Gastric electrical stimulation parameter dependently alters ventral medial hypothalamic activity and feeding in obese rats

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Zhang J, Maude-Griffin R, Zhu H, Sun Y, Starkebaum W, Firestone E, Chen JD. Gastric electrical stimulation parameter dependently alters ventral medial hypothalamic activity and feeding in obese rats. Am J Physiol Gastrointest Liver Physiol 301: G912–G918, 2011. First published August 18, 2011; doi:10.1152/ajpgi.00487.2010.—Gastric electrical stimulation (GES) has been used to treat obesity with unclear mechanisms and limited parameter ranges. This study explores effects of GES parameters on ventral medial hypothalamic (VMH) activity, feeding, and body weight in diet-induced obese (DIO) rats. For experiment 1, discharge rates were recorded in 39 gastric distension-responsive (GD-R) neurons in 12 DIO rats. Basal rates were compared with rates under GES using varied pulse amplitudes, widths, frequencies, and train-on times. For experiment 2, a crossover experiment in 16 DIO rats measured food intake and weight effects of GES pulse width, the parameter with the steepest neuronal response gradient in experiment 1. Treatments were sham and 0.5-, 2.0-, and 5.0-ms pulse GES. In experiment 1, 11 of 13 GES parameter sets tested produced significantly (P < 0.05) altered discharge rates of GD-R neurons. Increases in pulse amplitude (P < 0.05) and width (P < 0.0001) produced significant upward linear trends in response over the range tested, with the trend being strongest for pulse width. In experiment 2, over 4 days of 0.5-, 2.0-, and 5.0-ms GES treatment, food intake was 9.6% (P < 0.05), 21.0% (P < 0.0001), and 47.3% (P < 0.0001) lower than under sham-GES, whereas body weight changes were 0.7 (P = 0.48), 2.2 (P < 0.05), and 3.5 (P < 0.002) percentage points lower, respectively. We concluded that GES pulse width increases had the largest effect on VMH neuronal activity, and these effects were paralleled by pulse width-dependent reductions in food intake and body weight. Lengthening pulse width beyond the range used in prior clinical studies may be critical to making GES a viable obesity treatment. The prevalence of obesity is rising worldwide, and the majority of adults in the United States and many developed countries are overweight or obese. It is estimated that 1.6 billion adults worldwide are overweight [body mass index (BMI) > 25] and 400 million are obese (BMI > 30) (1). Obesity and its comorbidities have been estimated to cost over $100 billion annually in the US alone (11). Obesity is associated with higher rates of disability and all-cause mortality, and the obese have increased incidences of type 2 diabetes, cardiovascular disease, musculoskeletal disorders, sleep apnea, fatty liver disease, and certain cancers. Conventional obesity treatments fall into three categories: behavioral treatments focused on diet and exercise, pharmacotherapy, and surgical treatments. Although many patients achieve weight loss through behavioral treatments, only a small minority succeed in maintaining their losses. Similarly, Food and Drug Administration-approved antiobesity drugs have demonstrated only short-term effectiveness, and all of the approved medications have side effects that need to be considered, such as increased blood pressure and heart rate, or nausea and gastrointestinal symptoms (2). Surgical treatments have demonstrated satisfactory long-term weight loss and have been increasingly applied in clinic; however, their application is limited to a small percentage of morbidly obese patients. There is thus a need for additional obesity treatment options that are effective in producing long-term weight loss. Gastric electrical stimulation (GES) using implantable neurostimulators has been proposed as a safer, less invasive, and more tolerable alternative to existing bariatric surgeries. Several single-arm clinical trials (4–6, 18) found significant weight loss in GES-treated patients, but GES has yet to show efficacy in a randomized controlled trial. Most recently, a multisite, double-blind, randomized trial, conducted in 189 severely obese US patients, failed to show a significant difference in weight loss across active and sham GES treatments at 12 mo (19). Clinical studies of GES for obesity have been confined to a narrow range of stimulation parameters, imposed in part by limitations of the available implantable stimulators. For example, neurostimulators used in nearly all prior clinical GES studies to date were limited to pulse widths below 1.0 ms, despite animal evidence suggesting that longer pulse widths are more effective in modulating gastric motility (13). Other stimulation parameters in these studies were not so much a function of device limits as arbitrary choice. Nearly all of the clinical data on GES for obesity were collected using a single pulse frequency and duty cycle setting (40 Hz, 2 s on, 3 s off), the efficacy implications of which had never been systematically explored in animals or humans. If GES is to become a useful obesity therapy, further preclinical experiments are needed to identify more effective stimulation parameters than those previously tested in humans. The ventral medial hypothalamus (VMH) is closely related to feeding behavior and plays an important role in the mediating satiety. Bilateral VMH lesions cause hyperphagia and obesity, whereas electrical stimulation of VMH neurons has been found to have the opposite effect (7, 17). GES with appropriate parameters reliably produces gastric distension in fasted animals (24, 25, 27, 28), and this gastric tone effect is increasing pulse width beyond the range used in prior clinical studies may be critical to making GES a viable obesity treatment.
lean rats. This is consistent with GES-induced gastric distension having central neuronal effects that alter satiation and feeding. Before the present study, responses to the effects of gastric distension and GES on VMH neuronal activity have not been studied in an obese rat model.

