the short-term system control of feeding behavior.

Whether electrical stimulation at the stomach could affect the systemic release of these satiety-related peptides has not been carefully investigated. The present study was specifically designed to evaluate the effect of GES with various stimulation parameters on plasma ghrelin, leptin, insulin, and glucose in a canine model.

Methods

Animal preparations

After an overnight fast, nine healthy female hound dogs weighing 18–26 kg were anesthetized with an intravenous infusion of Pentothal (sodium thiopental, 11 mg/kg, Abbott Laboratories, North Chicago, IL, USA) and maintained with inhalation of 2% isoflurane (Abbott Laboratories) in oxygen (1 L/min) carrier gases delivered from a ventilator after endotracheal intubation. A midline laparotomy was performed. One pair of 28-gauge stainless-steel cardiac pacing wires (A&E Medical, Farmingdale, NJ, USA) were implanted into the seromuscular layer at the middle of the lesser curvature. The two electrodes in the pair were separated by approximately 1 cm. The electrodes were channeled to the back of the chest subcutaneously, exteriorized, secured, and numbered for attachment to the stimulation equipment.

Experiments were performed after the animals completely recovered from surgery, which normally took 10–14 days. The experimental protocol was approved by the Animal Research Committee of the Veterans Research Administration, Oklahoma City.

Gastric electrical stimulation

GES was applied by stimulating the lesser curvature electrodes with an adjustable electrical stimulator (Model A310; World Precision Instruments, Sarasota, FL, USA). Stimuli were composed of trains of short pulses that were contin-

uous rectangular pulses. The stimulation parameters evaluated in this study were as follows. (1) Implantable gastric stimulation (IGS) parameters for obese patients were 40 Hz, 0.3 msec, and 5 mA, on time of 2 sec, and off time of 3 sec and (2), for long pulses, 10 cpm, 300 msec, and 8 mA. (3) Modified IGS parameters used a pulse width increased from 0.3 to 2 msec, with the other parameters the same as for IGS (Fig. 1).

Collection of blood samples

Blood samples were collected from the same group of conscious, overnight-fasted animals. There were four sessions for each animal: a control session and three stimulation sessions using the parameters described above. Sessions were performed randomly, in the morning, on 4 separate days, spaced at least 2 days apart. An intravenous catheter was inserted into a foreleg vein and maintained with heparin lock during the course of the study. Blood samples were taken at the following time points: 15 and 0 min while fasting and 15, 30, and 60 min immediately after a test meal. Samples of 10 ml were collected in EDTA-pretreated tubes at each time point. On the day of electrical stimulation, GES was initiated 30 min before the first blood sample was collected and maintained until the end of the study session. Blood samples were immediately centrifuged at 3500 rpm and 4°C, and plasma aliquots were frozen at –20°C until analysis was performed.

The test meal was one can of 375 g solid food (Pedigree; Kal Kan Food, Inc., Vernon, CA, USA) containing at least 8% protein, 3% fat, and 1% carbohydrate.

Assay of hormones and plasma glucose

Concentrations of plasma ghrelin were measured with commercially available radioimmunoassay (RIA) kits (Linco Research, USA). Detection limit and intra- and interassay variations were 93 pg/ml and 4.3%, respectively.

Concentrations of plasma insulin were measured with commercially available RIA kits (Linco Research). Detection limit was 2 μU/ml.

Leptin levels in plasma were measured with ELISA systems, using a rat leptin antibody (Assay Designs, Ann Arbor, MI, USA). Preliminary experiments conducted in our lab have verified that this kit interacts with canine leptin, and another group has also used this kit to measure canine leptin [21]. Blood samples were concentrated first using Prevail Extract-clean C18 columns (Alltech, Deerfield, IL, USA). After conditioning of the columns with deionized water, 1-ml blood samples were loaded into and passed through the columns at a rate of 10 drops/min. Then the columns were washed with 3 ml of 1% trifluoroacetic acid (TFA) three times. Finally, the columns were eluted with 3 ml of 60% acetonitrile in 1% TFA, and the eluate was evaporated with compressed air at 50°C to remove the aqueous fraction completely, then stored at -80°C until assay. The dry extract was reconstituted in assay buffer and analyzed according to the protocol specified by the manufacturer. The extraction rate of standard leptin was between 78.9% and 85%. The detection limit of the assay was 131 pg/ml; the intra-assay variation was 4.1% within the range of concentrations measured, and the interassay variation was 7.5%.

