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ORIGINAL PAPER

Measurement of the rate of protein turnover and synthesis in the marsupial Honey possum (*Tarsipes rostratus*)

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Abstract Rates of protein turnover and synthesis were measured in wild-caught Honey possums (*Tarsipes rostratus*) in the southwest of Western Australia and compared between males and females with and without pouch young. Possums were injected with 50 μg of ^{15}N-glycine and ammonia collected within 24 h was used as the nitrogen end-product in a single-injection protocol. The overall mean rate of protein synthesis measured was 7.7 ± 0.5 g kg$^{-0.75}$ day^{-1}, which falls within the range of values reported for other marsupial species. Whole body rates of nitrogen flux and protein synthesis did not vary significantly between males and females with and without young, but females with pouch young showed significantly lower rates of protein synthesis when expressed in relation to metabolic body size. This difference was no longer apparent, however, if the mass of the females was corrected for the estimated mass of the young in the pouch averaging 9.3 ± 1.6 g kg$^{-0.75}$ day^{-1} and suggesting that the young should not be considered as part of the metabolic body pool. Whole body rates of protein degradation were significantly reduced in females carrying pouch young, suggesting that protein may be being diverted from the pool to milk production. Calculations indicate that the daily fraction of the female's nitrogen synthesis rate that needs to be diverted to pouch young to sustain their growth is less than 5%, and may not be detectable with the current methodology.

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Introduction

The tiny marsupial Honey possum, *Tarsipes rostratus,* is unique in being the only terrestrial vertebrate to feed exclusively on pollen and nectar from flowers (Vose 1973; Wooller et al. 1984). The extreme morphological specialisation of its skull and feeding apparatus (Gervais and Verreaux 1842; Richardson et al. 1986) attests to a long co-evolutionary relationship with specific Gondwanan plant families in the species-rich kwongan heathlands of south-west Australia, particularly Proteaceae, Myrtaceae and Epacridaceae (Hopper and Gioia 2004; Wooller et al. 1984). In an early study of the allometric relations of marsupials it was found that Honey possums have a particularly low offspring production rate when compared with other possums, and that this trend is shared with other exudate feeders, in contrast to folivorous species (Smith and Lee 1984). These authors speculated that the unusual high-carbohydrate diet of the Honey possum may be deficient in protein and essential amino acids, and that this may be a factor limiting their reproductive potential. A similar hypothesis has been advanced linking dietary quality with reproductive activity by Fisher et al. (2001) in a recent study of the causes of life-history variation in marsupials.

In order to test this hypothesis it is essential to have quantitative information on the feeding rates of Honey possums in their natural habitat and their daily rates of intake of both pollen and nectar (Van Tets 1998). The field metabolic rate (FMR) of Honey possums was first measured using doubly-labelled water by Nagy et al. (1995)

and averaged 34 kJ day^{-1} which is over 2.5 times the basal metabolic rate for this species measured under laboratory conditions (Withers et al. 1990). Although lower than similar-sized carnivorous marsupials (Nagy et al. 1978, 1988), the FMR of the Honey possum is still some 75% greater than that of an eutherian mammal of similar size (Nagy 2005; Nagy et al. 1999). A novel method, using a combination of both stable and radioactive isotopes, was developed by Bradshaw and Bradshaw (1999) to measure rates of intake of both nectar and pollen in free-ranging Honey possums. These were found to average 7 ml and 1 g per day respectively for a 9 g individual maintaining body mass. Given that mixed pollen has an average nitrogen content of 4% (Turner 1984), the consumption of 1 g of pollen per day equates to a nitrogen intake of 31 mg per day, after correcting for digestibility (Bradshaw and Bradshaw 2001). Without knowledge of the animal's minimum daily requirement for nitrogen balance, however, one has no way of knowing whether this level of nitrogen intake would constitute a limiting diet that might constrain reproduction.

The minimum nitrogen requirement for balance (MNR) for *T. rostratus* has been variously estimated at 147 mg N kg$^{-0.75}$ day^{-1} (Turner 1984) and 230 mg N kg$^{-0.75}$ day^{-1} (Wooller et al. 1999) but a detailed laboratory study by Bradshaw and Bradshaw (2001) measured an exceptionally low MNR of 89 $\pm$ 21 mg N kg$^{-0.75}$ day^{-1} for the Honey possum, almost identical to the figure of 87 mg N kg$^{-0.75}$ day^{-1} reported for the sugar glider, *Petaurus breviceps* by Smith and Green (1987) and similar to the figure of 50 mg N kg$^{-0.75}$ day^{-1} for the eastern pygmy possum, *Cercartetus nanus*, when maintained on a pollen diet (van Tets and Hulbert 1999). This figure for the Honey possum equates to a daily requirement of 2.6 mg N per day for a 9 g individual, which is less than 10% of their actual intake measured in the field. Bradshaw and Bradshaw (2001) concluded that their field diet was not deficient in nitrogen, especially as Bradshaw et al. (2000) were able to rear Honey possums successfully in a laboratory colony with a daily nitrogen intake of 30 mg, similar to that of field animals.

