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Determining biological tissue turnover using stable isotopes: the reaction progress variable

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Abstract The reaction progress variable is applied to stable isotope turnover of biological tissues. This approach has the advantage of readily determining whether more than one isotope turnover pool is present; in addition, the normalization process inherent to the model means that multiple experiments can be considered together although the initial and final isotope compositions are different. Consideration of multiple isotope turnover pools allows calculation of diet histories of animals using a time sequence of isotope measurements along with isotope turnover pools. The delayed release of blood cells from bone marrow during a diet turnover experiment can be quantified using this approach. Turnover pools can also be corrected for increasing mass during an experiment, such as when the animals are actively growing. Previous growth models have been for exponential growth; the approach here can be used for several different growth models.

Keywords Half-life · First-order rate constant · Carbon isotopes · Turnover · Stable isotopes

Introduction

Biologists have long used isotopes in studying turnover rates in biological tissues (e.g., muscle, heart, liver) or reservoirs contributing to ever-growing biological tissues (e.g., hair). Most recent studies have used a single exponential best-fit to the data to describe isotope turnover in biological tissues; this applies to earlier stable isotope studies (Fry and Arnold 1982; Tieszen et al. 1983; Hobson and Clark 1992) and to the most recent stable isotope studies (Podlesak et al. 2005). The ''exponential-fit'' approach has the underlying assumption that biological isotope exchange reactions are described by first-order rate kinetics. However, fitting data to an exponential function overemphasizes data at the extremes, and the possibility of multiple turnover pools is difficult to ascertain from fitting to an exponential function.

The purpose of this paper is to show that a reaction-progress variable (Criss et al. 1987; Criss 1999) is a more useful way to describe biological turnover experiments than the exponential-fit method, even though both follow first-order rate kinetics. The reaction progress variable normalizes isotope exchange and allows the system to be treated as linear functions rather than exponential functions. This method is analogous, in many ways, to considering a mixture of radionuclides of differing half-lives (Overman and Clark 1960; Friedlander et al. 1981). It has the advantage that it can readily determine whether more than one rate constant is being followed, whether a short- or

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long-term rate constant has been missed in the sampling, and can readily compare experiments having differing starting and ending isotope conditions. Early studies of metabolic turnover in mammals, using the radioisotope tritium, derived multiple rate constants (Thompson 1952a, b; Thompson and Ballou 1956). However, stable isotope notation does not lend itself readily to logarithmic functions and so the advantage gained in these earlier studies (that of determining multiple rate constants) was lost in later stable isotope studies. Here we show that the reaction progress variable regains, for stable isotopes, those valuable mathematical methods used in these much earlier studies using radioisotopes to study biological turnover.

First, we describe aspects of first-order reaction kinetics in isotope systems. We then give several examples, both theoretical and experimental, showing how the reaction progress variable yields additional information on stable isotope turnover in biological systems: examples include determining multiple rate constants and their fractional contributions in hair formation, comparison of experiments with different initial and final conditions, delayed release of red blood cells and the effect on calculated turnover rates. We also discuss the implications of multiple-turnover rate constants and show that detailed diet reconstruction using multiple turnover pools gives realistic scenarios whereas single turnover pools for the same system give impossible diet reconstruction scenarios. We then present a model for correcting for growth during the length of data collection that is not dependent on the growth rate model (e.g., constant, exponentially increasing, or sigmoidal). This method is most useful if the final equilibrium isotope ratios are known; if not explicitly measured, they must be estimated from related experimental data.

Methods and definitions

Terminology

The stable isotope ratio is described by:

$$\delta^{k}M_{A} = (R_{A}/R_{standard} - 1)1000, \quad (1)$$

where k is the rare isotope species of interest, M is the element of interest, A is the phase being of interest (e.g, water, diet, tissue), R_A is the ratio of the rare to abundant isotope (e.g., $^{13}C/^{12}C$) in the sample, and $R_{standard}$ the isotope standard. Isotope fractionation describes the ratio between two phases in equilibrium:

$$\alpha_{AB} = R_{A}/R_{B} = (1000 + \delta_{A})/(1000 + \delta_{B}) \quad (2)$$

and isotope enrichment is

$$\varepsilon_{AB} = (\alpha_{AB} - 1)1000. \quad (3)$$

To distinguish equilibrium and non-equilibrium processes involving R_A/R_B and to maintain mathematical integrity, we adopt the terminology α_{AB} and ε_{AB} for isotope fractionation (equilibrium) and α_{AB}* and ε_{AB}* for the isotope difference (non-equilibrium).

Isotope exchange experiment: *Neotoma cinerea*

We determined the rate of turnover for water in the body of bushy-tailed woodrats (*Neotoma cinerea*) by switching their drinking water from tap water($\delta^2H = -120 \pm 2\%$); ($\delta^{18}O = -16.0 \pm 0.3\%$) to highly-enriched drinking water($\delta^2H = 340 \pm 1\%$); ($\delta^{18}O = 15.0 \pm 0.1\%$) or vice versa. Woodrats were housed at the University of Utah's animal facilities in individual cages in the same room with a constant light cycle (12 h dark:12 h light) and temperature (25°C).

This experiment involved collecting breath samples and analyzing CO_2 for $\delta^{18}O$ over a 27-day period. Breath samples are non-invasive and allow repeated sampling of the same individual. Breath samples were collected by placing the woodrat in a metabolic chamber; supply air was scrubbed of CO_2 and water. An inline valve allowed sampling upstream to assure that no CO_2 was flowing into the chamber. The woodrat was in the chamber for 3–4 min to allow CO_2 levels to increase and stabilize. A 50-µl sample of breath was collected downstream of the chamber with a syringe and was immediately injected onto a gas chromatography column (Varian Poraplot Q, 25 m length, 0.32 mm internal diameter) attached to a Finnigan MAT 252 mass spectrometer. The isotopic value for each animal sampled is the mean of four injections and the reproducibility of multiple breath samples over approximately 10 min was ±0.2‰. Stable isotope ratios were calibrated to the Vienna standard mean ocean water scale for $\delta^{18}O$ using a working standard calibrated to NBS-19 ($\delta^{18}O$ = –2.19 Vienna PeeDee belemnite).

Modeling: the reaction progress variable

A typical turnover experiment involves changing the diet instantaneously and measuring the change in stable isotope ratio over time (e.g., Fry and Arnold 1982; Hobson and Clark 1992; Ayliffe et al. 2004; Podlesak et al. 2005). The typical fitting of the data is to a single exponential; however, the exponential fit has the

unstated assumption that the system is following a first-order rate constant. We accept the assumption of first-order rate kinetics and show that the reaction progress variable gives more information that the exponential fit method.

The ''exponential fit'' model has been used (e.g., Fry and Arnold 1982; Tieszen et al. 1983; Hobson and Clark 1992; Podlesak et al. 2005) to fit isotope data to the form:

$$\delta^t = a\mathrm{e}^{-\lambda t} + c, \tag{4}$$

where δ^t is the stable isotope of interest undergoing isotope exchange with time t, a and c are parameters derived from the ''best fit'', and λ is a first-order rate constant. Three important parameters result from this equation: λ is the data-derived rate constant from which the half-life is derived:

$$t_{1/2} = \ln(2)/\lambda, \tag{5}$$

c is the data-derived isotope equilibrium value δ^{eq}, a is the isotope difference between the initial and final equilibrium states (Tieszen et al. 1983), so that $c + a$ is the data-derived initial isotope value, δ^{init}. These have important implications and consequences: if the initial and equilibrium δ-values do not correspond to the observed values within the uncertainty of the data, the single exponential fit model is inappropriate to describe the system being studied. Likewise, with the reaction progress model being described here, the final equilibrium isotope ratio must be known either from related experiments or, as in the case with the exponential fit method, it must be estimated from the experiment itself.

A first-order rate constant is:

$$\frac{\mathrm{d}N}{\mathrm{d}t} = -\lambda N, \tag{6}$$

where N is the number of atoms or molecules in the system being described, t is time (s), and λ is the rate constant (s^{-1}). Integrated, this is

$$N = N_0\mathrm{e}^{-\lambda t}. \tag{7}$$

This equation is linearized by taking the natural logarithm:

$$\ln\left(\frac{N}{N_0}\right) = -\lambda t, \tag{8}$$

where N_0 is the initial number of atoms, and λ is the rate constant as in Eq. 5.

