
Nitrogen Metabolism and Requirements of Nitrogen and Energy in the Wombats (Marsupialia: Vombatidae)

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Abstract

The Wombats Vombatus ursinus and Lasiorhinus latifrons are grazing marsupials from mesic and xeric habitats, respectively. Four wombats of each species were fed pelleted isocaloric diets containing 1.0% and 0.6% N. Urea kinetics (^{14}C -urea and ^{15}N -urea) and whole-body protein turnover (^{15}N -glycine) were integrated into a model of N flow through pools of amino acids, protein, and urea in the body. Maintenance dietary N requirements were the lowest reported for herbivorous marsupials: 201 and 158 $\text{mg} \cdot \text{kg}^{-0.75} \cdot \text{d}^{-1}$ for Lasiorhinus and Vombatus, respectively. The mean maintenance energy requirement ($140 \text{ kJ} \cdot \text{kg}^{-0.75} \cdot \text{d}^{-1}$) was only 32% of the estimate for macropod marsupials. Urea was recycled extensively, with 64%–78% of synthesized urea degraded and 34%–42% reutilized in the body. Rates of creatinine excretion and whole-body protein turnover were similar to those for macropods ($4.06\text{--}6.66 \text{ g crude protein} \cdot \text{kg}^{-0.75} \cdot \text{d}^{-1}$), even though wombats excreted ^{15}N -glycine slowly, indicating that wombats have large protein pools, consistent with their muscular conformation. The low requirements for N and energy enable the wombats to utilize poor-quality pastures and to exploit habitats where food abundance may be limited seasonally.

Introduction

The maintenance requirement for N reflects whole-body metabolism of proteins and amino acids and physiological mechanisms for reducing net cat-

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abolic losses (e.g., urea reutilization). These traits may be adaptations to specific nutritional constraints or to phylogenetic attributes common to taxonomic groups. For example, marsupials generally require less N and energy for maintenance than eutherian mammals (Dawson and Hulbert 1970; Hume 1982). However, the requirements of herbivorous marsupials may also reflect adaptations to their natural diets; among macropod marsupials, browsers from mesic forests such as the parma wallaby (*Macropus parma*) and red-necked pademelon (*Thylogale thetis*) have higher N requirements than xeric-adapted species such as the tammar wallaby (*Macropus eugenii*), which often consumes fibrous grasses of low digestible N content (Hume 1986).

The Vombatidae are a distinct group of grazing marsupials that probably diverged from the other diprotodont groups 50–60 million years ago (Kirsch 1977). The wombats include two species from contrasting habitats: the common wombat *Vombatus ursinus*, from mesic areas of coastal heath to subalpine forest, and the southern hairy-nosed wombat *Lasiorbini latifrons*, of semiarid plains. *Vombatus* inhabits areas of low annual temperature and rainfall variability, whereas *Lasiorbini* is distributed in regions where these parameters are more variable and thus annual pasture growth is less consistent (Bridgewater 1987). The digestive physiology of the wombats is unlike that of the other herbivorous marsupials; they have an extensive microbial fermentation along a capacious proximal colon, with only a vestigial cecum and a small acidic stomach (Barboza and Hume 1992*a*, 1992*b*). The metabolic requirements of the wombats may thus reflect their common phylogeny, but these may also be specific adaptations to their contrasting habitats.

This article reports maintenance N and energy requirements of *Vombatus* and *Lasiorbini*. The metabolism of N was modeled through studies of urea kinetics with ¹⁴C-urea and ¹⁵N-urea and of whole-body protein turnover with ¹⁵N-glycine. Circulating levels of thyroid hormones, and the proportional masses of the heart and liver are also reported. These features reflect the extremely low maintenance requirements for both N and energy in the wombats.

Material and Methods

Experimental Procedure

General conditions and protocols for maintenance and balance experiments on wombats were described by Barboza and Hume (1992*a*). Two diets containing 1.0% N (high N) and 0.6% N (low N) of dry matter (DM) were

provided ad lib. These dietary N levels were similar to those of sedges and senescent grasses consumed by wombats in the wild (Barboza 1989). Experimental diets were pelleted and contained 70% milled oat (*Avena sativa*) straw and 3% mineral/vitamin supplement (Pig-Grower Premix, Fielder Gillespie, Tamworth, New South Wales) on an air-dry basis. In addition, the low N diet contained 27% ground corn (*Zea mays*), while the high N diet contained 24% corn and 3% hydrochloric casein. These diets were 88.6% DM, of which 66.3% was neutral detergent fiber (NDF) and 7.4% was ash; the gross energy content was 16.4 kJ · g⁻¹.

Four *Vombatus* and four *Lasiorbatus* were included in a crossover design with repeated measures for each animal on each diet. Wombats were acclimated to experimental conditions in metabolism cages for 4 wk. Two collection periods of 8 d were commenced after feed intakes were consistent for at least 7 d. Wombats were weighed at the beginning and end of the collection period, during which all feeds and feces were weighed daily. Urine was collected into glacial acetic acid to reduce pH below 3.5, thereby minimizing bacterial ureolysis and losses of ammonia. Feeds and excreta from each animal were subsampled (20%), stored at -20°C, and subsequently mixed to provide bulk samples for analysis of feed refusals, feces, and urine.

