Oecologia (2004) 139: 11–22
DOI 10.1007/s00442-003-1479-x

ECOPHYSIOLOGY

L. K. Ayliffe · T. E. Cerling · T. Robinson · A. G. West · M. Sponheimer · B. H. Passey · J. Hammer · B. Roeder · M. D. Dearing · J. R. Ehleringer

Turnover of carbon isotopes in tail hair and breath CO_2 of horses fed an isotopically varied diet

Received: 14 December 2002 / Accepted: 26 November 2003 / Published online: 17 January 2004

Abstract Temporal stable isotope records derived from animal tissues are increasingly studied to determine dietary and climatic histories. Despite this, the turnover times governing rates of isotope equilibration in specific tissues following a dietary isotope change are poorly known. The dietary isotope changes recorded in the hair and blood bicarbonate of two adult horses in this study are found to be successfully described by a model having three exponential isotope pools. For horse tail hair, the carbon isotope response observed following a dietary change from a C_3 to a C_4 grass was consistent with a pool having a very fast turnover rate ($t_{1/2}$~0.5 days) that made up ~41% of the isotope signal, a pool with an intermediate turnover rate ($t_{1/2}$ ~4 days) that comprised ~15% of the isotope signal, and a pool with very slow turnover rate ($t_{1/2}$ ~140 days) that made up ~44% of the total isotope signal. The carbon isotope signature of horse blood bicarbonate, in contrast, had a different isotopic composition, with ~67% of the isotope signal coming from a fast turnover pool ($t_{1/2}$ 0.2 days), ~17% from a pool with an intermediate turnover rate ($t_{1/2}$ ~3 days) and ~16% from a pool with a slow turnover rate ($t_{1/2}$ ~50 days). The constituent isotope pools probably correspond to one exogenous and two endogenous sources. The exogenous source equates to our fast turnover pool, and the pools with intermediate and slow turnover rates are thought to derive from the turnover of metabolically active tissues and relatively inactive tissues within the body, respectively. It seems that a greater proportion of the amino acids available for hair synthesis come from endogenous sources compared to the compounds undergoing cellular catabolism in the body. Consequently, the isotope composition of blood bicarbonate appears to be much more responsive to dietary isotope changes, whereas the amino acids in the blood exhibit considerable isotopic inertia.

Keywords Carbon isotopes · Hair · Breath CO_2 · Turnover · Model

L. K. Ayliffe · T. E. Cerling (✉) · B. H. Passey
Department of Geology and Geophysics, University of Utah, Salt Lake City, UT, 84112, USA
e-mail: tcerling@mines.utah.edu
Fax: +1-801-5817065

T. Robinson · J. Hammer · B. Roeder
Department of Animal Science, Brigham Young University, Provo, UT, 84112, USA

T. E. Cerling · A. G. West · M. Sponheimer · M. D. Dearing · J. R. Ehleringer
Department of Biology, University of Utah, Salt Lake City, UT, 84112, USA

Introduction

Carbon isotopes recorded in herbivore tissues such as hair, bone, and teeth have been used for dietary assessment in numerous modern isotope ecology and paleodietary studies. The general premise of such studies is that the isotope compositions of plants are recorded in animal tissues according to predictable fractionation factors (DeNiro and Epstein 1978; Lee-Thorp and van der Merwe 1987; Cerling and Harris 1999).

Recent improvements in analytical capabilities allow the study of detailed isotope variations within individual animal tissue samples. Many studies are now attempting to extract high resolution temporal records of dietary isotope variation using incremental sampling of animal tissues that have formed continuously. High-crowned or ever-growing teeth (Fricke et al. 1998; Sharp and Cerling 1998; Wiedemann et al. 1999; Gadbury et al. 2000; MacFadden 2000; Passey and Cerling 2002) and hair samples (Jones et al. 1981; White and Schwarcz 1994; Witt et al. 1998; O'Connell and Hedges 1999; Cerling et al. 2004) have been used. The potential for insights into dietary change at the seasonal scale, or finer, is tantalizingly apparent from the findings of these studies. However, the interpretation of high-resolution isotope records has been hampered by a limited understanding of assimilation of dietary isotope compositions into animals tissues on short to intermediate timescales (i.e., days to weeks). The interpretation of these field-based high-resolution isotope studies needs to be

placed on a firmer footing before detailed diet and climate reconstruction can be realized. Stable isotope signals that are damped by biological and/or physical processes (e.g., Passey and Cerling 2002) can be deconvoluted using forward or inverse modeling if the isotope systematics of tissue formation processes were better understood.

Here, we present the results of an experiment on adult horses designed to investigate the dynamics of the carbon isotope changes recorded in hair and blood bicarbonate (via breath CO_2) resulting from several controlled changes in dietary isotopes over a 12-month period. The behavior of the carbon isotope changes accompanying the dietary switches are explored using a multicomponent exponential model, with explicit examination for several different isotope turnover pools. This was accomplished by examining the approach to isotope equilibrium, rather than absolute isotope values, which is amenable to the extraction of several first-order rates constants.

Materials and methods

Study animals and feed

Two adult horses, one male "Dandy" and one female "Sassy", aged 19 and 20 years, respectively, at the beginning of our experiment, were the subjects of our feeding trials. These two animals were born and raised in the Spanish Fork area in central Utah and were fed exclusively on locally grown C_3 forages with occasional supplementation with alfalfa for most of their life. Accordingly, all of their body tissues should have formed exclusively from C_3 dietary sources as these horses had never been exposed to anything other than C_3 feeds throughout their life.

Three feed types were used in our experiment: a locally grown C_3 orchard meadow brome grass (*Bromus inermis*), a C_4 coastal bermuda grass (*Cynodon dactylon*), and the highly nutritious C_3 dicot alfalfa (*Medicago sativa*). Table 1 gives the $\delta^{13}C$ values and C/N of the feeds used in our experiment.

Diet history

The first step in our experiment was to establish an isotopic baseline for a diet of known isotopic composition. To do this, we continued feeding with the locally grown C_3 grass that had been the mainstay of the diet before the start of our experiment. The stable isotope compositions of the breath CO_2 and body hair of the two horses were closely monitored on this diet for a period of 7 weeks to assess equilibrium conditions. Establishing a solid isotopic baseline was especially important for our experiment as we needed to ensure that all animal tissues were in equilibrium with their diet before changing the diet to one with a different isotope composition. For tissues with extremely long turnover times, such as collagen (>10 years) it was obviously not feasible to ensure equilibration with our "baseline" diet by these means. However, we expect in this case that the long-term dietary average of these two horses, from which their collagen should have formed, will not be significantly different to our "baseline", locally growth C_3 grass.

The animals were equilibrated with a C_3 diet (*Bromus*) for 7 weeks and then switched to a C_4 diet for 21 weeks (*Cynodon*), after which they were returned to a different C_3 diet for 18 weeks (alfalfa). This was followed by a 2-week switch to a C_4 diet (*Cynodon*) and then a return to a C_3 diet (alfalfa). The dietary transition from C_4 grass to the highly nutritious alfalfa in week 28 took place gradually over a period of 4 days in order to prevent

Table 1 Carbon isotope compositions, C/N ratios, and macronutrient contents of the feeds used in this study along with the number of samples analyzed (*n*) for $\delta^{13}C$ and C/N ratios

		$\delta^{13}C$	C/N	Crude protein	Neutral detergent fiber	Acid detergent fiber	Lignin	Crude fat	Ash
Orchard meadow brome grass (C_3grass)	Mean	−26.5	31.4	9.7	59.7	41.9	5.1	2.2	9.5
	±1 SE	0.1	1.8						
	n	27	25						
Coastal bermuda grass (C_4grass)	Mean	−13.5	28.1	10.3	62.3	28.6	5.4	1.6	10.3
	±1 SE	0.1	0.8						
	n	22	22						
Alfalfa	Mean	−27.2	11.6	20.6	51.5	36.6	6.5	3.7	10.8
	±1 SE	0.1	0.6						
	n	14	14						

digestive upsets and the development of the condition laminitis in the two horses.

