

UC Davis

UC Davis Previously Published Works

Title

Influence of dietary protein level on body composition and energy expenditure in calorically restricted overweight cats

Permalink

<https://escholarship.org/uc/item/4xf925vz>

Journal

Journal of Animal Physiology and Animal Nutrition, 99(3)

ISSN

0931-2439

Authors

Courtis, X des
Wei, A
Kass, PH
et al.

Publication Date

2015-06-01

DOI

10.1111/jpn.12239

Peer reviewed

ORIGINAL ARTICLE

Influence of dietary protein level on body composition and energy expenditure in calorically restricted overweight cats

X. des Courtis¹, A. Wei¹, P. H. Kass², A. J. Fascetti¹, J. L. Graham^{1,3}, P. J. Havel^{1,3} and J. J. Ramsey¹

¹ Department of Molecular Biosciences, School of Veterinary Medicine, University of California, Davis, CA, USA

² Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, CA, USA, and

³ Department of Nutrition, University of California, Davis, CA, USA

Summary

High-protein (HP) diets help prevent loss of lean mass in calorie-restricted (CR) cats. However, it is not entirely known whether these diets also induce changes of energy expenditure during periods of CR. To investigate this issue, sixteen overweight cats were fed either a high-protein [(HP), 54.2% of metabolizable energy (ME)] or a moderate-protein [(MP), 31.5% of ME] diet at 70% of their maintenance energy intakes for 8 weeks, and energy expenditure, energy intake, body weight and composition, and serum metabolites and hormones were measured. While both groups of cats lost weight at a similar rate, only cats eating the HP diet maintained lean mass during weight loss. Indirect respiration calorimetry measurements revealed that both total and resting energy expenditure (kcal/d) significantly decreased during weight loss for both treatment groups. However, only cats eating the MP diet exhibited significant decreases of total and resting energy expenditures after energy expenditure was normalized for body weight or lean mass. Results from this study suggest that in addition to sparing the loss of lean mass, feeding HP diets to overweight cats in restricted amounts may be beneficial for preventing or minimizing decreases of mass-adjusted energy expenditure during weight loss.

Keywords obesity, weight loss, energy restriction, feline

Correspondence A. Wei, Department of Molecular Biosciences, School of Veterinary Medicine, University of California, Davis, CA 95616, USA.

Tel: +1 530 754 8177; Fax: +1 530 752 4698; E-mail: awei@ucdavis.edu

Received: 17 December 2013; accepted: 7 July 2014

Introduction

Obesity is the most common nutrition-related disease in companion animals in Western countries (Laflamme, 2006). It has been estimated that 28% (Freeman et al., 2006) to 52% (Russell et al., 2000) of the adult cat population is overweight or obese. Gonadectomy (Flynn et al., 1996; Fettman et al., 1997; Lund et al., 2005), indoor confinement (Lund et al., 2005) and *ad libitum* feeding of palatable diets that are high in fat content (Scarlett et al., 1994) contribute to the increased prevalence of overweight cats. Feline obesity is a major health concern as it is associated with diabetes mellitus, urinary tract disease, osteoarthritis and a number of other diseases (Scarlett et al., 1994; Lund et al., 2005; Freeman et al., 2006).

Dietary energy restriction is the primary approach used to induce weight loss. Several strategies have been used in diets intended to promote feline weight loss, such as (i) decreasing the energy density of the diet by reducing the dietary fat, enhancing the dietary fibre and/or the dietary water (Fekete et al., 2001;

Backus et al., 2007; Wei et al., 2011b); (ii) adding supplements, for example L-carnitine to facilitate β -oxidation of fatty acids (Center et al., 2000); (iii) increasing the dietary protein content (Laflamme and Hannah, 2005). Cats, as true carnivores, require a greater dietary protein intake compared with many other mammals. Their minimum requirement for protein is 16% of metabolizable energy (ME), which is double that required for an adult dog (N.R.C., 2006). However, commercial cat diets contain protein levels that are well above this minimum protein requirement. Thus, cats routinely consume relatively high-protein diets, and there is some question as to whether any weight loss advantage can be obtained by further increasing dietary protein levels.

One mechanism through which high-protein (HP) diets may promote weight loss is by increasing energy expenditure (EE). In humans, high-protein diets have been reported to increase total energy expenditure (TEE) (Halton and Hu, 2004; Lejeune et al., 2006) and diet-induced thermogenesis when compared with high-carbohydrate or high-fat diets (Westerterp et al.,

1999). Indeed, the thermic effect of protein (20–30%) is considerably higher than carbohydrate (5–15%) or fat (0–3%) (Tappy, 1996). It has been reported that increasing the protein content of the diet from 15% to 30% for women eating a 1760 kcal diet doubled diet-induced thermogenesis (Johnston *et al.*, 2002). High-protein diets may also affect resting energy expenditure (REE) because HP diets may help to maintain lean body mass (LBM) during weight loss, and LBM has a higher energy expenditure (kcal/g) than adipose tissue (Krebs, 1950; Elia, 1992).

