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Interpreting temporal variation in omnivore foraging ecology via stable isotope modelling

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Summary

1. The use of stable carbon (C) and nitrogen (N) isotopes ($\delta^{15}N$ and $\delta^{13}C$, respectively) to delineate trophic patterns in wild animals is common in ecology. Their utility as a tool for interpreting temporal change in diet due to seasonality, migration, climate change or species invasion depends upon an understanding of the rates at which stable isotopes incorporate from diet into animal tissues. To best determine the foraging habits of invasive rats on island ecosystems and to illuminate the interpretation of wild omnivore diets in general, I investigated isotope incorporation rates of C and N in fur, liver, kidney, muscle, serum and red blood cells (RBC) from captive rats raised on a diet with low $\delta^{15}N$ and $\delta^{13}C$ values and switched to a diet with higher $\delta^{15}N$ and $\delta^{13}C$ values.

2. I used the reaction progress variable method (RPVM), a linear fitting procedure, to estimate whether a single or multiple compartment model best described isotope turnover in each tissue. Small sample Akaike Information criterion (AIC_c) model comparison analysis indicated that 1 compartment nonlinear models best described isotope incorporation rates for liver, RBC, muscle, and fur, whereas 2 compartment nonlinear models were best for serum and kidney.

3. I compared isotope incorporation rates using the RPVM versus nonlinear models. There were no differences in estimated isotope retention times between the model types for serum and kidney (except for N turnover in kidney from females). Isotope incorporation took longer when estimated using the nonlinear models for RBC, muscle, and fur, but was shorter for liver tissue.

4. There were no statistical differences between sexes in the isotope incorporation rates. I also found that N and C isotope incorporation rates were decoupled for liver, with C incorporating into liver tissue faster than N.

5. The data demonstrate the utility of analysing isotope ratios of multiple tissues from a single animal when estimating temporal variation in mammalian foraging ecology.

Key-words: omnivore foraging ecology, invasive rats, stable isotopes, stable isotope incorporation rates, stable isotope turnover models

Introduction

The stable isotope ratios of nitrogen ($^{15}N/^{14}N$, reported as $\delta^{15}N$) and carbon ($^{13}C/^{12}C$, reported as $\delta^{13}C$) in consumer and prey tissues are used for many applications in ecology and their use is increasing dramatically. Predictable enrichment of ^{15}N with increasing trophic level allows for estimation of an animal's relative trophic position (Vanderklift & Ponsard 2003; Gannes *et al.* 1998). Carbon isotope ratios reflect sources of primary productivity and are thus strong indicators of an animal's habitat use (Rubenstein & Hobson 2004; Kurle & Gudmundson 2007) and can be used to distinguish dietary composition (Gannes *et al.* 1998). While useful as a tool in understanding trophic relationships, two of the problems in the application of stable isotopes have been: (i) modelling incorporation of diet into tissue in the best way to most accurately reflect the physiology of assimilation, and (ii) applying these models to understanding the temporal relationship between diet ingestion and reflection of that diet in the isotope composition of various animal tissues.

Comparing stable carbon (C) and nitrogen (N) isotopes in different tissues from individual animals can reveal temporal changes in diet because isotopic composition reflects an amalgamation of nutrients incorporated at different times depending on the growth, metabolism, protein turnover and

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synthesis of each tissue (Carleton & Martínez Del Rio 2005; Macavoy *et al.* 2005, 2006; Arneson *et al.* 2006; Tsahar *et al.* 2008). Such studies have tremendous potential to increase our understanding of animal ecology, without conducting long, costly and potentially damaging or lethal field studies. However, they are of limited utility unless validated by feeding experiments which can be used to populate models that determine isotope retention and turnover times for relevant tissues. These data can be used to inform interpretation of stable isotope studies estimating foraging ecology of wild animals over various time scales such as seasonal or annual changes in diet composition (Newsome *et al.* 2007). In this study, I determined the isotope retention times in six tissues from laboratory rats (*Rattus norvegicus*).

Rats were used in this study because they have been used as proxies for other mammals in a multitude of studies and therefore my data can be applied to estimating foraging ecology of other wild omnivores. Also, rats are the most common invasive animal on islands worldwide and estimating their diets is useful to conservation ecologists to adequately assess their alteration of native floral and faunal species through foraging (Kurle, Croll & Tershy 2008; C. M. Kurle & P. L. Koch, unpublished). As it is logistically difficult to monitor rats' diets year-round using traditional methods, especially in remote locations, stable C and N isotopes from multiple tissues can be used to estimate temporal variations in their diet.

There is some debate over the best approach to model nutrient absorption and isotope turnover in tissues. The most common method is a single compartment exponential equation model that includes isotope values from tissues collected at and after a diet switch (Tieszen *et al.* 1983; Hobson & Clark 1992; Podlesak *et al.* 2005; Mirón *et al.* 2006; Phillips & Eldridge 2006). This model assumes that food is ingested and isotopes from the diet merge into a single compartment or pool within a tissue and are replaced at the same rate by the ingestion of new dietary items. Recently, Cerling *et al.* (2007) developed a model using a reaction progress variable method (RPVM) allowing estimations of: (i) isotopic half-life and retention time (the time taken for an isotope introduced into a tissue from one diet to leave that tissue and be replaced by isotopes from other dietary sources), (ii) delays in dietary incorporation of isotopes into particular animal tissues, and (iii) the existence of multiple turnover pools within an organism that contribute to isotopic turnover in a particular tissue (see complete descriptions of the method in Cerling *et al.* 2007; Martínez del Rio & Anderson-Sprecher 2008; D. W. Podlesak, S. R. McWilliams & T. Cerling, unpublished). This may be a significant improvement because multiple turnover pools or compartments may be contributing to the isotopic turnover within a particular tissue and it is thought that the isotopic retention times of each pool must be considered separately to accurately determine the overall mean isotope retention time (Ayliffe *et al.* 2004; Cerling *et al.* 2007; D. W. Podlesak, S. R. McWilliams & T. Cerling, unpublished; Martínez del Rio & Anderson-Sprecher 2008).

Martinez del Rio and Anderson-Sprecher (2008) criticized the RPVM because it: (i) was too simplistic in the assumptions of how multiple pools contribute to isotopic turnover, (ii) ignored the pharmacological literature that indicates nonlinear fitting methods are best suited to modelling data such as isotope turnover times, (iii) does not provide a method to calculate error terms, and (iv) does not adequately address how to choose the most biologically relevant model from among single or multiple pool models. They recommend using the RPVM to estimate whether a multiple compartment model might be most appropriate to describe isotope incorporation, and then applying both a single and multiple compartment nonlinear model to estimate isotope retention time in tissues. They also recommend using a comparison of model goodness of fit with Akaike information criterion (AIC) values to quantitatively judge which model (the single or multiple compartment nonlinear model) best describes isotope incorporation rates (see also Burnham & Anderson 2002; Tsahar *et al.* 2008).

In this study, I establish the time required for $\delta^{15}N$ and $\delta^{13}C$ values of 6 tissues from laboratory rats to equilibrate between 2 diets, each with distinct $\delta^{15}N$ and $\delta^{13}C$ values. I use this data set to: (i) test for differences in estimations of isotope retention times between the RPVM and traditional nonlinear models, (ii) determine whether 1 or 2 compartment models best describe isotope turnover for each tissue, and (iii) provide for the best interpretation of temporal variation in wild omnivore diets using stable isotopes of N and C. Data examining the isotope discrimination factors between several diets and tissues from rats is published elsewhere (C. M. Kurle & P. L. Koch, unpublished).

