Urea kinetics of a carnivore, *Felis silvestris catus*

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The effect of two levels of dietary protein energy, moderate (20 %; MP) and high (70 %; HP), on urea kinetics in eleven domestic cats was studied. After a 3-week prefeed, a single dose of [$^{15}N^{15}N$]urea was administered, and urine and faeces collected over the subsequent 5 d. For each 24 h period, total urea and enrichment of [$^{15}N^{15}N$]- and [$^{15}N^{14}N$]urea in urine were determined, and a model applied to calculate urea production, entry into the gastrointestinal tract, recycling to urine or faeces and, by difference, retention by the body and potentially available for anabolism. Urea production and excretion increased with dietary protein level ($P < 0{\cdot}05$). Most of the urea produced was excreted, with only a small proportion entering the gut, and with the pattern of urea disposal not significantly different between the HP and MP diets. Thus, the percentages of urea production available to the gut were 15 % (MP) and 12 % (HP), of which 57 % (MP) and 59 % (HP) was recycled in the ornithine cycle, 40 % (MP and HP) was potentially available for anabolism and the rest lost as faecal N. As a percentage of urea produced the amount potentially available for anabolism was very low at 6·41 % (MP diet) and 4·79 % (HP diet). In absolute terms urea entering the gut, being recycled in the ornithine cycle and potentially available for anabolism was significantly higher on the HP diet ($P < 0{\cdot}05$). These results show that cats operate urea turnover, but at a lower rate, and with less nutritional sensitivity than has been reported for other species.

Protein: Nitrogen: Urea kinetics: Cat

Adaptation to dietary N intake is essential, because the capacity of the body to store N is limited (Millward, 1995). The mechanisms by which adaptation is achieved are principally dependent on hepatic enzyme systems, decreased dietary N intake being accommodated by decreases in both catabolism of amino acids and urea production. A further mechanism may involve salvage of urea-N by the gastrointestinal microflora, a process which is increased in human subjects when dietary N intake is low (Picou & Phillips, 1972; Tanaka *et al.* 1980; Jackson *et al.* 1990; Jackson, 1991; Danielsen & Jackson, 1992; Langran *et al.* 1992; Bundy *et al.* 1993), thus helping to maintain N balance. Urea freely enters the gut, where it is subject to hydrolysis by microbial urease to produce NH_3 (Walser & Bodenlos, 1959). NH_3-N is then available for synthesis of non-essential and essential amino acids by the microbes (Richards *et al.* 1967), and salvaged urea-N may be a functionally significant component of overall N supply (Jackson, 1983; Millward *et al.* 2000).

Cats have a high requirement for dietary protein compared with other species, which has been attributed to the apparent inability of the hepatic enzymes of this species to adapt to dietary protein intake (Rogers *et al.* 1977; Rogers & Morris, 1980). However, several groups have since produced *in vitro* evidence of the cat's ability to adapt to dietary protein in terms of ureagenesis, gluconeogenesis and protein turnover (Kettlehut *et al.* 1980; Silva & Mercer, 1985, 1991; Fau *et al.* 1987). There are currently no *in vivo* studies to confirm the ability of the cat to adapt or respond to dietary protein.

There is some suggestion that cats can salvage urea-N. Kornberg & Davies (1952) injected [^{15}N]urea subcutaneously into a single cat and demonstrated that only 83·5 % of the injected isotope was excreted in the urine. The remainder of the isotope remained in the body and was found to be widely distributed amongst body tissues, indicating that after 40 h 2·6 % of the [^{15}N]urea had been metabolised by the body. This finding suggested that the

Abbreviations: BW, body weight; HP, high dietary protein energy; MP, moderate dietary protein energy; *m*/*z*, mass:charge.

