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Effects of High-protein and High-fiber Diets on Weight and Glucose Regulation in Spiny Mice (*Acomys cahirinus*)

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Despite the long-term contributions of the spiny mouse (*Acomys cahirinus*) to research, basic knowledge of appropriate nutrition is lacking for this species. In the wild, spiny mice eat a high-fiber, high-protein food source. In the research setting, spiny mice are prone to obesity that can lead to diabetes mellitus. Common dietary modifications for weight control in humans with diabetes mellitus consist of increased fiber and protein. We hypothesized that increasing the dietary protein or fiber of spiny mice would reduce weight gain and improve their glycemic control, whereas the combination of protein and fiber in the diet would achieve optimal weight management and glycemic control without diet-related pathologic changes. We randomly assigned cages of young adult spiny mice (*n* = 34) to one of 4 diets: high protein (HP), high fiber (HF), a combination of both high protein and high fiber (HPF), or the base (control) diet (BD). Over the 8-wk study, spiny mice given HF diets maintained baseline weights despite the elevated dietary protein. None of the diets altered blood glucose levels; all diet groups maintained mean blood glucose levels within normal ranges. Spiny mice seem particularly sensitive to changes within their environment, as seen by increased food waste and transient elevated blood glucose levels when the spiny mice were transitioned to novel diets. The short-term elevations in protein and fiber that we tested were well tolerated by spiny mice. Although HF was effective in controlling weight, the ideal percentage of fiber still needs to be determined. The combination diet (HPF) maintained weight and body condition scores and showed a nonsignificant elevation of blood glucose that warrants a longer diet trial before our recommending this specific combination.

Abbreviations and Acronyms: BCS, body condition score; BD, base diet; HF, high fiber; HP, high protein; HPF, high protein and high fiber; DM, type 2 diabetes mellitus

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Introduction

Acomys cahirinus (Cairo spiny mice) have been used for over 20 y to study neonatal organogenesis, skin and organ regeneration,[24] social behavior,[12] and diabetes mellitus (DM).[32,35] Their use has increased in recent years after further characterization of their unique regenerative properties and applications for modeling tissue repair, including skin, organs, bone, and nervous tissue.[18,24,28,31] Despite long-term contributions, gaps in our knowledge about basic physiologic characteristics for spiny mice still exist (e.g., the optimal diet, which is a commonly overlooked research variable).

In many research settings, spiny mice are fed research diets developed for mice of the *Mus* genus, despite differing natural history, phylogenetic relationships, and disease predispositions. Spiny mice are desert animals, native to the North African deserts and, despite their name, are more closely related to gerbils (*Gerbillinae* spp.) than animals of the *Mus* genus.[1,7]

In the natural setting, spiny mice are omnivorous, favoring insects over plant-based food.[23] In a preference test among wild-caught Egyptian spiny mice, arthropods (crickets) were preferred followed by grains (barley), greens (alfalfa), and finally snails.[23] Other studies of spiny mice in the natural setting showed arthropods to be 40 to 65% of the diet.[2,23] The nutritional profile of insects varies depending on the species. In general, arthropods can provide 290 to 760 kcals/g. Protein content varies from 13 to 77% with common invertebrates like mealworms consisting of 50% protein. Locusts, which are a preferred food item of spiny mice, are about 77% protein, which is among the highest in arthropods. Furthermore, arthropods are covered in chitin, an indigestible fiber of about 2 to 135 mg/kg of dry weight depending on the species.[19,21,22] However, preference tests are often criticized because they are not considered to represent the true nutritional needs of a species,[29] which may be the case for spiny mice.

Type 2 diabetes mellitus, which occurs in captive spiny mice, is exacerbated by a high-fat diet and obesity. Spiny mice are prone to obesity when fed experimental high-fat diets.[16] In contrast, a diet of 50% sucrose resulted in no gain in adipose tissue.[33,34] The pathogenesis of (DM) in spiny mice is distinct from human type 2 DM and is characterized by low insulin release from pancreatic β-islet cells despite an increase of insulin production in the face of hyperglycemia; insulin retention leads to glucose intolerance, hypertrophy and lysis of the pancreatic β-islet cells, pancreatitis, and occasional glycogenic nephrosis.[14,32,35] Possibly because of the low circulating insulin levels, spiny mice do not show the characteristic peripheral insulin resistance seen with DM in other species, including

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humans. Hyperglycemia and hyperlipidemia also occur with advanced DM in spiny mice,[35] leading to downstream metabolic stress.[13,30]

This study aimed to narrow the current knowledge gap by providing insights into the dietary needs and physiologic responses of spiny mice by using common dietary recommendations for human DM. Human DM treatment often is accompanied by dietary guidelines that include dietary increases in either fiber or protein.[9,25,38,40] Dietary protein promotes satiety while increasing insulin circulation and supports the maintenance of lean muscle.[17] Although the effect dietary protein has on long-term blood glucose levels is still unclear, increased protein intake has been linked with improved cardiovascular indices in humans with DM.[5] In people, dietary fiber reduces mortality in patients with DM[6,15] and lowers the risk of cardiac disease, colon cancer, and obesity by promoting satiety while providing fewer calories and sugars.[8]

We hypothesized that increasing dietary protein or fiber will regulate weight gain and improve glycemic control in spiny mice, while the combination of protein and fiber in the diet would be the best option for weight management and glycemic control without diet-induced pathologic effects. We evaluated metabolic, hematologic, and physiologic parameters of spiny mice maintained on a commercially available rodent diet, base diet (BD), or fed a novel diet of high-fiber (HF), high-protein (HP), or a combination of both high-protein and high-fiber (HPF). A histopathologic analysis was performed to elucidate trends across primary organs resulting from these dietary modifications.

Materials and Methods

Animals. Young adult (7- to 29-wk-old) male (n = 27) and female (n = 7) spiny mice were obtained from 2 separate established closed colonies (University A, n = 2; University B, n = 22) and were also bred at Emory University (n = 10) in a breeding colony established using spiny mice obtained from University A. Spiny mice were weaned between 42 and 48 d of age[18] and were then separated into same-sex groups. Spiny mice obtained from external sources were maintained in their previously established same-sex groups. Groups were housed in clear polycarbonate cages (R20PC; Ancare, Bellmore, NY) with hardwood chipped bedding (Sani-chip; Envigo, Indianapolis, IN), reverse osmosis water in bottles ad libitum, and uniform environmental enrichment of one nondestructible (Bio-Huts; Bio-Serv, Flemington, NJ) and one destructible hide (Crawl Balls; Bio-Serv), a wooden block, and one Manzanita stick (Manzanita Wood Gnawing Sticks, Bio-Serv) per animal. Environmental parameters were within ranges recommended by the *Guide for the Care and Use of Laboratory Animals* for mice (*Mus* spp.): temperature 22 ± 2 °C (72 °F), humidity 55 ± 15%, and 12:12 light:dark photoperiod (on-off times of 0700 and 1900). Cages, bedding, food, and water bottles were changed weekly; enrichment was changed when visibly soiled, damaged, or no longer useful (destructible hide, Manzanita stick). Experimental protocols were approved by Emory University's IACUC. The animal facility at Emory University is AAALAC International accredited.

