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Cats Are Able to Adapt Protein Oxidation to Protein Intake Provided Their Requirement for Dietary Protein Is Met[1,2]

Alice S. Green, Jon J. Ramsey, Cecilia Villaverde, Danny K. Asami, Alfreda Wei, and Andrea J. Fascetti*

Department of Molecular Biosciences, School of Veterinary Medicine, University of California, Davis, CA 95616-8741

Abstract

Cats require more dietary protein than noncarnivorous species. Earlier work showed that cats lack the ability to regulate hepatic urea cycle enzymes in response to dietary protein concentration. We thus hypothesized that cats are unable to fully adapt protein oxidation to protein intake, particularly at low-protein concentrations. We used indirect respiration calorimetry to assess cats' ability to adapt substrate oxidation to diets containing different concentrations of protein, including 1 below their protein requirement. Nine cats (5 males and 4 females; 2.7 ± 0.5 y; 4.49 ± 0.19 kg) consumed each of 4 semipurified diets containing 7.5% [low protein (LP[3])], 14.2% [adequate protein (AP)], 27.1% [moderate protein (MP)], and 49.6% [high protein (HP)] of metabolizable energy from protein in a modified crossover design, beginning with the MP diet and then consuming the remaining diets in random order. After adaptation to each diet, cats completed a 5-d nitrogen balance trial and at least 2 12-h indirect calorimetry measurements. There was a significant effect of diet on protein oxidation ($P < 0.0001$), which measured 10.4 ± 0.5, 14.1 ± 1.0, 25.0 ± 1.7, and 53.2 ± 1.7% of total energy expenditure for the LP, AP, M,P and HP diets, respectively. The ratio of protein oxidation:protein intake was higher with the LP diet (1.39 ± 0.07) than the other 3 diets (AP, 1.00 ± 0.07; MP, 0.93 ± 0.06; HP, 1.07 ± 0.03; $P < 0.0001$), indicating a net loss of protein with the LP diet. Thus, cats are able to adapt protein oxidation to a wide range of dietary protein concentrations, provided their minimum protein requirement is met. J. Nutr. 138: 1053–1060, 2008.

Introduction

As carnivores, domestic cats have certain nutritional and metabolic idiosyncrasies that appear to be the result of evolutionary adaptations to a diet composed mainly of vertebrate tissue (1). These are manifested in the essentiality of some nutrients (vitamin D, niacin, taurine, and arginine) that can be synthesized in sufficient quantity in vivo by noncarnivorous species and in the higher maintenance requirement for dietary protein compared with noncarnivores. For example, the minimum requirement (MR)[3] of protein for maintenance for cats is 16% of metabolizable energy (ME) (2), whereas the omnivorous rat can maintain body weight (BW), nitrogen (N) balance, and carcass N while consuming diets containing 3.5–4.5% of ME as protein (3,4). The increased protein requirement of carnivores does not appear to reflect an increased requirement for essential amino acids (EAA) (5); instead, cats seem to have a high requirement for dispensable N. An explanation for this phenomenon was provided by a study showing that the activity of some hepatic aminotransferases and urea cycle enzymes did not differ in cats fed high- (54% of ME) and low-protein (14% of ME) diets (6). These results are in stark contrast to the dramatic changes in enzyme activity (often 3- to 4-fold greater with high- vs. low-protein diets) observed in omnivorous and herbivorous species (7–13) under similar experimental conditions. Metabolic inflexibility similar to the cat has been shown in other carnivores such as rainbow trout and barn owls (14,15). Thus, the high-protein requirement in cats and other carnivores seems to reflect a high obligatory rate of protein oxidation caused by an inability to downregulate the enzymes of protein catabolism to conserve N when consuming a low-protein diet. The finding that cats have higher endogenous N excretion on a protein-free diet compared with omnivores (16) provides further support for this hypothesis.

Protein, fat, and carbohydrate are oxidized by animals to provide energy for life. Total energy expenditure (TEE) and the contribution of each of these 3 substrates can be measured using indirect respiration calorimetry and urine collection. Protein oxidation is calculated based on urinary N (UN) excretion,

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[3] Abbreviations used: AA, amino acid; AP, adequate protein diet; BCAA, branched-chain amino acid; BW, body weight; CP, crude protein; DM, dry matter; EAA, essential amino acid; EE, energy expenditure; FQ, food quotient; FM, fat mass; HP, high-protein diet; LBM, lean body mass; LP, low-protein diet; ME, metabolizable energy; 3MH, 3-methyl-histidine; MP, moderate-protein diet; MR, minimum requirement; NEAA, nonessential amino acid; OX:IN, ration of substrate oxidation (percent of TEE) to substrate intake (percent of ME); RQ, respiratory quotient; TEE, total energy expenditure; UN, urinary N.

* To whom correspondence should be addressed. E-mail: ajfascetti@ucdavis.edu.

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whereas carbohydrate and fat oxidation are calculated from gas exchange measurements and the characteristic respiratory quotient (RQ) of each substrate. In an animal in energy balance and with stable body composition, substrate oxidation should theoretically adapt to match the composition of fuels in a nutritionally adequate diet. Indirect respiration calorimetry experiments in several species have demonstrated that adaptation to diet composition does occur (17–19).

Russell et al. (20) used indirect respiration calorimetry to investigate substrate oxidation when cats were fed moderate (35% of ME) or high-protein (52% of ME) diets, both well above the MR of protein (16% of ME) for maintenance (2). They found that protein oxidation increased when cats were fed the high-protein diet and concluded that perhaps cats are more capable of adjusting protein metabolism than previously thought based on the enzyme data (6). However, these authors only tested diets that were above the protein requirement and adaptation to these protein concentrations could be easily explained by allosteric and substrate/intermediate level regulation of the urea cycle and/or change in liver size (21).