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cat was capable of using urea-N for incorporation into body tissues, although the site of this mechanism was not described. This capability was confirmed by Deguchi (1985) who removed the liver, serum and muscle of cats fed a diet supplemented with [^{15}N]urea, and demonstrated that the enrichment of TCA precipitates of homogenised tissues and serum of these cats was higher than the natural abundance of ^{15}N in one control cat. There are currently no studies to confirm the ability of the cat to operate a urea salvage mechanism, and no indication of whether this process may show nutritional sensitivity and thus be a possible mechanism of adaptation to lower dietary protein intake.

In the present study urea kinetics were studied in the cat on a high protein intake (70 % of dietary energy as protein; HP) and a moderate protein intake (20 % of dietary energy as protein; MP). Jackson *et al.* (1993) developed a model that utilises a single dose of [^{15}N^{15}N]urea, measuring cumulative urinary excretion of isotope over 48 h. This model has been adapted for the current study, by providing the dose intravenously (since the cat has gastric urease; see Kornberg & Davies, 1952), and following excretion for 5 d.

Materials and methods

Animals, diets and experimental design

Twelve adult cats (six males, six females; mean age 4·5 years (SE 11 months); mean body weight (BW) 4·28 (SE 0·3) kg) were allocated to two treatment groups matched for age and sex. One female cat was removed from the study (HP group) because of an erratic urination pattern. The cats were housed individually in metabolism cages and exercised for approximately 1 h daily.

The cats received one of two diets only for 21 d before the beginning of the trial and for the 6 d trial duration, and were fed to appetite, being offered generous portions twice daily. 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 which is approximately 50 % of that found in standard commercial cat foods, but is sufficient to ensure good maintenance of intake and BW. The HP diet was formulated to provide 70 % energy from protein, a level at the top of the range of commercial cat foods.

Following the 21 d prefeed, urine and faeces collection commenced (09.00 hours on day 0) to establish baseline excreta ^{15}N values for each cat. [^{15}N^{15}N]urea (99·87 atom % Cambridge Isotope Laboratories Inc., Cambridge, MA, USA) was prepared as a 27·3 mg/ml stock solution in sterile physiological saline (9 g NaCl/l) immediately before use. At 10.00 hours on day 1, [^{15}N^{15}N]urea was administered via the cephalic vein of each cat, at a dose of 6·83 mg/kg BW. Two of the cats (both in the MP group) received their doses subcutaneously. Faeces and urine samples were collected for 5 d. Each defecation was immediately weighed and frozen at −20°C. Each 500 ml urine collection bottle contained 5 ml 5 M-HCl as preservative, and bottles were changed every 24 h, the urine being weighed and then frozen at −20°C.

It was subsequently found that urine recovery from this system was incomplete (88·3 (SE 0·98) %, *n* 23), calculated by pouring a known quantity of urine over a litter tray and calculating recovery after 1 min. All urine volumes from both diet groups were therefore multiplied by 1·136 to correct for this deficit, evaporation being unlikely to be influenced by diet. A correction to food intake data (assessed gravimetrically) was also made for loss of weight of diets due to evaporation, calculated by leaving an identical bowl of each diet in an empty cage for each meal. Evaporation during the morning meal (6 h) was 1·94 (SE 0·37) % (*n* 5) from the MP diet and 1·96 (SE 0·15) % (*n* 5) from the HP diet. Evaporation during the overnight meal (18 h) was found to be 6·97 (SE 0·31) % (*n* 5) from the MP diet and 7·64 (SE 0·48) % (*n* 5) from the HP diet.

Chemical analyses

N contents of diets, urine and faeces were determined by the Dumas procedure using an automated Leco FP428 analyser (The Leco Corporation, Saint Joseph, MI, USA). Urinary urea concentration was determined by the method of Marsh *et al.* (1965) using a Technicon AutoAnalyzer (Technicon Instruments Corporation, Tarrytown, NY, USA).