Breath and hair samples

Breath CO_2 samples were collected a minimum of every few weeks throughout the 50 weeks of our feeding trials. In addition, for the first dietary switch from the C_3 grass to the C_4 grass, breath samples were also collected much more intensively to outline the fine-scale features in the response of breath CO_2 carbon isotopes during this diet change. For the first two days, samples were collected every 2 h, and every 4 h for days 3 and 4. Breath collections were made by drawing ~30 cc quantities of expelled horse breath into plastic syringes placed in the horses' nostrils and then passing these through an alcohol trap at (~−90°C) to remove water. A sub-sample of the water-free breath sample was transferred into 10 cc head-space vials and sealed with a rubber septum held in place by a crimped aluminum cap. Breath samples collected using this method contained ~2–4% CO_2 by volume. These water-free breath samples were analyzed by injection into a GC-column connected to a Finnigan Mat 252 mass spectrometer (Finnigan Mat, Bremen, Germany) operated in continuous flow mode. A standard, comprising ~2% CO_2 in ~98% N_2, was used to calibrate the isotope results of the unknown samples. Due to the high concentrations of CO_2 in our breath samples, it was not considered necessary to correct for the small amount of atmospheric CO_2 (<400 ppm) likely to be present in each sample.

Tail hairs were sampled from the two horses at the end of the period of feeding with the C_4 grass, and at the very end of the experiment. Tail hairs in the active (anagen) growth phase, identified by the presence of adhering tissues of the internal root sheaf near the base of the hair, were preferentially selected for our isotopic measurements. Non-keratinous material (i.e., sheath and bulb tissues) was physically removed from each hair using a scalpel blade. The surface of the hairs was then repeatedly wiped with a tissue soaked in ethyl alcohol to remove surface contaminants and any lipids. Tail hairs were then serially sectioned using two main sampling strategies: the first consisted of sectioning the tail hair into 5 mm lengths (200–300 μg), to provide total coverage of the ~50 weeks of our feeding experiment and the previous ~25 weeks; the second sampling strategy was aimed at maximizing the resolution across the three dietary transitions. Two tail hairs were micro-sampled at ~1 mm increments (40–50 μg) across these dietary transitions. The lengths of each 1 mm segment were accurately measured using a micrometer. Cumulative errors in length estimates arising from our sampling strategies are thought to be <5 mm for every 10 cm length of hair sectioned. Different hairs from Dandy and Sassy are discussed in the text as D.1, D.2, and D.3, and S1, S2, and S3, respectively.

Stable isotope measurements and nomenclature

$\delta^{13}C$ of the hair samples were determined by combustion in a Carlo Erba elemental analyzer (Carlo-Erba, Milan, Italy) connected to the Finnigan Mat isotope ratio mass spectrometer operated in continuous flow mode. The reproducibility of measurement for $\delta^{13}C$ (both small and large samples sizes) was <±0.2‰.

According to the convention, all the stable isotope data in this paper are given as the per mil (‰) deviation of isotope ratios from a standard material, viz:

$$\delta^{13}C = (R_{sa}/R_{std} - 1) \times 1000 \quad (1)$$

where R_{sa} and R_{std} are the $^{13}C/^{12}C$ ratio of the sample and standard material, respectively. For carbon isotope ratios, the standard material is the Vienna Pee Dee Belemnite (VPDB).

The isotope enrichment factor (in ‰) between substances X and Y at equilibrium is defined as (Craig 1954)

$$\varepsilon_{XY} = (R_X/R_Y - 1) \times 1000 = ((\delta_X + 1000)/(\delta_Y + 1000) - 1) \times 1000 \quad (2)$$

and ε^*_{XY} is the apparent isotope enrichment when substance X is not in equilibrium with substance Y, such as might be expected to be the case for animal tissues immediately following a change in dietary isotope compositions. In the text, we use the subscripts B, D, H, and T for breath, diet, hair, and tissue, respectively for both the $\delta^{13}C$ and ε_{XY} notation. Thus, HD, BD, and TD refer to hair-diet, breath-diet, and tissue-diet, respectively.

Establishing the chronology for tail hair

To compare the isotope values recorded in the horse tail hairs that grew throughout the 50-week period of our experiment, it was first necessary to convert hair length measurements to time. This was achieved by using the position of the isotope changes recorded in the hairs themselves, by knowing the timing of collection of each tail hair and assuming constant growth rates. A number of other assumptions were made in establishing the chronology. The first was that, as observed for the blood bicarbonate pool, there was about a 3-h time lag between the dietary change and the incorporation of new dietary components (amino acids in this case) into the blood. Furthermore, it was assumed that, after entry into the blood, the hair-forming amino acids would first be laid down in the keratinous zone of the hair follicles in detectable quantities within ~8 h. This assumption is justified on the basis of the results of a study by Ryder (1958) who found that it took ~8 h for ^{35}S labeled cystine injected into the blood of lambs to appear in the prekeratinization zone of the hair follicles. If hair formation of horses is similar to that of sheep, then the overall time lag from the start of the dietary isotope change to incorporation of dietary amino acids into hair keratin will be ~12 h.

Errors were assigned to the chronologies derived for the horse tail hairs according to the uncertainty of correctly identifying the position of each isotope transition within the relevant tail hair segments. For instance, the mid-point of the tail hair segments that first showed a significant change in $\delta^{13}C$ from baseline values was initially chosen as the start (the time=0 point) of the first dietary switch to the C_4 grass. Since the actual time=0 point could lie anywhere within this hair segment, we assigned an error equal to half the total length of the hair segment when deriving the first estimates of the tail hair chronologies. Similar error estimates were made for identifying the location of the dietary change for the diet switch from C_4 grass to alfalfa for the two tail hairs sampled at high resolution.

Modeling

We model our results using first order rate constants, as has been used to determine isotope residence times in tissues (e.g., Tieszen et al. 1983; Hobson and Clark 1992, 1993). However, by transforming the data to the departure from instantaneous isotope equilibrium ($\varepsilon_{TD} - \varepsilon^*_{TD}$), where ε_{TD} is the equilibrium enrichment factor between the tissue in question (T) and new diet (D), it is possible to extract several first order rate constants from the data. For a system composed of multiple pools (with all pools initially at isotopic equilibrium with a dietary input) the progression to the new isotopic equilibrium state after a change in diet is given by:

$$(\varepsilon_{TD} - \varepsilon*_{TD}) = k_1 e^{-\lambda 1 t} + k_2 e^{-\lambda 2 t} + k_3 e^{-\lambda 3 t} + ... + k_n e^{-\lambda n t} \quad (3)$$

$\lambda_{1,2,3...}$ and $k_{1,2,3...}$ are the rate constant [$=\ln(0.5)/t_{1/2}$] and fractional contribution of pools 1, 2, 3..., respectively, and t is the time elapsed since the start of the change to the new diet. Where the difference in the rate constants of each of these pools is sufficiently large, and the number of pools not too great, it is possible to solve the above equation uniquely for all values of k and λ, using a "curve

stripping" approach. This is analogous to the standard procedure in radiochemistry where the longer-lived components are successively subtracted off to get both the fractions and the half-lives of all components of the mixture (Overman and Clark 1960; Faure 1977; Friedlander et al. 1981). As with radionuclides, this method is satisfactory for a small number of components (n<4) that have significantly different (ca. 2×) half-lives.

For a three-pool system where $\lambda_3<\lambda_2<\lambda_1$ it follows that, a certain amount of time after the diet switch, the isotopes of both the pools with the shorter half lives will have reached their equilibrium states, leaving only the contribution from the pool with the longest half life. Mathematically this equates to Eq. 3 reducing to:

$$(\varepsilon_{TD} - \varepsilon*_{TD}) \simeq k_3 e^{-\lambda 3t} \quad (4)$$

as

$$k_1 e^{-\lambda 1t} + k_2 e^{-\lambda 2t} \simeq 0 \quad (5)$$

The slope and intercept of the straight line fit through the data in the part of the curve, where the isotopes of the first two pools have reached their equilibrium states, will then yield an estimate of λ_3 and k_3. Once these are known, the contribution of the long-lived pool (pool 3) to the other two pools can be calculated and subtracted from the total isotope signal, leaving only the contribution of pools 1 and 2 to the residual isotope signal. Treating this residual data in the same fashion as before gives λ_2 and k_2, and ultimately λ_1 and k_1.

Our formulation of $\varepsilon_{TD}-\varepsilon^*_{TD}$ is similar to that used for isotope exchange in fluid-mineral systems (Criss et al. 1987; Criss 1999). To convert $\varepsilon_{TD}-\varepsilon^*_{TD}$ to their reaction progress, we divide by $\varepsilon_{TD(t=0)}-\varepsilon^*_{TD}$ to give:

$$(\varepsilon_{TD} - \varepsilon*_{TD})/(\varepsilon_{TD(t=0)} - \varepsilon*_{TD}) = (1 - F) \quad (6)$$

where $\varepsilon_{TD(t=0)}$is the initial value at t=0 and F is a progress variable that represents the fractional approach to isotope equilibrium (F=0 at t=0 and F=1 at equilibrium).

