Effects of weight loss with a moderate-protein, high-fiber diet on body composition, voluntary physical activity, and fecal microbiota of obese cats

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OBJECTIVE
To determine effects of restriction feeding of a moderate-protein, high-fiber diet on loss of body weight (BW), voluntary physical activity, body composition, and fecal microbiota of overweight cats.

ANIMALS
8 neutered male adult cats.

PROCEDURES
After BW maintenance for 4 weeks (week 0 = last week of baseline period), cats were fed to lose approximately 1.5% of BW/wk for 18 weeks. Food intake (daily), BW (twice per week), body condition score (weekly), body composition (every 4 weeks), serum biochemical analysis (weeks 0, 1, 2, 4, 8, 12, and 16), physical activity (every 6 weeks), and fecal microbiota (weeks 0, 1, 2, 4, 8, 12, and 16) were assessed.

RESULTS
BW, body condition score, serum triglyceride concentration, and body fat mass and percentage decreased significantly over time. Lean mass decreased significantly at weeks 12 and 16. Energy required to maintain BW was 14% less than National Research Council estimates for overweight cats and 16% more than resting energy requirement estimates. Energy required for weight loss was 11% more, 6% less, and 16% less than American Animal Hospital Association recommendations for weight loss (80% of resting energy requirement) at weeks 1 through 4, 5 through 8, and 9 through 18, respectively. Relative abundance of Actinobacteria increased and Bacteroidetes decreased with weight loss.

CONCLUSIONS AND CLINICAL RELEVANCE
Restricted feeding of a moderate-protein, high-fiber diet appeared to be a safe and effective means for weight loss in cats. Energy requirements for neutered cats may be overestimated and should be reconsidered. (Am J Vet Res 2018;79:181–190)

In 2015, 65% of US households owned a pet, and 42.9% of US households owned a cat.1 Unfortunately, there is an increasing incidence of obesity in companion animals in the United States, and obesity currently is considered the most common nutritional disorder in pets.2 Data from a 2014 survey conducted by the Association for Pet Obesity Prevention3 revealed that 57.9% of US cats (approx 55 million cats) are overweight (29.8%) or obese (28.1%). To further complicate the issue, there is a fat gap that inhibits owners from recognizing the degree to which their pets are overweight.4,5 A general classification defines an overweight cat as one that weighs 10% to 20% over its ideal BW and an obese cat as one that weighs > 20% over its ideal BW.6 Each unit increase in BCS above ideal (ideal BCS is 5 on a scale of 1 to 9) represents approximately 10% to 15% over ideal BW.7,8 Obesity in cats is associated with numerous metabolic abnormalities (eg, hyperlipidemia and glucose intolerance), endocrinopathies (eg, diabetes mellitus), skeletal stress, exercise intolerance, and many other detrimental effects on health, most of which are reversible with weight loss.2 Obesity develops as a result of a positive imbalance between energy intake and energy expenditure.2 Aspects of domestication and humanization of pets also contribute to obesity. These risk factors include neutering,4,5,9 decreased amounts of physical activity, increased food intake, and access to highly palatable high-fat or energy-dense diets.10–12 Although prevention of obesity would ideally avoid these conditions, it is necessary to develop effective and safe methods for treatment of obesity to improve health status. The recommendation for safe weight loss for cats is 1% to 1.5% of BW/wk.13 To safely avoid inducing hepatic lipidosis

ABBREVIATIONS
AOAC  Association of Official Analytical Chemists
BCS  Body condition score
BW  Body weight
DEXA  Dual-energy x-ray absorptiometry
ME  Metabolizable energy
MER  Maintenance energy requirement

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during weight loss, it is generally recommended that cats should eat at least 50% of their MER. However, experiments and clinical trials have included caloric restrictions between 59% and 80% of MER without evidence of hepatic lipidosis.

The feline gastrointestinal tract has a vast and diverse microbiota population, with the body containing as many (or more) bacteria than it does host cells. The community structure and function of the gastrointestinal microbiota (ie, gut microbiota) are influenced by diet through utilization of host nutrients and production of metabolites for uptake, which can promote both health and disease. Gut microbiota dysbiosis has been associated with obesity and is known to promote adiposity and influence peripheral organ function by altering satiety signals in the brain, hormone regulation in the gastrointestinal tract, and metabolism of lipids in the adipose tissue, liver, and muscle. Microbes may also affect energy harvest, influence intestinal permeability, or increase local or systemic inflammation, which can be associated with obesity and insulin resistance. Most of the studies have been conducted with humans or rodents. Although gut microbiota populations have been characterized in cats, to our knowledge, the gut microbiota of cats during weight loss have not been evaluated.