^{15}N analyses

A cation-exchange column containing 2 ml resin (AG-50, 100–200 mesh, ×8, H^+ form; Biorad, Richmond, CA, USA) prewashed with 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 5 ml water to remove any contaminants. Distilled water (55 ml) was then added, and the eluate collected and evaporated. The 250 μmol urea was resuspended in 10 ml water and mixed thoroughly, before being divided equally between twelve vials, each containing a known amount of N, and frozen before analysis.

For each sample, in duplicate, a portion was diluted by the addition of 2 ml N-free deionised water and de-gassed using a vacuum pump for 8 min at 1 Pa in a Louwers Hapert bottle (Louwers Hapert, PO Box 27, The Netherlands). The

Table 1. Diet composition and formulation at moderate (MP) and high (HP) protein levels

Diet	Chicken breast (g/kg)	Lard (g/kg)	Glucose (g/kg)	(1·5 %/v) Carob in water (g/kg)	Percentage of energy from: Protein	Carbohydrate	Fat	Predicted metabolisable energy (MJ/kg)
MP	164·30	29·60	79·80	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

sample was then frozen by lowering the tube slowly into liquid N_2, and 0·5 ml lithium hypobromite solution (10 % (v/v) LiOH in water plus 2 ml Br_2, bubbled with He) added. The sample was evacuated for 5 min at 10 mPa before incubating at 60°C for 15 min, or until ready for analysis. At this temperature hypobromite treatment of urea results in a Hoffman degradation, which under gaseous conditions produces N_2 gas, where both N atoms have arisen from the same urea molecule. By performing the reaction in a dilute liquid phase, the reaction that arises is not completely monomolecular, therefore a correction is required. To estimate this correction factor, standards were prepared by diluting labelled urea with natural-abundance urea to give a similar concentration and enrichment to that of the samples, and measured alongside each set of analyses. This procedure allowed corrections to be applied to the samples for the loss of ^{15}N from mass:charge (m/z) 30 and gain in m/z 29.

The sample was frozen by slowly lowering the tube into liquid N_2 and a dual-inlet isotope-ratio MS (SIRA 12; VG Isogas, Middlewich, Ches., UK) used to analyse the N arising from the doubly-labelled urea. The N_2 gas was ionised by electron impact ionisation, $^{15}N^{15}N$ producing m/z 30, $^{15}N^{14}N$ producing m/z 29 and $^{14}N^{14}N$ producing m/z 28. The ratios of ions 29:28 and 30:28 were compared with those of a known standard, and data corrected to ^{15}N atom % (i.e. 29 ion current was divided by 2 since only half the N is labelled).

Enrichment in faeces was determined from ion ratio 29:28 measured by combustion–continuous-flow MS of freeze-dried samples.

A correction was also applied to account for the proportion of [$^{15}N^{14}N$]urea present in the dose. This value was determined by GC–MS of the tertiary butyldimethylsilyl derivative of urea (Calder & Smith, 1988) and found to be 0·26 atom % of the total.

Model

The model used was developed from that of Jackson *et al.* (1993), incorporating three exits from the N pool and allowing for multiple entries of [$^{15}N^{14}N$]urea into the gut (Sarraseca *et al.*1998). In this model (Fig. 1) it is assumed that urea produced by the liver (P) can only have one of two fates; a portion (u) is excreted in urine (Eu), and the remainder (1−u) enters the gut (T). In the gut the urea is hydrolysed to NH_3, which has three possible fates; a portion (r) is returned to the ornithine cycle (Pr), a portion (f) is excreted in faeces (F), and the remaining portion (a) is retained in the body and so is potentially available for anabolism (A). Information on these fates is obtained by monitoring the appearance of [$^{15}N^{15}N$]urea (eu30) and [$^{15}N^{14}N$]urea (eu29) in urine and ^{15}N in faeces (F^*), following administration of a single dose of [$^{15}N^{15}N$]urea (d30).