Methods

To inform the debate regarding isotope turnover models, I used the RPVM to determine if a single or multiple compartment nonlinear model best estimated measured data on incorporation rates of N and C in multiple tissue types from captive rats switched from one isotopically distinct diet to another. I also calculated isotopic half-lives, retention times, and possible delays in isotopic incorporation for each tissue using the RPVM and compared model results with those obtained using nonlinear models. I fit 1 compartment nonlinear models to all of my data sets and 2 compartment RPVM and nonlinear models where it was indicated as appropriate by the RPVM results (Cerling *et al.* 2007; D. W. Podlesak, S. R. McWilliams & T. Cerling, unpublished). I then used small sample AIC (AICc) values to determine if a 1 or 2 compartment model was the best fit for the data (Martinez del Rio & Anderson-Sprecher 2008). Finally, I report the mean half-lives and retention times (±95% confidence intervals) of the N and C in tissues from female and male rats using the nonlinear model judged most appropriate. Retention time is thought to be the most useful parameter to describe isotope incorporation rate; I also report isotope half-lives as that is the parameter used historically and is helpful for comparison with past studies. The complete description of the models can be found in Appendix SI in the Supporting Information.

ISOTOPE TURNOVER

Female Sprague Dawley rats ($n = 13$) were impregnated at Charles River Laboratories and received 2 days later on 10 November 2005 at the University of California Santa Cruz vivarium. Rats were

randomly assigned to separate cages and fed an experimental, vegetarian, C_3 plant-based diet consisting of protein from wheat gluten, sucrose from beet sugar, and lipids from cotton seed oil (all components were from C_3 plants) along with cellulose and vitamin and mineral mixes (Table 1). All females gave birth over a 24 h period beginning on 8 December 2005 and all pups nursed from their mothers until weaning 26 days later on 3 January 2006. After weaning, 6 (3 female, 3 male) pups were euthanized and their tissues analysed for use as an isotopic baseline, and 54 (27 female, 27 male) randomly chosen rats were held on the vegetarian experimental diet. Ninety days post-weaning, 6 (3 female, 3 male) control rats continued to be held on the vegetarian diet and all remaining rats were switched to a second experimental, fish- and C_4 plant-based diet containing protein from Bering Sea walleye pollock (*Theragra chalcogramma*) fish meal, sucrose from cane sugar, lipids from corn oil (C_4 plants) and the same cellulose, vitamin, and mineral compositions as the C_3 vegetarian experimental diet (Table 1). This was referred to as day 0 and rats were approximately 16·5 weeks old and considered full grown. Rats were then euthanized via CO_2 asphyxiation according to a pre-determined schedule (days 0, 2, 5, 15, 30, 75, 120, 166, 187, 253, 258), sub-samples of blood, liver, kidney, muscle, and fur were removed, rinsed with water (except for blood), blood was separated into cellular and serum components, and all tissues analysed for stable C and N isotope ratios. Blood was taken by syringe from the heart, muscle was taken from the upper right leg, and fur was taken from the belly.

ISOTOPE ANALYSIS

Tissues were freeze dried for 24 h, and all lipids removed using petroleum ether (Dobush *et al.* 1985; Martinez del Rio & Anderson-Sprecher 2008) in a Dionex ASE-200 Accelerated Solvent Extractor. Samples were ground to a powder by hand and 0·7–1·0 mg were sealed into tin capsules and stable C and N isotope ratios analysed for one third of the samples using a Costech Analytical Technologies ECS4010 elemental analyser interfaced via a CONFLO II device to a Thermo-Electron Delta Plus XL mass spectrometer at the Colorado Plateau Stable Isotope Laboratory, Northern Arizona University Department of Biological Sciences. The $\delta^{15}N$ and $\delta^{13}C$ values from the remaining samples were analysed using a Carlo Erba CE1108 elemental analyser interfaced via a CONFLO III device to a Thermo-Electron Delta Plus XP mass spectrometer at the University of California Santa Cruz Department of Earth and Marine Sciences.

STATISTICAL ANALYSIS

Calculations of the reaction progress variable, the single and dual compartment RPVM and nonlinear models, the stable isotope retention times and half-lives, and AIC values are described in detail in Appendix S1 in the Supporting Information.

I used paired *t*-tests to determine differences in the $\delta^{15}N$ and $\delta^{13}C$ values between sexes for each tissue from the isotope turnover data. I used *t*-tests to determine differences in $\delta^{15}N$ and $\delta^{13}C$ values between sexes for each tissue type from rats held on the same diet (SYSTAT version 10·2; SYSTAT Software). Significance was tested at the $\alpha = 0{\cdot}05$ level. Differences in half-lives and retention times between the nonlinear and RPVM methods were determined by examining if the mean values from the RPVM fell within the mean ± 95% confidence intervals obtained using the nonlinear models.

Table 1. Rat dietary compositions and their macronutrient components. One percent of vitamins, 1·34% of minerals, and 5% of cellulose (Solka-Floc® wood fibre) were added to each diet. All diet pellets were manufactured by Harlan Teklad

Diet	Composition		Diet components and their isotope values (‰)							Whole diet isotope values (‰) and nitrogen and carbon concentration (±SE, $n = 3$)				
	Protein Source	Energy Source[a]	Protein (%)	Protein $\delta^{15}N$	Protein $\delta^{13}C$	Sucrose (%)	Sucrose $\delta^{13}C$	Oil (%)	Oil ^{13}C	$\delta^{15}N$	$\delta^{13}C$	% N	% C	C : N
Plant	C_3 plant	C_3	Wheat gluten (30·0)	4·7	–25·8	Beet (53·0)	–24·9	Cottonseed (7·0)	–28·5	4·8 ± 0·1	–25·3 ± 0·0	4·4 ± 0·2	44·1 ± 0·4	10·1 ± 0·5
Marine	Marine fish	C_4	Fish meal (40·0)[b]	12·5	–19·8	Cane (45·5)	–11·7	Corn (7·0)	–14·9	12·3 ± 0·1	–16·2 ± 0·2	5·4 ± 0·3	43·3 ± 0·3	8·1 ± 0·6

[a]Energy is comprised of sucrose and oil.
[b]Fish meal is comprised of wall eye pollock *Theragra chalcogramma* from the Bering sea and produced by Unisea, Inc.

Table 2. Mean (± SE) $\delta^{15}N$ and $\delta^{13}C$ values (‰) for control rats (n = 3 for each gender) held on the C_3-plant vegetarian diet for 276–277 days and the t- and P-values from the t-tests used to test for differences in isotope values between sexes; d.f. is 4 for all tests and an asterisk indicates a significant difference in isotope values between sexes

	Female		Male		$\delta^{15}N$	$\delta^{13}C$
Tissue	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	t- and P-values	t- and P-values
Fur	7·3 ± 0·1	–22·1 ± 0·1	7·1 ± 0·0	–22·4 ± 0·1	1·8, 0·16	2·5, 0·07
Kidney	7·6 ± 0·0*	–24·3 ± 0·1	7·0 ± 0·1*	–24·2 ± 0·1	6·6, 0·003	–1·4, 0·24
Liver	8·0 ± 0·2	–24·3 ± 0·0	7·8 ± 0·5	–24·5 ± 0·1	1·6, 0·19	0·4, 0·69
Muscle	7·3 ± 0·0*	–23·9 ± 0·0	6·8 ± 0·1*	–23·8 ± 0·1	5·8, 0·004	–0·6, 0·56
Plasma	8·5 ± 0·1*	–24·0 ± 0·1	8·3 ± 0·0*	–24·2 ± 0·0	3·5, 0·03	2·2, 0·09
RBC	7·0 ± 0·1*	–24·4 ± 0·1	6·5 ± 0·1*	–24·3 ± 0·1	3·5, 0·03	1·1, 0·33

Results

Mean isotope values ± SE for control animals held on the C_3 plant vegetarian diet for 276–277 days and for all animals in the diet changeover experiment are reported in Tables 2 and 3. The mean $\delta^{15}N$ values from females were higher than those of males in the control animals for all tissues except fur and liver (t-tests; Table 2) and for all tissues except fur in the diet changeover rats (paired t-tests; Table 3). There were no differences in mean $\delta^{13}C$ values between genders for the control rats (Table 2), but the mean $\delta^{13}C$ values from females were higher than those of males for kidney, liver, and red blood cells (RBC) for the diet changeover animals (Table 3).

