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RESEARCH ARTICLE

When fed foods with similar palatability, healthy adult dogs and cats choose different macronutrient compositions

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ABSTRACT

Dogs and cats make short-term food choices based on palatability. We hypothesized that, if palatability were masked, long-term food choices would be based on physiological requirements, and circulating metabolite concentrations would reflect those choices. Four experimental foods with similar palatability, but varying in macronutrient composition, were prepared for healthy adult dogs (n=17) and cats (n=27). Food 1 was high protein; food 2 was high fat; food 3 was high carbohydrates; and food 4 was balanced for macronutrients. By choosing any combination of foods, dogs and cats could individually set their macronutrient intake. Plasma metabolomic profiles were determined at baseline and after animals had consumed their food intake of choice for 28 days. Based on food intake calculations over 28 days, dogs on average chose to consume most of their calories from fat (41.1±4.3%) and then carbohydrate (35.8±3.7%), whereas cats on average chose to consume most of their calories from carbohydrate (43.1±4.0%) and then protein (30.3±3.9%; all P<0.001). Age and lean or fat body mass also influenced protein intake. Younger, leaner cats consumed more protein compared with older cats, whereas younger, leaner dogs consumed less protein compared with dogs having more fat body mass. Older cats with moderate protein intake had lower circulating docosahexaenoic acid (DHA) concentrations as well as higher concentrations of sulfated microbial catabolic products compared with younger, leaner cats. In summary, when fed foods with similar palatability, dogs and cats consume different macronutrient compositions, and concentrations of circulating metabolites in cats reflect food choices.

KEY WORDS: Cats, Dogs, Food intake, Macronutrient classes, Metabolomics, Palatability

INTRODUCTION

Palatability is defined as the momentary and subjective orosensory pleasantness of food consumption (Ramirez, 1990; Stubbs and Whybrow, 2004). In humans, it is assumed that volume, mass, energy content, macronutrient proportion and energy density are signals that control short-term food intake (Stubbs and Whybrow, 2004). However in the long-term, the perceived palatability of a food is strongly influenced by its post-ingestion consequences, and this effect can override sensory factors (Stubbs and Whybrow, 2004). Physiological changes occur as a result of ingesting food and, by conditioned association, the animal learns to associate certain foods with sensory and physiological consequences (Stubbs and Whybrow, 2004). Using animal models, it is possible to study the effects of food intake choices when palatability is normalized. Differences in age and body mass composition may affect macronutrient choices, with subsequent consequences on metabolism. Based on circulating concentrations of metabolic intermediates and end products, metabolism can also be investigated in these animal models.

Foods can be confirmed to have similar palatability by using a two-pan test over 2 days (Vondran, 2013). This test confirms equal consumption of food when two foods are offered in separate bowls for a short period of time. Once palatability is balanced, then other post-ingestion factors that drive food choices over the long term can be studied (Covasa, 2008; Olszewski and Levine, 2008; Stubbs et al., 2008).

Metabolic consequences related to macronutrient consumption likely contribute more to long-term preferences for fat, carbohydrates and protein than taste (Olszewski and Levine, 2008). Data suggests that there is a clear relationship between oral and gastrointestinal sensing systems for macronutrients, which allows for a proper selection of quality and amount of foods in relation to their macronutrient composition (Olszewski and Levine, 2008). Post-ingestive actions of nutrients can also modulate food preferences (Ackroff et al., 2005). For example, many different fat sources, particularly the highly polyunsaturated fats and/or the lower saturated fats, can condition flavor preferences (Ackroff et al., 2005). Certain neuropeptides, such as neuropeptide Y, also aid in integrating food-related information, and may lead to changes in meal patterns, preferences, reinforcement and other direct or indirect consequences of macronutrient consumption (Olszewski and Levine, 2008).

The feeding behaviors of dogs and cats are often described with reference to their wild ancestors (Bradshaw, 2006). Domestication has significantly changed the genotypes of dogs and cats, with the dog showing increased adaption to a carbohydrate-rich food (Axelsson et al., 2013). In the domestic cat, the predominate genotypic changes reported to date are associated with behavior modifications that occurred when farmers domesticated wild-born cats to control rodent populations consuming cultivated grains, thus losing some of their wild-born genetic imprints (Driscoll et al., 2007). In cats, there does not appear to be a similar genetic enhancement of carbohydrate metabolism as in dogs (Montague et al., 2014). That cats choose a higher percentage of protein compared with dogs is explained by ancestral characteristics of the cat family (Hewson-Hughes et al., 2011). However, it is apparent that the domestic cat is well positioned to successfully metabolize foods with the majority of the calories derived from carbohydrates (National Research Council, 2006). Recent experiments suggest that wild carnivores regulate food intake to balance macronutrients rather than relying on relatively invariant available prey (Hewson-Hughes et al., 2013, 2011). Nonetheless, wild carnivores have a dietary regime that is high in protein and fat relative to carbohydrate because preys are what are available as food sources.

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Cats are obligate carnivores. In general, they maintain body mass even if allowed *ad libitum* access to palatable foods, by eating small meals and adjusting intake according to the energy density of the food consumed (Bradshaw et al., 1996). Their taste buds are responsive to amino acids, but not sugars. Food choices can be modified by their relative abundance, their novelty and by adverse consequences associated with a flavor of food (Bradshaw et al., 1996).

The aim of this study was to compare species differences between dogs and cats regarding macronutrient consumption when fed foods with similar palatability. We also evaluated the relationships between food intake (macronutrient composition) and age, lean or fat body mass, and plasma metabolomics. We hypothesized that cats would choose different proportions of macronutrients compared with dogs when foods were balanced for palatability. We further hypothesized that, within a species, choices would differ based on age, and lean or fat body mass, and subsequently result in unique metabolic profiles in circulating blood, which could be characterized to study the effects of food intake on metabolism.

MATERIALS AND METHODS

Animals and ethics statement

All study protocols and this study were reviewed and approved by the Institutional Animal Care and Use Committee, Hill's Pet Nutrition, Inc., Topeka, KS, USA (permit numbers: canine 590 and feline 577), and complied with the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978). All studies were conducted at the Hill's Pet Nutrition Center.

Palatability equivalency testing

To determine that foods were of similar palatability, different groups of dogs and cats than those used in the macronutrient preference study were utilized (panels of 20 adult dogs or cats) for palatability equivalency testing. These animals were used to determine food preference in a 2 day test. Dogs were offered two bowls with each bowl containing an excess of the day's caloric requirement of food. Dogs were given up to 1 h to choose their daily food consumption and both food choices were removed when the calories calculated to maintain body mass had been consumed. Cats were given up to 23 h to consume the day's caloric requirement of food, but had the same restriction as dogs in that both foods were rendered unavailable when the caloric consumption calculated to maintain body mass was consumed.

Foods were subsequently balanced for palatability using palatability knobs (different types of palatability enhancers, different concentrations of palatability enhancers, or different ingredients) that were adjusted in order to equalize food intake between two foods in a two-pan test over 2 days (Vondran, 2013). Ultimately, palatability was masked both by changing macronutrient sources as well as concentrations of palatability enhancers (Table 1). For example, in order to reduce the palatability of the high-fat canine food, the concentration of palatability enhancers were reduced to 0.5% of the ingredient mix, whereas the maximum concentration of palatability enhancers that were added to the lower palatability foods were increased to as high as 2.5%. In order to mask the palatability of the high-protein food for cats, wheat gluten and pea protein were used as protein sources instead of chicken. All canine foods were cylindrical in shape, whereas all cat foods were disc shaped. When animals chose the same amount of each food in the short-term, this indicated balanced palatability. Within a species, there was no significant difference in individual food consumption, i.e. each food resulted in similar consumption when fed in a two-pan palatability test compared with each of the other available foods.