Criss (1999) has shown that for a trace isotope where the concentration of the rare isotope is significantly less than that of the abundant isotope, the system can be closely approximated as being characterized by changes only in the rare isotope. For a system following first-order rate constants, the following argument is made: controlled diet experiments are treated as an infinite reservoir where the isotope value of the food supply is unaffected by consumption. This leads to (see Criss 1999 for complete derivation):

$$\frac{R_{\mathrm{A}}^t - R_{\mathrm{A}}^{\mathrm{eq}}}{R_{\mathrm{A}}^{\mathrm{init}} - R_{\mathrm{A}}^{\mathrm{eq}}} = \mathrm{e}^{-\lambda t}, \tag{9}$$

where ${R_{\mathrm{A}}}^{\mathrm{init}}$ is the initial isotope ratio ($t = 0$), ${R_{\mathrm{A}}}^{\mathrm{eq}}$ is the isotope ratio at equilibrium (t = infinity), and ${R_{\mathrm{A}}}^{t}$ is the isotope ratio at time t, respectively. Using the δ notation Eq. 9 is:

$$\frac{\delta_{\mathrm{A}}^t - \delta_{\mathrm{A}}^{\mathrm{eq}}}{\delta_{\mathrm{A}}^{\mathrm{init}} - \delta_{\mathrm{A}}^{\mathrm{eq}}} = \mathrm{e}^{-\lambda t}, \tag{10}$$

where ${\delta_{\mathrm{A}}}^{\mathrm{init}}$, ${\delta_{\mathrm{A}}}^{\mathrm{eq}}$, and ${\delta_{\mathrm{A}}}^{t}$ are the δ-values at the initial time ($t = 0$), at equilibrium (t = infinity), and at time t during the reaction progress experiment. Reaction progress experiments are often described as the fractional approach to equilibrium (Criss 1999):

$$\frac{\delta_{\mathrm{A}}^t - \delta_{\mathrm{A}}^{\mathrm{eq}}}{\delta_{\mathrm{A}}^{\mathrm{init}} - \delta_{\mathrm{A}}^{\mathrm{eq}}} = (1 - F), \tag{11}$$

where $F = 0$ at the beginning of the exchange reaction and $F = 1$ at equilibrium (t = infinity). This scales the isotope difference between the initial and equilibrium conditions to be 1.0.

Equations 10 and 11 are particularly useful when cast as:

$$\ln(1 - F) = -\lambda t, \tag{12}$$

which has the property of being a straight line and because different related experiments can be plotted on the same figure (Fig. 1). For a single pool exchange experiment, this has the form:

$$y = mx + b, \tag{13}$$

where the intercept is 0.0 and the slope is $-\lambda$, and the the half life is Eq. 5. The intercept represents the fractional contribution of the pool; in this case the fractional contribution to the total is:

$$\mathrm{e}^b = \mathrm{e}^{0.0} = 1.00. \tag{14}$$

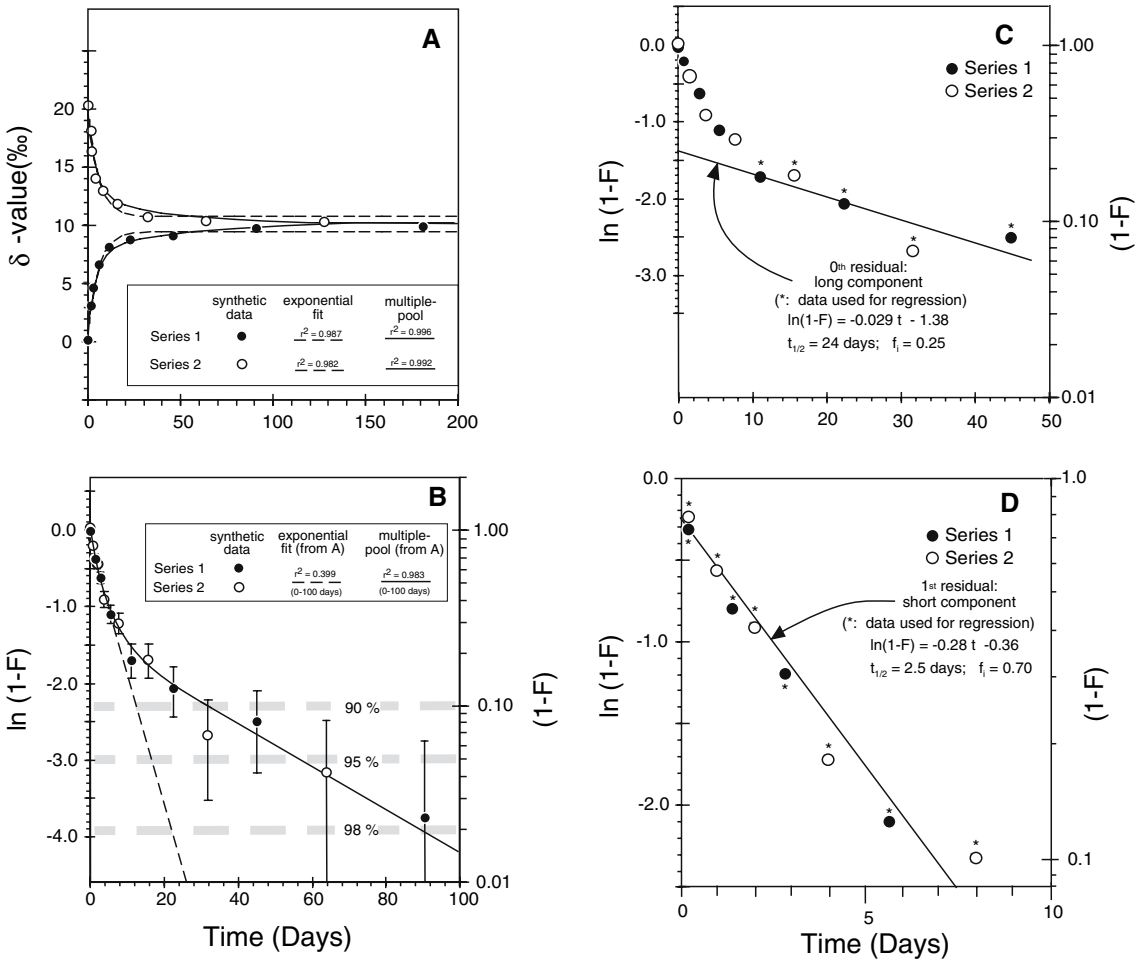

Fig. 1 **a** δ-Values for synthetic data set of Table 1 with two rate constants and a random uncertainty of ±0.2‰ for δ-values; rate constants are for half-lives of 2 and 20 days having fractions of 0.70 and 0.30, respectively. Initial conditions are data-derived initial isotope value (δ^{init}) and data-derived isotope equilibrium value (δ^{eq}) of 0 and +10‰ for Series 1 and +20 and +10‰ for Series 2; δ^{init} and δ^{eq} values have an uncertainty of 0.2‰ Exponential fit to data shown with *dashed lines*; reaction progress solution shown with *solid lines*. Correlation coefficients (r^2) for model compared to synthetic data given for each comparison. **b** Data of **a** presented as reaction progress variable [ln(1 – *F*)] with uncertainty propagated through calculations for a single simulation. *Dashed light line* shows the exponential fit of **a**. The *solid line* shows fit for the multi-pool modeled described in the text. *Thick dashed lines* show the reaction progress at 90 and 95% completion. **c** The Zeroth residual of the data of Table 1 as the reaction progress variable. *Solid line* shows the fit to the long component and gives a half-life of 23 days; *intercept* gives the contribution to the total signal($f_i = e^{-1.386} = 0.25$). Uncertainty estimates not shown to improve clarity. **d** The first residual of the data derived by subtracting (1 – *F*) values of the long component from the total (1 – *F*) values of **c**. *Solid line* shows the fit to the short component and gives a half-life of 2.5 days and a fractional contribution of 0.70 (calculated as in **c**). Uncertainty estimates not shown to improve clarity

Thus a single pool makes up 100% of the contribution to the whole.