When compared with diets containing a lower protein content, high-protein (>40% of ME) diets have been reported to maintain LBM while enhancing the loss of fat mass (FM) in dogs (Hannah, 1999; Diez *et al.*, 2002) and cats (Nguyen *et al.*, 2004; Laflamme and Hannah, 2005; Vasconcellos *et al.*, 2009). Similar results have also been reported in obese cats fed a HP diet *ad libitum* (Wei *et al.*, 2011a). Feeding a HP diet was also reported to decrease the energy restriction needed for weight loss in obese cats and resulted in an increase in their energy requirements during weight maintenance (Vasconcellos *et al.*, 2009). The results of previous studies (Nguyen *et al.*, 2004; Hoenig *et al.*, 2007) evaluating the effects of dietary protein levels on energy metabolism in non-obese cats fed at maintenance have been inconsistent. Nguyen *et al.* (2004) found that protein content (27% vs. 47% of ME) did not have an effect on EE. Hoenig *et al.* (2007) observed that for baseline measurements (cats were in a fasted state), protein content did not influence heat production per metabolic body size. Once fed, lean cats eating the HP diet (42% of ME) showed a significantly ($p < 0.05$) higher heat production than those consuming the MP diet (27% of ME). It has also been reported that mass-adjusted EE is decreased in obese cats during energy restriction when fed a diet containing 35% of protein on a ME basis (Villaverde *et al.*, 2008). However, it is not known whether feeding diets differing in dietary protein levels in restricted amounts will influence this change of EE during weight loss in overweight cats.

Thus, the purpose of this study was to compare the effects of two diets, a high-protein (HP, 54.2% of ME) diet and a moderate-protein (MP, 31.5% of ME) diet, on EE, body composition and weight loss in overweight cats undergoing CR. It was hypothesized that feeding the HP diet may mitigate the decreases of LBM and EE observed during energy restriction and increase weight loss in overweight cats when compared with cats consuming the MP diet. Fasting serum samples were also collected from the cats during each stage of the study to evaluate

the influences of diet on circulating metabolites and hormones.

Materials and methods

Cats

Sixteen specific pathogen-free overweight adult domestic shorthair cats [ten neutered males, six intact females; body condition score (BCS) ≥ 6.0 on a 9.0 scale (Laflamme, 1997)] were used. The cats had been overweight for at least 1 year and were considered overweight at BCS ≥ 6 and obese at BCS > 7 . Cats were housed in individual cages (1.17 m \times 1.83 m \times 2.41 m) at the University of California, Davis (USA). Cats had *ad libitum* access to water and a dry maintenance diet except during times when they were subjected to CR for weight loss promotion or fasting for blood collections. The mean (\pm SD) age of the cats was 5.2 (± 1.65) years, and the mean BW was 6.6 (± 1.37) kg. The mean BCS was 7.1 (± 0.60). Cats were provided with environmental enrichment (toys, scratching posts, daily petting and weekly brushing). Room temperature in the facility was maintained between 18–24 °C with a 14 h light/10 h dark cycle. The Institutional Animal Care and Use Committee of the University of California, Davis (USA), approved all experimental protocols (Animal Welfare Assurance Number A3433-01).

Diets

Three diets were used for the study: a dry expanded cat food (control diet), a moderate-protein (MP) diet and a high-protein (HP) diet. The control diet, a dry maintenance diet packaged exclusively for the University of California, Davis (USA), was the diet typically fed to cats at the facility, and it was routinely fed to the cats prior to the onset of the study. The nutrient composition of the control diet on an as-fed basis was 9.5% moisture, 39.8% crude protein, 12.5% crude fat, 28.8% nitrogen-free extract (NFE), 2.7% crude fibre and 6.7% ash. The calculated ME for the control diet was 14.59 kJ/g (3.49 kcal/g) on a dry-matter basis [calculated using the modified Atwater values of 14.63 kJ/g (3.5 kcal/g), 35.53 kJ/g (8.5 kcal/g) and 14.63 kJ/g (3.5 kcal/g) for protein, fat and NFE respectively (AAFCO, 2012)] This diet provided 40.5% of the energy from protein, 26.7% from fat and 30.8% from NFE and was formulated to meet the nutritional recommendations established by the Association of American Feed Control Officials (AAFCO, 2012) for nutrient profiles of all life stages of cats. The MP (31.5% crude protein, 29.0% fat, 39.5% NFE on a

ME basis) and HP (54.2% crude protein, 29.1% fat, 16.8% NFE on a ME basis) diets were both provided by TestDiet (St. Louis, MO, USA) (Table 1).

Study design

Before the start of the study, all 16 cats were given a physical exam. Individual maintenance energy intakes for each cat were then determined for at least 1.5 months from the feeding of the control diet. Maintenance EI determined during this time were used as reference baseline measurements for the rest of the study. A baseline BW measurement was also determined for each cat immediately prior to the start of CR, described below.

Cats were introduced to the calorimetry chamber for TEE and urinary nitrogen measurements, as described below. During this phase of calorimetry measurements, cats were provided with their allotted food amounts of the control diet as a single feeding in order to meet maintenance energy requirements determined at baseline. Body composition measurements were then performed on all animals within three days after the indirect respiration calorimetry measurements.

The cats were then divided into the HP diet group or the MP diet group by a stratification method whereby the heaviest cat was selected for the MP group, the second heaviest cat was selected for the HP group and so on until two groups of eight existed that were matched by gender, age and BW. Energy restriction was then instituted by feeding the cats the HP or MP diet at 70% of their

maintenance energy intake at baseline. Rather than a single meal, the allotted restricted food amount for each cat was divided into two feedings each day. The cats were maintained on this level of energy restriction for a period of 8 weeks. However, cats that reached a BCS of 5 before 8 weeks of weight loss were deemed to have completed the study, and indirect respiration calorimetry measurements and body composition measurements were immediately completed on these animals. Cats were weighed twice a week and their BCS was assessed every 2 weeks by the same investigator (J.J. Ramsey) for the duration of the study.

