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Refinement of Perioperative Feeding in a Mouse Model of Vertical Sleeve Gastrectomy

Carolyn M Doerning,[1,*] Lisa A Burlingame,[1] Alfor G Lewis,[2] Andriy Myronovych,[2] Randy J Seeley,[2] and Patrick A Lester[1]

Provision of liquid enteral nutrition (LEN) during the perioperative period is standard practice for rodents undergoing bariatric surgery, yet these diets are associated with several challenges, including coagulation of the liquid diet within the delivery system and decreased postoperative consumption. We investigated the use of a commercially available high-calorie dietary gel supplement (DG) as an alternative food source for mice during the perioperative period. C57BL/6J male mice were fed high-fat diet for 8 to 10 wk prior to surgery. The study groups were: vertical sleeve gastrectomy (VSG) +DG, VSG+LEN, sham surgery+DG, and sham+LEN. Food and water intakes, body weight, and body fat composition was monitored throughout the study. Mice that received DG lost significantly more weight preoperatively than those fed LEN. However, during the postoperative period, body weight, body fat composition, and water and caloric intake were similar among all experimental diet groups. Three mice in the VSG+LEN group were euthanized due to clinical illness during the course of the study. In summary, feeding a high-calorie DG to mice undergoing VSG surgery is a viable alternative to LEN, given that DG does not significantly affect the surgical model of weight loss or result in adverse clinical outcomes. We recommend additional metabolic characterization of DG supplementation to ensure that this novel diet does not confound specific research goals in the murine VSG model.

Abbreviations: DG, dietary gel; LEN, liquid enteral nutrition; VSG, vertical sleeve gastrectomy

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The obesity epidemic continues to grow throughout the United States, with an estimated prevalence of 39.8% in the adult population in 2016.[7] Bariatric surgery remains the most effective long-term treatment option, yet the underlying mechanisms responsible for resolution of obesity and associated comorbidities remains unclear.[1,6,19] Rodent models of bariatric surgery are the predominant means of testing mechanistic and molecular hypotheses regarding the benefits of metabolic surgeries.[1,19] The development and refinement of robust rodent surgical models requires paralleling the clinical treatment of humans, including pre- and postoperative nonsurgical care.

Liquid enteral nutrition (LEN) for the first 24 h after bariatric surgery followed by a gradual transition to solid foods is recommended clinical practice for human patients,[15] and similar guidelines are followed for rodents undergoing bariatric surgery.[2,3,16,22] Transitioning bariatric study animals off a solid, pelleted diet minimizes the risk of postoperative intestinal stasis and obstruction[2] and provides a concentrated source of nutrition and hydration during the immediate recovery period. Acclimation of mice to the LEN diet for several days prior to surgery is recommended, to diminish rodents' natural aversion to novel objects[14,21] and to avoid creating a conditioned taste aversion if the diet were introduced during the postoperative period, when the likelihood of the diet's association with surgical discomfort is high.[2,9,16] LEN is then continued as the sole source of nutrition during the immediate postoperative period, and solid food is reintroduced slowly, with the specific postoperative interval dependent on the surgical model.

At our institution, mice readily consume LEN during the preoperative period, indicating that the diet is palatable. However, laboratory and animal care personnel often report decreased LEN consumption by mice during the immediate postoperative period, with resultant dehydration and lethargy. The decrease in postoperative consumption of the LEN diet may be multifactorial in nature. Liquid diets are delivered in sipper tubes suspended from the wire food hopper, and the act of rearing or stretching to reach the sipper tube during the postoperative period may cause discomfort at the incision site, thus adversely affecting the amount of consumption. In addition, consistency of the LEN diet varies over time and can coagulate in the sipper tube prior to the 24-h change-out period, thus obstructing the outflow through the sipper tube. The increased physical effort required for mice to obtain the coagulated LEN may prohibit them from consuming sufficient nutrition to meet their postoperative requirements. In an effort to find an alternative nutritionally complete diet that mice will reliably consume during the postoperative period, we investigated a high-calorie dietary gel supplement (DG) and compared it with the currently supplied LEN. We hypothesized that mice that underwent a bariatric surgical procedure (here, vertical sleeve gastrectomy [VSG]) and received DG would maintain a more consistent body weight and clinical condition during the postoperative period than mice that underwent VSG and received LEN. To test this hypothesis, we evaluated both direct and indirect markers of rodent health throughout the study. Direct markers of postoperative health included body weight, surgical site condition, pain status, and overall attitude and appearance. Indirect assessments included MRI and measurement of food and water intakes.