The model assumes:

(1) the existence of two pools, a urea pool and a N pool;
(2) that labelled urea is handled in the same way as unlabelled urea;
(3) that there is a single input to the urea pool from urea synthesis (P), and two exits, to urinary excretion (Eu) and to the bowel (T);
(4) that N derived from the hydrolysis of urea enters a metabolic pool of N and has three fates, i.e. to urea synthesis (ornithine cycle; Pr), to anabolism (A) and faeces (F);
(5) a reasonably steady metabolic state throughout the study;
(6) that after [$^{15}N^{15}N$]urea has been hydrolysed in the gut, the ^{15}N returned to the ornithine cycle will only recombine with ^{14}N to form [$^{15}N^{14}N$]urea (i.e. the probability of two ^{15}N atoms recombining to form [$^{15}N^{15}N$]urea is negligible).

Monitoring the appearance of [$^{15}N^{15}N$]urea in urine gives us the proportion of urea production eliminated in urine:

$$u = eu30/d30.$$

Then, urea production follows from:

$$P = Eu/u,$$

$$\text{and } T = P - Eu.$$

Monitoring the appearance of [$^{15}N^{14}N$]urea in urine yields information on the amount of urea returned to the ornithine cycle. Let $R = eu29/(eu29 + eu30)$, which is the proportion of urea production originating from recycling. Then, the portion (r) of gut entry that is returned to the ornithine

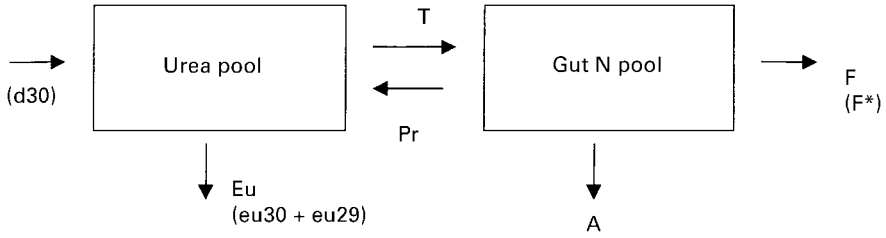

Fig. 1. The model used for measuring urea kinetics with a single dose of [$^{15}N^{15}N$]urea. Urea (P) enters a urea pool and is either excreted in the urine (Eu) or enters the gut (T). Urea entering the gut is hydrolysed by gut microbes, the nitrogen then either recycled to the ornithine cycle (Pr), excreted in the faeces (F) or remaining in the body potentially available for anabolism (A). eu29, eu30, [$^{15}N^{14}N$]urea and [$^{15}N^{15}N$]urea respectively in urine; d30, single dose of [$^{15}N^{15}N$]urea; F*, ^{15}N in faeces. (Modified from Jackson *et al.* 1993.)

Table 2. Nitrogen intake and excretion values (mmol N/kg body weight per d) for cats fed a moderate-protein diet (20 % protein energy; MP) or a high-protein diet (70 % protein energy; HP)*

(Values are means with their standard errors for five cats for HP diet and six cats for MP diet)

Diet…	MP		HP		Statistical significance of difference: *P*‡
	Mean	SE	Mean	SE	
N intake	28·2	3·25	75·7	4·90	0·01
N excretion in faeces	2·6	0·36	4·0	0·46	0·04
N excretion in urine	25·1	2·17	76·6	2·90	0·01
N balance†	0·6	3·14	−4·9	4·08	0·72

* For details of diets and procedures, see Table 1 and p. 598.
† Calculated as intake − (urine + faeces).
‡ By Kruskal–Wallis.

cycle is obtained from:

$$r = R/(1 - u).$$

This calculation allows for multiple entry of [$^{15}N^{14}N$]urea to the gut, and was not originally accounted for by Jackson *et al.* (1993), where r was derived from r = eu29/(d30(1 − u)). The proportion (f) of gut entry excreted in faeces is calculated as:

$$f = \frac{uF*}{(1 - u)(eu29 + eu30)}.$$

The proportion used for anabolism (a) is obtained by difference,

$$a = 1 - r - f.$$

Pr, F and A are obtained from multiplying T by r, f and a respectively.