Indirect respiration calorimetry

The cats completed measurements of TEE in the calorimetry chamber before and after weight loss. All cats were introduced to and housed in the chamber for at least two consecutive days prior to EE measurements for acclimation to the new environment and No-sorb cat litter (Catco, Cape Coral, FL, USA). The chamber dimensions were 0.60 m × 0.60 m × 0.76 m with a front Plexiglas window. Daily food intake and behaviour were recorded to assure that the cats were well acclimated to the chamber. It was previously reported that cats show no diurnal patterns of food intake (Kane *et al.*, 1981) or differences in daytime and night-time TEE measurements (Villaverde *et al.*, 2008); thus, 12 h TEE measurements were extrapolated to 24 h. Carbon dioxide (CO₂) production and oxygen (O₂) consumption were continuously measured from 8 AM to 8 PM. Cats completed at least two consecutive calorimetry measurements. An additional measurement was completed when TEE differed by more than 10% to ensure data consistency.

The calorimetry chamber was set up as an open circuit with a flow through design. A 6L/min flow of room air circulated into the chamber. Flow rate was measured with a mass flow controller (Flowkit 100; Sable Systems, Las Vegas, NV, USA). Oxygen and CO₂ were quantified by a paramagnetic O₂ analyser (AEI Technologies; Pittsburgh, PA, USA) and an infrared CO₂ analyser (AEI Technologies; Pittsburgh, PA, USA). The chamber temperature was maintained at 23 °C by fans blowing air over condenser coils circulating chilled water. An ethanol recovery was performed every week to check the system calibration. At the beginning of each 12-h run, analyses of two reference gases (100% N₂ and 1.90% CO₂) and room air were conducted for calibration of the

Table 1 Macronutrient composition of treatment diets on an as-fed basis and as a percentage of metabolizable energy (ME) intake

	Moderate-protein diet	High-protein diet
<i>Macronutrient composition</i>		
Crude protein, %	31.0	52.0
Crude fat, %	11.8	11.5
NFE, %	38.9	16.1
Moisture, %	10.0	10.0
Ash, %	6.3	7.8
Crude fibre, %	2.0	2.6
Protein, % of ME	31.5	54.2
Fat, % of ME	29.1	29.1
NFE, % of ME	39.5	16.8
ME*, kJ/g	16.1	15.7
ME*, kcal/g	3.85	3.76

*Calculated using 'modified' Atwater values of 14.63 kJ/g (3.5 kcal/g), 35.53 kJ/g (8.5 kcal/g) and 14.63 kJ/g (3.5 kcal/g) for protein, fat and NFE respectively (AAFCO, 2012).

analysers. Data were acquired from the analysers and the mass flow meter with a computer using LABVIEW software (National Instruments, Austin, TX, USA). The daily EE was calculated from the Weir equation (Weir, 1949). Resting EE was measured from stable periods lasting at least 30 min in duration where minimal spikes in EE were observed in plots of EE against time.

$$\text{EE (kJ)} = (16.5 \text{ kJ/L} \times \text{VO}_2) + (4.63 \text{ kJ/L} \times \text{VCO}_2) - (9.1 \text{ kJ/g} \times \text{g urinary nitrogen})$$

Initial TEE, REE, BW and LBM were plotted to obtain equations for regression lines from which predicted EE values were calculated. Energy expenditure values for each individual cat following weight loss was used to provide a visual indication of the relationship of the data to that obtained prior to weight loss. Predicted EE values were then subtracted from EE observed after weight loss.

Urinary nitrogen

After the 12-h calorimetry measurements, cats remained in the chamber for an additional 12 h. Total 24-h urine volume was collected and recorded. Total urine was then acidified with 5M hydrochloric acid until a final pH <3. Two 50 ml samples of the acidified urine were collected and frozen at -20°C until analyses. Nitrogen concentration was measured by the total Kjeldahl nitrogen method (Bremner and Mulvaney, 1983) at the Agriculture and Natural Resources Analytical Lab (University of California, Davis, USA).

Body composition measurements

Body composition was determined following completion of calorimetry measurements before and after CR. The deuterium oxide (D_2O) isotopic dilution method of (Backus *et al.*, 2000) was used to assess the LBM and body fat mass (FM). However, a few modifications were made to the procedure: food and water, respectively, were withheld for 12 h and 2 h before the D_2O injection and also during the 3-h equilibration period. A tracer dose (0.4 g/kg) of D_2O (Fisher Scientific; Pittsburgh, PA, USA) was injected subcutaneously instead of intravenously (Villaverde *et al.*, 2008). Blood samples (3 ml) were collected in Vacutainer[®] tubes (Becton Dickinson, Franklin Lakes, NJ, USA) and then kept at room temperature for 20 min for clotting. Tubes were centrifuged (2800 *g* for 15 min) to separate the serum, and serum was then stored at -20°C until analysis. An ATI Mattson Infinity Series Fourier transform infrared (FTIR) spectrometer equipped with a class 2A laser (Madison, WI,

USA) was used to analyse the condensed serum samples.

Biochemical determinations

Fasted whole blood samples were collected when body composition measurements were performed. Serum was collected from whole blood following separation by centrifugation (as described above in the body composition measurements section). Serum samples were then analysed for glucose, insulin, leptin, free fatty acids, triglycerides, adiponectin and ghrelin. Glucose and triglyceride concentrations were determined by enzymatic assay kits from Fisher Diagnostic (Middletown, VA, USA). Insulin analyses were performed using the porcine insulin radioimmunoassay (RIA) kit from Millipore (St. Charles, MO, USA). Leptin concentrations were determined by the multispecies leptin RIA kit from Millipore. Concentrations of free fatty acids were determined by enzymatic assay (Wako Diagnostics, Richmond, VA, USA). Adiponectin concentrations were determined using the rat/mouse adiponectin RIA kit (B-Bridge, Cupertino, CA, USA) that was validated for cats (Hoenig *et al.*, 2007). Ghrelin concentrations were determined using the human total ghrelin RIA kit from Millipore (previously validated for use in cats (Backus *et al.*, 2007). Analyses of blood metabolites and hormones were performed according to the directions of the commercially available analytical kits.