Materials and Methods

Animals. The study population (n = 28) comprised male C57BL/6J mice (age, 8 to 10 wk; Jackson Laboratory, Bar Harbor,

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[1]Unit for Laboratory Animal Medicine, and [2]Department of Surgery, University of Michigan, Ann Arbor, Michigan
[*]Corresponding author. Email: cdoernin@med.umich.edu

ME). All study mice were housed at an AAALAC-accredited animal facility at the University of Michigan. All procedures and housing were compliant with the *Guide for the Care and Use of Laboratory Animals*, 8th ed., and were approved by the University of Michigan's IACUC.[8] Mice were free of mouse hepatitis virus, minute virus of mice, mouse parvovirus, enzootic diarrhea of infant mice virus, ectromelia virus, Sendai virus, pneumonia virus of mice, Theiler murine encephalomyelitis virus, reovirus type 3, lymphocytic choriomeningitis virus, mouse adenovirus, polyomavirus, *Mycoplasma pulmonis*, fur mites, and pinworms. Mice had unrestricted access to water and a high-fat diet (catalog no. D12492, DIO High-Fat Diet, Research Diets, New Brunswick, NJ), according to a previously established protocol.[16,22]

Assessment of diet stability. To assess the 24-h viability of leaving study diets within the microenvironment of a ventilated cage, a single freshly opened (foil lid removed) cup of DG (2 oz., DietGel Boost, Clear H_2O, Westbrooke, ME) was placed onto the floor in each of 5 IVC. An additional 5 IVC each contained a freshly opened bottle of LEN (30 mL; Osmolite OneCal, Abbott Nutrition, Lake Forest, IL). LEN was provided by using a bottle apparatus consisting of a 50-mL tube (Falcon 50-mL Conical Centrifuge Tubes, Fisher Scientific, Pittsburgh, PA), metal sipper tube and rubber stopper. The sipper tube was positioned in the wire bar lid, as for the current postoperative feeding protocol (Figure 1). All cages were clean and contained the standard amount of corncob bedding and the 2 forms of enrichment (Cotton squares, Ancare, Bellmore, NY; EnviroPak, Animal Specialties and Provisions, Quakertown, PA) provided to singly housed mice at our institution. No animals were placed in the cages for the desiccation portion of the study. A data logger (EasyLog USB, Lascar Electronics, Erie, PA) was placed on the floor of each study cage and programmed to obtain hourly temperature and relative humidity data. Cages were returned to the housing room and placed on the IVC rack; microenvironmental data were collected every 1 h for 24 h, which was the standard amount of time between replenishing diets. Data were downloaded by using EasyLog USB software and transferred to Excel (Office 2016, Microsoft, Redmond, WA). Two of the 10 data loggers (one in each group) did not record data, but we did not repeat the experiment in light of the consistency of the temperature and humidity readings among the remaining cages. The final weights of the DG and LEN were recorded at the 24-h time point, and the amount of desiccation was calculated as weight difference.

Acclimation and baseline assessments. At 1 wk prior to surgery, mice were separated into individual cages and acclimated to single housing, to accurately track food and water consumption during the study period. According to institutional policy, 2 forms of enrichment were provided to all singly housed mice. Whole-body composition analysis was performed (Echo MRI, Echo Medical Systems, Houston, TX) during the week prior to surgery, after which the mice were assigned into 4 experimental groups, counterbalanced in terms of body fat composition. The groups were: VSG+DG (n = 8), VSG+LEN (n = 8), sham surgery+DG (n = 6), and sham+LEN (n = 6). Starting 3 d prior to surgery, mice were switched from high-fat diet to their respective study diet, in keeping with current laboratory practice.[16] DG was provided in the original plastic cup, with the foil lid removed, and set on the floor of the IVC; LEN was provided in a sipper-tube apparatus suspended from the wire food hopper. All study cages were disconnected from the automatic watering system and provided free access to water by using the sipper-tube set-up described for LEN.

Baseline data were collected for the 3-d acclimation period prior to surgery (that is, days –3 through –1), including the morning prior to surgery (day 0). The following parameters were assessed between 0700 to 0900 daily for each mouse: body weight, food intake, water intake, and general physical disposition. Daily food intake was calculated as the difference between the initial weight of the DG or LEN and that remaining after 24 h. Food-intake data were converted to caloric intake according to number of kilocalories per gram of each respective diet. Daily water intake was calculated by subtracting weight of the sipper-tube apparatus after 24 h from the initial weight. Remaining food and water were discarded at each 24-h time point and replaced with a new, preweighed portion.