The objective of the study reported here was to determine the effects of weight loss on body composition, voluntary physical activity, and fecal microbiota populations of overweight cats during consumption of a moderate-protein, high-fiber diet. We hypothesized that closely monitoring BW and adjusting feed intake accordingly would lead to steady weight loss and would increase fat loss while maintaining lean mass. In previous studies, weight gain was accompanied by a reduction in voluntary physical activity. Therefore, we hypothesized that the reverse would be true for weight loss in the cats of this study (ie, an increase in voluntary physical activity). Finally, we hypothesized that weight loss would reduce blood lipid concentrations, result in serum biochemical values within or close to reference ranges, and alter the fecal microbiota community (eg, increases in Bifidobacterium spp, Lactobacillus spp, and Faecalibacterium spp and decreases in Desulfovibrio spp).

Materials and Methods

Animals

Eight neutered male adult domestic shorthair cats were included in the study. All cats were half- or full-siblings. Mean ± SEM age at the start of the study was 7.78 ± 0.03 years. Mean BW was 7.7 ± 0.42 kg, and median BCS was 7.5 (range, 6.0 to 9.0) on a 9-point scale (increments of 0.5 were used if needed). Cats were housed at the University of Illinois in a room controlled for temperature (20°C) and lighting (16 hours of light [7:00 AM to 11:00 PM] and 8 hours of darkness [11:00 PM to 7:00 AM]). For 20 hours each day, cats were housed as a group and allowed to socialize with each other and exercise outside of their cages in the room. Cats were individually housed for two 2-hour periods each day during feeding to control and monitor food intake. Throughout the study, all cats were fed a dry commercial weight loss diet, which was formulated to meet nutrient requirements for adult domestic cats in accordance with recommendations from the National Research Council.

Water was available ad libitum at all times. All animal procedures were approved by the University of Illinois Institutional Animal Care and Use Committee.

Study design

The study was conducted in accordance with a repeated-measures design. The first 4 weeks of the study represented the baseline period (week 0 = last week of the baseline period). During this period, all cats were fed to maintain their starting BW. Mean ± SEM daily intake required to maintain BW was 79.5 ± 0.93 g/d (255.3 ± 2.74 kcal ME/d). Beginning the week after the baseline period (week 1), food intake was adjusted to achieve a target weight loss of approximately 1.5% of BW/wk. The American Animal Hospital Association has recommended 2 options for determining daily caloric requirements for weight loss.

One option is to feed an amount that would provide 80% of the current caloric intake. The second option is to calculate the resting energy requirement by use of the animal's estimated ideal weight (70 X ideal BW in kg) and then feed 80% of that amount. For the study reported here, feeding guidelines were based on current caloric intake. This method was used instead of the use of published equations because in our experience, those equations may have been effective for guiding the feeding of study populations but they did not accurately predict the needs of individual animals in our colony. Because this colony of cats had previously been fed to maintain a healthy BW and BCS, appropriate estimation of ideal BW and MER to maintain BW was known. The mean target BW (BCS = 5) for the cats was 4.60 kg. At that weight, mean body fat mass was 0.5 kg (fat percentage was 10.72%) and mean lean tissue mass was 4.0 kg.

Beginning at week 1, weight loss was initiated by reducing mean baseline caloric intake by 20%. Cats were placed in their assigned cages and fed individually twice each day (8:30 AM to 10:30 AM and 3:00 PM to 5:00 PM). Any uneaten food at the end of the 2-hour feeding period was weighed and recorded. Food intake (measured daily), BW (measured twice weekly), and BCS (measured weekly) were recorded throughout the study. The BCS was measured by 1 investigator (MRP) throughout the study to avoid interobserver variation. Analyzed outcome variables were BW, body composition analysis determined via DEXA, voluntary physical activity, results of serum biochemical analyses, and fecal microbiota populations.