The metabolism of urea and protein was measured by isotope dilution during each collection period. Wombats were injected with a single dose of 2.22 MBq ¹⁴C-urea (New England Nucleonics, Sydney), 50 mg ¹⁵N-urea (95 atoms ¹⁵N/100 atoms N; Australian Atomic Energy Commission, Lucas Heights, New South Wales), and 9.3 mg unlabeled urea in 3 mL physiological saline (9 g · L⁻¹ NaCl) at the start of the period. A second dose of 100 mg ¹⁵N-glycine (97 atoms ¹⁵N/100 atoms N; Australian Atomic Energy Commission) in 3 mL physiological saline was given 4 d later. Isotopes were given by intramuscular injection to the hind limbs of animals under manual restraint. At the end of the collection period, wombats were immobilized with 4% halothane (Fluothane; ACI, Sydney) in O₂ at 5 L · min⁻¹ via a face mask, and blood samples were taken from the brachial or saphenous veins.

Isotope levels were monitored in normally voided urine because regular sampling of blood plasma and catheterization of the urinary bladder were not feasible. This method was used in several recent studies of marsupials (Chilcott, Moore, and Hume 1985; Hume 1986; Foley and Hume 1987; White, Hume, and Nolan 1988) and was validated in rabbits (Regoezci et al. 1965). Urine was collected at hourly intervals for the first 24 h after dosing (average of seven urinations per animal) and at daily intervals thereafter. The time of urination was taken as the midpoint of the collection interval.

Analysis

Feed and feces were dried at 50°C and ground to pass through a 1-mm screen. Dry matter content was determined by drying to constant weight at 80°C in a convection oven. Ash content was determined by combustion in a muffle furnace for 3 h at 500°C. Neutral detergent fiber was extracted with the addition of sodium sulfite by the method of Van Soest, Robertson, and Lewis (1991).

Total N content of feeds, feces, and urine was determined by the Kjeldahl method as described by Barboza and Hume (1992*a*). Nitrogenous compounds in urine and plasma were measured as follows: urea content by the diacetyl monoxime technique (Marsh, Fingerhut, and Miller 1965), ammonia concentration by the salicylic acid technique (Bietz 1974), creatinine concentration by the picric acid method (Fabiny and Ertinghausen 1971), and uric acid content by a uricase procedure (Fossati, Prencipe, and Berti 1980).

We assayed ¹⁴C-urea by β-emission in a liquid scintillation spectrometer (Packard model 3255). Specific radioactivity was expressed on the basis of urea content (dpm · mmol⁻¹ urea) after correcting for quenching and for background radiation. The enrichment of urea nitrogen (urea-N) with ¹⁵N was determined with a single collector isotope ratio mass spectrometer (MS10; AEI, Manchester). Urea-N was prepared as ammonium borate by first hydrolyzing urea to ammonia with urease, then collecting ammonia into boric acid by steam distillation (Nolan and Leng 1972). This technique recovered 103% ± 2% of urinary ¹⁵N-urea when tested by adding small amounts of ¹⁵N-urea to bulk urine samples. Enrichments of urinary urea-N were corrected for the background level determined in urine collected before administration of ¹⁵N compounds.

Balance Calculations

The N composition of the bulk urine sample was used to estimate the daily outflow of each compound. Truly digestible N intake was calculated from the apparently digestible N intake plus the loss of N in the fecal NDF residue (Mason 1969; Smith and Green 1987). Dietary N was assumed to be completely digested except for that associated with plant cell walls. This assumption was shown to be valid in studies on horses (Fonnesbeck 1969) and ruminants (Van Soest 1982) and is supported by the sequences of N digestion along the digestive tract of wombats fed a similar pelleted diet (Barboza and Hume 1992*a*).

Maintenance N requirements were derived from the least-squares regressions of N balance on N intake and truly digestible N intake (Hume 1986).

Endogenous excretion of urinary N was derived from the regression of urinary N loss on truly digestible N intake (Smith and Green 1987). The maintenance energy requirement was derived from the regression of N balance on digestible energy intake. This procedure assumed that zero balance of N and energy were coincident in adult, nonreproducing animals (Hume 1974).

Isotope Kinetics

The kinetics of isotope excretion were estimated from the least-squares regressions of specific radioactivity or enrichment on time. Regressions were performed on the natural logarithms of concentrations after equilibration of the label with the body pool. Kinetics of ^{14}C -urea were estimated for the entire collection period, estimates of ^{15}N -urea kinetics were derived from the initial 4 d, while those for ^{15}N -glycine were based on the latter 4 d. Net enrichments of ^{15}N were calculated by subtracting extrapolated values from the regression of enrichment on time from the previous ^{15}N dose.

Urea excretion rates were measured directly from the discrete urinations. Rates of entry, degradation, reabsorption, and reutilization of urea were estimated from isotope kinetics by the methods of Cocimano and Leng (1967) and Chilcott and Hume (1984).

Parameters of protein turnover were calculated by the stochastic method described by White et al. (1988), which is 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 by Golden and Jackson (1981), Stein (1981), and Waterlow (1984). The daily irreversible loss of N from the amino acid pool was calculated as the daily urinary urea-N excretion divided by the fraction of the ^{15}N -glycine dose recovered in 87 h. Recycling of amino- ^{15}N from protein was assumed to be negligible during this period (87 h), and thus the irreversible loss was an estimate of daily protein turnover (Q) (White et al. 1988). Rates of protein synthesis (Z) and catabolism (B) were calculated from the model by the equation:

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

where E and I are the rates of amino-N excretion and intake, respectively. Daily dietary N intake and total N excretion were used as estimates of I and E , respectively. Parameters of protein metabolism were expressed on the basis of crude protein ($\text{CP} = 6.25 \times \text{N}$). The energetic cost of protein syn-

thesis was calculated on the basis of $4.5 \text{ kJ} \cdot \text{g}^{-1}$ CP for peptide bond synthesis (MacRae and Loblely 1986).