Results

Breath CO_2

The long-term changes of the breath CO_2 $\delta^{13}C(\delta^{13}C_B)$ values were very similar for both of the horses (Fig. 1a). The $\delta^{13}C_B$ values of both animals lag the actual timing of the diet switch by ~3 h. Such a delay is thought to be due to the time taken for digestion and passage of food stuffs into the part of the digestive tract where the bulk of the nutrient adsorption takes place, and transport of adsorbed dietary components to the sites of catabolism within the body where CO_2 is produced. The overall response of the $\delta^{13}C_B$ records of both horses was an initial very rapid change in $\delta^{13}C_B$ values, with ~71% of the expected total change in $\delta^{13}C_B$ values taking place within the first 24 h of the new dietary carbon isotopes first appearing in the breath CO_2. After the first week on the new C_4 grass diet, ~87% of the expected total change in $\delta^{13}C_B$ was observed, and after 21 weeks on the new diet ~97% of the expected total change in $\delta^{13}C_B$ had occurred (Fig. 1a). Similar rates of change in $\delta^{13}C_B$ values accompanied the other diet changes of our experiment although lack of sufficient sample resolution for these precludes a rigorous comparison here.

While good long-term agreement is observed in the $\delta^{13}C_B$records of both horses,some finer-scale differences did exist. The $\delta^{13}C_B$response of Dandy a couple of weeks after the first diet change to the C_4 grass was much more irregular than that observed for Sassy (standard deviation ±0.8 vs. ±0.4‰). Dandy's $\delta^{13}C_B$values plateaued numerous times for the first few weeks after the switch to the C_4 grass (Fig. 1b). This difference was attributed to slightly lower food intake for Dandy at these times compared to

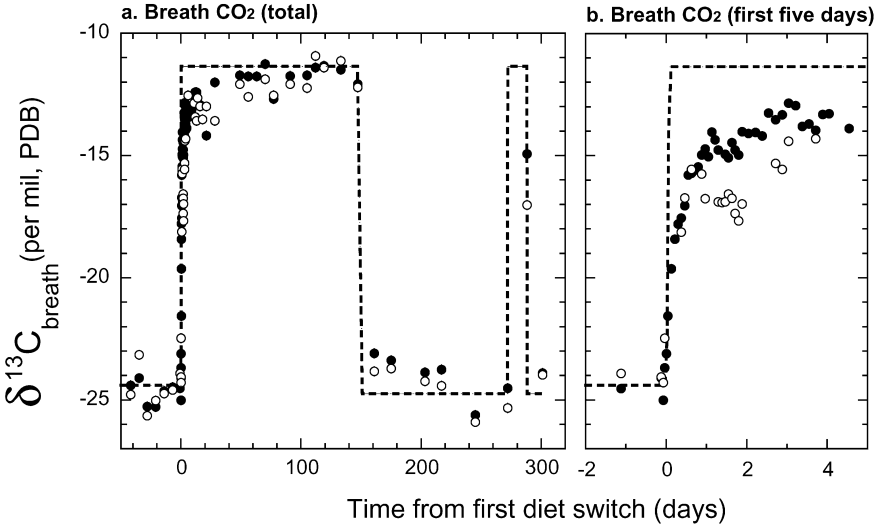

Fig. 1a, b Carbon isotope composition of breath CO_2 ($\delta^{13}C_B$) **a** for total period of collection and **b** for the first 5 days after the first dietary isotope change (Sassy *filled circles*; Dandy *open circles*). The timing of all breath samples have been corrected by −3 h to compensate for the time taken for isotope signatures to first appear in the blood after a diet change. The theoretical equilibrium $\delta^{13}C_B$ values (*dashed line*) calculated from the known dietary inputs assuming ε_{BD} (+2.1±0.2‰) for both the C_4 grass hay and the C_3 grass hay as observed before the first diet switch. The ε_{BD}we used to calculate this theoretical $\delta^{13}C_B$ curve for the alfalfa diet was +2.5 ±0.1‰ and was based on the results of measurements made on the hair of six horses, raised exclusively on C_3 forages, and then fed on alfalfa for 11 months

that of Sassy, with Dandy's lower $\delta^{13}C_B$ values reflecting the fact that he was accessing more of his internal body stores to meet metabolic demands than was Sassy at these times. While significant on the short time-scale, we do not think that these short-lived periods of slightly diminished food intake affected the longer-term isotope patterns recorded in Dandy's tissues.

Tail hair

Figure 2 shows the results for the six hairs analyzed using the rescaling method described above. For the two tail hairs (D.3 and S.3), it was also possible to compare the growth rates for two sections of each hair. Within the limitations of our ability to measure the absolute position of each hair segment, the growth rates calculated for the two periods are consistent with a constant linear growth rate (Fig. 2). The good correspondence between the rescaled isotope profiles of each of the hairs analyzed also suggests that all our tail hairs have maintained constant growth rates throughout the course of our feeding trial experiment.

$\delta^{13}C$, tail hair

Our composite tail hair $\delta^{13}C_H$ ($\delta^{13}C_H$) versus time curve (Fig. 2) exhibited a distinctly non-linear response to the first of the diet isotope switches. As for the breath CO_2 $\delta^{13}C$ data, there was a very rapid change in the $\delta^{13}C_H$ values within the first day of the switch to the C_4 grass being detectable in the hair, where between 37–46% of the expected total change in $\delta^{13}C_H$ values (calculated assuming the same $\varepsilon_{HD}$for the C_4 grass diet as for the C_3 grass diet) is estimated to have taken place. The limits placed on this estimate stem from the errors in our assigned chronology and are directly related to the size of our minimum sampling interval (of ~1 mm). One week after the switch to the C_4 grass diet, the change in $\delta^{13}C_H$values observed was ~55% of the expected total isotope change, and 21 weeks after the switch the change in $\delta^{13}C_H$was ~77% of the total expected. These rates of change for the $\delta^{13}C_H$ values on the C_4 diet were much slower than the corresponding rates for the $\delta^{13}C_B$ values for the same intervals. Clearly the horse tail hair carbon isotopes had not attained isotopic equilibrium with their new diet even after a period of 21 weeks.

The response of the $\delta^{13}C_H$ values following the diet switch from the C_4 grass to the highly nutritious alfalfa diet was similarly non-linear. Again, there was an initial rapid drop in $\delta^{13}C_H$ values, with $\delta^{13}C_H$ values changing by 28–50% of the total expected in the first day after the start of the transition to the alfalfa diet is first detectable in the hair. As the switch to the alfalfa diet was a gradual one, taking place over a 4-day period (Table 1), the estimates of rates of change in $\delta^{13}C_H$ values accompanying this dietary transition will in fact be minimum estimates. The observed change in $\delta^{13}C_H$ 1 week and 18 weeks after the start of the transition to an alfalfa diet were ~70% and ~89% of the

