Catheter-directed Gastric Artery Chemical Embolization Suppresses Systemic Ghrelin Levels in Porcine Model¹

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Purpose:

To prospectively test, in a porcine model, the hypothesis that catheter-directed gastric artery chemical embolization (GACE) can result in suppression of systemic ghrelin levels and affect weight gain.

Materials and Methods:

This study, which had Animal Care and Use Committee approval, was performed in healthy, growing swine (weight range, 40–45 kg; $n = 10$). GACE was performed in five swine with the infusion of sodium morrhuate (125 μ g) selectively into the gastric arteries that supply the fundus. Five control animals underwent a sham procedure with 5 mL of saline. Weight and fasting plasma ghrelin levels were obtained in animals at baseline and in weeks 1–4. Statistical testing for substantial differences in ghrelin blood levels over time and between treated and untreated animals was performed by using a cross-sectional time-series linear model with feasibility generalized least squares.

Results:

The pattern of the change in ghrelin levels over time was significantly different between control and treated animals ($P < .004$). In treated animals, ghrelin levels were significantly reduced at week 1 (mean, 664.1 pg/mL $\pm$ 103.1 [standard error of the mean], $P < .02$), week 2 (mean, 618.1 pg/mL $\pm$ 180.4, $P < .001$), week 3 (mean, 578.4 pg/mL $\pm$ 214.9, $P < .001$), and week 4 (mean, 876.6 pg/mL $\pm$ 228.6, $P < .03$) relative to baseline (mean, 1006.3 pg/mL $\pm$ 190.1). The percentage change in serum ghrelin values in swine treated with GACE decreased from baseline to –34%, –38.6%, –42.5%, and –12.9% during weeks 1–4, respectively. In control swine, percentage change in serum ghrelin was –1.7%, –9.7%, +2.6%, and +18.2% during weeks 1–4, respectively. At the end of 4 weeks, control swine continued to gain weight, with a 15.1% increase from their original weight, while the weight in swine treated with GACE plateaued at an increase of 7.8% from the original weight.

Conclusion:

Catheter-directed GACE can suppress the appetite hormone ghrelin and affect weight gain.

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Currently, there are three clinically viable surgical alternatives for the treatment of obesity: gastric bypass surgery (bariatric), gastric pacing, and endoscopic gastric banding (1–5). Although these modalities have varying success rates, these procedures are fairly invasive, require extensive gastric and/or bowel reconstruction or the use of external devices, and can have considerable surgical complications. Thus, there is a critical need for a minimally invasive image-guided intervention that targets this population.

Over the past decade, there has been a growing understanding of the role of the stomach as an endocrine organ that is critically involved in the maintenance of energy homeostasis (6,7). Although more than 40 hormones have been discovered that limit food intake, only one hormone, ghrelin, has been shown to be a very potent orexigenic—that is, a substance that is able to stimulate food intake (8). Ghrelin-producing cells are predominantly located in the fundus of the stomach and directly fuel appetite and induce positive energy balance, resulting in body weight gain (7). Owing to the unique nature of this hormone, multiple attempts to modulate ghrelin production have been attempted, but no feasible direct clinical technique has yet been achieved (9–11).

Recently, we developed a percutaneous procedure, gastric artery chemical embolization (GACE), that directly targets the gastric fundus (a major source of ghrelin) and its functionality with the targeted intraarterial delivery of a sclerosing agent (12). Thus, the purpose of our study was to prospectively test, in a porcine model, the hypothesis that catheter-directed GACE can modulate systemic ghrelin levels and influence weight gain.

Advances in Knowledge

- Gastric artery chemical embolization (GACE) can significantly suppress the appetite hormone ghrelin.
- The ability to suppress systemic ghrelin levels could impact appetite.

Materials and Methods

Animal Model and Baseline Blood Samples

The Animal Care and Use Committee of the Johns Hopkins Medical Institutes approved our study. We tested healthy, growing swine (weight range, 40–45 kg; $n = 10$). All were sedated with an intramuscular injection of a mixture of ketamine (22 mg per kilogram of body weight), acepromazine (1.1 mg/kg), and atropine (0.05 mg/kg). For preoperative care, one dose (300 000 U/mL) of an antibiotic, penicillin G benzathine/procaine (Dual-Cillin; Phoenix Pharmaceuticals, Belmont, Calif), was given intramuscularly just before the procedure. Intravenous pentobarbital (20 mg/kg) was administered to bring the animals to a surgical plane of anesthesia. Animals were intubated and were mechanically ventilated with 2% isoflurane.