The objective of our study was to investigate protein oxidation in cats fed diets with protein concentrations below, at, and above their requirement to test their ability to adapt substrate oxidation to dietary macronutrient concentration. We used indirect respiration calorimetry and N balance to measure substrate oxidation. We also measured plasma and urine amino acids (AA) as indicators of protein and AA metabolism. Our hypothesis was that cats would adapt protein oxidation to dietary intake provided their protein requirement was met, as shown by Russell et al. (20); however, we predicted that below their protein requirement, cats would be unable to decrease protein oxidation enough to maintain N balance.

Materials and Methods

Animals. Specific pathogen-free intact adult domestic short-hair cats (5 females and 5 males) from the University of California, Davis were used for the study. At the start of the study, mean age was 3.2 ± 0.6 y and mean BW was 4.52 ± 0.26 kg. Cats had normal body condition and were previously fed a commercial dry kibble diet for at least 1 y. Cats were housed individually in large runs (1.17 × 1.83 × 2.41 m) for most of the study, except as described for balance and calorimetry measurements. Rooms were temperature-controlled (21 ± 2°C) and maintained on a 14-h-light/10-h-dark cycle. Cats had free access to food and water and were provided with enrichment (scratching posts, toys, daily petting/brushing) throughout the study. The experimental protocol was approved by the Institutional Animal Care and Use Committee of the University of California, Davis.

Diets. Four semipurified diets (**Table 1**) containing 7.5% (LP), 14.2% (AP), 27.1% (MP), and 49.6% (HP) of ME from protein were used. Diets were uncooked and had a crumbly "cookie dough" texture. Diets were supplemented with purified EAA so that each diet met 150% of the MR of each EAA for growth in the cat, except for the LP diet, which was supplemented to meet 100% of the EAA requirements (2). Diet batches were sampled throughout the study for proximate analysis and moisture determination. ME of diets was calculated based on proximate analysis and standard ME values for protein, fat, and carbohydrate (Table 1).

Design. Cats were given fresh food between 0700 and 0900 h and had access to food throughout the day. Cats consumed all diets ad libitum, except in the case of 1 cat who was gaining BW on the HP diet. For this cat, intake was restricted to maintain a stable BW. Food intake was measured daily and BW twice per week.

All cats were first adapted to the MP diet until they maintained a stable BW for at least 14 d, after which time each cat completed a 5-d balance trial in a metabolic cage (0.60 × 0.60 × 0.60 m) during which all feces, urine, and unconsumed food were collected and frozen. Sufficient hydrochloric acid (5 mol/L) was added to the urine collection bottle so that the final volume had a pH < 3. All balance trial samples were collected once per day between 0700 and 0900. Urine was pooled in a large container and stored at 0°C until the end of the 5 d, when the total volume was measured in a graduated cylinder and subsamples frozen at −20°C. During the balance trial, cats also completed at least 2 12-h daytime (0800–2000 h) indirect respiration calorimetry measurements, described below. Additional calorimetry measurements were taken if the initial 2 had a CV > 5% for TEE. Some calorimetry experiments were extended to 24 h and TEE and RQ did not differ between day and night, a finding consistent with previous work showing that cats do not have

TABLE 1 Ingredient composition and macronutrient contribution to ME of treatment diets[1]

	Treatment diet			
	LP	AP	MP	HP
Ingredients, *g/kg (as-fed basis)*				
Soy[2]	25.0	76.5	181.8	343.1
Casein[3]	25.0	76.5	181.8	343.1
Glucose monohydrate	325.2	280.1	187.2	0
Cornstarch[4]	325.2	280.1	187.2	54.6
Poultry fat	175.0	175.0	175.0	175.0
Corn oil	19.0	19.0	19.0	19.0
Mineral mix[5]	50.0	50.0	50.0	50.0
Vitamin mix[6]	10.0	10.0	10.0	10.0
Choline bitartrate	3.8	3.8	3.8	3.8
L-Arginine	6.2	5.5	0	0
L-Histidine · HCl	1.8	0.9	0	0
L-Isoleucine	2.7	0	0	0
L-Leucine	7.8	6.1	0	0
L-Lysine · HCl	4.6	2.1	0	0
L-Methionine	6.6	8.0	2.8	0
L-Phenylalanine	2.3	0	0	0
L-Threonine	4.0	3.2	0	0
L-Tryptophan	0.9	0.5	0	0
L-Valine	3.3	1.3	0	0
Taurine	1.5	1.5	1.5	1.5
Water added, *g/kg diet*	100.0	200.0	325.0	1000.0
Protein,[7] *% of ME*	7.5	14.2	27.1	49.6
Fat,[8] *% of ME*	38.8	38.9	39.0	39.2
Carbohydrate,[9] *% of ME*	53.7	47.0	34.0	11.2
FQ[10]	0.88	0.86	0.84	0.80
ME, *MJ·kg DM*$^{-1}$	20.3	20.3	20.2	20.1