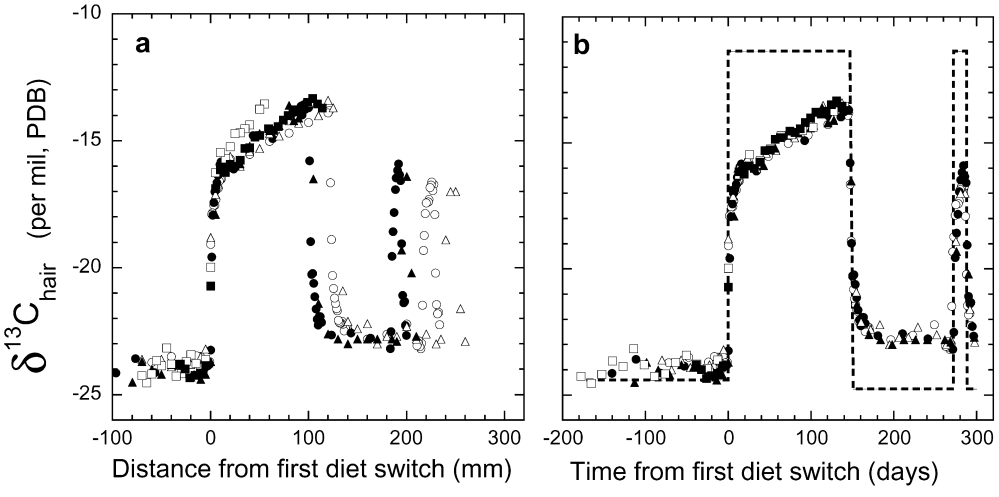

Fig. 2 **a** Tail hair $\delta^{13}C_H$ versus length for the six tail hairs analyzed. **b** Tail hair $\delta^{13}C_H$ versus time. The timing of all samples has been corrected by 12 h to compensate for the time taken for isotope signatures to first appear in the tail hair after a diet change is made. The isotope data of Sassy are shown as *closed* symbols and those for Dandy as *open*. Three tail hairs were analyzed for each animal: the first (S.1 and D.1) are represented by *squares*; the second (S.2 and D.2) by *triangles*; and the third (S.3 and D.3) by *circles*. The isotope data were rescaled according to the growth rates determined from the position of the isotope transitions and collection dates of each tail hair. The growth rates used in the rescaling are as follows: S.1 0.79 mm/day; D.1 0.39 mm/day; S.2 0.70 mm/day; D.2 0.87 mm/day; S.3 0.66 mm/day (week 50–28) and 0.69 mm/day (week 28–7); and D.3 0.76 mm/day (week 50–28) and 0.83 mm/day (week 28–7). Errors in absolute length measurements (and hence growth rates) are estimated to be <5%. Also shown is the theoretical equilibrium $\delta^{13}C_H$ curve (*dashed* line) calculated from the known dietary inputs. We assumed the same ε_{HD} (+2.7±0.1‰) for the C_4 grass that was observed for the animals on the C_3 grass hay in our calculations. This value is very similar to the ε_{HD} obtained in several field-based studies for grazers (Cerling and Harris 1999; Cerling et al. 2003). The ε_{HD}used to calculate the theoretical $\delta^{13}C_H$ curve for the alfalfa diet was +3.3±0.2‰ and was based on the results of measurements made on the hair of six horses, raised exclusively on C_3 forages, and then fed on alfalfa for 11 months

total expected change, respectively. The rates of isotopic change of the $\delta^{13}C_H$ values after the diet switch from the C_4 grass to the alfalfa diet appeared substantially faster than the rates of change observed after the switch from the C_3grass to C_4 grass diet.

Rates of isotopic change accompanying the ~2-week switch to the C_4 grass at week 46 were dampened compared to those observed for the other two dietary transitions. One week after the start of this 2-week C_4 diet switch, the change in $\delta^{13}C_H$ values was only ~47% of the total isotope change expected. This compared to the much larger changes observed for the corresponding time period for the C_3-C_4grass and C_4grass-alfalfa diet transitions of ~55% and ~70%, respectively.

The rates of change in $\delta^{13}C_H$ values following a dietary isotope change therefore would seem to be quite variable, and are possibly related to the nature of diet transition taking place.

Modeling of isotope pools

The observed isotope changes recorded in horse breath and tail hair that accompanied the various diet switches of our experiment indicate that several processes are operating on different time scales to determine the resulting isotopic compositions. We suggest that these processes could correspond to distinct isotope pools within these animals (from which blood bicarbonate and hair derive) equilibrating at different rates with the dietary input. Should these pools exchange isotopes with the dietary constituents in an exponential fashion, then it should be possible to calculate the rates of exchange (or half lives) and the relative sizes of each of these pools.

Tail hair

An examination of a plot of $\log(\varepsilon_{HD}-\varepsilon^*_{HD})$ versus time for our horse tail hair $\delta^{13}C_H$ data (Fig. 3) for the first diet switch of our experiment (where the isotopes of all horse tissues are initially fully equilibrated with the C_3 grass diet) suggests the presence of three major isotope pools whose behavior is consistent with an exponential function. There appeared to be a pool with a very fast turnover rate ($t_{1/2}$~1 day), one with an intermediate turnover rate ($t_{1/2}$~4 days) and another with a very slow turnover rate ($t_{1/2}$~140 days). These have fractional contributions of 0.40, 0.16, and 0.44, respectively, of the total change of $\delta^{13}C_D$ of 13.0‰.

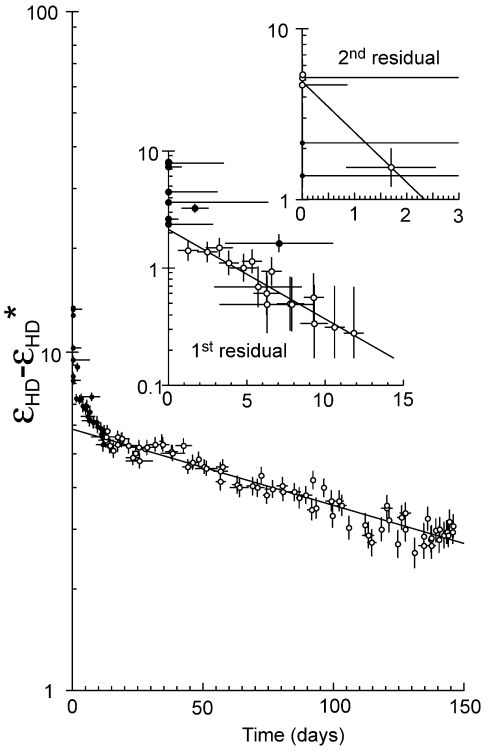

Fig. 3 $\log(\varepsilon_{HD}-\varepsilon^*_{HD})$ versus time for horse tail hair $\delta^{13}C_H$ data after the diet change from C_3 to C_4 grass. Prior to the diet change it was assumed that all horse tissues were fully equilibrated with the C_3 grass diet. Insets are the plots of residual data after subtraction of slower turnover pools (see text). Estimates of half lives and fractional pool sizes derived from these semi-log plot fits are shown in Table 2. *Open circles* are those data points used to determine rate constants; *closed circles* are those data points not used to determine rate constants

Given that the data are consistent with a system comprised of three pools equilibrating exponentially with a new dietary isotope composition, it is possible to improve upon our estimates for the location of the t=0 point within the first hair segment and ultimately, then, to improve the accuracy of our estimates of the half life and fractional contribution for the pool with the very fast turnover time. As outlined earlier, the tail hair chronologies were first estimated assuming that t=0 point was at the mid-point of the hair segments that first showed a significant change in $\delta^{13}C$ resulting from the change in dietary isotopes. However, it is obvious from the $\delta^{13}C_H$

Table 2 Half-life and fractions from Figs. 3, 4, and 5

	Pool 3	Pool 2	Pool 1	Figure	
Half life (days)	1.1±0.7	4.1±0.4	135±4	3	Hair
Fraction	0.40±0.02	0.16±0.02	0.44±0.01	3	
Half life (days)	0.5±0.1	4.3±0.7	136±5	4	Hair adjusted
Fraction	0.41±0.03	0.15±0.02	0.44±0.01	4	
Half life (days)	0.21±0.01	2.7±0.7	53±14	5	Breath
Fraction	0.67±0.05	0.17±0.02	0.16±0.03	5	

data that this is not likely to be an accurate assumption, i.e., the location of the t=0 point for those segments that give a $\delta^{13}C_H$ value just slightly different to the $\delta^{13}C_H$ value expected for the baseline diet is not going to be the same as that for segments which have $\delta^{13}C_H$ close to the theoretical equilibrium values with the new diet. To arrive at a better estimate for the position of the t=0 point, we assumed that the first segments could be thought of as being comprised of two parts: the first part being that which grew in equilibrium with the "baseline" C_3 grass diet, and the second part being that which grew during the exponential approach to isotopic equilibration with the new C_4 grass diet. Using the estimates of the half lives and fractional contributions derived from the initial semi-log plot fits, it is possible to calculate where along each hair segment the zero point should lie, such that a synthetically calculated $\varepsilon_{HD}-\varepsilon^*_{HD}$ value equals the observed $\varepsilon_{HD}-\varepsilon^*_{HD}$ value of the segment. With these improved estimates for the location of the t=0 point, it is possible to adjust the time scales of all the horse tail hairs and re-derive estimates for the $t_{1/2}$and k of each of the isotopic pools as before. These refined $t_{1/2}$and k values can then be used to again estimate the location of the t=0 point in the first hair segments, and the process repeated until no change in calculated values results. Adopting such an iterative approach, we derived improved estimates for the half life and fractional contributions of the three components comprising the tail hair carbon isotope system outlined in Fig. 4. The iterative model gives final half-lives of 0.5, 4.3, and 136 days with fractional contributions of 0.41, 0.15, and 0.44, respectively.