For our prospective study, the primary end point was a change in systemic ghrelin levels, and the secondary end point was weight change. Ten swine were randomized in a 1:1 fashion to undergo either GACE or a sham procedure. GACE-treated swine ($n = 5$) underwent embolization with 125 μ g of sodium morrhuate (American Regent, Shirley, NY), while control animals ($n = 5$) underwent a sham embolization with saline. The dosage was based on results of our prior experiments involving various dosages during GACE (12). After an overnight fast, two blood samples from each animal for baseline ghrelin analysis were obtained simultaneously immediately prior to the procedure. Blood samples were taken from either an ear vein or a femoral vein by one of the authors (A.A.M. or V.H.). Baseline weight was also obtained for each animal before the procedure.

Gastric Artery Embolization

All procedures (Movie, <http://radiology.rsnajnl.org/cgi/content/full/249/1/127>

Implication for Patient Care

- GACE may provide a minimally invasive image-guided alternative in targeting obesity and appetite.

/DC1) were performed by an interventional radiologist (A.A., with 7 years of experience). Percutaneous access to the right femoral artery was achieved by using ultrasonographic guidance and the Seldinger technique. After the femoral artery was accessed, a 5-F sheath (Cordis, Miami, Fla) was placed in the femoral artery. Angiographic procedures were performed with an interventional angiography system (Infinix CC-i; Toshiba, Tokyo, Japan). By using standard 5-F angiographic catheters (Omni Sos; Angiodynamics, Queensbury, NY), selective catheterization of the celiac artery was performed from the transfemoral approach. Digital subtraction angiography of the celiac and superior mesenteric arteries was performed to delineate vascular anatomy to the gastric fundus, liver, spleen, pancreas, and small bowel (Fig 1). After identification of the left gastric artery and other potential accessory gastric arteries to the fundus, these vessels were selectively catheterized by using a 3-F microcatheter system (SP3; Boston Scientific, Natick, Mass).

Prior to embolization, digital subtraction angiography was repeated through the microcatheters to confirm that these vessels provided flow to the gastric fundus. For embolization, sodium morrhuate (50 mg/mL, 5%) was delivered through the catheter at a dosage of 125 μ g. The sodium morrhuate dosage used

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Abbreviation:

GACE = gastric artery chemical embolization

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was based on results of our prior dose-escalating trial for GACE (12). GACE-treated swine that had multiple accessory gastric arteries to the fundus underwent injection of sodium morrhuate into all the accessory gastric arteries, with the volume of sodium morrhuate divided equally among arteries. After embolization, repeat angiography was performed to assess for stasis in gastric arteries and to determine whether nontarget embolization had occurred. Nontarget embolization was identified as stasis in other vessels that were not gastric arteries. After the procedure, all catheters and sheaths were removed, and the puncture site was closed by using 2.0 sutures (Monosof; Covidien, Mansfield, Mass).

Control Procedures

Control swine underwent an identical procedure to GACE-treated swine. After placement of a 5-F sheath in the femoral artery, the left gastric artery was selected in a similar fashion, followed by the infusion of 5 mL of 5% normal saline.

Postablation Protocol and Ghrelin Analysis

Animals were placed in standard housing and were fed an ad-libitum diet. Fasting serum ghrelin values were obtained in all animals at weekly intervals for 4 weeks. At each time point after the procedure, two ghrelin samples were drawn. After the procedure, all blood samples were drawn in the morning after an overnight fast. Blood was immediately transferred to a glass tube containing disodium edetic acid (1 mg/mL) and aprotinin (500 U/mL) and was centrifuged immediately. Ghrelin was measured by using a radioimmunoassay with iodine 125-labeled bioactive ghrelin as the tracer and a rabbit polyclonal antibody (Phoenix Pharmaceuticals). Animal weights were obtained at the same time points by two authors (T.T.P. and V.H.). At week 4, repeat angiography was performed, and animals were euthanized with intravenous sodium pentobarbital (100 mg/kg).

Statistical Analysis

The pre- and postprocedure ghrelin values (the mean of the four weekly postprocedure values for both GACE-

treated and control swine) were calculated and plotted. In addition, percentage changes from baseline ghrelin values were calculated and plotted at weeks 1–4 for both groups. Statistical testing for significant differences in ghrelin blood levels over time and between treated and untreated animals was performed by using a cross-sectional time-series linear model with feasibility generalized least squares (Stata, College Station, Tex). $P < .05$ was considered to indicate a statistically significant difference.