Macronutrient preference studies

Foods

Prior to beginning the study, all dogs and cats had been fed many types of commercial and non-commercial foods of varying nutrient compositions, including dry and canned foods, in palatability studies. All foods met the requirements established by the Association of American Feed Control Officials for complete and balanced pet foods for adult dogs and cats.

Dog foods were produced by Hill's Pet Nutrition, Inc., and met the nutritional requirements for adult dogs (≥1 year). Food was available in dry form only. Macronutrient composition of foods was determined by a commercial laboratory (Eurofins Scientific, Inc., Des Moines, IA, USA). Proximate analyses were completed using the following techniques: moisture – AOAC 930.15; protein – AOAC 2001.11; fat – AOAC 954.02; fiber – AOAC 962.09; and ash – AOAC 942.05. Carbohydrate composition was determined by

Table 1. Ingredients for each of the four equivalent palatability foods fed to dogs and cats

	Canine				Feline			
Ingredients (%)	High protein	High fat	High carbohydrate	Balanced	High protein	High fat	High carbohydrate	Balanced
Chicken	42.1	29.6	11.2	27.7	13.1	19.9	14.3	18.9
Pork fat	5.0	20.1	6.7	8.9	4.0	14.0	4.4	8.0
Corn	27.6	26.7	56.1	39.9	0.0	0.0	0.0	0.0
Brown rice	0.0	0.0	0.0	0.0	11.4	20.6	24.0	15.9
Brewers rice	10.0	10.0	10.0	10.0	11.4	20.6	24.0	15.9
Wheat	10.0	10.0	10.0	10.0	12.0	10.0	20.7	20.4
Wheat gluten	0.0	0.0	0.0	0.0	35.0	10.0	8.0	15.8
Pea protein	0.0	0.0	0.0	0.0	9.0	0.0	0.0	0.0
Natural chicken flavor	2.5	0.5	2.0	0.5	1.8	1.8	1.8	1.9
Methionine	0.2	0.2	0.2	0.2	0.3	0.5	0.3	0.3
Lysine	0.5	0.5	0.5	0.5	0.0	0.0	0.0	0.0
Threonine	0.2	0.1	0.1	0.1	0.0	0.0	0.0	0.0
Taurine	0.0	0.0	0.0	0.0	0.1	0.2	0.1	0.1
Vitamins	0.3	0.3	0.3	0.3	0.6	0.8	0.7	0.7
Minerals	1.7	2.0	2.9	1.9	1.3	1.6	1.7	2.1

Palatability was masked both by changing macronutrient sources as well as concentrations of palatability enhancers. All canine foods were cylindrical in shape, whereas all cat foods were disc shaped.

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Table 2. Macronutrient composition of each of the four foods offered to dogs[a]

Macronutrient	High-protein food	High-fat food	High-carbohydrate food	Balanced food
Moisture (%)	7.1	7.1	7.2	7.9
Protein (%)	34.4	24.6	16.4	25.2
Fat (%)	14.0	28.4	12.0	15.9
Carbohydrate (%)	36.9	33.8	58.4	44.9
Ash (%)	6.6	5.4	4.8	5.3
Crude fiber (%)	1.0	0.7	1.2	0.9
Energy (kcal kg^{-1})	368	445	364	380
Calories from protein (%)	32.7	19.3	15.8	23.2
Calories from fat (%)	32.3	54.1	28.0	35.6
Calories from carbohydrate (%)	35.1	26.6	56.2	41.3

[a]Macronutrient composition, expressed as percentage of food, as fed. Energy was calculated using the modified Atwater factors as described (National Research Council, 2006).

calculation. Macronutrient composition, expressed as percentage of food, as fed, is shown in Table 2.

Cat foods were produced by Hill's Pet Nutrition, Inc., and met the nutritional requirements for adult cats (≥1 year). Food was available in dry form only. Macronutrient composition of foods was determined by a commercial laboratory (Eurofins Scientific, Inc.). Carbohydrate composition was determined by calculation. Macronutrient composition, expressed as percentage of food, as fed, is shown in Table 3. No ingredient sources of eicosapentaenoic (EPA) or docosahexaenoic (DHA) acid were added to the food.

For dog and cat foods, changes to the macronutrient profile necessitated changes in ingredient composition. However, with the exception of macronutrients, the nutrient profiles of all foods were balanced such that similar amounts of vitamins and minerals were consumed without regard to macronutrient changes.

Dogs

These studies were conducted using 17 dogs randomly selected from a colony of Beagles (inclusion criteria were availability; exclusion criteria were preexisting illness and previously documented food bowl position bias) ranging in age from 2.1 to 11.8 years (mean±s.d.: 7.4±3.1 years): 12 ovariohysterectomized females and 5 neutered males, with varying lean body and fat body masses (mean±s.d.: body mass, 10.9±1.50 kg, range 7.6–13.6 kg; body lean, 6.70±1.16 kg, range 4.88–9.33 kg; body fat, 3.78±0.94 kg, range 2.4–6.2 kg). All dogs were exercised daily, and were provided with regular opportunities for socialization and environmental enrichment. Dogs were paired and housed in runs for 15 h each day, with space for sleeping and exercise. During the day they were housed together with 10 and 7 dogs per room, with 45 min intervals alternating between the group rooms and outside play in the dog park.

Table 3. Macronutrient composition of each of the four foods offered to cats[a]

Macronutrient	High-protein food	High-fat food	High-carbohydrate food	Balanced food
Moisture (%)	5.4	7.4	7.6	5.1
Protein (%)	44.1	26.6	24.9	31.4
Fat (%)	10.5	21.1	9.8	13.4
Carbohydrate (%)	34.8	39.8	52.6	44.1
Ash (%)	4.3	4.5	4.3	5.3
Crude fiber (%)	0.9	0.7	0.9	0.8
Atwater energy (kcal kg^{-1})	366	411	354	378
Calories from protein (%)	42.2	22.6	24.6	29.1
Calories from fat (%)	24.5	43.5	23.4	30.1
Calories from carbohydrate (%)	33.3	33.9	52.0	40.8

[a]Macronutrient composition, expressed as percentage of food, as fed.

Dogs were fed once daily, and were moved to individual stalls for a 1 h feeding period. Dogs were allowed to choose freely among any of the four foods, which were offered in a line at the same time, but were limited in food intake to a predetermined caloric allowance by restricting food intake once allowed caloric consumption was attained. Similar to the palatability studies defined above, the canine four-bowl test reported here had a bowl for each of the four foods available until the dogs had consumed the allotted daily calories. Multiple bowl testing is a common practice in the pet food industry to evaluate food intake choices. Each dog's caloric consumption was based on age, body mass and activity level. Dogs were micro-chipped and food disappearance was recorded from all food bowls, which were set on scales. A computer calculated change in food mass, and thus calories consumed. Thus, the total amount of food consumed was controlled to meet daily metabolic energy requirements and maintain body mass. The amount of each food consumed from each food source was used to calculate daily and average 28 day composite macronutrient intake for each dog.