Sample collection and analysis

A sample of the diet was collected each week and stored at 4°C until analysis. Diet samples were pooled and ground by use of a 2-mm screen in a laboratory-
scale machine for grinding materials in preparation for chemical analysis. Dry matter and organic matter were analyzed in accordance with AOAC methods (methods 934.01 and 942.05, respectively), fat concentration was analyzed by acid hydrolysis in accordance with the American Association of Cereal Chemists method followed by extraction in accordance with the method of Budde or an AOAC method (method 922.06), crude protein was analyzed in accordance with an AOAC method (method 992.15) by use of a nitrogen-measuring device; gross energy was analyzed by use of a bomb calorimeter, and total dietary fiber content was analyzed in accordance with the methods of Prosky et al and an AOAC method (method 985.29).

To avoid conflicts with other measurements that can affect typical physical activity, voluntary physical activity was measured at weeks 0, 6, 12, and 18 by use of activity monitors that were attached to neck collars for a 7-day measurement period at each time point. Human interference was limited to only the daily feedings to ensure physical activity of the cats was voluntary. The monitors contained an omnidirectional sensor that integrated the amplitude and frequency of motion and produced an electrical current that varied in magnitude. As the intensity of motion increased, voltage also increased. Once the activity monitors were removed, activity monitoring software was used to analyze the data, which were converted into arbitrary numbers (referred to as activity counts). Mean activity was represented as activity counts per epoch (epoch duration, 15 seconds). Activity monitoring software reported the mean activity counts per epoch during the entire day, the 16-hour period of light, and the 8-hour period of darkness. The light-to-darkness ratio of activity counts was also analyzed.

Body composition was analyzed via DEXA scans, which were obtained at weeks 0, 4, 8, 12, and 16. This technique has been validated for use in dogs and it has been used in a variety of clinical and research applications at the University of Illinois Veterinary Teaching Hospital. Before each DEXA scan was obtained, cats were administered an IM injection of a combination of butorphanol tartrate (0.3 mg/kg), dexmedetomidine (0.02 mg/kg), and atropine (0.04 mg/kg). This caused immobilization and sedation to enable DEXA scanning. Sedation was reversed by injection of atipamezole (0.2 mg/kg, IM). Sedated cats were placed in ventral recumbency, and body composition was analyzed with a densitometer. The 4 limbs, trunk, and head of each cat were scanned separately. Measurements of fat content, lean nonbone tissue, and bone mineral content were obtained for each body region. Body fat percentage was calculated for each region and for the entire body. Cats were monitored until fully recovered from sedation.

Whereas detection of changes in body composition and physical activity requires several weeks, a reduction in food intake and consequent weight loss would be expected to alter blood metabolite concentrations and fecal microbiota after a much shorter period. Therefore, food was withheld from cats overnight (≥12 hours), and blood samples (5 mL) were collected via radial, femoral, or jugular venipuncture at weeks 0, 1, 2, 4, 8, 12, and 16. Cats were manually restrained, but sedation was not needed because the cats were familiar with the blood collection procedures and stress was minimal. Blood samples were collected into evacuated tubes and allowed to clot at room temperature. All tubes were centrifuged at 13,000 X g for 15 minutes at 4°C. Serum then was harvested with a pipette and placed into cryogenic vials. Serum samples were stored at −80°C until biochemical analysis was performed at a veterinary medical diagnostic laboratory.

To determine fecal microbial populations throughout the period of weight loss, fresh fecal samples (collected within 15 minutes after defecation) were obtained at weeks 0, 1, 2, 4, 8, 12, and 16. Samples were collected into 2.0-mL cryogenic vials, immediately snap-frozen in liquid nitrogen, and stored at −80°C until analysis.

Bacterial DNA was extracted, and the concentration of extracted DNA was quantified. Quality of DNA was assessed with precast agarose gels, which was followed by electrophoresis sequencing. The 16S rRNA gene amplicons were generated, and sequencing was performed. Sequence data were analyzed in accordance with methods described elsewhere. Briefly, electrophoresis sequencing was a high-throughput method of parallel sequencing by use of a proprietary method that detected single bases incorporated into growing DNA strands. In each sequencing cycle, a fluorescently labeled reversible terminator was imaged as each deoxynucleotide was added and then cleaved to allow incorporation of the next base. Because all 4 reversible terminator-bound deoxynucleotides were present during each sequencing cycle, natural competition minimized incorporation bias. The end result was base-by-base sequencing that provided high accuracy for many applications, including microbiota analysis.

An open-source bioinformatics pipeline was used to process the resulting sequence data. Briefly, high-quality (quality value > 25) sequence data derived from the sequencing process were demultiplexed (ie, assigned to sample of origin in silico). Sequences were then clustered by use of closed-reference operational taxonomic units that were selected against a reference database (similarity threshold, 97%). An even sampling depth of 3,466 sequences/sample was used for assessing measures of α and β diversity.