Weights (in pounds) were obtained on a weekly basis. The weight at baseline and at weeks 1–4 for both GACE-treated and control swine were calculated. To minimize variability in the weights of swine, the mean percentage changes in weight, rather than absolute values, from baseline to weeks 1–4 were calculated and plotted. Statistical testing for significant differences in weight over time and between treated and untreated animals was performed in a similar fashion to that described above, with $P < .05$ considered to indicate a statistically significant difference. Data are reported as means $\pm$ standard errors of the mean.

Results

All animals survived the 4-week protocol with no apparent clinical sequelae. GACE was performed with 100% technical success. All GACE-treated swine required selective injection of at least two gastric arteries arising from the celiac axis and hepatic artery. One swine required injection of three arteries that fed the gastric fundus. Nontarget embolization, as determined by stasis in nongastric arteries, was seen most frequently in hepatic, splenic, and esophageal arteries in almost all animals. At the end of 4 weeks, repeat angiography prior to euthanasia demonstrated that all arteries were patent in GACE-treated and control swine.

Postprocedure Ghrelin Values

Control swine.—After sham embolization with saline, the serum ghrelin levels changed from a baseline of 1078 pg/

mL $\pm$ 161.9 to a mean postprocedure value of 1104 pg/mL $\pm$ 74.8 (mean of all postprocedure ghrelin samples) (Fig 2).

GACE swine.—GACE resulted in a decrease in serum ghrelin values from a baseline of 1006.3 pg/mL $\pm$ 190.1 to a mean postprocedure value of 684.3 pg/mL $\pm$ 88.1 (mean of all postprocedure ghrelin samples) (Fig 2).

Figure 1

Figure 1: Catheter-directed GACE technique. Celiac artery angiography is performed in anteroposterior projection to identify the celiac artery, two left gastric arteries (black arrow), and a left accessory gastric artery (white arrow). Superselective catheterization of the arteries that supply the gastric fundus was performed. For the GACE procedure, sodium morrhuate (50 mg/mL, 5%) was infused into the arteries that supply the fundus.

Figure 2

Figure 2: Bar graph shows mean serum ghrelin levels (mean of all postprocedure ghrelin samples over the 4-week period) before and after the procedure in five control swine (teal bars) and five GACE-treated swine (red bars). Error bars = standard errors of the mean.

Figure 3

Figure 3: Graph shows mean ghrelin values in control and GACE-treated swine at baseline and at weeks 1–4. The pattern of the change in ghrelin levels over time was significantly different between control and treated animals ($P < .004$). * = $P < .02$, + = $P < .001$, ** = $P < .001$, # = $P < .03$ (ghrelin levels were significantly reduced at all time end points relative to baseline). Error bars = standard errors of the mean.

Weekly Ghrelin Values

The pattern of the change in ghrelin levels over time was significantly different between control and treated animals ($P < .004$) (Fig 3). In treated animals, ghrelin levels were significantly reduced at week 1 ($664.1 \text{ pg/mL} \pm 103.1$, $P < .02$), week 2 ($618.1 \text{ pg/mL} \pm 180.4$, $P < .001$), week 3 ($578.4 \text{ pg/mL} \pm 214.9$, $P < .001$), and week 4 ($876.6 \text{ pg/mL} \pm 228.6$, $P < .03$) relative to baseline ($1006.3 \text{ pg/mL} \pm 190.1$). Ghrelin values in control animals remained relatively constant over 3 weeks (week 1, $1060.1 \text{ pg/mL} \pm 155.8$; week 2, $973.3 \text{ pg/mL} \pm 96.4$; week 3, $1107.1 \text{ pg/mL} \pm 107.2$), with a significant increase seen only at week 4 ($1275 \text{ pg/mL} \pm 228.3$ vs baseline level of $1078 \text{ pg/mL} \pm 161.9$, $P < .004$).

Percentage Change in Ghrelin Values

In control swine, percentage change in serum ghrelin was -1.7% , -9.7% , $+2.6\%$, and $+18.2\%$ during weeks 1–4, respectively (Fig 4). The serum ghrelin values in GACE-treated swine decreased from baseline by -34% , -38.6% , -42.5% , and -12.9% . In GACE-treated swine, there was a substantial decrease in ghrelin values at all weeks compared with baseline.