The relationship between endogenous urinary nitrogen excretion (EUN) and the BMR has also been found to be substantially lower in marsupials than eutherians and the relationship described by Smuts (1935) whereby approximately 2 mg of nitrogen is excreted for each kilocalorie (=4.184 kJ) of heat does not hold for marsupials. Bradshaw and Bradshaw (2001) found that the relationship between EUN and BMR for eight species of marsupials was described by the equation EUN (mg N kg^{-1} day^{-1}) = 0.08 BMR (kJ kg^{-1} day^{-1}) + 12.92 ($r^2 = 0.96$) with the production of 4.184 kJ of heat being associated with the excretion of only 0.34 mg N, rather than 2 mg. White et al. (1988) suggested that the reduced EUN of marsupials could mean that they also have lower rates of protein turnover. This factor could impact on an animal's reproductive potential when the increased burden of producing milk for the young is added to what may be an already low rate of protein synthesis in the mother. To date, rates of nitrogen turnover and protein synthesis have only been measured in man and other large mammals in laboratory situations and an attempt to estimate these in field animals poses a number of challenges, not the least when dealing with an animal as small as the Honey possum. The aim of the present study was to compare rates of protein turnover and synthesis in males and females with and without pouch young in Scott National Park in the extreme southwest of Western Australia.

Materials and methods

Study area, capture and treatment of animals

The study was carried out in Scott National Park (34°17′S, 115°13′E) in the extreme southwest of Western Australia. The vegetation is predominantly low swamp/sedge/heath, punctuated by deep sandy ridges supporting sparse woodlands dominated by *Banksia ilicifolia* trees growing to 5–6 m, which are the primary food source of Honey possums (Bradshaw et al. 2007). Trapping extended over a period of 6 years from 2001 to 2007 with field trips in summer (1), winter (1) and spring (3). Honey possums may have up to three reproductive periods during 1 year and trapping for this study was focused on spring when previous work has shown that the Scott National Park females are most likely to have medium ($\sim$1 g) to large sized ($\sim$2 g) young in the pouch. Animals were captured overnight in pitfall traps constructed from 50 cm lengths of 15 cm diameter PVC piping set into the ground with the rim flush with ground level. Traps were set 5–8 m apart in 50–200 m grid lines and closed with metal lids when not in use and full details of the study site and its vegetation are given in Bradshaw et al. (2007). Traps were cleared at first light and animals were transported in calico bags to a nearby mobile field laboratory where they were individually marked, weighed ($\pm$0.1 g) and brushed for identification of pollen. They were then injected with ^{15}N-glycine and placed in small purpose-built metabolism cages for the collection overnight of urine and faeces (see Fig. 1). Urine collectors contained 0.5 ml of Ondina® oil (Shell) to prevent evaporation plus 0.4 ml of 1 N H_2SO_4 to prevent the loss of nitrogen in the form of ammonia. The metabolism cage was fitted with a 10 ml plastic syringe containing an artificial diet composed of sand-plain pollen, Banksia honey, water and supplements as detailed in Bradshaw et al.

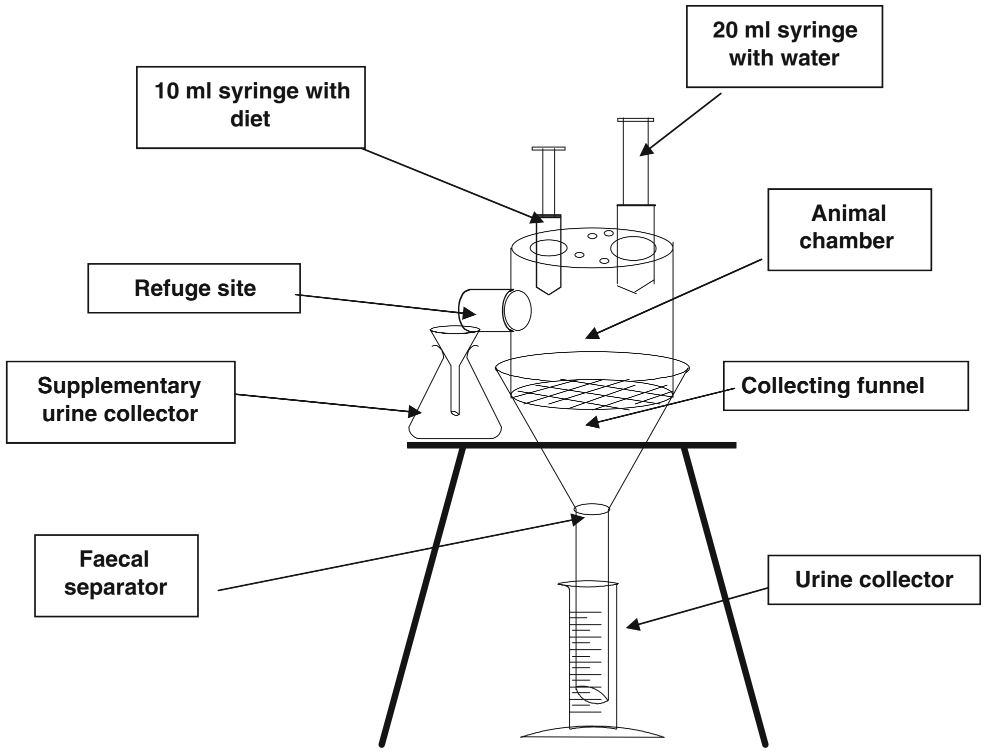

Fig. 1 Diagram of a purpose-built metabolism cage used to collect urine and faeces from the 10 g marsupial Honey possum, *Tarsipes rostratus*

(2000). The energy and nitrogen content of this diet closely approximated measured intakes of Honey possums in the field as determined by Bradshaw and Bradshaw (1999) with an intake of 10 ml of the diet providing the possum with 38 mg of nitrogen. The metabolism cages were calibrated for loss of urine on the walls of the chamber after spraying with silicone and urine volumes were corrected using the following equation: $y = 0.9703x - 0.0959$ ($r^2 = 0.999$) where $y =$ volume recovered and $x =$ volume added in millilitres. The possums were held in the cage for a maximum of 24 h and the urine produced overnight was collected and measured and the quantity of diet consumed noted. The possums were re-weighed and then allowed to feed on a 20% honey solution before being released in the field at their site of capture.