Statistical analysis

The results are presented as mean \pm SD. All variables were tested for normality using the Shapiro–Wilk test. The Mann–Whitney test was used for comparisons when the normality assumption was violated. Body composition, hormone and metabolite data were compared using repeated measures analysis of variance (JMP, SAS Institute, Cary, NC, USA) with diet and time as variables as well as their interaction. Within-diet comparisons were completed using a paired *t*-test. For the EE data, regression lines relating LBM or BW to EE were determined from baseline data calculated for all cats. Equations were used to calculate a predicted value of EE after weight loss for all cats. Pearson correlation coefficients associated with each equation line were calculated, along with their significance. A paired *t*-test was used to test the difference between the observed-minus-predicted values for each cat. Predicted values were considered significantly different from the observed value when $p < 0.05$.

Results

Body weight and body composition

There were no differences in BW, body composition or BCS between the two groups of cats prior to the start of CR. Caloric restriction resulted in significant decreases of BW and FM ($p < 0.001$) for both groups. The overall percentage of weight loss was similar for both groups ($12.2 \pm 2.29\%$ and $9.9 \pm 3.92\%$ for the MP and HP groups respectively). No differences were observed in the rate of weight loss between cats in each diet group. LBM was significantly decreased ($p = 0.033$) following CR in the cats consuming the MP diet, while no significant change of LBM was observed in the cats consuming the HP diet (Table 2). Both groups exhibited significant decreases of FM ($p = 0.004$ and $p = 0.017$ for the HP and MP groups respectively).

Energy intake and energy expenditure

No differences in baseline maintenance energy intakes were observed between the MP group (1098 ± 127.4 kJ/day) and the HP group (1177 ± 209.3 kJ/day). No differences were observed in TEE and REE between the two groups of cats prior to the start of CR (Table 2). Initial TEE, REE, BW and LBM were plotted to obtain the regression lines from which predicted EE values were then calculated. Total EE was positively correlated with LBM [TEE = 150.79 (LBM) + 488 , $r^2 = 0.65$, $p < 0.001$] and BW [TEE = 92.028 (BW) + 524.43 , $r^2 = 0.54$, $p = 0.001$]. Also, REE was positively

correlated with LBM [REE = 127.39 (LBM) + 469.43 , $r^2 = 0.48$, $p = 0.003$] and BW [REE = 79.964 (BW) + 485.71 , $r^2 = 0.42$, $p = 0.006$]. Total EE and REE significantly decreased after weight loss for all cats (Table 2). Weight loss in the MP group was associated with significant decreases in observed vs. predicted values for TEE adjusted for BW ($p < 0.001$) or LBM ($p = 0.009$) (Fig. 1). Observed REE values were also significantly lower than predicted values when adjusted for BW ($p = 0.002$) or LBM ($p = 0.010$). In contrast, observed vs. predicted values for TEE or REE adjusted for either BW or LBM were not significantly ($p > 0.05$) different for the cats fed the HP diet (Fig. 1).

Serum chemistry

No differences in circulating ghrelin, insulin, leptin, free fatty acids, triglycerides or glucose concentrations were observed between the HP and MP diet groups prior to or following CR (Table 3). Adiponectin levels were increased ($p < 0.05$) in female compared with male cats both prior to and after CR. However, the sex*diet interaction was not significant, indicating that both sexes showed similar responses to diet. Both groups of cats showed significant ($p < 0.05$) decreases of serum leptin concentrations during CR (Table 3). No other CR-related changes of serum hormone concentrations were observed for cats consuming the HP diet. In contrast, cats on the MP diet also showed significant ($p < 0.05$) decreases of serum triglyceride

Table 2 Measurements of body weight, body composition and energy expenditures in two groups of cats fed at their maintenance energy intake (MEI, control diet) and then at 70% of their MEI with a moderate (MP)- or high-protein (HP) diet*

	MEI		70% MEI	
	MP group	HP group	MP group	HP group
<i>Characteristics</i>				
BW, kg	6.51 ± 1.482^a	6.60 ± 1.346^a	5.69 ± 1.307^b	5.91 ± 1.192^b
BCS	7.1 ± 0.62	7.3 ± 0.60	6.3 ± 0.96	6.4 ± 0.99
<i>Body composition</i>				
LBM, kg	4.22 ± 0.820^a	4.26 ± 1.060	3.87 ± 0.843^b	4.03 ± 0.976
FM, kg	2.29 ± 0.889^a	2.34 ± 0.600^a	1.82 ± 0.551^b	1.88 ± 0.537^b
LBM, % BW	66 ± 9.0	64 ± 6.0	68 ± 4.9	68 ± 7.0
FM, % BW	34 ± 9.0	36 ± 6.0	32 ± 4.9	32 ± 7.0
<i>Energy expenditure</i>				
TEE, kJ/d	1125 ± 124.3^a	1130 ± 217.6^a	981 ± 133.5^b	1047 ± 179.1^b
REE, kJ/d	1005 ± 124.7^a	1015 ± 212.2^a	860 ± 122.6^b	945 ± 173.0^b

BW, body weight; BCS, body condition score; LBM, lean body mass; FM, fat mass; TEE, total energy expenditure; REE, resting energy expenditure.

*Values with different superscripts denote significant ($p < 0.05$) differences between the calorie restriction phase and the maintenance phase within each (MP or HP) treatment group. Values are given as mean \pm SD, where $n = 8$ cats per group.