For a system with multiple turnover pools, Eq. 9 is:

$$\frac{\delta_{\text{A}}^{t} - \delta_{\text{A}}^{\text{eq}}}{\delta_{\text{A}}^{\text{init}} - \delta_{\text{A}}^{\text{eq}}} = (1 - F) = \sum_{j}^{n} f_j e^{-\lambda_j t}, \tag{15}$$

where f_j represents the fraction of each isotope pool j and δ_j is the rate constant for each pool j. From Eq. 10 each isotope turnover pool changes as

$$\delta_j^{t} = \delta_j^{\text{init}} e^{-\lambda_j t} + \delta^{\text{eq}}(1 - e^{-\lambda_j t}). \tag{16}$$

To illustrate the solution to a multiple-pool turnover problem, Eq. 16 becomes for a three-pool system:

$$\frac{\delta_{\text{A}}^{t} - \delta_{\text{A}}^{\text{eq}}}{\delta_{\text{A}}^{\text{init}} - \delta_{\text{A}}^{\text{eq}}} = f_1 e^{-\lambda_1 t} + f_2 e^{-\lambda_2 t} + f_3 e^{-\lambda_3 t}. \tag{17}$$

This equation is always concave upwards because the shorter-lived components become less important

with time and the long half-lived components dominate at long time periods (Friedlander et al. 1981).

The graphical approach is to identify the longest-lived component and solve for the slope and intercept using a least-squares approach; the slope of the longest component gives the rate constant (and therefore the half-life) and the intercept gives the fractional contribution to the whole ($f_i = e^b$). The $(1 - F)$ values of the longest component are subtracted from the total $(1 - F)$ values to give a first residual plot of $\ln(1 - F)$ versus time. Successively shorter half-lives and their overall contribution to the summed turnover pool are then determined in an analogous fashion (first and second residual plots). Overman and Clark (1960) and Friedlander et al. (1981) point out that for radioisotopes, from which this analogy was derived, it is difficult to derive more than three rate constants (turnover pools).

Results: examples of isotope exchange and the reaction progress variable

In this section we provide examples of the reaction progress variable in biological isotope turnover studies. The first is a hypothetical example to illustrate the advantages over the exponential fit method; this system that has two half-lives of 2 and 20 days, respectively. Second, we examine an example of a real system incorporating a controlled diet change (Ayliffe et al. 2004) with a very small sampling interval to illustrate that up to three turnover pools can be identified using the reaction progress variable approach. Third, we show that related experiments, such as approaching isotope change from opposite directions can be treated together because of the normalization procedure, and that it brings out new information difficult to extract from the exponential fit method. Fourth, we use forward modeling to calculate diet history from a time sequence of samples using the isotope turnover parameters derived from experiment. Fifth, we show that the reaction progress variable is useful in identifying a delayed response, such as release of red blood cells, during a diet-switch experiment. Sixth, we present a model for isotope turnover for animals undergoing growth during the experimental turnover study that is not dependent on the growth model.

Multiple turnover pools with differing half-lives

Consider a system where the tissue of interest has two turnover pools, one with a half-life of 20 days comprising 30% of the total and one with a half-life of 2 days comprising 70% of the total. Figure 1a shows one simulated result for two experiments conducted in opposite directions: one with $\delta^{init} = 0‰$ and $\delta^{eq} = +10‰$, and the other with $\delta^{init} = +20$ and $\delta^{eq} = +10‰$. We introduced random error with a one sigma distribution of ±0.2‰ for each δ^{init} value, and assume ±0.2‰ for δ^{eq} and δ^{t}; Fig. 1a, b is a single example of this system with propagated uncertainties. Examination of Fig. 1a, which is the conventional method of plotting turnover rate experiments, does not distinguish whether one or two isotope turnover pools are present. However, the fact that the data in Fig. 1b, which uses the reaction progress method, are distinctly curvilinear indicates that more than one isotope pool is present. Table 1 shows the synthetic data for Fig. 1. We calculated half-lives and correlation coefficients for a one-pool model for the synthetic data of Table 1, and for a two-pool model using 20 random error simulations. The longer pool was calculated using the longest linear portion of the time versus $\ln(1 - F)$ (i.e., Fig. 1c); the short pool was calculated using the residual values of $(1 - F)$ as shown in Fig. 1d.

The results for the single exponential fit are give in Table 2 for a variety of combinations of the data. This exercise shows that data collected at the beginning of the experiment favor the short pool, whereas data collected at the end of the experiment favor the long pool, and the conclusions are biased towards any particular sampling strategy. Correlation coefficients (r^2) are high for all fits and shows that high correlation coefficients alone are not a good indicator of goodness of fit for exponential fits to δ-values if more than one isotope turnover pool are present. Likewise, the δ^{init} and δ^{eq} values using the single exponential fit determined are dependent on which sample intervals are used and are incorrect, indicating a problem with these results. Results from the 20 simulations for the two-pool model were $t_{1/2} = 19.0 \pm 3.2$ and 1.9 ± 0.7 days, having fractions of 0.33 ± 0.04 and 0.68 ± 0.04, respectively and an overall r^2 of 0.966. We emphasize that the exponential fit method is satisfactory if only a single pool is present; the reaction progress approach is a way to test for multiple pools, and offers a strategy for solving for up to three distinct isotope pools (e.g., Example 2).

Reaction progress model applied to carbon turnover in controlled diet study of mammals

Ayliffe et al. (2004) conducted a controlled diet experiment where two domestic horses (*Equus caballas*), which had been fed C_3 hay for their entire lives were placed on a C_4 hay diet for 150 days. Hair was collected from two different horses; three hairs from

Table 1 Synthetic data set used in Fig. 1 and Example 6. Two different pools are used with half-lives of 2.0 and 20.0 days, and contributing 70 and 30%, respectively. Values are given for two exchange reactions: one with initial and final δ-vales of 0.0 and 10.0‰ respectively (*Series 1*), and the other with initial and final δ-values of 20.0 and 10.0‰, respectively (*Series 2*). A random error with 1 σ of ± 0.2‰ is assigned to each δ-value for both data sets and for the δ^{init} and δ^{eq} values. Propagated uncertainty for $(1 - F)$ is 1 σ ± 0.04; therefore reaction progress computations in text are done with samples for $(1 - F) > 0.05$ [$\ln(1 - F) > -3.0$]

Series	Time (days)	Example 1						Example 6[a]		
		Series 1			Series 2			δ-value	Mass (k = 0.02 day^{-1})	δ-value (k = 0.02 day^{-1})
		δ-value	$1 - F$	$\ln(1 - F)$	δ-value	$1 - F$	$\ln(1 - F)$	No growth		
Series 1	0.00	0.15	0.98	–0.02	20.33	1.03	0.03	0.00	1.00	0.00
Series 2	1.00				18.14	0.81	–0.21	2.15	1.02	2.31
Series 1	1.41	3.10	0.69	–0.37				2.86	1.03	3.05
Series 2	2.00				16.40	0.64	–0.45	3.70	1.04	3.95
Series 1	2.83	4.65	0.53	–0.63				4.65	1.06	4.95
Series 2	4.00				14.02	0.40	–0.91	5.64	1.08	5.97
Series 1	5.66	6.65	0.33	–1.09				6.55	1.12	6.92
Series 2	8.00				12.96	0.30	–1.22	7.29	1.17	7.69
Series 1	11.31	8.16	0.18	–1.70				7.83	1.25	8.27
Series 2	16.00				11.83	0.18	–1.70	8.25	1.38	8.73
Series 1	22.63	8.73	0.13	–2.06				8.63	1.57	9.13
Series 2	32.00				10.69	0.07	–2.67	9.01	1.90	9.48
Series 1	45.25	9.18	0.08	–2.50				9.37	2.47	9.75
Series 2	64.00				10.43	0.04	–3.15	9.67	3.60	9.91
Series 1	90.51	9.77	0.02	–3.75				9.87	6.11	9.98
Series 2	128.00				10.26	0.03	–3.64	9.96	12.94	10.00
Series 1	181.02	9.92	0.01	–4.82						
Series 2	256.00				10.21	0.02	–3.89			

[a] Data for the experiment for growth (see final figure below) have no uncertainty; they show δ-values for the no-growth case for the Series 1 conditions, and relative mass and δ-values for exponential growth with k = 0.02 day^{-1}

each horse were sectioned and analyzed and the results treated together. The analysis of Ayliffe et al. (2004) was similar to that of the reaction progress model presented here except that they did not normalize the results to1.0; however, they noted that such normalization would result in the reaction progress formulation. We present their results here in the reaction progress formulation because that study, to date, has the most detailed sampling yet conducted in diet turnover studies. The results of their study are presented in Fig. 2 and show that three isotope turnover pools can be identified, with half-lives of 0.5, 4.3 and 140 days and with the fractions 0.41, 0.15, and 0.44. Figure 2 shows the results with the slope giving the half-life and the intercept giving the fraction of the longest pool. Likewise, for the first-residual plot the slope gives the half-life and the intercept gives the fraction of the intermediate pool. Lastly, in an analogous fashion, the second-residual plot gives the half-life and fraction of the shortest pool. Significantly, the three fractional pools sum to 1. In conclusion, the multiple pool method gives three pools with half-lives of 0.5, 4.3 and 140 days and with the fractions 0.41, 0.15, and 0.44, respectively.