The RPVM indicated that 2 compartment models might be appropriate for the best estimation of N and C isotope incorporation in liver, serum, and kidney from both male and female rats. However, examination of the AICc values demonstrated that 2 compartment models provided the best estimation of N and C isotope incorporation only for serum and kidney tissues, whereas a single compartment model was best for liver tissue (Table 4). The RPVM indicated that isotope incorporation in all other tissues was best described by a single compartment model.

The isotope integration rates of C and N varied substantially across the different tissues (Figs S1–S6, Table 5), but the order of isotope retention times in the tissues were the same regardless of sex and isotope type when using the nonlinear models. The order (from shortest to longest) of the overall mean retention times for N and C in tissues from female and male rats using either the single or the dual compartment nonlinear models was: liver < serum < kidney < RBC < muscle < fur (Figs S1–S6, Table 6). N isotope incorporation took longer than that for C in liver tissue. There were no statistically significant differences in the isotope half-lives or retention times between sexes as indicated by the 95% confidence intervals (Table 5).

The RPVM demonstrated intercepts for serum, liver, and kidney <0·0 indicating multiple source pools may have been contributing to the $\delta^{15}N$ and $\delta^{13}C$ values from those tissues (See example in Fig. 1 and Figs S1–S3). The intercepts for RBC, muscle, and fur were >0·0 indicating delays before the diet switch was apparent in the isotope values of the tissues (Figs S4–S6). Mean N and C isotope half-lives, retention times, and delays (where applicable) in days in tissues from rats using the RPVM are in Table 5. The orders of mean isotope retention times were different than those obtained using the nonlinear models and varied between isotope type and sex (Table 6).

I compared overall mean half-lives and retention times as they were determined with the nonlinear models and the RPVM using the 95% confidence intervals from the nonlinear models. For N and C from females and for N from males, the pattern was that half-lives and residence times were shorter using the nonlinear models for liver tissue, the same between both methods for serum and kidney (except for N in kidney from females where the RPVM indicated faster turnover), and shorter using the RPVM for RBC, muscle, and fur tissues. The pattern was slightly different for half-lives and retention times of C from males. Again the values from the RPVM were shorter for RBC and fur, and there were no differences between methods for serum and kidney as above, but values for liver and muscle were also the same (Table 5). When a delay in the time between diet ingestion and a corresponding change in isotope values was indicated by the RPVM, adding the delay time onto the half-lives and retention times estimated using the RPVM accounted for the difference relative to the nonlinear models in most cases (Table 5).

Discussion

The verification of the isotope turnover times from various animal tissues provides an important tool for ecological studies. These data enable scientists to interpret temporal variation in foraging ecology of wild animals with the relatively simple collection and analyses of a host of tissues from the animals of interest. This study demonstrates that these temporal interpretations of animal diet can be made with the non-lethal collection of tissues such as serum, RBC, and fur that provide information about an animal's recent, intermediate, and long term dietary habits, respectively. Determination of the foraging ecology of endangered or otherwise protected species is especially important, and the use of non-lethal means to do so is invaluable. The exponential models provided a consistent order in the timing of both N and C isotope turnover from the

Table 3. The mean $\delta^{15}N$ and $\delta^{13}C$ values (‰, ±SE) for select rat tissues held on the C_3 plant diet for 90 days post-weaning and switched to the C_4 plant and marine diet. Time is in days; –90 indicates the day rats were weaned and started on the C_3 plant diet; 0 indicates the day rats were switched to the C_4 plant and marine diet 90 days after they weaned. An asterisk indicates a significant difference in values between sexes as demonstrated by paired *t*-tests; test results are footnoted beneath the table

		Fur				Kidney				Liver			
		Female†		Male†		Female‡		Male‡		Female§		Male§	
Time	*N*	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$*	$\delta^{13}C$*	$\delta^{15}N$*	$\delta^{13}C$*	$\delta^{15}N$*	$\delta^{13}C$*	$\delta^{15}N$*	$\delta^{13}C$*
–90	3	7·2 ± 0·1	–21·9 ± 0·0	7·4 ± 0·0	–21·8 ± 0·0	6·2 ± 0·1	–24·1 ± 0·1	6·0 ± 0·0	–24·2 ± 0·2	7·1 ± 0·1	–23·9 ± 0·0	7·1 ± 0·1	–24·0 ± 0·0
0	2	7·1 ± 0·1	–22·0 ± 0·2	6·8 ± 0·1	–22·7 ± 0·6	7·7 ± 0·1	–23·9 ± 0·2	6·9 ± 0·1	–24·1 ± 0·1	8·4 ± 0·0	–23·5 ± 0·2	7·7 ± 0·1	–23·6 ± 0·1
2	2	6·8 ± 0·0	–21·9 ± 0·0	6·8 ± 0·0	–22·0 ± 0·1	8·8 ± 0·3	–22·4 ± 0·3	8·4 ± 0·0	–22·7 ± 0·1	9·9 ± 0·0	–20·6 ± 0·1	9·7 ± 0·0	–21·0 ± 0·1
5	2	7·1 ± 0·0	–22·2 ± 0·3	6·8 ± 0·1	–22·2 ± 0·1	10·0 ± 0·1	–21·3 ± 0·1	9·3 ± 0·1	–21·6 ± 0·1	11·2 ± 0·3	–19·0 ± 0·5	10·5 ± 0·1	–20·2 ± 0·4
15	2	7·2 ± 0·0	–21·8 ± 0·1	6·9 ± 0·0	–22·1 ± 0·0	11·9 ± 0·1	–20·2 ± 0·2	11·4 ± 0·0	–19·7 ± 0·0	13·4 ± 0·1	–17·4 ± 0·0	13·0 ± 0·0	–18·4 ± 0·1
30	2	10·6 ± 0·8	–19·5 ± 0·6	7·0 ± 0·0	–22·0 ± 0·0	13·1 ± 0·0	–18·8 ± 0·1	12·6 ± 0·1	–19·0 ± 0·1	14·5 ± 0·1	–17·7 ± 0·0	13·9 ± 0·2	–18·2 ± 0·1
75	2	12·3 ± 0·0	–18·8 ± 0·0	11·7 ± 0·2	–19·1 ± 0·2	14·2 ± 0·0	–18·4 ± 0·0	13·8 ± 0·0	–19·0 ± 0·6	15·4 ± 0·1	–17·7 ± 0·4	14·7 ± 0·1	–18·9 ± 0·2
120	2	12·5 ± 0·1	–18·4 ± 0·1	14·0 ± 1·0	–17·6 ± 0·4	14·9 ± 0·1	–17·8 ± 0·0	14·3 ± 0·1	–18·2 ± 0·3	15·4 ± 0·0	–17·6 ± 0·3	15·0 ± 0·0	–18·0 ± 0·2
166	2	15·1 ± 0·0	–16·8 ± 0·1	14·6 ± 0·3	–17·0 ± 0·2	14·8 ± 0·0	–17·5 ± 0·1	14·4 ± 0·0	–17·5 ± 0·2	15·4 ± 0·0	–17·8 ± 0·2	14·9 ± 0·2	–17·6 ± 0·4
187	3	14·2 ± 0·3	–17·2 ± 0·1	14·9 ± 0·1	–16·5 ± 0·1	15·1 ± 0·1	–17·5 ± 0·0	14·7 ± 0·0	–17·5 ± 0·0	15·3 ± 0·0	–16·9 ± 0·0	15·3 ± 0·0	–19·2 ± 0·2
253	2	15·3 ± 0·2	–16·1 ± 0·0	15·3 ± 0·4	–16·5 ± 0·4	14·9 ± 0·0	–17·5 ± 0·5	14·8 ± 0·1	–17·4 ± 0·3	15·0 ± 0·4	–17·0 ± 0·7	15·1 ± 0·2	–17·4 ± 0·1
258	2	15·7 ± 0·4	–16·1 ± 0·2	14·6 ± 0·3	–16·4 ± 0·4	15·1 ± 0·2	–17·6 ± 0·2	14·8 ± 0·1	–17·8 ± 0·1	15·1 ± 0·1	–17·0 ± 0·1	14·6 ± 0·2	–17·6 ± 0·2

