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ORIGINAL ARTICLE

Influence of a high-protein diet on energy balance in obese cats allowed *ad libitum* access to food

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Summary

The influence of a high-protein [HP, 47% of metabolizable energy (ME)] diet on energy balance was evaluated in obese cats allowed *ad libitum* access to food. Energy intake, body weight, body composition, energy expenditure, and concentrations of hormones and metabolites associated with carbohydrate and lipid metabolism (glucose, insulin, free fatty acids, triglycerides and leptin) were measured in cats after consuming either a moderate protein (MP, 27% of ME) or HP diet for 4 months. Indirect respiration calorimetry showed that resting and total energy expenditure (kJ/day) adjusted for either body weight or lean body mass was increased in cats consuming the HP in relation to MP diets. However, voluntary energy intake also was increased in the HP treatment and, thus, there was no difference in body weight between animals consuming the two diets. Body composition measurements using deuterium oxide dilution showed that dietary protein content did not alter amounts of either lean body mass or fat mass. No significant differences ($p > 0.05$) were observed between the two treatment groups for blood glucose, free fatty acid or leptin concentrations, although there was a trend ($p = 0.054$) towards an increase of serum insulin concentrations in the cats eating the HP diet. This study showed that short-term *ad libitum* feeding of an HP diet did not reduce food intake or promote weight loss in obese cats. However, energy expenditure was increased in the HP diet group and it is possible that this effect of HP might help promote weight loss when energy intake is restricted.

Introduction

The prevalence of obesity has increased in the human population over the past several decades and a similar increase in the incidence of obesity has been observed in cats (German, 2006). Accordingly, obesity-related diseases such as type 2 diabetes have increased in both humans and domestic cats (Gonzalez et al., 2009; Prahl et al., 2007). Restriction of energy intake by dietary means is the most common treatment method used by owners to promote weight loss, but this method often has limited

success (Butterwick and Hawthorne, 1998). As such, dietary strategies based on human weight-reducing regimens are being considered for use in overweight cats.

In humans, positive results have been reported for the use of high-protein (HP) diets to promote weight loss (Weigle et al., 2005). However, it has also been argued that these diets may only be effective at inducing short-term changes in body weight (BW) (Foster et al., 2003). Nonetheless, HP diets appear to promote weight loss by increasing satiety and energy expenditure (Halton and Hu, 2004). In human studies comparing the thermic effects of diets of differing protein compositions, increased energy expenditures were observed following consumption of higher protein meals (Dauncey and Bingham, 1983; Westerterp et al., 1999). Although it is known that protein induces a greater thermic effect (20–35% of energy consumed) than the other macronutrients, the contribution of this effect to body weight in the long term is questionable and requires further investigation. Human studies have demonstrated HP diets to be more satiating than diets high in other macronutrients (Westerterp-Plantenga et al., 1999, Smeets et al., 2008). As a consequence, satiety may affect subsequent energy intake and promote weight loss (Weigle et al., 2005). Therefore, it is possible that HP diets may induce weight loss by both increasing energy expenditure and decreasing voluntary energy intake.

The minimum requirement of crude protein for adult cats at maintenance is 16% of metabolizable energy (ME) (N.R.C., 2006). In contrast, the maintenance requirement of protein for omnivores varies from approximately 5% of ME for the rat (NRC, 1995) to 8% of ME for the dog (NRC, 2006). It has been shown that both weight gain and energy intake are decreased in rats consuming a HP diet (Kinzig et al., 2007). In obese cats, the effect of *ad libitum* consumption of HP diets on energy balance is unknown. Cats, as carnivores, have evolved to consume diets that are relatively high in protein, and it is possible that they may be insensitive to some of the cues that limit energy intake and promote weight loss in omnivores consuming HP diets. Additional studies are needed to determine if HP diets are efficacious for the treatment of obesity in cats.

The purpose of this study was to determine if energy intake, energy expenditure, body weight and body composition are altered in obese cats given *ad libitum* consumption of a HP (47% of ME) or moderate protein (MP) (27% of ME) diet. We hypothesized that cats consuming a HP diet would lose body weight and have less body fat than cats eating a MP diet.