Routine disease surveillance was performed using dirty bedding (Emory University, University A), mouse sentinels exposed to dirty mouse bedding (University B), and EAD filter testing (University A). Although the following agents are not know to affect spiny mice, the vivarium they were housed at Emory University excludes lymphocytic choriomeningitis, lactate dehydrogenase elevating virus, mouse adenovirus, mouse cytomegalovirus, mouse hepatitis virus, mouse parvovirus, minute virus of mice, mousepox, mouse rotavirus, mouse polyoma virus, pneumonia virus of mice, mouse reovirus, Sendai virus, Theiler murine encephalomyelitis virus, *Mycoplasma pulmonis*, mites (*Myobia*, *Myocoptes*, *Radfordia* spp.), and pinworms (*Syphacia* and *Aspiculuris*). Although not excluded, animals housed in the vivarium routinely test negative for *Streptococcus* spp., *Bordetella pseudohinzii*, *Corynebacterium* spp., *Klebsiella* spp., *Pneumocystis*, *Pseudomonas aeruginosa*, *Salmonella* spp., *Streptococcus pneumoniae*, *Giardia*, *Helicobacter* spp., *Spironucleus muris*. *Rodentibacter heylii*, *Helicobacter ganmani*, *Trichomonas* spp., and *Entamoeba* spp. were detected on routine surveillance in this spiny mouse population with no associated clinical signs noted.

Study design. Social housing was prioritized because of the highly social nature of this species; however, we also used some spiny mice that were being housed singly because of a lack of compatible social groups (n = 8). Cages of 1 to 3 same-sex siblings were assigned by computerized simple randomization to one of 3 experimental diets (HP, HF, and HPF) or the BD. Veterinarians, data collectors, and the pathologist were blind to the diet assignments. The diet groups were comprised of mixed ages and sexes (Table 1).

The base diet (5P07 Rodent/Mouse/Hamster 1000; LabDiet, St. Louis, MO) is a closed-formula, natural-ingredient, pelleted diet and was used as the foundation for the formulation of all experimental diets. The experimental diets (TestDiet, St. Louis, MO) were formulated by a nutritionist and were nutritionally different only in protein and fiber content (Table 1). The BD contained 16.4% protein and 3.5% fiber, the HP diet 30% protein and 4.3% fiber, the HF diet 17.5% protein and 14.8% fiber, and the combination HPF diet 22.9% protein and 12% fiber. Because of the nature of natural ingredient diets, the nutritional compositions could not be matched exactly across diets, which caused other slight differences among the diets in addition to fiber and protein. Differences in fat (%) and caloric value are minimal and were not expected to significantly alter measures of interest (Table 1). Nutrient sources were similar for all diets and included porcine products, alfalfa, soybean, wheat, and corn in various percentages to achieve appropriate protein and fiber proportions. Spiny mice received the assigned diets exclusively for 8 wk; food-based enrichment was not provided during this time.

Food grinding (manipulation of food into small or fine particles) resulted in food waste and reduced the accuracy of measuring food consumption. Food and food waste were weighed weekly to track food consumption, which was monitored throughout the study by using the following formula: (dry weight of collectable food waste present at the weekly cage change) – (total weight of food provided since the previous weekly cage change).

Data collection. Spiny mice were physically examined weekly by a veterinarian at 1200 ± 2 h The same veterinarian performed physical exams of all the spiny mice in a cage throughout the study. Physical exams consisted of body weights (Adam Equipment LBK6a calibrated scale, Hogentogler, Columbia, MD), assigning a body condition score (BCS), and evaluating external physical parameters including gait and posture, haircoat, eye and ear position, and activity level. All spiny mice had been acclimated to these collection procedures, which were routinely performed during weekly cage changing activities (Figure 1).

BCSs were assigned from 1 to 5, with half steps as deemed appropriate, by the veterinarian in a blind fashion using parameters similar to those commonly assessed in other species. A comparison of BCS assigned by different veterinarians for individual spiny mice did not reveal any clinically relevant

Table 1. Diet descriptions and key differences in composition

Appearance	Diet	Group composition	Protein (%)	Fiber (max, %)	Fat (ether extract %)	Calories (kCal/g)
	BD	*n* = 8; 0 females	16.4	3.5	6.2	3.53
	HP	*n* = 9; 0 females	30	4.3	6.5	3.42
	HF	*n* = 9; 3 females, 6 males	17.5	14.8	6.0	3.01
	HPF	*n* = 8; 3 females, 5 males	22.9	12.0	7.1	3.13

Fiber is total fiber, combining both dietary and functional fiber.