Weight Change

Although the mean weight of the GACE-treated animals was slightly higher than that of the control animals at baseline, the difference was not statistically significant ($P = .13$). However, the pattern of weight gain over 4 weeks was significantly different between GACE-treated and control swine ($P < .04$) (Fig 5). Control animals showed a steady increase in weight: $0\% \pm 6.8$ at week 1, $6.6\% \pm 4.8$ at week 2, $10\% \pm 6.1$ at week 3, and $15.1\% \pm 6$ at week 4, with significant weight gains at 2–4 weeks relative to baseline ($P < .001$). In swine that underwent GACE, the average increase in body weight was $0.2\% \pm 4.4$ at week 1, $5.3\% \pm 5.1$ at week 2, $6.5\% \pm 4.4$ at week 3, and $7.8\% \pm 5.5$ at week 4, with significant gains relative to baseline seen only at 3 and 4 weeks ($P < .02$).

Figure 4

Figure 4: Bar graph shows mean percentage change from baseline in serum ghrelin levels in control and GACE-treated swine over a 4-week period. In control swine, there was no noteworthy change in systemic ghrelin levels from baseline until week 4. In GACE-treated swine, there was a significant decrease in ghrelin levels at all weeks. Maximum suppression of ghrelin was noted at week 3. * = $P < .05$. Error bars = standard errors of the mean.

Discussion

Our study results demonstrate, with serologic evidence, that using a catheter-based procedure, GACE, in a porcine model can suppress systemic levels of ghrelin, thereby substantially impacting weight gain compared with weight gain in control animals over the time course in our study. GACE may offer a potentially minimally invasive approach to the temporary suppression of systemic ghrelin levels.

Although there are multiple hormones (to date, more than 40) that limit food intake, there is only one hormone, ghrelin, that has been shown to be orexigenic—that is, to stimulate food intake. Discovered in 1999 by Kojima et al (13), in the rat stomach, ghrelin was shown to induce growth hormone release by acting on the growth hormone secretagogue receptor (GHS-R) in the hypothalamus or anterior pituitary gland. Extensive study of this hormone has shown that it is well preserved across species and is produced mostly by the oxyntic cells of the gastric fundus. Identifying GHS-R localization has shown that ghrelin has widespread systemic and physiologic effects. GHS-Rs have been identified within the myocardium, small intestine, stomach, liver, and pancreas (6,11,14). In addition, various studies have demonstrated that administration of ghrelin has a variety of effects within each target organ (15–18).

The strongest link, however, exists between ghrelin and its effect on appetite formation and weight regulation. Since the identification of this neuropeptide, extensive data in both animals and humans have shown the potent orexigenic effects of this hormone. In multiple rat studies, peripheral or central administration of ghrelin results in a very potent, short-term increase in food intake and growth hormone secretion (10). Mechanisms to antagonize or suppress the effects of ghrelin on the central nervous system have resulted in dramatic weight loss and change in appetite in various studies (10,19). In studies performed in volunteers (20), intravenous ghrelin stimulates meal ini-

Figure 5

Figure 5: Graph shows percentage change in weight over the 4-week period for control and GACE-treated swine. Control swine continued to grow over the time period, while GACE-treated swine plateaued in growth. The pattern of weight gain over 4 weeks was significantly different between GACE-treated and control swine. * = $P < .04$. Error bars = standard errors of the mean.

tiation, with volunteers describing a sensation of hunger and increase in caloric intake. Further evaluation has shown that there is a pattern to ghrelin secretion, which begins to rise before every meal. Therefore, with weight loss, a compensatory increase in ghrelin levels may explain the difficulty in maintaining body weight (8). In obese patients, suppression of ghrelin is not present, which impairs postprandial satiety and causes overeating (14,20). Owing to these potent orexigenic effects, ghrelin has been a target for the treatment of obesity and weight loss. Although various reports of ghrelin suppression have been published, no techniques are clinically practical; these include intraventricular and large intraperitoneal dosage of agents in rats (10,19,21). On the basis of these findings, achieving low systemic ghrelin levels has become a potential strategy to control obesity and maintain weight loss.

Anatomically, the left gastric artery, which arises from the celiac axis, provides the dominant flow to the fundus of the stomach, the richest source of ghre-

lin (14). Furthermore, the vascular supply to the gastric fundus is distinct, is usually identifiable, and can be accessed with a catheter from a percutaneous approach. In our prior work (12), we had previously demonstrated that GACE can be used to overexpress or to suppress ghrelin production. We have built on this prior study to demonstrate that, with a single larger dose, suppression of ghrelin is now feasible in a preclinical model. For this reason, gastric arteries can now be explored as a target for fundal functionality and hormonal suppression.