Treatment of this data set as a single pool gives interesting results. Fitting the data to a single exponential gives: $y = -14.5 - 8.25\ e^{-0.1608t}$; this is a half-life of 4.3 ± 0.5 days ($r^2 = 0.87$). This fit gives δ^{eq} and δ^{init} values of –14.5 and –22.8‰ allowing calculation of $\varepsilon_{hair\text{-}diet}$*; this gives –1.0 and 3.9‰ for the isotope enrichment for the final and initial conditions, respectively. The measured $\varepsilon_{hair\text{-}diet}$* at the beginning of the experiment was 2.7‰.

Fitting the data to a single correlation on the time versus $\ln(1 - F)$ plot (the ''0th residual'') in Fig. 2 gives a single half-life of 111 days with an r^2 value of 0.81. While this seems to be a good fit (high r^2), the intercept shows that only 0.52 of the total signal is actually accounted for.

Water turnover using $\delta^{18}O$ in breath

One water turnover experiment on the bushy tailed woodrat (*Neotoma cinerea*) was carried out in which the $\delta^{18}O$ of water supply changed and food source was kept constant.

Table 3 gives data for $\delta^{18}O_{breath}$ for individual woodrats changed from an ^{18}O enriched to an ^{18}O

Table 2 Sigmaplot results for exponential fitting of the Series 1 and the Series 2 synthetic data sets (Table 1) to the form $\delta^t = ae^{-\lambda t} + c$ with correlation coefficients derived for successive data points of each series[a]

	Total days	λ	$t_{1/2}$	c	a	δ^{init}	n	r^2	P
Series 1									
0–181	181	0.215	3.2	9.4	–8.9	0.43	9	0.987	<0.0001
1.4–181	180	0.169	4.1	9.5	–7.9	1.57	8	0.982	<0.0001
2.8–181	178	0.143	4.8	9.5	–7.0	2.47	7	0.968	0.0010
5.7–181	185	0.071	9.8	9.7	–4.3	5.45	6	0.950	0.0111
11.3–181	170	0.027	25.6	9.9	–2.4	7.56	5	0.994	0.0056
22.6–181	158	0.023	29.6	10.0	–2.1	7.83	4	0.993	0.0835
45.3–181	136	0.035	20.1	9.9	–3.6	6.37	3	1.000	<0.0001
0–2.8	3	0.460	1.5	6.3	–6.2	0.15	3	1.000	<0.0001
0–5.7	6	0.322	2.2	7.9	–7.7	0.18	4	0.999	0.0369
0–11.3	11	0.279	2.5	8.4	–8.3	0.23	5	0.998	0.0017
0–22.6	23	0.264	2.6	8.7	–8.4	0.26	6	0.998	<0.0001
0–45.3	45	0.247	2.8	8.9	–8.6	0.31	7	0.996	<0.0001
0–90.5	91	0.227	3.1	9.2	–8.8	0.38	8	0.990	<0.0001
0–181	181	0.215	3.2	9.4	–8.9	0.43	9	0.987	<0.0001
Series 2									
0–256	256	0.216	3.2	10.6	9.4	20.0	10	0.982	<0.0001
1–256	255	0.174	4.0	10.5	8.5	19.1	9	0.975	<0.0001
2–256	254	0.125	5.5	10.4	7.1	17.5	8	0.971	0.0001
4–256	252	0.075	9.2	10.3	5.0	15.3	7	0.998	<0.0001
8–256	248	0.072	9.7	10.3	4.8	15.1	6	0.996	0.0002
16–256	240	0.079	8.8	10.3	5.5	15.7	5	0.989	0.0108
32–256	224	0.024	28.8	10.2	1.0	11.2	4	0.999	0.0101
64–256	192	0.023	30.8	10.2	0.9	11.1	3	1.0000	<0.0001
0–2	2	0.230	3.0	9.7	10.7	20.3	3	1.000	<0.0001
0–4	4	0.248	2.8	10.3	10.0	20.3	4	1.000	0.0031
0–8	8	0.361	1.9	12.4	8.0	20.4	5	0.996	0.0036
0–16	16	0.321	2.2	12.0	8.4	20.4	6	0.995	0.0003
0–32	32	0.266	2.6	11.3	8.9	20.2	7	0.986	0.0002
0–64	64	0.241	2.9	11.0	9.1	20.1	8	0.983	<0.0001
0–128	128	0.225	3.1	10.8	9.3	20.0	9	0.982	<0.0001
0–256	256	0.216	3.2	10.6	9.4	20.0	10	0.982	<0.0001

[a] δ^{eq} (=c) and δ^{init} (=$c + a$) are determined by the exponential fit to the successive data points as shown

depleted water source, and in the complementary experiment for a switch from an ^{18}O depleted to an ^{18}O enriched water source. Table 3 shows that some individual animals have consistent offsets over long periods of time compared to other individuals with identical histories (e.g., no. 3 was consistently 2‰ enriched relative to no. 29 in spite of identical water and food source histories). Therefore, for the reaction progress calculations we use the initial and final $\delta^{18}O$ values measured for each individual rather than an average for all individuals with identical histories. Figure 3a shows the $\delta^{18}O$ values for breath for both directions of water switch; Fig. 3b shows the data plotted as the reaction progress variable $\ln(1 - F)$ for those points of <95% exchange. Figure 3b illustrates that the uncertainties become very large for near the end of the experiment (90–100% exchange) and therefore we use only the data from 0 to 90% exchange to calculate rate constants. Figure 3b also shows that the forward and reverse experiments can be used together to calculate rate constants. In this case, only one rate constant is present and Fig. 3b gives an overall rate constant of $\lambda = 0.22$ day^{-1}, which corresponds to a half-life $t_{1/2} = 3.1$ days. Treated separately, the three individuals (nos. 21, 23, 29) give half-lives of 3.2, 3.4, and 2.9 days, respectively.

Use of turnover rate constants to model animal diets

The isotope composition of each pool changes with time incrementally as (from Eq. 16):

$$\delta_j^t = \delta_j^{(t-1)} e^{-\lambda_j(\Delta t)} + \delta^{eq}\left(1 - e^{-\lambda_j(\Delta t)}\right), \quad (18)$$

where $\delta_j^{(t-1)}$ is the isotope value of pool j at time $(t - 1)$, and Δt is the difference in time between $t - 1$ and t. For a change in isotope input value, the isotope value δ_j^t can be calculated from Eq. 18 as a function of time if δ^{eq} is known.

For the case where the δ^{eq} can be related to a known fractionation factor α, such as in the relatively simple case of diet reconstruction from hair with three isotope turnover pools, a dietary history can be reconstructed from a sequence of hair samples by (Cerling et al. 2004):