Thyroid Hormone and Organ Measures

Measures of thyroid hormones and internal organs were performed on four captive wombats of each species in conjunction with experiments described by Barboza and Hume (1992a). Blood plasma was sampled by cardiac puncture from anesthetized animals and stored at -20°C . Total thyroxine (T_4) was assayed by radioimmunoassay (Amerlex-M T_4 kit; Amersham, Sydney) with human antisera. The binding capacity of serum proteins for T_4 was assessed from triiodothyronine (T_3) uptake by human antisera (MAA kit; Amersham, Sydney). Total T_3 was assayed with rabbit antisera (Tri-Tab RIA kit; Organon Teknika, Durham, N.C.).

The heart and liver were dissected from each carcass and weighed after removing mesenteric membranes and trimming the blood vessels to their points of origin. Organ mass was related to empty body mass, that is, the carcass mass minus the wet gastrointestinal contents (Dawson and Needham 1981).

Statistics

Results from the balance experiments were compared by ANOVA on repeated measures, with species as a factor between groups and treatment as a factor within groups (Winer 1971; Tabachnick and Fidell 1983). Regression relationships were compared between species with the multivariate general linear model (Wilkinson 1990). Organ measures and hormone levels were compared between species by *t*-test (Winer 1971). All percentages were arcsine-transformed before statistical analysis (Zar 1974). Means are reported with the standard deviation of the sample.

Results

Balance Measures

Although body mass changes during the collections were small, the wombats lost mass slowly and had lower masses on the low N diet (table 1). Feed intakes and the digestibilities of DM (39%), energy (39%), and NDF (26%–27%) were similar between species and diets (table 1). However, N digest-

TABLE 1

Measures of intake and excretion in wombats fed high (1.0%) and low (0.6%) N diets

Measure and Species	High N	Low N	Statistic
Body mass (kg)			S*, D**
<i>Vombatus</i>	28.3 ± 2.9	27.4 ± 2.7	
<i>Lasiorbini</i>	23.3 ± 1.6	22.9 ± 1.7	
Dry matter intake (g · kg ^{-0.75} · d ⁻¹)			NS
<i>Vombatus</i>	32.6 ± 10.4	25.7 ± 6.6	
<i>Lasiorbini</i>	28.6 ± 7.4	25.1 ± 8.3	
Digestible energy intake (kJ · kg ^{-0.75} · d ⁻¹)			NS
<i>Vombatus</i>	199.7 ± 70.3	166.6 ± 44.8	
<i>Lasiorbini</i>	183.2 ± 42.9	156.5 ± 49.8	
N (%) digestibility			D***
<i>Vombatus</i>	51.5 ± 6.7	26.4 ± 4.7	
<i>Lasiorbini</i>	57.1 ± 7.7	29.8 ± 7.7	
N balance parameters (mg · kg ^{-0.75} · d ⁻¹):			
Intake			D**
<i>Vombatus</i>	333 ± 105	167 ± 42	
<i>Lasiorbini</i>	285 ± 73	159 ± 53	
Urinary loss			S**, D**
<i>Vombatus</i>	87 ± 25	42 ± 11	
<i>Lasiorbini</i>	132 ± 23	74 ± 12	
Fecal loss			NS
<i>Vombatus</i>	162 ± 60	122 ± 25	
<i>Lasiorbini</i>	124 ± 40	110 ± 30	
Apparent balance			D***
<i>Vombatus</i>	84 ± 60	3 ± 18	
<i>Lasiorbini</i>	45 ± 35	-25 ± 24	
Fecal NDF-N loss			NS
<i>Vombatus</i>	31 ± 10	24 ± 4	
<i>Lasiorbini</i>	26 ± 6	26 ± 7	
Truly digestible intake			D***
<i>Vombatus</i>	202 ± 65	69 ± 21	
<i>Lasiorbini</i>	187 ± 46	75 ± 32	

Note. Values are means ± SD for four animals of each species. Statistics are effects of species (S), diet (D), and species-by-diet interaction (I) with the significance of the *F* statistic. NS, Not significant.

* *P* < 0.05.

** *P* < 0.01.

*** *P* < 0.001.

ibilities were lower in wombats on the low N diet, reflecting the greater digestibility of casein included in the high N diet (table 1).

Fecal N losses were similar between the species, with only 19%–24% associated with the NDF fraction. Urinary N losses were greater in *Lasiorbini* than in *Vombatus* for both diets, with a trend toward lower N balances for *Lasiorbini* (table 1). The higher urinary N losses of *Lasiorbini* were reflected in greater outflows of ammonia and creatinine than those of *Vombatus* (table 2), with a similar trend for urea. Excretion of residual urinary N was also greater for *Lasiorbini* than for *Vombatus*. Uric acid was only a minor component (<0.005%) of this residual N in both species. Reduced urinary N losses from both species on the low N ration were mainly due to lower urea outflows (tables 1, 2). Losses of urinary N during the collection procedures were probably minor, as the N composition of bulk urine samples was similar to that of samples collected directly from the urinary bladder of both species in captivity (Barboza 1989).