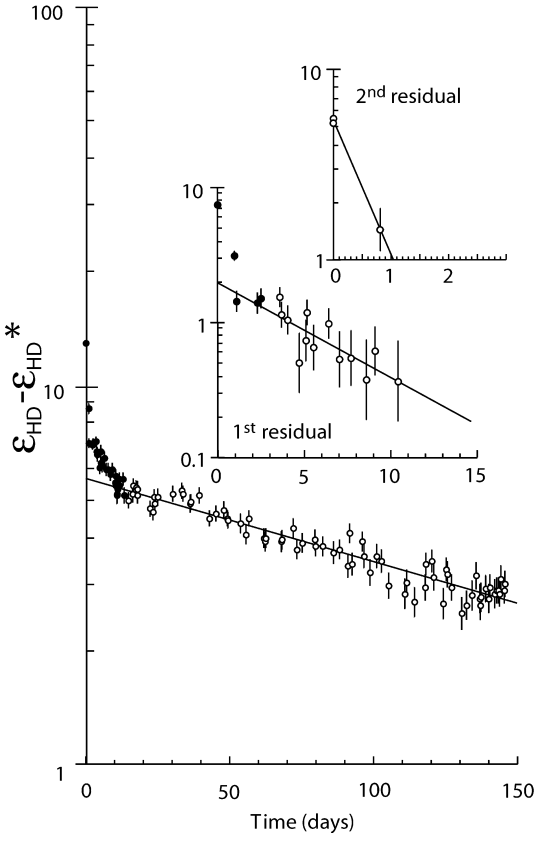

Fig. 4 $\log(\varepsilon_{HD}-\varepsilon^*_{HD})$ versus time for horse tail hair $\delta^{13}C_H$ data. Tail hair chronologies have been adjusted slightly according to the procedures outlined in the text. The improved estimates of half lives and fractional pool sizes are shown in Table 2. Symbols as in Fig. 3

Breath CO_2

The $\delta^{13}C_B$ data exhibited a very different response than the horse tail hair $\delta^{13}C_H$ data for the first diet switch. Although the breath CO_2 carbon isotopes also appeared to be comprised of three pools with slow, intermediate and fast turnover rates, the relative sizes of each of these pools was very different to that of the hair carbon isotopes (Fig. 5). Compared to that of the hair, it seemed that a significantly larger fraction of the isotope contribution for the breath carbon isotopes was coming from the pool with the fastest turnover rate. The multi-component model gives half-lives of 0.21, 2.7, and 53 days, with fractional contributions of 0.67, 0.17, and 0.16, respectively, to the total isotope signal.

Discussion

The tissues of all animals are in a continual state of degradation and renewal. For animal proteins, such turnover manifests itself in the exchange of the amino acids required for protein synthesis, and those resulting from protein breakdown, between the tissue in question and the surrounding body fluids. Rates of turnover appear to be correlated with the metabolic activity of specific tissues (Waterlow et al. 1978; Tieszen et al. 1983; Bender 1985; Simon 1989). In mammals, for instance, collagen, connective tissue, and muscle turnover at much slower rates than the more metabolically active tissues such as adipose tissue, liver, kidney, pancreas, and components of the digestive tract. There is also evidence that individual proteins within each tissue have characteristic turnover times depending on the metabolic functions each performs (Waterlow et al. 1978). Furthermore, total body protein turnover in mammals exceeds dietary protein intake by approximately five times, and there is extensive reutilization of the amino acids liberated from protein breakdown for de novo protein synthesis in the body. For humans and rats, it is estimated that amino acid reutilization is 80–90% (Waterlow et al. 1978). The amino acids of the body seem to have the ability to exhibit considerable "isotope inertia" on the time scales required to completely turnover constituent tissues.

Amino acids entering the blood from the food supply mix with those arising from the breakdown of proteins from the various tissues throughout the body. After a change in dietary isotopes, therefore, it may take some time for all the amino acids in the blood to reach isotopic equilibrium with the new diet as the exchange of amino acids between the new dietary input and the various endogenous protein reserves will not be instantaneous. For

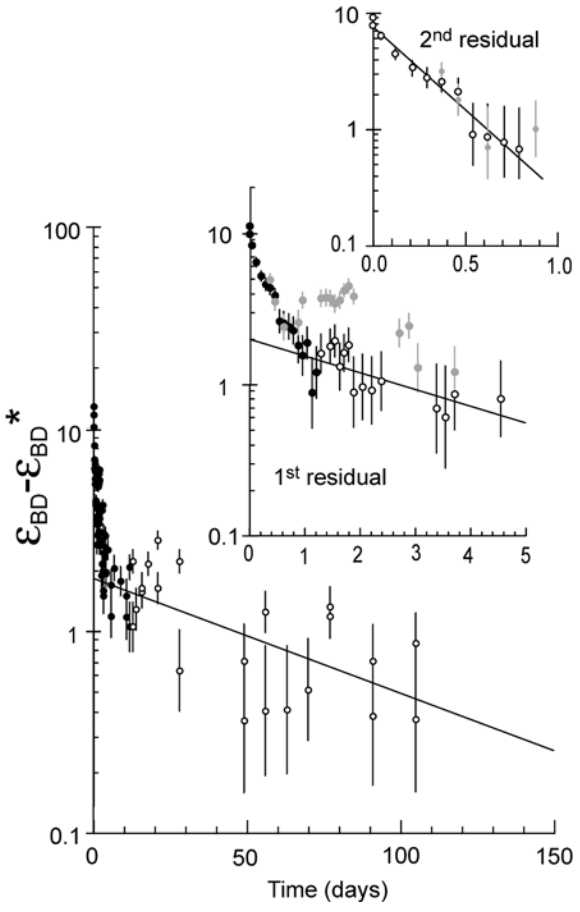

Fig. 5 $\log(\varepsilon_{BD}-\varepsilon^*_{BD})$ versus time for horse breath CO_2 $\delta^{13}C_B$ data. The estimates of half lives and fractional pool sizes are shown in Table 2 and symbols as in Fig. 3. Data points from Dandy not used in the fits, for reasons outlined in the text, are shown as *filled gray circles*

tissues such as hair, which can be more or less thought of as sampling the hair-forming amino acids present in the blood at any given instant, it is important to be aware of the time constants of these processes when attempting to extract a record of short-term dietary isotope change from the isotope records laid down in this tissue.

Potential sources of isotope pools

Several studies have attempted to determine the rates of tissue turnover for a variety of animals (i.e., Simon 1989; Hobson and Clarke 1992, 1993; Tieszen and Fagre 1993; Hilderbrand et al. 1996). In these studies, the progression of isotopic change was monitored in individual tissues over time after a change in dietary isotope compositions, or perfusion of an isotopically labeled compound into the blood. These studies in general suggest that the constituent tissues of the animals investigated to date fell into three broad categories: those with turnover rates on the time-scale of a few days (i.e., liver, kidney, components of the gastrointestinal tract, adipose tissue, blood plasma proteins); those with turnover rates of 10's to 100's of days (i.e., skeletal muscle, heart muscle, and brain tissue) and those that take many years to completely turnover (i.e., connective tissue and collagen). As each of these tissues undergoes turnover, a certain percentage of the degradation products enter the blood and mix with the assimilated constituents of recently ingested foodstuffs. The amino acids present in the blood that are ultimately involved in hair synthesis, therefore, most likely originate from a number of different sources. The exact proportions of these sources will be determined by the size and turnover rate of endogenous tissues (endogenous flux) and the amounts of amino acids entering directly from diet (exogenous flux). Similarly, the bicarbonate present in the blood can be regarded as having multiple origins: catabolism of recently assimilated dietary components, and catabolism of endogenous reserves liberated during tissue turnover.