Processing of urine

Evolved ammonia was collected in 2% boric acid, following the addition of 0.25–0.5 ml of 8 N KOH to measured aliquots of urine (1–2 ml) in Conway micro-diffusion plates. The borate was dried at 60°C and the powder collected and stored in tared Eppendorf vials for subsequent analysis of ^{15}N enrichment levels.

Measurement of ^{15}N enrichment in ammonia samples

Samples were loaded into high purity tin cups and measured for $\delta^{15}N$ and N%. The samples were measured, along with a range of suitable enriched $\delta^{15}N$ normalisation standards, using a Eurovector model EA3000 elemental analyser and an Isoprime mass spectrometer. Additionally, check standards were also included in each run to allow confirmation of the normalisation and linearisation methodology and the R_R value used for Air was 0.0036765. Typical precision for $\delta^{15}N$ using this arrangement was <1 per mil.

Measurement of rate of protein synthesis

Rates of protein turnover and synthesis are normally measured using fed or injected amino acids labelled with ^{15}N and methods have been developed that have been applied routinely to man and other domestic and farm animals (Jeevanandam et al. 1985; Waterlow 1984; Waterlow et al. 1978a, b). The small size of the Honey possum and their sensitivity to handling and disturbance required investigation into the various possible methodological approaches.

Pilot studies

1. The first pilot study attempted to achieve a desired equilibrium in the body by feeding to three male Honey possums artificial diet to which had been added ^{14}C-labelled glycine (Amersham). The possums were maintained in metabolism cages over a 3-day period and samples of blood, urine and ear tissue were

collected daily. Whole blood samples (20 μl) and urine samples (100 μl) were counted directly in Picofluor scintillant (Packard) after bleaching with 30% H_2O_2. Ear tissue samples (0.5–1.0 mg) were dissolved in Soluene (Packard) prior to counting in a Packard Liquid Scintillation Spectrometer Model 400CD.

2. In a second pilot study three Honey possums (two females without pouch young and one male) were injected with tritiated glycine, and urine was collected in metabolism cages over a 3-day period to compare the excretory profiles of both ammonia and urea as potential end-products. The dosage of radioactivity injected intraperitoneally was 1.85 MBq (50 μCi) of ^{3}H-glycine (Amersham) in a total volume of 50 μl. The possums were provided with access to 10 ml of artificial diet per day and 0.4 ml of 1 N H_2SO_4 was added to Ondina oil in the urine collecting vials to prevent breakdown of the nitrogenous end-products. Urine aliquots were first counted for total radioactivity and then analysed for both dissolved ammonia and urea content, counted for tritium and expressed as percentages.
3. A third pilot study compared the use of both ammonia and urea as the nitrogenous end-product for the estimation of rates of protein synthesis. Seven adult Honey possums of both sexes (males and females without pouch young, mean body mass = 10.2 g) were injected intraperitoneally with 50 μg of ^{15}N-glycine and voided urine collected over a 3-day period. Urine was collected and analysed as described above with urea levels measured after hydrolysis to ammonia with urease (Sigma) (Fawcett and Scott 1960).

Protein turnover protocol

Previous studies with the Honey possum (Bradshaw and Bradshaw 2001) have shown that it is not possible to maintain females with pouch young in small metabolism cages for more than 24 h if the females are to retain their young. The single-injection approach, with NH_3 as the nitrogen end-product, was thus the appropriate method of choice for measuring the rate of protein synthesis, rather than the constant infusion method. (Waterlow 1981; Waterlow et al. 1978a, b; Young et al. 1991). Ammonia is almost completely eliminated within 24 h after injection of the ^{15} N-glyciine, compared with 72 h for urea elimination. Possums were injected intraperitoneally with 0.125 ml of a sterile saline solution containing 50 μg of ^{15}N-glycine (>97% enrichment, ANSTO) and placed in metabolism cages for the collection overnight of urine and faeces. A total of 38 adult possums with a mean body mass of 12.02 ± 0.63 g was used in the study. The mean mass of males was 8.48 ± 0.41 g ($n = 10$), females without pouch young 9.44 ± 0.98 g ($n = 7$), and the total mass of females carrying pouch young 14.4 ± 0.81 g ($n = 21$).

In calculating rates of nitrogen flux and protein metabolism we have followed the protocol of White et al. (1988) based on a model of two pools for amino acids and proteins in the body (Picou and Taylor-Roberts 1969). The assumptions of this model have been discussed extensively (Golden and Jackson 1981; Stein 1981; Stein et al. 1986) and (Waterlow 1984) and the irreversible loss rate of amino nitrogen from the pool (IL) is calculated from:

$$\mathrm{IL} = \text{urinary nitrogen excretion rate } (\mathrm{g\ N\ day^{-1}}) / \text{fraction of } {}^{15}\mathrm{N} \text{ dose recovered in urinary end product}$$

If there is no recycling of the isotope, then $\mathrm{IL} = Q$ where Q is the total flux or turnover of nitrogen through the metabolic pool and:

$$Q = S + E = D + 1$$

where S rate of protein synthesis, E ammonia nitrogen excretion, I dietary nitrogen intake and D rate of protein degradation.

Thus: $D = Q - I$ (rate of protein degradation) and $S = Q - E$ (rate of protein synthesis). Rates of protein metabolism were expressed on the basis of crude protein ($\mathrm{CP} = 6.25 \times N$).