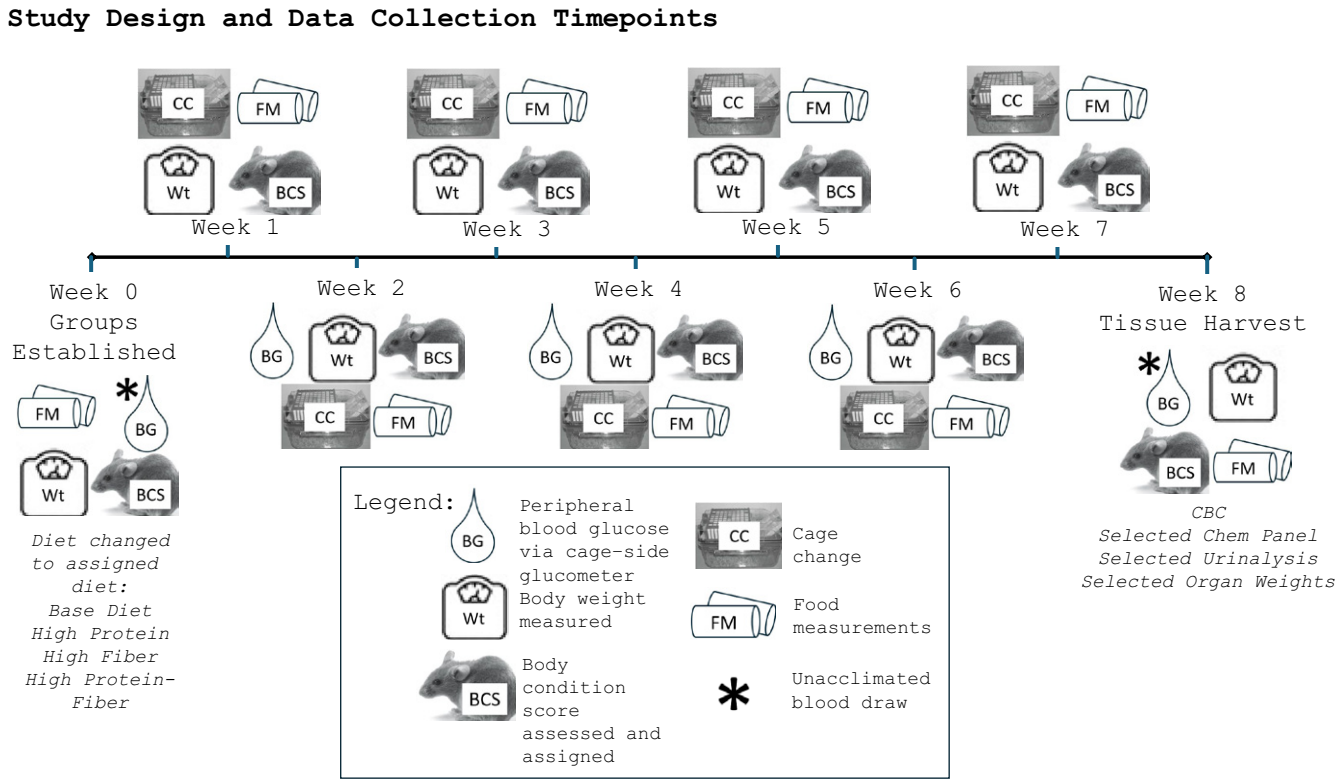

Figure 1. Timeline showing key time points over the 8-wk diet trial. Most data were collected weekly, except for blood glucose cage-side collection, which was collected at baseline and then every other week.

differences in assigned scores. A BCS of 1 was defined as emaciated, 2 was underweight, 3 was ideal, 4 was overweight, and 5 was obese. Palpation of fat deposits over bony prominences (ribs, vertebral processes, pelvis), around the neck, and those affecting the silhouette of the mouse were the primary factors influencing the assigned BCS (Figure 2). A species-specific attribute used as a secondary parameter was the crowding of the spines along the dorsum when the spiny mouse was in a relaxed position, with all 4 paws on the ground. The skin between the spines became increasingly visible as the spiny mouse approached a BCS of 5/5. Dorsal skin was not visible in spiny mice with an ideal BCS of 3/5.

Blood was collected every other week at 1400 ± 2 h across all groups. Because of their skin fragility during handling, spiny mice were not acclimated before the baseline blood collection to minimize the overall number of times they were restrained. Approximately 50 µL of blood was collected from the lateral saphenous vein using a 25- or 23-gauge needle; the resulting drop of blood was collected directly onto the glucometer strip. A disposable bouffant cap was used for gentle restraint to facilitate conscious collections (a modified version of the "spiny mouse bouquet" technique[10]) (Figure 3). A calibrated Accu-Chek Aviva Plus Meter (Roche Diabetes Care, Indianapolis, IN) was

Figure 2. Appearance of spiny mice with a body condition score (BCS) of (A) 3/5, (B) 4/5), and (C) 5/5. The dorsal spinal splay increases as the BCS approaches 5/5. When viewing the spiny mouse from the front, the silhouette starts to resemble a teardrop shape as the BCS increases because of the deposition of fat around the neck and shoulders. A prominent shawl of fat deposition can be seen around the neck for an obese (5/5) animal.

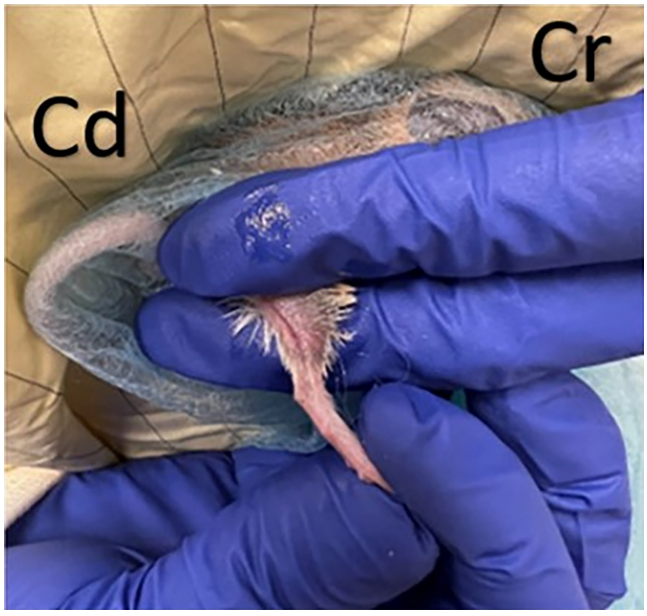

Figure 3. Exposure of the right lateral saphenous vein using a modified "spiny mouse bouquet" restraint technique employing a disposable bouffant cap for primary gentle restraint. Cr and Cd are, respectively, the cranial and caudal aspect of the spiny mouse.

used exclusively for cage-side blood glucose measurement. The Accu-Chek Aviva Plus Meter was chosen based on the consistent and accurate data it provides in mice.[26] At the end of the 8-wk study period, euthanasia was performed by administration of carbon dioxide into the home cage. Blood was collected at euthanasia via cardiocentesis and blood glucose was immediately measured using the cage-side glucose monitor. Selected blood chemistry parameters were evaluated using a point-of-care-testing platform (iSTAT Alinity v; Zoetis, Louisville, KY). The remaining blood was distributed between an EDTA tube (MiniCollect EDTA Tubes; Greiner Bio-One, Monroe, NC) for a complete blood count (Vetscan HM5; Zoetis) that was performed within 16 h after collection and a lithium heparin tube (MiniCollect Plasma Tubes; Greiner Bio-One) that was stored at −80 °C until processed for blood chemistry. Thawed plasma was separated by spinning the lithium heparin-treated samples for 20 min at 3,000 rpm. Biochemical analysis (Vet Axcel; Alfa Wassermann, West Caldwell, NJ) was performed for alanine aminotransferase, alkaline phosphatase, cholesterol, triglycerides, albumin, total protein, amylase, and creatine kinase.

Spiny mice with blood glucose greater than 300 mg/dL at euthanasia (determined by the cage-side glucose monitor), had urine collected if present (n = 22) via cystocentesis during gross necropsy. Urine was evaluated using a urine reagent strip (Urocheck 7; Clarity Diagnostics, Boca Raton, FL); analyzing

glucose, protein, ketones, erythrocyte presence, and pH measurements were prioritized in the order as written here until the sample was depleted.

The liver, spleen, pancreas, and kidneys were collected from all spiny mice. The liver, spleen, and left and right kidneys were individually weighed during necropsy and normalized to body weight by the following formula: (organ weight/body weight) × 100, expressed as a percentage of body weight. All tissues were placed in 10% neutral buffered formalin. Tissues were embedded in parafilm wax, sectioned into 4-µm slices, and mounted on positively charged glass slides. Histologic sections were stained with hematoxylin and eosin for routine histopathologic analysis and evaluated by a board-certified veterinary pathologist who was blind to the diet groups.