Materials and methods

Cats

Twenty specific pathogen-free overweight adult domestic short-hair cats [five neutered males and 15 spayed females; body condition score (BCS) > 6 on a 9-point scale (Laflamme, 1997)] were housed at the University of California, Davis. At the beginning of the study, the mean age of the cats was 4.3 ± 1.2 years and the mean body weight was 6.0 ± 1.4 kg. Cats were housed in individual cages with *ad libitum* access to food and water, except during daily periods of 7–8 h in which they were enclosed in group cages for socializing and exercise. During these times, cats had access to water but not food. The facility maintained room temperatures between 18 and 24°C and a 14-h light/10-h dark cycle. Approval of experimental protocols was granted by the Institutional Animal Care and Use Committee of the University of California, Davis and complied with the Guide for the Care and Use of Laboratory Animals (NRC, 1996).

Diets

Two experimental dry diets were used (Table 1), a MP diet (27.1% crude protein, 44.1% crude fat, 28.8% carbohydrates on a ME basis) and an HP diet (47.3% crude protein, 44.5% crude fat, 8.2% carbohydrates on a ME basis). The ME content for each diet was reported by the manufacturer to be 16.3 kJ/g for the MP diet and 16.2 kJ/g for the HP diet.

Study design

Prior to the start of the study, the health of each cat was assessed by physical examination and blood collection for serum chemistry and complete blood count measurements. For baseline, all cats were fed the MP diet *ad libitum* for 3–4 months. Once the cats reached stable body weights and were adapted to the MP diet, they were introduced to the indirect calorimetry chamber for total daily energy expenditure (TEE) measurements, described below. At the end of baseline, blood was collected from cats by jugular venipuncture for measurements of body composition and analyses of glucose, insulin, free fatty acids, triglycerides, and leptin (described below). Prior to blood collection, food was removed

Table 1 Ingredient and macronutrient composition of treatment diets and their percentage contributions on an as fed basis

	Moderate protein diet	High protein diet
Ingredient (%)		
Chicken meal	31.32	67.66
Potato product	31.79	0.00
Chicken fat	13.15	8.64
Potato starch	7.50	7.50
Dried egg product	5.00	5.00
Fish meal	5.00	5.00
Beet pulp	3.00	3.00
Natural flavours	1.00	1.00
Potassium chloride	0.83	0.60
Premium cat vitamin premix*,†	0.65	0.68
Salt	0.25	0.25
Fish oil	0.23	0.36
Premium cat mineral premix*,‡	0.15	0.15
Dried chickory root	0.10	0.10
Dried natural antioxidant	0.04	0.06
Macronutrient composition		
Crude protein, %	30.2	52.2
Crude fat, %	20.2	20.2
Carbohydrate (NFE), %	32.0	9.1
Moisture, %	7.5	7.5
Ash, %	8.0	10.0
Crude fibre, %	2.7	2.5
Protein, % of ME	27.1	47.3
Fat, % of ME	44.1	44.5
CHO, % of ME	28.8	8.2
ME, kJ/g	16.3	16.2
ME§, kcal/kg	3894.6	3861.5
Food quotient¶	0.83	0.79

*Trouw Nutrition (Highland, IL, USA).

†Vitamin premix composition (g/kg mix): pea fibre carrier, 728.4; $CaCO_3$, 170.9; vitamin E (50% adsorbate), 40.0; betaine, 26.0; soybean oil carrier, 10.0; nicotinic acid, 9.6; vitamin A, 4.0; d-calcium pantothenate, 2.7; vitamin B_1 thiamine mononitrate, 2.7; vitamin B_2 riboflavin, 1.25; β-carotene, 1.0; vitamin B_{12}, 1.0; vitamin D_3, 0.8; biotin, 0.7; vitamin B_6 pyridoxine, 0.7; folic acid, 0.2.