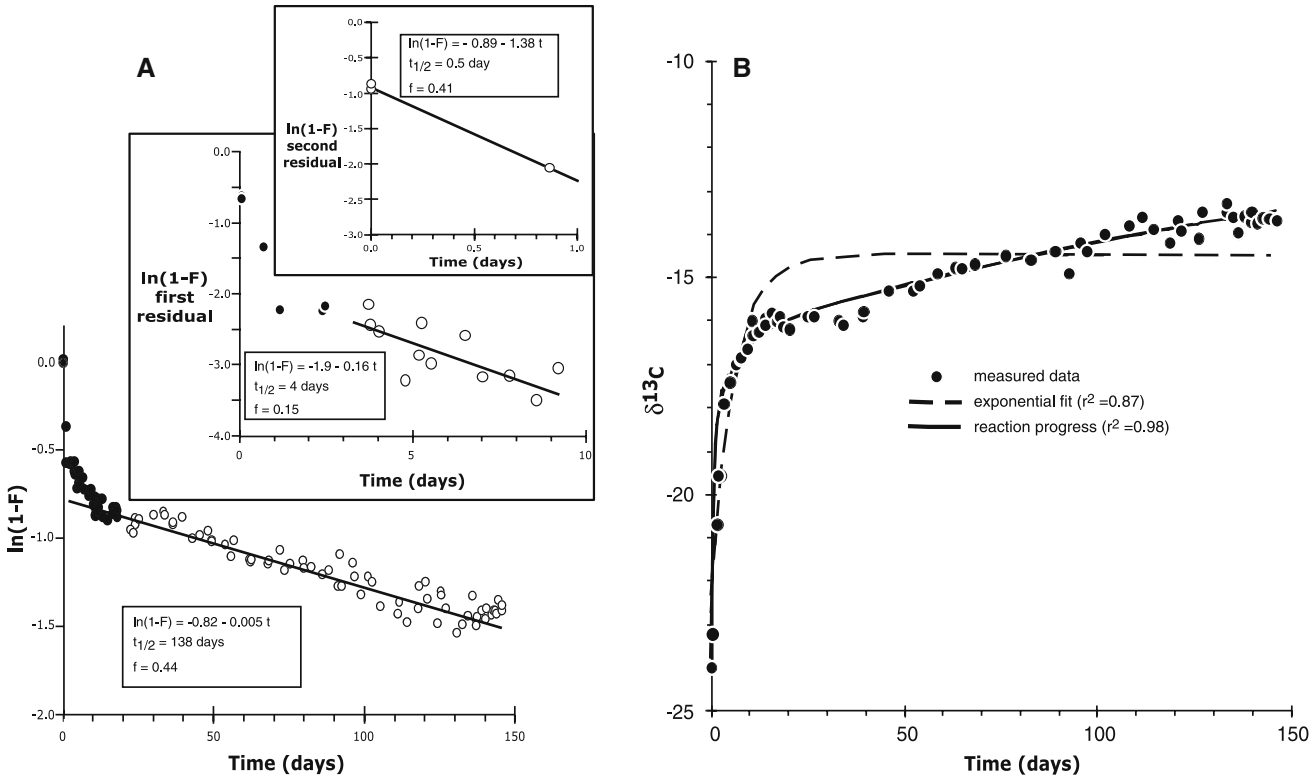

Fig. 2 **a** Reaction progress [ln(1 – *F*)] for data from Ayliffe et al. (2004) for diet change from C_3 to C_4 grass for horses (*Equus caballus*). *Insets* are the plots of residual data after subtraction of slower turnover pools (see text). *Open circles* are those data points used to determine rate constants; *closed circles* are those data points not used to determine rate constants in each panel. **b** Original $\delta^{13}C$ data and model results for both the exponential fit model and for the reaction progress model

$$\delta_{\mathrm{D}}^{(t)} = \frac{\left(\frac{\delta_{\mathrm{H}}^{(t)}+1000}{\alpha_{\mathrm{HD}}} - 1000\right) - \left(\Sigma_{j=1}^{3} f_j \delta_j^{(t-1)} \mathrm{e}^{-\lambda_j \Delta t}\right)}{\left(\Sigma_{j=1}^{3} f_j (1 - \mathrm{e}^{-\lambda_j \Delta t})\right)}, \qquad (19)$$

where $\delta_{\mathrm{D}}{}^{(t)}$ is the isotopic composition of diet at time *t*, $\delta_{\mathrm{H}}{}^{(t)}$ is the isotopic composition of an individual hair segment in a sequence of hair samples at time *t*, α_{HD} is the isotope fractionation factor between hair and diet (written as enrichment), f_j is the fractional contribution of each isotope turnover pool, $\delta_j{}^{(t\ -\ 1)}$ is the isotope composition of the hair segment older than the one being measured ($t - 1$), and Δt is the difference in time between segment *i* and segment *i*, $t - 1$. Initial conditions for each turnover pool must be specified for diet reconstructions: for short half-lived pools the approach to equilibrium is rapid so that, by discarding the first few samples in a sequence, the initial conditions do not influence the longer term history; for long pools a choice must be made for initial conditions that are consistent with the known history of the animal. For the case discussed below, the long pool is chosen to be representative of known long-term history of the individual animal: a constant diet. In other cases, a more representative choice might be the long-term diet estimated from all measurements comprising the dietary history (see Cerling et al. 2004, 2006).

We reconstructed the diet of a horse from the Ayliffe et al. (2004) study from a single horse hair using the parameters obtained in Fig. 2 (data used to derive turnover parameters were from six hairs from two different horses with identical diet histories, for the period 0–147 days). Figure 4 shows the reconstructed diet compared to known diet changes for the horses used above to determine rate constants related to hair growth. Figure 4 shows data for both the calibration period (days 0–147) and for time outside the calibration interval where the animals underwent significant dietary change. Figure 4a, b is for time intervals where the δ_{hair} and δ_{diet} values are over a time interval of >8 or >1 days. The reconstructed diet history was very similar to the known diet, with correlation coefficients (r^2) between 0.92 and 0.94, respectively. Using a single turnover pool (exponential fit model) with a half-life of 4 days gave required diet changes, ranging from –11.6 to –31.9‰ for the $\delta^{13}C$ value of diet; these had large ''overshoots'' at the periods of diet switch.

Table 3 $\delta^{18}O$ values for breath[a] in water turnover experiments. Initial $\delta^{18}O_{water}$ ($\delta^{18}O_{w\text{-}initial}$) and final $\delta^{18}O_{water}$ ($\delta^{18}O_{w\text{-}final}$) are given for each animal. Feed, and therefore, $\delta^{18}O$ of feed, was constant throughout the experiment

Days	Animal ID no.						
	21[b]	23[c]	28[b]	25[d]	29[d]	3[d]	30[d]
–0.3	44.7	27.8					
0.0	44.9	27.3	45.5				
0.2	43.4	28.8	45.0				
0.5	42.5	29.6	44.2				
0.8	42.3	30.1	44.0				
1.0				28.3	45.6		
1.2	41.7	31.7	42.1				
1.5	41.3	31.9	42.0				
1.8	41.1	32.2	41.2				
2.0				29.1	45.6		
2.2	39.6	33.3	39.0			47.9	
3.2	36.7	35.5	37.3				
5.2	33.7	38.7	33.0				
7.2	32.3	41.0	31.2			47.3	
12.2	29.7	43.7	28.9				
15.2	29.1	44.1	28.2				
20.2	29.0	44.8	27.9	28.6	45.5		
26.2	28.9	44.6	28.0	28.7	45.5	46.9	46.5
$\delta^{18}O_{w\text{-}initial}$	15.0	–16.0	15.0	–16.0	15.0	15.0	15.0
$\delta^{18}O_{w\text{-}final}$	–16.0	15.0	–16.0	–16.0	15.0	15.0	15.0

[a] Uncertainties in $\delta^{18}O_{breath}$ values are ± 0.2‰ based on multiple injections

[b] Animals 21 and 28 were switched from ^{18}O-enriched water to ^{18}O-depleted water

[c] Animal no. 23 was switched from ^{18}O-depleted water to ^{18}O-enriched water

[d] Animals 25, 29, 3 and 30 were maintained on constant water throughout the experiment

A more independent test of diet reconstruction is based on the analysis of a single horse hair from the study of West et al. (2004). We use the parameters derived in Ayliffe et al. (2004) and reconstruct the diet of a horse which was not used in the study of Ayliffe et al. (2004) to determine the half-lives and fractions of turnover pools. The West et al. (2004) study consisted of a single 7-day diet switch; $\delta^{13}C$ of the C_3 and C_4 diets were –26.8 and –13.5‰, respectively. Hair was cut into 1-mm segments and the chronology established as in the original paper (approximately 1.4 days per segment). Diet calculated from the $\delta^{13}C$ of individual hair segments using Eq. 19. Figure 5 shows the reconstructed diet for both the three-pool and the one-pool exponential-fit model. The total calculated range in $\delta^{13}C_{diet}$ for the three-pool was –27.7 to –13.6‰; for the one-pool model the calculated range was –35.1 to –13.1‰. No diet estimates for the three-pool model were outside the range of –30 to –10‰ (0 of 32), whereas two of 32 diet estimates for the one-pool model were outside the range of –30 to –10‰.