Immunohistochemistry was performed on formalin-fixed, paraffin-embedded pancreatic sections. Sections were deparaffinized by 2 washes in xylene, then rehydrated in graded alcohol (100%, 95%, and 70%) solutions. Antigen retrieval was performed by exposing the sections to Proteinase K (Dako 53020; Dako, Carpinteria, CA) digestion solution for 5 min at room temperature. Endogenous peroxidase activity of the sections was blocked by incubation of the sections in 3% H_2O_2 (Fisher H324-500; Fisher Scientific, Hampton, NH) for 5 min at room temperature. The sections were then blocked with 1:10 diluted Universal Blocking Reagent (BioGenex HK085-5K; BioGenex Laboratories, Fremont, CA) for 5 min at room temperature. Sections were incubated with insulin antibody (Sigma 18510; Sigma-Aldrich, St. Louis, MO) at 1:2000 dilution for 30 min at room temperature and again with biotinylated anti-guinea pig antibody (Vector BA7000; Vector Laboratories, Newark, CA) diluted to 1:300 for 10 min at room temperature. Finally, sections were incubated with 4+ Streptavidin HRP Label (BioCare Medical HP604H; BioCare Medical, Pacheco, CA) for 10 min at room temperature and again with Betazoid DAB Chromogen Kit (BioCare Medical DBD2004L) for 12 min at room temperature.

Statistical analysis. Group size was guided by a priori power analysis. For differences between 2 group means on independent *t* tests with equal variance, a large effect size (minimum significant difference divided by standard deviation) of 1.5 and a significance level of 0.05, 9 animals per diet group, were found to be sufficient to achieve a power of 0.80.[11] For differences between 2 group medians, with a large (0.9) probability of an observation being greater in one group, 8 mice per diet group would be sufficient to achieve a power of 0.79.[27]

All data analysis was conducted via R-Studio (version 4.1.0) and SAS (version 9.4). All tests were 2-sided, and significance levels were set at 0.05. Continuous variables were reported as mean ± SD if normally distributed, and median IQR if nonnormally distributed. Statistical testing was conducted based on diet grouping. Because of nonnormal distributions of biomarker outcomes, the Kruskal–Wallis test was used to test for any difference in the 4 diet group medians, and Bonferroni-adjusted Mann–Whitney *U* tests were used to perform all pairwise comparisons of group medians.

Results

Weight. Spiny mice consuming high-fiber diets (HF, HPF) maintained weight closer to their respective starting weight over time. BD spiny mice were significantly heavier than spiny mice on HPF and HF diets ($P = 0.01$ and 0.0004, respectively). Similarly, HP spiny mice were significantly heavier by week 8 as compared with HPF and HF spiny mice ($P = 0.0003$ and <0.0001, respectively) (Figure 4). The weights of BD and HP spiny mice were not significantly different from each other at the end of the 8-wk experimental diet trial, nor were the weights of HF and HPF spiny mice.

On average, spiny mice consuming HF had a slight weight loss from their starting weight (week 0) yet maintained a BCS between 2.5 and 5. The least amount of weight gain over time

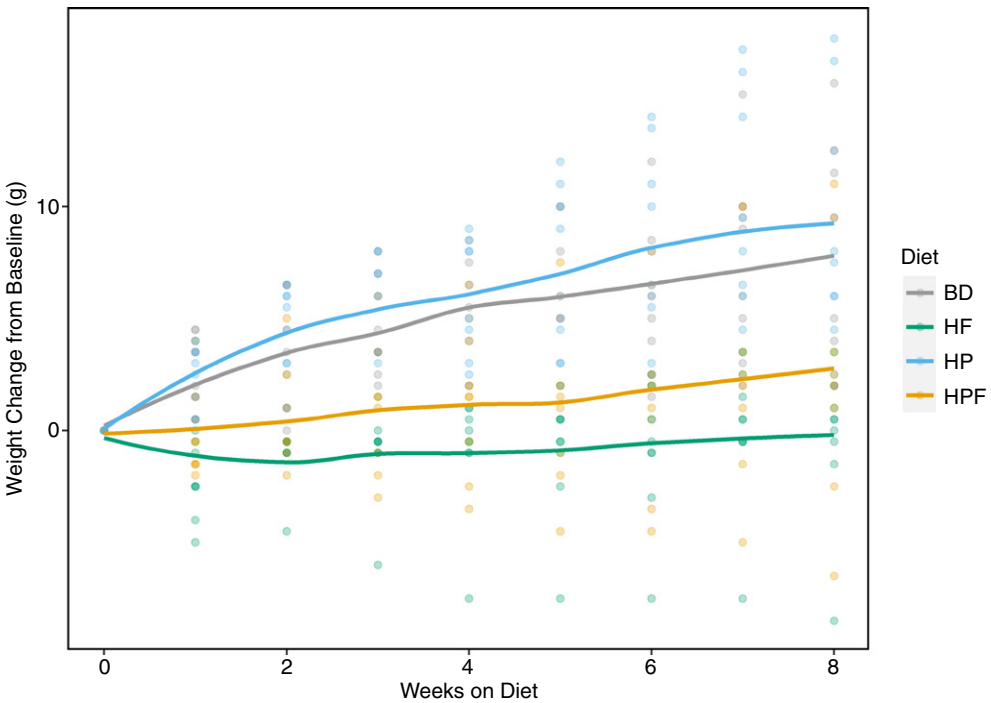

Figure 4. Weight change from baseline over time by diet. Weekly weights for all animals are shown by a solid line representing the mean of all data points (●) for the diet group. The mean baseline weights for HP (37.61 g), HPF (39.63 g), HF (39.44 g), and BD (37.5 g) were not significantly different ($P = 0.896$). Weight change was significantly different by diet ($P < 0.0001$) over the 8-wk study period.

occurred in HF spiny mice. Likewise, spiny mice that received the HP diet showed the most weight gain over time (Figure 5).

Published guidelines are not available for the average body weight of spiny mice. Therefore, both weight and BCS were evaluated to assist in diet comparison across age groups. Among all diet groups, the average weight at the end of the 8-wk experimental period of spiny mice that were within 0.5 points of an ideal BCS (2.5 to 3.5) was 40.7 g, and the average age was 132 d.

All novel diets were consumed at similar rates and a faster rate than BD (Figure 6). Initially, food waste increased for all novel diets, perhaps because the spiny mice were not familiar with experimental diets. Food waste was present in 72% of cages (15 of 18) after the first week of the study and fell to 55% of cages (10 of 18) by week 8. Before week 6, BD was consumed at a significantly higher rate than were the novel diets (BD-HP $P < 0.02$; BD-HPF $P < 0.05$; BD-HF $P < 0.016$), concurrent with periods with high food wastage. Significant differences in food consumption were not detected among diets after week 6.

