Whole-body protein turnover of a carnivore, *Felis silvestris catus*

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The cat (*Felis silvestris catus*) has a higher dietary protein requirement than omnivores and herbivores, thought to be due to metabolic inflexibility. An aspect of metabolic flexibility was examined with studies of whole-body protein turnover at two levels of dietary protein energy, moderate protein (MP; 20 %) and high protein (HP; 70 %), in five adult cats in a crossover design. Following a 14 d pre-feed period, a single intravenous dose of [^{15}N]glycine was administered and cumulative excretion of the isotope in urine and faeces determined over 48 h. N flux increased ($P<0.005$) with dietary protein, being 56 (SE 5) mmol N/kg body weight (BW) per d for cats fed the MP diet and 146 (SE 8) mmol N/kg BW per d for cats fed the HP diet. Protein synthesis was higher ($P<0.05$) on the HP diet (75 (SE 10) mmol N/kg BW per d; 6·6 (SE 1) g protein/kg BW per d) than the MP diet (38 (SE 5) mmol N/kg BW per d; 3·4 (SE 0·4) g protein/kg BW per d). Protein breakdown was higher ($P<0.05$) on the HP diet (72 (SE 8) mmol N/kg BW per d; 6·3 (SE 0·7) g protein/kg BW per d) than the MP diet (44 (SE 3) mmol N/kg BW per d; 3·9 (SE 0·3) g protein/kg BW per d). Compared with other species the rate of whole-body protein synthesis in the well-nourished cat (9·7 (SE 1·3) g protein/kg BW^{075} per d) is at the lower end of the range. These results show that feline protein turnover adapts to dietary protein as has been shown in other species and demonstrates metabolic flexibility. Further work is required to determine exactly why cats have such a high protein requirement.

Cat: Nitrogen: [^{15}N]glycine endproduct method: Protein metabolism: Protein intake

The maintenance of N balance in the face of varying protein intake is achieved by a variety of adaptive mechanisms including changes in ureagenesis, protein oxidation and protein turnover (Waterlow, 1999). Because the capacity of the body to store N is limited, adaptation of pathways of amino acid oxidation to variation in dietary protein intake is essential. Adaptation in rates of protein turnover also occurs in many species (Waterlow *et al.* 1978*a*) although less so in man (Pacy *et al.* 1994; Millward, 1995).

Cats (*Felis silvestris catus*) are generally thought to have a high requirement for dietary protein compared with both omnivores and herbivores (Rogers & Morris, 1980) and this has been attributed to the apparent inability of the hepatic catabolic, ureagenic and gluconeogenic enzymes of the feline to adapt to dietary protein intake (Rogers *et al.* 1977; Morris, 2002). However, several groups have since reported that the cat does adapt to dietary protein *in vitro* in terms of both ureagenesis and

gluconeogenesis (Kettlehut *et al.* 1980; Silva & Mercer, 1985). These findings are supported by recent evidence from studies *in vivo* of adaptation of feline ureagenesis (Russell *et al.* 2000) and protein oxidation (Russell *et al.* 2002) to variation in protein intake in which clear changes in the rates of each process occurred in response to changes in protein intake.

Furthermore, a study *in vitro* suggested that feline protein degradation may be sensitive to dietary protein intake (Silva & Mercer, 1991). The potential activity of the lysosomal pathway of proteolysis (and hence protein degradation) was found to be greater in the liver of cats fed a high-protein diet compared with a low-protein diet. However, there has been no reported study *in vivo* to confirm these findings.

In view of the purported requirement of the cat for a high-protein diet it is important to know if similar mechanisms of adaptation exist as for omnivores and herbivores,

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Abbreviations: BW, body weight; HP, high-protein; MP, moderate-protein.
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or whether specialised processes have evolved. The inability of the cat to adapt protein turnover to variation in intake might result in a high protein requirement.

In the present study, whole-body protein turnover was studied in the cat on a high protein intake (70 % of dietary energy as protein) and a moderate protein intake (20 % of energy). The [^{15}N]glycine endproduct method was used (Fern *et al.* 1981) with the isotope provided as a single intravenous dose and excreta collected for 48 h.

Materials and methods

Animals, diets and experimental design

The Waltham ethical review committee approved the study. Cats were housed individually in environmentally enriched lodges and group-socialised for several hours each day except during periods of excreta collection. The lodges were equipped with litter trays designed to maximise urine collection. These were 0.61×0.21 m in size and constructed of sheet aluminium coated with polytetra-fluoroethylene, also known as TEFLON™ (PTFE). The base sloped steeply into one corner, where a hole drained into the collection bottle suspended below. Recovery from this system was found to be 99.4 (SE 0.07) % (n 12) after a simulation exercise. Water was poured quantitatively over the surface of the tray and collected within 1 min, using volumes within the range commonly voided by cats (for example, 100–300 ml). This system does not separate urine and faeces but cross-contamination is negligible facilitated by the rapid draining of urine from the tray. Following familiarisation, all cats demonstrated reliable urination habits using these trays. Careful collection of urine is particularly important in the feline since urination frequency is low, often only once daily.