TABLE 2

Partitioning urinary N loss ($\text{mg} \cdot \text{kg}^{-0.75} \cdot \text{d}^{-1}$) in wombats fed high (1.0%) and low (0.6%) N diets

Measure and Species	High N	Low N	Statistic
Urea			D***
<i>Vombatus</i>	70.8 ± 21.7	24.6 ± 7.3	
<i>Lasiorbini</i>	88.2 ± 16.5	40.8 ± 4.3	
Ammonia			S*
<i>Vombatus</i>	3.8 ± 1.5	2.1 ± 1.6	
<i>Lasiorbini</i>	9.0 ± 6.4	7.4 ± 2.8	
Creatinine			S*
<i>Vombatus</i>	9.1 ± 2.1	8.3 ± 1.8	
<i>Lasiorbini</i>	12.1 ± 4.3	13.3 ± 1.7	
Residual N			S**
<i>Vombatus</i>	3.3 ± 9.0	7.5 ± 3.1	
<i>Lasiorbini</i>	22.5 ± 16.7	12.6 ± 12.5	

Note. Values are means ± SD for four animals of each species. Statistics: effects of species (S), diet (D), and species-by-diet interaction (I) with the significance of the *F* statistic. NS, Not significant.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

Estimates of maintenance N requirements were higher in *Lasiorhinus* than in *Vombatus* on both a dietary (201 vs. 158 $\text{mg N} \cdot \text{kg}^{-0.75} \cdot \text{d}^{-1}$; fig. 1) and truly digestible basis (71 vs. 116 $\text{mg N} \cdot \text{kg}^{-0.75} \cdot \text{d}^{-1}$; fig. 2). However, the intercepts and slopes of these regressions were not significantly different ($P > 0.05$) between species, and the combined estimates were 182 $\text{mg dietary N} \cdot \text{kg}^{-0.75} \cdot \text{d}^{-1}$ (fig. 1) and 93 $\text{mg truly digestible N} \cdot \text{kg}^{-0.75} \cdot \text{d}^{-1}$ (fig. 2). The dietary N requirement was probably overestimated because the digestibility of N decreased with dietary N content (table 1). That is, zero balance would have been indicated at lower apparent N intakes if N digestibility had been consistent between diets.

The estimate of endogenous urinary N excretion in *Lasiorhinus* was 42 $\text{mg} \cdot \text{kg}^{-0.75} \cdot \text{d}^{-1}$ from the following regression relationship between urinary loss (y) and truly digestible intake (x): $y = 42.1 + 0.46x$, $R^2 = 0.850$, $P < 0.001$. The regression relationship for *Vombatus* was $y = 31.3$ mg

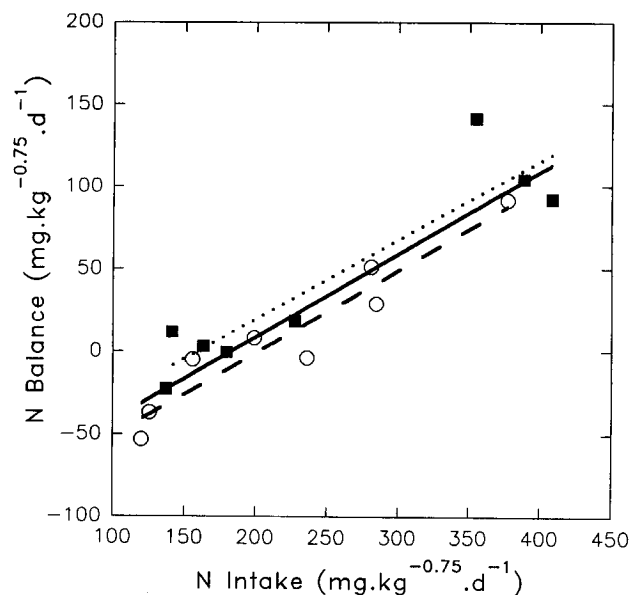


Fig. 1. Regression relationships between N balance and N intake in wombats. *Vombatus* (closed squares, dotted line): $y = -75.99 + 0.48x$, $R^2 = 0.844$, $P < 0.001$. *Lasiorhinus* (open circles, dashed line): $y = -100.46 + 0.50x$, $R^2 = 0.915$, $P < 0.001$. Intercepts and coefficients were not significantly different between species ($P > 0.05$). Relationship for both species combined (solid line): $y = -91.20 + 0.50x$, $R^2 = 0.849$, $P < 0.001$. N intake required for maintenance ($\text{mg} \cdot \text{kg}^{-0.75} \cdot \text{d}^{-1}$): *Vombatus* 158; *Lasiorhinus* 201; species combined 182.