Surgery and postoperative care. Mice were anesthetized for surgery by using isoflurane, placed on a heat source, and received analgesics (buprenorphine, 0.1 mg/kg SC; meloxicam, 0.5 mg/kg SC), prophylactic antibiotic (gentamicin, 8 mg/kg SC), and warm sterile saline (1 mL 0.9% NaCl SC) for intraoperative fluid support. Two designated lab members with extensive experience in rodent bariatric surgery performed all VSG and sham surgeries by using aseptic technique and as previously described.[16,22] In brief, both surgeries consisted of a laparotomy at the cranial abdomen through a midline incision (approximate length, 1 to 2 cm). Sham surgery involved an analogous midline laparotomy, with manual pressure applied to the stomach by using blunt forceps. The abdominal muscle and skin were closed in 2 separate layers. Upon recovery, mice were placed into clean cages and provided with their respective study diet once they were observed to be alert and fully ambulatory. All mice received warm sterile saline SC at 24 h after surgery and meloxicam SC every 24 h for 3 d after surgery, with the option to continue analgesia as indicated on the basis of clinical assessment.

Postoperative assessments. The first postoperative time point was at 0700 to 0900 on the morning after surgery (that is, day 1). Fresh food and water were provided to the designated experimental groups as previously detailed for during preoperative acclimation. Evaluation of parameters during the postoperative phase was analogous to preoperative data collection, with the addition of daily pain assessment. Pain status was evaluated subjectively by cageside observation of nesting behavior,[5,10] physical appearance,[12,13] body condition,[20] and reactivity to handling. Postoperative assessment continued for days 1 through 3, and on the morning of day 4, mice were transitioned back to the high-fat diet. Daily monitoring of body weight and postoperative condition was continued during days 5 through 8, at which time surgical monitoring was considered complete. MRI scans were performed on days 3, 14, and 35 after surgery. Mice were transferred to a secondary study after day 35, in an effort to reduce the use of additional animals.

Statistical analysis. Prism 7 (GraphPad, San Diego, CA) was used for all statistical analysis. Unpaired t tests were performed during diet stability assessment to analyze temperature, humidity, and weight data. Two-way repeated-measures ANOVA followed by Sidak posthoc multiple-comparison tests were conducted on body weight, food and water intake, and MRI data, with main effects of treatment group and time. VSG and sham groups were compared to ensure that body weight and body fat composition behaved as expected for the surgical model. The experimental diet groups were compared within each surgical group to detect changes attributable to diet alone. P values of 0.05 or less were considered significant.

Results

Assessment of diet stability. Daily room temperature and humidity measurements, as well as the blower parameters

Figure 1. Initial set-up of study cages. LEN was provided alongside the water bottle in the low-profile food hopper (left). DG was provided on the cage floor, with the water bottle in the low-profile food hopper (right). During assessment of diet stability, the data logger was placed on the cage floor.

for the ventilated rack, remained consistent from the start of assessment through the 24-h endpoint. At the 24-h endpoint, the mean weight change was significantly ($P < 0.0001$) greater for DG than LEN (Table 1). Relative humidity over 24 h was significantly ($P = 0.0167$) greater in cages containing DG than those with LEN (Table 1). Ambient cage temperatures did not differ between diets. These differences in microenvironment remained within acceptable ranges for laboratory mice housing.[8] None of the sipper tubes contained coagulated liquid diet at the 24-h endpoint of the stability assessment. However, several instances of coagulation within LEN tubes occurred throughout the surgical study (Figure 2). Data regarding the frequency of or surgical group with coagulated LEN were not collected.

Body weight and fat composition. Baseline body weight and fat composition (that is, day –3) did not differ significantly between mice. Preoperatively, the individual body weight of each animal was assessed for change from baseline weight allowing for each mouse to act as its own control. Time and diet had significant effects ($P = 0.0128$ and $P < 0.0001$, respectively) on the DG and LEN groups; post hoc testing confirmed significant differences at all time points (Figure 3). The interaction of time and diet was nonsignificant. Mice that consumed DG averaged a greater negative weight change daily as compared with mice that consumed LEN.

We compared the postoperative change in body weight relative to baseline between sham and VSG surgery groups to ensure that VSG created a weight-loss model, as intended. Significant effects of time, treatment group, and their interaction (all $P < 0.0001$) were present, with posthoc testing confirming significance during days 4 through 21; both treatment groups experienced similar weight loss during postoperative days 1 through 3. Weight loss began to increase significantly in VSG mice as compared with sham mice during days 4 through d 21 (Figure 4).