Six adult cats (four males, two females; mean age 3.7 (SE 0.58) years; mean body weight (BW) 5.0 (SE 0.4) kg; all neutered) were allocated to two treatment groups. Measurements were conducted when cats were fed each of two diets (a moderate-protein (MP) diet and a high-protein (HP) diet) in a balanced crossover design. The cats were offered the allocated diet alone and were fed to appetite, being offered generous portions twice a day. The isoenergetic diets were preparations of boiled chicken breast, lard and glucose, with vitamins and minerals added to meet requirements (National Research Council, 1986), and carob solution to thicken (Table 1). The MP diet was formulated to provide 20 % energy from protein, a level that is approximately 50 % that found in standard commercial cat foods but is usually thought sufficient to ensure good maintenance of intake and BW (this was achieved in

previous studies (Russell *et al.* 2000)). The HP diet was formulated to provide 70 % energy from protein, a level at the top of the range of commercial cat foods. A correction to food-intake data (assessed gravimetrically) was made for loss of weight of diets due to evaporation, calculated by leaving an identical bowl of each diet in an empty lodge for each meal. Evaporation during the morning meal (6 h) was 2.03 (SE 0.18) % (n 4) from the MP diet and 3.24 (SE 0.06) % (n 4) from the HP diet. Evaporation during the overnight meal (18 h) was found to be 9.30 (SE 1.4) % (n 4) from the MP diet and 12.75 (SE 0.18) % (n 4) from the HP diet. Energy intake was calculated using the corrected food intake and calculated dietary predicted metabolisable energy (Table 1).

Each phase consisted of a 13 d pre-feed period followed by a 72 h study. Following the pre-feed, urine and faeces collection commenced (9.00 hours, day 0) to establish baseline excreta ^{15}N values for each cat. Each defecation was immediately weighed and frozen at −20°C. Each 500 ml urine collection bottle contained 5 M-HCl (5 ml) as preservative, and bottles were changed every 24 h, the urine being mixed, weighed and then frozen at −20°C.

Food was withdrawn at 8.00 hours on day 1 and cats fasted for 2 h to standardise their metabolic states. At 10.00 hours on day 1 the cats were weighed and a venous (jugular) blood sample was taken (into heparin) for measurement of plasma urea, followed immediately by an intravenous dose of [^{15}N]glycine into the cephalic vein. The [^{15}N]glycine (99.5 at% (atom percent); Cambridge Isotope Laboratories Inc., Cambridge, MA) was prepared as a stock solution (50.0 mg/ml) in sterile physiological saline (9 g NaCl/l) immediately before use, and administered at a dose of 5.0 mg/kg BW. Each cat was immediately fed on return to its lodge following these procedures, and urine and faeces collected for the subsequent 48 h. Food was continuously available throughout the collection period. On day 3, food was again withdrawn at 8.00 hours and cats fasted for 2 h and weighed before withdrawal of a venous blood sample for measurement of plasma urea. There was a 4 d rest period between the two phases of the study.

Chemical analyses

N content of diets, urine and faeces were determined by the Dumas procedure using an automated Leco FP428 analyzer (The Leco Corporation, Saint Joseph, MI). Urinary urea concentrations were determined by the method of Marsh *et al.* (1965) using a Technicon Auto analyzer (Technicon Instruments Corporation, Tarrytown, NY). Plasma urea concentrations were determined using a Kone Selective

Table 1. Diet composition and formulation for moderate-protein diet (MP) and high-protein diet (HP)

Diet	Chicken breast (g/kg)	Lard (g/kg)	Glucose (g/kg)	Carob (1.5 %, w/v) in water (g/kg)	Protein (MJ %)	Carbohydrate (MJ %)	Fat (MJ %)	Predicted metabolisable energy* (MJ/kg)
MP	164.30	79.80	29.60	726.40	20.00	10.20	69.80	3.76
HP	576.80	17.10	26.00	380.20	70.00	10.00	20.00	3.76

* Calculated from standard Atwater factors applied to proximate analysis of ingredients.

Delta analyser (ThermoClinical Labsystems Oy, Espoo, Finland). Urinary NH_3 concentration was determined by the Berthelot reaction using a Technicon Auto analyzer (Technicon Instruments Corporation, Tarrytown, NY).

^{15}N analyses

Ammonia-N and urea-N were extracted from urine following a method adapted from that published previously (Read *et al.* 1982). A cation exchange resin (AG-50, 100–200 mesh, x8, H^+ form; Biorad, Richmond, CA) was converted to the Na/K form by stirring resin (100 g) for 15 min with each of three 600 ml batches of 0·1 M-NaOH. The resin was then washed to neutrality with deionised water and stirred for 15 min with each of three 600 ml batches of 0·2 M-sodium potassium phosphate buffer (KH_2PO4 (27·2 g) dissolved in deionised water (800 ml), pH adjusted to 7·4 with 10 M-NaOH, and made up to 1 litre). The resin was again washed to neutrality before making up to a total weight of 400 g with deionised water and storage at 4°C. Before use, the resin was drained and washed with HPLC-grade water (Sigma Aldrich Co., Gillingham, Kent, UK).