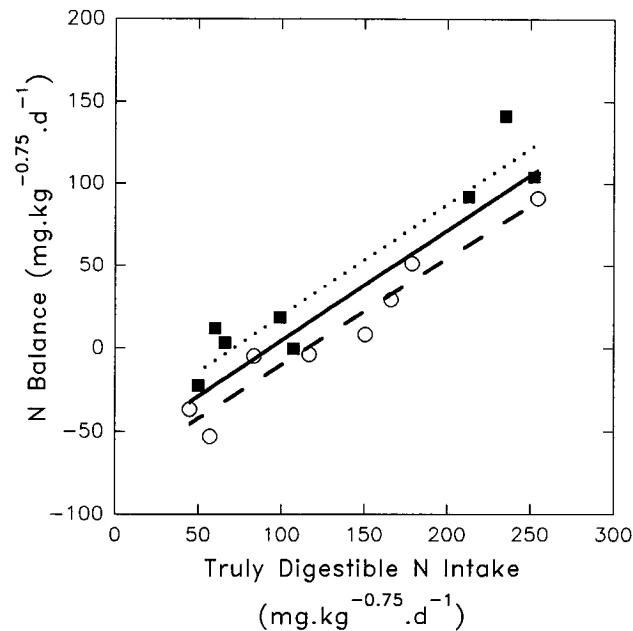


Fig. 2. Regression relationships between N balance and truly digestible N intake in wombats. *Vombatus* (closed squares, dotted line): $y = -48.19 + 0.68x$, $R^2 = 0.905$, $P < 0.001$. *Lasiorhinus* (open circles, dashed line): $y = -74.45 + 0.64x$, $R^2 = 0.940$, $P < 0.001$. Intercepts and coefficients were not significantly different between species ($P > 0.05$). Relationship for both species combined (solid line): $y = -62.44 + 0.67x$, $R^2 = 0.842$, $P < 0.001$. Truly digestible N intake required for maintenance ($\text{mg} \cdot \text{kg}^{-0.75} \cdot \text{d}^{-1}$): *Vombatus*, 71; *Lasiorhinus*, 116; species combined, 93.

$\text{N} \cdot \text{kg}^{-0.75} \cdot \text{d}^{-1} + 0.247x$, $R^2 = .488$, $P = 0.054$. As the intercepts of these regressions were not significantly different, the endogenous urinary N loss of the two species combined was $40 \text{ mg} \cdot \text{kg}^{-0.75} \cdot \text{d}^{-1}$.

The maintenance energy requirement was estimated at $140 \text{ kJ} \cdot \text{kg}^{-0.75} \cdot \text{d}^{-1}$ for both species from the regression shown in figure 3. The slow weight loss of animals on the low N diet (table 1) suggests that wombats were probably in negative energy balance as well as near zero N balance on this diet (table 1). Since net protein deposition requires both energy and N, it is unlikely that negative energy balance would accompany positive N balance in an adult, nonreproducing animal. Therefore, the decline in body mass in wombats on the low N diet probably reflects declines in lean mass rather than fat loss, and the digestible energy intake at zero N balance approximated the energy required to maintain lean body mass in the wombats. This as-

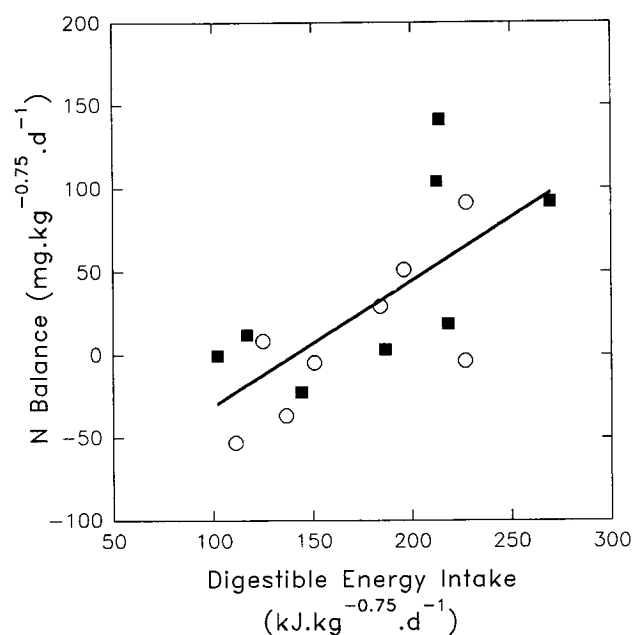


Fig. 3. Regression relationship between N balance and digestible energy intake in *Vombatus* (closed squares) and *Lasiorhinus* (open circles). Relationship for both species combined: $y = -106.68 + 0.76x$, $R^2 = 0.487$, $P < 0.01$. Digestible energy intake required for maintenance at zero N balance: $140 \text{ kJ} \cdot \text{kg}^{-0.75} \cdot \text{d}^{-1}$.

sumes that lean mass was the principal determinant of whole-body energy expenditure (Blaxter 1989).

Urea Kinetics

Lower dietary N resulted both in reduced plasma urea levels (table 3) and a proportional reduction in urinary urea outflows (table 2) in the wombats. Consequently, the ratios of urinary urea to plasma urea were similar between diets (table 3). The fractional turnover of the urea pool was similar between dietary N levels, whereas urea pool size and all urea flow rates were smaller on the low N diet (table 3). However, changes in absolute flow rates were directly proportional to the rate at which urea was formed and were therefore similar between diets when expressed as a percentage of entry rate. These proportional rates of urea flow tended to be higher for *Vombatus* than for *Lasiorhinus* (78% vs. 64% degraded, 36% vs. 29% reabsorbed, 42% vs. 34% reutilized).