Statistical analysis

As the data infringed the assumptions for parametric statistical analysis, the data were analysed using Kruskal–Wallis Test. Results are expressed as means with their standard errors, and $P < 0{\cdot}05$ was considered significant.

Results

BW changes were minimal over the 4-week study period (mean 1·9 (SE 1·5) % for MP cats, mean −3·2 (SE 0·8) % for HP cats). Over the final week of the study when data were being collected, BW changes were 1·8 (SE 0·7) % for MP cats and 0·2 (SE 0·7) % for HP cats. The majority of cats (both diet groups) were found to be close to zero N balance (Tables 2 and 3), and there was no significant effect of diet on N balance. The minimal BW changes and N balance indicate that the cats were in a reasonably steady metabolic state.

Although the sample size was small, no significant difference ($P > 0{\cdot}05$) was found between dosing regimens (intravenous *v.* subcutaneous) in the MP cats for any part of the model, so the data were combined (for individual data, see Table 3).

The urea production rates and urinary urea excretion rates of HP cats were significantly higher than those for MP cats, although there was no significant difference in urea-N lost through faecal excretion (Table 4). Urea-N formed a greater proportion of total urinary N for HP cats than for MP cats (75 (SE 1·3) % for HP cats and 65 (SE 1·6) % for MP cats, $P < 0{\cdot}05$). Urea production expressed on per kg metabolic body weight (kg $BW^{0{\cdot}75}$) basis was 92·7 (SE 4·5) mmol N/d for HP cats and 27·6 (SE 2·6) mmol N/d for MP cats ($P < 0{\cdot}05$).

More urea-N was available for hydrolysis in the gut and more urea-N was returned to the ornithine cycle of HP cats than MP cats. More urea-N was potentially available for anabolism in HP cats compared with MP cats (Table 4). Intake of N plus that available to the gut was significantly greater ($P < 0{\cdot}05$) in HP cats (83·6 (SE 5·1) mmol N/kg BW per d) compared with MP cats (32·3 (SE 3·5) mmol N/kg BW per d).

Despite the significant difference between HP cats and MP cats in absolute values (Table 4), as a proportion of urea produced there was little difference between the diets. As a percentage of urea produced, there was no significant difference in urea entering the gut, or that available for anabolism in MP and HP cats (Table 5). As a percentage of urea entering the gut there was also no significant difference between the diets in terms of urea recycled, or that available for anabolism (Table 5).

Table 3. Individual values (mmol N/kg body weight per d) for urea production (P), urinary urea excretion (Eu), urea entering gut (T), urea recycled (Pr) and urea retained for anabolism (A) for cats fed a moderate-protein diet (20 % protein energy; MP) or a high-protein diet (70 % protein energy; HP)*

Cat no.	Diet	Dose route*	N balance	P	Eu	T	Pr	A
1	MP	sc	−1·1	13·9	11·8	2·1	0·9	1·2
2	MP	iv	−6·9	17·7	16·9	0·9	0·4	0·4
3	MP	iv	−2·6	24·7	19·7	5·0	2·9	2·0
4	MP	iv	15·3	21·7	15·8	5·9	2·7	3·1
5	MP	sc	−2·6	19·8	17·2	2·6	1·8	0·7
6	MP	iv	1·2	16·2	14·7	1·5	1·1	0·3
7	HP	iv	−2·1	57·8	52·0	5·7	3·0	2·7
8	HP	iv	−20·6	62·4	54·3	8·1	4·9	3·1
9	HP	iv	3·0	65·9	56·8	9·1	4·5	4·5
10	HP	iv	−3·9	68·3	59·9	8·4	4·5	3·8
11	HP	iv	−0·8	72·9	65·1	7·8	6·2	1·5

iv, intravenous; sc, subcutaneous.
* For details of diets and procedures, see Table 1 and p. 598.