Statistical analysis

Data were analyzed by use of statistical software. The experimental design consisted of a single factor (week) with repeated measures, with week as a fixed effect and cat as a random effect. Dif-
ferences among weeks were determined by use of the Fisher-protected least significant difference test with a Tukey adjustment to control for experiment-wise error. Values of $P \leq 0.05$ were considered significant.

**Results**

**Food intake**

Composition of the diet was determined (Table 1). The diet consisted of 93.0% dry matter and contained 3,207 kcal ME/kg of diet.

During the 4-week baseline period, there was no change in BW or food intake. Food intake was significantly lower for weeks 1 through 18 than during the baseline period. Food intake was not different from weeks 8 to 18 (47.7 to 44.7 g/d [153.2 to 143.7 kcal ME/d]), but cats continued to lose weight (Figure 1).

**Table 1**—Proximate analysis of a diet fed to induce weight loss in 8 obese neutered male adult cats.

<table>
<thead>
<tr>
<th>Variable</th>
<th>%*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic matter</td>
<td>92.3</td>
</tr>
<tr>
<td>Crude protein</td>
<td>35.9</td>
</tr>
<tr>
<td>Acid hydrolyzed fat</td>
<td>8.9</td>
</tr>
<tr>
<td>Total dietary fiber</td>
<td>16.8</td>
</tr>
<tr>
<td>Insoluble</td>
<td>14.9</td>
</tr>
<tr>
<td>Soluble</td>
<td>1.9</td>
</tr>
</tbody>
</table>

*Results are reported on a dry-matter basis.

The diet contained chicken, pea protein, brewers rice, chicken meal, whole brown rice, split peas, dehydrated alfalfa meal, oat flour, potato protein, dried plain beet pulp, flaxseed, chicken fat (preserved with mixed tocopherols), natural flavors, rice bran, salmon meal, potassium chloride, choline chloride, thiamine mononitrate (vitamin B1), riboflavin supplement (vitamin B2), niacin supplement, selenium yeast, manganese proteinate, taurine, vitamin E supplement, pyridoxine hydrochloride (vitamin B6), calcium pantothenate, niacinamide, vitamin B12 supplement, copper proteinate, vitamin B12, biotin, riboflavin supplement (vitamin B2), calcium pantothenate, potassium iodide, thiamine mononitrate (vitamin B1), vitamin A supplement, pyridoxine hydrochloride (vitamin B6), vitamin D3, supplement, folate, rosemary extract, decaffeinated green tea extract, and spearmint extract.

All cats ate all the food provided to them at each meal. Therefore, reduced food intake was the result of a reduced amount of food provided rather than to a voluntary reduction in intake.

**BW and body composition**

All cats lost weight and body fat as a result of caloric restriction (Table 2). Mean BW (7.7 vs 6.2 kg) and mean BCS (7.6 vs 6.0) decreased significantly from week 0 to week 16. Mean fat mass was significantly less at weeks 8, 12, and 16 (2.42, 2.10, and 1.81 kg, respectively) than at week 0 (2.92 kg). Body fat percentage also was significantly less at weeks 8, 12, and 16 (36.8%, 34.0%, and 30.7%, respectively) than at week 0 (40.9%). Mean lean body mass was significantly less at weeks 12 and 16 (3.67 and 3.66 kg, respectively) than at week 0 (3.87 kg). Mean bone mineral content was significantly less at weeks 12 and 16 (92.7 and 92.4 g, respectively) than at week 0 (108.2 g).

**Voluntary physical activity**

Mean daily level of voluntary physical activity did not differ significantly throughout the study (Figure 2). Although mean activity during the 16-hour period of light increased with weight loss, there were no significant changes in mean activity during the periods of light or darkness. However, the mean light-to-darkness ratio of activity was significantly greater at week 18 than at weeks 0, 6, and 12.

**Serum biochemical analysis**

All serum biochemical results remained within the respective reference ranges of the clinical laboratory throughout the study (Supplementary Table 1, available at avmajournals.avma.org/doi/suppl/10.2460/ajvr.79.2.181, except for significantly higher creatinine concentrations (reference range, 0.4 to 1.6 mg/dL) from weeks 1 (1.74 mg/dL) through 16 (1.91 mg/dL), compared with the concentration at week 0 (1.59 mg/dL). Mean triglycerides concentrations were significantly lower for weeks 1 through 16, compared with the concentration at week 0 (56.0 mg/dL).

