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Efficacy of Supplemental Diet Gels for Preventing Postoperative Weight Loss in Mice (*Mus musculus*)

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This study investigated whether the use of commercially available diet gels prevented the postoperative weight loss associated with major survival surgery in mice. C57BL/6 mice were divided into 3 groups (*n* = 9 per group) that received moistened chow pellets alone or with one of 2 commercially available diet gels. Mice began receiving the test diets 3 d before surgery (baseline) and were weighed daily for 7 d after surgery. On day 0, mice underwent ventral midline laparotomy, during which the intestines were manipulated for 2 min and a segment of jejunum was briefly clamped. Compared with the baseline value for the same group, body weights for the mice that received moistened chow only were significantly lower on all postoperative days (days 1 through 7). In contrast, body weights of mice that received both moistened chow and diet gel differed from baseline only on days 2 and 3 for one product and were never different from baseline for the other product. This study indicates that the combination of diet gel and moistened chow prevented or mitigated postoperative weight loss after a laparotomy procedure in mice.

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Introduction

Weight loss is commonly used as an indirect indicator of pain and distress. In addition, postoperative weight loss is an important concern regarding mice used for major survival surgical procedures.[27] Given the small size and high metabolic rate of mice, even a small loss of weight can lead to increased morbidity and mortality.[27] Many IACUCs require weight monitoring, with a loss of 15% to 20% of baseline body weight[32] or otherwise severe weight loss constituting a humane endpoint.[27] Some recent studies suggest that weight loss greater than 10% should be considered as a humane endpoint.[32] However, the need to euthanize animals for humane reasons prior to the experimental endpoint can increase the number of mice needed to finish a project. Although weight loss is a common postsurgical complication that should be prevented or minimized, information on how to accomplish this goal is scarce.

Enteral nutritional support is known to enhance postoperative recovery and reduce complications associated with weight loss in humans[28,33] and other species.[36] In humans, enteral nutritional support is commonly used in patients who are at risk of malnutrition, either at home or in the hospital, including postoperatively.[1] Many animal facilities[2] encourage the use of moistened chow or diet gel on the cage floor to increase ease of access, hydration, and food consumption during the postoperative recovery period in mice.[20] Although these nutritional support techniques are widely applied, their effectiveness for preventing postoperative weight loss has not been evaluated. In mice, diet gels are the most widely used enteral nutritional support and vary widely in their macronutrient, micronutrient, and moisture contents. Prior research has found that supplementation with commercially available diet gels is a procedural refinement for the prevention of weight loss in radiation[22] and gastric bypass[6] models. Diet gels are most often categorized as hydration supplements, nutritional supplements, or nutritionally complete.[3] For example, HydroGel (ClearH2O, Westbrook, ME) and PureWater Gel (BioServ, Flemington, NJ) primarily focus on providing an alternative method of hydration;[19,22] DietGel Boost (ClearH2O) is a nutritional supplement that includes vitamins and electrolytes;[11] and NutraGel (BioServ) is a nutritionally complete (AIN 93G) purified diet.[7] AIN 93G is the current standardized formula from the American Institute of Nutrition (AIN) for a rodent diet that is intended to support early growth and reproduction.[25] Although moistened rodent chow and diet gels are used widely for nutritional support, little is known about their efficacy for preventing postsurgical weight loss in mice.

In this study, our goal was to determine whether supplementing moistened rodent chow with diet gel decreased postoperative weight loss after mouse laparotomy. We hypothesized that the addition of either of the 2 diet gels evaluated would attenuate weight loss after laparotomy in mice.