Discussion

The cat has a requirement for dietary protein of 10 % dietary energy, a level that will just allow it to maintain N balance (Burger *et al.* 1984). This level is more than twice

Table 4. Absolute urea production, excretion, gut entry, recycling and retention rates (mmol N/kg body weight per d) for cats fed a moderate-protein diet (20 % protein energy; MP) or a high-protein diet (70 % protein energy; HP)*

(Values are means with their standard errors for five cats for HP diet and six cats for MP diet)

Diet…	MP		HP		Statistical significance of difference: *P*†
	Mean	SE	Mean	SE	
Urea production	19·0	1·59	65·4	2·57	0·01
Urinary urea excretion	16·0	1·08	57·6	2·28	0·01
Urea to gut	3·0	0·82	7·8	0·57	0·01
Urea recycled to ornithine cycle	1·6	0·41	4·6	0·51	0·01
Urea excreted in faeces	0·1	0·03	0·1	0·03	0·86
N retained for anabolism	1·3	0·44	3·1	0·50	0·03

* For details of diets and procedures, see Table 1 and p. 598.
† By Kruskal–Wallis.

the requirement of the dog, human subject and rat, which have been reported to require 4 % dietary energy from protein (Rogers & Morris, 1980). The high protein requirement of the cat has been attributed to the apparent inability of the hepatic enzymes of this species to adapt to dietary protein intake (Rogers *et al.* 1977; Rogers & Morris, 1980). In their classic study, Rogers *et al.*(1977) examined several feline hepatic enzymes involved in ureagenesis. Six adult cats were fed for 5 weeks on each of two diets in a crossover design; a 70 % (w/w) protein diet (700 g soyabean protein/kg diet) and a 17·5 % (w/w) protein diet (175 g soyabean protein/kg diet). Liver biopsies taken at the end of each phase were assessed for the activity of six major enzymes involved in urea metabolism. Few differences were found in the activities of these enzymes from cats that had previously been fed high-protein or low-protein diets. The authors concluded that the hepatic enzymes of the cat appeared to be permanently set to a very high rate and failed to adapt to low dietary protein as seen in the rat (Schmike, 1962). However, it should be noted that the enzyme assays employed in this study were those optimised for the rat, without validation for the cat, and this factor could possibly have affected the absolute enzyme activities.

Subsequent studies have raised questions about these findings. Thus, Silva & Mercer (1985) used parenchymal hepatocytes isolated from cats fed the same high (70 %, w/w)- or low (17·5 %, w/w)-protein diets for 6 weeks. The cats were fasted for 16 h before removal of hepatocytes, then *in vitro* ureagenesis was assessed following incubation with lactate and pyruvate. The rate of urea production was significantly higher in the cells of high-protein-fed cats, in contrast to the results of Rogers *et al.* (1977). The present study supports the findings of Silva & Mercer (1985), with a significant increase in urea production in HP cats compared with MP cats, and no increase in urea recycling to the ornithine cycle in MP cats. Increased urea production in response to increased dietary protein intake might be expected since the urea cycle is very sensitive to substrate supply, and supports findings in the healthy adult human subject (Langran *et al.* 1992; Forslund *et al.* 1998), although the results of the latter did not reach significance. Findings by other research groups (for example, see Jackson *et al.* 1990) of no such change in urea production rate with protein intake are surprising, and may be a result of the use of diets which did not differ sufficiently in protein level (8·8 *v.* 10·8 % protein energy). In any case, recent measurements in infants do show marked changes in urea production when dietary protein energy intakes range from 2·5 to 11·9 % during both catch-up and normal growth (Badaloo *et al.*1999).