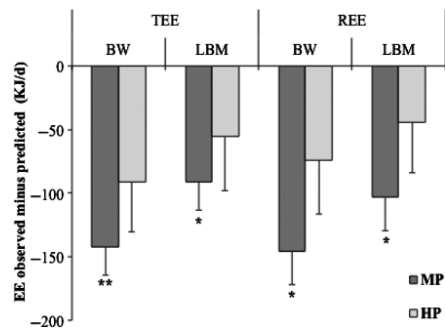


Fig. 1 Measures of 24-h observed-minus-predicted total and resting energy expenditure in calorically restricted (70% MER) cats eating a moderate-protein (MP) or high-protein (HP) diet, after adjustments with body weight or lean body mass. Differences from the maintenance phase within each treatment group are denoted by asterisks (*), where $p < 0.01$ is represented by * and $p < 0.001$ is represented by **.

concentrations and increased adiponectin concentrations during CR (Table 3).

Discussion

It is estimated that 28–52% of adult cats in Westernized countries are overweight or obese. To reduce BW in companion animals, dietary strategies combining restrictions on EI and adjustments to dietary protein and fibre content have been employed (Fekete et al., 2001; German et al., 2010). In cats, restricted feeding of HP diets has been shown to maintain LBM and enhance fat loss (Laflamme and Hannah, 2005; Vasconcellos et al., 2009). In addition, there is insufficient data to determine whether HP diets, when fed in restricted amounts as part of a weight loss regimen, would increase EE, promote diet-induced thermogenesis and affect body composition in the short term. Thus, the objectives of this study were to

investigate the short-term effects of two diets differing in protein content on TEE, REE, body composition and weight loss in overweight cats undergoing CR.

The main goal of a successful feline weight loss programme is to lose FM while maintaining or limiting loss of LBM. In the present study, there was a significant decrease ($p < 0.05$) of LBM in cats consuming the MP diet, while LBM was maintained in the HP diet group following CR. These results are consistent with previous studies reporting that HP diets spare the loss of LBM in cats (Laflamme and Hannah, 2005; Vasconcellos et al., 2009), dogs (Hannah, 1999; Diez et al., 2002) and humans (Piatti et al., 1994; Layman et al., 2003) undergoing weight loss induced by CR.

However, there are at least two major factors that need to be considered when comparing studies to determine the extent to which HP diets influence LBM during weight loss. First, the duration of the study and the magnitude of the weight loss will clearly influence changes of LBM. Although statistically significant, the magnitude of the changes of mean LBM between the two diet groups in the present study was relatively small (slightly over 100 g). This likely reflects the fact the duration of the weight loss portion of the study was only 8 weeks and total weight loss was approximately 10% of initial BW. In contrast, the weight loss phases in other feline studies using HP diets have been more than twice as long as the present study and have resulted in loss of 20% or more of initial BW (Nguyen et al., 2002; Laflamme and Hannah, 2005; Vasconcellos et al., 2009). It is possible that the influence of the HP diet on LBM would have been more pronounced if the present study had been of longer duration. Nonetheless, the results of the study indicate that the effects of HP diets on body composition can be observed even during relatively short periods of weight loss.

	MEI		70% MEI	
	MP group	HP group	MP group	HP group
<i>Serum chemistry</i>				
Glucose, mg/dl	82.5 ± 10.72	84.9 ± 11.21	84.9 ± 10.05	83.4 ± 7.24
Insulin, µU/ml	15.4 ± 8.24	16.6 ± 6.41	9.9 ± 2.88	15.6 ± 7.01
Leptin, ng/ml	10.2 ± 4.02 ^a	10.1 ± 2.70 ^a	6.6 ± 2.65 ^b	7.2 ± 2.10 ^b
Adiponectin, (µg/ml)	0.9 ± 0.64 ^a	1.4 ± 1.02	1.6 ± 0.66 ^b	1.3 ± 1.12
Ghrelin, pg/ml	960 ± 145.2	1073 ± 314.7	918 ± 133.1	1112 ± 182.7
Triglycerides, mg/dl	57 ± 17.8 ^a	54 ± 29.6	33 ± 13.3 ^b	39 ± 19.7
Free fatty acids, mEq/l	0.4 ± 0.16	0.4 ± 0.25	0.4 ± 0.12	0.3 ± 0.08

*Values with different superscripts denote significant ($p < 0.05$) differences between the calorie restriction phase and the maintenance phase within each (MP or HP) treatment group. Not significant if $p \geq 0.05$. All trends, $\alpha = 0.10$, are reported. Values are given as mean ± SD, where $n = 8$ cats per group.

Table 3 Fasted concentrations of serum hormones and metabolic substrates. Values were derived from two groups of overweight cats that were fed at their maintenance energy intake (MEI, control diet) and then at 70% of their MEI with a moderate (MP)- or high-protein (HP) diet*

Second, the level of protein in the diets being compared could influence changes of body composition with weight loss. There is variability (42–51% of ME) in the level of proteins used in HP [and control (27–44% of ME)] diets in existing feline weight loss studies (Nguyen *et al.*, 2002; Laflamme and Hannah, 2005; Hoenig *et al.*, 2007; Vasconcellos *et al.*, 2009), and it is not possible at this time to determine whether differences between studies are due primarily to dietary protein level or other differences in study conditions. Additional research is needed to determine whether there is an optimum level of protein to maintain LBM during weight loss.