Cats

Studies were conducted using 27 cats randomly selected from a colony of cats (inclusion criteria were availability; exclusion criteria were preexisting illness) ranging in age from 2.3 to 6.6 years (mean±s.d.: 4.0±1.6 years): 14 ovariohysterectomized females and 13 neutered males, with varying lean body and fat body masses (mean±s.d.: body mass, 5.42±0.93 kg, range 3.6–7.1 kg; body lean, 3.92±0.54 kg, range 2.9–4.8 kg; body fat, 1.36±0.46 kg, range 0.3–2.2 kg). Cats were housed in indoor rooms of 10 and 17, with access to glass-enclosed porches and toys at all times.

Cats were housed together, with 13 or 14 cats per room. There were four feeding stations in each room. From the beginning of the study, cats were offered food *ad libitum* from any of the four feeding stations in the room. Multiple bowl testing is a common practice in the pet food industry to evaluate food intake choices. Similar to dogs, cats were micro-chipped such that food disappearance was recorded from each feeding station after every meal. Cats were allowed to choose freely among any of the four foods offered, but were limited in food intake to a predetermined caloric allowance based on age and body mass. Thus, the total amount of food consumed was controlled to meet daily metabolic energy requirements and maintain body mass. The amount of each food consumed from each food source was used to calculate daily and average 28 day composite macronutrient intake for each cat.

Study design and measurements

For a 28 day period, dogs and cats were given the opportunity to choose from any of four completely balanced foods that differed in concentration of macronutrients as a percent of total calories fed. Dogs and cats were allowed to choose among the four food sources until the amount of food consumed met the daily established caloric allowance to maintain a healthy mass. Based on the amount of food consumed from each feeding station, the total amount of protein, fat and carbohydrate consumed each day were calculated and summed over the 28 day period. The percent of each macronutrient

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consumed relative to total caloric intake over the same 28 day period was then determined.

All foods had been previously balanced for palatability. Animals that exhibited a bowl position bias in previous studies were excluded from this study. Furthermore, bowl position was changed daily such that animals needed to move their consumption position in order to maintain the macronutrient intake mixture of choice. Their macronutrient intake mixture stabilized within the first week, and was stable for the remainder of the study.

Body mass and composition were assessed by dual-energy X-ray absorptiometry (DXA-QDR-4500, Hologic, Inc., Waltham, MA, USA) scan analysis during the third week of the feeding trial. Total, fat and lean mass were determined using software supplied by the manufacturer.

Blood was collected at 28 days after animals had consumed their food intake composition of choice in order to determine plasma metabolomic profiles. Blood was collected from dogs before the next day's meal, resulting in a 23–24 h food withholding period before the blood sample was drawn. For blood collection in cats, food bowls were removed at the end of the day and blood was collected before food was replaced the next day such that food was withheld 15–16 h before blood was collected. Plasma metabolites were measured by a commercial laboratory (Metabolon, Durham, NC, USA). Extracted supernatant was split and run on gas chromatography and liquid chromatography mass spectrometer platforms (Evans et al., 2009) in a randomized order. Gas chromatography (for hydrophobic molecules) and liquid chromatography (for hydrophilic molecules) were used to identify and provide relative quantification of small metabolites present in plasma samples. Endogenous biochemicals included amino acids, peptides, carbohydrates, lipids, nucleotides, cofactors and vitamins. Data for each individual compound were normalized by calculating the median values for each run-day block (block normalization). This minimized any inter-day instrument gain drift, but did not interfere with intra-day sample variability.

Statistical analyses

For palatability equivalency testing, food preference was calculated using a consumption ratio, which was calculated by dividing the amount of test food consumed by total food consumed. The ratio varies from zero (only one food is consumed) to one (only the other food is consumed). For example, if the consumption of one of the test foods is defined as A and the other as B, then the ratio is A/(A+B). A *t*-test was then performed with the null hypothesis being that the ratio equals 0.5. Foods were subsequently balanced for palatability by changing macronutrient sources as well as concentrations of palatability enhancers until consumption ratios were non-significant ($P>0.05$).

For macronutrient preferences studies, analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC, USA). To investigate the relationship between food macronutrients, Pearson correlation coefficients were measured between the response variables using PROC GLM. Statistical significance was declared at $P\leq0.05$ and a tendency at $0.05<P\leq0.10$.

Because the total amount of calories consumed is divided among calories from protein, carbohydrate and fat, caloric intake data is presented as compositional. Aitchison's centered log ratio transformation was performed on a composition consisting of protein, carbohydrate and fat, and used in the subsequent statistical analysis as described by Pawlowsky-Glahn et al. (2015). In brief, the centered log ratio of each macronutrient intake over 28 days, expressed per calories consumed over 28 days, was calculated by taking the ratio of each macronutrient intake to the geometric mean of the three-component composition, and then calculating the natural log of each ratio. Centered log ratio transformed data were subsequently used to calculate *r* values. For visual purposes, data are presented in figures as untransformed data for percent calories from protein, fat and carbohydrate. Macronutrient intake comparisons between species were completed using the centered log transformed data and the PROC GLM program.

To investigate the relationship between protein intake from calories (the outcome variable) and other variables [age, fat (g), lean (g), bone mineral (g) and gender], a regression tree analysis was conducted using the predictive modeling platform in JMP 12 (SAS Institute). The 5-fold cross-validation stopping rule was used to terminate stepping when improvement in the cross-validation r^2 was minimal. The groups defined by the regression tree analysis for protein intake, based on age and lean body mass (cats), and fat body mass and age (dogs), divided cats into four groups and dogs into three groups. We used these groups to determine the effect of consuming macronutrients of choice for 28 days on selected metabolites of the major macronutrient classes (essential fatty acids and long-chain elongation and desaturation products, essential and nonessential amino acids, glucose and tricarboxylic acid cycle intermediates, urea cycle intermediates, and sulfated microbial catabolic products), using MULTTEST. Animal was considered the experiment unit. For metabolites, an adjusted $P\leq0.05$ for type 1 error was used.

RESULTS

Palatability equivalency testing

Initially, there was a significant effect of macronutrient content on food intake preference. In the dog, there was a preference for high-fat food, whereas, in the cat, there was a preference for high-protein food. Food preference was calculated using a consumption ratio, which was calculated by dividing the amount of test food consumed by total food consumed (values are grams of test food/grams of total food consumed). In dogs, consumption of high-fat food (defined as A) was compared individually to consumption of each of the other foods (defined as B) such that the ratio of A/(A+B) was 0.74 for B=high-protein food, 0.60 for B=high-carbohydrate food, and 0.46 for B=balanced food. A *t*-test was significant at $P<0.05$ for B=high-protein food and B=high-carbohydrate food. The amount of palatability enhancer (natural chicken flavor) was then titrated from 0.5 to 2.5% so as to offset basic changes in palatability of macronutrients (Table 1). The high-fat and balanced foods had the least amount of added natural chicken flavor (0.5%), whereas the high-protein food had the most (2.5%). Subsequent calculation of food consumption ratios comparing high-fat food (A) to each of the other foods (tested individually as B), resulted in non-significant ($P>0.05$) ratios.