The rate of ureagenesis is a function of the activity of urea cycle enzymes (controlled by alterations in mRNA expression), and also substrate availability (the concentration of urea cycle intermediates and of NH_3). If feline hepatic enzymes do not genetically adapt to dietary protein level (as suggested by Rogers *et al.*1977), and are set to an intermediate or high level, the concentration of urea cycle intermediates may be more important in the control of ureagenesis (Morris & Rogers, 1986). In reviewing the

Table 5. Percentage of urea produced or urea entering the gut that was recycled or retained by cats fed a moderate-protein diet (20 % protein energy; MP) or a high-protein diet (70 % protein energy; HP)*

(Values are means with their standard errors for five cats for HP diet and six cats for MP diet)

Diet…	MP		HP		Statistical significance of difference: *P*†
	Mean	SE	Mean	SE	
Urea to gut:urea produced	15·0	3·23	11·9	0·73	0·47
Urinary urea excretion:urea produced	85·1	3·23	88·1	0·73	0·47
Urea recycled to ornithine cycle:urea produced	8·1	1·52	7·0	0·58	0·58
Urea recycled to ornithine cycle:urea to gut	56·6	5·30	59·3	5·47	0·58
Available for anabolism:urea to gut	40·0	5·70	39·6	5·40	0·86
Available for anabolism:urea produced	6·4	1·96	4·8	0·77	0·71

* For details of diets and procedures, see Table 1 and p. 598.
† By Kruskal–Wallis.

mysteries of N balance regulation Waterlow (1999) discusses the dual regulation of the urea cycle by substrate supply and by adaptive changes in the urea cycle enzymes. He argues that given the instantaneous and automatic regulation of carbamoylphosphate synthase 1, it should be able to handle many times the normal input to the cycle, so that it is not clear why adaptive changes in the urea cycle enzymes should be necessary. If this regulation process is viewed in terms of reactive regulation (substrate supply) and adaptive regulation (urea cycle enzyme adaptation), then it may be that the cat reacts rather than adapts to dietary protein level, but with the same net result. It is not clear then exactly why the cat has a higher protein requirement than other species.

Salvage of urea-N is a mechanism essential to the N economy of ruminants, where the high rumen microbial urease activity promotes synthesis of large amounts of proteins. The proteins previously undigested are hydrolysed when they reach the microbes in the small intestine, and the amino acids are absorbed and utilised by the host. This process allows them to maintain N balance on very-low-protein diets (for example, see Sarraseca *et al.* 1998). Urea also enters the gut of omnivores such as rats (Torrallardona *et al.* 1996), pigs (Torrallardona *et al.* 1994) and human subjects, but to a lesser extent than in herbivores. Less urea-N is retained for anabolism (salvaged) in non-ruminants and, although it has been shown in human studies that some of the retained N includes essential amino acids (Millward *et al.* 2000), the physiological importance of such salvage is as yet unclear. Several studies of urea kinetics in human subjects have demonstrated that on adequate but low-protein diets urea entering the gut was often increased in both absolute terms and as a proportion of production; e.g. the percentage of urea produced that entered the gut was 60–70 % on low-protein diets and 30–40 % on high-protein diets (Picou & Phillips, 1972; Jackson *et al.* 1990; Langran *et al.* 1992). Such large differences between low- and high-protein diets are not always observed, however, as shown in recent studies in malnourished infants (Badaloo *et al.* 1999). Nevertheless, in all these studies subjects fed low-protein diets may retain up to 90 % of the N entering the gut that is then potentially available for anabolism (for example, see Jackson *et al.* 1990; Langran *et al.* 1992; Badaloo *et al.* 1999). Urea kinetics have not been studied in a carnivorous species, and we were concerned with two issues, i.e. the extent and nutritional sensitivity of urea hydrolysis in the gut, and whether urea entering the cat gut is retained for anabolism, thus forming a possible mechanism of adaptation to low dietary protein.