This study explores the effects of varying GES parameters on neuronal activity in the VMH and on food intake and body weight in diet-induced obese (DIO) rats. The parameter range tested extends beyond that used in prior clinical studies in an attempt to identify GES settings that may prove more effective in treating obesity than those already applied in humans. Experiment 1 explores the effects of varying GES parameters on spontaneous unit discharge rates of GD-R neurons in the VMH. Beginning from a basal set of GES parameters typical of device settings in prior clinical studies, four stimulation parameters (pulse amplitude, width, frequency, and train-on time) were individually varied while holding all remaining parameters fixed. Effects on GD-R VMH neurons were recorded at each parameter value. The strongest effects on neuronal activity were generated by variations in GES pulse width, with longer pulse widths increasing both the proportion of GD-R neurons activated and the magnitude of absolute changes in discharge rates. Drawing on the results of experiment 1, experiment 2 investigates whether variations in GES pulse width produce changes in food intake and body weight that parallel the effects on VMH neuronal activity.

MATERIALS AND METHODS

 Experiment 1. Effects of GES on VMH Neuronal Activity of DIO Rats

Animal use committee approval. The animal care and procedures used in experiments 1 and 2 were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at the Oklahoma City Veterans Affairs Medical Center and carried out in accordance with IACUC guidelines.

Establishing a DIO rat model. Three-week-old male Sprague-Dawley rats weighing 45–55 g were shipped from Charles River Laboratories (Kingston, NY). Animals were singly housed in Plexiglas shoebox cages in a temperature controlled room (22 ± 2°C) on a 12-h:12-h light/dark cycle with 24-h food and water access. The rats accelerated undisturbed in their home cages for 1 wk after arrival while continuing a lab Chow diet (Lab Diet 5002; PMI Nutrition International, Brentwood, MO). The animals were then randomized into two groups: a control group (n = 10) that continued the lab Chow diet and a high-fat group (n = 20) that was switched to a standardized high-fat diet (D12451, 4.73 kcal/g, 45% from fat; Research Diets, New Brunswick, NJ). After feeding for 14 wk, high-fat group rats with body weights greater than the heaviest lab chow-fed control rat had unlimited access to high-fat diet (D12451, 4.73 kcal/g, 45% from fat; Research Diets) from weaning. Consistent with their obesity-prone phenotype and prolonged exposure to high-fat diet, their mean fat; Research Diets) from weaning. Consistent with their obesity-

Experiment 2. Effects of GES on Food Intake and Body Weight in DIO Rats

Animal preparation. Sixteen male Sprague Dawley rats of the DIO-prone phenotype originally derived by Levin et al. (9) were shipped from Charles River Laboratories at 10–11 wk of age. The rats had unlimited access to high-fat diet (D12451, 4.73 kcal/g, 45% from fat; Research Diets) from weaning. Consistent with their obesity-prone phenotype and prolonged exposure to high-fat diet, their mean
body weight (512 g, range: 439–630 g) on arrival was ~75 g (~50%) greater than that of comparably aged, lab chow-fed, Charles River Sprague Dawley males. Animals were singly housed in Plexiglas shoebox cages in a temperature controlled room (22 ± 2°C) on a 12-h:12-h light/dark cycle (lights on 6:00 AM-6:00 PM) with 24-h access to food and water. The rats recovered from shipping for 7 days before implant surgery.