**Fecal microbial populations**

Sequencing identified 6 phyla, 13 classes, 15 orders, 33 families, and 59 genera of bacteria. The dataset was rarefied (ie, all samples were evenly subsampled) to 3,466 sequences for analysis of diversity and species richness. The $\alpha$ diversity (species richness) was not affected by weight loss (data not shown). Principal coordinates analysis of weighted and unweighted distances measured by use of the UniFrac metric with the 97% operational taxonomic unit abundance matrix revealed high variability of the microbiota community structure at week 0 (Figure 3). Even though principal coordinates analysis revealed some clustering for weeks 12 and 16 of weight loss, $\beta$ diversity was not significantly different during weight loss. Microbiota with a total abundance > 0.1% were considered...
for statistical analysis, and results were expressed as the mean percentage of sequences (Supplementary Table 2, available at avmajournals.avma.org/doi/suppl/10.2460/ajvr.79.2.181); those with significant values were summarized. Relative abundance of the phylum Actinobacteria was significantly higher at week 12 than at week 0. An undefined genus from the family Bifidobacteriaceae increased significantly from week 0 to 16, as did bacteria of the genera Bifidobacterium and Collinsella from week 0 to week 12 (Figure 4). Relative abundance of the phylum Bacteroidetes was significantly lower at week 12 than at week 2. Bacteria from the genus Prevotella of the Prevotellaceae and Paraprevotellaceae families decreased significantly with weight loss (Figure 5). Firmicutes did not change over time, but several genera within that phylum were altered with weight loss. Genera that increased significantly over time included Blautia, Dorea, Eubacterium, Oscillospira, Peptococcus, and Ruminococcus. Genera that decreased significantly over time included Lactobacillus, Butyricicoccus, and Pbsoclarctobacterium.

Table 2—Values for BW, BCS, and body composition during weight loss for 8 obese adult cats.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Week 0</th>
<th>Week 4</th>
<th>Week 8</th>
<th>Week 12</th>
<th>Week 16</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean BW (kg)</td>
<td>7.74a</td>
<td>7.41b</td>
<td>7.06c</td>
<td>6.67d</td>
<td>6.34e</td>
<td>0.42</td>
</tr>
<tr>
<td>BCS&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>7.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.38</td>
</tr>
<tr>
<td>Median</td>
<td>7.5</td>
<td>7.5</td>
<td>7.0</td>
<td>6.5</td>
<td>6.0</td>
<td>NA</td>
</tr>
<tr>
<td>Range</td>
<td>6.0–9.0</td>
<td>6.0–9.0</td>
<td>5.5–9.0</td>
<td>5.5–9.0</td>
<td>5.0–8.0</td>
<td>NA</td>
</tr>
<tr>
<td>Mean BMC (g)</td>
<td>108.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>105.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>104.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>92.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>92.4&lt;sup&gt;de&lt;/sup&gt;</td>
<td>4.44</td>
</tr>
<tr>
<td>Mean total fat (kg)</td>
<td>2.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.78&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.81&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.06</td>
</tr>
<tr>
<td>Mean total lean (kg)</td>
<td>3.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.75&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.78&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.67&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.05</td>
</tr>
<tr>
<td>Mean fat (%)</td>
<td>40.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40.4&lt;sup&gt;e&lt;/sup&gt;</td>
<td>36.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>34.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>30.7&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.90</td>
</tr>
</tbody>
</table>

<sup>a</sup>The BCS was scored on a scale of 1 to 9; increments of 0.5 were used if needed.
<sup>b</sup>BMC = Bone mineral content. NA = Not applicable.

*Within a row, means with different superscript letters differ significantly (P ≤ 0.05).

Figure 2—Mean ± SEM daily, 16-hour period of light, and 8-hour period of darkness counts of voluntary physical activity (A) and the light-to-darkness ratio of activity counts (B) for 8 obese adult cats during weight loss. Data represent activity counts per epoch (epoch duration, 15 seconds). a,b Means with different letters differ significantly (P ≤ 0.05).