Statistics

The distribution of all data was assessed for normality by constructing probability plots (Gnanadesikan 1977) and, where appropriate, variables were logarithmically transformed prior to statistical analysis. Patterns of variation in the data were explored initially through analysis of variance (ANOVA), using the SYSTAT statistical package, coupled with either a Student–Neuman Keul's test (SNK), a Bonferroni Test or Tukey HSD post hoc multiple test comparisons. The significance of differences between selected group means was also assessed, where appropriate, by paired and unpaired Student's t test. A probability of less than 0.05 indicated significance.

Results

A total of 38 animals were processed in this study but data from only 18 have been retained. The selection was based on those animals that consumed more than 5 ml of diet and produced more than 2 ml of urine in the 24 h period—a combination that was established in previous experiments with Honey possums held in metabolism cages for longer periods (Bradshaw and Bradshaw 2001). Four of these

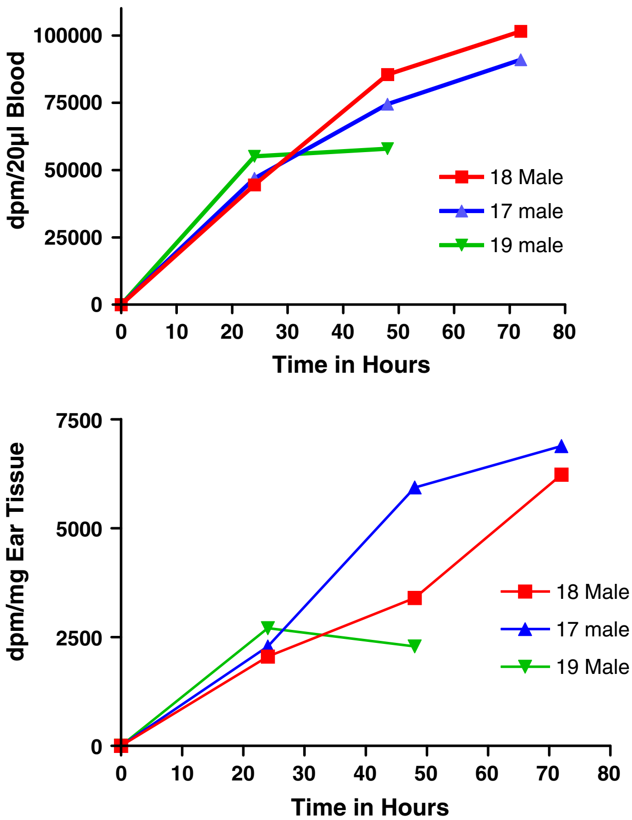

Fig. 2 Time course over a 3-day period of accumulation of orally introduced ^{14}C-glycine in the blood and ear tissue of three male Honey possums (*Tarsipes rostratus*)

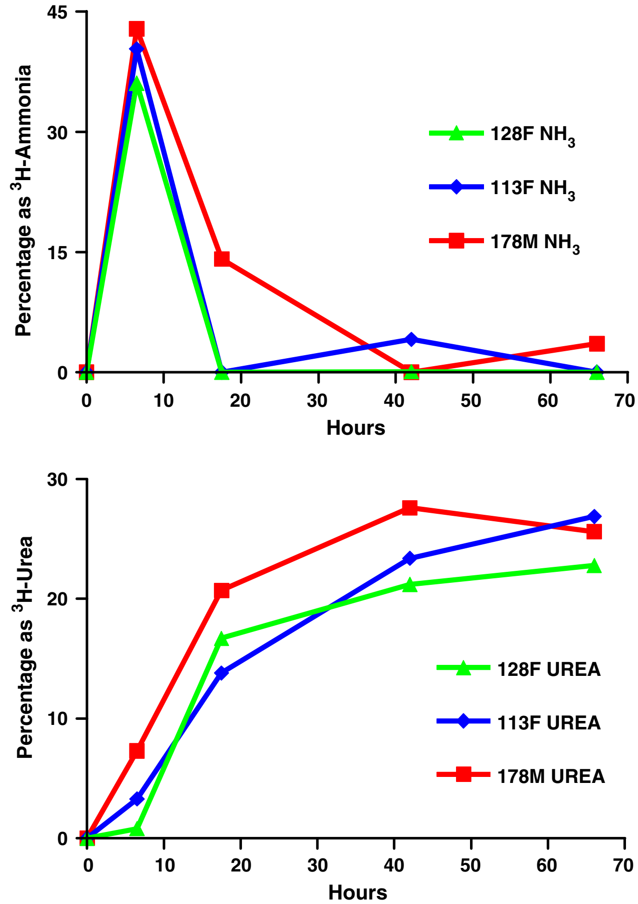

Fig. 3 Time course showing the excretion of intraperitoneally-injected ^{3}H-glycine in the form of labelled ammonia ($^{3}HNH_3$) or urea over a 3-day period in two females and one male Honey possum (*Tarsipes rostratus*)

were males and five were females not carrying pouch young. Of the remaining nine females with pouch young, five had medium-sized young (∼1 g) and four had large-sized young (∼2 g).

Results of the first pilot study (1) are shown in Fig. 2 and it is evident that levels of ^{14}C-glycine had not reached a plateau in either blood (dpm 20 μl^{-1}) or ear tissue (dpm mg^{-1}) after 72 h on the labelled diet and a longer period of perhaps 5 days would be needed to reach equilibrium. The certain loss of pouch young from the females held for this length of time precluded this approach.