The rats were anesthetized with isoflurane inhalation (2–3%) by mask. A midline abdominal incision was made, and one pair of stranded stainless steel myocardial pacing wires (A&E Medical 025–100 Myowire or Medtronic 6494 Streamline) was implanted below the serosa of the lesser curve of the gastric antrum. The proximal ends of the lead wires were externalized percutaneously on the back of the neck for GES delivery.

Acclimation to restricted feeding. All 16 rats survived the implant surgery without complication and recovered undisturbed for 7 days with 24-h access to food and water. After recovery, all food was removed from their cages, and the rats began a 19-day period of acclimation to time-restricted feeding in custom-built restrainers with access to a surplus of high-fat diet pellets (Research Diets, D12451) for 2 h each day (10:00 AM-12:00 noon). The restrainer was adjustable to the rat’s body size and limited movement to maintain the connection between the percutaneous leads and the external pulse generator used for GES delivery during the experiment.

The restricted feeding schedule produced a marked decline in the sample rats’ food intake during the initial days of acclimation. Body weights, which had recovered to presurgery levels, declined by 11.5% during the first 7 days of acclimation and were stable thereafter. Because of the lack of renewed weight gain, the rats were given supplemental access to 10 g of high-fat food for 2 h daily in their home cages immediately after restrainer feedings during the last week of acclimation and throughout the experiment. The timing of this access was such that the rats were fasted for 20 h before restrainer feeding. Consumption of supplemental food was minimal, averaging 1.2 g/day, and accounting for less than 10% of daily intake. Restrainer food intake continued to increase, gradually leading to renewed weight gain during the experiment.

Crossover experiment design. After restrainer feeding acclimation, the rats were randomized into a 4 × 4 Latin Square crossover design in which each rat received a sequence of four treatments in one of four assigned orders. Four sample rats were allocated to each treatment sequence. Treatments included active GES with three different pulse widths (0.5, 2.0, and 5.0 ms) and a sham-stimulation control. Remaining GES parameters were fixed at 6 mA, 40 Hz, 2 s on, 3 s off. The GES pulse widths tested were chosen to begin within and extend beyond the range tested in the VMH neuronal recording experiment. This experiment was performed at the completion of experiment 1. In experiment 1, we tested pulse width from 0.1 ms to 3.0 ms and found a linear correlation between the excitation rate of neurons and the pulse width with the highest rate of excitation at 3.0 ms. That is, the pulse width of 3.0 ms might not be optimal pulse width. Accordingly, in this experiment, we chose to expand the pulse width to a higher value of 5.0 ms. Because the number of pulse width values we could test was limited as a result of technical and logistical issues, the test on the pulse width of 3.0 ms was skipped. Stimulation was delivered only during the 2-h restrainer feedings using constant current pulse generators (models A365 and DS8000 with model A395D and DLS100 stimulus isolators; World Precision Instruments, Sarasota, FL) connected to the externalized leads with alligator clip wires. The rats had access to a surplus high-fat diet pellets during feedings. Each treatment was delivered on four consecutive days and was separated from the next treatment by a 3-day washout period. The rats continued the restrainer feeding regimen without GES during washouts. Food intake was measured daily, and changes in body weight over each treatment period were calculated from body weights taken before feeding on the first day of each treatment period and on the first day of the subsequent washout.

**Behavioral monitoring during GES.** The rats were monitored during GES administration, and any unusual behaviors were recorded. In addition to any marked changes in routine behaviors (grooming, sleeping, sniffing, and locomotion), occurrences of any behaviors specifically linked to visceral pain in rats (flinching, writhing, squashing, and arching) were also recorded (22).