Figure 3—Principal coordinates analysis plots of unweighted (A) and weighted (B) UniFrac distances performed on the 97% operational taxonomic unit abundance matrix for fecal microbial communities of 8 obese adult cats during weight loss. Percentages represent the percentage variation explained by each coordinate. PC1 = Principal coordinate 1. PC2 = Principal coordinate 2. PC3 = Principal coordinate 3.
Obesity is the most common nutritional disorder in cats, with 57.9% of US cats reportedly being overweight or obese. Clearly, efforts to prevent and treat obesity have become a challenge for veterinarians and owners, which makes this issue a major area for research. A successful weight loss program can correct malnutrition, reduce prevalence or risk of disease, and improve quality of life. This success depends heavily on diet selection as well as owner compliance to carefully track weight and modify food intake while attempting to increase an animal’s physical activity.

The first step to a weight loss program is identifying body condition of the cat. For the 9-point BCS scale, each unit increase over ideal BCS (ie, BCS = 5) represents approximately 10% to 15% over ideal BW. A general classification defines an overweight cat as one that weighs 10% to 20% over its ideal BW, and an obese cat is one that weighs > 20% over its ideal BW. The baseline BCS and body fat percentage of the cats in the present study indicated that they were overweight. There was no change in BW or food intake during the 4-week baseline period. Previous weight records were used to determine the appropriate ideal target weight for each cat.

Metabolic differences exist among cats, dogs, and humans, but maintenance of lean body tissue is an essential goal of safe weight loss across species because it is directly related to total energy expenditure, may prevent weight regain, and is an important reservoir to support protein turnover. However, some loss of lean body tissue almost always results during substantial weight loss and may even be considered physiologically necessary to maintain a ratio of lean tissue to fat mass. Investigators of another study reported that after caloric restriction for 18 weeks, 90.5% of weight loss was from fat, 8.2% was from lean tissue, and 1.3% was from bone mineral content, as determined with DEXA results. For a case study of weight loss in client-owned cats, it was reported that 86% of weight loss was from fat, 13% was from lean tissue, and 0.9% was from bone mineral content over a mean of 40 weeks. Those data are extremely similar to the DEXA results for the present study, whereby weight loss by week 16 was 84.03% from fat, 14.75% from lean tissue, and 1.21% from bone mineral content. The present study also confirmed the notion that weight loss in a research setting is often accomplished at a faster rate than is weight loss in a clinical setting. This likely would be attributable to better compliance with the dietary plan in research settings.

As expected, caloric restriction was a successful means for inducing weight loss by the cats of the present study. Even though initial caloric intake at week 1 was established to be a 20% reduction from baseline intake, additional restriction was required to maintain weight loss over time. By weeks 5 and 10, caloric intake had been restricted by 33% and 40% of baseline intake, respectively. These decreases were not a voluntary reduction in intake, but instead were the result of a reduction in food offered during the study. Food intake was not different from weeks 8 to 18, but cats continued to lose weight, with mean caloric intake being restricted by 42% of the baseline intake. Compared with the baseline BW, mean ± SEM BW at week 18 was reduced by 19.5 ± 3.35%, with a mean weekly weight loss of 1.26 ± 0.16%. These results are comparable to those for another study in which cats lost 18.1 ± 3.53% of their starting BW over an 18-week period of caloric restriction.

Metabolic differences exist among cats, dogs, and humans, but maintenance of lean body tissue is an essential goal of safe weight loss across species because it is directly related to total energy expenditure, may prevent weight regain, and is an important reservoir to support protein turnover. However, some loss of lean body tissue almost always results during substantial weight loss and may even be considered physiologically necessary to maintain a ratio of lean tissue to fat mass. Investigators of another study reported that after caloric restriction for 18 weeks, 90.5% of weight loss was from fat, 8.2% was from lean tissue, and 1.3% was from bone mineral content, as determined with DEXA results. For a case study of weight loss in client-owned cats, it was reported that 86% of weight loss was from fat, 13% was from lean tissue, and 0.9% was from bone mineral content over a mean of 40 weeks. Those data are extremely similar to the DEXA results for the present study, whereby weight loss by week 16 was 84.03% from fat, 14.75% from lean tissue, and 1.21% from bone mineral content. The present study also confirmed the notion that weight loss in a research setting is often accomplished at a faster rate than is weight loss in a clinical setting. This likely would be attributable to better compliance with the dietary plan in research settings.