‡Mineral premix composition (g/kg mix): $CaCO_3$, 360.2; $ZnSO_4$, 208.3; OPTiMIN (Trouw Nutrition) Zn proteinate (15% Zn), 166.7; $FeSO_4$, 77.4; OPTiMIN Fe proteinate (15% Fe), 53.3; $CuSO_4$, 35.7; OPTiMIN Cu proteinate (10% Cu), 3.0; $MnSO_4$, 23.4; OPTiMIN Mn proteinate (15% Mn), 16.7; Se, 12.0; soybean oil carrier, 10.0; OPTiMIN Co proteinate (2.5% Co), 3.8; I, 1.8; $CoCO_3$, 0.6.

§Calculated using 'Modified' Atwater values assuming protein, fat and carbohydrate contain 3.5, 8.5 and 3.5 kcal ME/g diet respectively (AAFCO, 1998).

¶Calculated assuming FQ of fat, protein and CHO are 0.71, 0.835 and 1.0 respectively (Elia, 1992).

from the cats' cages for 16 h and water was removed for 2 h.

The experimental phase started immediately after the blood collections for body composition measurements. For this phase of the study, 10 cats were switched to the HP diet, whereas the other 10 remained on the MP diet. Cats were assigned to either the HP or MP diets based on BW at the end of baseline in an effort to distribute the cats so that one group did not have a mean starting body weight greater than the other. Cats were fed these diets *ad libitum* for 4 months. At the end of the experimental phase, indirect respiration calorimetry was again used to measure the TEE of cats consuming either the MP or HP diets. Blood was also collected after a 16-h fast for body composition measurements and analyses of hormones and metabolic substrates. Cats were weighed twice per week and individual food intakes were recorded daily for the duration of the study. Assessments of BCS based on a 9-point system (Laflamme, 1997) were performed at the start of the study and then monthly until the end of the study by one investigator. Cats were considered overweight with BCS > 5 and obese with BCS > 7 (Laflamme, 1997). All cats in the study had a BCS of 6.5 or greater at the start of the study.

Indirect respiration calorimetry

Indirect respiration calorimetry measurements were conducted on cats using the methods and calorimetry chamber system described by Villaverde et al. (2008). Briefly, cats were acclimated to the calorimetry chamber for at least three consecutive days before TEE measurements were performed at the end of each diet phase (baseline or experimental). At least two TEE measurements were completed for each cat. Cats had continuous access to food and water during the calorimetry measurements. As TEE and respiratory quotients (RQ) data obtained from 12 and 24 h experiments did not differ between day and night, data collected for 12 h were extrapolated to 24 h. Data from the flowmeter, oxygen analyser and carbon dioxide analyser were collected using a data acquisition system (National Instruments, Austin, TX, USA) with a PC using Labview software (National Instruments). Energy expenditure was calculated using the Weir equation (Weir, 1949). Resting energy expenditure (REE) was also estimated during the calorimetry runs. REE was determined by plotting energy expenditure against time and selecting stable periods (at least 30 min in duration) where there were minimal spikes in energy expenditure. The analysers were calibrated daily with nitrogen, standard gas (1.90% carbon dioxide) and dry outside air. Sample gas, outside air and standard gas were also continually cycled through the analysers during the calorimetry runs to allow any

adjustments for drift. Calibration of the entire calorimetry system was evaluated weekly using an ethyl alcohol recovery.

Body composition determination

Estimation of body fat mass (FM) and lean mass was accomplished using the deuterium oxide (D_2O) isotopic dilution method of Backus et al. (2000), with modifications (Villaverde et al., 2008). Deuterium oxide was purchased from Fisher Scientific (Pittsburgh, PA, USA). Condensed serum water samples were analysed on an ATI Mattson Infinity Series Fourier transform infrared spectrometer equipped with a class 2A laser (Madison, WI, USA). Body composition measurements were performed at the end of baseline and the experimental phase after completion of indirect calorimetry runs. However, instead of withholding food from the cats for 24 h as described by Villaverde et al. (2008), food was withheld for 16 h.