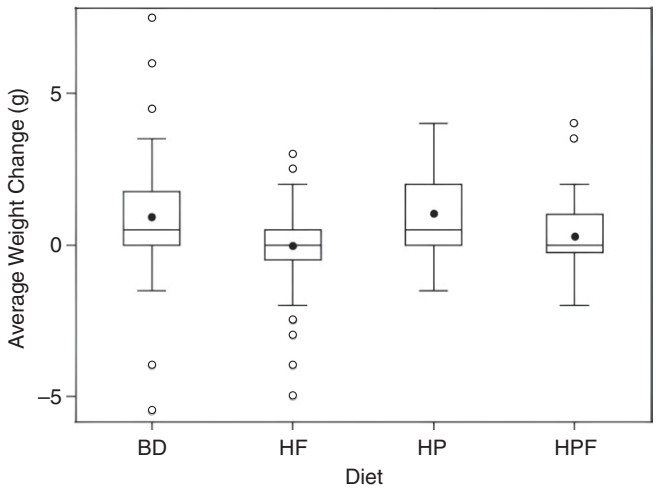

Figure 5. Average weight change in grams per week compared by diet, showing mean (●), minimum-[lower quartile-median-upper quartile]-maximum, and outliers (o) for diet groups.

Blood glucose. Blood glucose levels were not significantly affected by diet (Figure 7). The range of blood glucose values during experimental weeks 2 to 6 was 80 to 193 mg/dL across all diets. The median blood glucose level in BD spiny mice was between 100 and 125 mg/dL. Among the 4 groups, BD spiny mice showed the least variability over the 6-wk experimental period.

Blood glucose levels were not significantly different across diets. The novel diet groups (HP, HPF, and HF) had mean blood glucose above 125 mg/dL at the baseline (week 0) sampling time point, with a range of 102 to 247 mg/dL. By week 2, the second blood collection, the mean blood glucose levels in spiny mice fed novel diets fell below 125 mg/dL. Spiny mice that were fed BD and not transitioned to a novel diet maintained blood glucose levels under 125 mg/dL for all conscious blood collection procedures.

Spiny mice fed HP had slightly higher blood glucose levels by week 6 because of higher minimum levels that developed in the cohort over time. Spiny mice fed the HF diet had the broadest range of blood glucose levels at baseline; the range decreased starting in the second week, giving this group the smallest range in blood glucose levels at week 6.

Spiny mice fed HPF had the largest range in blood glucose levels, with a difference of 103 mg/dL between the lowest and highest reading by the last acclimated blood collection (week 6). There was a simultaneous decrease in the minimum and increase in the maximum blood glucose within the cohort. Animals receiving this combination diet were unable to consistently maintain blood glucose levels under 120 mg/dL, with 75% of the sampling points showing blood glucose levels above 120 mg/dL. Within acclimated samples (weeks 2 to 6),

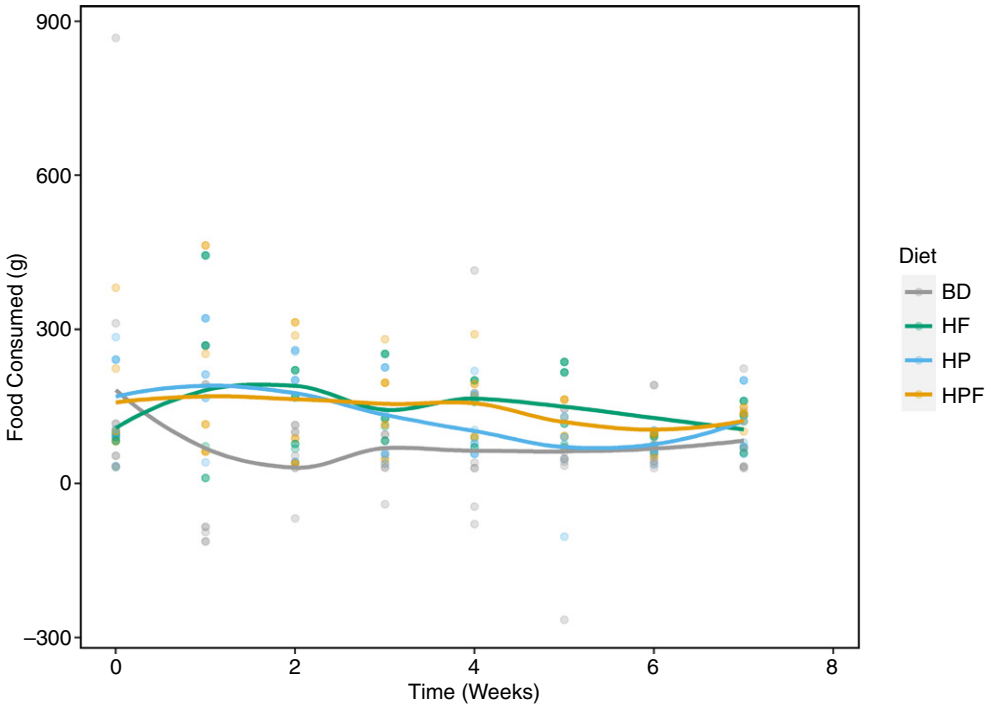

Figure 6. Food consumed (g) per week by diet. As compared with the BD, consumption of all novel diets was significantly higher ($P < 0.05$) at weeks 1 and 3, HP and HF ($P < 0.015$) at week 2, and HF ($P = 0.0123$) at week 5. Food waste from grinding activity resulted in variability from week to week; wasted food was decreased by week 6.

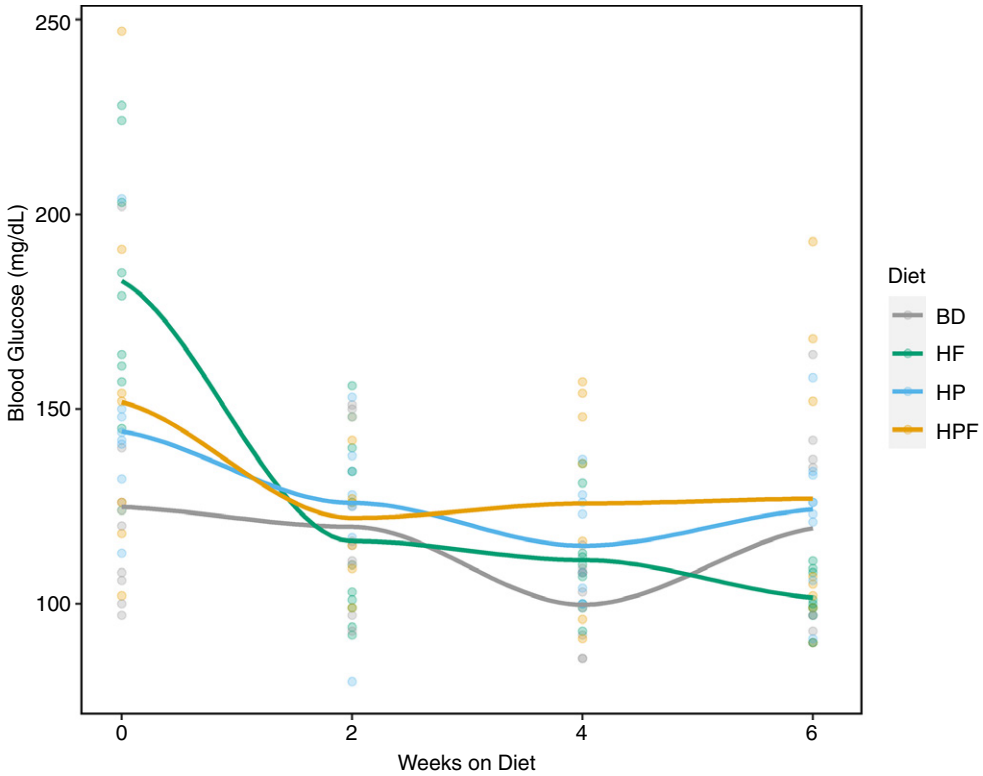

Figure 7. Blood glucose (mg/dL) over time by diet. Biweekly blood glucose values from conscious spiny mice are shown as a solid line representing the mean of all data points (●) for the diet group.