There is evidence that dietary protein increases EE in humans consuming energy-restricted diets and thus may facilitate weight loss (Halton and Hu, 2004; Brehm and D'Alessio, 2008; Westerterp-Plantenga *et al.*, 2009, 2012). In cats, there is little information regarding the influence of dietary protein levels on EE during weight loss. In their study, Vasconcellos *et al.* (2009) found that obese cats fed a HP diet (42% of ME) at the beginning of weight loss required less of an energy restriction than that needed by the MP diet (31% of ME) group. Once the cats achieved a 20% loss of initial BW, the authors found that the metabolizable EI required for BW maintenance on a commercial diet (41% of ME was provided by protein) was the same for both groups during the first 40 days of maintenance, but increased after 120 days to a greater extent (31% vs. 18% of initial ME for the MP diet group) in cats that had lost weight eating the HP diet. Their findings suggest that protein intake during weight loss influences the energy needed to promote weight loss and subsequent weight maintenance in obese cats. The results of the present study indicate that EE, adjusted for either BW or LBM, was significantly decreased during weight loss in cats consuming a MP diet, but not in cats consuming a HP diet. This result indicates that HP diets may help prevent or mitigate decreases of weight-adjusted EE with CR. This contradicts the conclusions of a previous study which reported that changes in EE during weight loss do not differ between MP and HP diets (Nguyen *et al.*, 2002).

There are several possible reasons for the differences between the studies performed in cats. First, the statistical approach used to normalize EE for body size or composition differed between the studies, with Nguyen *et al.* (2002) using ratios (kJ/kg BW or kJ/kg LBM^{0.89}) to adjust their data, while the present study used a regression approach to adjust EE for BW or LBM. The validity of using ratios to adjust EE data has been questioned and it is recommended that a regres-

sion approach should be used to compare EE in individuals that differ in body size or composition (Allison *et al.*, 1995; Arch *et al.*, 2006; Tschop *et al.*, 2012). It is possible that the approaches used to adjust EE data could contribute to the different conclusions drawn by the two studies. Second, the level of protein in the HP diets compared with MP diets differed between the studies. The present study showed a wider difference in protein levels between diet groups (21% by weight vs. 11% by weight in the previous study) and a relatively large difference in protein between diets is likely needed to observe significant differences in EE. Third, the study by Nguyen *et al.* (2002) did not compare baseline and post-weight loss EE values. In the present study, the diets differed in their ability to prevent a significant decrease in mass-adjusted EE following weight loss. However, this pre- vs. post-weight loss comparison was not performed in the previous study (Nguyen *et al.*, 2002), and thus, the investigators were not capable of determining whether diet influenced changes of EE in response to weight loss. Nonetheless, the results of the present study support the idea that HP diets may help facilitate weight loss by mitigating decreases of EE in response to CR.

The mechanism through which the HP diet prevented a significant decrease in mass-adjusted EE in the cats during weight loss is not entirely clear. Diet-induced thermogenesis is higher for protein than other nutrients (Tappy, 1996), and it is likely that increased diet-induced thermogenesis helped maintain TEE in cats consuming the HP diet. However, mass-adjusted REE also did not show a significant decrease with weight loss in the HP diet group. The REE was recorded at times during the day when EE was at a minimum, and frequently well after the meal had been consumed. Thus, these values would be expected to include little or no contribution from diet-induced thermogenesis. The maintenance of REE is consistent with data in humans which shows that dietary protein increases sleeping EE during weight loss (Westerterp-Plantenga *et al.*, 2009, 2012). It has been proposed that dietary protein-related increases in EE in humans may be at least partially due to stimulation of gluconeogenesis (Veldhorst *et al.*, 2009) and increased protein turnover (Westerterp-Plantenga *et al.*, 2009). Similar metabolic changes in response to dietary protein would also be expected to occur in cats, although additional studies are needed to determine the specific mechanisms through which high-protein diets influence both TEE and REE.

Although the HP diet prevented decreases of mass-adjusted TEE and REE, it did not produce a greater

percentage weight loss or rate of weight loss than the MP diet. This likely reflects the fact that the differences in energetic response to weight loss were not large between the two diets. It has been calculated that increasing dietary protein in humans from 15% to 30–35% of ME during weight loss will only increase weight loss by approximately 40 g per week (Buchholz and Schoeller, 2004). Thus, the modest changes in EE induced by dietary protein would be expected to require studies of longer duration in order to observe clear differences in BW.

For both diet groups, circulating leptin concentrations decreased with weight loss as a result of CR. These results were consistent with those observed in obese cats undergoing weight loss in Vasconcellos et al. (2009). In the present study, cats on the MP diet exhibited changes of hormone concentrations that were consistent with improved insulin sensitivity (decreased serum insulin and triglyceride concentrations, and increased adiponectin concentration). In contrast, cats on the HP diet either did not exhibit changes in hormones indicative of improved insulin

sensitivity or the observed responses in hormone concentrations were blunted in comparison with the MP group. Other reports (Hoenig et al., 2007; Wei et al., 2011a) have found that dietary protein intake does not affect insulin sensitivity in cats.

In conclusion, results from this study indicate that short-term feeding of HP diets to overweight cats undergoing CR may be supportive to weight loss by maintaining LBM and mitigating decreases of EE in response to CR. Long-term investigations into the effects of feeding a HP diet with CR on weight loss, weight management, and maintenance of LBM and mass-adjusted EE in overweight cats are warranted and deserve further attention.

Acknowledgements

The authors would like to thank Deborah Bee and her staff for their assistance and care of the cats, Dr. Zengshou Yu for technical assistance, and the staff at the Agriculture and Natural Resources Analytical Lab for their assistance with urinary nitrogen analyses.