From these considerations, it seems reasonable to suggest that the three components of isotopic change observed in our horse tail and breath carbon isotope data correspond to an exogenous (very fast turnover pool) and two main endogenous (slow and intermediate turnover pools) sources. The intermediate turnover pool is most likely composed of the liver, pancreas, kidney, and gastrointestinal tract, all of which have been shown to have half lives of several days (Simon 1989; Hobson and Clarke 1992; Tieszen and Fagre 1993). 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 (Simon 1989). 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. The exogenous dietary component is expected to be determined by the processes governing the assimilation and release of dietary constituents into the blood. The rate of isotope change for this exogenous component is probably determined by a number of different metabolic processes taking place in the liver. The liver acts as a temporary store of dietary amino acids and glucose/glycogen between meals. For amino acids, it not only attenuates diurnal fluctuations in concentrations in the blood by gradual release of its temporary stores, but also sometimes modifies the non-essential amino acid composition of ingested foodstuffs released to better meet the body's needs. We speculate that the half lives observed for the fast turnover pool of breath CO_2 and hair amino acids are probably principally governed by this regulating capacity of the liver. The turnover time of the free amino acid and glucose pools throughout the body are most likely too rapid to contribute significantly to determining the half life of the fast turnover pools observed in the horse breath CO_2 and hair isotope compositions. Turnover times for the free amino acid pool in the body, at least, are estimated to be of the order of several minutes (Waterlow et al. 1978). The different apparent relative sizes of the pools contributing to the breath $CO_2$and tail hair isotope compositions suggest that fundamentally different pro-

cesses may be governing the protein and "energy" pathways in the body. During equilibration with the C_4 grass diet of our experiment, a much larger fraction of the breath CO_2 appeared to come directly from assimilated dietary components and only minor amounts from endogenous tissue turnover compared with the hair forming amino acids.

When interpreting the fractional sizes of the constituent pools derived for our breath CO_2 and hair data derived from the $\log(\varepsilon_{TD}-\varepsilon^*_{TD})$ versus t plots, it should be noted that they are probably not reflecting the actual size of the pool in question. This is because we have assumed in our data fits that each of the constituent pools in a given tissue has the same enrichment factor as observed for the bulk tissue. Should this not be the case, then the fractional pool sizes derived in our data fits will not be equivalent to the actual fractional size of the pool in question. It is well established that dietary isotopes are not uniformly fractioned throughout all tissues in the body (DeNiro and Epstein 1978). In fact certain tissue types, such as adipose tissue, can be up to several per mil more depleted in carbon isotopes compared to the bulk isotope compositions of the body (DeNiro and Epstein 1977). The other potential complicating factor in the interpretation of our data fits is that we have assumed that the various components of our feed samples (i.e., proteins and carbohydrates, etc) have a uniform isotope composition. Should this also not be the case, and should there be preferential routing of one component of the feed into one of the isotope pools, then our derived estimates for the fractional size of that pool will not equate with the actual size of the pool. Fortunately, for carbon isotopes at least, this is not likely to be a major problem for herbivores as the proteins and carbohydrates of plants are thought not to vary greatly in terms of their carbon isotope compositions (Ambrose and Norr 1993). Given these considerations, it is perhaps more appropriate to view the fractional sizes derived from our data fits as being equivalent to "apparent" fractional pool sizes.

Numerical model predictions

To assess the isotopic response of the second half of our feeding trial experiment, we constructed a numerical model based on the three component exponential system outlined above. This enabled the estimation of the response of the horse breath CO_2 and hair carbon isotope compositions to a variable dietary isotope input. Figures 6 and 7 show the results of this numerical model for the breath CO_2 and tail hair isotope compositions using the half life estimates and fractional contributions derived from the fits of the $\log(\varepsilon_{HD}-\varepsilon^*_{HD})$ versus t plots of the C_3-C_4 grass diet switch.

A comparison of the model predictions and data for the breath CO_2 and tail hair carbon isotopes in the modeling results diverged from the measured data in several places (Figs. 6 and 7). For the tail hair isotope data, the model under-predicts the change observed in the $\delta^{13}C_H$ data after

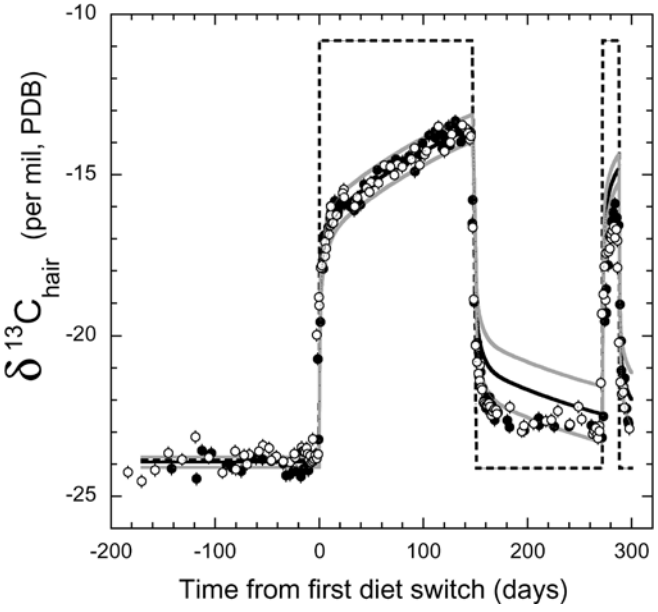

Fig. 6 Carbon isotope composition of horse tail hair ($\delta^{13}C_H$) (Sassy *filled circles*; Dandy *open circles*). The *thick black curve* is the predicted $\delta^{13}C_H$ response assuming a three component exponential model (see text) with the parameters derived from the fits of the log $(\varepsilon_{HD}-\varepsilon^*_{HD})$ versus time data for tail hair carbon isotopes outlined in Fig. 4. The two *finer gray curves* give the estimates of the ±1 S.E. errors of the model predictions. *Dashed curve* shows the $^{13}C_H$values in isotopic equilibrium with the diet. Analytical errors are indicated

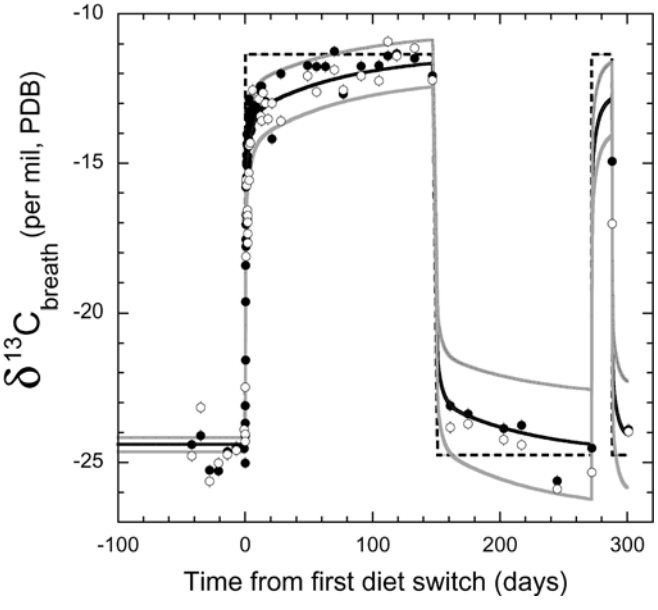

Fig. 7 Carbon isotope composition of horse breath CO_2 ($\delta^{13}C_B$) (Sassy *filled circles*; Dandy *open circles*). The *thick black curve* is the predicted $\delta^{13}C_B$ response assuming a three component exponential model (see text) with the parameters derived from the fits of the $\log(\varepsilon_{BD}-\varepsilon^*_{BD})$ versus t data for horse breath CO_2 carbon isotopes outlined in Fig. 5. The two *finer gray curves* give the estimates of the ±1 S.E. errors of the model predictions

the switch to the alfalfa diet and over predicts the observed change for the 2-week switch back to the C_4 grass diet near the end of the experiment. The situation for the breath CO_2 isotopes in the second half of our experiment is not as

clear due to the infrequent sampling and inherently noisier data. If the two data points at the end of the 2-week change to the C_4 grass diet are real, it does seem that the model predictions also overestimate the isotope response during this short dietary change.

Should our three component exponential model be an accurate representation of the processes operating to determine the isotope compositions of horse breath CO_2 and tail hair, the divergences of the model predictions and observed isotope data in the second half of our experiment must indicate that some of the parameters used in the model formulations are not appropriate. Assuming that the half lives of the various pools in our model are determined by fundamental aspects of the animal's physiology and are not likely to change, then this suggests that the fractional contributions of each pool may be different in the second half of our experiment. Given that the alfalfa diet contains approximately three times the nitrogen (and hence protein) content of the C_4 grass diet (Table 1), it would be expected that the total amount of amino acids from the diet (fast turnover pool) present in the blood might go up on this diet, increasing the overall concentration of amino acids in the blood but decreasing the relative contributions from the two endogenous pools to hair production. The likely effect on the exogenous supply of compounds undergoing catabolic oxidation resulting from a change to an alfalfa diet is not as easy to predict. It might be expected, then, that proportionately more dietary proteins will be catabolized on this diet, but this is not likely to contribute significantly to total energy production. Given that the carbohydrate contents of the C_4 grass and alfalfa diets are probably not substantially different from one another, this suggests that the exogenous contribution to the blood bicarbonate isotopes will probably not change substantially on the alfalfa diet.