		Muscle				Serum				RBC			
		Female¶		Male¶		Female**		Male**		Female††		Male††	
Time	*N*	$\delta^{15}N$*	$\delta^{13}C$	$\delta^{15}N$*	$\delta^{13}C$	$\delta^{15}N$*	$\delta^{13}C$	$\delta^{15}N$*	$\delta^{13}C$	$\delta^{15}N$*	$\delta^{13}C$*	$\delta^{15}N$*	$\delta^{13}C$*
–90	3	6·8 ± 0·1	–23·8 ± 0·0	6·7 ± 0·1	–24·1 ± 0·0	NA	NA	NA	NA	NA	NA	NA	NA
0	2	7·4 ± 0·1	–24·0 ± 0·4	6·7 ± 0·2	–23·3 ± 0·5	8·9 ± 0·1	–23·8 ± 0·1	8·3 ± 0·1	–23·9 ± 0·1	6·9 ± 0·0	–23·8 ± 0·1	6·6 ± 0·1	–24·2 ± 0·0
2	2	7·5 ± 0·1	–23·4 ± 0·2	7·0 ± 0·0	–23·4 ± 0·1	11·9 ± 0·7	–20·3 ± 0·8	10·6 ± 0·2	–21·2 ± 0·0	7·1 ± 0·1	–23·6 ± 0·0	6·7 ± 0·0	–24·0 ± 0·0
5	2	8·0 ± 0·0	–23·5 ± 0·7	7·3 ± 0·1	–22·9 ± 0·0	12·4 ± 0·6	–19·7 ± 0·6	12·2 ± 0·0	–19·8 ± 0·1	7·5 ± 0·1	–23·2 ± 0·0	7·1 ± 0·0	–23·7 ± 0·0
15	2	9·1 ± 0·1	–22·1 ± 0·3	8·6 ± 0·0	–21·9 ± 0·0	14·5 ± 0·0	–18·0 ± 0·0	14·0 ± 0·1	–18·2 ± 0·2	8·6 ± 0·3	–22·3 ± 0·2	8·5 ± 0·1	–22·1 ± 0·3
30	2	10·3 ± 0·1	–20·6 ± 0·0	9·6 ± 0·3	–20·9 ± 0·0	14·9 ± 0·1	–17·8 ± 0·1	14·7 ± 0·1	–17·8 ± 0·1	11·0 ± 0·4	–20·5 ± 0·2	9·9 ± 0·1	–21·1 ± 0·1
75	2	12·8 ± 0·2	–19·0 ± 0·1	12·0 ± 0·1	–19·4 ± 0·5	15·7 ± 0·1	–17·4 ± 0·0	15·2 ± 0·0	–17·3 ± 0·0	14·7 ± 0·4	–18·1 ± 0·1	13·9 ± 0·2	–18·2 ± 0·0
120	2	14·2 ± 0·3	–17·8 ± 0·0	13·4 ± 0·2	–18·3 ± 0·3	16·0 ± 0·0	–17·4 ± 0·0	15·6 ± 0·0	–17·1 ± 0·1	15·2 ± 0·0	–17·6 ± 0·1	14·7 ± 0·0	–17·8 ± 0·2
166	2	14·5 ± 0·0	–17·9 ± 0·2	13·9 ± 0·0	–17·5 ± 0·0	15·8 ± 0·0	–17·1 ± 0·1	15·4 ± 0·1	–17·1 ± 0·1	15·3 ± 0·0	–17·3 ± 0·1	14·9 ± 0·1	–17·4 ± 0·0
187	3	15·2 ± 0·2	–17·4 ± 0·1	14·4 ± 0·2	–17·4 ± 0·1	16·0 ± 0·1	–17·3v0·0	15·6 ± 0·1	–16·9 ± 0·0	15·4 ± 0·1	–17·2 ± 0·0	15·0 ± 0·1	–17·4 ± 0·1
253	2	14·8 ± 0·0	–17·1 ± 0·1	14·5 ± 0·2	–17·3 ± 0·2	15·6 ± 0·4	–17·1 ± 0·0	15·6 ± 0·1	–17·2 ± 0·2	15·1 ± 0·3	–17·3 ± 0·1	15·0 ± 0·1	–17·3 ± 0·0

†$\delta^{15}N$: $t_{32} = 1{\cdot}5$, $P = 0{\cdot}14$; $\delta^{13}C$: $t_{32} = 1{\cdot}5$, $P = 0{\cdot}13$; ‡$\delta^{15}N$: $t_{38} = 12{\cdot}0$, $P = 0{\cdot}00$; $\delta^{13}C$: $t_{38} = 2{\cdot}0$, $P = 0{\cdot}05$; §$\delta^{15}N$: $t_{31} = 6{\cdot}6$, $P = 0{\cdot}00$; $\delta^{13}C$: $t_{31} = 4{\cdot}5$, $P = 0{\cdot}00$;
¶$\delta^{15}N$: $t_{38} = 11{\cdot}0$, $P = 0{\cdot}00$; $\delta^{13}C$: $t_{38} = -0{\cdot}4$, $P = 0{\cdot}72$; **$\delta^{15}N$: $t_{35} = 7{\cdot}2$, $P = 0{\cdot}00$; $\delta^{13}C$: $t_{29} = 0{\cdot}4$, $P = 0{\cdot}70$; ††$\delta^{15}N$: $t_{29} = 7{\cdot}3$, $P = 0{\cdot}00$; $\delta^{13}C$: $t_{29} = 3{\cdot}8$, $P = 0{\cdot}00$.