Details of the second pilot study (2) are shown in Fig. 3 where excretory profiles for injected glycine, labelled with tritium, are shown. Sampling times were at 6.5, 17.5, 42 and 66 h following injection, and 39.7% of the tritium was excreted as NH_3 by 6.5 h with a large decrease to 4.7% by 17.5 h. Urea excretion on the other hand showed a delayed pattern with tritium excretion peaking at 42 h and remaining constant until 66 h. Cumulative excretion of ^{3}H-NH_3 is shown in Fig. 4 and it is evident that collection of urine produced up to 24 h will capture virtually all the ammonia produced from the injected glycine, and justifying the use of this time frame.

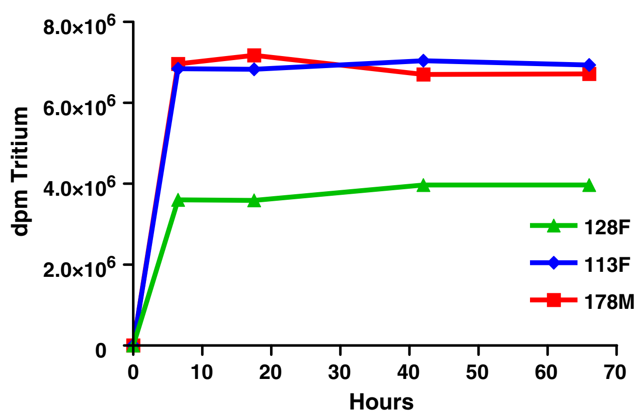

Fig. 4 Time course showing the cumulative excretion of injected ^{3}H-glycine in the form of ^{3}H-ammonia over a 3-day period in two females and one male Honey possum (*Tarsipes rostratus*)

In the third pilot study (3), no significant difference was found between the rate of protein turnover and synthesis estimated with either urea or ammonia as the nitrogen end-product (8.31 ± 0.56 vs. 8.24 ± 0.81 g $kg^{-0.75}$ day^{-1} respectively, paired $t_6 = 0.617$, $P = 0.56$). Ammonia was the end-product of choice, however, because of its total excretion within the first 24 h.

The mean rate of protein synthesis from the 18 possums was 271 ± 17 mg day^{-1} or 7.7 ± 0.5 g $kg^{-0.75}$ day^{-1} ($n = 18$) when expressed as a function of metabolic body size (see Table 1). The rate of protein degradation was lower, averaging 1.8 ± 0.5 g $kg^{-0.75}$ day^{-1}, and the net change in the protein pool was thus positive and averaged 5.8 ± 0.4 g $kg^{-0.75}$ day^{-1}. The rate of protein synthesis was also found to be independent of dietary nitrogen intake, as seen in Fig. 5 with $r^2 = 0.003$. Females carrying pouch young were heavier than both females without pouch young, and males ($F_{2,15} = 10.708$, $P = 0.0011$). Dietary intake of nitrogen varied significantly, being higher in females carrying pouch young than in males ($F_{2,15} = 3.851$, $P = 0.0431$) and NH_3 excretion rates were also significantly higher in females with pouch young than in males ($F_{2,15} = 5.006$, $P = 0.0205$). There was no significant difference, however, between measured nitrogen turnover rates (Q), the rate of nitrogen synthesis (S), or the whole body rate of crude protein synthesis in mg day^{-1} in the three groups (see Table 1). Mean fractional recoveries of the injected label were: males = 0.00825 ± 0.001403; females without young = 0.01878 ± 0.003428; females with pouch young = 0.02583 ± 0.004418 ($P = 0.027$ Kruskal–Wallis test).

When the rate of protein synthesis is expressed as a function of metabolic body size (i.e. to the power 0.75), however, a significant difference emerges between the three groups of possums, with the rate being lower in females carrying pouch young than in males (6.4 ± 0.7 vs. 9.7 ± 0.8 g $kg^{-0.75}$ day^{-1} $F_{2,15} = 4.357$ $P = 0.031$). The rate of protein degradation is also lower in females with pouch young than in males (0.7 ± 0.5 vs. 3.9 ± 0.6 g $kg^{-0.75}$ day^{-1} $F_{2,15} = 5.41$, $P = 0.017$) when expressed as a function of metabolic body size. There was no significant difference, however, between the rate of protein synthesis in females with and without pouch young. If the body mass of females carrying pouch young is corrected for the estimated mass of the young (medium = 1 g, large = 2 g) then the difference between males and females with pouch young, expressed as a function of metabolic body size, is no longer significant, averaging 9.3 ± 1.6 g $kg^{-0.75}$ day^{-1} in females with pouch young versus 9.6 ± 0.6 g $kg^{-0.75}$ day^{-1} in males, with $P = 0.6391$.

Although the whole-body rate of crude protein synthesis (S) in mg day^{-1} does not differ significantly between the groups, the rate of degradation (D) does, with the rate in females carrying young being significantly lower than that of both the females without young and the males

Table 1 Comparative data on rates of nitrogen turnover and protein metabolism in Honey possums (*Tarsipes rostratus*) where M = males, F-PY = females without pouch young and F + PY = females with pouch young (n)