Plasma levels of glucose were assessed with a commercially available glucometer (MediSense Precision Xtra; Abbot Laboratories, MA, USA). A drop of blood was applied to the tip of the test strip and the readings of plasma glucose were displayed automatically.

Data and statistical analysis

The changes in the total area under the curve (AUC) were calculated for each peptide and plasma glucose on each occasion. The changes in plasma glucose relative to the fasting state at each time point during the postprandial period were also calculated. One-way repeated-measures ANOVA followed by Dunnett’s test was used to compare changes in total AUC and postprandial plasma glucose between control and various GES sessions. All data are expressed as mean ± SE. A *P* value of <0.05 was considered statistically significant.

Results

Electrical stimulation on plasma ghrelin

In the control session (no stimulation), plasma concentrations of ghrelin fluctuated around the fasting basal level without a clear trend of decrease or increase after the test meal (Fig. 2). The same pattern was also observed in the other three sessions with GES (Fig. 2). A trend of increase in plasma ghrelin levels was noticed during stimulation with IGS and modified IGS parameters during both the fasting and the postprandial periods (Fig. 2), but there was no sta-

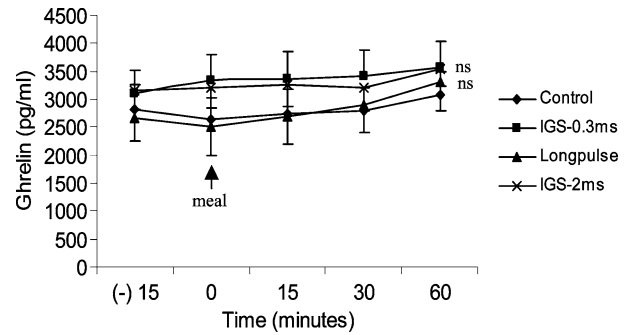


Fig. 2 Effect of GES with various stimulation parameters on plasma ghrelin (*n* = 8). A trend of increase in plasma ghrelin levels was noticed during stimulation with IGS and modified IGS parameters in the fasting and postprandial periods. However, there was no statistical significance between control and various stimulation sessions when AUC was compared. One-way repeated-measures ANOVA, versus control: ns, not significant

tistical significance between control and various stimulation sessions when total AUC was compared (Table 1).

Electrical stimulation on plasma leptin

In the control session (no stimulation), plasma concentrations of leptin were quite stable during fasting, then peaked at 15 min after the test meal and declined gradually afterward (Fig. 3). This release pattern of leptin seemed to be flattened during stimulation with short pulse trains (IGS and modified IGS) and was disrupted completely with long pulse stimulation (Fig. 3). Despite these changes, there was no statistical significance between the control and the various stimulation sessions when total AUCs were compared (Table 1).

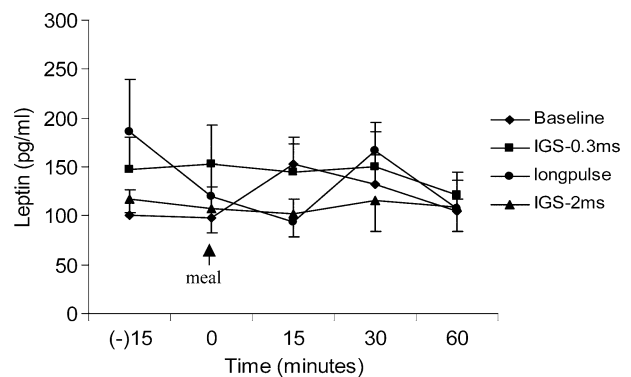


Fig. 3 Effect of GES with various stimulation parameters on plasma leptin (*n* = 6). Plasma concentrations of leptin peaked at 15 min after the test meal and declined gradually afterward in the control session. This release pattern of leptin was interrupted with electrical stimulation, though there was no statistical significance between the control and the various stimulation sessions when AUC was compared. One-way repeated-measures ANOVA