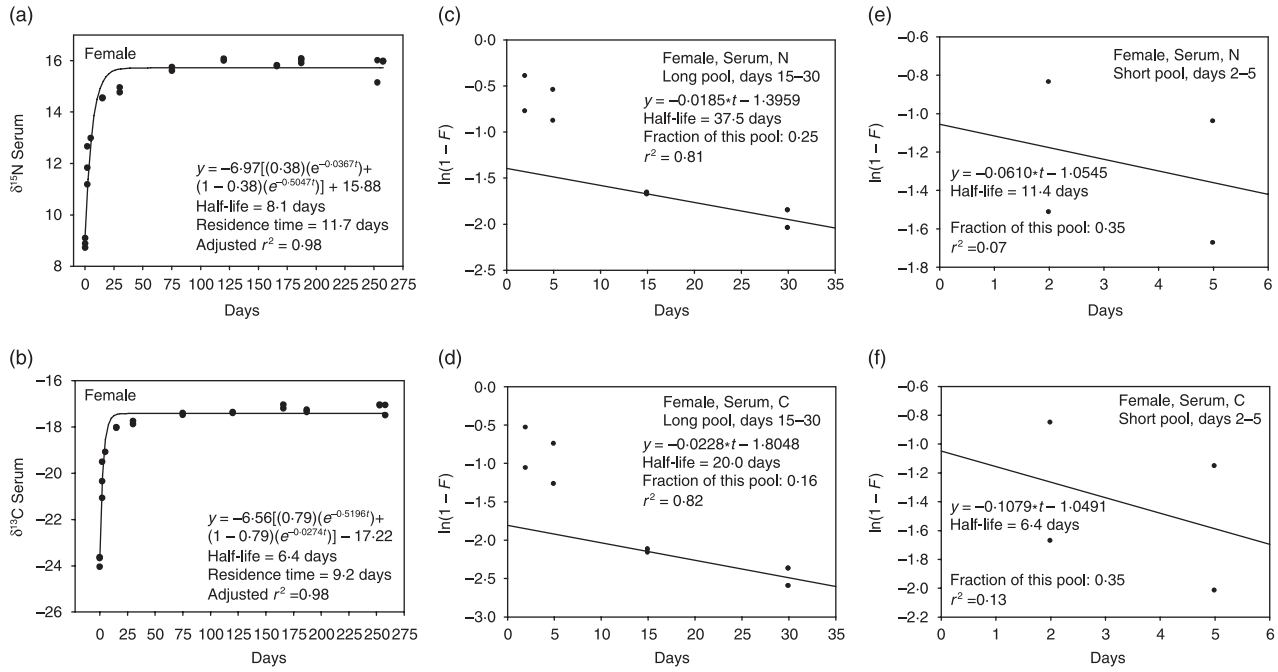

Fig. 1. (a, b) Nonlinear, 2 pool models for N and C isotope incorporation rates in serum from captive female rats switched on day 0 from a C_3 plant-based diet to a fish and C_4 plant-based diet. (c–f) Reaction progress variable method 2 pool models for the same data. (c, d) Graphs show data from days 2–30 from the RPVM, but only the data from days 15 to the turnover equilibrium on day 30 are included in the regression line to compute the half-lives and pool fractions for the long isotope turnover pools. (e, f) Graphs use data from days 2 to 5 to compute the half-lives and pool fractions of the short isotope turnover pools; data are the first residuals derived by subtracting (1–F) values of the long component from the total (1–F) values shown in the companion graphs c and d. Data are graphed as individual rats; $n = 2$ rats for each sex on each day except day 187 where $n = 3$. See Figs S1–S6 in the Supporting Information for isotope turnover models for all tissues.

Table 4. The overall mean half-lives and retention times in days with 95% confidence intervals and AICc values from 1 and 2 compartment nonlinear models describing nitrogen and carbon isotope turnover in the 3 tissues that the reaction progress variable method indicated may best be described by 2 compartment models. Models with the lowest AICc value are judged to be the best fit; the AICc values from these data demonstrate that 2 compartment models are appropriate only for serum and kidney tissues, whereas a 1 compartment model is best for liver. See Appendix S1 in the Supporting Information for equations for the models and computing AICc

Tissue	Gender	Isotope	Number of compartments *n* model	AICc	Overall mean half-life (95% confidence interval)	Overall mean retention time (95% confidence interval)	Model chosen
Liver	Female	Nitrogen	1	11·4	7·7 (6·7, 9·0)	11·1 (9·7, 13·0)	
			2	13·2	8·5 (4·8, 29·4)	12·2 (6·8, 42·3)	1
		Carbon	1	23·6	2·4 (1·9, 3·5)	3·5 (2·7, 5·0)	
			2	29·3	3·0 (1·8, 9·5)	4·4 (2·6, 13·7)	1
Liver	Male	Nitrogen	1	17·767	7·8 (6·6, 9·6)	11·3 (9·5, 13·9)	
			2	17·762	10·0 (5·2, 214·2)	14·5 (7·5, 309·1)	1
		Carbon	1	24·7	3·1 (2·1, 5·9)	4·5 (3·1, 8·6)	
			2	35·1	4·8 (2·4, 460·9)	6·9 (3·5, 664·9)	1
Serum	Female	Nitrogen	1	25·7	4·2 (3·2, 6·3)	6·1 (4·6, 9·1)	
			2	21·4	8·1 (4·5, 51·8)	7·3 (6·4, 74·7)	2
		Carbon	1	19·7	2·1 (1·7, 2·7)	3·0 (2·5, 3·9)	
			2	18·7	6·4 (3·1, 19·0)	9·2 (4·4, 27·4)	2
Serum	Male	Nitrogen	1	19·3	4·9 (4·1, 6·3)	7·1 (5·8, 9·0)	
			2	10·0	8·4 (5·2, 22·5)	12·0 (7·5, 32·5)	2
		Carbon	1	24·6	3·2 (2·5, 4·3)	4·6 (3·6, 6·2)	
			2	20·0	6·4 (3·5, 35·1)	9·2 (5·1, 50·7)	2
Kidney	Female	Nitrogen	1	16·2	13·3 (11·4, 15·9)	19·2 (16·4, 23·0)	
			2	7·1	19·4 (14·0, 31·2)	27·8 (20·3, 45·0)	2
		Carbon	1	23·5	11·1 (8·7, 15·6)	16·1 (12·5, 22·5)	
			2	19·2	16·5 (10·1, 44·3)	23·8 (14·6, 64·0)	2
Kidney	Male	Nitrogen	1	20·2	9·1 (7·1, 12·8)	13·2 (10·3, 18·4)	
			2	3·9	22·3 (17·2, 31·7)	32·1 (24·8, 45·7)	2
		Carbon	1	24·1	13·1 (11·0, 16·2)	18·9 (15·9, 23·4)	
			2	23·2	37·1 (15·7, 77·5)	53·5 (22·6, 111·7)	2

Table 5. Mean half-lives and residence times of N and C estimated from single or dual compartment [denoted as 1 (short pool) and 2 (long pool)] nonlinear models reported in days with 95% confidence intervals in parentheses from 6 tissues from rats. Values derived using the reaction progress variable method are also reported, but without confidence intervals as the method does not allow for calculation of error. See Appendix S1 in the Supporting Information for calculations

