Gastric Electrical Stimulation (GES) Reduces Food Intake After Stimulation is Stopped in Obese Rats.

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BACKGROUND

The severely obese (BMI>35) population in the US numbers >20 million adults, and rises by more than one million persons each year. Severe obesity is a leading cause of chronic disease and death, and accounts for most of the >$100 billion in annual obesity-related medical costs. This study is part of an ongoing preclincal research program that is using multiple animal models, including rodents, to identify modes of GES that may prove more effective for obesity treatment in humans than those used in prior clinical studies.

OBJECTIVES

The primary objectives of this study were: (1) to test whether GES delivered just before (but not during) feeding reduces food intake in an obese rat model; and (2) to test whether the food intake suppression effects of pre-feeding GES depend on the duration of GES applied.

ANIMAL PREPARATION

Sixteen male Sprague-Dawley rats, ~16 weeks of age, weighing an average of 550g , were implanted with pairs of 0.37mm stainless steel heart wires (A&E Medical Myowires) in the muscularis of the distal gastric antrum. Lead wires were externalized percutaneously on the animals' backs for connection to an external pulse generator. Prior to implant, the rats were conditioned to obesity with 6 weeks of 24 hour *ad libitum* access to a standardized high fat diet (Research Diets D12451, 45% of kcal from fat).

After surgical recovery, the rats were conditioned to feed exclusively in a BioDaq continuous food intake monitoring system for 3 hours each day (FIGURE 1). The BioDaq monitor provided a complete record of both the quantity and timing of each rat's food intake over each 3 hour feeding.

The rats were also acclimated to spend 60-90 minutes in a custom built restrainer with alligator clip wires attached to the externalized lead wires just prior to daily BioDaq feedings (FIGURE 2); in the subsequent experiment, these restrainers were used for pre-feeding GES delivery.

FIGURE 1. BioDAQ Continuous Food Intake Monitoring System
The system includes a rack of 16 cages (A) with food hoppers mounted on a load cell. Hopper weights are transmitted to a computer (B) via a central controller hub (C). Specialized software on the computer identifies and records feeding events. Detailed images of the load cell and hopper are shown in (D) and (E).

FIGURE 2. Restrainers Used for GES Delivery

DESIGN AND ANALYSIS

After 14 days of restrainer and BioDaq-feeding acclimation, the sample rats entered a 4-week study of post-stimulation food intake with a Latin Square cross-over design. Each rat received 5 days of each comparison treatment in a randomly assigned order, with treatment periods separated by 2-day washouts. Treatments were a sham GES control and 3 durations of active GES (15, 30 and 60 minutes) administered just before feeding. GES parameters were fixed at values effective in reducing food intake when delivered during feeding: 6mA, 4ms, 40Hz, 2s On-3s Off.

Areas under the 3 hour cumulative food intake profile for each feeding were calculated from the BioDaq monitor data.The resulting 320 rat-day area under curve (AUC) observations were analyzed using a repeated measures regression with fixed effects for treatment, time and rat. Primary outcomes of interest were the differences in mean AUC between each duration of pre-feeding GES and the sham-GES control, and the presence or absence of a significant linear trend in AUC with pre-feeding GES duration. The detailed feeding data collected by the BioDaq were also used for exploratory analyses of GES effects on the number and timing of meals, their size, and their duration.

Additionally, Western Blot assays of the hunger-inducing gut peptide ghrelin were conducted in harvested gastric tissue for use in exploratory correlation analyses. Ghrelin has been implicated in both the long- and short-term regulation of feeding and body weight, and is produced almost exclusively in gastric tissue.

RESULTS

As shown in FIGURE 3, the mean area under the 3 hour cumulative food intake curve was reduced relative to the sham GES control following all 3 durations of active GES treatment, falling by 14.8%, 13.3% and 13.1% after 15, 30, and 60 minutes of pre-feeding GES. These reductions were statistically significant in all cases ($p<0.01$ for 15 minutes, $p<0.05$ for 30 and 60 minutes) There was, however, no significant difference in the AUC measure across active treatments.