Extraction and analysis of urinary ammonia-nitrogen

Urinary NH_3-N was extracted in duplicate by the addition of the amount of urine containing 600 μg NH_3-N to a cation exchange column containing AG50W-X8 resin (2 ml) previously converted to the Na/K form. All liquid was allowed to pass through the column followed by HPLC-grade water (3 ml) to wash. The ammonium bound to the column was eluted using 1 M-KOH (1 ml) then HPLC-grade water (1 ml), and immediately acidified by collection into a tube containing 2 M-H_2SO_4 (180 μl) and bromphenol blue (20 μl; Sigma Aldrich Co., Gillingham, Kent, UK, 1 % in HPLC-grade water) indicator. The samples were freeze-dried before hypobromite treatment.

Hypobromite treatment was necessary since the extracted ammonium sulfate samples were found to contain a large amount of N from an unidentified source. Hypobromite would be expected to produce N_2 gas only from the NH_3-N in the sample. Each sample was dissolved in N-free deionised water (250 μl) and transferred to a Louwers Hapert bottle (PO box 27, The Netherlands). The sample was frozen by lowering the tube slowly into liquid N, and lithium hypobromite solution (0·5 ml; 10 % (w/v) lithium hydroxide in water plus bromine (2 ml), bubbled with He) was added. The tube was evacuated for 5 min at 0·0001 mbar before incubating at 60°C for 15 min, or until ready for analysis. The sample was frozen by slowly lowering the tube into liquid N and a duel inlet isotope ratio mass spectrometer (SIRA 12; VG Isogas, Middlewich, Ches., UK) was used to analyse the N_2 gas. The N_2 gas was ionised by electron impact ionisation and the ratio of ions 29:28 and 30:28 were compared with those of a known standard.

Extraction and analysis of urinary urea-nitrogen

Urinary urea-N was extracted by a two-step procedure, with urea first extracted from the urine matrix before conversion to ammonium sulfate. This was necessary due to the presence of inhibitors in cat urine that prevented direct reaction with urease. A cation exchange column containing resin (2 ml) (AG-50, 100–200 mesh, x8, H^+ form; Biorad, Richmond, CA) pre-washed with HPLC-grade water was used to separate urea from urine. The volume of urine containing 250 μmol urea was placed on top of the resin and all liquid allowed to pass through the column, followed by HPLC-grade water (5 ml) to remove any contaminants. Then HPLC-grade water (55 ml) was added, and the eluate collected.

The urea was converted to NH_3 by treatment with urease before conversion of NH_3 to ammonium sulfate. Urease (type III from jack beans; Sigma Aldrich Co., Gillingham, Kent, UK) solution was prepared by dissolving urease (25 mg) in sodium phosphate buffer (10 ml) (17·3 g Na_2HPO_4 + 10·8 g NaH_2PO_4 dissolved in HPLC-grade water (800 ml), adjusted to pH 7 and made up to 1 litre). The volume of urea solution (adjusted to pH 5·5–7·5) containing 600 μg N was added to a bottle containing urease solution (170 μl; 7 units activity) and sodium phosphate buffer (5 ml). The bottle was placed on a roller-mixer and incubated for 2 h at room temperature, before the addition of two drops of H_2SO_4 to acidify. The incubation mixture was added to a column containing AG50W-X8 resin (2 ml) previously converted to the Na/K form. All liquid was allowed to pass through the column followed by HPLC-grade water (3 ml) to wash. The ammonium (derived from urea) bound to the column was eluted using 1 M-KOH (1 ml) then HPLC-grade water (1 ml), and immediately acidified by collecting into a tube containing 2 M-H_2SO_4 (180 μl) and bromphenol blue (20 μl) (1 % in HPLC-grade water) indicator. The samples were then freeze-dried before analysis in triplicate by combustion–continuous flow-MS to determine mass/charge 29:28 and 30:28 (Tracermass 20/20; Europa Scientific, Crewe, Ches., UK).

The level of enrichment obtained in the two endproducts (NH_3 and urea) should be similar. Any differences $> \pm 25$ % were investigated by hypobromite treatment (as above) of the urea solution to rule out contamination.

Mass/charge 29:28 and 30:28 in freeze-dried faeces was determined by combustion–continuous flow-MS.

Model

N flux, protein synthesis and degradation were determined in *ad libitum*-fed cats using the single intravenous dose, [^{15}N]glycine endproduct model (Waterlow *et al.* 1978*b*; Fern *et al.* 1981). This was modified for use in the present study, with 48 h allowed for excretion of isotope. This time-frame was selected following a previous study (Russell *et al.* 2000) that showed that 48 h was required for complete excretion of isotope following an intravenous dose of [$^{15}N^{15}N$]urea (Zuur *et al.* 2000). This was also necessary due to the infrequent urination of the cats.