[1] Calculated assuming protein, carbohydrate, and fat contain 16.736, 16.736, and 37.656 MJ/kg, respectively.
[2] Supro 661, 87% CP, as-fed basis; Solae Company.
[3] Alacid Lactic Casein, 87% CP, as-fed basis, 30 mesh; NZMP Fonterra.
[4] Melojel refined cornstarch; National Starch.
[5] Provided (g/kg diet): calcium phosphate dibasic (anhydrous) 39.0; potassium phosphate dibasic (anhyd.) 9.0; calcium carbonate 11.0; magnesium sulfate (anhyd.) 4.5; potassium chloride 10.0; potassium bicarbonate 10.0; sodium bicarbonate 14.0; (mg/kg diet): manganese sulfate · H_2O 38.4; copper sulfate · $5H_2O$ 80; ferric citrate 1000; potassium iodide 3.5; stannous chloride · $2H_2O$ 100; sodium selenate 30; ammonium molybdate · $4H_2O$ 4.0; chromic chloride · $6H_2O$ 26; nickel chloride · $6H_2O$ 30; sodium fluoride 14; ammonium vanadate · $4H_2O$ 2.0.
[6] Provided (mg/kg diet): retinyl palmitate 11.0; cholecalciferol, 0.05; DL-α-tocopheryl acetate, 160; menadione, 15; thiamin-HCl, 25; riboflavin, 10; pyridoxine, 10; nicotinic acid, 100; calcium pantothenate, 20; myoinositol, 200; folic acid, 10; cobalamin, 0.05; biotin, 1; ascorbic acid, 200.0; sucrose, 843.4.
[7] Determined by Kjeldahl analysis ($N \times 6.25$).
[8] Determined by ether extraction.
[9] Calculated by subtraction: 100 − % CP − % ether extract − % ash.
[10] Calculated assuming FQ of fat, protein, and carbohydrate are 0.71, 0.835, and 1, respectively (29).

clearly defined circadian rhythms (2). Therefore, all data obtained during 12-h measurements were extrapolated to 24 h.

After the MP diet phase, all cats completed phases on the remaining 3 diets in random order. Cats were given at least 14 d to adapt to a new diet. When cats gained weight with a new diet, they were given additional adaptation time to attain a stable BW. When cats lost weight, they were given exactly 14 d of adaptation to prevent excessive weight loss. After each adaptation period, cats again completed 5-d balance trials, including at least 2 12-h periods of indirect calorimetry measurements.

During each phase, blood samples were collected from cats after at least 7 d of adaptation to each diet for plasma AA analysis. Samples were collected into sodium heparin tubes (Vacutainer) 3 h after offering fresh food and immediately centrifuged (2817 × *g*; 20 min). An equal volume of 60 g/L 5-sulfosalicylic acid was added to plasma aliquots and samples were frozen at −20°C.

Indirect respiration calorimetry measurements. The custom-built calorimetry chamber (UC Davis Metal Shop) is described in Villaverde et al. (22). All cats were introduced to the chamber prior to the measurements for at least 3 consecutive d for acclimation. Behavior and food intake were observed during the training period to assess adaptation. The gas analyzers were calibrated daily using room air and a reference gas. Weekly alcohol recoveries were conducted by measuring gas exchange resulting from the burning of a known mass of ethanol and these results were used to calibrate the system.

Sample analyses. Moisture content of feces and diet samples was determined using lyophilization for 4 d and oven drying at 100°C for 24 h, respectively. Samples were ground with mortar and pestle, and hair was removed from feces samples. Total N was determined in urine, feces, diet, and spilled food samples using the Kjeldahl method (23). Creatinine and ammonia in urine were determined (24,25) using a UV-visible spectrophotometer (Cary 1E, Varian). All assays were completed in duplicate.

Samples of plasma and urine were filtered (0.45 μm, Millex–FH, Millipore) and analyzed for free AA with an automated AA analyzer (Biochrom 30) using cation-exchange HPLC and ninhydrin-reactive colorimetric detection. The felinine peak in urine samples was identified by retention time and relationship to other AA peaks compared with published data (26). Felinine concentration was estimated based on peak area compared with that of equimolar solutions of norleucine determined on a similar AA analyzer system by Dr. Wouter Hendriks (Wageningen University; personal communication, 2007).

Body composition measurement. Body composition was estimated in cats using a modification (22) of the isotope dilution procedure described by Backus et al. (27), except that the deuterium dilution space was assumed to be 1.0459 × body water volume (28). This procedure was completed with each cat within 5 d of the end of the balance trial for each diet.

Calculations and statistical analysis. Mean changes per min in CO_2 and O_2 were calculated for each calorimetry experiment and were extrapolated to 24 h (CO_{2total} and O_{2total}). Substrate oxidation was calculated based on the equations of Elia and Livesey (29) for a 3-substrate mixture adjusted for urinary creatinine and ammonia concentrations (see Appendix). The difference between total UN and ammonia + creatinine was assumed to be urea (16) for the purposes of calculations.

The experimental design was a variation of a crossover design and a linear mixed effects model was used to assess the effects of treatment on metabolic parameters. Diet, sex, and diet × sex interaction were included as fixed effects and cat ID was included as a random effect. The Tukey's adjustment for multiple comparisons was used to perform post hoc tests of treatment effects. Similar ANCOVA models with BW and lean body mass (LBM) as covariates were used to assess the effects of diet treatment on variables related to TEE. We used simple linear regression to assess the association of protein intake and oxidation. Data were transformed for statistical analyses when necessary to meet requirements for normality and homogeneity of variance. Data are presented as means ± SEM. Differences were considered significant at $P < 0.05$. All analyses were performed using SAS version 9.1 (SAS Institute).

Results

All cats decreased in BW during the initial purified diet adaptation phase on the MP diet. Cats were generally weight stable after 6 wk of adaptation, during which time their BW decreased by 357 ± 54 g or 8.5 ± 1.6%. One female cat was removed from the study due to chronic diarrhea while consuming the MP and HP diets; it was excluded from the study before trying the other 2 diets. After exclusion, the initial age of the cats was 2.7 ± 0.5 y and the BW was 4.49 ± 0.19 kg. All results are presented for the 9 remaining cats only. The treatment diets were generally well accepted by the remaining cats, although loose feces were occasionally observed in cats consuming the LP diet.