Because cats initially preferred high-protein food, this food was defined as A, and a consumption ratio was subsequently calculated to individually compare consumption of food A to consumption of each of the other foods (defined as B) such that the ratio of A/(A+B) was 0.72 for B=high-fat food, 0.60 for B=high-carbohydrate food, and 0.67 for B=balanced food. A *t*-test was significant at $P<0.05$ for B=high-fat food, high-carbohydrate food and balanced food. Ingredient changes were used to offset basic changes in palatability of macronutrients (Table 1). Because cats like chicken more than pea protein, pea protein (9.0%) and wheat gluten (35%) were used as protein sources instead of as much chicken (13.1%) in the high-protein food. More wheat (20.7%) and brown rice (24.0%) were added to the high-carbohydrate food compared with the high-protein food (12.0 and 11.4%, respectively). Subsequent calculation of food consumption ratios comparing high-protein food (A) to each

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Table 4. Composite macronutrient intake (percentage) over the 28 day feeding period for dogs and cats

	Dogs		Cats	
% Calories from	Mean	s.d.	Mean	s.d.
Protein	23.1[a]	1.6	30.3[b]	3.9
Carbohydrate	35.8[a]	3.7	43.1[b]	4.0
Fat	41.1[a]	4.3	26.6[b]	2.3

[a,b]Means with different superscripts within a row are significantly different (P<0.001).

of the other foods (tested individually as B), resulted in non-significant (P>0.05) ratios.

Macronutrient preference studies

Healthy adult cats on average chose to consume most of their calories from carbohydrate (43%) and protein (30%), whereas healthy adult dogs on average chose to consume most of their calories from fat (41%) and carbohydrate (36%). Macronutrient percentages were based on calculations of composite macronutrient intake over the 28 day feeding period (Table 4). Overall, when fed foods with similar palatability, healthy adult dogs and cats chose different macronutrient intakes (P<0.001). Cats chose a broader concentration of protein intake compared with dogs, as evidenced by the standard deviations for protein intake. No dog or cat, by choice, maximized protein intake by feeding solely from the high-protein feeding station.

There was not an equal response to change in protein intake between cats and dogs (Figs 1A and 2A). In cats, when protein intake increased, carbohydrate intake decreased (r=−0.77; P<0.001; Fig. 1B), and fat intake decreased as well (r=−0.47; P=0.01). In dogs, variation in protein intake was less than observed for cats and was not offset by carbohydrate intake (r<0.03; P=0.87; Fig. 2B), although increased protein intake was offset by decreased fat intake

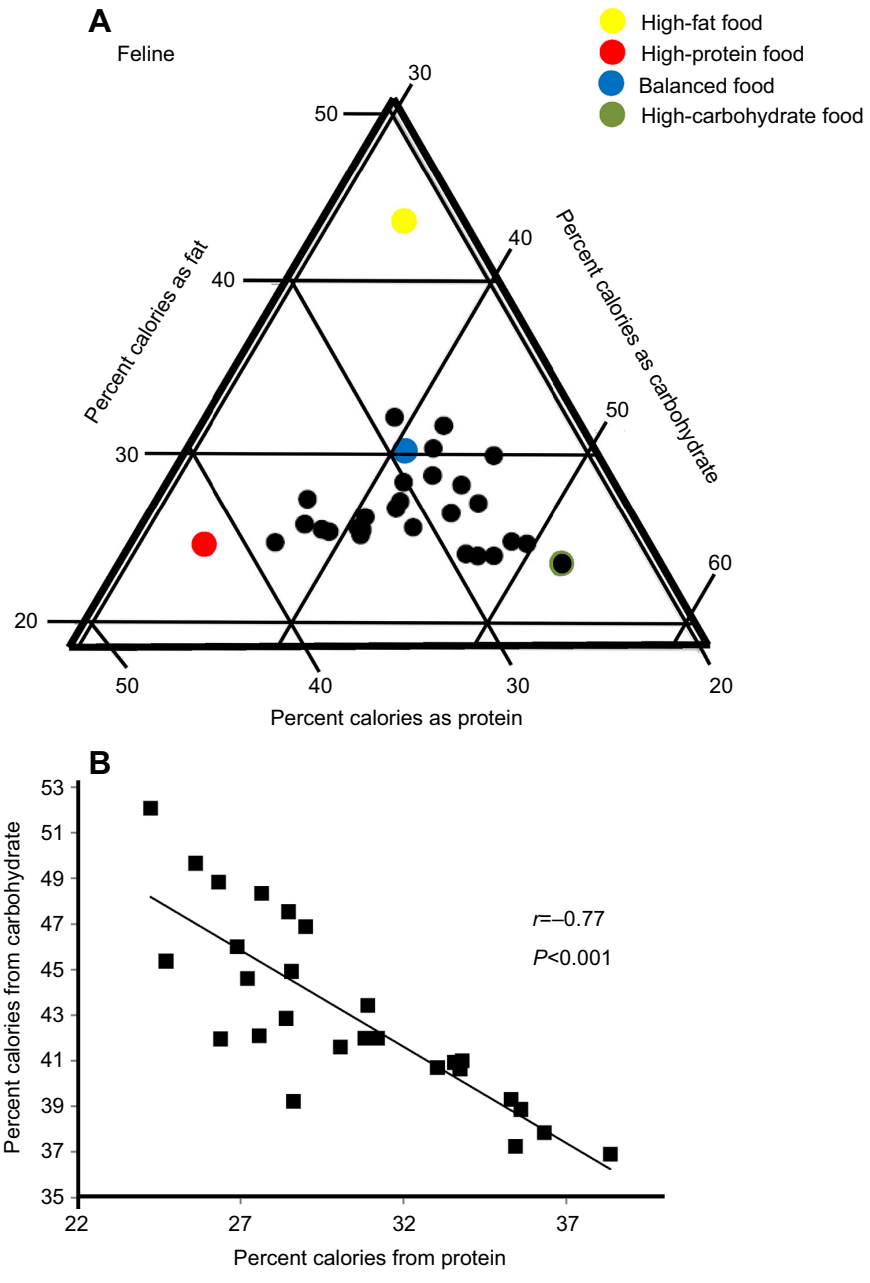

Fig. 1. The effect of protein, fat or carbohydrate intake on intake of other macronutrients in cats is illustrated using geometric techniques. (A) Based on the amount of food consumed from each feeding station, the total amount of protein, fat and carbohydrate consumed each day was calculated and summed over the 28 day period. The percent of each macronutrient consumed relative to total caloric intake was then determined. Each cat (n=27) is represented only once as a black circle in the figure. Also shown are colored dots that represent where the circle would have been located if cats had chosen only high-fat food (yellow), high-protein food (red), high-carbohydrate food (green) or the balanced food (blue). (B) Cats chose a broader concentration of protein intake compared with dogs. When protein intake increased, carbohydrate intake decreased (r=−0.77; P<0.001) and fat intake decreased as well (r=−0.47; P=0.01).