The model (Fig. 1) assumes the existence of two pools of N in the body, i.e. a free amino acid pool and a protein pool. Introduction of isotope-labelled N (dose) into the free amino acid pool is assumed to mix homogeneously within the pool. Amino-acid N leaves the free pool and
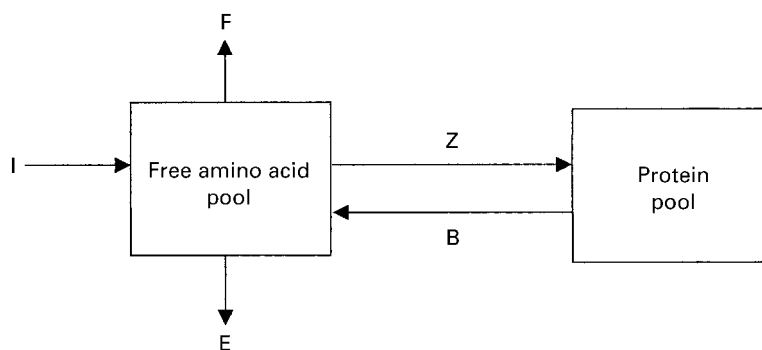
Fig. 1. The model used for calculation of whole-body protein turnover using a single dose of [^{15}N]glycine. Amino acids enter the free amino acid pool from dietary protein (I). Amino acids leave the free pool via three routes: into protein synthesis (Z); into protein oxidation (E); into faeces (F). Once in the protein pool, amino acids can be returned to the free amino acid pool upon protein degradation (B), assumed to be negligible over the time-course of the study.

enters the protein pool via protein synthesis (Z). There is assumed to be no re-entry of isotope from the protein pool into the free amino acid pool (i.e. protein degradation; B) within the time-course of the study. N also leaves the free pool due to amino acid oxidation (E), and this is traced by monitoring excretion of N in urine. A further exit from the free amino acid pool incorporated into the model for the present study was into faeces (F).

The single dose model assumes that the fraction of label excreted in either endproduct is the same as the contribution of unlabelled N excreted in that endproduct to the total flux (dose/e_x = Q/E_x) (Waterlow *et al.* 1978*a*). The flux (Q) was calculated separately for NH$_3$ and urea excretion and the 'endproduct average' technique (Fern *et al.* 1981) employed to calculate N flux for each sample:

$$Q = E_x \times dose/e_x,$$

where Q is the rate of N flux (mmol N/48 h), E_x is the excretion of NH$_3$ or urea (mmol N/48 h), dose is the dose of isotopic N (mmol ^{15}N), e_x is the amount of isotope excreted in NH$_3$ or urea (mmol ^{15}N/48 h).

Urea excretion was corrected for changes in the size of the urea pool over the 48 h collection period, as well as for further loss of N into faeces (F). The change in size of the pool (mmol N/48 h) was calculated from the plasma urea concentration (mmol N/litre), which was converted to total amount in plasma water (assumed to be 92 % of plasma volume) assuming body water content to be 0·6 × BW kg:

$$change\ in\ urea\ pool = (urea_{end} \times (100/92) \times 0.6\ BW_{end})$$
$$- (urea_{start} \times (100/92)$$
$$\times 0.6\ BW_{start}).$$

Changes were added to the observed urinary urea excretion.

Ammonia excretion was uncorrected for any changes in blood levels. The proportion of total faecal N likely to originate from the body (rather than food) and therefore constituting an exit (F) from the free amino acid pool

was calculated from (faecal ^{15}N/urinary urea ^{15}N) × total urinary urea–N. This was added to the urea excretion (E_x), and faecal ^{15}N excretion added to isotope excretion (e_x) during calculation of urea flux. This principle assumed no contribution to faecal ^{15}N by food, and that label entered the gut at the same enrichment as urinary urea.

Rates of protein synthesis and breakdown were calculated from the arithmetic mean of NH$_3$ and urea fluxes (Q) using the expression:

$$Q = E + Z = I + B,$$

where E is the total urinary N excretion, corrected for changes in urea pool size as earlier, Z is the rate of whole–body synthesis, I is the rate of intake of N from the diet, B is the rate of whole–body breakdown (degradation), all expressed as mmol N/48 h, and converted to protein using 14 × 6·25 as the conversion factor.

Although there was large variation between measurements, the distribution of within-cat differences did not show clear signs of non-normality and so, given the modest sample size, the greater power of a *t* test was adopted rather than a non-parametric test. In any case, because HP measurements of protein kinetics were always greater than the corresponding MP values, the non-parametric *P* values would be 1/(2^5) (i.e. 0·03). The data are presented as mean values with their standard errors, with $P < 0.05$ by paired *t* test considered significant. Data are expressed as per 24 h, derived by dividing the 48 h measured value by 2.

Results

One cat (male, age 5 years 10 months, BW 6·1 kg) produced anomalous results when fed the HP diet and no explanation could be found for this result although possibilities include an inaccurate dose. This was evident as very low flux and derived values (i.e. NH$_3$ and urea flux values of 48 % and 62 % of the remaining five cats). Since this meant that the calculated protein breakdown rate was impossibly negative this cat was omitted from the subsequent analysis.