BW, body composition, and ME intake. Mean BW, measured at the time of the calorimetry experiments, was lower ($P < 0.0001$) when cats were in the LP and AP phases compared with the MP and HP phases (**Table 2**). Diet did not affect body composition. Cats consumed 41.5 ± 4.3, 58.5 ± 7.5, 54.0 ± 2.4, and 57.8 ± 5.0 g dry matter (DM) per cat per day on the LP, AP, MP, and HP diets, respectively. There was a effect of diet on both DM and ME intake ($P = 0.0087$; Table 2), which was lower when cats were in the LP phase, although not different from the MP phase ($P = 0.0920$). The effect of diet on ME intake remained significant when BW ($P = 0.0176$) or LBM ($P = 0.0180$) was included in the statistical model.

Energy expenditure. There was an effect of diet on TEE when the data were analyzed either with LBM as a covariate ($P = 0.0005$) or without a covariate ($P = 0.0020$; Table 2). However, when BW was included as a covariate, the effect of diet became nonsignificant ($P = 0.1171$; Table 2).

N balance. As expected, N intake varied with treatment diet ($P < 0.0001$), increasing from the LP to HP diets (**Table 3**). UN and fecal N also increased in this order, both affected by diet ($P < 0.0001$ and $P = 0.0102$, respectively; Table 3). There was also an effect of diet on N balance ($P = 0.0009$; Table 3). In the LP phase, cats were in negative N balance, whereas balance was positive with the other 3 diets. The excretion of urinary creatinine N (mg/d) was not significantly affected by diet, but because UN increased with dietary protein, creatinine N as a percent of UN decreased as dietary protein increased ($P < 0.0001$; Table 3). Urinary ammonia N increased with dietary protein when expressed in mg/d ($P = 0.0023$) and decreased when expressed as a percent of UN ($P < 0.0001$; Table 3).

Substrate oxidation and RQ. Oxidation of protein, carbohydrate, and fat generally shifted with intake on the treatment diets (**Table 4**). For all 3 substrates, there was a significant effect of diet on oxidation. Linear regression analysis demonstrated a strong relationship between protein intake and protein oxidation (**Fig. 1**; $R^2 = 0.946$; $P < 0.0001$). For each substrate, the ratio of oxidation (percent of TEE) to intake (percent of ME) (OX:IN) was used to evaluate how well the 2 were matched, a ratio of 1 indicating a perfect match. For protein, there was an effect of diet on OX:IN ($P < 0.0001$), the value for LP being significantly higher than for the other 3 diets. When cats consumed LP, protein oxidation exceeded intake by ~39% on an energy basis, whereas protein oxidation and intake were relatively well matched for AP, MP, and HP. There was also an

TABLE 2 Body mass and composition and energy balance for cats fed 4 diets varying in protein concentration[1]

	LP	AP	MP	HP	*P*-value
BW,[2] *kg*	3.84 ± 0.29[a]	3.99 ± 0.31[a]	4.20 ± 0.29[b]	4.17 ± 0.28[b]	<0.0001
LBM,[3] *% of BW*	90.4 ± 2.4	87.1 ± 2.8	83.4 ± 4.0	88.3 ± 3.0	NS
Fat mass,[4] *% of BW*	9.6 ± 2.4	12.9 ± 2.8	16.6 ± 4.0	11.7 ± 3.0	NS
BW change,[5] *g/d*	−15.5 ± 2.45[a]	−2.0 ± 1.2[b]	2.6 ± 2.2[b]	0.0 ± 1.7[b]	<0.0001
Food intake, *g DM/d*	41.5 ± 4.3[a]	58.5 ± 7.5[b]	54.0 ± 2.4[ab]	57.8 ± 5.0[b]	0.0087
ME intake,[2] *MJ/d*	0.83 ± 0.09[a]	1.19 ± 0.15[b]	1.09 ± 0.05[ab]	1.16 ± 0.10[b]	0.0087
TEE, *MJ/d*	0.91 ± 0.06[a]	1.00 ± 0.09[ab]	1.15 ± 0.06[c]	1.09 ± 0.07[bc]	0.0020
TEE_{BW},[6] *MJ/d*	0.91 ± 0.06	1.00 ± 0.09	1.15 ± 0.06	1.09 ± 0.07	NS
TEE_{LBM},[7] *MJ/d*	0.91 ± 0.06[a]	1.00 ± 0.09[ab]	1.15 ± 0.06[c]	1.09 ± 0.07[bc]	0.0005
E balance,[2] *kJ/d*	−67 ± 50[a]	186 ± 86[b]	−57 ± 47[a]	72 ± 53[ab]	0.0155

[1] Values are means ± SEM, $n = 9$. Means in a row with superscripts without a common letter differ, $P < 0.05$. NS, $P \geq 0.05$. *P*-value refers to the ANOVA for effect of diet treatment.
[2] At time of calorimetry measurements.
[3] LBM, measured using deuterium oxide dilution method.
[4] Calculated as BW-LBM.
[5] Calculated for last 20 d on diet.
[6] TEE adjusted for BW using ANCOVA.
[7] TEE adjusted for LBM using ANCOVA.

effect of diet on the OX:IN ratio for fat ($P = 0.0021$), these values being higher for LP and MP and lower for AP and HP.