	Female																									
	Nitrogen													Carbon												
	Nonlinear Models						RPVM							Nonlinear Models						RPVM						
	Half-lives			Residence times			Half-lives			Residence times				Half-lives			Residence times			Half-lives			Residence Times			
Tissue	1	2	Overall	1	2	Overall	1	2	Overall	1	2	Overall	Delay	1	2	Overall	1	2	Overall	1	2	Overall	1	2	Overall	Delay
Liver	7·7 (6·7, 9·0)	NA	NA	11·1 (9·7, 3·0)	NA	NA	10·2	NA	NA	14·7	NA	NA	NA	2·4 (1·9, 3·5)	NA	NA	3·5 (2·7, 5·0)	NA	NA	7·3	NA	NA	10·5	NA	NA	NA
Serum	1·4 (0·9, 3·3)	18·9 (10·2, 129·5)	8·1 (4·5, 51·8)	2·0 (1·3, 4·7)	27·3 (14·7, 186·9)	11·7 (6·4, 74·7)	11·4	37·5	16·0	16·4	54·1	23·0	NA	1·3 (1·0, 2·1)	25·3 (11·0, 82·5)	6·4 (3·1, 19·0)	1·9 (1·4, 3·0)	36·5 (15·8, 119·0)	9·2 (4·4, 27·4)	6·4	20·0	8·0	9·3	43·9	16·9	NA
Kidney	4·8 (3·5, 7·7)	33·9 (24·5, 54·7)	19·4 (14·0, 31·2)	6·7 (5·1, 11·1)	48·9 (35·4, 78·9)	27·8 (20·3, 45·0)	9·6	20·0	9·5*	13·9	28·9	13·8*	NA	2·6 (1·6, 7·3)	29·3 (18·0, 78·4)	16·5 (10·1, 44·3)	3·8 (2·3, 10·6)	42·3 (26·0, 113·2)	23·8 (14·6, 64·0)	9·6	32·4	11·9	13·9	46·7	32·1	NA
RBC	29·3 (25·3, 34·9)	NA	NA	42·2 (36·4, 50·3)	NA	NA	15·2	NA	NA	22·0	NA	NA	8·4	28·1 (26·0, 30·7)	NA	NA	40·7 (37·5, 44·2)	NA	NA	23·0	NA	NA	33·2	NA	NA	4·2
Muscle	40·8 (36·3, 46·4)	NA	NA	58·8 (52·4, 67·0)	NA	NA	31·1	NA	NA	44·8	NA	NA	4·8	31·6 (24·9, 43·1)	NA	NA	45·5 (36·0, 62·1)	NA	NA	24·5	NA	NA	35·3	NA	NA	4·6
Fur	65·1 (46·2, 110·1)	NA	NA	93·5 (66·6, 158·8)	NA	NA	42·3	NA	NA	61·0	NA	NA	14·0	85·4 (59·7, 150·4)	NA	NA	123·5 (86·1, 217·0)	NA	NA	59·2	NA	NA	85·5	NA	NA	3·8
	Male																									
	Nitrogen													Carbon												
	Nonlinear models						RPVM							Nonlinear models						RPVM						
	Half-lives			Residence times			Half-lives			Residence times				Half-lives			Residence times			Half-lives			Residence times			
Tissue	1	2	Overall	1	2	Overall	1	2	Overall	1	2	Overall	Delay	1	2	Overall	1	2	Overall	1	2	Overall	1	2	Overall	Delay
Liver	7·8 (6·6, 9·6)	NA	NA	11·3 (9·5, 13·9)	NA	NA	11·4		NA	16·5	NA	NA	NA	3·1 (2·1, 5·9)	NA	NA	4·5 (3·1, 8·6)	NA	NA	3·4	NA	NA	4·9	NA	NA	NA
Serum	2·5 (1·8, 3·8)	19·2 (11·5, 57·3)	8·4 (5·2, 22·5)	3·6 (2·6, 5·5)	27·7 (16·6, 32·7)	12·0 (7·5, 32·5)	2·1	15·9	10·4	3·0	22·9	14·9	NA	1·2 (0·7, 3·3)	14·7 (8·0, 86·3)	6·4 (3·5, 35·1)	1·7 (1·0, 4·8)	21·2 (11·6, 124·6)	9·2 (5·1, 50·7)	2·6	23·3	15·1	3·7	33·7	21·8	NA
Kidney	4·7 (3·8, 6·3)	42·4 (32·5, 60·7)	22·3 (17·2, 31·7)	6·8 (5·4, 9·0)	61·1 (46·9, 87·6)	32·1 (24·8, 45·7)	4·5	32·7	17·6	6·5	47·2	25·4	NA	4·2 (2·9, 7·9)	93·2 (37·6, 196·0)	37·1 (15·7, 77·5)	6·1 (4·1, 11·4)	134·4 (54·2, 282·7)	53·5 (22·6, 111·7)	15·5	33·2	21·7	22·4	87·7	51·2	NA
RBC	33·7 (29·0, 40·2)	NA	NA	48·5 (41·9, 58·0)	NA	NA	21·9	NA	NA	31·6	NA	NA	7·3	30·6 (27·1, 35·1)	NA	NA	44·1 (39·1, 50·6)	NA	NA	22·4	NA	NA	32·4	NA	NA	4·8
Muscle	45·3 (39·6, 52·9)	NA	NA	65·4 (57·1, 76·3)	NA	NA	36·1	NA	NA	52·1	NA	NA	2·6	36·4 (31·3, 43·5)	NA	NA	52·6 (45·2, 62·7)	NA	NA	39·2	NA	NA	56·5	NA	NA	0·9
Fur	70·2 (48·4, 127·1)	NA	NA	101·0 (69·9, 183·3)	NA	NA	29·0	NA	NA	41·8	NA	NA	22·9	79·9 (53·4, 158·7)	NA	NA	114·6 (77·1, 228·9)	NA	NA	40·5	NA	NA	58·5	NA	NA	17·4

*The overall mean half-life and residence time for N in kidney tissue from females were shorter than the first pool turnover time. This is because the fraction of the first turnover pool was 91% and the fraction for the second was only 4%. See Appendix S1 in the Supporting Information for the full description of how these terms are calculated and see Results for description of why the fractions do not equal 100%

Table 6. Order of overall mean retention times from shortest (top) to longest (bottom) were consistent between sexes and isotope type when estimated with the nonlinear models, but varied between sexes and isotope type when estimated using the reaction progress variable method. There were also differences in the order between the two model types

Nonlinear models				RVPM			
Female		Male		Female		Male	
N	C	N	C	N	C	N	C
Liver	Liver	Liver	Liver	Kidney	Liver	Serum	Liver
Serum	Serum	Serum	Serum	Liver	Serum	Liver	Serum
Kidney	Kidney	Kidney	Kidney	RBC	Kidney	Kidney	RBC
RBC	RBC	RBC	RBC	Serum	RBC	RBC	Kidney
Muscle	Muscle	Muscle	Muscle	Muscle	Muscle	Muscle	Muscle
Fur	Fur	Fur	Fur	Fur	Fur	Fur	Fur

Table 7. Comparison of mean nitrogen (N) and carbon (C) isotope half-lives (days) in tissues from rodents of varying sizes in order from smallest (top) to largest (bottom). All half-lives were calculated using the nonlinear method

Rodent	Tissue	*N* half-life	C half-life	Study
Mouse (*mus musculus*)	Liver	7·3	NA	MacAvoy *et al*. 2005
Mouse (*mus musculus*)	Muscle	24·8	23·9	MacAvoy *et al*. 2005
Mouse (*mus musculus*)	Liver	12 to 15·1	7·7	Arneson *et al*. 2006
Mouse (*mus musculus*)	Muscle	18·2 to 21·7	18·2	Arneson *et al*. 2006
Gerbil (*Meriones unguienlatus*)	Liver	NA	6·4	Tieszen *et al*. 1983
Gerbil (*Meriones unguienlatus*)	Muscle	NA	27·6	Tieszen *et al*. 1983
Gerbil (*Meriones unguienlatus*)	Fur	NA	47·5	Tieszen *et al*. 1983
Rat (*R. norvegicus*)	Liver	7·7 to 7·8	2·4 to 3·1	This study
Rat (*R. norvegicus*)	Muscle	40·8 to 45·3	31·6 to 36·4	This study
Rat (*R. norvegicus*)	Fur	65·1 to 70·2	79·9 to 85·4	This study

various tissues. This suggests that a general pattern of short, intermediate, and long-term isotope retention times can be applied to the appropriate tissue types regardless of the study animal. Finally, I recommend the use of the RPVM to estimate the need for multiple compartment models and to estimate the potential delays in isotope incorporation. Single or multiple compartment nonlinear models are best for estimating isotope incorporation rates.

The rate at which C and N integrated into the rat tissues varied substantially among tissues regardless of the model used. Short (liver and serum), intermediate (kidney, RBC, and muscle), and long (fur) half-lives and retention times were demonstrated for both N and C from the various tissues. These general trends were similar for those found using a single compartment exponential equation model for C turnover from liver, kidney, and muscle tissue from gerbils (*Meriones unguienlatus*) (Tieszen *et al*. 1983), for N turnover from liver and muscle tissue from mice (*Mus musculus*) (MacAvoy *et al*. 2005), and for N and C turnover from liver, kidney, and muscle tissue from mice (Arneson *et al*. 2006) (Table 7). Isotope incorporation rates vary among tissues due to a variety of factors including tissue metabolism. Tissues are constantly broken down and regenerated and the amino acids in body fluids that are utilized to build proteins come both from the tissues that are degrading (endogenous sources) and dietary protein, carbohydrate, and lipid intake (exogenous sources). All amino acids are mixed in the blood and the speed at which a tissue reaches equilibrium with a dietary amino acid source is correlated with the metabolic activity of that particular tissue and the overall metabolic rate of the animal in question (Waterlow *et al.* 1978; Tieszen *et al*. 1983; Ayliffe *et al*. 2004). Some tissues such as collagen can take years to equilibrate with dietary sources while other, more metabolically active tissues such as liver and serum take only a few days (Ayliffe *et al*. 2004), hence the differences in isotope turnover times observed between tissue types.