Table 1. Change in diet weight relative to baseline (g), cage temperature (°F), and cage humidity (%) over 24 h

	DG	LEN	*P*
Change in diet weight	1.11 ± 0.10	−0.04 ± 0.10	<0.0001
Temperature	71.7 ± 0.2	71.9 ± 0.1	0.2563
Humidity	38.5 ± 1.2	36.5 ± 0.3	0.0167

Data are given as mean ± 1 SD.

We also evaluated the postoperative change in body weight relative to baseline within each surgical group to compare the effect of the experimental diet. For sham mice, significant effects of time ($P < 0.0001$), treatment group ($P = 0.0284$), and their interaction ($P = 0.0201$) were present. Posthoc testing detected significance at day 21, which was 17 d after reintroduction of solid food (Figure 5). Postoperative changes in the body weight of VSG mice behaved similarly to those before surgery, with a significant effect of time ($P < 0.0001$) and interaction ($P = 0.0004$); post hoc testing confirmed a significant difference at d 21 (Figure 6).

Figure 2. LEN has congealed within the tube within 24 h; flow through the sipper tube was obstructed.

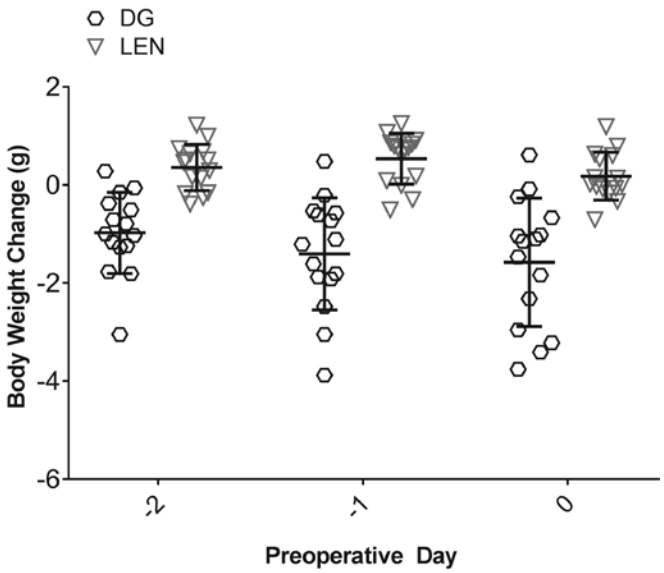

Figure 3. Mean daily change in body weight (g) relative to each mouse's baseline weight. Weight change differed significantly between mice receiving DG and LEN on day –2 ($P = 0.0002$), day –1 ($P < 0.0001$), and day 0 ($P < 0.0001$). Bar, 1 SD.

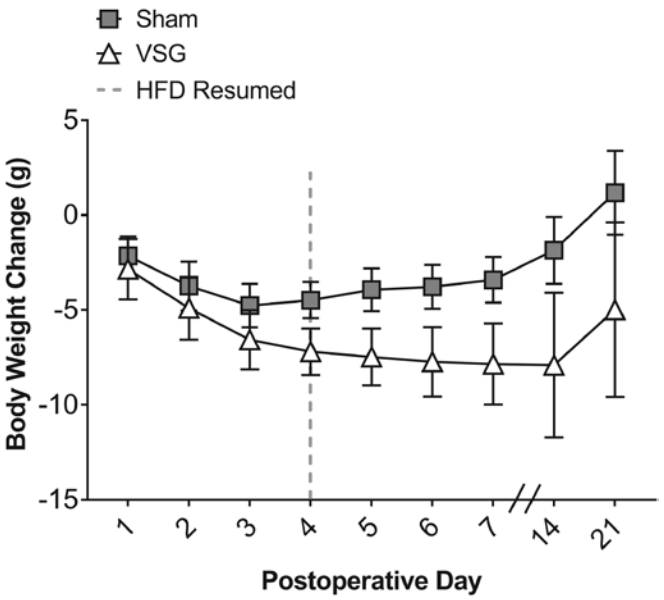

Figure 4. Average daily change in group weight (g) relative to baseline. Significant differences between VSG and sham groups were detected on day 4 ($P = 0.0078$), day 5 ($P = 0.0001$), and days 6–21 ($P < 0.0001$). The dashed line indicates the day on which high-fat diet (HFD) was reintroduced. Bar, 1 SD.