RQ varied with diet ($P = 0.0056$) and measured within ± 0.01 of the calculated food quotient (FQ) (Table 1) for each diet except LP. The RQ for cats consuming LP was 0.83 and the FQ was 0.88. This indicates agreement with the substrate oxidation data that cats were oxidizing more fat and protein and less carbohydrate than they were consuming when consuming LP.

Plasma and urine AA. The plasma AA patterns varied with diet (**Table 5**; **Fig. 2**). There was an effect of diet ($P < 0.0001$) on plasma total AA, total EAA, and total nonessential AA (NEAA), each increasing with dietary protein. With the HP diet, the increase in EAA exceeded the increase in NEAA, as evidenced by the higher EAA:NEAA ratio on this diet compared with the other diets. Plasma branched-chain AA (BCAA) increased dramatically with dietary protein ($P < 0.0001$). The effect of diet remained on EAA minus BCAA ($P = 0.0004$) (data not shown), indicating that the EAA pattern is independent of BCAA.

In urine (**Table 6**), diet did not affect the excretion of EAA, but NEAA excretion increased with dietary protein ($P = 0.0208$), mainly driven by Gly, Ala, and Asp. Felinine was usually the most abundant AA present in urine. There was an effect of diet on felinine excretion ($P = 0.0302$), but the effect of sex was also significant ($P < 0.0001$) and there was a sex × diet interaction ($P = 0.0140$), males having higher excretion and a greater response to increasing dietary protein than females. With the inclusion of BW in the statistical model, the effect of sex, diet, and sex × diet remained significant whereas BW was nonsignificant. After felinine, taurine was the next most abundant AA excreted in urine, but there was no effect of diet or sex on this value. There was also no effect of diet on the excretion of 3-methyl-histidine (3MH), but there was a significant effect of sex, males having greater excretion of 3MH than females. However, if BW was included in the statistical model, the *P*-value for BW was significant ($P = 0.0120$) and the effect of sex became nonsignificant.

Discussion

The NRC (2) recommends a MR for protein for cats of 16% of ME [16% crude protein (CP)] as high-quality protein. The data from our study is in agreement with this MR, as our LP diet (8% of ME, 9% CP) was clearly inadequate, whereas our AP diet (14% of ME, 17% CP) may have been marginal to some cats. Our cats did well with the MP (27% of ME, 33% CP) and HP (50% of ME, 60% CP) diets, which are representative of the range of dietary protein concentrations commonly fed to and known to sustain health in domestic cats.

TABLE 3 N balance and components of excreted N for cats fed 4 diets varying in protein concentration[1]

	LP	AP	MP	HP	*P*-value
N intake, *g/d*	0.60 ± 0.06[a]	1.61 ± 0.21[b]	2.82 ± 0.13[c]	5.52 ± 0.48[d]	<0.0001
Total UN, *g/d*	0.76 ± 0.07[a]	1.16 ± 0.15[a]	2.29 ± 0.17[b]	4.58 ± 0.27[c]	<0.0001
Ammonia N, *mg/d*	117.21 ± 16.10[a]	152.34 ± 22.18[ab]	156.76 ± 7.61[ab]	197.66 ± 14.61[b]	0.0023
Ammonia N, *% of UN*	15.11 ± 1.36[d]	13.09 ± 0.42[c]	7.14 ± 0.47[b]	4.37 ± 0.30[a]	<0.0001
Creatinine N, *mg/d*	48.71 ± 5.06	47.06 ± 4.28	56.54 ± 5.62	54.52 ± 2.65	NS
Creatinine N, *% of UN*	6.38 ± 0.40[c]	4.32 ± 0.32[b]	2.51 ± 0.19[a]	1.21 ± 0.07[a]	<0.0001
Fecal N, *g/d*	0.09 ± 0.01[a]	0.12 ± 0.02[ab]	0.15 ± 0.02[ab]	0.17 ± 0.03[b]	0.0102
N balance, *g/d*	−0.25 ± 0.05[a]	0.33 ± 0.07[ab]	0.39 ± 0.14[b]	0.76 ± 0.25[b]	0.0009

[1] Values are means ± SEM, $n = 9$. Means in a row with superscripts without a common letter differ, $P < 0.05$. NS, $P \geq 0.05$. *P*-value refers to the ANOVA for effect of diet treatment.

TABLE 4 Substrate oxidation in cats fed 4 diets varying in protein concentration[1]

	LP	AP	MP	HP	*P*-value
Protein					
Intake, *g/d*	3.8 ± 0.4[a]	10.0 ± 1.3[b]	17.6 ± 0.8[c]	34.5 ± 3.0[d]	<0.0001
Oxidation, *g/d*	5.7 ± 0.5[a]	8.6 ± 1.2[a]	17.1 ± 1.3[b]	34.6 ± 2.1[c]	<0.0001
Oxidation, *% of EE*	10.4 ± 0.5[a]	14.1 ± 1.0[a]	25.0 ± 1.7[b]	53.2 ± 1.7[c]	<0.0001
OX:IN	1.39 ± 0.07[b]	1.00 ± 0.07[a]	0.93 ± 0.06[a]	1.07 ± 0.03[a]	<0.0001
Carbohydrate					
Intake, *g/d*	27.1 ± 2.8[bc]	33.3 ± 4.3[c]	22.2 ± 1.0[b]	7.8 ± 0.7[a]	<0.0001
Oxidation, *g/d*	21.6 ± 2.8[b]	27.1 ± 1.7[b]	20.3 ± 2.2[b]	9.2 ± 1.8[a]	<0.0001
Oxidation, *% of EE*	38.9 ± 3.7[bc]	46.8 ± 3.5[c]	29.3 ± 2.6[b]	13.9 ± 2.6[a]	<0.0001
OX:IN	0.72 ± 0.07	1.00 ± 0.07	0.86 ± 0.08	1.24 ± 0.23	NS
Fat					
Intake, *g/d*	8.7 ± 0.9[a]	12.3 ± 1.6[b]	11.3 ± 0.5[ab]	12.1 ± 1.1[b]	0.0075
Oxidation, *g/d*	12.1 ± 0.9[ab]	10.7 ± 1.5[ab]	13.9 ± 0.9[b]	9.5 ± 1.2[a]	0.0308
Oxidation, *% of EE*	50.7 ± 3.7[b]	39.1 ± 3.0[ab]	45.6 ± 1.6[b]	32.9 ± 3.5[a]	0.0026
OX:IN	1.3 ± 0.1[b]	1.0 ± 0.1[ab]	1.2 ± 0.0[b]	0.8 ± 0.1[a]	0.0021
RQ	0.83 ± 0.01[ab]	0.86 ± 0.01[b]	0.82 ± 0.01[a]	0.81 ± 0.01[a]	0.0056