Serum chemistry

Serum was separated by centrifugation from whole blood samples collected for body composition determinations before and after the dietary intervention. These serum samples were then analysed for glucose, free fatty acids, triglycerides, insulin and leptin concentrations. Measurements were conducted as described in the directions of commercially available kits. Glucose concentrations were measured by a colorimetric assay kit (Thermo, Louisville, CO, USA). Leptin was analysed by radioimmunoassay using a multi-species leptin assay kit (Millipore, St Charles, MO, USA). Insulin concentrations were determined using a porcine insulin radioimmunoassay kit from Millipore. Free fatty acids were analysed by the Wako HR series NEFA-HR(2) *in vitro* enzymatic colorimetric assay from Wako Chemicals (Richmond, VA, USA). Triglyceride concentrations were determined by enzymatic colorimetric means using the L-Type TG M test kit from Wako Chemicals. All radioimmunoassay were analysed using a Packard COBRA gamma counter (Perkin-Elmer Life and Analytical Sciences, Waltham, MA, USA). Colorimetric assays were performed on a Perkin-Elmer VICTOR2 plate reader (Perkin-Elmer Life and Analytical Sciences).

Statistical analysis

All values are presented as mean ± SD for cats in each dietary group, with *n* indicating the number of cats. Results were analysed with the GLM procedure of SAS Version 9.1 (SAS Institute, Cary, NC, USA). Diet was included in the model as a classification factor. An additional model that includes metabolic BW or lean body mass (LBM) of the animal as covariates to analyse the effect of diet on energy expenditure is presented. The MIXED procedure of SAS was used to analyse food and energy intake values by week, with diet and week being included as classification factors. The alpha level used for determination of significance for all analyses was 0.05 and trends were reported when alpha level was <0.10. The statistical methods used were selected to allow for comparisons between the HP and MP groups at the end of baseline or the experimental phase, while accounting for variations in environment and time.

Results

All 20 cats completed measurements for energy intake, BW, energy expenditure, body composition and blood chemistry during baseline of the experiment on the MP diet (Table 2). There were no significant differences in the baseline measurements between the cats assigned to each diet group, with the exception of free fatty acids which were higher ($p < 0.009$) in the MP group. For the experimental phase, only a subset of cats from each group was evaluated for energy expenditure, body composition and blood chemistry because of repairs of the indirect calorimetry system, which extended beyond our time limits for this phase of the experiment. Measurements were collected from seven of the 10 cats in the HP group and six of the 10 cats in the MP group. As a consequence (and for uniformity in statistical analyses), values shown in Tables 3–5 for energy expenditure, body composition, and blood hormones and metabolites for both phases were only derived from measurements collected from the 13 cats. Energy intakes, BW, and BCS were still measured for all 20 cats for the experimental phase.

Energy intake, body weight and body composition

Energy intake and food intake was increased in the HP group in relation to the MP group (Table 3, $p = 0.026$). Energy intake was 1.043 ± 97.8 kJ/day for the MP group in relation to 1.212 ± 242.3 kJ/day for the HP group (Table 3). When food and energy intakes were measured for the subset group of cats eating the HP or MP diets, no differences were observed. Average food intake calculated from all 20

Table 2 Baseline measurements of food and energy intakes, body weight, body composition, energy expenditure, hormones and metabolic substrates in two groups of obese cats prior to feeding of a moderate protein (MP) or high protein (HP) diet