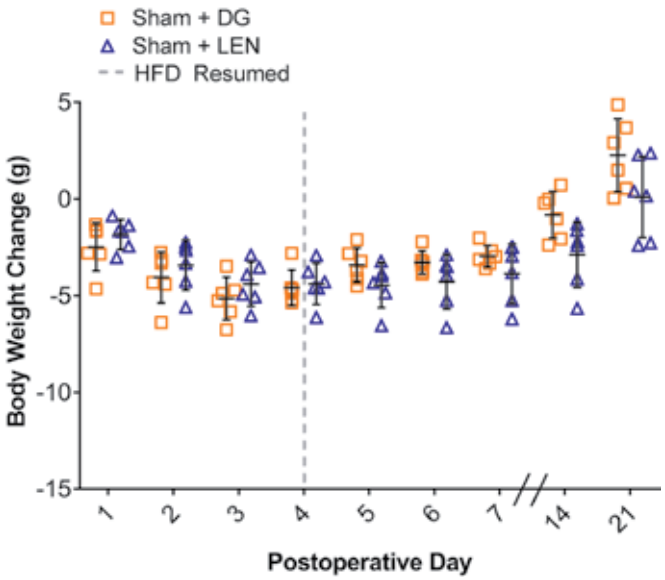

Figure 5. Mean daily change in body weight (g) relative to each mouse's baseline weight: sham surgery group. A significant difference between DG and LEN groups was detected on day 21 ($P = 0.0371$). Bar, 1 SD.

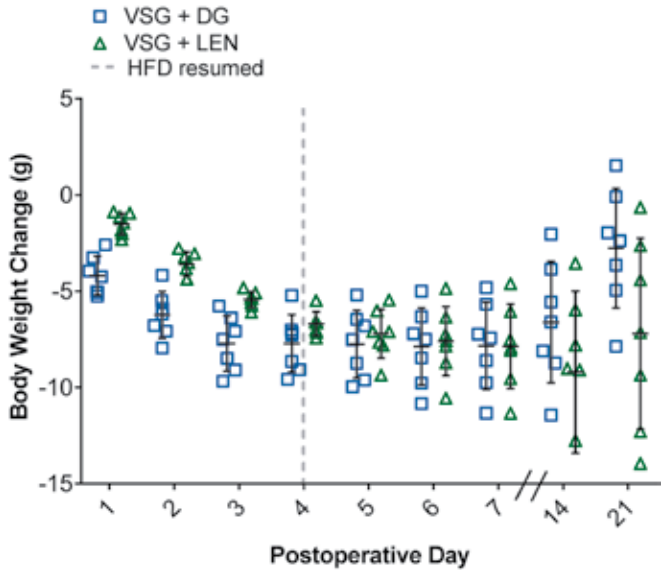

Figure 6. Mean daily change in body weight (g) relative to each mouse's baseline weight: VSG surgery group. A significant difference between DG and LEN groups was detected on day 21 ($P = 0.0033$). Bar, 1 SD.

Total-body MRI results showed significant effects of time ($P < 0.0001$), treatment group ($P = 0.0021$), and their interaction ($P = 0.0113$). Posthoc testing confirmed a significant difference between day-14 fat tissue mass in sham mice compared with VSG mice (Figure 7). No significant difference in fat tissue mass was detected between mice that received LEN compared with DG within their respective surgical groups. Lean tissue mass did not differ significantly between treatments groups at any time point.

No significant differences in caloric intake within a surgical treatment group emerged when DG was compared with LEN.

Postoperative clinical condition. All mice ($n = 28$) recovered from surgery uneventfully. Two mice from the VSG+LEN group were euthanized at d 2 due to postoperative morbidity as determined by clinical assessment; these mice were excluded

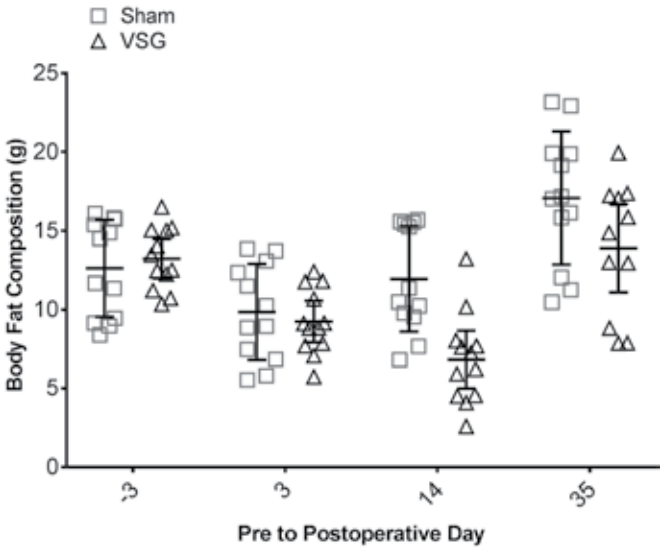

Figure 7. Individual body fat tissue composition (g) as determined by total-body MRI. A significant difference between sham and VSG groups was detected on day 14 (P = 0.0007). Within each surgery group, body fat composition did not differ significantly between DG and LEN groups (data not shown). Bar, 1 SD.