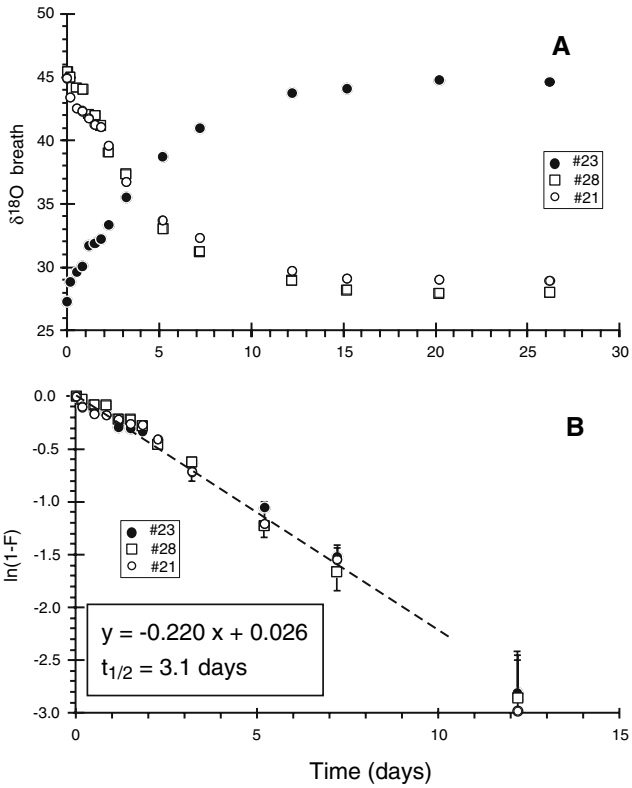

Fig. 3a, b Isotope data and reaction progress variable for isotope turnover in woodrats (*Neotoma cinerea*) using breath. **a** $\delta^{18}O$ of breath for three different individuals going from ^{18}O-depleted to ^{18}O-enriched water source (no. 23) and from ^{18}O-enriched to ^{18}O-depleted water source (nos. 21 and 28). **b** Reaction progress plot [ln (1 – *F*) vs. time] for data of **a** for <95% isotope exchange. Uncertainties are propagated through calculations and shown by *vertical uncertainty bars*. Slope (*dashed line*), which gives the first-order rate constant for isotope turnover, is determined from data <90% turnover

Quantification of delayed release during diet turnover

In this section we show that the delayed release of red blood cells from bone marrow is determined using the reaction progress plot. Podlesak et al. (2005) studied turnover of breath, blood, red blood cells, and feces in the yellow-rumped warblers (*Dendroica coronata*). Plotting the change in $\delta^{13}C$ values for red-blood cells after a change in diet illustrates that the incorporation of carbon from a new dietary source is not isotopically measurable in red-blood cells for up to 3 days (Podlesak et al. 2005). This delay in a measurable change in $\delta^{13}C$ may be due to hematopoiesis. Red blood cells are produced primarily in the bone marrow from stem cells through differentiation and maturation. Mature cells are subsequently released into the circulatory system. The delay in a measurable change in the $\delta^{13}C$ values of the red blood cells may be a measure of the

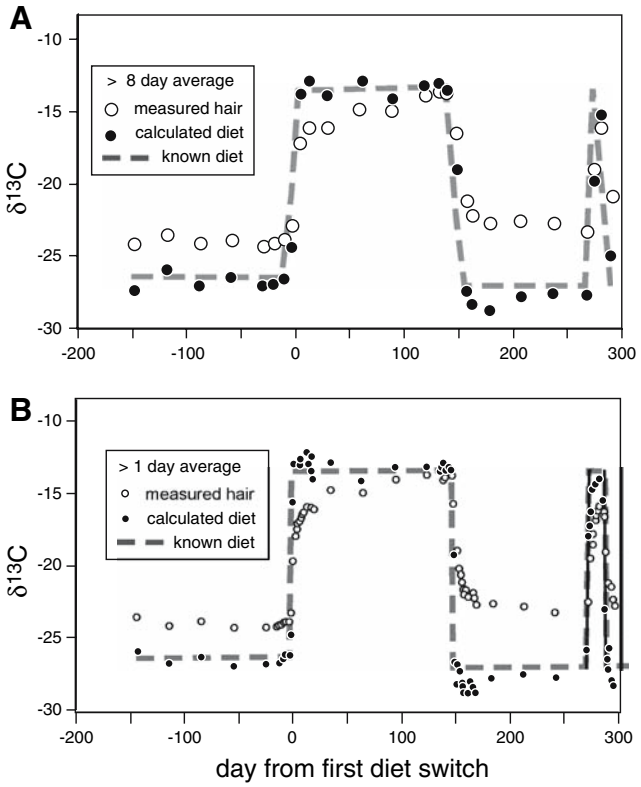

Fig. 4a, b Diet history reconstructed from stable isotope measurements of hair segments from a single hair of a horse with a known diet history. Calibration of turnover pools (Fig. 2 and Discussion) was for days 0–147 on six hairs (including the one shown in this figure) from two different horses with identical diet histories. **a** Data from Ayliffe et al. (2004) averaged to >8-day intervals. *Dashed line* is known diet history; *open circles* are the measured δ^{13}C values of hair; *closed circles* are the reconstructed diet using Eq. 20. **b** Data from Ayliffe et al. (2004) but all segments used in diet reconstruction. Symbols as in **a**

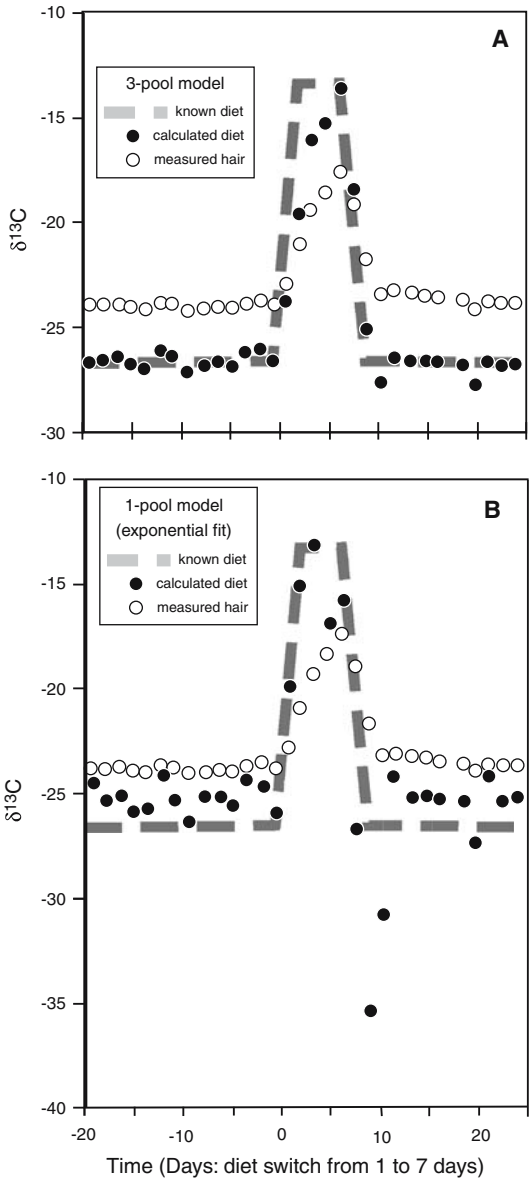

Fig. 5a, b Diet history reconstructed from stable isotope measurements of horse hair. Data from West et al. (2004). **a** Diet reconstructed using a three-pool model and using the parameters from Ayliffe et al. (2004). **b** Diet reconstructed using one-pool model, using a single exponential best-fit model. This reconstruction has a poor match between the known and reconstructed diet

length of time for stem cells to differentiate, mature and be released into the blood stream as red blood cells; it should be subtracted from the time used to calculate the half-life. Figure 6 gives a delay time of 84 h for hematopoiesis, and a half-life of 149 h for turnover.