Materials and Methods

Animals. Equivalent numbers of adult male and female C57BL/6 mice (*Mus musculus*; *n* = 27 [*n* = 9 per group]; The Jackson Laboratory, Bar Harbor, ME) were used. Sentinel mice were free of minute virus of mice, mouse hepatitis virus, mouse rotavirus, Theiler murine encephalomyelitis virus, Sendai virus, murine adenovirus 1 and 2, ectromelia virus, lymphocytic choriomeningitis virus, pneumonia virus of mice, respiratory enteric virus 3 (Reovirus 3), *Mycoplasma pulmonis*, endo- and ectoparasites, and pinworms via routine serology, necropsy, and PCR testing. Mice were singly housed in IVC caging (Innovive, San Diego, CA) that was pre-filled with corncob bedding, fed an irradiated commercial diet (Teklad

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Global 18% Protein Rodent Diet 2018, Harlan Laboratories, Madison, WI), provided with acidified bottled water (Innovive, San Diego, CA) and Enviro-dri (Lab Supply, Fort Worth, TX) for bedding and enrichment. Rooms were maintained on a 12:12-h dark:light cycle at 68 to 79 °F (20 to 26 °C) and 30% to 70% relative humidity. Experiments were approved by Stanford University's Administrative Panel for Laboratory Animal Care. All mice were treated in accordance with the *Guide for the Care and Use of Laboratory Animals*.[14] Mice were acclimated to the facility for a minimum of 3 d before baseline testing. Mice were weighed daily from 3 d prior to surgery until euthanasia at 7 d after surgery. Mice were euthanized by carbon dioxide asphyxiation, followed by cervical dislocation.

Study design. Mice were randomly assigned to 1 of 3 diet groups to receive moistened chow pellets alone or supplemented with one of 2 commercially available diet gels (a vitamin and nutritional supplement, DietGel Boost, ClearH2O; a nutritionally complete product, NutraGel, BioServ; Table 1). To acclimate mice, they were provided with their assigned diet beginning 3 d before surgery. Chow was moistened by using acidified water and placed on the floor of the cage (Figure 1). Diet gels were placed in paper huts (Shepherd Shacks, Shepherd Specialty Papers, Watertown, TN) to prevent the mice from upending the containers (Figure 1), and approximately 10 to 12 g of diet gel (an amount calculated to provide a mouse's daily caloric requirement) was provided and refreshed daily to prevent desiccation. Mice were weighed every morning between 0800 and 0900 beginning 3 d before surgery. In addition, mice were observed daily for other clinical signs of pain and distress.

Surgical procedure. On day 0, isoflurane-anesthetized mice underwent laparotomy under aseptic technique. Mice were induced in an induction chamber at 3% to 5% isoflurane and then maintained by nose cone on 1% to 2% isoflurane in 100% O_2 (0.5 L/min) on a circulating water blanket (Stryker, Kalamazoo MI). The eyes were protected with ophthalmic lubricant (OptixCare, Aventix Animal Health, Burlington, Ontario, Canada). The abdomen was shaved and then aseptically prepped with alternating povidone–iodine betadine and alcohol swabs. The mice then were transferred to receiving isoflurane via a nose cone, placed on a heat pad set to 38 °C, and given sustained-release buprenorphine (0.6 mg/kg SC; Wedgewood Pharmacy, Swedesboro, NJ) and cefazolin (30 mg/kg SC; Hikma Pharmaceuticals, London, UK).

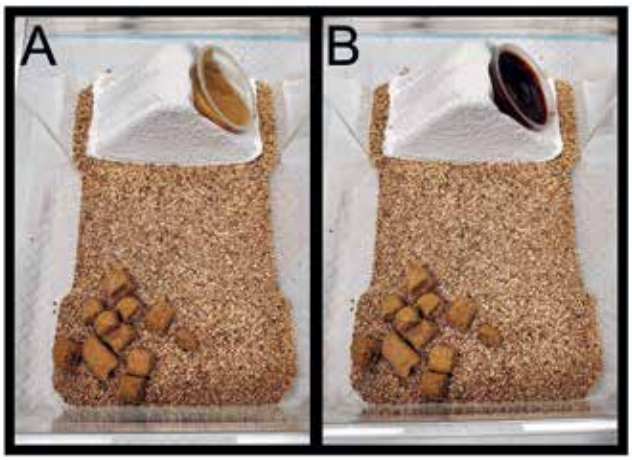

Figure 1. (A) The nutritionally complete diet gel and (B) the vitamin and nutritional supplement were placed in the paper huts. Moistened chow was distributed on the bottom of the cage