Our results show that urea hydrolysis as a percentage of production is low, with no significant difference between cats fed the MP diet (15 %) or HP diet (12 %). This finding supports some previous findings that there is no significant difference in the proportion of urea entering the gut on low- and high-protein diets (Langran *et al.* 1992; Forslund *et al.* 1998), but is in contrast to other previous studies (for example, see Jackson *et al.* 1990). The MP value for hydrolysis in percentage or absolute terms reported here can be compared with the 46 % or 6–8·1 mmol N/kg BW per d reported for healthy adult human subjects given an equivalent diet relative to requirement (Langran *et al.* 1992; Forslund *et al.* 1998). It is also very low when compared with the child recovering from malnutrition who retained even more, at 8·9–17·5 mmol N/kg BW per d (Jackson *et al.* 1990). These results confirm that the cat has a low gut entry rate of urea compared with the human.

The factors that determine the entry rate of urea into the gut are currently unknown, but might include diffusion and transport mechanisms (given the recent discovery of the urea transporters; Hediger *et al.* 1996), and the effectiveness of such mechanisms would be related to gut size. If a gut urea transporter is responsible for the control of gut entry, the lower gut entry in the MP-fed cat may indicate fewer gut urea transporters in this species. Alternatively, if gut entry rate were determined by removal of urea by microbial hydrolysis (i.e. maintenance of a diffusion gradient), a low gut entry rate might be expected in a species with a relatively small microbial mass. The significantly increased ($P = 0{\cdot}01$) absolute gut entry in HP cats compared with MP cats is in contrast to findings in healthy adult human subjects (Langran *et al.* 1992; Forslund *et al.*1998), and may be a reflection of diet design, since the HP diet was higher in protein energy than those used in human studies. It is likely that the increased urea production of HP cats increased urea diffusion into all compartments of the body, including the gut.

The lower amount and proportion of urea entering the gut of the cat compared with the human subject may be a species-specific difference, although it could also be a reflection of diet design. It is possible that substantial urea hydrolysis is only measurable at protein intakes below that required for maintenance. For the cat the maintenance requirement for protein is 10 % energy (Burger *et al.* 1984), and at 20 % energy, the MP diet used in the current study was twice that level. However, due to the dynamic nature of the system it is probable that some difference between the two protein levels (MP and HP) would be expected. Such differentiation was observed in human studies utilising diets with protein levels above the maintenance requirement (Bundy *et al.* 1993; Forslund *et al.* 1998).

As far as the extent to which urea entering the cat gut is retained for anabolism, our results show that the absolute amounts were very small (probably negligible) in comparison with those of healthy adult human subjects (e.g. 5·9–6·8 mmol N/kg BW per d; Langran *et al.* 1992), and malnourished children, who retained even more (8·9–17·5 mmol N/kg BW per d; Jackson *et al.* 1990). We found a lower percentage (only 40 %) of N entering the gut is retained in the cat compared with the 76–89 % retained in human studies (Jackson *et al.* 1990; Langran *et al.* 1992). Also we found no dietary sensitivity of the percentage of urea production available for anabolism, in contrast to the human infants (up to 50 % on a low-protein diet compared with 30 % on a high-protein diet; Jackson *et al.* 1990). Although care should be taken in the interpretation of results obtained by difference, when errors due to faecal losses can have a large effect on the outcome, the human findings are interpreted as an adaptation to low dietary protein to increase the functionally significant overall N supply (Jackson, 1983). It is clear that the cat retains very little urea-N for anabolic purposes.

In conclusion, therefore, the current study has shown that whilst ureagenesis in a carnivore is sensitive to dietary protein intake, there is a low level and lack of nutritional sensitivity of urea entry and hydrolysis into the gut, and subsequent retention of urea-N for anabolism. This very low rate of gut entry and salvage of urea-N for anabolism in the cat may be due to a very low microbial urease activity in a species with a very short gastrointestinal tract and habitual diet low in fermentable substances. Alternatively, it could be due to a lack of evolutionary pressure to conserve N in a species that consumes a diet habitually high in protein.

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