COMPARISON OF ISOTOPE RETENTION TIMES BETWEEN MODELS

Overall mean N and C isotope incorporation in serum and kidney tissue was not significantly different between the RPVM and nonlinear models (except for N in kidney from females), but significant differences between models were apparent in all other tissue types, some of which can be amended by accounting for delays in isotopic incorporation (see below). Interestingly, serum and kidney were the only tissues for which 2 compartment models were indicated as the best fit, but the physiological reasons for this are not apparent. Regardless, it may be acceptable to use either a 2-compartment nonlinear or RPVM model to estimate isotopic incorporation rates for kidney or serum. However, I recommend the use of

single or multiple compartment nonlinear models to estimate isotope turnover in all tissues because: (i) the nonlinear models provide measurements of error (confidence intervals) and the RPVM does not, (ii) the nonlinear models have fewer restrictions in the inclusion of the isotope values than the RPVM at the start and end of the isotope turnover experiment that come with the limitations imposed by linearizing the data with a ln function (see Martinez Del Rio & Anderson-Sprecher 2008), and (iii) the nonlinear models provided consistent results across sexes and N and C isotopes whereas the RPVM did not, and there is no clear physiological reason why the order of isotope retention times would differ across sexes and isotopes with the RPVM.

MEASURING MULTIPLE POOLS

The RPVM models for serum and kidney indicated that multiple pools were contributing to the N and C isotope incorporation rates. Fitting the dual compartment model to these data demonstrated that both faster and slower turnover pools were contributing to isotopic incorporation rates. When multiple pools are present and their incorporation rates quantified, fast turnover pools representing direct uptake of dietary components following digestion can have half-lives on the order of minutes to hours to a few days (Cerling *et al*. 2007; D. W. Podlesak, S. R. McWilliams & T. Cerling, unpublished) and can make-up a significant percentage of the C or N in a particular tissue. For example, serum is the liquid component of blood that carries RBC and other components and absorbs nutrients directly from the stomach and intestines to transport them to the rest of the body. Therefore, some of the serum is expected to exhibit a change in isotope values related to a diet switch very quickly. Other components of the serum such as urea and hormones that are created via mechanisms other than nutrient absorption and the breakdown/turnover of much larger pools with slower half-lives such as muscle and bone, would likely contribute to a slower turnover pool. Future diet changeover studies would benefit from sampling schemes designed to document isotope turnover in pools with very short isotope retention times. To best accomplish this, tissue sampling on the order of minutes to hours following a diet switch is vital so as not to miss these potentially important data. This is exemplified in the isotope data presented in this study. When using the 2-pool RPVM for isotope turnover in serum and kidney tissue, there were several instances where the fractional contribution of each pool did not add to 100% (see Figs 1 and S3E–L). This is likely because I missed important turnover data that occurred between days 0 and 2.

DELAY IN ISOTOPE INCORPORATION

The concept of a delay in the isotopic value change of tissues following a diet switch may be an important component to consider when modelling isotopic turnover. In this study, the RPVM indicated delays of a few hours to many days in the isotopic change of RBC, muscle, and fur after the diet switch (Table 5). Cerling *et al*. (2007) and D. W. Podlesak, S. R. McWilliams & T. Cerling (unpublished) found delays in the isotope value change of blood in birds and attributed them to the time it takes for the cellular components of blood to be made and released into the blood stream (erythropoiesis). This is likely causing the delay observed in RBC in this study as RBC in postnatal mammals are formed in the bone marrow and fed by arteries designed to transport nutrients to the centre of the marrow, a process taking approximately 12 days (Parmley 1988). Mature RBC take up nutrients as they circulate in the bloodstream (Sherwood 2004), but D. W. Podlesak, S. R. McWilliams & T. Cerling (unpublished) speculate that incorporation from a new diet after a switch may not be observable on a large scale until newly formed RBC are released into the blood stream. Myogenesis likely accounts for the delay observed in muscle tissue.

My data demonstrate a delay in the isotopic change of fur tissue when analysed with the RPVM; however Cerling *et al*. (2007) found no delay in hair from horses and instead found a system with multiple turnover pools. Rat hair growth can be up to 1 mm a day, occurs in waves across the body such that portions of the skin are actively growing hair while others are not, and varies seasonally even in captive rats held at constant temperatures (Durward & Rudall 1949; Ishiguro *et al*. 1993). I chose a random sample of well-mixed fur for analysis in this study. Therefore, the fur likely had hairs in different stages of growth, rest, or shed, which could account for the variation in isotope turnover time in fur samples and likely accounts for the delay observed using the RPVM.

The nonlinear models do not allow for the explicit modelling of a delay in isotopic change following a diet switch. The delays in isotope incorporation found in this study varied from 2·6 to 22·9 days and could potentially be important when trying to reconstruct animal diets in the wild. Therefore, when devising an experiment using one of the tissues that exhibit isotope delays in this study, it may be useful to use the RPVM to acquire an estimate of that delay. Finally, the time encompassing any possible delay is incorporated into the overall half-life and retention times generated by the nonlinear models. For example, RBC, muscle, and fur all demonstrated delays and shorter isotopic half-lives and retention times when quantified using the RPVM versus the nonlinear models. The addition of the delay onto the half-lives and retention times estimated using the RPVM accounts for most of the time differences exhibited between methods.

COMPARISON OF ISOTOPE INCORPORATION RATES FOR OTHER RODENTS

The trends in turnover times of isotopes from our tissues mirrored those from multiple species of rodents analysed in other studies; however the actual half-lives for the isotopes in our study were different. A tissue's turnover depends somewhat on the overall metabolic rate of the animal in question (Waterlow *et al*. 1978; MacAvoy *et al*. 2006). Tissues from animals with higher metabolic rates should have shorter isotope half-lives and thus faster isotope incorporation rates than animals with slower metabolisms. Therefore, tissues from animals of

different sizes and thus different metabolic rates would logically exhibit variations in isotope turnover times. This has been demonstrated experimentally between rats and mice (MacAvoy *et al*. 2006). Comparisons of the mean half-lives from muscle and fur in this study to those from gerbils and mice, smaller species with higher metabolic rates, mostly support this conclusion; however the results from liver tissue do not (Table 7). Tieszen *et al*. (1983) demonstrated longer half-lives of C in liver in gerbils than in this study, and Arneson *et al*. (2006) also found longer half-lives of C and N in liver tissue from mice. Finally, MacAvoy *et al*. (2005) found nearly identical half-lives for liver N from mice as those from rats in this study. The differences between rodents indicate that caution should be followed when using general rodent isotope incorporation rates to estimate diets of particular rodents in the wild.

SEX DIFFERENCES

Examination of the mean $\delta^{15}N$ and $\delta^{13}C$ values obtained from the tissues throughout the diet changeover experiment revealed some differences between sexes (Table 3). When there were significant differences in mean values between sexes for specific tissues, females always had higher $\delta^{15}N$ and $\delta^{13}C$ values than males (Table 3). However, the differences were very small and well below what could be considered ecologically significant when applying these data to interpretation of wild rat foraging ecology.

Isotope incorporation rates were not significantly different between sexes, but the trend was for shorter half-lives and retention times for females (except for C in fur which was the opposite). As stated above, size influences metabolism and the male rats in this study were on average 315 g heavier than female rats (mean female weight: 398·3 ± SD 65·2 g; mean male weight: 713·7 ± SD 106·5 g). As smaller animals tend to have higher metabolic rates, and the smaller females trended towards faster isotope incorporation rates, size disparity was likely driving this trend between the sexes. Studies of wild animal trophic ecology attribute differences in stable isotope values between sexes to variations in diet or foraging location (i.e. Kurle & Worthy 2001; Awkerman *et al.* 2007). My data suggest that sex differences in isotope values and isotope turnover times may be due to physiological factors not related to diet type and may need to be considered when analysing wild animal isotope data, especially in species where sexual dimorphism is pronounced.

COUPLING OF N AND C ISOTOPE TURNOVER

The hypothesis that isotopic turnover rates of N and C in bird and mammal tissues are coupled and therefore the same is supported by several studies, but only for some tissues and/or for some tissues from animals held on certain diet types (Haramis *et al*. 2001; Bearhop *et al*. 2002; Evans-Ogden *et al.* 2004; Macavoy *et al*. 2005; Mirón *et al*. 2006). On the other hand, several studies have rejected this hypothesis (Haramis *et al*. 2001; Hobson & Bairlein 2003; Pearson *et al*. 2003; Voigt & Matt 2004; MacAvoy *et al*. 2005; Carleton *et al*. 2005; Mirón *et al*. 2006). Existing data on isotopic turnover times for both N and C from mammals are scarce and results reporting which isotope turns over faster are variable, even for the same tissue from the same type of study animal. The majority of data from this study followed a trend of shorter half-lives and retention times for C, but this trend was only significant for liver tissue.