Group	Body mass (g)	NH_3 excretion rate mg N day^{-1} (E)	Nitrogen turnover mg N day^{-1} (Q)	Rate of nitrogen synthesis mg N day^{-1} ($S = Q - E$)	Rate of protein synthesis mg day^{-1}	Rate of protein synthesis g $kg^{-0.75}$ day^{-1}	Dietary intake mg N day^{-1} (I)	Rate of protein degradation g $kg^{-0.75}$ day^{-1} ($D = Q - I$)	Net change to protein pool g $kg^{-0.75}$ day^{-1}
M (4)	8.4 ± 0.4^a	0.36 ± 0.08^c	43.4 ± 4.7	43.1 ± 4.7	269.2 ± 29.5	9.7 ± 0.8^d	26.0 ± 2.1^e	3.9 ± 0.6^f	5.8 ± 0.3
F − PY (5)	10.8 ± 1.1^b	0.84 ± 0.12	47.3 ± 6.1	46.4 ± 6.1	290.4 ± 38.6	8.7 ± 0.8	35.1 ± 2.0	2.1 ± 0.9	6.5 ± 0.3
F + PY (9)	14.6 ± 0.8^{ab}	1.0 ± 0.12^c	42.9 ± 4.1	41.9 ± 4.1	262.3 ± 26.2	6.4 ± 0.7^d	38.4 ± 2.9^e	0.7 ± 0.5^f	5.7 ± 0.5
Stat. sig.	$P = 0.0011$	$P = 0.02$	NS	NS	NS	$P = 0.03$	$P = 0.04$	$P = 0.02$	NS

Data expressed as Mean ± SE. Statistically significant differences between means within columns are indicated by similar superscripts i.e. aa, bb, cc, etc. For definitions of S, Q, E, D and I, see text

(13.4 ± 15.6 mg N day^{-1} in females with pouch young vs. 76.1 ± 36.7 mg N day^{-1} in females without pouch young and 108.8 ± 20.8 mg day^{-1} in males; $F_{2,15} = 4.55$, $P = 0.028$). This difference from the males is also apparent when the rate of protein degradation is expressed as a function of metabolic body size in females carrying pouch young when their body mass is corrected for the estimated mass of pouch young (0.4 ± 0.4 vs. 3.9 ± 0.6 g kg$^{-0.75}$ day^{-1} $F_{2,15} = 5.76$, $P = 0.014$).

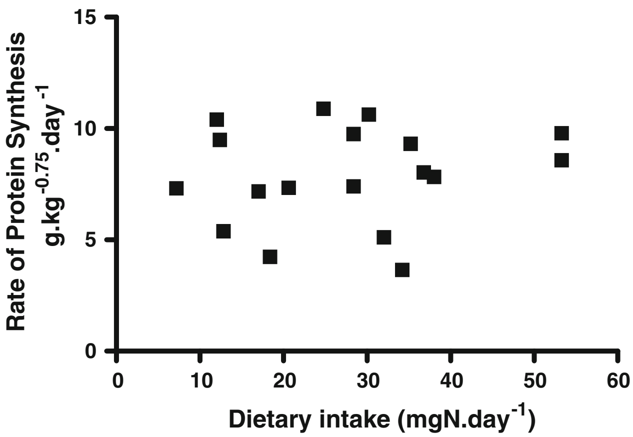

Fig. 5 Regression of the rate of protein synthesis in g kg$^{-0.75}$ day^{-1} on dietary intake in milligram of nitrogen per day of Honey possums (*Tarsipes rostratus*). The equation of the regression is $y = 0.0226x + 7.289$, $r^2 = 0.0188$, $P = 0.587$ where y = rate of protein synthesis and x = dietary intake

The net positive change in the protein pool ($S - D$ = synthesis − degradation) also differs between the groups when calculated in milligram of crude protein per day with that of females carrying pouch young, being significantly greater than males (244 ± 16.6 mg day^{-1} in females carrying pouch young versus 214 ± 12.0 mg day^{-1} in females without young and 160.2 ± 13.3 mg day^{-1} in males; $F_{2,15} = 5.942$, $P = 0.0126$).

Figure 6 gives details of an attempt to construct a model for nitrogen metabolism in the Honey possum, calculated on a whole body basis in mg N per day. The model was calculated from the measured rates of intake of nectar and pollen for mass balance of a 9 g adult of 7 ml and 1 g, respectively, reported by Bradshaw and Bradshaw (1999) for free-ranging Honey possums in Scott National Park. One gram of sand-plain pollen with an average of 4% nitrogen content provides a daily intake (I) of 40 mg N (Bradshaw and Bradshaw 2001) that is taken as 100% in the model. The mean rate of nitrogen synthesis to the body