Cats of the study reported here were fed to maintain BW for 4 weeks prior to feeding to induce weight loss. During the baseline period, mean energy intake was 255.3 kcal ME/d. The National Research Council MER for overweight cats is esti-
mated by use of the following equation: 130 X BW in kg^{0.40}. Mean weight at baseline was 7.7 kg. Therefore, we estimated by use of the National Research Council equation that the baseline MER was 293.9 kcal ME/d, which was an overestimation by 14.1% of the food intake required to maintain BW in the colony of cats. Although an additional 38.4 kcal ME/d may not appear to be a substantial number of calories, it is estimated that a 4-kg cat consuming an excess of 10 kcal/d can gain almost 0.5 kg of adipose tissue (energy content = 7,920 kcal/kg of adipose tissue) or 12% of its BW in 1 year. By use of the mean food intake and metabolic BW (ie, kg^{0.40}), MER during the baseline period of the present study was calculated by use of the following equation: 113 X BW in kg^{0.40}.

The American Animal Hospital Association has recommended 2 options for determining the daily caloric requirements for weight loss. One option is to feed an amount that will provide 80% of the current caloric intake. The second option is to calculate the resting energy requirement by use of the pet’s estimated ideal weight (70 X ideal BW in kg^{0.75}) and then feed 80% of that amount. To initiate weight loss in the present study, cats were fed 80% of the baseline MER (203.0 kcal ME/d). Throughout weeks 1 through 4, 5 through 8, and 9 through 18, the energy required for target weight loss was 11% more, 6% less, and 16% less, respectively, than that for the American Animal Hospital Association recommendation. Alterations in food intake that were needed to sustain weight loss over time indicated that energy requirements and consequent weight loss occurred in stages as the body adjusted. This stresses the importance of closely monitoring BW and adjusting food intake carefully, which may be better than use of a constant amount of restriction over time, and will confirm that weight loss occurs and help to avoid extreme weight loss that may increase risk of hepatic lipidosis and other health issues.

A combination of decreased energy expenditure and increased energy intake leads to an energy imbalance that promotes weight gain. It can be difficult to encourage physical activity of cats, but physical activity can help promote fat loss and may prevent a drastic decrease in lean tissue during weight loss. Items of environmental enrichment (eg, climbing towers, window perches, or scratching posts) can be effective for increasing physical activity. Voluntary physical activity was increased when cats were fed 4 meals or a random number of meals each day, rather than feeding 1 meal each day. Cats of the study reported here had access to environmental enrichment items such as scratching posts and climbing towers and were fed twice daily. Daily voluntary physical activity was not altered, but the light-to-darkness ratio of voluntary physical activity was greater at week 18 than at week 0. Physical activity during the 16-hour period of light numerically increased over time with weight loss, but at no time points did values differ significantly from the baseline value. It is likely that weight loss promotes voluntary physical activity, thereby assisting the negative energy balance necessary to sustain weight loss.

Creatinine was the only serum metabolite that had a concentration higher than the reference range, as determined by the clinical laboratory. Serum cre-
atine concentration is used as an indicator of renal function, and high concentrations often are used to diagnose chronic renal failure. Elevated BUN, potassium, calcium, and phosphorus concentrations are also considerations for the diagnosis of renal disease, but all remained within their respective reference range throughout the study. Furthermore, healthy geriatric cats have a serum creatinine concentration reference range of 0.7 to 2.1 mg/dL, as established by the American Association of Feline Practitioners. Because cats of the study reported here had a creatinine concentration of 1.6 mg/dL, prior to weight loss, maintained creatinine concentrations between 1.6 and 2.1 mg/dL in previous studies, and remained in our colony without issue, we do not believe there was a decline in renal function with weight loss in the present study.

Obese and type II diabetic cats have hypertriglyceridemia. However, development of hypertension and atherosclerosis has not been observed. Results of the present study agree with those of another study in which it was reported that mean ± SD cholesterol concentrations did not differ between lean and obese cats (132 ± 36 mg/dL and 139 ± 28 mg/dL, respectively), but mean triglyceride concentrations were lower in lean than in obese cats (21 ± 8 mg/dL and 48 ± 19 mg/dL, respectively). We also observed no change in cholesterol concentrations at week 16, compared with concentrations at week 0, but detected a lower mean ± SEM triglyceride concentrations at week 16 than at week 0 (37 ± 3.99 mg/dL and 56 ± 3.99 mg/dL, respectively). Values for phospholipids, free fatty acids, plasma protein subclasses, and particle size were not determined for the present study, but results for those variables may be helpful for determining overall improvements in dyslipidemia with weight loss.