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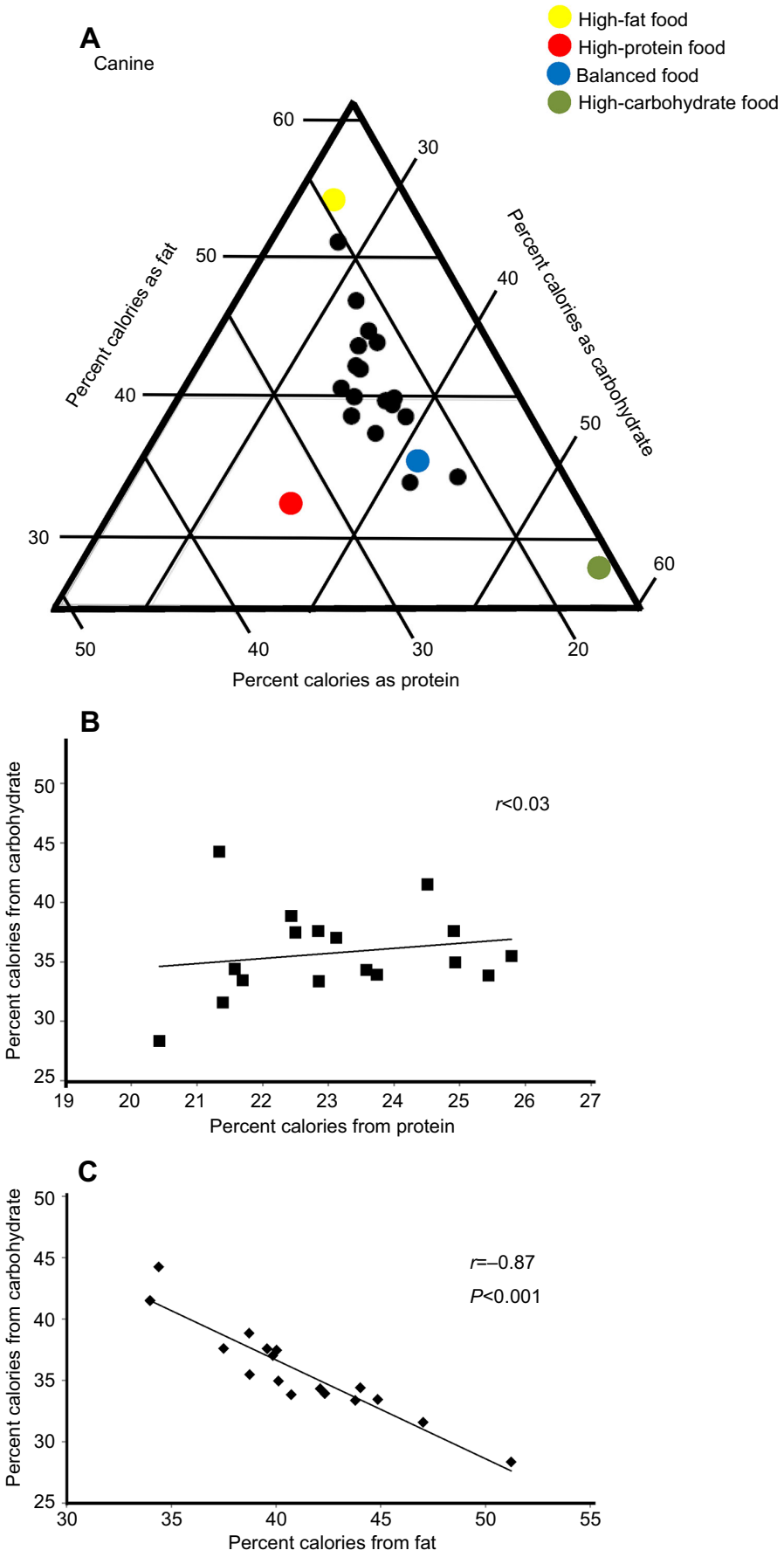

Fig. 2. The effect of protein, fat or carbohydrate intake on intake of other macronutrients in dogs is illustrated using geometric techniques. (A) Based on the amount of food consumed from each feeding station, the total amount of protein, fat and carbohydrate consumed each day was calculated and summed over the 28 day period. The percent of each macronutrient consumed relative to total caloric intake was then determined. Each dog (n=17) is represented only once as a black circle in the figure. Also shown are colored dots that represent where the circle would have been located if dogs had chosen only high-fat food (yellow), high-protein food (red), high-carbohydrate food (green) or the balanced food (blue). (B) Dogs chose a more narrow range of protein intake compared with cats. In dogs, variation in protein intake was not offset by carbohydrate intake (r<0.03; P=0.87), although increased protein intake was offset by decreased fat intake similar to cats (r=–0.52; P=0.03). (C) Dogs chose a broader concentration of fat intake compared with cats. In dogs, unlike cats, there was an inverse relationship between fat and carbohydrate intake: as fat intake increased, carbohydrate intake decreased (r=–0.87; P<0.001).

similar to cats (r=−0.52; P=0.03). In dogs, unlike cats, there was an inverse relationship between fat and carbohydrate intake; as fat intake increased, carbohydrate intake decreased (r=−0.87; P<0.001; Fig. 2C). In cats, as fat intake increased, there was no change in carbohydrate intake (r=−0.20; P=0.32).

Cats, on average, had more variation in protein intake (range 23–38%; 78% of the range between the highest and lowest protein food available to cats) compared with dogs (range 20–26%; 37% of the range between the highest and lowest protein food available to dogs).

Age, lean body mass and fat body mass influenced the percent of calories chosen from protein (Fig. 3). In cats, there was a negative relationship between percent of calories consumed from protein and age and lean body mass (r^2=0.57; P<0.001). Overall, younger cats showed a greater preference for dietary calories from protein. All cat groups over 3.2 years of age consumed less protein than younger cats. Cats aged 3.2–3.4 years consumed the least percent of calories from protein. Younger cats with less lean body mass had a greater preference for dietary calories from protein (34.4%) compared with younger cats with more lean body mass (>3.8 kg). In dogs, there was a relationship between percent of calories chosen from protein and age and fat body mass (r^2=0.55; P<0.001). Overall, dogs with greater fat body mass (>4.2 kg) had a greater preference for dietary calories from protein (24.5%). Age also had a significant effect on protein intake in that younger dogs (<7.8 years) with less fat body mass chose to consume fewer calories from protein (21.5%).

Cats and dogs were divided into groups (cats four groups; dogs three groups) defined by the regression tree analysis for protein intake, based on age and lean body mass (cats), and fat body mass and age (dogs) (Fig. 3). These groups were studied to determine the effect of consuming macronutrients of choice for 28 days on selected metabolites of the major macronutrient classes. The older cats (>3.4 years) had significantly lower (P=0.01) plasma concentrations of DHA compared with younger cats (<3.2 years) (Table 5). None of the foods contained ingredient sources of EPA or DHA. Older cats had significantly higher (P=0.02) plasma concentrations of phenylalanine. Metabolomic analysis of urea cycle intermediates showed that increased protein intake in younger cats was associated with increased concentrations of arginine (P=0.05) and homoarginine (P=0.01). Plasma urea concentrations were not increased with protein intake. In older cats, concentrations of sulfated microbial catabolic products were significantly increased for p-cresol sulfate (P=<0.01) and 4-ethyl phenyl sulfate (P=0.02).

We found no significant differences between the three dog groups for essential fatty acids, essential and nonessential amino acids, glucose and tricarboxylic acid cycle intermediates, urea cycle intermediates, and sulfated microbial catabolic products after adjusting for false discovery rate (data not shown). There was a trend toward higher concentrations of several urea cycle intermediates when they were individually compared to protein intake.