**Statistical analysis.** Food intake and body weight change outcomes were analyzed with repeated-measures regressions including fixed effects for treatment, time period, and animal. Results are reported as least-squares mean values for food intake and weight change by treatment and differences in these means across the active GES conditions and the sham-stimulation control. The statistical significance of differences between treatment and control were assessed with model-based t-tests. Linear contrast F-tests were used to assess dose responses of food intake and body weight to increases in GES pulse width as evidenced by a statistically significant linear trend. Reported P values incorporate the Holm step-down Bonferroni adjustment for multiple comparisons, with adjusted P < 0.05 considered statistically significant.

**RESULTS**

Effects of GES on GD-R VMH Neurons

All but the GES settings delivering the lowest levels of stimulation energy significantly affected the discharge rates of the identified GD-R neurons in the VMH. This was true both in terms of the share of GD-R neurons tested that increased or decreased their firing rates by at least 20% relative to pre-GES baseline levels (Table 1) and in terms of the mean absolute changes in discharge rates from pre-GES levels (Fig. 1).

Only increases in pulse amplitude and pulse width were associated with statistically significant positive linear trends in

Table 1. Response of GD-R VMH neurons to GES with different pulse frequencies, widths, amplitudes and train-on times in DIO rats

<table>
<thead>
<tr>
<th>GES Parameters</th>
<th>No./Total Tested</th>
<th>%</th>
<th>Linear Trend Test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pulse Frequency</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 Hz</td>
<td>2/19</td>
<td>10.5</td>
<td></td>
</tr>
<tr>
<td>20 Hz</td>
<td>2/23</td>
<td>8.7</td>
<td></td>
</tr>
<tr>
<td>40 Hz</td>
<td>9/21</td>
<td>42.9*</td>
<td></td>
</tr>
<tr>
<td>100 Hz</td>
<td>10/24</td>
<td>41.7*</td>
<td>P = 0.0682</td>
</tr>
<tr>
<td><strong>Pulse Width</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 ms</td>
<td>0/23</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>0.3 ms</td>
<td>9/21</td>
<td>42.9†</td>
<td></td>
</tr>
<tr>
<td>0.6 ms</td>
<td>13/24</td>
<td>54.2‡</td>
<td></td>
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<tr>
<td>1.2 ms</td>
<td>13/20</td>
<td>65.0‡</td>
<td></td>
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<tr>
<td>3.0 ms</td>
<td>18/23</td>
<td>78.3‡</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td><strong>Pulse Amplitude</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 mA</td>
<td>5/23</td>
<td>21.7*</td>
<td></td>
</tr>
<tr>
<td>6 mA</td>
<td>9/21</td>
<td>42.9*</td>
<td></td>
</tr>
<tr>
<td>10 mA</td>
<td>11/23</td>
<td>47.8‡</td>
<td>P = 0.0307</td>
</tr>
<tr>
<td><strong>Train-On Time</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 s</td>
<td>3/20</td>
<td>15.0</td>
<td></td>
</tr>
<tr>
<td>0.5 s</td>
<td>4/21</td>
<td>19.0</td>
<td></td>
</tr>
<tr>
<td>1.0 s</td>
<td>6/19</td>
<td>31.6†</td>
<td></td>
</tr>
<tr>
<td>2.0 s</td>
<td>9/21</td>
<td>42.9‡</td>
<td>P = 0.0934</td>
</tr>
</tbody>
</table>

Tested neurons are defined as responsive to gastric electrical stimulation (GES) if their unit discharge rate increased or decreased by more than 20% from the baseline recording period. GES parameters other than the one being tested were fixed at 6 mA, 0.3 ms, 40 Hz, 2 s on, 3 s off. Percentages of responsive neurons that are significantly different from zero are indicated as *P < 0.01, †P < 0.001, and ‡P < 0.0001. GD-R, gastric distension-responsive; VMH, ventral medial hypothalamus; DIO, diet-induced obese.
the degree of GES-induced neuronal response across the entire range of parameter values tested. Whereas higher levels of train-on time and pulse frequency both evoked significant changes in the activity of GD-R neurons, the degree of neuronal response reached a plateau within the parameter range tested. In contrast, both the proportion of neurons responding and the mean absolute changes in discharge rates increased progressively with each increase in pulse width and pulse amplitude.