66% of the elevations in blood glucose levels among animals fed HPF were toward the end of the study period showing a trend toward consistently elevated blood glucose levels.

Blood glucose levels at week 8 were significantly higher than those measured at week 6. At week 8, blood was collected immediately after euthanasia, a stressful procedure. On average, the blood glucose values at week 8 were 229 mg/dL higher than at 6 wk across all diets (Figure 8). None of the dietary modifications significantly affected blood glucose levels measured at euthanasia. Animals fed protein-laden diets, HP and HPF, had a difference in median blood glucose levels of 215.6 and 210.9 mg/dL, respectively, between the acclimated (week 6) and high-stress (week 8) blood collections. HF spiny mice had an increase of 247.3 mg/dL in median blood glucose levels, and BD spiny mice had an increase in median blood glucose levels of 255.6 mg/dL in response to the stressful event.

Blood glucose levels at week 8 were measured on both the handheld glucometer and a point-of-care machine. No significant difference was noted between the 2 methods ($P = 0.0615$) across all diet groups (Figure 9). The point-of-care machine consistently had higher readings (398 ± 78 mg/dL) than the handheld monitor (357 ± 92 mg/dL).

Blood parameters. Biomarker parameters measured from spiny mice fed the novel diets (HF, HP, and HPF) were compared with BD, and the HF and HP diets were compared with the HPF diet (Table 2). In all 3 novel diets, plasma sodium levels were significantly lower than BD values BD ($P = 0.05$). Spiny mice on high-fiber diets (HF, HPF) had significantly lower plasma amylase levels than did spiny mice on BD ($P = 0.003$). Spiny mice fed HP had significantly higher hematocrit values as compared with both BD and HPF ($P = 0.002$). Spiny mice fed the HF diet had significantly lower hematocrit values than did BD spiny mice ($P = 0.010$).

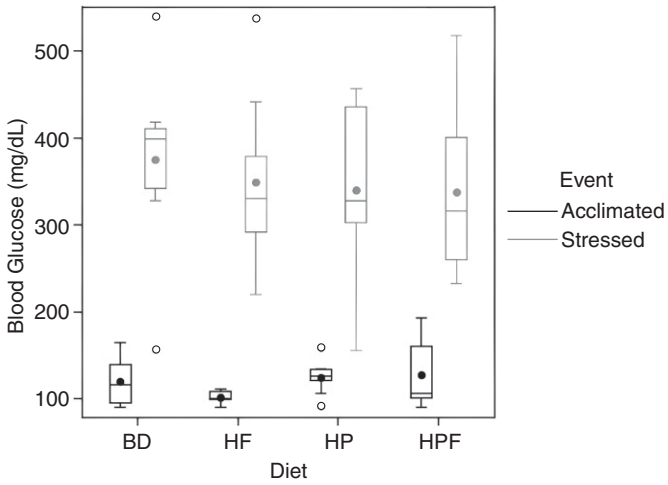

Figure 8. Blood glucose (mg/dL) after euthanasia as compared with in vivo collection at week 6, showing mean (●), minimum-[lower quartile-median-upper quartile]-maximum, and outliers (o) for diet groups. Blood glucose was measured using the same calibrated cage-side glucometer for each time point.

HF was the only diet that significantly reduced plasma total protein values ($P = 0.017$), even though BD had a similar protein content. Spiny mice fed HF also had significantly lower plasma albumin values as compared with all other diets ($P = 0.026$). Thus, high dietary fiber was associated with low circulating protein levels regardless of whether the spiny mice received additional dietary protein. As compared with the other diet groups, BUN was significantly higher in spiny mice fed the HP diet ($P = 0.003$).

Histopathology. None of the spiny mice showed significant histopathologic changes in the liver, pancreas, spleen, or kidneys. In contrast to other rodents, all spleens grossly appeared to

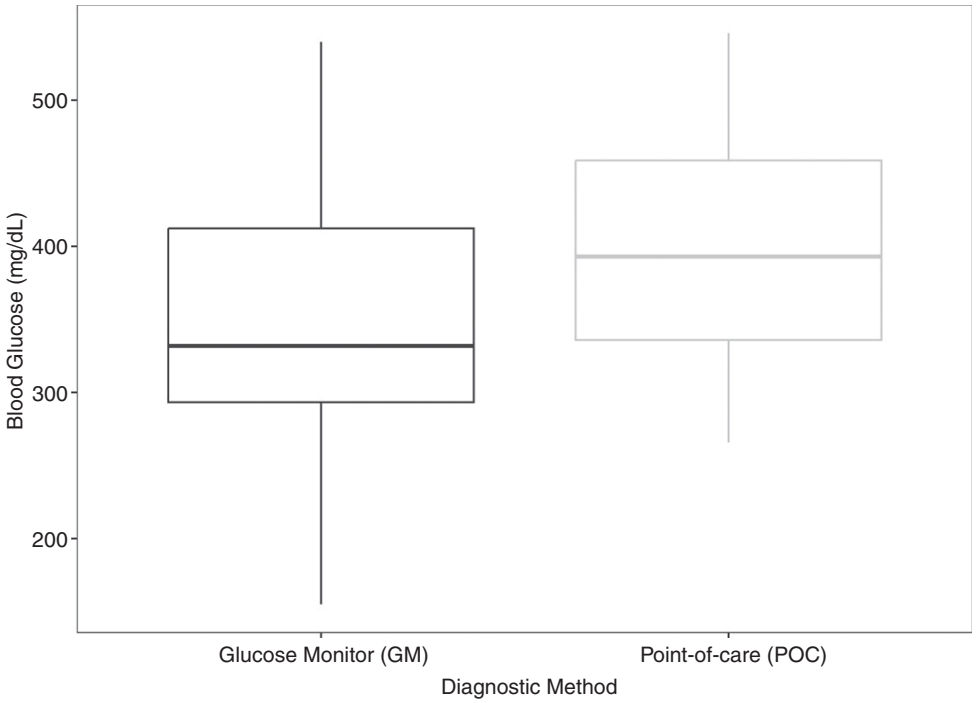

Figure 9. Blood glucose measurement compared by diagnostic method showing minimum-[lower quartile-median-upper quartile]-maximum for each diagnostic method.