	Baseline		
	MP diet	HP diet	p-value*
Characteristics			
Food intake†, g/day	69 ± 10	72 ± 12	NS
Food intake‡, g/day	70 ± 9	71 ± 12	NS
Energy intake†, kJ/day	1124 ± 162.9	1173 ± 195.5	NS
Energy intake‡, kJ/day	1141 ± 146.7	1157 ± 195.5	NS
BW†, kg	6.1 ± 1.5	6.0 ± 1.4	NS
$BW^{0.75}$†, kg	3.9 ± 0.7	3.8 ± 0.6	NS
BW‡ (D_2O), kg	5.5 ± 0.8	6.3 ± 1.4	NS
BCS†	7.6 ± 0.8	7.7 ± 0.8	NS
Body composition‡			
FM, kg	1.6 ± 0.8	2.2 ± 0.8	NS
LBM, kg	3.9 ± 0.5	4.1 ± 0.8	NS
FM, % BW	28 ± 10.9	34 ± 7.7	NS
LBM, % BW	72 ± 10.9	66 ± 7.7	NS
Energy expenditures‡			
REE, kJ/day	1043 ± 102.1	1040 ± 121.1	NS
TEE, kJ/day	1120 ± 95.8	1155 ± 128.9	NS
Adjusted TEE§, kJ/day	1146 ± 39.0	1133 ± 35.9	NS
Adjusted REE§, kJ/day	1071 ± 34.7	1015 ± 32.0	NS
Adjusted TEE¶, kJ/day	1025 ± 45.0	1151 ± 41.6	NS
Adjusted REE¶, kJ/day	1049 ± 40.9	1034 ± 37.9	NS
RQ	0.84 ± 0.05	0.86 ± 0.05	NS
Serum chemistry‡			
Glucose (mg/dl)	73.3 ± 3.7	78.0 ± 6.8	NS
Insulin (μU/ml)	9.1 ± 3.1	10.1 ± 3.0	NS
Free fatty acids (mEq/l)	0.51 ± 0.06	0.41 ± 0.04	0.009
Triglycerides (mg/dl)	33.8 ± 15.7	31.8 ± 23.3	NS
Leptin (ng/ml)	6.5 ± 2.6	7.4 ± 3.5	NS

BW, body weight; $BW^{0.75}$, metabolic body weight; BW (D_2O), body weight at time of D_2O body composition evaluation; D_2O, deuterium oxide; BCS, body condition score; FM, fat mass; LBM, lean body mass; REE, resting energy expenditure; TEE, total energy expenditure; RQ, respiratory quotient.
*NS if $p \geq 0.05$.
†Values are expressed as mean ± SD, n = 10 cats/treatment group.
‡Values are expressed as mean ± SD, where n = 7 cats for the HP diet and n = 6 cats for the MP diet.
§BW at time of D_2O measurement included as a covariate.
¶LBM included as a covariate.

cats consuming either the MP or HP diets from week −6 to week 8 showed that introduction of the HP diet to cats at week 0 resulted in immediate increases in food intake (Fig. 1). Average energy intake for cats eating the HP diet peaked at 1.320 ± 282.0 kJ/day ($p = 0.002$) during week 1 and continued to be greater than average energy intake for cats consuming the MP diet for the duration of the study.

Table 3 Measurements of food and energy intakes, body weights and body compositions taken from two groups of obese cats during consumption of either a moderate protein (MP) or a high-protein (HP) diet

	Experimental phase		
	MP diet	HP diet	p-value*
Characteristics			
Food intake†, g/day	64 ± 6	75 ± 15	0.026
Food intake‡, g/day	65 ± 5	78 ± 13	NS
Energy intake†, kJ/day	1043 ± 97.8	1212 ± 242.3	0.026
Energy intake‡, kJ/day	1059 ± 81.5	1260 ± 210.0	NS
BW†, kg	6.0 ± 1.4	6.3 ± 1.4	NS
$BW^{0.75}$†, kg	3.8 ± 0.6	3.9 ± 0.7	NS
BW‡ (D_2O), kg	5.5 ± 0.9	6.6 ± 1.4	NS
BCS†	7.5 ± 0.9	7.5 ± 0.9	NS
Body composition‡			
FM, kg	1.9 ± 0.7	2.4 ± 0.7	NS
LBM, kg	3.6 ± 0.4	4.2 ± 0.9	NS
FM, % BW	34 ± 8.0	36 ± 6.1	NS
LBM, % BW	66 ± 8.0	64 ± 6.1	NS

BW, body weight; $BW^{0.75}$, metabolic body weight; BW (D_2O), body weight at time of D_2O body composition evaluation; D_2O, deuterium oxide; BCS, body condition score; FM, fat mass; LBM, lean body mass.
*NS if $p \geq 0.05$.
†Values are expressed as mean ± SD, n = 10 cats/treatment group.
‡Values are expressed as mean ± SD, where n = 7 cats for the HP diet and n = 6 cats for the MP diet.