For the remaining cats, BW was well maintained on both

diets, with mean (n 5) change over the 16 d study being $-5\cdot4$ (SE $1\cdot7$) % for the MP diet and $2\cdot4$ (SE $1\cdot0$) % for the HP diet. During the 48 h study period following the dose, absolute mean (n 5) BW changes were $2\cdot7$ (SE $14\cdot0$) g/d for the MP diet and $-0\cdot1$ (SE $23\cdot2$) g/d for the HP diet, although this was not significantly different ($P>0\cdot05$).

There was no difference ($P>0\cdot05$) in energy intake between the two diets, either during the 14 d pre-feed ($113\cdot8$ (SE $21\cdot7$) kJ/kg BW per d (MP) and $162\cdot5$ (SE $25\cdot6$) kJ/kg BW per d (HP)) or 2 d test phase ($102\cdot5$ (SE $27\cdot1$) kJ/kg BW per d (MP) and $148\cdot6$ (SE $17\cdot2$) kJ/kg BW per d (HP)). However the lower mean value for MP reflected a low food intake for cats 1, 2 and 5 (Table 2), which was reflected in BW loss in these cats. Therefore the observed responses in protein turnover reflect to some extent the lowered energy as well as protein intake. There was poor correlation between protein synthesis and BW change (linear regression, $R^2 = 9\cdot4\%$, intercept $= 356\cdot9$, slope $= 0\cdot79$, $P = 0\cdot6$) indicating that energy intake was probably not driving the observed changes in protein synthesis. However, it must be accepted that in such a small sample size it is not possible to determine with certainty the effect this negative energy balance would have upon the protein turnover of the MP cats. Individual results for energy balance and protein turnover are provided for comparison, and it can be seen that cats 1, 2 and 5 had similar N balance to the other cats during the MP diet phase (Table 2 and Table 3).

The 48 h change in urea pool size was different between the two diets ($P<0\cdot05$) (Table 4). Faecal N excretion for these diets was low, amounting to $34\cdot5$ (SE $5\cdot6$) mmol N/ 48 h (n 11 faeces samples), although higher on the HP diet ($P<0\cdot001$) (Table 4).

During the period of feeding the HP diet, the cats had a greater N intake ($P<0\cdot05$) and greater urinary N excretion ($P<0\cdot05$) than during the MP phase. Mean N balance over the 48 h study period was $-34\cdot2$ (SE $2\cdot9$) and $-4\cdot8$ (SE $21\cdot1$) mmol N/d for MP and HP diets respectively (Table 3). For the MP diet group, N balance was less than zero (i.e. the MP cats were in negative N balance) (one variable t test, $P<0\cdot001$), although the N balance of the HP group was not different from zero (one variable t test, $P>0\cdot05$).

There were some large differences in enrichment (atom % excess) of urea and NH_3, with NH_3 often (although not always) the greater. Ammonia enrichment was greater than urea for cat 4 (MP diet) that only produced a 24 h urine sample. Samples with large differences were not related to diet, and although sometimes occurring in early voided samples (i.e. <24 h after the dose), this was not always the case. The urea extractions were repeated and subjected to hypobromite oxidation, and replicates for both NH_3 and urea were good indicating that the problem was not analytical. This resulted in a difference ($P<0\cdot05$) in the N flux calculated using the two endproducts, with mean (n 10, both diets) NH_3 flux $421\cdot7$ (SE $75\cdot7$) and urea flux $531\cdot9$ (SE $93\cdot4$) mmol N/24 h.

There was a significant effect of diet on protein turnover, with the MP diet resulting in lower flux ($P<0\cdot005$), protein synthesis ($P<0\cdot05$) and protein breakdown ($P<0\cdot05$) than the HP diet (Table 3).

When the data for both diets were combined (n 10), the absolute rate of protein synthesis was $23\cdot4$ (SE $3\cdot7$) g protein/d or $4\cdot9$ (SE $0\cdot7$) g protein/kg BW per d, or if related to metabolic tissue mass, $7\cdot3$ (SE $1\cdot1$) g protein/kg $BW^{0\cdot75}$ per d.

Discussion

Whole-body protein turnover was measured in five adult cats at maintenance, fed either a moderate-protein diet or a higher-protein diet. Flux, protein synthesis and protein breakdown were all significantly lower on the moderate-protein diet. The measured rates of protein turnover synthesis and breakdown were corrected for BW (Table 3) but not for variation in fatness and consequent lean body mass. However, it is unlikely that differences in body composition could confound the results since marked changes are unlikely to have occurred during the test periods.

The whole-body protein turnover of adults at maintenance has been little studied in non-human species. From the data available (Table 5), the whole-body rate of protein synthesis ranges from $12\cdot6$ to 17 g/kg $BW^{0\cdot75}$. Assuming

Table 2. Energy intake (kJ/kg body weight per d) of cats fed diets moderate in protein (MP) or high in protein (HP) during the 14 d pre-feed and 2 d test phases*

(Individual values, and mean values with their standard errors)

	Cat						
	1	2	3	4	5	Mean	SE
MP							
Mean intake during pre-feed (n 14)	93·1	66·7	195·1	111·1	102·9	113·8	21·7
Mean intake during test (n 2)	36·8	74·3	191·2	134·2	75·9	102·5	27·1
Test/pre-feed (%)	39·6	111·4	98·0	120·7	73·8	88·7	14·6
HP							
Mean intake during pre-feed (n 14)	88·8	161·7	196·4	129·1	236·3	162·5	25·6
Mean intake during test (n 2)	115·2	135·3	182·8	113·6	195·8	148·6	17·2
Test/pre-feed (%)	129·8	83·7	93·1	88·0	82·9	95·5	8·8
MP/HP during test phase (%)	32·0	54·9	104·6	118·1	38·8	69·7	17·6

* For details of diets and procedures see Table 1 and p. 30.