DISCUSSION

Palatability among foods was made similar by adjusting palatability knobs before the study began using different groups of dogs and cats than those used in this study in a two-pan test over 2 days (Vondran, 2013). Initially, there was a significant effect of macronutrient content on food intake preference. In the dog, there was a preference for high-fat food, whereas, in the cat, there was a preference for high-protein food. Preference for high-fat food in dogs was masked by changing the amount of palatability enhancer (natural chicken flavor) added to the other foods. The dog foods with similar palatability were subsequently used in the canine macronutrient preference study. Preference for high-protein food in cats was masked using ingredient changes, e.g. pea protein and wheat gluten were added as protein sources instead of chicken. The cat foods with similar palatability were subsequently used in the feline macronutrient preference study. Although palatability enhancers may include digests (animal tissues enzymatically altered by proteolytic enzymes), salt, fat, L-lysine, L-cysteine, monosodium

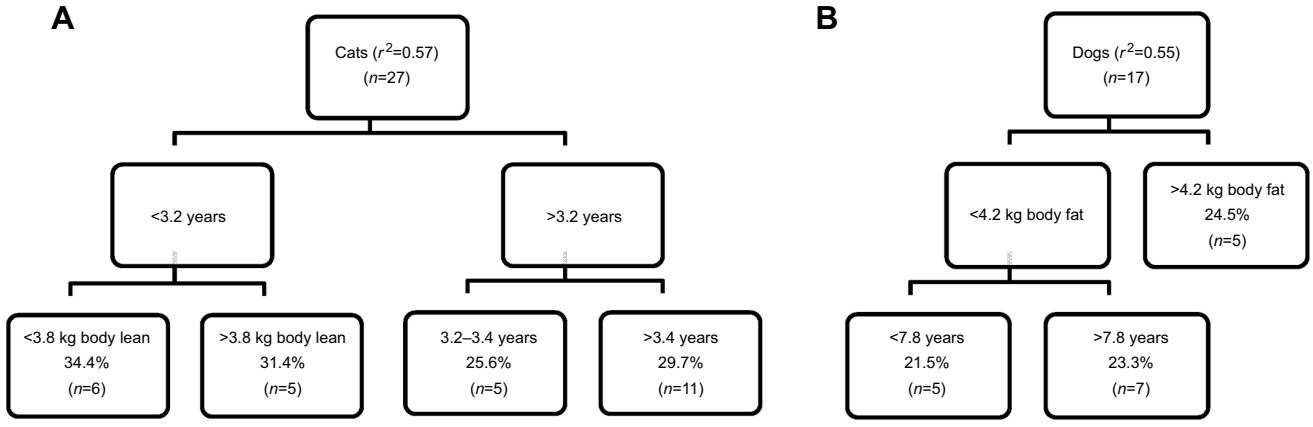

Fig. 3. Age, lean body mass and fat body mass of cats and dogs influence the percent of calories chosen from protein. (A) Cats (n=27); (B) dogs (n=17). To investigate the relationship between protein intake (calories) and other variables [age, fat (g), lean (g), bone mineral (g) and gender], a regression tree analysis was conducted using the predictive modeling platform in JMP 12 (SAS Institute). The 5-fold cross-validation stopping rule was used to terminate stepping when improvement in the cross-validation r^2 was minimal. The groups defined by the regression tree analysis for protein intake, based on age and lean body mass (cats), and fat body mass and age (dogs), divided cats into four groups and dogs into three groups. In cats, there was a negative relationship between percent of calories consumed from protein and age and lean body mass (r^2=0.57; P<0.001). Overall, younger cats showed a greater preference for dietary calories from protein. All cat groups over 3.2 years of age consumed less protein than younger cats. Cats aged 3.2–3.4 years consumed the least percent of calories from protein. Younger cats with less lean body mass had a greater preference for dietary calories from protein (34.4%) compared with younger cats with more lean body mass (>3.8 kg). In dogs, there was a relationship between percent of calories chosen from protein and age and fat body mass (r^2=0.55; P<0.001). Overall, dogs with greater fat body mass (>4.2 kg) had a greater preference for dietary calories from protein (24.5%). Age also had a significant effect on protein intake in that younger dogs (<7.8 years) with less fat body mass chose to consume fewer calories from protein (21.5%).

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Table 5. Effect of cats consuming food intake of choice for 28 days on selected metabolites of the major macronutrient classes

Variables	Group classification based on regression tree analysis of protein consumption				s.e.m.[1]	*P*-values[2]
Age (years)	<3.2	<3.2	3.2–3.4	>3.4		
Body lean (kg)	<3.8	>3.8				
Protein intake (%)	34.4	31.4	25.6	29.7		
Number of cats (*N*)	6	5	5	11		
Essential fatty acids						
Linoleic acid [18:2 (*n*-6)]	1.02	1.21	0.97	0.90	0.09	0.20
Linolenic acid [18:3 (*n*-3)]	0.97	1.08	1.27	1.03	0.13	0.92
Arachidonic acid [20:4 (*n*-6)]	1.06	1.20	1.05	0.92	0.20	0.27
EPA [20:5 (*n*-3)]	4.94	5.53	5.03	4.60	0.70	0.96
DHA [22:6 (*n*-3)]	3.73[a]	3.94[a]	2.99[a,b]	2.07[b]	0.44	0.01
Essential amino acids[3]						
Threonine	0.93	1.02	0.84	0.90	0.08	0.95
Lysine	0.78	0.68	0.94	1.28	0.18	0.12
Methionine	0.84	0.81	0.79	0.91	0.07	0.98
Tryptophan	0.97	0.74	0.91	0.88	0.06	0.72
Leucine	0.90	1.03	0.99	1.00	0.07	0.95
Isoleucine	0.95	1.04	1.05	1.04	0.07	0.94
Valine	1.02	1.09	1.07	1.05	0.07	1.00
Tyrosine	1.01	0.97	1.06	1.14	0.06	0.37
Phenylalanine	0.98[b]	0.97[b]	1.03[b]	1.16[a]	0.05	0.02
Histidine	0.92	0.94	0.95	1.04	0.05	0.35
Non-essential amino acids						
Alanine	1.14	1.19	1.12	1.04	0.13	0.99
Asparagine	1.09	1.05	1.00	1.23	0.08	0.88
Aspartate	1.26	1.16	0.93	1.24	0.13	0.99
Cysteine	0.97	0.98	1.15	1.08	0.11	0.44
Cystine	0.30	0.46	0.43	0.34	0.13	0.99
Glutamate	1.26	1.33	1.05	1.11	0.08	0.12
Glutamine	0.91	1.00	0.93	0.87	0.05	0.86
Glycine	1.11	1.08	1.11	1.31	0.08	0.30
Proline	0.96	1.08	1.03	1.09	0.05	0.54
Serine	1.03	1.22	0.98	1.21	0.07	0.90
Glucose and tricarboxylic acid cycle						
Glucose	1.10	1.03	0.99	0.99	0.05	0.22
Lactate	1.34	1.36	1.11	1.18	0.12	0.39
Pyruvate	1.16	0.76	1.17	0.97	0.20	1.00
Citrate	0.99	0.98	0.98	0.91	0.04	0.33
α-Ketoglutarate	1.01	0.82	1.04	1.03	0.17	0.99
Succinate	0.87	0.92	0.92	0.96	0.10	0.95
Fumarate	1.18	1.22	1.28	1.34	0.19	0.94
Malate	1.24	1.27	1.31	1.44	0.18	0.87
Urea cycle						
Arginine	1.17[a]	1.03[a,b]	0.94[b]	0.99[b]	0.07	0.05
Homo arginine	5.48[a]	6.27[a]	1.57[b]	0.26[b]	0.61	0.01
Ornithine	0.94	0.79	0.80	1.05	0.15	0.94
Citrulline	0.94	0.77	0.74	0.90	0.08	0.96
Urea	1.21	1.07	1.11	1.20	0.15	0.94
Sulfated microbial catabolic products						
p-Cresol sulfate	0.50[c]	5.28[c]	30.0[b]	58.8[a]	9.72	<0.01
o-Cresol sulfate	1.59	1.58	0.40	0.83	0.48	0.24
Indoxyl sulfate	0.87	0.81	0.36	0.94	0.22	1.00
Catechol sulfate	0.43	0.87	1.15	0.62	0.21	0.24
4-Ethyl phenyl sulfate	0.15[b]	0.22[b]	0.39[b]	4.44[a]	1.13	0.02