Warm 0.9% NaCl (10 mL/kg SC) was administered; the heating pad was used throughout anesthesia. Mice were draped in sterile cling wrap (Press 'N Seal, Glad, Oakland CA), and a small window was made to expose the surgical site. The depth of anesthesia was confirmed via toe pinch before the surgical incision was made. A 2-cm cutaneous ventral midline incision was followed by a 1.5-cm incision through the abdominal wall. The intestines were manipulated for 2 min by using closed tissue forceps, followed by clamping of a segment of the midjejunum for 5 s.[17] The abdominal wall was closed with 5-0 absorbable monofilament suture (Monocryl, Ethicon, Rariton, NJ), and the skin was closed with 2 wound clips (Alzet, Cupertino, CA) and 2 drops of sterile surgical glue (VetBond, 3M, Two Harbors, MN). Mice were recovered in an empty cage lined with paper towels and on a heat pad. Once the mice were ambulatory, they were transferred to their home cage.

Statistical analysis. To examine differences within groups over time and between groups at the same time point, body weights were analyzed by using the F test in ANOVA with repeated measures followed by Bonferroni correction for multiple comparisons. In addition, data were tested for normality by using R software (R Core Team, Vienna, Austria). Data are expressed as mean ± SEM. A P value of less than 0.05 was considered significant.

Table 1. Macro- and micronutritional contents (per 100 g of diet) of chow and diet gels used in this study, as reported in the manufacturers' catalogs

	Chow	Vitamin and nutritional supplement[a]	Nutritionally complete diet gel[b]
Calories	310	369	155.7
Protein, g	18.6	9.9	10.8
Carbohydrates, g	44.2	37.8	20.8
Fat, g	6.2	21.6	3.2
Moisture, %	not reported	25–30	< 70
Calcium, mg	1000	93.9	203.5
Phosphorus, mg	700	264.0	110.6
Potassium, mg	600	558.4	146.0
Sodium, mg	200	185.3	49.1

[a]DietGel Boost, ClearH2O, Westbrook, ME
[b]NutraGel, BioServ, Flemington, NJ

Results

Because baseline body weights (that is, weights on day -3) varied among individuals, all weights were normalized to each subject's baseline weight. Data for male and female mice were then combined.

Weights did not differ significantly among the 3 groups on days −3, −2, and −1 (laparotomy performed on day 0). Compared with the day −3 value, significant ($P < 0.05$) decreases in weight occurred on days 1 through 7 after surgery in mice that received moistened chow only and on days 2 and 3 in those that received a combination of moistened chow and vitamin-containing diet gel. Mice that received moistened chow supplemented with the nutritionally complete diet gel showed no significant differences in weight after surgery (Figure 2).

The weights of mice fed moistened chow and the vitamin supplement differed significantly from those of mice fed moistened chow alone on days 2, 3, 6, and 7 after surgery. In addition, as compared with the chow-only controls, mice that received