References

- AAFCO, 2012: 2012 Official Publication of American Feed Control Officials Incorporated. Association of American Feed Control Officials.
- Allison, D. B.; Paultre, F.; Goran, M. I.; Poehlman, E. T.; Heymsfield, S. B., 1995: Statistical considerations regarding the use of ratios to adjust data. *International Journal of Obesity and Related Metabolic Disorders* **19**, 644–652.
- Arch, J. R.; Hislop, D.; Wang, S. J.; Speakman, J. R., 2006: Some mathematical and technical issues in the measurement and interpretation of open-circuit indirect calorimetry in small animals. *International Journal of Obesity* **30**, 1322–1331.
- Backus, R. C.; Havel, P. J.; Gingerich, R. L.; Rogers, Q. R., 2000: Relationship between serum leptin immunoreactivity and body fat mass as estimated by use of a novel gas-phase Fourier transform infrared spectroscopy deuterium dilution method in cats. *American Journal of Veterinary Research* **61**, 796–801.
- Backus, R. C.; Cave, N. J.; Keisler, D. H., 2007: Gonadectomy and high dietary fat but not high dietary carbohydrate induce gains in body weight and fat of domestic cats. *British Journal of Nutrition* **98**, 641–650.
- Brehm, B. J.; D'Alessio, D. A., 2008: Benefits of high-protein weight loss diets: enough evidence for practice? *Current opinion in endocrinology, diabetes, and obesity* **15**, 416–421.
- Bremner, J. M.; Mulvaney, C. S., 1983: Nitrogen - total. In: A. L. Page, R. H. Miller, D. R. Keeney (eds), *Methods of Soil Analysis*, Vol. 9 (Part 2). American Society of Agronomy, Soil Science Society of America, Madison, WI, pp. 595–624.
- Buchholz, A. C.; Schoeller, D. A., 2004: Is a calorie a calorie? *American Journal of Clinical Nutrition* **79**, 899S–906S.
- Center, S. A.; Harte, J.; Watrous, D.; Reynolds, A.; Watson, T. D. G.; Markwell, P. J.; Millington, D. S.; Wood, P. A.; Yeager, A. E.; Erb, H. N., 2000: The clinical and metabolic effects of rapid weight loss in obese pet cats and the influence of supplemental oral L-carnitine. *Journal of Veterinary Internal Medicine* **14**, 598–608.
- Diez, M.; Nguyen, P.; Jeusette, I.; Devois, C.; Istasse, L.; Biourge, V., 2002: Weight loss in obese dogs: evaluation of a high-protein, low-carbohydrate diet. *Journal of Nutrition* **132**, 1685S–1687S.
- Elia, M., 1992: Organ and tissue contribution to metabolic rate. In: M. Elia, J. M. Kinney, H. N. Tucker (eds), *Energy Metabolism: Tissue Determinants and Cellular Corollaries*. Raven Press, New York, pp. 61–80.
- Fekete, S.; Hullár, I.; Andrásófszky, E.; Rigó, Z.; Berkényi, T., 2001: Reduction of the energy density of cat foods by increasing their fibre content with a view to nutrients' digestibility. *Journal of Animal Physiology and Animal Nutrition* **85**, 200–204.
- Fettman, M. J.; Stanton, C. A.; Banks, L. L.; Hamar, D. W.; Johnson, D. E.; Hegstad, R. L.; Johnston, S., 1997: Effects of neutering on bodyweight, metabolic rate and glucose tolerance of domestic cats. *Research in veterinary science* **62**, 131–136.
- Flynn, M. F.; Hardie, E. M.; Armstrong, P. J., 1996: Effect of ovariectomy on maintenance energy requirement in cats. *Journal of the American Veterinary Medical Association* **209**, 1572–1581.
- Freeman, L. M.; Abood, S. K.; Fascetti, A. J.; Fleeman, L. M.; Michel, K. E.; Laflamme, D. P.; Bauer, C.; Kemp, B. L.; Van Doren, J. R.; Willoughby, K. N., 2006: Disease prevalence among dogs and cats in the United States and Australia and proportions of dogs and cats that receive therapeutic diets or dietary supplements. *Journal of the American Veterinary Medical Association* **229**, 531–534.
- German, A. J.; Holden, S. L.; Bissot, T.; Morris, P. J.; Biourge, V., 2010: A high