Table 3. Protein turnover on moderate-protein diet (MP) and higher-protein diet (HP). Nitrogen flux is provided for each endproduct (Q ammonia, Q urea) as well as the arithmetic mean (Q average). Nitrogen intake (I) and urinary nitrogen excretion (E) are provided along with synthesis (Z) and breakdown (B) (all mmol N/kg body weight (BW) d) together with synthesis and breakdown* converted to protein

(Individual values and mean values with their standard errors)

Diet...	MP							HP							
Cat...	1	2	3	4	5	Mean	SE	1	2	3	4	5	Mean	SE	P†
Q ammonia	36.9	58.7	70.2	52.9	47.9	53.3	5.5	137.1	93.3	147.2	108.3	129.7	123.1	9.8	<0.005
Q urea	43.5	59.7	69.6	72.5	44.8	58.0	6.1	182.1	163.2	156.7	145.0	193.8	168.2	8.8	<0.005
Q average	40.2	59.1	69.9	62.7	46.4	55.7	5.4	159.6	128.2	152.0	126.7	161.8	145.6	7.6	<0.005
I	4.0	8.1	21.1	14.7	8.7	11.3	3.0	57.3	67.3	89.8	56.1	96.8	73.5	8.4	<0.005
E	12.9	14.2	24.0	16.6	19.1	17.4	2.0	45.6	72.2	80.2	52.0	101.7	70.3	10.1	<0.005
Z	27.2	45.0	45.9	46.1	27.3	38.3	4.5	114.0	56.0	71.8	74.6	60.0	75.3	10.3	<0.05
B	36.1	51.1	48.9	48.0	37.7	44.3	3.1	102.3	60.9	62.1	70.5	65.0	72.2	7.7	<0.05
N balance (mmol N/d)	−43.2	−34.1	−32.5	−25.3	−35.8	−34.2	2.9	51.4	−53.2	36.8	−13.7	−45.2	−4.8	21.1	NS
BW change (g)	−42.5	−13.5	29.5	8.0	32.0	2.7	14.0	33.5	−53.0	35.0	−60.5	44.5	−0.1	23.2	NS
Protein synthesised (g/kg BW per d)	2.4	4.0	4.0	4.0	2.4	3.4	0.4	9.9	4.9	6.3	6.5	5.3	6.6	0.9	<0.05
Protein synthesised (g/kg $BW^{0.75}$ per d)	3.4	5.7	6.3	6.0	3.2	4.9	0.7	14.2	7.1	10.0	9.9	7.2	9.7	1.3	<0.05
Protein breakdown (g/kg BW per d)	3.1	4.5	4.2	4.2	3.3	3.9	0.3	8.9	5.3	5.5	6.2	5.7	6.3	0.7	<0.05
Protein breakdown (g/kg $BW^{0.75}$ per d)	4.5	6.5	6.7	6.3	4.4	5.7	0.5	12.8	7.7	8.7	9.3	7.8	9.3	0.9	<0.05

*These data are reported for 24 h, derived by dividing the 48 h values by 2. For details of diets and procedures see Table 1 and p. 30.
†Difference between diets analysed by paired *t* test.

the rate on the high-protein diet to reflect the 'normal' rate for this species the cat appears at the lower end of the range for omnivores and herbivores (Table 5). A low overall rate of protein turnover may be related to physiological factors unique to the cat. Carnivores have a shorter gastrointestinal tract than omnivores and herbivores, and since the gut may account for around 25 % of the total body protein synthesis (Waterlow *et al.* 1978*a*), this may reduce the cost to the cat and would correlate with a lower overall protein turnover.

This low N flux rate may be a species-specific difference although confounding factors introduced by the experimental design may contribute. These include the dose administration and period of excreta collection.

The dose was provided as a single rapid injection rather than the slow (for example, 1 h) injection used previously (Fern *et al.* 1985). Although a rapid dose would avoid stressing the cats, it could potentially flood the free amino acid pool with a large dose of glycine, and stimulate immediate removal by oxidation. Rapid disposal of isotope by oxidation would lead to an underestimate of flux (by increasing e_x). The dose rate administered in the present study was selected to be similar to those used in human studies, relative to BW (Fern *et al.* 1981; Grove & Jackson, 1995). Furthermore, supplying a larger oral dose did not influence flux in human subjects (Fern *et al.* 1981), nor did the route of administration (Fern *et al.* 1985).