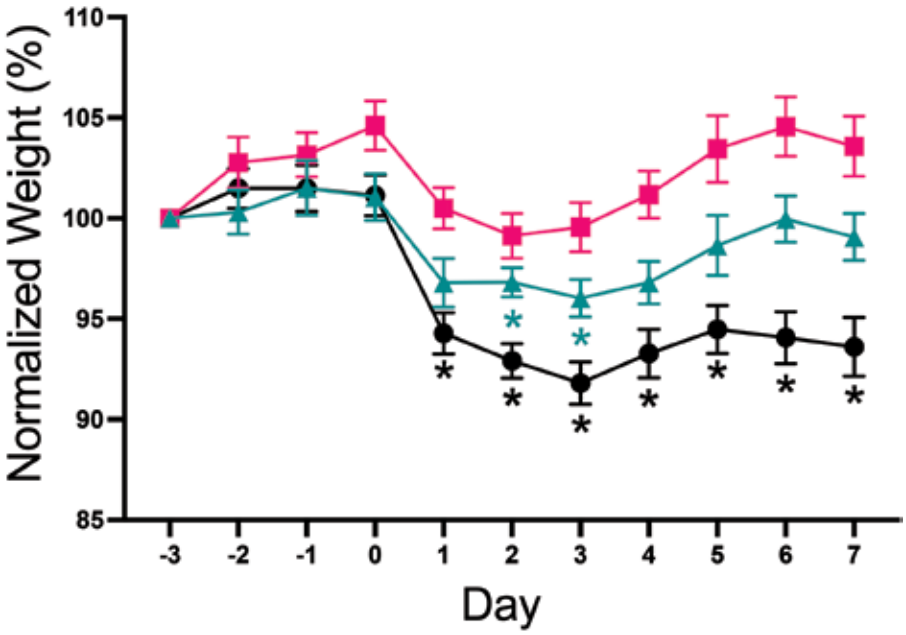

Figure 2. Time course of mouse weights, individually normalized to their body weights on day -3. Normalized weights of mice fed moistened chow only (black) differed between day −3 (the preoperative baseline) and all 7 d after surgery, Mice fed both moistened chow and the vitamin supplement (teal) differed from baseline only on days 2 and 3 after surgery. Normalized weights of mice fed moistened chow and the nutritionally complete diet gel (pink) did not differ from that on day −3 (preoperative baseline) throughout the 7 d postoperative period. *, Value significantly ($P < 0.05$) different from that on day −3; $n = 9$ mice per group.

moistened chow and the nutritionally complete diet gel showed no decrease in weight on any of the 7 d of the postoperative observation period (Table 1).

Discussion

The goal of our study was to examine whether adding a diet gel to moistened chow decreased postoperative weight loss after a laparotomy in mice. When mice were given a nutritionally complete diet gel, their postoperative weight on days 1 through 7 did not differ from that at baseline (day −3; laparotomy performed on day 0). Adding a diet gel providing additional vitamins and nutrition limited postoperative weight loss (4% to 5%) to days 2 and 3, with a return to baseline weight on D4. Daily body weight assessment has been used as a measure of animal wellbeing.[17] The current data support our hypothesis that the combination of a diet gel and moistened chow decreases the amount of postoperative weight loss after a laparotomy procedure in mice.

A surgical model was necessary to achieve significant and prolonged weight loss sufficient for evaluation of the effectiveness of supplemental diet gels for the study. We explicitly used a laparotomy with intestinal clamping that was kmown to induce significant weight loss.[17] A pilot study showed that this model induced weight loss in mice for at least 7 d and is very translatable to a variety of laparotomy-based studies. In addition, we provided preemptive analgesia by using a single dose of sustained-release buprenorphine, as in previous studies.[17,23] This mode of pain management is standard at our institution and is broadly considered an important refinement to rodent surgeries.[8,23,34] For the control group and to increase ease of access, we placed moistened chow on the cage floor rather than in a food hopper, thus following current recommendations for postoperative enteral support at our institution and others. Placing moistened chow or mash on the cage floor is a common practice in a variety of mouse surgical and other models.[10,15,26] In the chow-only control group, the laparotomy procedure resulted in at least 7 d of postoperative weight loss. Because we did not continue to record weight loss beyond 7 d in the current study, we do not know how long weight loss might persist. Our anecdotal experience indicates that weight commonly rebounds after 14 d, thus corresponding with published data.[27]

Nutrition is critically important to recovery after surgery.[5,35] Postoperative nutritional support has been studied extensively in humans, for whom enteral support practices more invasive than those used in our mice are commonly implemented to improve outcomes.[4,12] Providing sufficient nutrition postoperatively is an essential refinement to for mouse surgery. The most critical factor in wound healing is sufficient calories,[9] but the complete nutritional profile should be assessed. Among the chow and diet gels we evaluated, the macronutritional breakdowns were markedly different with regard to the moisture, carbohydrate, and fat contents, and the micronutritional constituents of calcium, phosphorus, potassium, and sodium (Table 2). However, the protein content, which is a key factor wound healing,[35] was similar in the 2 diet gels. Human research has shown beneficial effects of specific amino acids,[13,31] but similar comparisons in mice are beyond the scope of our study.