Table 4 Energy expenditure measurements collected from two groups of obese cats during consumption of either a moderate protein (MP) or a high-protein (HP) diet using indirect respiration calorimetry

	Experimental phase		
	MP diet	HP diet	p-value*
Energy expenditures†			
REE, kJ/day	892 ± 72.0	1070 ± 130.4	0.013
TEE, kJ/day	975 ± 85.0	1146 ± 125.5	0.017
Adjusted TEE‡, kJ/day	1024 ± 26.3	1103 ± 24.1	0.063
Adjusted REE‡, kJ/day	942 ± 23.6	1027 ± 21.7	0.032
Adjusted TEE§, kJ/day	1007 ± 31.2	1118 ± 28.8	0.030
Adjusted REE§, kJ/day	925 ± 29.4	1042 ± 27.1	0.018
RQ	0.85 ± 0.04	0.83 ± 0.04	NS

REE, resting energy expenditure; TEE, total energy expenditure; RQ, respiratory quotient.
*NS if $p \geq 0.05$. All trends, $\alpha = 0.10$, are reported.
†Values are expressed as mean ± sd, where n = 7 cats for the HP diet and n = 6 cats for the MP diet.
‡BW at time of D_2O measurement included as a covariate.
§LBM included as a covariate.

There were no significant differences in BW between the MP and HP cats (Table 3). Body weight measured during D_2O procedures for the MP and HP

Table 5 Serum chemistry measurements of hormones and metabolic substrates collected from two groups of obese cats during consumption of either a moderate protein (MP) or a high-protein (HP) diet

	Experimental phase		
	MP diet	HP diet	p-value*
Serum chemistry†			
Glucose (mg/dl)	71.0 ± 5.9	77.2 ± 8.2	NS
Insulin (μU/ml)	7.1 ± 3.1	11.3 ± 3.8	0.054
Free fatty acids (mEq/l)	0.48 ± 0.10	0.44 ± 0.12	NS
Triglycerides (mg/dl)	32.3 ± 10.6	63.3 ± 38.7	0.085
Leptin (ng/ml)	7.2 ± 2.9	8.5 ± 4.7	NS

*NS if $p \geq 0.05$. All trends, $\alpha = 0.10$, are reported.
†Values are expressed as mean ± SD, where $n = 7$ cats for the HP diet and $n = 6$ cats for the MP diet.

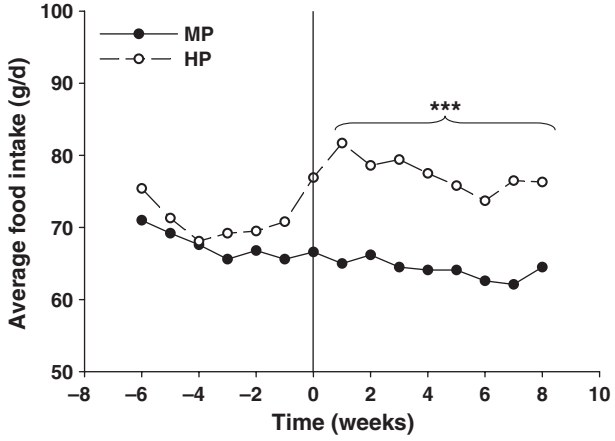

Fig. 1 Average weekly food intakes for cats eating the moderate protein (MP) or high-protein (HP) diets. Values are derived from 10 cats per treatment group. Week 0 represents the start of the experimental phase. Asterisks over the bracket covering week 1 to week 8 denote significant differences in food intakes between the MP and HP groups of cats, $p < 0.05$.

cats that underwent body composition and calorimetry measurements were also not significantly different (5.5 ± 0.9 and 6.6 ± 1.4 kg respectively). Measurements of FM and LBM were determined following calorimetry experiments for the thirteen cats. No significant differences were observed between the MP and HP groups for these measurements.