[1] Values are means ± SEM, $n = 9$. Means in a row with superscripts without a common letter differ, $P < 0.05$. NS, $P \geq 0.05$. *P*-value refers to the ANOVA for effect of diet treatment.

The BW loss observed in cats consuming LP was at least partly explained by decreased ME intake and negative energy balance. Other studies measuring food intake in cats consuming low-protein diets have found mixed results (30,31). The decreased food intake observed in our study may be due to lack of palatability or could be a metabolic response to the low-protein or very high-carbohydrate concentration of the LP diet.

The decrease in ME intake and energy balance in the MP phase may be an effect of treatment order, because the MP phase was the first of the experimental sequence. Whereas cats were previously acclimated to the calorimetry chamber and appeared to be exhibiting normal behavior and food intake, a small increase in TEE and/or decrease in food intake could have occurred during these first days of calorimetry measurements. Support for this explanation is the fact that cats had stable BW once adapted to the MP diet, so it is unlikely that they were indeed in negative energy balance for any substantial amount of time.

We did not observe a change in body composition associated with the treatment diets. Although cats consuming the LP diet were losing BW, the substrate oxidation data demonstrated that both protein and fat oxidation exceeded intake to roughly the same extent, so weight loss may have been balanced in both LBM and fat mass. The relatively short treatment periods and the errors associated with the body composition measurement (28) also may have impacted our ability to detect a small change in body composition related to diet treatment.

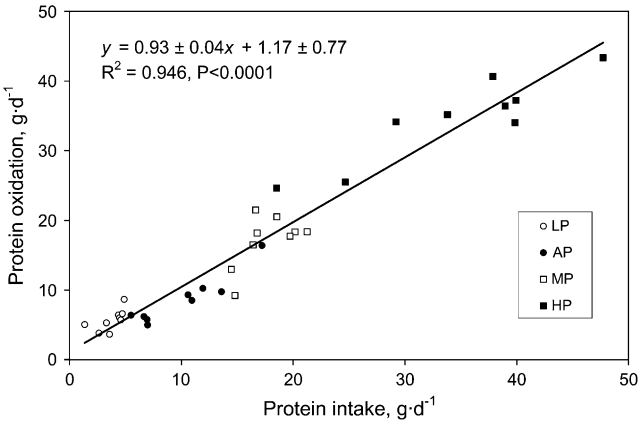

FIGURE 1 Linear regression of protein intake and protein oxidation in cats, $n = 9$. Each point represents a cat consuming 1 of 4 treatment diets varying in protein concentration.

When consuming LP, cats were unable to maintain N balance, whereas N balance was positive with the other diets. We measured a N balance on LP (7.5% protein) of −250 mg/d, in close agreement with the −264 mg/d measured by Burger et al. (32) in cats fed an 8% protein diet. Our finding that the N balance was positive when the higher protein diets were fed, a condition that is theoretically unsustainable in weight-stable animals, was also observed by these authors and consistently found in other studies (33,34). This is likely a consequence of the balance technique tending to overestimate N balance (35).

With the exception of the LP treatment, protein oxidation was closely matched to intake. Thus, provided the diet contained sufficient protein to meet their MR, cats were able to adjust protein oxidation to maintain N balance while consuming diets with a wide range of protein concentrations (14–50% of ME). However, when fed a diet below their MR, protein oxidation exceeded intake and N balance was negative. Our data are thus consistent with Russell et al. (20), but their study tested only diets with moderate- and high-protein concentrations. Our study design allowed us to define a range in which cats were able to adapt protein oxidation while also finding that there is a limit to this adaptation with the LP diet.

It could be argued that protein oxidation exceeded intake with the LP diet, because cats were in negative energy balance when they consumed this diet. To address this question, we conducted a follow-up study with 3 cats in which we fed the MP diet in amounts restricted to the same percent of ME to which these cats voluntarily decreased their food intake (75 ± 8%) when switched to the LP diet during the main study. BW loss did not differ between the LP- and MP-restricted treatments. However, protein oxidation for the MP-restricted treatment [25.53 ± 3.07% of energy expenditure (EE)] was similar to that of the MP treatment (27.57 ± 1.90% of EE) and not the LP treatment (9.84 ± 0.23% of EE; $P = 0.002$). The ratio of OX:IN

TABLE 5 Plasma AA concentrations in cats fed 4 diets varying in protein concentration[1]