Cats were divided into four groups based on a regression tree analysis of the variables affecting percent of calories chosen from protein. Significant variables in the regression tree analysis for cats were age and lean body mass. Shown are mean values for individual metabolites in each group of cats. Mean values are adjusted such that, in the complete data set for dogs and cats, data for each individual compound had a median value of 1.

[1]The largest s.e.m. of the four treatment groups is shown.

[2]The *P*-values are adjusted for multiple comparisons.

[3]Arginine is an essential amino acid as well, but it is included below as a urea cycle intermediate.

[a,b]Means with different superscripts within a row are significantly different ($P \leq 0.05$).

DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

glutamate, sugar, yeasts, whey, cheese powder, meat slurries and hydrolyzed vegetable protein, in this study, the amount of palatability enhancer or ingredient changes were the primary means used to make palatability similar among foods.

The two-pan preference test measures ‘choice’ between a pair of test foods that are fed simultaneously side by side. The two-pan test is currently the most reliable method for determining pet food preference (Hutton, 2002), although it has limitations (Aldrich and

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Koppel, 2015). Houpt and Smith (1981) observed that preferences of dogs in laboratory situations is relatively uniform with regards to food intake. It has also been shown that short-term palatability studies performed in a subset of dogs have relevance to the larger population of dogs (Vondran, 2013). Waterhouse and Fritsch (1967) also studied the effect of position of the food dishes and reported that only 5% of dogs tested had a preference for a particular bowl position.

In the two-pan test, the animal has the opportunity to freely choose between two foods. Preference is evaluated through the use of an intake ratio, or the ratio of the amount of test food consumed divided by the total amount of foods consumed (Araujo and Milgram, 2004). Foods are placed in individual bowls and presented simultaneously to the animal. The caloric amount of food allowed to be consumed is based on the amount needed to maintain constant body mass. Total consumption of each food is recorded and specific ratios are calculated. An intake ratio of 0.50 would indicate no preference between the two foods, i.e. the animal ate exactly the same amount of each food. In-home testing for a panel of foods showed that results were the same on day one as for seven subsequent days of testing (Vondran, 2013). In addition, previous exposure to a food did not alter subsequent preference for that food (Vondran, 2013). In our study, no food had more than a 5% difference in consumption between that individual food and the average consumption of all other foods in the test.

The major findings in the macronutrient preference study reported here show that, given the opportunity to choose among foods with similar palatability over a 28 day period, healthy adult cats on average chose to consume most of their calories from carbohydrate (43%) and protein (30%), whereas healthy adult dogs on average chose to consume most of their calories from fat (41%) and carbohydrate (36%). Cats choose a broader concentration of protein intake compared with dogs, as evidenced by the standard deviations for protein intake. No dog or cat, by choice, maximized protein intake by feeding solely from the high-protein feeding station.

Previous studies have shown that, if palatability is not balanced between foods, cats prefer to eat very high levels of protein (Hewson-Hughes et al., 2011) and dogs prefer to eat very high levels of fat (Hewson-Hughes et al., 2013). In the short term, when offered complete and balanced foods formulated with varying concentrations of protein, fat and carbohydrate, cats first choose based on palatability (D.E.J., unpublished results; Hill's Pet Nutrition, Inc.). Cats choose a higher-protein food compared with dogs (Bradshaw, 2006; Hewson-Hughes et al., 2016, 2011). Conversely, under similar conditions, dogs choose a high-fat food (D.E.J., unpublished results; Hill's Pet Nutrition, Inc.) (Hewson-Hughes et al., 2013). Other researchers have previously suggested that, if dogs and cats are offered a range of nutritionally variable foods, they make food choices that maintain a specific ratio of macronutrients. For example, using nutritional geometric analysis studies, the macronutrient content of dog food was regulated to maintain 30% protein, 63% fat and 7% carbohydrate intake (Hewson-Hughes et al., 2013), and cat food was regulated to maintain 52% protein, 36% fat and 12% carbohydrate intake (Hewson-Hughes et al., 2011). These studies implied that food choices reflected a physiological need rather than choices based on palatability or food availability. However, palatability issues (including food properties such as energy density, water content, flavor, aroma and texture) were not accounted for and, thus, confounded macronutrient choices (Hewson-Hughes et al., 2016). In addition, the total amount of food and energy consumed was far greater than daily metabolic energy requirements for adult dogs (Hewson-Hughes et al., 2013), raising the question as to whether these ratios would remain the same if animals were fed to meet their metabolic energy requirements. After assuring that palatability of foods was balanced, we found that macronutrient content of dog and cat intake choices were different than what was previously reported when palatability was not balanced (Hewson-Hughes et al., 2013, 2011). Because both dogs and cats preferred significantly different macronutrient content than what they choose based on palatability, our study shows the importance of removing palatability as a determinant of food intake. Although our study did not offer the previously published macronutrient targets, cats never chose to eat as much protein as was possible, nor dogs as much fat as was possible in our study. Therefore, the span of macronutrient choices offered in our study allowed animals to achieve the macronutrient mix desired when palatability was balanced.

There was not an equivalent response to change in protein intake between cats and dogs. In cats, when protein intake increased, carbohydrate intake decreased, and fat intake decreased as well. In dogs, variation in protein intake was less than observed for cats and was not offset by carbohydrate intake. In dogs, increased protein intake was offset by decreased fat intake similar to cats. In dogs, unlike cats, there was an inverse relationship between fat and carbohydrate intake: as fat intake increased, carbohydrate intake decreased. In cats, as fat intake increased, there was no change in carbohydrate intake.