Energy expenditure

Resting and total energy expenditures were increased in the HP in relation to the MP group of cats. Total energy expenditure for the cats eating the MP diet was 975 ± 85.0 kJ/day, which differed ($p = 0.017$) from the TEE of cats consuming the HP diet (1146 ± 125.5 kJ/day) (Table 4). REE was increased ($p = 0.013$) in the HP in relation to the MP group. Energy expenditure was also increased in the HP in relation to MP group after adjusting for BW or LBM. REE adjusted with BW as a covariate was increased ($p = 0.032$) in the HP in relation to the MP group, and there was a trend towards an increase in TEE in the HP group after adjusting for BW ($p = 0.063$). When TEE and REE were adjusted by LBM, there was also a significant increase in the HP in relation to the MP group ($p = 0.030$ and 0.018 respectively). RQ did not differ between the two diet groups (Table 4).

Serum chemistry

There were no significant differences in glucose, leptin or free fatty acids between the HP and MP groups (Table 3). There were trends towards increases in insulin ($p = 0.054$) and triglyceride ($p = 0.085$) concentrations in the HP in relation to MP group (Table 5).

Discussion

This study assessed the effects of a HP diet on energy balance in *ad libitum* fed obese cats by monitoring changes of energy intake, energy expenditure, body weight and body composition. In humans, HP diets have been reported to increase satiety which, at least in the short term, leads to decreased energy intake (Halton and Hu, 2004). There is some evidence, however, that cats may behave differently than humans, and other omnivores, and show no decrease in energy intake in response to HP diets. No difference in food intake was observed between cats consuming either MP (27% of ME) or HP (50% of ME) diets in a recent study (Green *et al.*, 2008). Similarly, Russell et al. (2002) also did not observe a difference in energy intake in adult cats consuming either a moderate (35% of ME) or high (52% of ME) protein diet. In contrast to these findings, an increase of energy intake was observed in the present study in the HP in relation to MP groups of cats. The reason for this increase in energy intake is not entirely clear, although it is likely that the cats consuming the HP found the diet more palatable and this could lead to the increase in energy intake. It is important to note that energy intake increased immediately after switching the cats to the HP diet, and this is consistent with the idea that palatability played a major role in the increased energy intake. It has been shown that cats like the flavour

components in meats and peptides (N.R.C., 2006), and thus it may be possible to stimulate food intake in cats fed a HP diet if the ingredients used to raise the protein content also increase the palatability of the diet. The results of the present study are consistent with other studies indicating that HP diets do not induce a decrease in energy intake in cats. This is supportive of the idea that cats, as carnivores, have evolved to consume HP diets, and survival could be jeopardized if energy intake were decreased by elevated dietary protein concentrations.

Short-term human investigations suggest that HP diets enhance weight loss when compared with lower protein diets (Halton and Hu, 2004; Westerterp-Plantenga et al., 2009). In rats, long-term consumption of a HP diet (46% of ME) has also been shown to promote weight loss (Lacroix et al., 2004). There is little information, however, about the influence of HP diets on BW in cats. Laflamme and Hannah (2005) showed that feeding a HP (46% of ME) diet in restricted amounts to overweight cats induced greater fat loss and preserved lean tissue when in relation to animals on a normal protein (37% of ME) diet. In another study, non-obese cats were fed either a MP (27% of ME) or HP (47% of ME) diet in amounts intended to maintain optimal BW (Nguyen et al., 2004). Cats consuming the HP diet did not show any change in BW but gained LBM whereas cats fed the MP diet lost BW without losing LBM. In contrast to this finding, Green et al. (2008) observed that BW and body composition were not altered in cats allowed *ad libitum* consumption of an HP diet when in relation to a MP diet. The results of the present study are consistent with those of Green et al. (2008) and support the idea that HP diets are not capable of altering BW or body composition when fed in unrestricted amounts to cats.