Flux would also be underestimated if there were significant recycling of isotope from protein breakdown into the free amino acid pool. Within the model, recycling of isotope into the free amino acid pool is assumed to be negligible. However, the longer the period of excreta collection, the more likely it is that isotope will be recycled. The present data are based on a 48 h collection, and extending the collection period to 48 h with human subjects only decreased flux by 20 % compared with that measured at 24 h (Grove & Jackson, 1995). This would therefore be unlikely to account for the very low flux measured in the present study.

A major assumption of the model is that there is a single homogeneous pool of metabolic N (the free amino acid pool) into which the isotope is instantly distributed, and from which N is taken for protein synthesis and oxidation (excretion). If this were true the turnover (flux) derived from either endproduct should be the same. In the present study the flux rate obtained using urea was higher than that obtained using NH_3, supporting previous work in human subjects (Fern *et al.* 1981, 1985; Grove & Jackson, 1995). This may be due to the different sites of endproduct synthesis (different free amino acid pools), since the precursors for urea (aspartate, glutamate and NH_3) originate in the liver whilst urinary NH_3 is derived from glutamine in muscle (Waterlow, 1984).

Cat 4 failed to urinate between 24 and 48 h after the dose when fed the MP diet. This sample may be expected to have a high NH_3 enrichment compared with urea, and hence urea flux $> NH_3$ flux. This was the case, but was also seen in other cats that provided complete urine collections. Individual differences in patterns of urination behaviour are likely to contribute to the variability of all results since urine was collected for a standard 48 h after each dose. Although the majority of cats produced a

Table 4. Changes (/48 h) in urea pool, dose and excretion of isotope in cats fed moderate-protein diet (MP) and higher-protein diet (HP)*

(Mean values with their standard errors)

Diet	MP (n 5)		HP (n 5)		P†
	Mean	SE	Mean	SE	
Urea pool change (mmol N/48 h)	−3·1	2·2	−0·7	1·6	<0·05
Dose administered (mmol ^{15}N)	0·29	0·03	0·32	0·04	NS
Urinary excretion of dose (%)	26·7	3·1	41·2	4·6	<0·05
Faecal N excretion (mmol N/48 h)	19·6	2·6	56·2	2·5	<0·0001
Faeces enrichment (^{15}N atom % excess)	0·0070	0·0023	0·0031	0·0014	NS
Faecal N originating from body/total faecal N (%)	44·2	23·0	15·6	6·8	NS
Faecal excretion of dose (%)	0·4	0·1	0·5	0·2	NS

* For details of diets and procedures, see Table 1 and p. 30.
† Difference between diets analysed by paired t test.

sample between 24 and 48 h, this was not always at the end of the 48 h collection period. This means that the end-point of the collection varied between 24 and 48 h, introducing unavoidable variability into the kinetics data.

The effect of nutrition on protein turnover has been studied at length in the growing rat and in the adult human subject, but rarely in other adult animals at maintenance. In the growing rat there are marked reductions in protein turnover in skeletal muscle with protein deficiency (Garlick et al. 1975; Millward et al. 1975) and reductions in hepatic protein synthesis mainly due to a fall in the hepatic protein mass (Garlick et al. 1975). These changes were associated with marked hormonal responses to dietary protein, including reduced insulin and triiodothyronine (Jepson et al. 1988) and insulin-like growth factor-1 (Yahya et al. 1990). In adult human subjects there is less evidence for marked changes in turnover, and variation in protein intakes over a wide range are not associated with measurable changes in insulin-like growth factor-1 or triiodothyronine levels (Pacy et al. 1994). However the response of protein synthesis and breakdown to feeding is sensitive to the dietary protein level, an effect mediated in part directly by variation in amino acid intake (Pacy et al. 1994; Fereday et al. 1998). Thus increasing dietary protein

induces an increasing inhibition of protein breakdown and, to a lesser extent, an increasing stimulation of synthesis (Waterlow, 1999). These effects may be difficult to detect, however, probably due to variability introduced by study design, subject, diet or methodology, resulting in a variety of outcomes that are often non-significant and challenging to interpret (Hoffer et al. 1985; Goulet et al. 1993; El Khoury et al. 1995; Cayol et al. 1997; Forslund et al. 1998).

Responses to changes in the prevailing diet have been little studied using the endproduct technique, despite its applicability. A rigorous study using a 1 h infusion of [^{15}N]glycine in normal adult human subjects failed to find a significant effect of prevailing diet on protein synthesis or breakdown (Pacy et al. 1994). Thus, although mean values did increase by 60 % from a very low to a generous protein intake level, the variability of the data was such that the change was not significant. In contrast, studies using single oral doses of [^{15}N]glycine found reduced synthesis and breakdown in elderly and young human subjects adapted to lower-protein diets (Pannemans et al. 1995a,b, 1997). The present results showing decreased protein synthesis and breakdown in cats adapted to a lower-protein diet support these latter findings and small-animal studies. Cats therefore exhibit metabolic flexibility with an ability to adapt protein metabolism to dietary protein, supporting previous work (Kettlehut et al. 1980; Silva & Mercer, 1985, 1991; Russell et al. 2000, 2002). It would seem reasonable to conclude that adaptation to a lower-protein diet involves a coordinated decrease in protein breakdown to match the decreased protein synthesis to facilitate the maintenance of N balance.