TABLE 3

Measures of urea kinetics in wombats fed high (1.0%) and low (0.6%)
N diets

Measure and Species	High N	Low N	Statistic
Plasma urea (mmol · L ⁻¹)			D***
<i>Vombatus</i>	5.28 ± 1.75	2.43 ± 1.28	
<i>Lasiorbinius</i>	4.57 ± 1.61	3.14 ± 1.21	
Urea N ratio urine:plasma			NS
<i>Vombatus</i>	22.4 ± 14.7	26.3 ± 15.7	
<i>Lasiorbinius</i>	36.9 ± 26.9	24.0 ± 18.9	
Pool size (mmol · kg ^{-0.75})			D**
<i>Vombatus</i>	8.90 ± 4.90	3.27 ± 1.33	
<i>Lasiorbinius</i>	8.94 ± 1.89	4.65 ± .76	
Pool turnover (d ⁻¹)			NS
<i>Vombatus</i>	1.34 ± .31	1.30 ± .26	
<i>Lasiorbinius</i>	1.01 ± .19	1.01 ± .19	
Flow rates (mmol · kg ^{-0.75} · d ⁻¹):			
Entry			D**
<i>Vombatus</i>	11.30 ± 5.78	4.16 ± 1.43	
<i>Lasiorbinius</i>	9.12 ± 2.85	4.70 ± 1.00	
Excretion			S*, D***
<i>Vombatus</i>	2.31 ± .81	.75 ± .26	
<i>Lasiorbinius</i>	3.27 ± .60	1.62 ± .35	
Degradation			D*
<i>Vombatus</i>	9.00 ± 5.57	3.41 ± 1.48	
<i>Lasiorbinius</i>	5.85 ± 2.60	3.07 ± .71	
Reabsorption			D*
<i>Vombatus</i>	4.41 ± 2.85	1.42 ± .76	
<i>Lasiorbinius</i>	2.34 ± .92	1.57 ± .70	
Reutilization			D*
<i>Vombatus</i>	4.57 ± 3.22	1.99 ± 1.13	
<i>Lasiorbinius</i>	3.51 ± 2.34	1.50 ± .51	

Note. Values are means ± SD for four animals of each species. Statistics: effects of species (S), diet (D), and species-by-diet interaction (I) with the significance of the *F* statistic. NS, Not significant.

* *P* < 0.05.

** *P* < 0.01.

*** *P* < 0.001.

Protein Turnover

Excretion of the ^{15}N -glycine dose in urinary urea was slow at both levels of dietary N. The fractional turnover rates (per hour) of the labeled amino acid pool were 0.0110 ± 0.0036 and 0.0140 ± 0.0051 for the low and high N diets, respectively. Recoveries of the ^{15}N -glycine dose were also low, especially at the lower rates of urea-N excretion on the low N diet (low N, $4.1\% \pm 1.9\%$; high N, $8.1\% \pm 2.3\%$). This slow rate of turnover suggests that recycling of ^{15}N from the protein pool back into the amino acid pool would have been minimal during the 87 h following dosing. It is also unlikely that a significant portion of the dose remained in the amino acid pool after 87 h, as ^{15}N excretion declined progressively from 60 h after dosing. Rates of whole-body protein turnover, catabolism, and synthesis were similar between diets, with a consistent trend toward lower estimates in *Vombatus* than in *Lasiorbatus* (table 4). The proportion of the digestible energy intake required for protein synthesis was similar between diets and species. Protein

TABLE 4
Measures of whole-body protein metabolism (g crude protein \cdot kg $^{-0.75}$ \cdot d $^{-1}$) and the energetic cost of protein synthesis (as % digestible energy intake) in wombats fed high (1.0%) and low (0.6%) N diets

Measure and Species	High N	Low N
Turnover (<i>Q</i>):		
<i>Vombatus</i>	5.77 \pm 2.02	4.06 \pm 1.57
<i>Lasiorbatus</i>	6.56 \pm .54	6.66 \pm 1.08
Catabolism (<i>B</i>):		
<i>Vombatus</i>	3.69 \pm 2.67	3.01 \pm 1.74
<i>Lasiorbatus</i>	4.77 \pm .68	5.66 \pm 1.23
Synthesis (<i>Z</i>):		
<i>Vombatus</i>	4.21 \pm 2.37	3.03 \pm 1.67
<i>Lasiorbatus</i>	4.96 \pm .58	5.51 \pm 1.16
Energy for synthesis:		
<i>Vombatus</i>	13.0 \pm 13.6	8.8 \pm 4.6
<i>Lasiorbatus</i>	12.9 \pm 4.2	17.1 \pm 6.3

Note. Values are means \pm SD for four animals of each species. Effects of species (S), diet (D), and species-by-diet interaction (I) were not significant ($P > 0.05$).

synthesis and catabolism would have been underestimated if N intake and excretion included some nonamino N, but this was probably a minor component of these artificial diets.

Thyroid Hormones and Organ Measures

Dissections were performed on wombats of similar total mass (*Vombatus* 27.4 ± 1.4 kg, *Lasiorbhinus* 24.3 ± 2.9 kg) and empty body mass (*Vombatus* 23.4 ± 1.8 kg, *Lasiorbhinus* 21.6 ± 2.4 kg). However, the masses of the liver and the heart were greater in *Vombatus* than in *Lasiorbhinus*: liver 355 ± 59 g versus 259 ± 39 g, $P < 0.05$; heart 99 ± 13 g versus 60 ± 7 g, $P < 0.01$.

Plasma concentrations of T_4 in these animals were similar between *Vombatus* ($0.44 \pm 0.24 \mu\text{g} \cdot \text{dL}^{-1}$) and *Lasiorbhinus* ($0.49 \pm 0.15 \mu\text{g} \cdot \text{dL}^{-1}$). The concentrations of T_3 were also similar between the species but near the limits of sensitivity for the assay ($<10 \text{ ng} \cdot \text{dL}^{-1}$). Conversely, T_3 uptakes were similar to those recommended for euthyroid humans and indicated a similar binding affinity of serum proteins for T_4 (*Vombatus* $42.5\% \pm 1.4\%$, *Lasiorbhinus* $43.5\% \pm 1.5\%$). This also suggested that the antisera were appropriate for wombat plasma and that the low concentrations were not an artifact of the assay.