Table 2. Selected biomarkers compared across diets

Biomarkers	BD (n = 8)[a]	HF (n = 9)[a]	HP (n = 9)[a]	HPF (n = 8)[a]	P value[b]
White blood cells (10^9/L)	11.30 (7.35, 12.88)	8.13 (6.99, 9.54)	11.54 (10.85, 15.65)	9.72 (8.65, 11.93)	0.067
Lymphocytes (10^9/L)	10.45 (7.13, 11.94)	7.88 (6.74, 9.26)	10.64 (10.04, 15.12)	9.35 (8.34, 10.61)	0.082
Hematocrit (%)	46.5 (44, 48)	44 (39, 44)[c]	49 (47, 49)[a,b,c,d]	46 (45.5, 47)	0.002
Liver weight: body weight	1.46 (1.18, 1.55)	1.25 (1.15, 1.39)	1.53 (1.50, 1.60)[b,d]	1.37 (1.16, 1.38)	0.026
Total protein (g/dL)	7.8 (7.6, 8.1)	6.2 (5.6, 6.8)*[,c,d]	7.6 (6.65, 8.5)	7.6 (7.1, 8.4)	0.017
Albumin (g/dL)	3.6 (3.5, 3.8)	2.8 (2.6, 3.3)*[,c,d]	3.7 (3.3, 4.1)	3.4 (3.2, 4.0)	0.026
Alkaline phosphate (U/L)	148 (139, 162)	126 (44, 148)	173 (142, 198)	132 (118, 163)	0.048
Blood urea nitrogen (mg/dL)	28 (27.5, 31)	27 (25, 29)[b,d]	35 (32, 41)[a,b,c,d]	32 (30, 33)[a,c]	0.003
Amylase (U/L)	1,541 (1,453, 1,766)	972 (700, 1,186)[a,c]	1,272 (506, 1,679)	1,173 (1,075, 1,409)[a,c]	0.005
Sodium (mmol/L)	153 (151.2, 153)	149 (149, 152)[a,c]	150 (150, 151)[a,c]	150 (147.5, 151.5)[a,c]	0.05

The P value listed is for all groups. Novel diets (HF, HP, and HPF) were compared with BD; HF and HP were in addition compared with HPF in Bonferroni-adjusted Mann–Whitney U all-pairwise comparison tests with significance shown for each comparison. Biomarkers not shown here were not significantly different between diets.
[a]Median (IQR).
[b]Kruskal–Wallis test.
[c]$P < 0.05$ compared with BD.
[d]$P < 0.05$ compared with HPF.

be striped, showing an interrupted linear pattern of prominent white pulp with no microscopic pathology (Figure 10).

Grossly, the livers of all spiny mice had a prominent reticular pattern that was consistent with glycogen storage on histopathologic review. This pattern can occur postprandially and often resolves with normal metabolism.[37] Histologically, the livers of all spiny mice had mild hepatocytic anisokaryosis and binucleation (a histologic feature of both mice and gerbils).[36] Among all livers evaluated, 23.5% showed evidence of scant lipid storage within the liver. The presence of hepatic lipidosis did not correlate with any specific variable of interest, including age (P = 0.36), sex (P = 0.51), or diet (P = 0.65). Spiny mice with hepatic lipidosis had significantly lower plasma ALT (62 ± 20 U/L) than did spiny mice without hepatic lipidosis (ALT 126 ± 137 U/L) (P = 0.033). None of the other evaluated parameters had a similar pattern. The average liver-to-body weight ratio of all spiny mice was 3.2. Spiny mice with evidence of hepatic lipidosis did not have a significant elevation of the liver-to-body weight ratio (3.3).

None of the pancreas samples in any of the spiny mice showed microscopic abnormalities, evidence of hypertrophic β-cells, or insulin retention that has been associated with the initial stages of DM in spiny mice (Figure 11).[14,33,35]

Discussion

The goal of this study was to determine the effect of HP, HF, and HPF on weight and glucose management in spiny mice

while evaluating for any diet-induced pathology. These diet modifications attempted to mimic a diet similar to their natural diet and the diet prescribed for a similar species—the gerbil (16 to 22% protein[4]).

Spiny mice that were fed diets high in fiber (HF and HPF) maintained their baseline weights, even in the presence of elevated dietary protein. Fiber had the most effect on weight, maintaining the average weight within 2 g of the baseline weight. The average weight change from baseline over the 8 wk was the least for diets high in fiber.

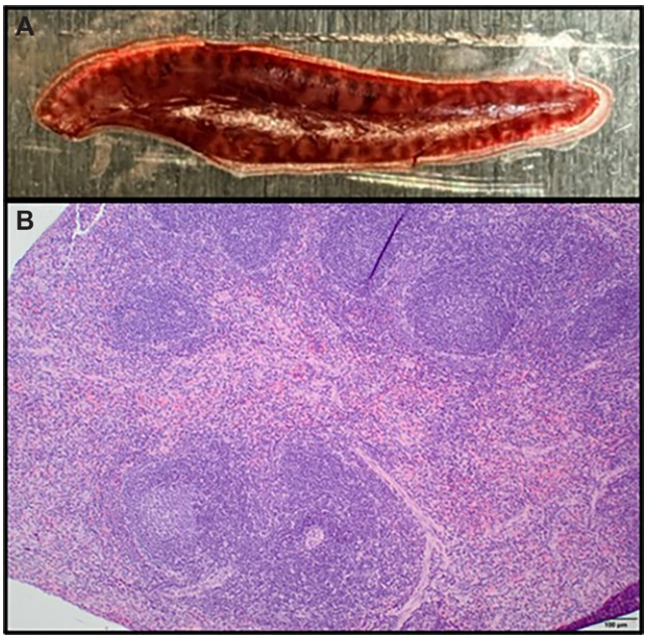

Figure 10. (A) Gross appearance and (B) histologic appearance of a representative spleen. Spleen shows a prominent striped pattern with regular distribution and characteristics of the white pulp. Hematoxylin and eosin staining at 100× original magnification. Scale bar = 100 µm.

Overall weight gain patterns follow the expected trend when considering the kilocalorie variations of the diets. The most and least calorically dense diets were BD and HF, respectively, with an approximate difference of 520 calories per gram. A significant difference in food consumption between diets coincided with periods of high food waste; however, food consumption was similar by week 6 for the experimental groups, such that spiny mice on high-calorie diets would be expected to gain more weight. However, the pattern was not conserved when evaluating weight differences caused by protein-laden diets. HP had 110 fewer calories per gram than BD (3.1% less) but on average, spiny mice gained 14% more weight over the 8 wk. Fiber likely contributed to satiety because both fiber-laden diets were associated with less weight gain despite a similar quantity of food consumed as compared with HP and BD over the 8-wk experimental period. The pattern of protein-induced weight gain was also seen when comparing both high-fiber diets. HPF had only 120 more calories per gram than HF (3.8% more), but the addition of protein to this fiber-laden diet resulted in an 87% higher average weight gain. Protein may be more efficient for energy metabolism than are other nutrients, but adding fiber appears to counteract the weight gain related to protein.