from the postoperative data set. Gross necropsy of both animals revealed signs of intraabdominal hemorrhage associated with the surgical site. A 3rd mouse from the VSG+LEN group was euthanized at day 17 due to abscess formation at the incisional site; the data collected on this mouse were included through d 14. In addition, 3 mice (2 from VSG+DG and 1 from VSG+LEN) were noted on day 16 to have moderate scabbing at the incisional line. Because we attributed these lesions to chewing at the incision, all affected mice received carprofen (5 mg/kg SC once daily for 3 d). The incisions healed without complication; data from these mice were included in the data set, because none of the animals demonstrated significant changes to body weight or hydration status during the course of treatment. No adverse clinical conditions were noted in the remaining 22 mice throughout the course of the study.

Discussion

The assessment of diet stability indicated that DG desiccated more than LEN over a 24-h period in an IVC. This effect can be expected, given that the DG is exposed directly to cage air and therefore subject to an evaporative effect from the cage air-exchange system. Conversely, LEN is protected within the sipper tube and does not evaporate noticeably. Because the initial stability assessment was performed in cages without mice, note that the intracage temperature and humidity likely will vary due to several factors, including the number of mice, presence of shelters, and type of nesting material.[8]

Although the observed weight loss of DG over 24 h averaged 1.5% of the initial diet weight, researchers should consider the rate of desiccation when deciding how to provide any diet and the frequency of replacement. We considered providing the gel directly on the floor of the cage to encourage consumption but opted to provide it in the original container to obtain a more accurate 24-h weight. The manufacturer recommends changing DG every 48 h, and our findings support this recommendation, because the amount of desiccation over 48 h likely will not notably affect the moisture content (25% to 30%) or palatability.

Preoperative changes in body weight for both groups fluctuated within 2 g daily; this variability can be expected when initiating a change in diet. The clinical condition of all mice remained within normal limits during this phase. The finding that mice provided DG had greater weight loss daily than those fed LEN may be due to a variety of reasons. First, acclimation to a gel-based diet might take longer than the current 3-d period. Mice are already accustomed to using a sipper on the automatic watering system, which may contribute to faster acclimation to a liquid food source provided by a similar method. Although both diets are anecdotally reported to be readily consumed by rodents, a standardized preference test performed in prestudy animals may be beneficial in evaluating palatability across study diets. Also of note was the tendency for mice to completely avoid or bury the gel cups during the study (Figures 8 and 9). The burying of novel objects has previously been noted as a stress response in rodents[11,14] and may be responsible for this reaction to the gel. Providing gel directly on the cage floor may facilitate consumption but would interfere with weighing the diet, due to dispersal throughout the cage. In addition, the gel will desiccate more rapidly when the surface area exposed to the cage environment is increased. Furthermore, we noted no chewing of the plastic container throughout the study, but caution should be taken when providing diets in plastic packaging. Pica behavior of rats has been well documented in conjunction with buprenorphine administration, and an alternative method of providing DG should be considered when using it in this species.[4,17] Further investigation into diet preferences and how the diets are supplied within the cage may shed more light on these differences in preoperative consumption, although the presurgical weight fluctuations in the DG group did not appear to result in surgical morbidity.

The postoperative data indicate that DG and LEN achieve similar results in daily body-weight change until day 21, when mice in the sham+DG and VSG+DG had greater average weight gains than mice in both LEN groups. Body composition results did not differ between study diets and showed that weight loss after VSG was attributable to the loss of fat tissue, a finding consistent with previous rodent bariatric studies.[6,18] A larger sample size would be required to determine whether this loss was a long-term effect of the diet or due to interindividual variation. The DG and LEN formulations we used in the current study did not have equivalent nutritional profiles, and DG contained higher protein, fat, and carbohydrate concentrations than LEN. However, total calorie intake during the immediate postoperative period did not differ between both sham and VSG dietary groups. Although calorie intake and body composition remained equivalent between the 2 diets, metabolic changes in lipid handling and glucose tolerance are important factors in the rodent bariatric model, and ensuring that the differences in the dietary composition do not alter the metabolic outcome of the surgical model requires further investigation.[1,6,9]

In terms of postoperative clinical condition, the 2 mice euthanized within the immediate postoperative period showed signs consistent with postoperative hemorrhage, which is a documented adverse consequence of VSG surgery. The mouse euthanized for a subcutaneous abscess was 17 d past surgery, and we detected no definitive communication between the abdomen and the abscess, therefore suggesting superficial contamination of the surgical site. The loss of 3 of 16 mice after invasive abdominal surgery is not uncommon, and we initially planned to have 8 mice in each VSG group, compared with 6 animals for each sham group. Whether the euthanasia of 3 mice from the VSG+LEN group but none from the VSG+DG

Figure 8. The DG container has been buried with bedding and nesting material, suggesting a possible stress response to the novel diet. Photograph taken 24 h after placement in cage.