Implications for isotope turnover studies of growing animals

The equations used here can be used to unravel the effects of isotope exchange and that of newly added tissue. Previous workers have treated this scenario as a case of regular metabolic tissue turnover with superimposed isotope dilution owing to the material added during growth (Fry and Arnold 1982; Hesslein et al. 1993) and we follow this approach here. If the mass at the beginning of the exchange experiment is considered to undergo isotope exchange and change as in Eqs. 15 and 16, and the added mass is considered to be formed in equilibrium with the current diet, then the system is resolved as:

$$\delta^{\mathrm{meas}(t)} M^{\mathrm{meas}(t)} = \delta^{\mathrm{eq}} M^{t}_{\mathrm{new}} + \delta^{t} M^{\mathrm{init}}. \quad (20)$$

Mass balance requires that:

$$M^{\mathrm{meas}(t)} = M^{t}_{\mathrm{new}} + M^{\mathrm{init}}, \quad (21)$$

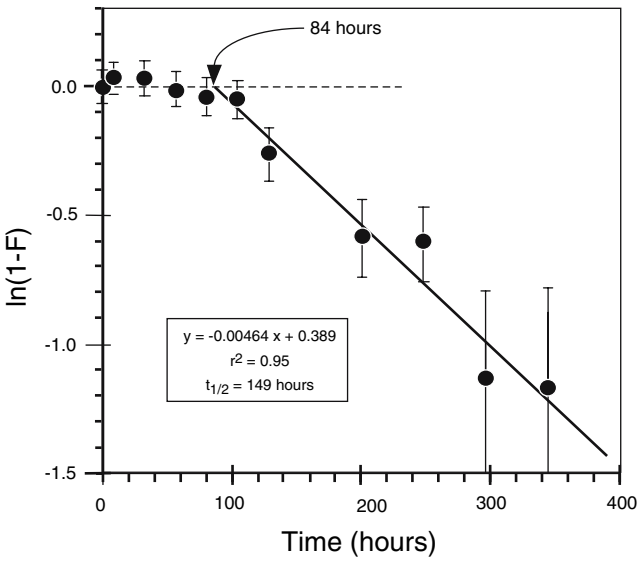

Fig. 6 Reaction progress plot for $\delta^{13}C$ in blood cells in diet switch experiment of Podlesak et al. (2005). Red blood cells were separated from plasma by centrifugation. We use the isotope data of Podlesak et al. (2005) assuming a 5% contamination of red blood cells by blood plasma. Isotope shift begins 84 h after the diet switch, representing the time delay from diet switch to release of newly formed blood cells from bone marrow. The slope gives a half-life of 149 h for carbon turnover after 84 h

where $M^{\text{meas}}(t)$, $M_{\text{new}}{}^{t}$, and M^{init} are the measured masses at time t, the new mass added since time 0, and the initial mass at time 0. $\delta^{\text{meas}}(t)$ is the δ-value measured at time t. Therefore, the turnover for any pool, with growth removed, is described as (combining Eqs. 16, 20 and 21):

$$\delta^{(t)} = \left[\delta^{\text{init}} e^{-\lambda t} + \delta^{\text{eq}}(1 - e^{-\lambda t})\right] = \frac{(\delta^{\text{meas}(t)} - \delta^{\text{eq}})M^{\text{meas}(t)} + \delta^{\text{eq}} M^{\text{init}}}{M^{\text{init}}}. \quad (22)$$

This model is compatible with various (e.g., constant, exponentially increasing, or sigmoidal) growth models and each turnover pool can be considered separately. Therefore it differs from growth models used previously (e.g., Fry and Arnold 1982; Hesslein et al. 1993; Suzuki et al. 2005; Trueman et al. 2005) which assume exponential growth.

As an example we consider the theoretical experiment shown in Fig. 1. We assume the same fractional contributions (0.70 and 0.30) and half-lives (2.0 and 20.0 days), respectively. In addition, we now include an exponential increase in mass of approximately 2% per day using

$$M^{\text{meas}(t)} = M^{\text{init}} e^{kt}, \quad (23)$$

where k is the rate constant for growth (here we use 0.02), and time is in days. Table 1 gives the synthetic data for this example (with no random error). Figure 7 shows the ''apparent'' and corrected reaction progress plot for this hypothetical experiment, plotted as $\ln(1 - F)$ versus time. The uncorrected solution gives half-lives and relative fractions of 1.7 and 11.5 days, with fractions of 0.68 and 0.33, respectively; the corrected reaction mass progress equation (Eq. 22) give half-lives and relative fractions of 2.0 and 20.0 days, with fractions of 0.70 and 0.30, respectively.

Discussion

The reaction progress model

The reaction progress variable has an enormous advantage over the traditional approach of fitting a single exponential to data in turnover experiments using stable isotopes. The principal advantage lies in the linearization of the reaction progress variable:

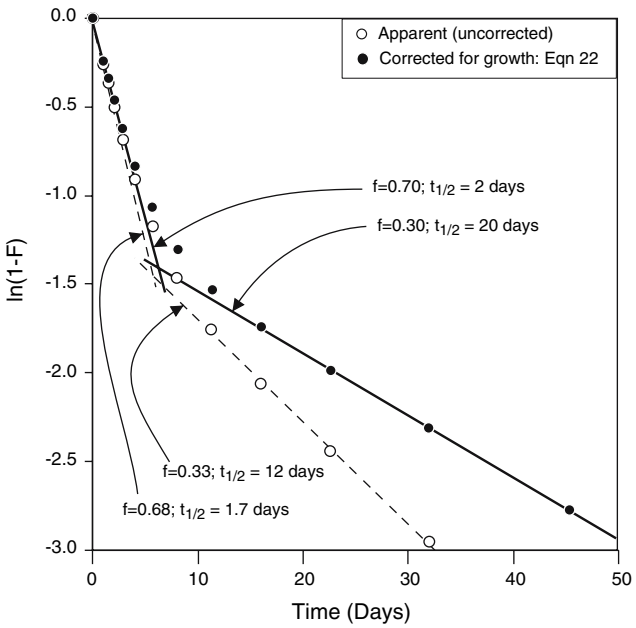

Fig. 7 Corrections for increase in mass during a turnover tissue experiment. The two-pool model of Fig. 1 is used, but where tissue increases exponentially with a rate constant of 0.02 day^{-1}. *Open symbols* show ''apparent'' reaction progress model; *closed symbols* corrected for additional mass as described in text; *crosses* show ''dilution only'' equation of Fry and Arnold (1982). *Lines through open symbols* show two pools identified by reaction progress model for the ''apparent'' turnover; *lines through solid symbols* show the two pools identified by the reaction progress model after correction for added mass due to growth; *dashed line through crosses* is the ''dilution only'' equation of Fry and Arnold (1982)

$$\ln(1 - F) = -\lambda t$$

and the relatively simple recognition of multiple turnover pools. A second advantage is in the planning of sample intervals: from the data shown in Figs. 1, 2 and 3 it is clear that the simple geometric progression 1, 2, 4, 8, 16, 32, 64, 128, 256 is too sparse to adequately describe isotope turnover and more frequent sampling is desired. The reaction progress model can be useful in planning sampling frequencies if rough estimates of half-lives for tissue turnover are known. A geometric sample frequency where time increases as $(2)^{1/2}$ captures more of the detail needed to determine multiple half-lives. A third advantage is that multiple experiments can be considered together to derive rate constants, an important factor when a limited number of samples are available.

Multiple turnover pools with differing half-lives

Figure 1 illustrates the advantage of using the reaction progress approach rather than the exponential fit to isotope turnover data. Although the correlation coefficient is very high for a single exponential fit to the data of Fig. 1a (r^2 = 0.98 for all data) data collected during different intervals give different half-lives (Table 2). The multiple-pool model, gives two half-lives of 19.0 ± 3.2 and 1.9 ± 0.7 days with fractions of 0.33 ± 0.04 and 0.68 ± 0.04. This is indistinguishable from the known model which had half-lives of 20.0 and 2.0 days with fractions of 0.30 and 0.70, respectively. The summed intercepts account for 1.01 ± 0.06 of the total turnover pools and is indistinguishable from 1.00. This illustrates the validity of using the reaction progress model, where isotope parameters are reduced to $\ln(1 - F)$ and plotted versus time; the reaction progress method easily determines multiple half-lives, whereas the traditional exponential fit method does not readily show whether more than one turnover pool is present. Earlier studies on tissue turnover (Thompson 1952a, b; Thompson and Ballou 1956) found multiple turnover pools using radioisotopes as tracers. We anticipate that stable isotope studies will also do so in the future.

When only one rate constant is present, the two approaches will give the same result. However, when multiple pools are present this is not the case. Table 2 shows that samples collected near the beginning of the experiment accurately predict the short-term pool, whereas those collected after a longer time are dominated by the long-term component. The initial and final values derived from the exponential fit also are incorrect if more than a single pool is present.

Reaction progress model applied to carbon turnover in controlled diet study of mammals

We reconsidered the data of Ayliffe et al. (2004) using the reaction progress model; Ayliffe et al. (2004) had noted the similarity of their approach to the reaction progress model but their results were plotted as the absolute isotope difference between the initial and equilibrium conditions (i.e., not normalized to 100%). We obtain identical half-lives and contributing fractions as does Ayliffe et al. (2004) and note that both methods give identical results when only a single isotope experiment is under consideration.

Figure 2b compares the model fits for both the exponential fit model and the reaction progress model to the original data. The exponential fit model, although it has a high correlation coefficient (r^2 = 0.87) clearly underestimates change on long time scales. On the other hand, the reaction progress pool closely fits the measured data with a very high r^2 (=0.98).