	LP	AP	MP	HP	*P*-value
Total AA,[2] *μmol/L*	1827 ± 308[a]	2579 ± 123[a]	4461 ± 413[b]	5363 ± 443[b]	<0.0001
Total EAA,[3] *μmol/L*	755 ± 84[a]	1044 ± 56[a]	1779 ± 221[b]	2659 ± 308[b]	<0.0001
Total NEAA,[4] *μmol/L*	1072 ± 228[a]	1536 ± 76[a]	2682 ± 204[b]	2705 ± 169[b]	0.0001
EAA:NEAA, *(mol·100)·mol*$^{-1}$	74 ± 7[a]	68 ± 3[a]	66 ± 5[a]	98 ± 8[b]	0.0100
BCAA,[5] *μmol/L*	154 ± 36[a]	221 ± 22[a]	564 ± 85[b]	1286 ± 178[c]	<0.0001

[1] Values are means ± SEM, $n = 4$. Means in a row with superscripts without a common letter differ, $P < 0.05$. Data were log-transformed to meet assumptions of equal variance prior to analysis, but untransformed values are presented. *P*-value refers to the ANOVA for effect of diet treatment.
[2] Total free plasma AA, including all EAA and NEAA.
[3] Total EAA, including arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, taurine, threonine, tryptophan, and valine. EAA also include tyrosine and cysteine (measured as 1/2 cystine), because these can only be synthesized when EAA are available.
[4] Total NEAA, including alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, and serine.
[5] BCAA (μmol/L), including valine, leucine, and isoleucine.

for protein in cats during the MP-restricted phase (0.94 ± 0.11) did not differ from that of the MP phase (1.01 ± 0.07) but was lower ($P = 0.047$) than with the LP diet (1.28 ± 0.03). Thus, cats' inability to adapt protein oxidation to low concentrations of dietary protein appeared to be independent of energy balance in our experiment, where ME intake was moderately restricted when cats consumed the LP diet.

We found that total plasma AA, EAA (including EAA minus BCAA), and NEAA increased with dietary protein in cats. These results are in contrast to data from rats adapted to high-protein diets, where EAA minus BCAA are similar to or less than control concentrations (36,37) and total NEAA often decrease with increasing dietary protein (38). Plasma NEAA in rats is also negatively correlated with the activities of hepatic aspartic transaminase and threonine dehydratase, which increase dramatically in rats fed high-protein diets (38). These enzymes are among those that do not adapt to changes in dietary protein in cats (6), which is likely the explanation for the different responses in rats and cats. The dramatic increase in BCAA with dietary protein is consistent with work with cats (39) and rats (36,38).

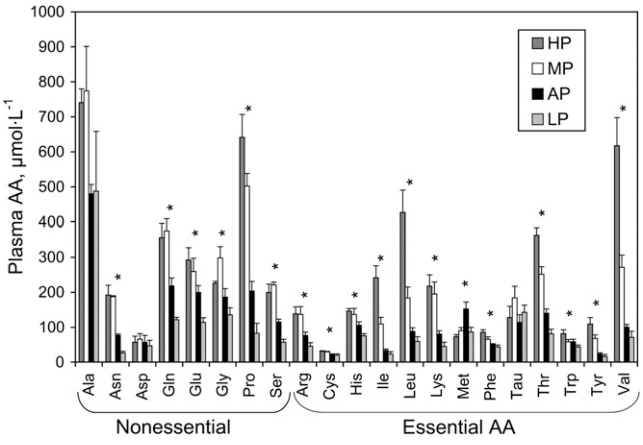

FIGURE 2 Plasma AA measured in cats consuming 4 diets varying in protein concentration. Each bar represents the means ± SEM for each diet, $n = 4$. Data were log-transformed to meet assumptions of equal variance prior to analysis, but untransformed values are presented. AA for which there was a significant effect of diet are marked with as asterisk above the set of bars. AA are grouped as NEAA and EAA for the domestic cat (see Table 5, footnotes 3 and 4 for definitions).

Our finding that felinine excretion was higher in male cats than female cats is consistent with the literature (40). To our knowledge, the significant effect of dietary protein on felinine excretion observed in our study has not been shown before. However, a positive effect of dietary cystine on felinine excretion has been observed (41).

Urinary excretion of 3MH has been used as an indicator of muscle protein breakdown, because it is almost exclusively found in skeletal muscle and cannot be reused for protein synthesis. The quantitative excretion of 3MH has been validated in cats (42), but to our knowledge, data on the effect of dietary protein on 3MH excretion in cats have not been published. In other species, including humans (43,44) and rats (45), 3MH excretion decreases with protein malnutrition and increases upon repletion with an adequate- or high-protein diet, presumably caused by the positive effect of protein intake on protein turnover (46,47). Interestingly, we found no effect of diet on 3MH excretion in our study, perhaps an indication that the cat has a limited ability to adapt protein breakdown to rather acute changes in dietary protein intake. However, 1 study has shown changes in protein breakdown in cats fed moderate- vs. high-protein diets (48). More research will be required to determine the relevance of 3MH excretion to protein turnover in cats.

The major finding of our study was that cats have the ability to adapt whole-body protein oxidation to a wide range of dietary protein concentrations, provided their high-protein requirement is met. How an animal responds metabolically to dietary changes is the net sum of the adaptive mechanisms available. Rogers et al. (6) showed that adaptation of hepatic enzymes of the urea cycle is 1 mechanism unavailable to the cat. Our data and that of Russell et al. (20) imply that perhaps other mechanisms [i.e. substrate and allosteric regulation of the urea cycle and liver size (21)] do allow the cat to adapt protein oxidation to a wide range of dietary protein concentrations. The significant changes we observed in plasma AA associated with diet treatment provide evidence that substrate supply to the urea cycle is indeed affected by dietary protein. However, when cats consumed the LP diet, they were unable to decrease protein oxidation enough to match intake, implying that the capacity of adaptive mechanisms had been exceeded.