Table 1 Effect of GES on systemic peptide release and postprandial plasma glucose, presented as total AUC($\times 10^3$)

	Control	IGS	Long pulse	Modified IGS
Ghrelin ($n = 8$)	210.7 \pm 30.9	253.8 \pm 34.7 (<i>ns</i>)	213.2 \pm 36.9 (<i>ns</i>)	245.9 \pm 25.9 (<i>ns</i>)
Leptin ($n = 6$)	9.5 \pm 1.7	10.8 \pm 2.2 (<i>ns</i>)	10.8 \pm 2.2 (<i>ns</i>)	8.2 \pm 1.1 (<i>ns</i>)
Insulin ($n = 8$)	7.5 \pm 0.8	5.2 \pm 0.5*	4.7 \pm 0.7*	5.9 \pm 0.9 (<i>ns</i>)
Glucose ($n = 7$)	6.3 \pm 0.1	6.3 \pm 0.2 (<i>ns</i>) ^s	5.9 \pm 0.1*	6.0 \pm 0.1*

Note. Data are mean \pm SE. One-way repeated-measures ANOVA, versus control: * $P < 0.05$; ns, not significant.

Electrical stimulation on plasma insulin

Plasma concentrations of insulin increased dramatically and peaked at 30 min after the test meal in the control session. A similar pattern was also observed in the other three sessions with GES (Fig. 4). It seemed that GES exerted an inhibitory effect on the release of plasma insulin in both the fasting and the postprandial periods. A trend of decrease in AUC was also observed with modified IGS parameters (Fig. 4). The total AUCs were significantly lower in sessions with IGS and long pulse parameters ($P < 0.05$) compared with the control (Table 1).

Electrical stimulation on plasma glucose

GES affected postprandial plasma levels of glucose, and the influence seemed to be stimulation parameter related (Fig. 5). At the end of the 60-min postprandial period, the concentration of plasma glucose was increased by 6.14 ± 2.0 g/ml relative to that in the fasting period in the control session. While in GES sessions, the same variable was decreased by 0.14 ± 2.2 g/ml with IGS, 3.1 ± 2.1 g/ml with long pulses ($P < 0.05$ vs. control), and 6.4 ± 2.9 g/ml with modified IGS parameters ($P < 0.05$ vs. control). The AUCs

were also significantly lower in sessions with long pulses and modified IGS parameters in comparison with the control ($P < 0.05$; $n = 7$) (Table 1).

Discussion

In this study, we have found that acute GES with different stimulation parameters has no significant effect on plasma ghrelin and leptin. The effect of GES on plasma insulin was stimulation parameter related. Conventional IGS and long pulses significantly reduced the release of insulin into the systemic circulation. A similar trend was also found with modified IGS parameters. A significantly lower plasma level of glucose was also noticed with long pulse and modified IGS parameters during the postprandial period.

Obesity is a serious health problem around the world. In the United States alone, about 65% of adults are obese or overweight and 5% are severely obese [22]. There are approximately 300,000 deaths every year caused by obesity and more than \$100 billion is being spent each year for the treatment of obesity and its primary comorbidities [23–26]. Despite its significant impact on health, the management of this disease remains challenging. Surgery has been the only treatment that can lead to satisfactory long-term weight loss, but its use is limited due to substantial risks and complications related to operative procedures. GES therapy