The compositions of the diets used in the present study (20 % and 70 % protein energy) were outside the range normally encountered by domestic cats, this being approximately 40 % dietary energy. These diets were selected at the extremes to maximise the power of the study and so may not reflect protein turnover on a more usual protein intake. Both diets provided adequate protein, the minimum protein requirement of the adult domestic cat being 10 % dietary energy (Burger et al. 1984). However, even when adequate, lower-protein diets can be unpalatable to cats (Zentek et al. 1998) when lack of appetite can result in BW loss. BW loss occurred during the MP diet phase

Table 5. Comparison of rate of whole-body protein synthesis in different adult animals at maintenance

Species	BW (kg)	Protein synthesis		
		g/d	g/kg BW per d	g/kg BW$^{0.75}$ per d
Rat*	0·35	7·7	22·0	16·9
Rabbit*	3·6	33	9·2	12·6
Sheep*	63	351	5·6	15·7
Man†	67	310	4·6	12·6
Man‡	67	296	4·4	12·6
Cow*	575	1740	3·0	14·8
Cat (MP)§	4·6	15·8	3·3	4·9
Cat (HP)§	4·8	31·4	6·6	9·7

BW, body weight; MP, moderate-protein diet; HP, high-protein diet.
* Data provided using precursor method (Reeds & Harris, 1981).
† Data provided using endproduct method in the fed state (Pacy et al. 1994).
‡ Data provided using precursor method (Pacy et al. 1994).
§ Data from present study.

and was more pronounced in three of the five cats (cat 1, 2 and 5; Table 2). Thus while an effect of the reduced energy intake on protein turnover cannot be entirely ruled out, it was the case that the kinetic response of these cats was the same as the rest of the group.

BW changes also occurred during the pre-feed period and these may have influenced responses during the study periods. During the pre-feed period for the MP diet, all five cats lost BW, although three of the five cats had stabilised by the time they were dosed. A catabolic state produced by negative energy balance and negative N balance would be expected to result in decreased protein synthesis. During the pre-feed period for the HP diet, the majority (4/5) of cats gained BW and continued in positive energy balance, maintaining N balance during the 48 h study period. These cats were in an anabolic state and would be expected to have an increased rate of protein synthesis. BW changes during the pre-feed and test phases therefore contribute to the effect of diet on protein synthesis and breakdown in the present study.

The high protein requirement of the feline, thought to be for N rather than essential amino acids, is reported to be due to a permanently high hepatic capacity for amino acid catabolism and ureagenesis (Rogers *et al.* 1977; Morris, 2002). This confers a limited ability to adapt to a low-protein diet with a consequent negative N balance. However, as we have shown, rates of ureagenesis, urea excretion and protein oxidation do adapt to varying levels of protein intake, increasing 3-fold as protein intakes are increased from 20 % (moderate) to 70 % (high) protein energy (Russell *et al.* 2000, 2002). Indeed, were these adaptations not to occur there would be an inability to eat the very-high-protein diets that are a normal part of the obligate carnivore's food intake. Similarly, adult cats fed decreasing or protein-free diets reduce both total N and urea-N excretion. The crucial difference from other species may be the more limited extent to which this N excretion can be reduced and the consequent higher obligatory N loss (Hendriks *et al.* 1997). Thus, the key feature of feline amino acid metabolism may be a higher minimum or basal rate of amino acid oxidation and N excretion. In other species, obligatory N loss reflects catabolism of amino acids at a rate determined by the metabolic needs of the rate-limiting amino acid (usually considered to be methionine) provided from body protein. On this basis, the higher obligatory N loss in felines might suggest that maintenance requirements for one, or more, essential amino acids are increased. Whilst it is usually argued that cats do not have an unusual balance in their requirements for essential amino acids (apart from arginine; Rogers & Morris, 1979), this is based on their growth requirement, not the requirement for adult maintenance. However, the observation that the lowest protein requirement reported for adult cats involved a mixture of soya protein and essential amino acids (Burger *et al.* 1984; Burger & Smith, 1987) suggests that the increased overall requirement of the feline is an increased maintenance need for specific essential amino acids, rather than for non-essential N.

An increased maintenance requirement for amino acids could possibly result from a higher rate of protein turnover and the present study was designed to test the hypothesis that the cat exhibits either an increased rate or an inability to adapt the rate to variation in intake. The results indicate that neither of these hypotheses is correct. The rate of protein turnover in the cat is at the lower end of the range for omnivores and herbivores. Also, as with other small animal species, the cat does exhibit metabolic flexibility in terms of adaptation of its protein turnover rate to variation in protein intake. This rules out the characteristics of protein turnover as an explanation for the higher protein requirement.

In conclusion, therefore, the apparently high feline protein requirement remains unexplained and is probably not a simple reflection of an inability to adapt hepatic catabolic capacity to variation in protein intake. Further work is required, therefore, to establish exactly why cats appear to need so much dietary protein with studies aimed particularly at responses to the lower range of protein intakes (for example, around 6 % protein energy, as tolerated by omnivores and herbivores) and determining the maintenance essential amino acid requirements in more detail.

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