Figure 9. Various levels of DG manipulation among 4 mouse cages. Photograph taken 24 h after placement in cage.

was coincidental or influenced by the diet remains to be determined and likely requires a larger sample size. A critique of the study design may be our use of 2 surgeons for performing all 28 surgeries, given that it can be a source of variability between animals. However, both surgeons were senior lab members who were experienced in bariatric rodent surgery and capable of completing a VSG procedure within 15 to 20 min. Analogous materials and techniques were used, and we believe the consistency and skill of the surgeons considerably minimized surgery-associated variability.

In summary, use of DG as a perioperative food source for a bariatric surgical mouse model does not lead to changes in body weight or composition significantly different from those associated with the current standard of LEN. Additional benefits of a gel-based diet include the relative ease of providing the diet as compared with obtaining the equipment and manually filling the bottles for providing a liquid food source. In addition, using a gel formulation that can be offered without a delivery system and thus directly within the cage avoids the complication of obstructed sipper tubes, which can occur with liquid diets. Consequently, gel formulations may provide a more reliable and less labor-intensive alternative to the traditional liquid diet. The consistency of the gel diet parallels the recommended clinical practice guidelines of providing human postoperative patients liquefied, pureed, or soft foods,[15] and given that we saw no adverse consequences in the mice that received DG, opting for a gel over a liquid diet does not appear to markedly increase the risk of postoperative stasis or obstruction. We conclude that the use of DG is a clinically safe alternative to a liquid diet for mice recovering from gastrointestinal surgery and results in a similar model of weight loss. Further characterization of the nutritional effects of DG is recommended to ensure that this formulation does not alter the desired disease phenotype in the mouse VSG model.

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References

1. **Arble DM, Sandoval DA, Seeley RJ.** 2014. Mechanisms underlying weight loss and metabolic improvements in rodent models of bariatric surgery. Diabetologia **58:**211–220. https://doi.org/10.1007/s00125-014-3433-3.
2. **Bruinsma BG, Uygun K, Yarmush ML, Saeidi N.** 2015. Surgical models of Roux-en-Y gastric bypass surgery and sleeve gastrectomy in rats and mice. Nat Protoc **10:**495–507. https://doi.org/10.1038/nprot.2015.027.
3. **Chambers AP, Wilson-Perez HE, McGrath S, Grayson BE, Ryan KK, D'Alessio DA, Woods SC, Sandoval DA, Seeley RJ.** 2012. Effect of vertical sleeve gastrectomy on food selection and satiation in rats. Am J Physiol Endocrinol Metab **303:**E1076–E1084. https://doi.org/10.1152/ajpendo.00211.2012.
4. **Clark JA Jr, Myers PH, Goelz MF, Thigpen JE, Forsythe DB.** 1997. Pica behavior associated with buprenorphine administration in the rat. Lab Anim Sci **47:**300–303.
5. **Gaskill BN, Karas AZ, Garner JP, Pritchett-Corning KR.** 2013. Nest building as an indicator of health and welfare in laboratory mice. J Vis Exp **82:**51012. doi:10.3791/51012.
6. **Grayson BE, Gutierrez-Aguilar R, Sorrell JE, Matter EK, Adams MR, Howles P, Karns R, Seeley RJ, Sandoval DA.** 2017. Bariatric surgery emphasizes biological sex differences in rodent hepatic lipid handling. Biol Sex Differ **8:**1–12. https://doi.org/10.1186/s13293-017-0126-x.
7. **Hales CM, Carroll MD, Fryar CD, Ogden CL.** 2017. Prevalence of obesity among adults and youth: United States, 2015–2016. NCHS Data Brief **288:**1–8.
8. **Institute for Laboratory Animal Research**. 2011. Guide for the care and use of laboratory animals, 8th ed. Washington (DC): National Academies Press.
9. **Jackness C, Karmally W, Febres G, Conwell IM, Ahmed L, Bessler M, McMahon DJ, Korner J.** 2013. Very low-calorie diet mimics the early beneficial effect of Roux-en-Y gastric bypass on insulin sensitivity and β-cell function in type 2 diabetic patients. Diabetes **62:**3027–3032. https://doi.org/10.2337/db12-1762.
10. **Jirkof P.** 2014. Burrowing and nest building behavior as indicators of wellbeing in mice. J Neurosci Methods **234:**139–146. https://doi.org/10.1016/j.jneumeth.2014.02.001.
11. **Kedia S, Chattarji S.** 2014. Marble burying as a test of the delayed anxiogenic effects of acute immobilisation stress in mice.