Several studies have evaluated the influence of HP versus low/MP diets on energy expenditure (Westerterp-Plantenga et al. 2009). In relation to other macronutrients, dietary protein induces a greater increase in diet-induced energy expenditure (Crovetti et al., 1997; Johnston et al., 2002). In addition to increasing diet-induced energy expenditure, a few studies in humans also have reported that sleeping metabolic rate is increased in individuals consuming HP diets (Mikkelsen et al., 2000; Lejeune et al., 2006). In rats, increased energy expenditure has also been observed following consumption of HP diets (Petzke et al., 2007), although results have been conflicting (Petzke et al., 2005). Similar to studies in humans, the results of the present study showed that energy expenditure is increased in obese cats consuming the HP in relation to MP diet. This result differs from two studies in non-obese cats which reported no change in energy expenditure between animals on HP and MP diets (Russell et al., 2002; Green et al., 2008). There are at least two possible explanations for this difference between studies in cats. First, the cats in our study showed an increase in energy intake in the HP in relation to MP diet groups while the other studies (Russell et al., 2002; Green et al., 2008) found no change in intake between diet groups. The magnitude of the diet-induced energy expenditure is dependent on both energy intake and nutrient composition of the diet, and increases in energy and protein intake would both be expected to increase diet-induced energy expenditure (Blaxter, 1989; Westerterp-Plantenga et al., 2009). Second, the cats used in our study were obese and very sedentary as shown by the relatively small difference between resting and total energy expenditure observed in these animals. It is possible that normal daily variations in physical activity in non-sedentary animals may obscure the relatively small increases in diet-induced energy expenditure caused by HP diets. The fact that the animals in our study were very sedentary may have made it easier to detect changes in energy expenditure induced by the HP diet. Another related factor that could explain the small (sometimes negligible) variations in energy expenditure observed in cats and the more pronounced changes in energy expenditure observed in humans after consumption of MP vs. HP diets is that of meal size. In humans, measurements of post-prandial thermogenesis after eating meals of varying protein concentrations shows pronounced effects on energy expenditure because of distinct and defined meal patterns. For cats, food consumption occurs as many small meals during a 24-h period (N.R.C., 2006). Energy expenditure associated with consumption of small meals over a 24-h cycle would be less pronounced and more difficult to quantify. Overall, the results of our study are consistent with many of the studies in humans and support the idea that HP diets can lead to increases in TEE in cats.

Studies to evaluate the effect of HP diets on blood glucose and insulin concentrations in humans have produced conflicting results (Promintzer and Krebs, 2006; Layman et al., 2008). Although there is general agreement that weight loss increases insulin sensitivity, it is less certain if long-term consumption of HP diets have positive or negative influences on blood glucose and insulin concentrations, independent of

any changes induced by weight loss (Promintzer and Krebs, 2006). A few studies in non-obese (Leray et al., 2006; Hoenig et al., 2007) and obese cats (Hoenig et al., 2007) have reported that insulin sensitivity is not altered in animals consuming HP in relation to MP diets. In the present study, no change in insulin sensitivity was observed between the two groups of cats (HP vs. MP diet) (as estimated by the insulin/glucose ratio). However, future studies using more animals and a more accurate measurement of insulin sensitivity, such as the glucose tolerance test, may be necessary to truly determine if the increase in insulin concentrations is indicative of insulin resistance. Fasting serum glucose concentrations were not different in cats consuming the HP or MP diets, although there was a trend towards an increase ($p = 0.054$) in insulin concentrations in the HP group. It has been shown that amino acids potently stimulate insulin secretion from the cat pancreas (Curry et al., 1982), and it is possible that HP diets may promote insulin secretion if they lead to increases in circulating amino acid concentrations. Additional studies, however, will be needed to determine if long-term consumption of HP diets do truly alter insulin concentrations in cats.

Overall, the results of this study indicate that energy expenditure is increased in overweight cats allowed *ad libitum* access to a HP diet. However, this change in energy expenditure is accompanied by an increase in voluntary energy intake and does not lead to a decrease in BW or alteration in body composition. Results from this study show that *ad libitum* consumption of a HP diet does not effectively promote weight loss in obese cats. However, as energy expenditure was increased in cats eating the HP diet, it is possible that this effect of HP diets may help promote weight loss when energy intake is restricted.

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