BCS did not indicate underconditioning at any time point for any diet group. BCS were evaluated and scored by veterinarians based on parameters commonly assessed in other species. Specific parameters assessed were fat deposits around the neck, fat coverage over the ribs, spine, pelvis, and the silhouette of the animal. None of the spiny mice scored below 2.5/5 during the study, and none scored under the ideal BCS (3/5) after the first 2 wk of the study. Spiny mice on high-fiber diets (HPF and HF) scored between ideal (3/5) and overweight (4/5) by the end of week 8, in contrast to spiny mice consuming the BD or HP diets. Spiny mice on HP were scored overweight (4/5) to obese (5/5), whereas spiny mice on BD scored between trending overweight (3.5/5) to obese (5/5) by week 8.

The modified diets were equivalent with regard to control of blood glucose; spiny mice on all diets were able to maintain

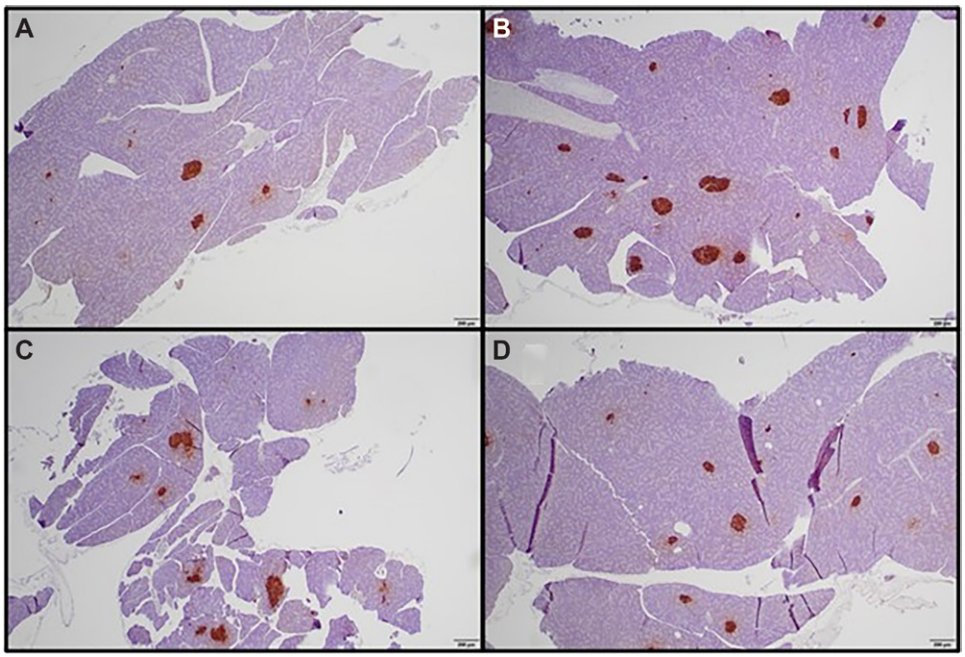

Figure 11. Low-magnification photomicrograph of representative pancreas: (A) HF, (B) HP, (C) BD, (D) HPF with insulin immunostaining. The pancreatic islands stain strongly. The size variation and random distribution of the pancreatic islands are normal. Peroxidase method, DAB chromogen, with hematoxylin counterstaining at 40× original magnification. Scale bar = 200 µm.

mean blood glucose levels within normal ranges reported in gerbils.[4,20,39] None of the spiny mice had developed DM during the study period based on histopathologic evaluation. Although no diet showed a significant difference in blood glucose, HF showed a trending decrease in blood glucose levels, deviating from the remaining diet groups by week 6. Similarly, in studies of wild-caught spiny mice fed either a high-fat or high-sucrose diet, blood glucose was not a sensitive marker for determining DM progression. Intermittently elevated blood glucose levels were evident at 3 mo, and elevations in peripheral insulin were not noted until 18 mo, after the onset of marked weight gain. Obesity was the first clinical sign of the progression of DM and a strong marker for future pancreatic pathology.[32-35]

Spiny mice in our study were not fasted before any blood glucose collection; postprandial blood glucose levels may have been captured at some time collection points. However, this situation mimics presentations of clinical conditions or study restrictions that may not allow the collection of a fasted blood sample. Furthermore, fasting the spiny mice would have required periods of time during which food was not available, thereby potentially altering food consumption and blood values and creating metabolic stress. Published ranges of blood glucose for normal spiny mice are not available, thereby lessening the value of fasted samples for comparison. The published upper range of blood glucose for gerbils ranges from 117 to 135 mg/dL,[4,20,39] which we used as a guide. Nonfasted glucose readings were all taken within 4 h of each other across the sampling days. This strategy shifts the emphasis from individual data points to evaluating trends across all readings.

Hyperglycemia alone may not be diagnostic for DM but can be an indicator of acute stress. In many species, acute, transient hyperglycemia can occur during stressful events or procedures.[3] We observed hyperglycemia multiple times during novel procedures or when spiny mice were first presented with a novel diet. Spiny mice that were changed to a novel diet had a higher blood glucose level at baseline sampling even though all of the spiny mice, regardless of diet, were new to the blood collection procedure. Similarly, spiny mice receiving a novel diet showed an increase in food waste that was not seen in those spiny mice that experienced similar procedures while being maintained on the BD.

Hyperglycemia was also noted in the blood collected after euthanasia. This expected stress-induced hyperglycemia showed blood glucose 211 to 256 mg/dL higher than the prior in vivo collections. If urine was available at euthanasia, a urinalysis was performed (n = 22). None of the spiny mice tested were glucosuric, indicating that the blood glucose elevation was acute and therefore likely transient.

BUN was also higher in spiny mice on HP diets. BUN increases in response to protein metabolism or decreased renal clearance of protein. Proteinuria (renal protein clearance) was seen in all urine samples collected, regardless of diet. Histopathologic evaluation confirmed that the kidneys had a normal appearance and architecture after 8 wk on diets high in protein (HP and HPF), indicating that the spiny mouse kidneys appropriately managed the elevated circulating protein.

Species-specific considerations are important in the management of spiny mice. One such consideration is a focus on early intervention to prevent obesity, which is a critical component of maintaining colony health. In spiny mice at risk for DM, monitoring for both glucosuria and hyperglycemia may increase the sensitivity of disease detection. The sensitivity of spiny mice to environmental changes may also promote a high incidence of hyperglycemia that is not necessarily because of underlying pathology. The effects of environmental change can also be seen behaviorally and underline strong recommendations for an acclimation period before data collection in this species.

None of the tested diets were optimal for both reducing weight gain and controlling blood glucose. Although HF was effective in controlling weight, the ideal percentage of fiber is yet to be determined. A HP diet without additional fiber will predispose spiny mice to obesity within a short amount of time. None of the diets differed significantly with regard to blood glucose levels, none of the spiny mice were clinically diagnosed with DM, and no clinically relevant variations were noted in the hematology or histopathology data. Thus, we conclude that in the short term (8-wk), dietary fiber and protein are well tolerated by spiny mice. The HPF diet slowed weight gain but warrants a longer diet trial before recommending this specific intervention.

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Conflicts of Interest

The author(s) have no conflict(s) of interest related to this publication treat.

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