There is much speculation on why there may or may not be differences in the incorporation rates of C and N. Two non-exclusive hypotheses may explain these differences: (i) N incorporation rates are faster because they are only linked to protein turnover, whereas C rates are linked to protein, carbohydrate, and fat turnover, and (ii) N may be re-used endogenously, slowing its incorporation rate compared to C. The former hypothesis has been speculated to result in both faster and slower turnover C; faster because carbohydrates and fats from the diet are taken up more quickly than protein (Hobson & Bairlein 2003), and slower because C is present in body protein, nucleic acids, carbohydrates, and lipids thus representing total tissue turnover, whereas N is present mostly in protein and nucleic acids and thus reflects principally protein turnover (MacAvoy *et al*. 2005; Mirón *et al*. 2006). The latter hypothesis occurs when 'waste' endogenous N is re-used via transamination to amino acid C skeletons that have been synthesized from dietary carbohydrates and lipids. These non-essential amino acids with recycled N can then be incorporated into body protein. Essential amino acids cannot be created this way, and come directly from dietary C and nitrogen. This re-using of endogenous N works to slow down the incorporation of purely dietary N into body proteins compared to the incorporation of dietary C. This process would be more pronounced for animals fed a protein-poor diet, as they would be more pressed to re-use endogenous N, and that animals on a protein-rich diet would have closer coupling of C and N isotope turnover rates (Carleton *et al*. 2005).

The rats in my study were switched from a diet with protein from wheat gluten to a diet with protein from fish meal. Neither diet was protein poor, but I still saw turnover of C and N that varied from statistically the same (but with a trend of faster C isotope incorporation) to significantly faster for C in liver tissue, which is the tissue with the fastest isotope incorporation rate. Either hypothesis may apply; C may turn over faster than N because carbohydrates and fats from dietary sources are taken up more quickly into tissues than proteins (Hobson *et al*. 2003) and re-use of endogenous 'waste' N could be slowing down N isotope turnover (Carleton *et al*. 2005). Further studies are needed to determine which process is actually causing decoupling of N and C turnover in liver. When estimating diets of wild animals over time, any differences in C and N isotope incorporation rates could be very important ecologically, and should be considered when pairing stable C and N isotopes to investigate long term changes in foraging.

APPLICATION OF CAPTIVE RAT STUDIES TO WILD RATS

The isotope incorporation rates in this study reflect those from sedentary rats with activity levels that are considerably

less than those from rats in the wild. Previous studies have assumed that measuring isotopic turnover in non-exercised captive animals underestimates the turnover rates from wild animals. Hobson and Yohannes (2007) examined C turnover in Rosy Starlings (*Sturnus roseus*) while mimicking migration via flight in a wind tunnel. They found no differences in C turnover in blood between exercised and non-exercised birds. They concluded that estimates of isotopic turnover in tissues from sedentary birds could reasonably be applied to wild birds. No studies comparing isotopic turnover in exercising and sedentary mammals have been performed, but are recommended for future study.

CONCLUSIONS

The trends in isotope half-lives in this study were similar to those from other studies of rodents, but the actual half-life estimates varied from previous work. There were no differences between sexes in isotope incorporation rates. There was significant decoupling of C and N incorporation rates in liver tissue with C demonstrating a faster turnover than N. The AIC_c values indicated the use of 2 compartment models for estimation of N and C incorporation into serum and kidney tissues; single compartment models were indicated for all other tissues. There were no differences in overall isotope incorporation rates between model types for serum and kidney tissues (except for N in kidney tissue from females), and there were differences between the incorporation rates estimated between the RPVM and nonlinear models for all other tissues. I recommend the use of the RPVM to estimate the need for multiple compartment models and to estimate isotope incorporation delays for tissues with slower isotope turnover rates. The use of single or multiple compartment nonlinear models with one of the many computer programs available is the easiest method for estimating isotope incorporation rates and they avoid several of the restrictions inherent to the RPVM (see Martinez del Rio & Anderson-Sprecher 2008). If the RPVM does indicate a delay in isotope incorporation, my data indicate that the nonlinear models can still be used as an estimate of isotope turnover because the turnover times found using the nonlinear models are all longer than those found with the RPVM thereby accounting for this delay. My results allow for estimates of temporal variability in diets of wild omnivores in general and invasive rats in particular using stable isotopes of C and N.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figs S1–S6. Stable isotope turnover model graphs for six tissues from captive rats.

Fig. S1. (A–D) Nonlinear, 1 compartment models for N and C isotope incorporation rates in liver from captive rats switched on day 0 from a C_3 plant-based diet to a fish and C_4 plant-based diet. (E–H) Reaction progress variable method models for the same data. Data are graphed as individual rats; $n = 2$ rats for each sex on each day except day 187 where $n = 3$.

Fig. S2. (A–D) Nonlinear, 2 pool models for N and C isotope incorporation rates in serum from captive rats switched on day 0 from a C_3 plant-based diet to a fish and C_4 plant-based diet. (E–L) Reaction progress variable method 2 pool models for the same data. (E–H) Graphs show data from days 2–30 from the RPVM, but only the data from days 15 to the turnover equilibrium on day 30 are included in the regression line to compute the half-lives and pool fractions for the long isotope turnover pools. (I–L) Graphs use data from days 2–5 to compute the half-lives and pool fractions of the short isotope turnover pools; data are the first residuals derived by subtracting (1–F) values of the long component from the total (1–F) values shown in the companion graphs E–H. Data are graphed as individual rats; $n = 2$ rats for each sex on each day except day 187 where $n = 3$.

Fig. S3. (A–D) Nonlinear, 2 pool models for N and C isotope incorporation rates in kidney from captive rats switched on day 0 from a C_3 plant-based diet to a fish and C_4 plant-based diet. (E–L) Reaction progress variable method 2 pool models for the same data. (E–H) Graphs show data from days 2–120 from the RPVM, but only the data from days 30 to the turnover equilibrium on day 120 are included in the regression line to compute the half-lives and pool fractions for the long isotope turnover pools. (I–L) Graphs use data from days 2–15 to compute the half-lives and pool fractions of the short isotope turnover pools; data are the first residuals derived by subtracting (1–F) values of the long component from the total (1–F) values shown in the companion graphs E–H. Data are graphed as individual rats; $n = 2$ rats for each sex on each day except day 187 where $n = 3$.

Fig. S4. (A–D) Nonlinear, 1 compartment models for N and C isotope incorporation rates in RBC from captive rats switched on day 0 from a C_3 plant-based diet to a fish and C_4 plant-based diet. (E–H) Reaction progress variable method models for the same data. Data are graphed as individual rats; $n = 2$ rats for each sex on each day except day 187 where $n = 3$.

Fig. S5. (A–D) Nonlinear, 1 compartment models for N and C isotope incorporation rates in muscle from captive rats switched on day 0 from a C_3 plant-based diet to a fish and C_4 plant-based diet. (E–H) Reaction progress variable method models for the same data. Data are graphed as individual rats; $n = 2$ rats for each sex on each day except day 187 where $n = 3$.

Fig. S6. (A–D) Nonlinear, 1 compartment models for N and C isotope incorporation rates in fur from captive rats switched on day 0 from a C_3 plant-based diet to a fish and C_4 plant-based diet. (E–H) Reaction progress variable method models for the same data. Data are graphed as individual rats; $n = 2$ rats for each sex on each day except day 187 where $n = 3$.

Appendix S1. Modelling methods for estimating stable isotope turnover times and AIC values

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