By increasing the relative contribution of the fast turnover pool on the alfalfa diet in our numerical model formulations, and keeping the contributions of the intermediate and slow turnover pools in the same relative proportion to one another as that observed during the C_3-C_4 grass switch, it is possible to obtain a much better agreement between model output and the data for the horse tail hair isotope compositions. For the tail hair carbon isotope model formulations, increasing the fractional contribution of the fast turnover pool from 0.42 to ~0.65 results in a good match of model predictions and the data (Fig. 8a).

A significant difference also exists between the model predictions and the observed isotope data of the tail hairs during the 2-week change to the C_4 grass diet. In this case, the model over-predicts the change in tail hair isotope compositions. We suggest that this difference might reflect the fact that the two horses built up body condition (i.e., body protein mass and fat stores) while on the previous, highly nutritious alfalfa diet. If this was in fact the case, then this would have resulted in an increase in the fractional contributions of these two endogenous pools when the diet was switched back to the C_4 grass, compared to the fractional contributions that existed during the C_3-C_4 grass diet switch. Since this endogenous supply will have much more negative carbon compositions

Table 3 Half-life and fractions from Fig. 8

	Pool 3	Pool 2	Pool 1	Diet change	Figure
Half life (days)	0.5	4.3	136		8
Fraction	0.42	0.15	0.44	C_3-C_4grass	8
Fraction	0.65	0.09	0.26	C_4 grass-alfalfa	8b
Fraction a	0.42	0.15	0.44	C_4 grass-alfalfa	8a
Fraction b	0.53	0.12	0.35	C_4 grass-alfalfa	8a
Fraction c	0.65	0.09	0.26	C_4 grass-alfalfa	8a
Fraction d	0.77	0.06	0.17	C_4 grass-alfalfa	8a
Fraction e	0.42	0.15	0.44	C_4 grass-alfalfa	8b
Fraction f	0.31	0.18	0.52	C_4 grass-alfalfa	8b
Fraction g	0.20	0.20	0.60	C_4 grass-alfalfa	8b
Fraction h	0.10	0.23	0.68	C_4 grass-alfalfa	8b

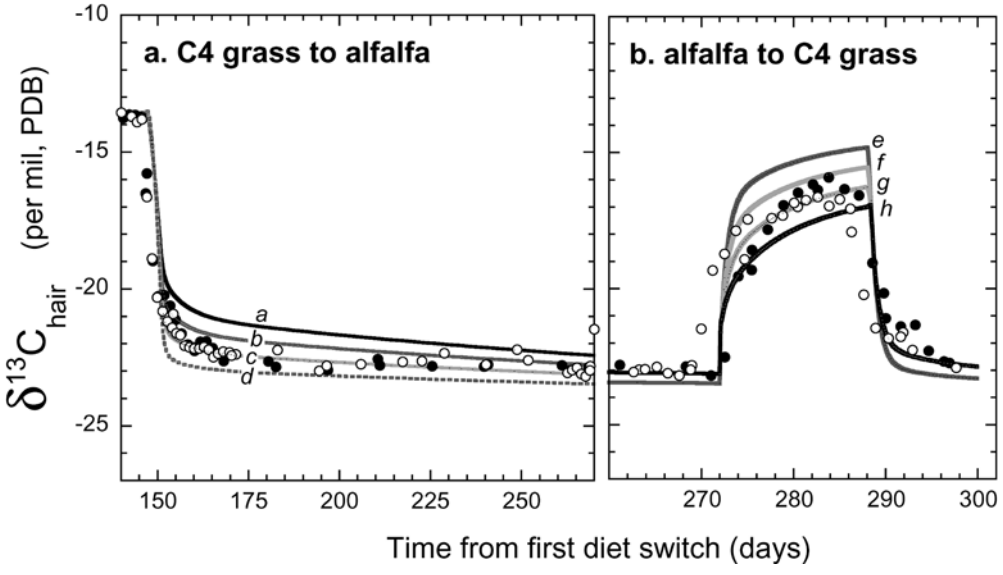

Fig. 8a, b $\delta^{13}C$ tail hair data and model predictions using various parameters (see Table 3) for diet changes **a** C_4 grass to alfalfa and **b** alfalfa to C_4 grass. Symbols as in Fig. 6

than the input from the C_4 grass diet, this will result in the model over-predicting the isotope change recorded in the tail hair. By increasing the fractional contributions of the two endogenous pools for this period by ~30% in our model, a much better agreement is obtained with the observed tail hair isotope data, (Fig. 8b).

Comparisons of the numerical modeling results of the breath CO_2 data with the measured data for the second half of our experiment are tenuous due to the fact that our breath collections for this period were separated by long intervals, and the data themselves are quite noisy. During the period of equilibration with the alfalfa diet, the model predictions using the same parameters as that derived from the C_3-C_4 grass diet switch appear to agree well with the observed data, Fig. 9a. However it should be noted that several combinations of input parameter will generate output model curves with just as good agreement with the data. We cannot be sure whether or not there are changes in the relative fractional contributions of the constituent pools accompanying the diet switch to alfalfa. For the 2-week switch back to the C_4 grass near the end of the experiment, our data points are too sparse to draw solid conclusions. Despite this, these data suggest that the relative fractional contribution of the slow turnover pool in the numerical model calculations has to be substantially increased (from 0.16 to ~0.48) to obtain a reasonable agreement with the data (Fig. 9b). This also suggests that the horses may be accessing larger amounts of isotopically depleted endogenous reserves (i.e., proteins and fats laid down during the alfalfa diet) for catabolism than they were on the previous C_3 or C_4 grass diets. Together with the tail hair findings, these results suggest that the body condition of an animal has an influences on the isotope signatures recorded in animal tissues accompanying dietary isotope changes.

Table 4 Half-life and fractions from Fig. 9

	Pool 3	Pool 2	Pool 1	Diet change	Figure
Half life (days)	0.21	2.70	53		9
Fraction	0.67	0.17	0.16	C_3-C_4grass	9
Fraction	0.67	0.17	0.16	C_4 grass-alfalfa	9b
Fraction A	0.84	0.09	0.08	C_4 grass-alfalfa	9a
Fraction B	0.67	0.17	0.16	C_4 grass-alfalfa	9a
Fraction C	0.51	0.26	0.24	C_4 grass-alfalfa	9a
Fraction D	0.67	0.17	0.16	alfalfa-C_4 grass	9b
Fraction E	0.01	0.51	0.48	alfalfa-C_4 grass	9b
Fraction F	0.36	0.16	0.48	alfalfa-C_4 grass	9b
Fraction G	0.52	0.00	0.48	alfalfa-C_4 grass	9b

Conclusions

We have shown that detailed temporal isotope measurements on breath CO_2 and on hair give three isotope reservoirs that can be modeled to predict the isotope composition of hair from known diet histories. Previous studies of hair and other tissues have estimated only one isotope reservoir and one turnover rate, in a large part because of less temporal resolution in those studies (e.g., Tieszen et al. 1983; Hobson and Clarke 1992, 1993). For both hair and breath CO_2, we find two very short-lived pools, and one long-lived pool that is more than one order of magnitude longer than the intermediate pool.