The neuronal response gradient was greatest for increases in pulse width. Under the highest GES pulse width (3.0 ms), 78.3% of GD-R neurons tested altered their discharge rates by 20% or more from basal levels under GES delivery, and the mean absolute change in discharges per second was more than three times higher than that observed under the highest tested values of pulse amplitude, frequency, or train-on time.

Figure 2 contains a sample recording of neuronal activity in a GD-R neuron within and around 1-min periods of GES administration with varying pulse widths. In the case shown, activity of the GD-R neuron is unaffected by 0.1-ms pulse width GES but is inhibited by 0.6-, 1.2-, and 3.0-ms pulse widths, and the degree of inhibition during the 1-min GES treatment periods increases with increasing pulse width.

Food Intake Effects of Increasing GES Pulse Width

Mean food intake was significantly lower under the active GES treatments than under sham stimulation (Fig. 3). Consistent with a pulse width dose response, there was a clear downward trend in food intake with increasing pulse width. Compared with the off control condition, mean restrainer food intake was 9.6%, 21.0%, and 47.3% lower during the 0.5-, 2.0-, and 5.0-ms treatment periods. The observed reductions in food intake were statistically significant for all three pulse widths (\(P < 0.0001\) for 0.5 ms; \(P < 0.0001\) for 2.0 and 5.0 ms), as was the linear trend in food intake with pulse width (\(P < 0.0001\)).

Body Weight Effects of Increasing GES Pulse Width

Food intake suppression during active GES treatment was associated with a suppression of weight gain in a pulse-width-dependent fashion (Fig. 3). Whereas body weight increased an average of 3.2% over the 4-day GES off treatment periods, the mean gains over the 0.5- and 2.0-ms GES treatment periods amounted to only 2.5% and 1.0% of body weight, and under 5.0 ms GES the rats’ body weights actually declined by an average of 0.3%. Percentage weight change was significantly different from the off control treatment under the 2.0-ms (\(P < 0.05\) and 5.0-ms (\(P < 0.001\)) GES treatments but not under the 0.5-ms treatment (\(P = 0.4811\)). The downward trend in percentage weight change with increasing GES pulse width was also statistically significant in the linear trend test (\(P < 0.001\)).

Behavioral Observations

Other than the reduction in food intake under GES treatment, no remarkable changes in routine behaviors (grooming,
GES ALTERS VMH ACTIVITY AND FEEDING IN RATS

Fig. 2. Sample recording of the effects of GES with varying pulse widths on unit discharge rates in a GD-R VMH neuron. **Bottom:** raw trace of extracellular voltage for a GD-R VMH neuron recorded with a stereotactically placed micropipette electrode. **Top:** corresponding histogram of mean discharges per second within 10-s intervals. The arrows point to 1-min periods of GES administration with varying GES pulse widths (0.1, 0.6, 1.2, and 3.0 ms), which are also identifiable by the 40-Hz stimulation artifact. All remaining GES parameters were fixed at 6 mA, 40 Hz, 2 s on, 3 s off. Cell activity and discharge rates in this case were unaffected by 0.1-ms pulses but inhibited by 0.6-, 1.2-, and 3.0-ms pulses. This inhibition notably persists 20–30 s after stimulation is discontinued.

**DISCUSSION**

This study investigated the effects of GES with different parameters on GD-R VMH neurons in DIO rats. VMH neuronal responses were screened for 13 different GES parameter sets by individually varying pulse amplitude, width, frequency, or train-on time away from a base parameter set (6 mA, 0.3 ms, 40 Hz, 2 s on, 3 s off) typical of that used in prior clinical studies. It was found that, although all but the lowest energy GES parameter sets tested produced significant changes in VMH neuronal activity, the largest changes by far occurred with increases in pulse width. We then further investigated the effects of GES with different pulse widths on food intake in DIO rats, finding that GES with 5.0-ms pulses reduced food intake by 47% from sham-GES control levels without inducing any behaviors indicative of pain or discomfort.