The limited metabolic flexibility in cats and other carnivores likely represents an evolutionary adaptation to a consistently abundant supply of dietary protein. The moderate to high and nonadjustable activity of the urea cycle provides a safeguard against ammonia toxicity after a high-protein meal. In addition, it allows the ready utilization of the carbon backbones of AA for

TABLE 6 Free AA excreted in urine from cats fed 4 diets varying in protein concentration[1]

	LP	AP	MP	HP	*P*-value
			μmol/d		
Total EAA[2]	353 ± 96	479 ± 297	329 ± 70	330 ± 95	NS
Total NEAA[2]	65 ± 15[a]	84 ± 20[ab]	129 ± 19[ab]	197 ± 58[b]	0.0208
EAA:NEAA, *(mol·100)·mol*$^{-1}$	613 ± 175	483 ± 199	276 ± 72	168 ± 22	NS
Felinine[3]	388 ± 168	458 ± 215	1009 ± 477	919 ± 452	0.0302
Felinine, males[4]	(559, 776)	(561, 1017)	(1496, 2115)	(1582, 1811)	
Felinine, females[4]	(71, 144)	(55, 193)	(151, 227)	(129, 152)	
Taurine	207 ± 77	312 ± 216	203 ± 76	131 ± 58	NS
3MH	22 ± 5	24 ± 5	26 ± 3	23 ± 3	NS

[1] Values are means ± SEM, $n = 4$, except for felinine. Means in a row with superscripts without a common letter differ, $P < 0.05$. NS, $P \geq 0.05$. Data were log-transformed to meet assumptions of equal variance prior to analysis, but untransformed values are presented. *P*-value refers to the ANOVA for effect of diet treatment.
[2] See Table 5 for definitions.
[3] In ANOVA, significant effects of sex, $P < 0.0001$, and sex × diet interaction, $P = 0.014$.
[4] Values for $n = 2$ per sex are given.

energy via direct oxidation or as a precursor for gluconeogenesis. It is only when a cat is fed a low-protein diet, a condition that would likely only occur in captivity, that the high rate of protein oxidation becomes a disadvantage. Cats thus exceed their ability to adapt to low-protein diets and are faced with negative N balance when many omnivores could continue to thrive. A rat subjected to our experiment would likely adapt protein oxidation to the LP diet, given this species' ability to decrease enzyme activity to conserve N (7,8).

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Appendix

Equations used to calculate EE and substrate oxidation:

$$\mathrm{L\,O_2 \cdot g\,UN^{-1}} = (\%\,\mathrm{UN_{urea}} \times 6.48) + (\%\,\mathrm{UN_{ammonia}} \times 6.48) + (\%\,\mathrm{UN_{creatinine}} \times 4.84)$$

$$\mathrm{L\,CO_2 \cdot g\,UN^{-1}} = (\%\,\mathrm{UN_{urea}} \times 5.35) + (\%\,\mathrm{UN_{ammonia}} \times 6.15) + (\%\,\mathrm{UN_{creatinine}} \times 4.02)$$

$$\mathrm{kJ \cdot g\,UN^{-1}} = (\%\,\mathrm{UN_{urea}} \times 126.24) + (\%\,\mathrm{UN_{ammonia}} \times 127.61) + (\%\,\mathrm{UN_{creatinine}} \times 92.46)$$

$$\mathrm{O_2\ consumed\ by\ protein\ oxidation\ (O_{2protein},\ L)} = (\mathrm{L\,O_2 \cdot g\,UN^{-1}}) \times \mathrm{g\,UN}$$

$$\mathrm{CO_2\ produced\ by\ protein\ oxidation\ (CO_{2protein},\ L)} = (\mathrm{L\,CO_2 \cdot g\,UN^{-1}}) \times \mathrm{g\,UN}$$

$$\mathrm{Energy\ (kJ)\ produced\ by\ protein\ oxidation\ (EE_{protein})} = (\mathrm{kJ \cdot g\,UN^{-1}}) \times \mathrm{g\,UN}$$

$$\mathrm{O_2\ consumed\ by\ CHO+fat\ oxidation\ (O_{2CHO+fat})} = \mathrm{O_{2total} - O_{2protein}}$$

$$\mathrm{CO_2\ released\ by\ CHO+fat\ oxidation\ (CO_{2CHO+fat})} = \mathrm{CO_{2total} - CO_{2protein}}$$

$$\mathrm{Energy\ (kJ)\ produced\ by\ CHO+fat\ oxidation\ (EE_{CHO+fat})} = (\mathrm{O_{2CHO+fat}} \times 15.913) + (\mathrm{CO_{2CHO+fat}} \times 5.207)$$

$$\mathrm{Nonprotein\ respiratory\ quotient\ (NPRQ)} = \mathrm{CO_{2CHO+fat} \cdot O_{2CHO+fat}^{-1}}$$

$$\mathrm{Energy\ (kJ)\ produced\ by\ CHO\ oxidation\ (EE_{CHO})} = \frac{21.12(\mathrm{NPRQ} - 0.71) \times \mathrm{EE_{CHO+fat}}}{21.12(\mathrm{NPRQ} - 0.71) + 19.61(1 - \mathrm{NPRQ})}$$

$$\mathrm{Energy\ (kJ)\ produced\ by\ fat\ oxidation\ (EE_{fat})} = \mathrm{EE_{CHO+fat} - EE_{CHO}}$$