By using a single one-time injection of a sclerosing agent into the gastric artery that supplies the fundus, we were able to directly suppress systemic ghrelin levels for a 4-week period. Using this approach, we were able to achieve a dramatic decrease in systemic ghrelin levels from baseline. On a weekly basis, the maximum suppression of ghrelin occurred at weeks 2 and 3 and started to reverse at week 4. Notably, the suppressed ghrelin levels in our study were similar to postoperative ghrelin levels in patients who underwent gastric bypass

surgery (9,22). These results suggest that ablative techniques, which have been used for a variety of other conditions, may now be applied to the stomach.

The slow recovery of ghrelin production may be explained by two potential causes. First, revascularization and therefore recovery of the gastric mucosa could have allowed the renewal of ghrelin production from the fundus. The alternative option for this pattern is the potential for altered regulation of other sites of ghrelin production. For example, alternative sites of ghrelin production, such as the small bowel and pancreas, may have been upregulated and thus compensated for fundal suppression. In light of the finite temporal effect of embolization on suppressing ghrelin levels, future studies could repeat GACE therapy to determine whether the loss of suppression is due to replacement of cells within the fundus or to upregulation in other organs. Because the gastric arteries are patent at follow-up, this should be technically feasible.

Our study had limitations. Because weight was not the primary end point, our study was not adequately powered to completely evaluate weight changes. However, the pattern of weight gain between GACE-treated and control swine during the 4 weeks was substantially different. As expected, control swine continued on a regular growth curve, gaining 11.6 lbs, or 15% of their weight. On the other hand, GACE-treated swine gained only 6.5 lbs, or 7.8% of their weight, and appeared to reach a plateau by week 4. Like the weekly ghrelin levels, weight growth appeared to lag behind by a week, as demonstrated by a stabilization of growth during weeks 3 and 4, which may be a resultant effect of the ghrelin levels. Finally, we did not notice any weight loss in our study, except for a plateau effect on further gain that was seen up to 4 weeks. These findings may be related to our animal model, in which young, healthy swine can be expected to grow on an ad-libitum diet, and to the selection of only a 4-week follow-up. Therefore, follow-up studies that include a larger number of animals, swine that have reached maximum weight, a monitoring of dietary

intake, and a longer follow-up period could provide further insight. Finally, because we selectively embolized the gastric fundus, and not selectively for ghrelin-producing cells, we could not evaluate the resultant functionality of the gastric fundus in our study. Future studies should include evaluation of the embolization effect on other hormones produced in the fundus, as well as on gastric motility.

In our study, several important technical problems should be highlighted: (a) It was difficult to identify, with conventional angiography, the vascular supply to the gastric fundus because of overlapping vessels from the liver and spleen; (b) nontarget embolization to the liver, esophagus, spleen, and other portions of the stomach was often seen in almost all animals, as documented with postprocedure angiography (however, it should be noted that no sequelae were observed); (c) maximum suppression of the ghrelin level was only up to 50%; (d) there was no histopathologic correlation to document gastric erosions or ulcers; and (e) there was an apparent reversal of suppression that began at week 4.

To address some of these limitations, improved imaging techniques could enhance this procedure. Because GACE uses a potentially caustic agent, nontarget embolization due to inadequate imaging with conventional two-dimensional x-ray techniques could have sequelae. Therefore, to minimize the dosage, a high-spatial-resolution imaging technique to maximize targeted embolization and the suppressive effect of GACE is needed. In addition, a method to deliver sodium morrhuate in a slow controlled release over several days may enable a sustained effect with less caustic injuries and greater suppression of ghrelin than the 50% we achieved. In addition, a slow release mechanism may have a suppressive effect that lasts longer than the 3 weeks we observed in our study. Last, having the capability to visualize this or other embolic agents would have considerable impact on the safety of this procedure, since this would enhance targeting of the fundus.

Practical application: On the basis

of our findings, there are several potential avenues of further investigation. As shown by our results, using the gastric arteries to deliver therapeutic agents for locoregional control of ghrelin expression may become a feasible strategy. Various agents to suppress ghrelin have been described but are limited in their applicability, either because of a high dosage requirement or because of the necessity to deliver these agents directly into the central nervous system (10,19). With the minimally invasive nature of the GACE procedure, further refinements could provide the ability to deliver a variety of novel agents directly to the gastric fundus that would provide sustained suppression of ghrelin. By implementing such steps, an alternative minimally invasive technique may emerge that could affect energy balance and potentially influence obesity.

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