Discussion

Measures of the kinetics and balance of N were integrated in a quantitative model of N flow in *Vombatus* and *Lasiorbhinus* fed the high N diet (fig. 4).

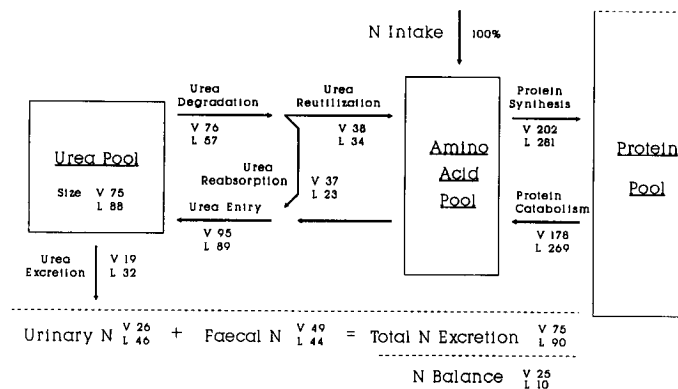


Fig. 4. Model of N flow in relation to dietary intake (100%) for *Vombatus* (V) and *Lasiorbhinus* (L) fed the high (1.0%) N diet.

The model includes three pools of N within the body: amino acids, proteins, and urea. Nitrogen enters the body as dietary protein, which is digested and absorbed into the amino acid pool. Amino acid N is continuously exchanged with the protein pool during synthesis and catabolism of proteins. The amino acid and protein pools in the model include all tissues and organs in the body as well as microbes in the hindgut (Barboza and Hume 1992*b*). Any exchanges between the organs (Felig 1981) or differences in turnover rates between tissues occur within these pools (Bier, Matthews, and Young 1985). Amino acid N is catabolized to ammonia and is subsequently incorporated into urea, which is either excreted in the urine or degraded to ammonia by microbes in the hindgut. Urea enters the hindgut by diffusion across the mucosa from the blood and in secretions from the small intestine (Mason 1984; Stevens 1988). Ammonia produced from the degradation of urea is converted to microbial amino acids or directly absorbed from the gut as reported in ruminants (Nolan, Norton, and Leng 1976; Dixon and Nolan 1982) and macropod marsupials (Dellow, Nolan, and Hume 1983). The absorbed ammonia is carried to the liver and is either reutilized by the body in the synthesis of nonessential amino acids or reabsorbed into the urea pool (Wrong and Vince 1984).

Barboza and Hume (1992*a*) reported low concentrations and slow production *in vitro* of ammonia in digesta from the hindgut of wombats. Therefore, ammonia produced from urea degradation was probably absorbed rapidly by microbes in the wombat hindgut. However, ureolytic microbes associated with the gut mucosa also produce ammonia that may be rapidly absorbed by the body (Egan, Boda, and Varady 1986). The large proportion of urea reutilized and reabsorbed (fig. 4) in the wombats suggests that ammonia was probably absorbed across the gut wall after ureolysis by microbes associated with the gut mucosa.

A small proportion of the apparently reutilized urea-N may have been from absorption of microbial amino acids from the hindgut. This suggestion is supported by the apparent accumulation of N and fermentation of amino acids in the distal colon of wombats (Barboza and Hume 1992*a*, 1992*b*). Absorption of amino acids from the hindgut has been suggested by Slade et al. (1971) in the horse and by Niiyama et al. (1979) in the pig. However, conclusive evidence for active transport of amino acids across the adult mammalian hindgut mucosa has not been produced (Hume, Karasov, and Dakin 1993), and studies of the colon of sheep and pigs suggest that amino acid absorption from the hindgut is of little consequence to the N balance of the animal (Mason 1984). Therefore, any amino acid absorption from the hindgut in wombats may only serve to reduce fecal N loss.

Since only a small proportion of the digestible dietary N enters the wombat hindgut (Barboza and Hume 1992a), endogenous urea must be an important source of N for microbes in the colon. In animals fed diets low in N content, endogenous urea was indicated as an important source of N to microbes in the ruminant forestomach (Kennedy and Milligan 1980) and the colon of the horse (Prior et al. 1974; Wooton and Argenzio 1975). In the wombats, similar fiber digestibilities between the two levels of dietary N suggest that cellulolytic microbes in the hindgut were not limited by a shortage of N on the low N diet. Therefore, an endogenous N source such as urea probably contributed significantly toward maintaining microbial activity in the colon.

Although urea metabolism accounted for a large proportion of the N economy, protein turnover dominated the flow of N (fig. 4). *Lasiorbhinus* probably had a higher rate of protein metabolism than *Vombatus*, as indicated by greater creatinine outflows and a trend toward higher estimates of protein turnover and endogenous urinary N loss. In *Lasiorbhinus*, these losses were exacerbated by lower rates of urea recycling than in *Vombatus*. This may be related to the smaller surface area of the proximal colon in *Lasiorbhinus* than in *Vombatus* (Barboza and Hume 1992a), which may limit the mucosal exchange of urea N. These differences are reflected in the higher estimates of N requirements for *Lasiorbhinus* (figs. 1, 2).