In humans, macronutrients with the same caloric content exert different effects on satiety, with protein having the most potent action on satiety, then carbohydrate and lastly fat (Stubbs et al., 2000). In contrast, energy-dense foods are more palatable, with a frequent preference in humans for high-fat foods (Drewnowski, 1998). Thus, reducing energy density while maintaining palatability is a constant challenge for the food industry (Drewnowski, 1998). In our study, when animal models were fed foods with varying macronutrient composition but similar palatability, we found that cats, on average, had more variation in protein intake (range 23–38%; 78% of the range between the highest and lowest protein food available to cats) compared with dogs (range 20–26%; 37% of the range between the highest and lowest protein food available to dogs). Increased protein intake in cats was associated with decreased carbohydrate and fat intake. There was no ceiling effect for carbohydrate intake as has been suggested (Hewson-Hughes et al., 2011); rather, carbohydrate (and fat) intake were inversely correlated to protein intake. Carbohydrate, and to a lesser degree fat, were simply chosen as replacements for a decrease in the amount of protein intake. This suggests that the previously observed carbohydrate ceiling effect was the result of a food preference rather than a physiological benefit. Interestingly, there was no correlation between fat and carbohydrate intake in cats. In contrast, dogs had less variability in protein intake compared with cats, such that protein intake was maintained within a narrower range. Variation in protein intake was offset by fat, but not carbohydrate, intake. Furthermore, increased fat intake in dogs was associated with decreased carbohydrate intake. In humans, macronutrient-specific satiety may develop after consumption of a given macronutrient and this may suppress intake of that macronutrient for up to 2 days (de Castro, 1999). In animal models, macronutrient intake may be less affected by sensory-specific satiety if palatability is masked.

Although there were species differences, in our study, age, lean body mass and fat body mass influenced the percent of calories chosen from protein. Because the relative choice of macronutrient concentration was influenced in both dogs and cats by age and either

lean body mass (cats) or fat body mass (dogs), this suggests a physiological basis for ingestion behavior. Although it is unclear whether this is for a health benefit, cats with less lean body mass and dogs with greater fat body mass preferred a higher protein intake.

The groups of cats and dogs defined by the regression tree analysis for protein intake, based on age and lean body mass (cats), and fat body mass and age (dogs), divided cats into four groups and dogs into three groups. We used these groups to determine the effect of consuming macronutrients of choice for 28 days on selected metabolites of the major macronutrient classes. There were significant differences in selected metabolites of the major nutrient classes for cats.

The older cats were less able to elongate and desaturate fatty acids and, thus, synthesize DHA, such that older cats (>3.4 years) had lower plasma concentrations of DHA compared with younger cats (<3.2 years). None of the foods contained ingredient sources of EPA or DHA. Cats make limited amounts of arachidonic acid from linoleic acid presumably because they lack the Δ-6-desaturase enzyme (Sinclair et al., 1979), which makes arachidonic acid an essential dietary fatty acid in the cat. Nonetheless, cats are able to elongate and desaturate fatty acids, but less efficiently in older cats.

Older cats had higher plasma concentrations of phenylalanine. In humans, phenylalanine plasma concentrations were higher in the elderly compared with younger subjects (Bancel et al., 1994). Also, in humans, circulating concentrations of phenylalanine were shown to decrease during the first year of life and then tended to increase throughout childhood and adolescence (up to 18 years of age) (Lepage et al., 1997).

Metabolomic analysis of urea cycle intermediates showed that increased protein intake in younger cats was associated with increased concentrations of arginine and homoarginine. This suggests that excess protein is excreted through the urea cycle. Plasma urea concentrations were not increased with protein intake, confirming that urea concentrations are maintained within the normal range. Specific adaptive processes, including increased activities of catabolic pathways such as the urea cycle, are involved in rats adapting to a long-term high-protein diet (Jean et al., 2001). Homoarginine is the methylene homolog of arginine and, in adult humans, reduced concentrations are generally considered a cardiovascular risk factor (Tsikas and Wu, 2015).

In older cats, concentrations of sulfated microbial catabolic products were increased for p-cresol sulfate and 4-ethyl phenyl sulfate. It is well known that modification of the diet affects the colonic microbiota, although, in our study, the older cats chose a moderate level of protein intake. Studies in humans show that, with increasing age, changes occur in the composition of the intestinal microflora, with a reduction in species diversity in most bacterial groups (Mariat et al., 2009; Woodmansey, 2007). Thus, older cats in our study may have had a different colonic microbiome than younger groups of cats, resulting in alteration of microbial metabolic activities (Woodmansey, 2007). The colonic bacteria produce many compounds that are absorbed and normally excreted in the urine (Tanaka et al., 2015). Evidence suggests that some of the colon-derived uremic solutes are toxic (Tanaka et al., 2015). p-Cresol sulfate is derived from p-cresol, an end product of protein catabolism, which is produced in the intestine from tyrosine and phenylalanine by intestinal bacteria (Vanholder et al., 1999). p-Cresol is sulfated to produce p-cresol sulfate in the intestinal wall (Martinez et al., 2005; Schepers et al., 2007; Vanholder et al., 2011). In humans, high concentrations of p-cresol sulfate have been correlated with cardiovascular diseases and mortality in patients with chronic kidney disease (Bammens et al., 2006; Liabeuf et al., 2010; Wu et al., 2012). The renal toxicity of p-cresol sulfate results from its intracellular accumulation, leading to the production of reactive oxygen species, which then trigger induction of inflammatory cytokines that are involved in renal fibrosis (Watanabe et al., 2013). 4-Ethyl phenyl sulfate is also a microbiome-derived product absorbed from the colon and normally excreted in the urine (Tanaka et al., 2015). Future studies are planned to investigate the effect of age on intestinal dysbiosis (Rehman, 2012) and microbial production of these microbial catabolic products.

There were no differences in selected metabolites of the major nutrient classes for dogs, after adjusting for false discovery rate. We found no differences between the three dog groups for essential fatty acids, essential and nonessential amino acids, glucose and tricarboxylic acid cycle intermediates, urea cycle intermediates, and sulfated microbial catabolic products. This was most likely because the difference in mean protein intake among the three groups of dogs defined by the regression tree analysis for protein intake was only 3%. Although urea cycle metabolites did not show a significant response in our analysis, there was a trend toward higher concentrations of many urea cycle intermediates when they were individually compared to protein intake, suggesting that excess protein is excreted through the urea cycle. The study is limited in that only ovariohysterectomized females and neutered males were used, and not all animal ages were represented.

Conclusions

When fed foods with similar palatability, healthy adult dogs and cats choose different macronutrient intakes (P<0.0001). Dogs and cats chose to consume a specific protein content (dogs on average 23% and cats on average 30% of calories as protein) based on factors not associated with food palatability. Overall, dogs with more fat body mass had a greater preference for dietary calories from protein, and younger dogs with less fat body mass consumed fewer calories from protein. Overall, younger cats showed a greater preference for dietary calories from protein, and younger cats with less lean body mass had a greater preference for dietary calories from protein. Older cats with moderate protein intake had lower circulating DHA concentrations as well as higher concentrations of circulating phenylalanine and sulfated microbial catabolic products compared with younger, leaner cats. Thus, in cats, concentrations of circulating metabolites reflected food choices. In dogs, there were no differences in selected metabolites of the macronutrient classes based on food choices.

Competing interests

Hill's Pet Nutrition, Inc. provided support in the form of salaries for authors (J.C.V., M.A.V., D.E.J.), but did not have any additional role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author contributions

Conceptualization: D.E.J.; Validation: J.A.H., D.E.J.; Formal analysis: J.A.H., D.E.J.; Investigation: J.C.V., M.A.V.; Writing - original draft: J.A.H.; Writing - review & editing: J.C.V., M.A.V., D.E.J.; Project administration: D.E.J.; Funding acquisition: D.E.J.

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