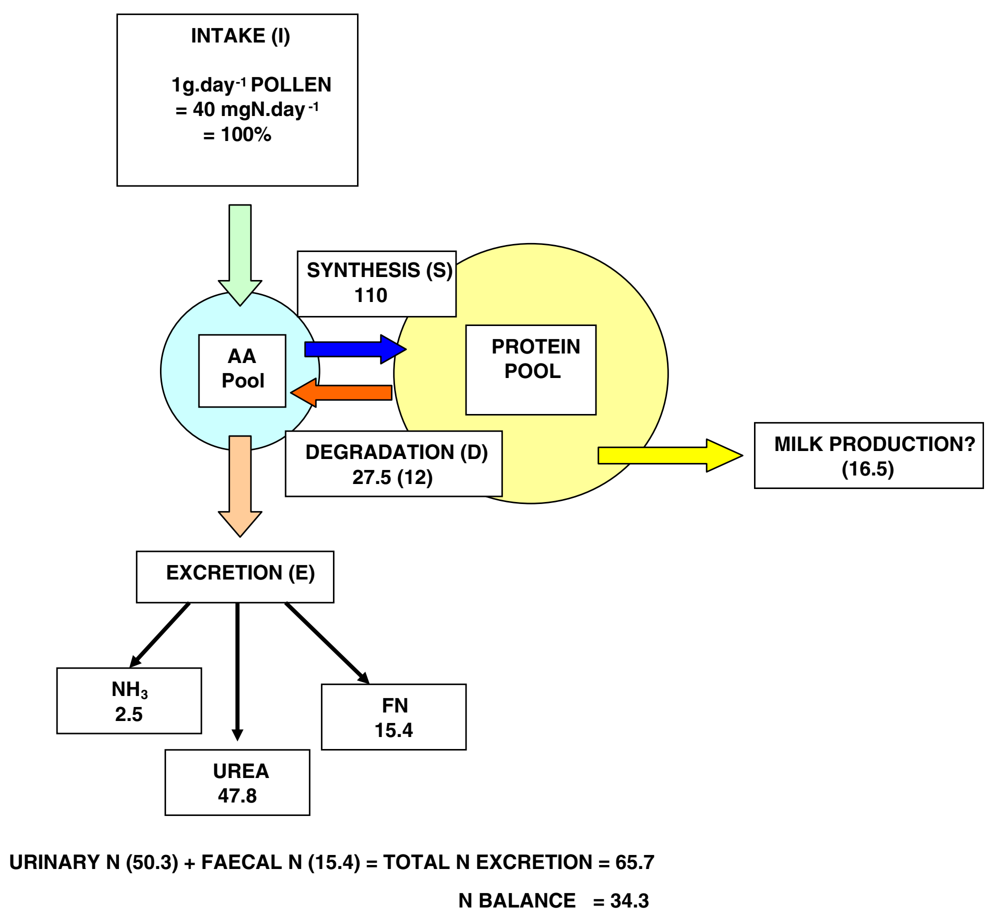

Fig. 6 A model of nitrogen flow in relation to dietary intake of 1 g of pollen of 4% nitrogen content for the Honey possum *Tarsipes rostratus*. Dietary intake is set at 40 mg per day and taken as 100% with all other parameters shown as percentage values of this. *I* nitrogen intake, *E* nitrogen excretion, *S* rate of nitrogen synthesis to the protein pool and *D* rate of nitrogen degradation form the protein pool. *FN* faecal nitrogen excretion, *urea* urinary urea excretion and *NH*$_3$ urinary ammonia excretion. The value for *D* in parentheses (12) represents the lower whole body degradation rate measured in females carrying pouch young

protein pool (*S*) was estimated at 44 mg N day^{-1} (110%) and the rate of degradation (*D*) at 11 mg N day^{-1} (27.5 %). Faecal nitrogen excretion (FN) and total urinary nitrogen excretion (UN) figures for the model were calculated from the study of (Bradshaw and Bradshaw 2001) by regressing both parameters against daily nitrogen intake and extrapolating the curve to an intake of 40 mg N day^{-1}. Excretion of ammonia in the urine was measured at 1 mg N day^{-1} (2.5%) in this study and urea excretion was taken as the difference between total urinary nitrogen excretion from Bradshaw and Bradshaw (2001) and ammonia excretion (47.8%). Overall nitrogen excretion (*E*) thus equalled 65.7% of intake in the model and the nitrogen balance is 34.3. The significantly lower rate of nitrogen degradation measured in females carrying pouch young (12%) is shown in parentheses.

Discussion

Rates of protein turnover and synthesis have only been measured in a small number of marsupial species to date and, as expected from their lower basal metabolic rate (BMR) (Welle and Nair 1990), they are generally some 30–40% lower than those of similar-sized eutherian mammals (Hume 1982, 1999). There can be substantial differences, however, even between subspecies as reported by Freudenberger and Nolan (1993). In their study comparing two subspecies of kangaroo (the eastern wallaroo—*Macropus robustus robustus*—and the western euro—*Macropus robustus erubescens*) with feral goats (*Capra hircus*), the crude protein turnover of the euro kangaroo at 14.8 g kg$^{-0.75}$ day^{-1} was similar to that of the goat at 13.8 g kg$^{-0.75}$ day^{-1} with only the wallaroo recording a lower rate at 10.4 g kg$^{-0.75}$ day^{-1}. The mean rate of whole body protein turnover reported in six species of eutherian mammals by Waterlow (1984), however was, 13.2 g kg$^{-0.75}$ day^{-1}, which is substantially higher than the range of 4.1–6.7 g kg$^{-0.75}$ day^{-1} reported by Barboza et al. (1993) for the large wombat, *Lasiorhinus latifrons*, which has one of the lowest BMRs recorded for any marsupial, and above the figure for Honey possum, measured here of 7.9 ± 0.6 g kg$^{-0.75}$ day^{-1}.

The mean rate of protein synthesis reported here for the Honey possum of 7.7 ± 0.5 g kg$^{-0.75}$ day^{-1} (males = 9.7 ± 0.8 and females without pouch young = 8.7 ± 0.8 g kg$^{-0.75}$ day^{-1}, respectively) is comparable with published rates for other marsupial species, which range from 5.1 ± 0.7 g kg$^{-0.75}$ day^{-1} in the tammar wallaby, *Macropus eugenii* (White et al. 1988) to 8.5 g kg$^{-0.75}$ day^{-1} in the Brushtail possum, *Trichosurus vulpecula* (Dellow and Harris 1984) and 10.8 ± 0.3 g kg$^{-0.75}$ day^{-1} in the euro kangaroo (Freudenberger and Nolan 1993). Protein metabolism is thought to account for approximately 20–30% of the BMR in mammals (Welle and Nair 1990) and, although unaffected by activity and thermoregulation, is assumed to increase during periods of growth and reproduction. Wallis and Hume (1992), for example, found that the nitrogen requirements of breeding female rufous rat-kangaroos (*Aepyprymnus rufescens*) at peak lactation were four times those of adult males. Munks and Green (1995) used the doubly-labelled water technique to measure the field metabolic rate (FMR) of ringtail possums, *Pseudocheirus peregrinus*, and found that energy expenditure in lactating females was increased by some 30% when compared with males and non-lactating females. The peak metabolisable energy allocated during lactation was estimated at 759 kg day^{-1} which was lower than recorded in other herbivores and Munks and Green (1995) concluded that the ringtail possum has a relatively low overall energy investment in reproduction. Data are not available on nitrogen investment for this species but one imagines that it would also be low.