The gut microbiome has a relationship with obesity in many host species, including humans and rodents. Anatomic, dietary, and metabolic differences exist among host species, but many of the microbial taxa and their biological activities are similar among species; thus, comparative studies have relevance, especially because of the lack of research conducted with companion animals. Calorie restriction and increased physical activity of obese adolescent humans influence the gut microbiota and correlate with weight loss and reductions in body mass index. Obesity is linked to a reduction in bacterial diversity and altered representation of bacterial populations and genes that correspond to numerous metabolic pathways. Genetically obese leptin-deficient (ob/ob) mice have an increased abundance of Firmicutes and a reduction of Bacteroidetes in the distal portion of the gastrointestinal tract, compared with results for their lean (ob/+ or +/+ ) littermates. Similar dysbiosis in the fecal microbiota has been observed for obese humans. When comparing the fecal microbiota of lean and obese human twins, a lower proportion of Bacteroidetes and higher proportion of Actinobacteria were associated with obesity.

Results of the study reported here differed from results of previous studies. In the present study, weight loss was associated with a greater proportion of Actinobacteria and lower proportion of Bacteroidetes, with Firmicutes being unchanged, although many genera within this phyla shifted with weight loss. The reduction in Bacteroidetes with weight loss was primarily attributable to a reduction in Prevotella spp. The increase in Actinobacteria with weight loss was primarily attributable to an increase in Bifidobacterium spp and Collinsella spp. Obese Zucker rats (fa/ fa) have reduced counts of Bifidobacterium spp as quantified by use of fluorescence in situ hybridization. In agreement with those results, cats of the present study had a greater proportion of Bifidobacterium spp at certain times during weight loss, but it was not consistently different than the baseline values at all time points. Although gut microbiota populations change with weight loss, it is also possible that the food restriction necessary for weight loss contributed to the shifts observed in the study reported here. A reduction in food would be expected to reduce the amount of fermentable substrate delivered to the colon, which would force microbes to use alternative energy sources (eg, mucins). Given that the microbiome field is in its infancy and there is a lack of data for cats, further studies will be necessary to understand the potential metabolic or health implications of shifted microbiota populations with weight loss or food restriction.

The present study had some limitations. The study included a small number (n = 8) of mature (> 7 years old) neutered male cats and was conducted in an extremely controlled environment. Although this control reduced variability, application of these results to the general cat population must be more limited than for results of larger studies that include client-owned animals of various ages, sexes, neuter status, and living conditions. Energy needs of the cats of the study reported here may also differ from energy needs of cats living outdoors that likely would have higher amounts of physical activity and would need to deal with temperature fluctuations throughout the seasons.

For the present study, restricted feeding of a moderate-protein, high-fiber diet was a safe and effective means for weight loss in overweight adult domestic cats, which resulted in a decrease in body fat percentage. Although total voluntary physical activity was not altered, shifts in activity occurred so that the light-to-darkness ratio for physical activity was higher with weight loss. Concentrations for all blood metabolites remained within reference ranges, except for the mean creatinine concentration. Mean triglyceride concentrations decreased throughout weight loss. Several shifts in the fecal microbiota were noted with weight loss, but more studies are necessary to determine how these shifts contribute to the metabolic processes or health benefits of weight loss.
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Footnotes

a. The Nutro Company, Franklin, Tenn.
b. Wiley mill, model 4, Thomas Scientific, Swedesboro, NJ.
d. Oxygen bomb calorimeter, model 1261, Parr Instruments Co, Moline, Ill.
e. Actial physical activity monitor, Mini Mitter Co, Bend, Ore.
f. Actical physical activity software, Mini Mitter Co, Bend, Ore.
h. BD vacutainer serum separator tubes, BD Medical Technology, Franklin Lakes, NJ.
i. Hitachi 911 clinical chemistry analyzer, Roche Diagnostics, Palo Alto, Calif.
j. Veterinary Medicine Diagnostics Laboratory, College of Veterinary Medicine, University of Illinois, Urbana, Ill.
k. MO BIO PowerSoil kit, MO BIO Laboratories, Carlsbad, Calif.
l. Qubit 3.0 fluorometer, Life Technologies, Grand Island, NY.
m. E-Gel EX gel 1%, Invitrogen, Grand Island, NY.


r. PROC mixed, SAS, version 9.3, SAS Institute Inc, Cary, NC.


x. PROC MIXED, SAS, version 9.3, SAS Institute Inc, Cary, NC.


References