Why are there different isotope pools? Ayliffe et al. (2004) suggest that these pools correspond to an exogenous (very fast turnover pool) and two main endogenous (slow and intermediate turnover pools) sources. The fastest pool is likely to be dominated by essential amino acids that cannot be made by the animal. The intermediate turnover pool is likely related to functions of the liver, pancreas, kidney and gastrointestinal tract, all of which have been shown to have half-lives of several days (Tieszen and Fagre 1993; Hobson and Clark 1992; Simon 1989). The slow turnover pool is most likely composed principally of the skeletal muscle tissue with minor contributions from organs like heart muscle and the brain. These tissue types are known to turnover at much slower rates than the more metabolically active tissues. Furthermore skeletal muscle tissue is the only one large enough to produce the high flux/slow turnover pool of amino acids observed in the hair. Once it is possible to analyze individual amino acids, it may be possible to more directly answer this question.

Turnover rates from an isotope exchange experiment

The breath turnover experiments gave oxygen isotope turnover in woodrats, with only a single pool identified, and with a half-life of 3.1 days. A few individuals were sampled for blood in a related experiment (to be published separately); the isotope composition of breath compared to blood gives an isotope enrichment of 39.1 ± 1.3 and 38.3 ± 1.2‰ for the ^{18}O-depleted and ^{18}O-enriched experiments. The average, 38.8‰, is the

equilibrium fractionation factor $\varepsilon_{CO_2-H_2O}$ at 38 °C which is close to the body temperature of the animals. This suggests that the CO_2 in breath is in isotopic equilibrium with the aqueous blood solution. In this case, we were able to achieve a zero blank of CO_2 by stripping CO_2 out of the air entering the chamber so that there was no correction for atmospheric CO_2 as in Ayliffe et al. (2004) for large mammals.

This example, however, shows the distinction between the Ayliffe et al. (2004) and the reaction progress model because all isotope experiments can be plotted on the same axes because all experiments are normalized to 1.00 (100%).

Use of turnover rate constants to model animal diets from time-sequential stable isotope measurements

The study by Ayliffe et al. (2004) was used to calibrate isotope turnover in tissues resulting in hair formation. The mathematical formulation of the multiple turnover pool model is used to calculate diet from a sequence of tissue samples if the turnover parameters are known. To test the diet estimates from a sequence of hair samples we examined a single hair from two different horses with known diet histories: one was one of the individuals used to calibrate the turnover pools and the second was part of a different diet experiment (West et al. 2004). In the latter study, the horse being studied underwent a 7-day diet shift from C_3 alfalfa to C_4 grass and back to the C_3 alfalfa. Figures 4 and 5 show that Eq. 20 predicts the diet very well (correlation coefficients > 0.90 for estimated diet compared to known diet for all comparisons; the range in estimated $\delta^{13}C$ for the three-pool model was similar to the known range in diet; in contrast, a one-pool model (a single exponential fit to the Ayliffe et al. 2004 data, as discussed above) required a range in $\delta^{13}C$ of diet that was well outside the possible input values. In addition, the exponential fit model predicted isotope enrichment values that were significantly different than the known value, resulting in a systematic offset under the long-term diet conditions (Fig. 5b). The exponential fit method predicted isotope enrichment values of +3.7 and –1.0‰ for the initial and final conditions; feeding trials for this experiment gave an isotope enrichment of +2.7‰ for the initial conditions (Ayliffe et al. 2004). Figure 2b shows that the isotope enrichment determined by the single exponential fit method (the long-term asymptote) does not fit the data and does not give a reasonable estimate of the final equilibrium conditions.

The agreement between the calculated and the known diet shows that this mathematical formulation to calculate diet from hair very closely reproduces the known diet history. This will have great utility in wildlife ecology, because diet estimates can be obtained for animals even with no direct observational history. Growth rates can be established using overlapping isotope patterns for hair collected at different times from the same or from related individuals (Cerling et al. 2004; Cerling and Viehl 2004). This will be particularly useful when coupled with traditional observational data and new technology such as satellite-tracking using the Global Positioning System (Cerling et al. 2006). To be sure, such studies will have to make assumptions about isotope fractionation factors, and the fractional pool contributions and half-lives of turnover. Sponheimer et al. (2003) and West et al. (2004) show that large differences in feed quality have only a minor effect on isotope fractionation factors and turnover pool components. Passey et al. (2005) show that some differences in isotope fractionation factors are related to digestion strategies (e.g., hindgut versus foregut digestion) and we anticipate that further research will better refine the parameters needed to model wildlife behavior.

Initial studies that calculate a dietary history from hair show that detailed short-term diet changes can be related to vegetation changes associated with rainfall or migration, and that crop-raiding can be quantified in some cases (Cerling et al. 2006). Using hair as a dietary recorder allows information to be obtained from animals that have no visual observations and from animals whose behavior is difficult to observe.

Delayed response in tissue measurements to diet change

Processes such as hematopoiesis take place in regions where sampling for stable isotopes is not readily done: in this case the bone marrow. The delay time gives the time for red blood cell formation which, for yellow-rumped warblers, is 84 h. If the time for red-blood cell formation is not subtracted from the calculation of half-lives, the calculated half-life will be inaccurate. For example, using the exponential fit method of calculating half-lives, the half-life of carbon in red-blood cells for yellow-rumped warblers was approximately 262 h (Podlesak et al. 2005) whereas, the half-life of carbon in red-blood cells calculated using the reaction progress method was 149 h (Fig. 6). This difference in half-life calculation has important ramifications when half-lives of tissues such as red-blood cells are used for diet reconstruction.

Hobson and Clark (1993) studied American crows (*Corvus brachyrhnchos*) and found no change in the $\delta^{13}C$ of red blood cells after 2 days, but a significant change by 4 days. Applying the reaction progress variable approach to their data gives a time for hemato-

poiesis of 90 h; the half-life for this data set is 356 h assuming that the isotope fractionation factor is constant for both the initial and final diets . This compares to a half-life of 715 h in the original paper; the difference results in large part because of the difference in fractionation factor required by the exponential fit to an experiment that did not reach equilibrium.

A similar result would be found for hair follicle eruption through skin: this confounds previous studies of hair from samples that had not been plucked (e.g., shaved or cut).

Implications for isotope turnover studies of growing animals

Many isotope turnover studies have been done on animals that were growing during the experimental period. The approach outlined here places limits on the rate constants derived for animals undergoing mass change during the experimental period. The uncorrected mass assumes that the new tissue is identical to the existing tissue in its isotopic history, whereas the mass-corrected rate constants assume that the new tissue is in isotopic equilibrium with the new diet. These two cases represent the end-members for rate constant calculations. This places bounds on the slopes (and therefore half-lives) and fractional contributions of single or multiple isotope pools. Improved understanding of tissue will lead to improvements in this formulation. The Fry and Arnold (1982) approach is valid for cases where there is exponential growth and there is only one rate constant.

The equation given here (Eq. 22) is independent of the growth model (i.e., constant, exponential, sigmoidal). This method has the advantage of correcting each sample independently of all other samples, and can distinguish multiple turnover pools. Application of this method to the organisms that increase in mass during the experiment would be best accomplished if initial masses of individual specimens is known; otherwise assumptions of absolute growth must be made for each specimen.

Conclusions

In this study we demonstrate that the reaction progress variable $\ln(1 - F)$ is a useful way to determine whether an isotope turnover experiment has one or more rate constants, and therefore has advantages over the traditional method of curve-fitting with a single exponential function. Initial and equilibrium isotope compositions must be known, or closely estimated, to use the reaction progress variable. Although differences in the fractionation factors have been found for different conditions, often they can be well constrained by existing experimental (Sponheimer et al. 2003; Passey et al. 2005) or field (Cerling and Harris 1999) data. Multiple pools and their contribution to the whole can be determined using an approach commonly used in radiochemistry.

Use of isotope turnover parameters can be used to calculate diet histories of animals if a sequential sequence of isotope measurements (such as in hair) is available. Application of multiple turnover pools in hair formation gives results similar to known diet histories, whereas inadequate description of multiple turnover pools can give improbable diet input values. This will have important applications in wildlife ecology where diet histories may be reconstructed using sequential isotope records.

The delayed appearance of an isotope shift can be used to determine the residence time of a tissue before being released to an environment where it is measured. We provide an example of hematopoiesis, the residence time of blood cells in bone marrow before being released to the blood stream.

Modeling animal growth can place bounds on possible rate constants of different isotope pools, and their fractional contribution to the whole.

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