- protein high fibre diet improves weight loss in obese dogs. *The Veterinary Journal* **183**, 294–297.
- Halton, T. L.; Hu, F. B., 2004: The effects of high protein diets on thermogenesis, satiety and weight loss: a critical review. *Journal of the American College of Nutrition* **23**, 373–385.
- Hannah, S., 1999: Role of dietary protein in weight management. *Compendium on Continuing Education for the Practicing Veterinarian* **21**, 32–33.
- Hoening, M.; Thomaseth, K.; Waldron, M.; Ferguson, D. C., 2007: Insulin sensitivity, fat distribution, and adipocytokine response to different diets in lean and obese cats before and after weight loss. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* **292**, R227–R234.
- Johnston, C. S.; Day, C. S.; Swan, P. D., 2002: Postprandial thermogenesis is increased 100% on a high-protein, low-fat diet vs. a high-carbohydrate, low-fat diet in healthy, young women. *Journal of the American College of Nutrition* **21**, 55–61.
- Kane, E.; Rogers, Q. R.; Morris, J. G., 1981: Feeding behavior of the cat fed laboratory and commercial diets. *Nutrition Research* **1**, 499–507.
- Krebs, H. A., 1950: Body size and tissue respiration. *Biochimica et Biophysica Acta* **4**, 249–269.
- Laflamme, D., 1997: Development and validation of a body condition score system for cats: a clinical tool. *Feline Practice* **25**, 13–18.
- Laflamme, D. P., 2006: Understanding and managing obesity in dogs and cats. *Veterinary Clinics of North America. Small Animal Practice* **36**, 1283–1295, vii.
- Laflamme, D. P.; Hannah, S. S., 2005: Increased dietary protein promotes fat loss and reduces loss of lean body mass during weight loss in cats. *International Journal of Applied Research in Veterinary Medicine* **3**, 62–68.
- Layman, D. K.; Boileau, R. A.; Erickson, D. J.; Painter, J. E.; Shiue, H.; Sather, C.; Christou, D. D., 2003: A reduced ratio of dietary carbohydrate to protein improves body composition and blood lipid profiles during weight loss in adult women. *Journal of Nutrition* **133**, 411–417.
- Lejeune, M. P.; Westerterp, K. R.; Adam, T. C.; Luscombe-Marsh, N. D.; Westerterp-Plantenga, M. S., 2006: Ghrelin and glucagon-like peptide 1 concentrations, 24-h satiety, and energy and substrate metabolism during a high-protein diet and measured in a respiration chamber. *American Journal of Clinical Nutrition* **83**, 89–94.
- Lund, E. M.; Armstrong, P. J.; Kirk, C. A.; Klausner, J. S., 2005: Prevalence and risk factors for obesity in adult cats from private US veterinary practices. *International Journal of Applied Research in Veterinary Medicine* **3**, 88–96.
- Nguyen, P.; Dumon, H.; Martin, L.; Siliart, B.; Ferrier, L.; Humbert, B.; Diez, M.; Breul, S.; Biourge, V., 2002: Weight loss does not influence energy expenditure or leucine metabolism in obese cats. *Journal of Nutrition* **132**, 1649S–1651S.
- Nguyen, P.; Leray, V.; Dumon, H.; Martin, L.; Siliart, B.; Diez, M.; Biourge, V., 2004: High protein intake affects lean body mass but not energy expenditure in nonobese neutered cats. *Journal of Nutrition* **134**, 2084S–2086S.
- N.R.C., 2006: *Nutrient Requirements of Dogs and Cats*. National Academies Press, Washington, D.C.
- Piatti, P. M.; Monti, F.; Fermo, I.; Baruffaldi, L.; Nasser, R.; Santambrogio, G.; Librenti, M. C.; Galli-Kienle, M.; Pontiroli, A. E.; Pozza, G., 1994: Hypocaloric high-protein diet improves glucose oxidation and spares lean body mass: comparison to hypocaloric high-carbohydrate diet. *Metabolism* **43**, 1481–1487.
- Russell, K.; Sabin, R.; Holt, S.; Bradley, R.; Harper, E. J., 2000: Influence of feeding regimen on body condition in the cat. *The Journal of small animal practice* **41**, 12–17.
- Scarlett, J. M.; Donoghue, S.; Saidla, J.; Wills, J., 1994: Overweight cats: prevalence and risk factors. *International Journal of Obesity and Related Metabolic Disorders* **18**(Suppl 1), S22–S28.
- Tappy, L., 1996: Thermic effect of food and sympathetic nervous system activity in humans. *Reproduction, nutrition, development* **36**, 391–397.
- Tschop, M. H.; Speakman, J. R.; Arch, J. R.; Auwerx, J.; Bruning, J. C.; Chan, L.; Eckel, R. H.; Farese, R. V. Jr; Galgani, J. E.; Hambly, C.; Herman, M. A.; Horvath, T. L.; Kahn, B. B.; Kozma, S. C.; Maratos-Flier, E.; Muller, T. D.; Munzberg, H.; Pfluger, P. T.; Plum, L.; Reitman, M. L.; Rahmouni, K.; Shulman, G. I.; Thomas, G.; Kahn, C. R.; Ravussin, E., 2012: A guide to analysis of mouse energy metabolism. *Nature methods* **9**, 57–63.
- Vasconcellos, R. S.; Borges, N. C.; Goncalves, K. N.; Canola, J. C.; de Paula, F. J.; Malheiros, E. B.; Brunetto, M. A.; Carciofi, A. C., 2009: Protein intake during weight loss influences the energy required for weight loss and maintenance in cats. *Journal of Nutrition* **139**, 855–860.
- Veldhorst, M. A.; Westerterp-Plantenga, M. S.; Westerterp, K. R., 2009: Gluconeogenesis and energy expenditure after a high-protein, carbohydrate-free diet. *American Journal of Clinical Nutrition* **90**, 519–526.
- Villaverde, C.; Ramsey, J. J.; Green, A. S.; Asami, D. K.; Yoo, S.; Fascetti, A. J., 2008: Energy restriction results in a mass-adjusted decrease in energy expenditure in cats that is maintained after weight regain. *Journal of Nutrition* **138**, 856–860.
- Wei, A.; Fascetti, A. J.; Liu, K. J.; Villaverde, C.; Green, A. S.; Manzanilla, E. G.; Havel, P. J.; Ramsey, J. J., 2011a: Influence of a high-protein diet on energy balance in obese cats allowed ad libitum access to food. *Journal of Animal Physiology and Animal Nutrition* **95**, 359–367.
- Wei, A.; Fascetti, A. J.; Villaverde, C.; Wong, R. K.; Ramsey, J. J., 2011b: Effect of water content in a canned food on voluntary food intake and body weight in cats. *American Journal of Veterinary Research* **72**, 918–923.
- Weir, J. B., 1949: New methods for calculating metabolic rate with special reference to protein metabolism. *Journal of Physiology* **109**, 1–9.
- Westerterp, K. R.; Wilson, S. A.; Rolland, V., 1999: Diet induced thermogenesis measured over 24 h in a respiration chamber: effect of diet composition. *International Journal of Obesity and Related Metabolic Disorders* **23**, 287–292.
- Westerterp-Plantenga, M. S.; Nieuwenhuizen, A.; Tome, D.; Soenen, S.; Westerterp, K. R., 2009: Dietary protein, weight loss, and weight maintenance. *Annual Review of Nutrition* **29**, 21–41.
- Westerterp-Plantenga, M. S.; Lemmens, S. G.; Westerterp, K. R., 2012: Dietary protein - its role in satiety, energetics, weight loss and health. *British Journal of Nutrition* **108**(Suppl 2), S105–S112.