J Neurosci Methods **233:**150–154. https://doi.org/10.1016/j.jneumeth.2014.06.012.

12. **Langford DJ, Bailey AL, Chanda ML, Clarke SE, Drummond TE, Echols S, Glick S, Ingrao J, Klassen-Ross T, Lacroix-Fralish ML, Matsumiya L, Sorge RE, Sotocinal SG, Tabaka JM, Wong D, van den Maagdenberg AM, Ferrari MD, Craig KD, Mogil JS.** 2010. Coding of facial expressions of pain in the laboratory mouse. Nat Methods **7:**447–449. https://doi.org/10.1038/nmeth.1455.
13. **Leach MC, Klaus K, Miller AL, Scotto di Perrotolo M, Sotocinal SG, Flecknell PA.** 2012. The assessment of postvasectomy pain in mice using behaviour and the Mouse Grimace Scale. PLoS One **7:**1–9. https://doi.org/10.1371/journal.pone.0035656.
14. **Londei T, Valentini AMV, Leone VG.** 1998. Investigative burying by laboratory mice may involve nonfunctional, compulsive behaviour. Behav Brain Res **94:**249–254. https://doi.org/10.1016/S0166-4328(97)00162-9.
15. **Mechanick JI, Youdim A, Jones DB, Garvey WT, Hurley DL, McMahon MM, Heinberg LJ, Kushner R, Adams TD, Shikora S, Dixon JB, Brethauer S, American Association of Clinical Endocrinologists; Obesity Society; American Society for Metabolic & Bariatric Surgery.** 2013. Clinical practice guidelines for the perioperative nutritional, metabolic, and nonsurgical support of the bariatric surgery patient—2013 update: cosponsored by American Association of Clinical Endocrinologists, the Obesity Society, and American Society for Metabolic and Bariatric Surgery. Endocr Pract **19:**337–372. https://doi.org/10.4158/EP12437.GL.
16. **Pressler JW, Haller A, Sorrell J, Wang F, Seeley RJ, Tso P, Sandoval DA.** 2014. Vertical sleeve gastrectomy restores glucose homeostasis in apolipoprotein A-IV KO mice. Diabetes **64:**498–507. https://doi.org/10.2337/db14-0825.
17. **Schaap MW, Uilenreef JJ, Mitsogiannis MD, van 't Klooster JG, Arndt SS, Hellebrekers LJ.** 2012. Optimizing the dosing interval of buprenorphine in a multimodal postoperative analgesic strategy in the rat: minimizing side effects without affecting weight gain and food intake. Lab Anim **46:**287–292. https://doi.org/10.1258/la.2012.012058.
18. **Stefater MA, Perez-Tilve D, Chambers AP, Wilson-Perez HE, Sandoval DA, Berger J, Toure M, Tschop M, Woods SC, Seeley RJ.** 2010. Sleeve gastrectomy induces loss of weight and fat mass in obese rats, but does not affect leptin sensitivity. Gastroenterology **138:**2426–2436.e3. https://doi.org/10.1053/j.gastro.2010.02.059.
19. **Stefater MA, Wilson-Perez HE, Chambers AP, Sandoval DA, Seeley RJ.** 2012. All bariatric surgeries are not created equal: insights from mechanistic comparisons. Endocr Rev **33:**595–622. https://doi.org/10.1210/er.2011-1044.
20. **Ullman- Culleré MH, Foltz CJ.** 1999. Body condition scoring: a rapid and accurate method for assessing health status in mice. Lab Anim Sci **49:**319–323.
21. **van Gaalen MM, Steckler T.** 2000. Behavioural analysis of 4 mouse strains in an anxiety test battery. Behav Brain Res **115:**95–106. https://doi.org/10.1016/S0166-4328(00)00240-0.
22. **Wilson- Pérez HE, Chambers AP, Ryan KK, Li B, Sandoval DA, Stoffers D, Drucker DJ, Perez-Tilve D, Seeley RJ.** 2013. Vertical sleeve gastrectomy is effective in 2 genetic mouse models of glucagon-like Peptide 1 receptor deficiency. Diabetes **62:**2380–2385. https://doi.org/10.2337/db12-1498.