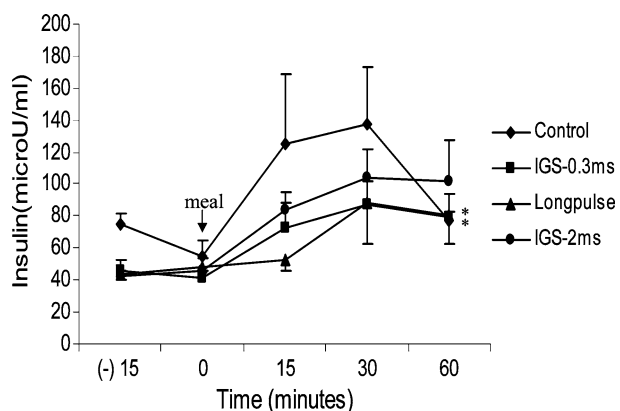


Fig. 4 Effect of GES with various stimulation parameters on plasma insulin ($n = 8$). GES exerted an inhibitory effect on the release of plasma insulin in both the fasting and the postprandial periods. The AUCs were significantly lower in sessions with IGS and long pulse parameters ($P < 0.05$) compared with the control. A trend of decrease in AUC was also observed with modified IGS parameters. One-way repeated-measures ANOVA, versus control: * $P < 0.05$

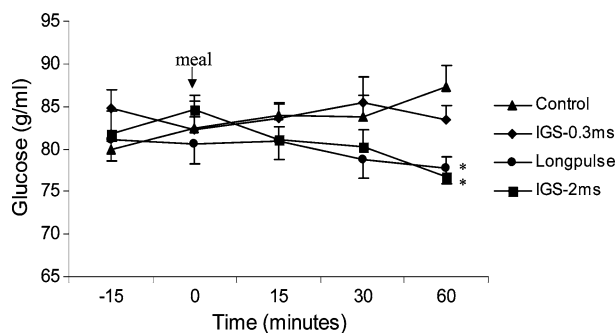


Fig. 5 Effect of GES with various stimulation parameters on plasma glucose ($n = 7$). GES affected postprandial plasma levels of glucose, and the influence seemed to be stimulation parameter related. AUCs were significantly lower in sessions with long pulses and modified IGS parameters in comparison with the control. One-way repeated-measures ANOVA, versus control: * $P < 0.05$

was initially based on the observations that gastric pacing could affect gastric contractions and caused changed eating behavior and weight loss in pigs [3, 4]. Subsequent clinical trials have shown that this therapy can indeed induce considerable weight loss in morbid obese patients [5–8]. As a novel option, it is far less aggressive than the usual surgical procedures and is safe and effective, with no significant side effects, compared with long-term drug therapy or bariatric surgery.

Leptin, the protein hormone encoded by the *ob* gene, is secreted primarily from adipose tissue and plays an important role in the regulation of food intake [12]. This hormone acts on the hypothalamus to reduce food intake and increase energy expenditure. Leptin was initially thought to be secreted only by adipocytes. But recent evidence has shown that leptin also occurs in the stomach of rats [14] and humans [15]. Leptin messenger RNA and leptin protein are present in the gastric epithelium, and cells in the glands of the gastric fundic mucosa are immunoreactive for leptin [14, 15]. Both feeding and administration of biologically active CCK-8 result in a rapid and large decrease in both leptin cell immunoreactivity and the leptin content of the fundic epithelium, with a concomitant increase in the concentration of leptin in the plasma [14]. These results indicate that gastric leptin may be involved in early CCK-mediated effects activated by food intake, possibly including satiety. Intravenous infusions of pentagastrin and secretin also stimulate the release of gastric leptin simultaneously into the blood and into the gastric juice [16]. All these observations suggest that gastric leptin may interact synergically with other short-term satiety peptides and plays a role in the regulation of satiety and food intake [27].

Though overall GES with various parameters did not significantly affect plasma concentrations of leptin in the fasting and postprandial periods, it seems to affect the pattern of release of this hormone. Under control conditions, it was observed that plasma leptin levels usually peaked at 15 min after a meal, while with stimulation the peak level was no longer observed, especially during stimulation with short pulse trains. The reason for this is unclear. This phenomenon may suggest that GES can indeed affect the secretion of leptin from the stomach, while peripheral levels of leptin may not adequately reflect such a change in the gastrointestinal lumen.