This model will allow the calculation of diet from a temporal sequence of hair and therefore will be useful in wildlife studies to understand the diet history of mammals. Likewise, understanding of the isotope pools and turnover times in breath CO_2, and therefore blood bicarbonate, from which teeth are formed (LeGeros 1981) can be used to model tooth enamel formation. Inverse modeling of isotope profiles in tooth enamel, along with the parameters describing the isotopic composition of blood bicarbonate and the details of enamel maturation (Passey and Cerling 2002) will allow diet reconstruction of both fossil and

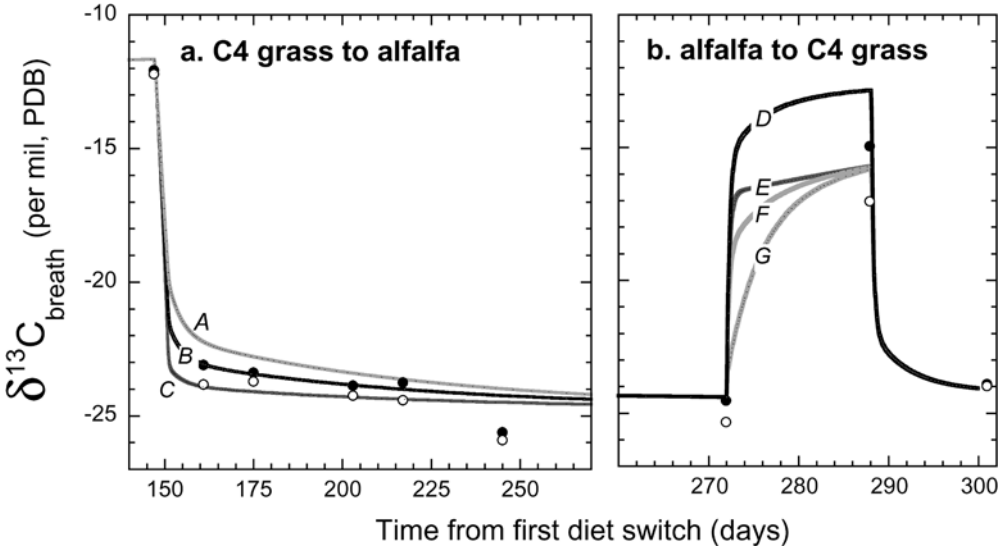

Fig. 9a, b $\delta^{13}C$ horse breath data and model predictions using various parameters (see Table 4) for diet changes **a** C_4 grass to alfalfa and **b** alfalfa to C_4 grass. Symbols as in Fig. 7

extant mammals. We look forward to the application of compound-specific studies to determine the routing of carbon and other isotopes in the formation of hair and other tissues.

How representative the diet-tissue relationships derived in this study are for other mammalian herbivores remains to be explored. It is probably the case that the isotope signatures of the tissues of other herbivores will also be controlled by the same basic partitioning between exogenous and endogenous contributions to such things as hair forming amino acids and blood bicarbonate. However, species differences in rates of endogenous tissue turnover and metabolic efficiencies are likely to affect the rates of equilibration with new dietary isotope compositions. The influence of such factors as the presence of ruminal microflora on responses to changes in dietary isotopes recorded in animal tissues also remains largely unknown at this stage. Additional experiments on other species are obviously needed to investigate these aspects.

Acknowledgements Funding to support this research was provided by the Packard Foundation, Brigham Young University (BYU), and The University of Utah. The authors are much indebted to the students and staff of the BYU animal facility and Y. Rahman for help with sample collection and animal care. C. Cook and M. Lott are thanked for providing technical support in the SIRFER mass spectrometer lab.

References

Ambrose SH, Norr L (1993) Carbon isotopic evidence for routing of dietary protein to bone collagen, and whole diet to bone apatite carbonate: purified diet growth experiments. In: Lambert J, Grupe G (eds) Molecular archaeology of prehistoric human bone. Springer, Berlin Heidelberg New York, pp 1–37

Bender DA (1985) Amino acid metabolism. Wiley, New York

Cerling TE, Harris JM (1999) Carbon isotope fractionation between diet and bioapatite in ungulate mammals and implications for ecological and palaeoecological studies. Oecologia 120:347–363

Cerling TE, Harris JM, Passey BH (2003) Dietary preferences of East African bovidea based on stable isotope analysis. J Mammal 84:456–470

Cerling TE, Passey BH, Cook CS, Ehleringer JR, Harris JM, Kasiki SM, Dhidha MB (2004) An orphan's tale: seasonal dietary changes in elephants from Tsavo National Park, Kenya. Palaeogeog Palaeoclimatol Palaeoecol (in press)

Craig H (1954) Carbon-13 in plants and the relationships between carbon-13 and carbon-14 variations in nature. J Geol 62:115–149

Criss RE (1999) Principles of stable isotope distribution. Oxford University Press, Oxford

Criss RE, Gregory RT, Taylor HP (1987) Kinetic theory of oxygen isotope exchange between minerals and water. Geochim Cosmochim Acta 51:1099–1108

DeNiro MJ, Epstein S (1977) Mechanism of carbon isotope fractionation associated with lipid synthesis. Science 197:261–263

DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. Geochim Cosmochim Acta 42:495–506

Faure G (1977) Principles of isotope geology. Wiley, New York

Fricke HC, Clyde WC, O'Neil JR (1998) Intra-tooth variations in $\delta^{18}O(PO_4)$ of mammalian tooth enamel as a record of seasonal variations in continental climate variables. Palaeogeogr Palaeoclimatol Palaeoecol 126:91–99

Friedlander G, Kennedy JW, Macias ES, Miller JM (1981) Nuclear and radiochemistry. Wiley, New York

Gadbury C, Tood L, Jaren AH, Amundson R (2000) Spatial and temporary variations in the isotopic composition of bison tooth enamel from the early Holocene Hudson-Meng bone bed. Palaeogeogr Palaeoclimatol Palaeoecol 157:79–93

Hilderbrand GV, Farley SD, Robbins CT, Hanley TA, Titus K, Servheen C (1996) Use of stable isotopes to determine diets of living and extinct bears. Can J Zool 74:2080–2088

Hobson KA, Clarke RG (1992) Assessing avian diets using stable isotopes I: turnover of ^{13}C in tissues. Condor 94:181–188

Hobson KA, Clarke RG (1993) Turnover of ^{13}C in cellular and plasma fractions of blood: implications for nondestructive sampling in avian dietary studies. Auk 110:638–641

Jones RJ, Ludlow MM, Troughton JH, Blunt CG (1981) Changes in the natural carbon isotope ratios of the hair from steers fed diets of C_4, C_3 and C_4 species in sequence. Search 12:85–87

Lee-Thorp J, van der Merwe NJ (1987) Carbon isotope analysis of fossil bone apatite. S Afr J Sci 83:712–715

LeGeros RZ (1981) Apatites in biological systems. In: Pamplin B (ed) Inorganic biological crystal growth, progress in crystal growth, and characterization, vol 4. Pergamon, New York, pp 1–45

MacFadden BJ (2000) Middle Pleistocene climate change recorded in fossil mammal teeth from Tarijia, Bolivia, and upper limit of the Ensenadan Land-Mammal Age. Quat Res 54:121–131

O'Connell TC, Hedges REM (1999) Investigation into the effect of diet on modern human hair isotopic values. Am J Phys Anthropol 108:409–425

Overman RT, Clark HM (1960) Radioisotope techniques. McGraw-Hill, New York

Passey BH, Cerling TE (2002) Tooth enamel mineralization in ungulates: implications for recovering a primary isotopic time-series. Geochim Cosmochim Acta 66:3225–3234

Ryder ML (1958) Nutritional factors influencing hair and wool growth. In: Montagna W, Ellis RA (eds) The biology of hair growth. Academic Press, New York, pp 305–334, 520

Sharp ZD, Cerling TE (1998) Fossil isotope records of seasonal climate and ecology: straight from the horse's mouth. Geology 26:219–222

Simon O (1989) Metabolism of proteins and amino acids. In: Bock HD, Eggum BO, Low AG, Simon O, Zebrowska T (eds) Protein metabolism in farm animals. Evaluation, digestion, adsorption, and metabolism. Oxford University Press, Oxford, pp 273–363

Tieszen LL, Fagre T (1993) Effect of diet quality and composition on the isotope composition of respiratory CO_2, bone collagen, bioapatite, and soft tissues. In: Lambert J, Grupe G (eds) Molecular archaeology of prehistoric human bone. Springer, Berlin Heidelberg New York

Tieszen LL, Boutton TW, Tesdahl KG, Slade NA (1983) Fractionation and turnover of stable carbon isotopes in animal tissue: implications for $\delta^{13}C$ analysis of diet. Oecologia 57:32–37

Waterlow JC, Garlick PJ, Millward DJ (1978) Protein turnover in mammalian tissues and in the whole body. Elsevier/North-Holland Biomedical Press, Amsterdam

White CD, Schwarcz HP (1994) Temporal trends in stable isotopes for nubian mummy tissues. Am J Phys Anthropol 93:165–187

Wiedemann FB, Bocherens H, Mariotti A, von den Driesch A, Grupe G (1999) Methodological and archaeological implications of intra-tooth variations ($\delta^{13}C$, $\delta^{18}O$) in herbivores from Ain Ghazal (Jordan, Neolithic). J Archaeol Sci 26:697–704

Witt GB, Moll EJ, Beeton RJS, Murray PJ (1998) Isotopes, wool, and rangeland monitoring: let the sheep do the sampling. Environ Manage 22:145–152