The recent failure of a rigorous, double-blind randomized clinical trial (19) to demonstrate the efficacy of GES as an obesity treatment clearly indicates that the viability of the therapy will depend on identifying GES variants that are more effective than those tested in previous clinical studies. This effort requires investigation of the mechanisms and effects of GES with varying electrode configurations and stimulation parameters in animal models. The present study contributes to this effort by using recording of neuronal responses to GES in the VMH, a brain nucleus with well-established links to feeding, to identify which of a range of stimulation parameters had the strongest central neuronal effects. The working hypothesis underlying this exercise was that the GES parameter with the strongest VMH neuronal response gradient would have parallel effects on food intake and body weight. Our neuronal recording and crossover experiment results are consistent with this working hypothesis; increases in pulse width away from the 0.1-to 0.6-ms range used in prior clinical studies produced the largest changes in VMH neuronal activity, and similar increases in pulse width also strongly increased the food intake and body weight-suppressing effects of GES in obese rats.

The findings of the present study suggest that GES with expanded pulse widths may be a viable therapy for obesity; according to the relationship between the weight loss and pulse width shown in Fig. 3, a recommended pulse width would be >3 ms. Possible mechanisms by which expanded pulse width GES may reduce food intake and body weight can be usefully categorized as mechanical, neuronal, and hormonal although it should be kept in mind that these pathways are closely intertwined in the regulation of energy balance.

Mechanically, GES with expanded parameters has been reported to induce gastric distention in rats (31) and dogs (21). The induced gastric distention has been found to correlate with GES-induced reductions in food intake in both lean canines (12) and in DIO rats (unpublished data). GES with expanded parameters has also been found to alter gastric slow waves, induce gastric dysrhythmia, and inhibit antral motility in canines (20, 21). GES with 2.0-ms pulses reduced the percentage of normal slow waves by 23% in healthy dogs and, in a recent study in our laboratory, decreased the postprandial antral motility index in healthy dogs by 34%. GES with 2.0-ms pulses was also found to significantly delay both liquid and solid gastric emptying in dogs (20).

Neuronally, in lean rats GES with pulse widths ≥2.0 ms has been reported to modulate the activity of spinal afferent neurons at T9 and T10 that are implicated in gastrointestinal nociception (15). It has also been shown to modulate neuronal activity in brain nuclei with well-established roles in the regulation of feeding, including vagal afferent neurons at the nucleus tractus solitarius (NTS) in lean rats (16) and in the VMH in both lean (23) and DIO rats (present study). The neuronal effects elicited by expanded pulse width GES were similar to those induced by gastric distention, and more potent than those recorded under GES with pulse widths ≤1.0 ms.

Hormonally, expanded pulse width GES was found to reduce expression of the orexigenic peptides orexin and ghrelin...
in the hypothalamus of lean rats (26, 30) and to increase expression of the anorexigenic peptides oxytocin in the hypothalamus (26) and cholecystokinin in the hippocampus (10). Expanded pulse width GES was also found to reduce gastric tissue ghrelin in DIO rats (29).

Taken together with the present study, these previous studies of the mechanisms by which GES may alter feeding behavior support the potential of expanded pulse width GES to be an effective treatment for obesity. At present the main obstacle to testing the efficacy of expanded pulse width GES in humans is the lack of suitable implantable pulse generators; no presently available for its application.

In conclusion, GES with appropriate parameters alters the activity of GD-R neurons in the VMH, an established brain satiety center. Increases in GES pulse width produced the largest effect on VMH neuronal activity, and these neuronal effects are paralleled by pulse width dose-dependent reductions in food intake and body weight in DIO rats. Lengthening pulse width beyond the range of commercial implantable pulse generators may be critical to making GES an effective obesity treatment.

FIG. 3. Mean food intake and body weight changes over 4-day treatment periods. Plotted values are least-squares means (± SE) of food intake and percentage changes in body weight over 4-day treatment periods from repeated-measures regressions including fixed treatment, time, and rat effects, and estimated using 256 rat × day observations on food intake and 128 pre- and posttreatment observations on body weight. GES parameters other than pulse width were fixed at 6 mA, 40 Hz, 2 s on, 3 s off. The statistical significance of differences between the active GES and GES-off control treatments are indicated in the inset tables: *P < 0.05, **P < 0.001, ***P < 0.0001.

DISCLOSURES
This study was funded by Medtronic. Medtronic employees (Maude-Griffin, Firestone and Starkebaum) participated in the study design, analysis, and manuscript preparation. No conflicts of interest exist for any authors.

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