The maintenance N requirements estimated here for the wombats are lower than published estimates for any other herbivorous marsupial (Smith and Green 1987). However, parameters of whole-body protein metabolism were similar to values published for macropod marsupials (White et al. 1988). The values for creatinine and endogenous urinary N excretion from the wombats were also similar to those reported from macropod marsupials (Fraser and Kinnear 1969; Smith and Green 1987). The slow excretion of the ^{15}N -glycine dose from wombats suggested that protein turnover was slower than in wallabies (White et al. 1988); thus, the similar whole-body estimate reflects a larger protein pool. A proportionately large lean body mass is consistent with the heavily muscled conformation of the Vombatidae and is probably related to their fossorial habit and the ability to excavate extensive burrow systems.

The low maintenance N requirements of the wombats were partly related to greater recycling of urea than in wallabies (Dellow and Hume 1982; Chilcott et al. 1985). Low N requirements in the wombats were accompanied by a low maintenance energy requirement (fig. 3), which was only 32% of that for kangaroos (*Macropus rufus* and *Macropus robustus*) ($435 \text{ kJ} \cdot \text{kg}^{-0.75} \cdot \text{d}^{-1}$) and just 25% of the requirement for sheep ($569 \text{ kJ} \cdot \text{kg}^{-0.75} \cdot \text{d}^{-1}$) (Hume 1974). Estimated basal energy expenditures of kangaroos and sheep

were approximately half their maintenance energy requirements (Hume 1974); thus, the standard metabolic rate for the wombats is probably also significantly lower than that of other marsupials (Wells 1978).

A low rate of metabolism in the wombats was also indicated by the plasma concentrations of thyroid hormones that are the lowest reported for any mammal (Hulbert and Augee 1982). Elevated thyroid function increases many metabolic processes, including basal metabolism and body temperature (Hulbert 1978; Summers, McBride, and Milligan 1988). Although plasma concentrations of the hormones do not directly indicate their rates of secretion or absorption, they have been correlated with basal rates of metabolism among species of rodents (Scott, Yousef, and Johnson 1976).

The low plasma concentrations of T_4 in captive wombats were confirmed in wild animals of both species and in wombats from other colonies (P. S. Barboza, unpublished data) and therefore were not due to the diet or to the management of wombats in this study. Furthermore, it is unlikely that these low thyroid hormone levels are due to insufficient dietary iodine as all rations included a supplement to provide at least $0.7 \text{ mg I} \cdot \text{kg}^{-1}$ (on an air-dry basis) in the finished diet (Hume and Barboza 1993). This level exceeds the dietary requirements of most domestic species, including dairy cattle (Hetzel and Maberly 1986).

Rectal body temperatures in captive wombats were similar to those of other marsupials (*Vombatus* $35.1^\circ \pm 0.4^\circ\text{C}$, *Lasiorbini* $33.9^\circ \pm 1.8^\circ\text{C}$; Barboza 1989) and consistent with measures in wild and captive wombats by Wells (1978) and Brown (1984). Thermoregulation in the wombats is characterized by a labile body temperature and a low conductance (Wells 1978; Brown 1984). The energetic cost of thermoregulation is minimized by an increase in body temperature while actively foraging and a gradual decline while resting (Wells 1978).

The proportional mass of the heart was only 38% and 58% of the marsupial mean for *Lasiorbini* and *Vombatus*, respectively, or 51% and 77% of the eutherian mean, by the allometric relationships of Dawson (1983). The relatively small hearts of the wombats suggest that their stroke volumes are smaller than those of other marsupials. Since heart rates in wombats are similar to those of other marsupials (Baudinette 1978; Brown 1984), the small stroke volumes suggest that the rate of blood flow must be lower in the wombats.

Similarly, the proportional masses of the liver in wombats were only 55% (*Vombatus*) and 44% (*Lasiorbini*) of those predicted from their body weights by the allometric relationship of Munro (1969). The small liver weights are consistent with low rates of protein and energy metabolism in

the wombats and suggest that the total metabolic activity of the liver is lower in the wombats than in other mammals. However, this suggestion requires confirmation from direct measures of hepatic enzyme activities and energy expenditure.

Wombats may require less energy for blood circulation, hepatic activity, and thermoregulation than most marsupials but may expend more energy on protein metabolism. Protein synthesis accounted for 13% of the digestible energy intake of wombats fed the high N diet, and this was 14%–16% of the estimated maintenance energy requirement. This estimate is higher than those reported for other marsupials such as wallabies (5%–7% of digestible energy intake) (White et al. 1988) and the brushtail possum (*Trichosurus vulpecula*; Dellow and Harris, 1984) but similar to those for eutherians (15%–25% of energy expenditure; Reeds, Nicholson, and Fuller 1987; Summers et al. 1988). The higher estimate for the wombats compared with the other marsupials studied is directly related to their lower digestible energy intakes and lower maintenance energy requirements rather than to higher rates of protein synthesis.

Low requirements for maintenance enable the wombats to subsist on low digestible intakes of protein and energy. This would facilitate utilization of poor-quality feeds high in fiber and low in protein content such as the sedges and senescent grasses consumed by *Vombatus* (Barboza and Hume 1992a). In addition, these low requirements may also allow the wombats to subsist in habitats where plant availability is often low and feed intake is seasonally limited, as in the semiarid range of *Lasiorbatus* (Barboza and Hume 1992a).

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