Meal pattern analysis showed that reduced food intake following pre-feeding GES was due to reductions in the size and duration of meals, with the number meals, time to first meal and inter-meal interval being unaltered (TABLE 1).

Food intake responses to pre-feeding GES were highly correlated with ghrelin concentrations in gastric tissue, with larger reductions in food intake occurring in rats with lower tissue ghrelin levels (FIGURE 4).

TABLE 1. Effects of Pre-Feeding GES on Meal Patterns

	Pre-Feeding GES Duration:				
	0 minutes	15 minutes	30 minutes	60 minutes	F-test H_0: Tx Effects=0
Time to Initial Meal (minutes)	7.4	7.8	6.3	10.4	p=0.6527
Number of Meals	1.9	2.0	2.0	2.0	p=0.2981
Meal Duration (minutes)	55.6	39.7**	48.2	42.5*	p=0.0240
Meal Size (grams)	9.2	6.8***	6.8***	7.4***	p<0.0001
Intermeal Interval (minutes)	82.4	85.7	87.3	77.8	p=0.5580

FIGURE 3. Mean Cumulative Food Intake Profiles by Treatment and Area Under Curve (AUC) Comparisons.

Each curve is an average across 80 daily observations from 16 sample rats. The AUC differences shown in the inset table are least squares means from a repeated measures regression controlling for time period and subject effects. Reported p-values include a Bonferroni adjustment for multiple comparisons.

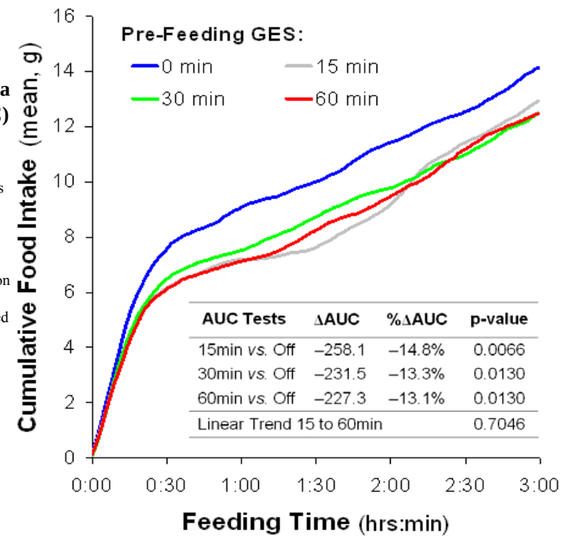
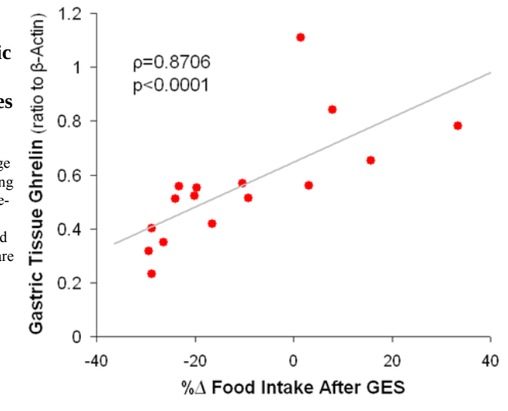


FIGURE 4. Correlation of Gastric Ghrelin Levels with Food Intake Responses

Figures show ghrelin measurements from gastric tissue plotted against percentage changes in food intake following active as compared to sham pre-feeding GES. Tissue ghrelin concentrations were determined by Western Blot analysis and are expressed as ratios to β-actin control loading..



CONCLUSIONS

- Fifteen minutes of GES administered prior to feeding is sufficient to suppress food intake over the subsequent 3 hours in obese rats.
- Pre-feeding GES treatment reduces food intake by reducing the size and duration of meals, with no apparent effect on the number and timing of meals.
- The strong correlation of the magnitude of food intake reduction with ghrelin levels in gastric tissue points to suppression of ghrelin as a possible GES mechanism of action.
- Persistence of GES-induced food intake suppression after stimulation is stopped may allow intermittent GES delivery without a loss of overall efficacy in reducing food intake.