Ghrelin is a 28-amino acid peptide with a structural resemblance to motilin [17]. The ghrelin gene is predominantly expressed in the stomach. Ghrelin is secreted into the circulatory system, acting in the hypothalamus, affecting food intake and satiety [9–11]. Expression and secretion of ghrelin are increased by fasting and are reduced by feeding [10, 11]. In addition, diet-induced weight loss increases the plasma ghrelin level in humans [28]. Recently, different forms of ghrelin have been found, such as acylated

ghrelin and desacyl ghrelin. Acylated ghrelin can induce a positive energy balance. Administered acylated ghrelin induces body weight gain and adiposity by promoting food intake and decreasing fat use or energy expenditure [10, 11]. On the other hand, desacyl ghrelin induces a negative energy balance by decreasing food intake and delaying gastric emptying [29]. It seems that plasma total ghrelin levels were not affected by electrical stimulation, as no significant changes were observed during stimulation with various parameters. The trend of decrease in plasma ghrelin after a meal under normal conditions, however, was also not clearly observed in this study. This is probably related to the test meal we used, which was rich in protein. It has been reported that meals rich in carbohydrate or fat tend to decrease plasma ghrelin levels, while protein-rich meals have the opposite influence [30]. Also, it would be more appropriate to assay the different forms of ghrelin separately in future studies, rather than the measurements of total ghrelin, as different molecular forms of ghrelin seem to affect food intake differently.

The most significant change induced by GES was observed in plasma insulin. Plasma insulin levels seem to be lower than the control in the fasting and postprandial periods with all tested stimulation parameters. It is known that systemic insulin circulates through the blood-brain barrier into the brain, where it gains access to neurons containing specific insulin receptors that are important in the control of feeding and metabolism. Its levels in the blood have been considered a reliable indicator of adiposity [13]. However, the significance of such a change of plasma insulin in the induction of satiety by acute GES needs further investigation. Insulin is normally secreted from the pancreatic islets, and the release of endogenous insulin is under the influence of circulating nutrients and various neural factors, including vagal and parasympathetic pathways [18]. It has been reported that vagal nerve stimulation can significantly increase insulin secretion for a prolonged period of time in intact and in partly dysfunctional pancreas, while splanchnic nerve (sympathetic) stimulation can reduce insulin secretion [19, 20]. The lower levels of plasma insulin observed in this study may be associated with the activation of sympathetic pathways, as we have recently found that electrical stimulation indeed can activate α - and β -adrenergic pathways in the stomach [1].

The plasma level of glucose was observed to be significantly lower in the postprandial period with long pulse and modified IGS parameters but not with IGS parameters. This is probably due to delayed release of food from the stomach, as the rate of gastric emptying is a major determinant of postprandial glycemia. We have previously reported that higher energy stimulation tends to delay gastric emptying [31, 32]. On the other hand, the link between lower levels of glucose and plasma insulin cannot be excluded, because the passage

of glucose through the intestine is one of the major stimuli for postprandial release of insulin.

Our findings from this study suggest that electrical stimulation at the stomach can affect the release of some satiety-related hormones from the gastrointestinal tract. This is consistent with a previous report by Cigaina *et al.* [8]. They reported that IGS can inhibit the meal-related response of CCK and somatostatin, and basal levels of GLP-1 and leptin were also significantly reduced compared with the tests before stimulation [8]

The mechanisms by which IGS benefits obese patients are more likely to be multifactorial, and include the following.

1. The vagovagal reflex. IGS may activate local vagal afferents, which transmits coded information to the control center of the brain. There is limited information suggesting that IGS may activate the central neuronal activity recorded from neurons of the nucleus tractus solitarius (NTS) in rats, the relay center of signals from the gastrointestinal system [33].
2. Activation of local myenteric neurons, which is associated with alterations of visceral perception and gastric motility
3. Modulation of autonomic nerve activity, especially the parasympathetic pathway [1, 2]
4. Alterations in gastric motility [2]
5. Release of satiety-related gastrointestinal hormones.

Alterations in the release of gastrointestinal hormones may play a certain role in the changed eating behavior and weight loss associated with gastric electrical stimulation, and further studies are needed to elucidate associations among gastrointestinal hormones, satiety, and weight loss in patients on long-term GES therapy.

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