An important difference between the 2 diet gels evaluated is that one product (DietGel Boost, ClearH_2O) is a vitamin and

Table 2. Daily normalized weights of mice that received diet gel and moistened chow compared with that of mice fed moistened chow only

	Experimental Day										
	−3	−2	−1	0	1	2	3	4	5	6	7
Vitamin supplement	–	–	–	–	–	*	*	–	–	*	*
Nutritionally complete gel	–	–	–	–	*	*	*	*	*	*	*

*,Value significantly ($P < 0.05$) different from that of the chow-only mice on the same day; –, no difference in weight compared with that of chow-only mice on the same day. All groups contained 9 mice.

calorically dense nutritional supplement for weanling, aged, and postoperative mice and cannot be used to replace a complete rodent diet. The manufacturer of this supplementary diet gel does offer other diet gels that are nutritionally complete relative to various standard rodent diets (for example, 93M, 31M, 76A, GEM). The lower moisture content and higher fat content of the vitamin and nutritional supplement results in a thick paste-like texture rather than a gel-like consistency. In comparison, the nutritionally complete diet gel that we evaluated (NutraGel, BioServ) has more traditional gel consistency and was slightly easier to distribute. In addition, the flavors of the 2 diet gels are different (DietGel Boost, peanut-butter flavor; NutraGel, bacon flavor);[21] in our experience, mice readily accepted both of these. Both diet gels provided effective postoperative support; the diet gel should be selected based on preferences of the researcher and the mice. We recommend testing a diet gel before use to determine whether mice show any preference. If neither of the gel diets that we evaluated is palatable to particular mouse strains, other flavors (for example, molasses, pumpkin) are available but were not evaluated in the present study.

A novel component of this experiment was providing the diet gels to the mice beginning 3 d before surgery to reduce neophobia.[18] A common complaint of many researchers is that mice or rats do not eat supplemental diet gels after surgery. When our study mice were acclimated to the diet gels, they gained weight prior to surgery. Another common problem with diet gels is that they can become quickly soiled. We recommend using a device to elevate containers holding the diet gel, such as the paper huts we used in the current study (Figure 2). The paper huts prevented the diet gel from getting contaminated with bedding and feces. Neither the bedding nor paper huts we used are sterilized by the manufacturer, so we recommend autoclaving them before use.

Several limitations to our study should be considered. For this study, mice were housed singly so that we could evaluate individual consumption of the diet gel and chow. We do not recommend routine individual housing of mice due to their social nature.[16,24] When mice are socially housed, we recommend providing a full container of diet gel in each cage to reduce competition and to ensure sufficient diet gel for all mice. Another limitation is that we assessed only one strain of mouse; taste preferences can vary among strains of mice and rats.[21,29,30] For the current study, the diet gel was replaced daily to ensure that sufficient clean product was available. Although replacing the diet gel daily does increase labor somewhat, this factor may be negligible considering that daily postoperative rechecks are often required; providing additional diet gel can be coordinated with these evaluations. Future research should determine whether supplementation of moistened chow with diet gels is effective in other mouse or rat surgical models (orthopedic, cardiovascular, craniotomies) as well as other disease models (neoplasia, neurologic, degenerative). Other commercially available diet gels should be compared as well.

In summary, our data indicate that providing a supplemental diet gel prevents weight loss after a laparotomy procedure in mice. We recommend providing the supplemental diet gel beginning 3 d before surgery as a refinement to the postoperative care and support of rodents.

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