Our expectation in this study, that the rate of protein synthesis in lactating females would be greater than that of males and non-lactating females, was not supported by the data. Only when the mass of the young was included with the weight of the mother was there a difference, and then negative rather than positive. Our conclusion is that the mass of the young, that are living outside the mother in the pouch and connected with her only via milk production, should not be included when calculating metabolic body size and, as a result, there is no difference between the groups in their rate of protein synthesis. The whole body rate of degradation of crude protein was significantly lower, however, in females carrying pouch young when compared with either males or females without young. This difference in protein dynamics suggests that the protein pool is increasing more in females carrying pouch young than in males and females without young. This may reflect protein being diverted to milk production and, if this were the case, we can estimate this at approximately 6.6 mg N day^{-1}, representing 16.5% of the daily nitrogen intake. There are no data on the composition of Honey possum milk but the milk of marsupials varies between approximately 3–9% protein concentrations throughout pouch life in a number of species (Hume 1999) with an estimated nitrogen concentration ranging from 5 to 14 mg N ml^{-1}.

A further calculation puts this result into some context. Honey possums at birth weigh only 4 mg (Russell and Renfree 1989) and grow to a final mass of 2.5 g at weaning (Bradshaw et al. 2000) after a period of 9–10 weeks being fed by the mother (Russell 1982). This equates to an average growth rate of 35.7 mg day^{-1} and, assuming that the joey is composed of 16% protein, of which 16% is nitrogen, this is equal to an investment of 0.91 mg of nitrogen per day per joey. The rate of nitrogen synthesis measured in this study, however, averages approximately 45 mg day^{-1} (see Table 1) and thus on average a hypothetical 2% of this needs to be diverted to the young to sustain its growth in the pouch, or a total of 4.04% if the female successfully weans two young as is usually the case (Russell and Renfree 1989). Our expectation that we would be able to discern this difference was probably far too optimistic, given the errors involved in estimating rates of nitrogen and protein synthesis with a mean coefficient of variation (CV) of 23.2% (again see Table 1)

The tentative model constructed to describe the kinetics of nitrogen metabolism in the Honey possum (see Fig. 6) is similar to that developed by Barboza et al. (1993) for the wombat, *Vombatus ursinus*. Our model is very preliminary but it does raise the possibility that the difference detected in degradation rates of crude protein in females carrying pouch young may reflect protein that is being diverted to milk production. If this were the case then the estimated figure of 6.6 mg N day^{-1} would correspond to milk with a protein concentration of approximately 4% which is the norm for marsupials early in pouch life (Munks et al. 1991). If we assume from the kinetic model that females with pouch young are able to divert 6.6 mg N day^{-1} towards milk production, this would result in 0.52 ml day^{-1} of milk at an average nitrogen concentration of 4% (assuming 50% incorporation of the nitrogen into milk protein). The total amount of nitrogen needed to produce two young of body mass 2.5 g at weaning is 128 mg which would require a total of 20 ml of milk of 4% protein concentration. This would be achieved in a little over 38 days at the above rate which suggests that the rate of milk production and nitrogen transfer would be lower than this in the Honey possum with a 60–70 day period of pouch life (Russell 1982). The point of the calculation, however, is to show that the diversion of quite small amounts of nitrogen is adequate to support the growth of the young in the pouch.

Estimates of the energy intake at peak lactation of Honey possum young, using the allometric equations of Riek (2008), also indicate that less than 5 kJ per day, or approximately 14% of the mother's FMR (Bradshaw and Bradshaw 1999), is diverted to each joey when it reaches its peak mass of 2.5 g. The energetic cost of protein synthesis, estimated at 4.5 kJ g^{-1} crude protein, is also very low, averaging only 4.3% of the FMR, less than the 7–8% estimated for three marsupial wallabies by (White et al. 1988) and well below the figure of 20% usually cited for eutherian mammals (MacRae and Lobley 1986).

Overall, our data suggest because the Honey possum has such a high daily intake of nitrogen in relation to its very low minimum requirement for balance (MNR), coupled with a high level of urinary excretion of nitrogen compared with other marsupials (Bradshaw and Bradshaw 2001), that it can readily divert nitrogen to milk production by reducing the rate of degradation of protein in the protein pool, rather than by increasing its rate of synthesis.

It is perhaps appropriate to also comment on the validity of the hypothesis that initiated this study—that of Smith and Lee (1984) who suggested that the relatively low offspring production rate in grams per month of Honey possums, when compared with folivorous species, could result from their high carbohydrate diet that may be deficient in protein. We suggest that, because an animal has a lower rate of reproduction than another does not necessarily indicate that its diet is limiting. Rates of reproduction of different species are attuned to their particular environment as a result of natural selection and, unless the population is in obvious decline, the reproductive rate will be adequate to balance mortality and emigration (Stearns 1992). The Honey possum is also unusual amongst marsupials in retaining its young with the mother for a longer period of time than similar-sized phalangeroids (Russell 1982; Wooller et al. 1984) and this may indicate a longer learning experience that enhances their probability of survival and thus compensates for a lower rate of offspring production than other small possums.

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