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Should we use one-, or multi-compartment models to describe ^{13}C incorporation into animal tissues?

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Understanding rates of isotopic incorporation and discrimination factors between tissues and diet is an important focus of ecologists seeking to use stable isotopes to track temporal changes in diet. We used a diet-shift experiment to measure differences among tissues in ^{13}C incorporation rates in house sparrows (*Passer domesticus*). We predicted faster incorporation rates in splanchnic than in structural tissues. We also assessed whether isotopic incorporation data were better supported by the one-compartment models most commonly used by ecologists or by multi-compartment models. We found large differences in the residence time of ^{13}C among tissues and, as predicted, splanchnic tissues had faster rates of isotopic incorporation and thus shorter retention times than structural tissues. We found that one-compartment models supported isotopic incorporation data better in breath, excreta, red blood cells, bone collagen, and claw tissues. However, data in plasma, intestine, liver, pectoralis muscle, gizzard, and intestine tissues supported two-compartment models. More importantly, the inferences that we derived from the two types of models differed. Two-compartment models estimated longer ^{13}C residence times, and smaller tissue to diet differences in isotopic composition, than one-compartment models. Our study highlights the importance of considering both one- and multi-compartment models when interpreting laboratory and field isotopic incorporation studies. It also emphasizes the opportunities that measuring several tissues with contrasting isotopic residence times offer to elucidate animal diets at different time scales. Copyright © 2008 John Wiley & Sons, Ltd.

Tieszen *et al*.[1] observed that the rate of isotopic incorporation differed between tissues and associated this variation with differences in metabolic activity. Their observation is useful because it gives ecologists a variety of temporal windows through which they can observe changes in an organism's diet. Some tissues, such as liver and plasma proteins, have faster rates of incorporation and track isotopic changes in diet closely, whereas tissues with slow incorporation rates (such as red blood cells, muscle, and bone collagen) integrate inputs from a larger temporal window.[1–3] In spite of the usefulness of this observation, their conjecture of an association between metabolic rate and isotopic incorporation has led to confusion. They assumed that a tissue's respiration rate (measured by its rate of oxygen consumption) is directly related to the rate at which the tissue incorporates and loses materials. They supported their conjecture by showing that *in vitro* oxygen consumption rates were negatively correlated with the half-lives of $\delta^{13}C$ in different tissues. Understandably, the results of their study have come to be interpreted to mean that organisms and tissues with high rates of oxygen consumption should have faster rates of isotopic incorporation.[4–6]

Carleton and Martínez del Rio[7] tested this hypothesis by increasing the oxygen consumption rates of house sparrows by exposing them to chronic cold. Despite a doubling in $\dot{V}O_2$ (rate of oxygen consumption), the incorporation of the new diet into blood tissue did not change between sparrows housed at two different temperatures. Their result demonstrates that tissue isotopic turnover can be uncoupled from changes in metabolic rate. They suggested that the hypothesis of Tieszen *et al*.[1] must be interpreted more narrowly, and that 'metabolic rate' should be construed as the rate of macromolecular synthesis and catabolism. More specifically, Carleton and Martínez del Rio[7] hypothesized that the rates of isotopic incorporation into the tissues most widely studied by isotopic ecologists should be proportional to protein turnover.[8] Their hypothesis is consistent with the observation that protein turnover differs among different tissues.[9–12]

A large number of studies have revealed that splanchnic tissues (visceral organs) such as liver, stomach, and gastrointestinal tract have faster rates of protein turnover

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than structural tissues such as skeletal muscle and bone collagen.[13] Most studies of isotopic incorporation, however, have only analyzed one or a few tissues from the same organism.[14] We tested whether the rates of carbon isotopic incorporation between several splanchnic and structural tissues from the same organism differed and whether we could predict the rank order in their incorporation rates based on the results of protein turnover studies. We hypothesized that (1) splanchnic tisues would have higher rates of incorporation than structural tissues and (2) the magnitude of the rates of isotopic incorporation found in different tissues would be ranked in the same order as the rates of protein synthesis.[14] We examined these hypotheses in ten different splanchnic and structural tissues obtained from house sparrows following a diet switch.

Ecologists have traditionally estimated isotope incorporation rates using one-compartment models with first-order kinetics.[7,15] In contrast, since the late 1950s, physiologists studying protein turnover typically rely on multi-compartment models.[13] Independently from these physiological studies, Sponheimer *et al.*,[16] and, more recently, Cerling *et al.*,[17] have called for the use of multi-compartment models when calculating isotopic incorporation rates in tissues. Cerling *et al.*[17] argued that by using one-compartment models in isotopic incorporation studies, ecologists have over-simplified a complex process. Implicit in their contention[17] is the observation that, by using one-compartment models, we may be biasing the estimates of how long isotopes stay in tissues. We used our data on the isotopic incorporation of ^{13}C into several tissues of house sparrows to ask whether (1) isotopic incorporation data are best described by one- or multi-compartment models and (2) whether one draws different inferences when using one- or multi-compartment models. To answer these questions we used the approach proposed by Martínez del Rio and Anderson-Sprecher.[18] This approach uses the Akaike's Information Criterion (AIC) to compare the relative support of different models given the data, and allows the estimation of the average retention time of an isotope in a tissue and the error associated with this estimated value.[18]

EXPERIMENTAL

Sparrow maintenance and experimental design

Sixty house sparrows (body mass ± standard deviation (SD) = 21.51 ± 0.14 g) were captured with mist nets in Laramie, Wyoming, USA (41°18′50.71″N 105°35′31.56″W) in February, 2003, and housed at 21°C (±1°C), on a 12L:12D photoperiod, in 1 × 1 × 1 m wire screen cages. The birds were fed whole wheat ($\delta^{13}C = -25.36‰ \pm 0.19$ VPDB, n = 5, Table 1) for 120 days, and then shifted to cracked corn ($\delta^{13}C = -11.28‰ \pm 0.21$ VPDB, n = 5). A mineral and vitamin supplement was mixed with the birds' water (4 mg L^{-1}, $\delta^{13}C = -25.54‰ \pm 0.23$ VPDB, n = 5). On days 0, 1, 2, 4, 8, 16, 32, 64, and 128 after switching to the corn diet, two or four birds were randomly chosen for isotopic measurements. Forty-eight hours prior to sample collection, birds were housed individually in 30.5 × 15.2 × 20.3 (length × width × height) cm cages to allow them to acclimate to the experimental conditions. The bottom of each cage contained a wire mesh, with a removable tray to collect excreta.

Sample collection and stable isotope analysis

To ensure that samples were taken on fasted birds, food was removed 24 h prior to sample collection. To measure the $\delta^{13}C$ of exhaled CO_2, individual birds were placed in 500 mL environmental chambers (Nalgene®, Rochester, NY, USA) connected to a gas line on one end and fitted with a one-way stopcock valve on the other. The chamber was flushed with CO_2-free air for 30 s to remove ambient CO_2. After allowing exhaled CO_2 to accumulate in the chamber for 4 min, a 30 mL air sample was extracted with a syringe. This sample was gathered within 3 min after birds had been taken from their cages. We transferred air samples to pre-evacuated gastight vials (Exetainer®, Labco Ltd., High Wycombe, UK) and then measured the isotopic composition of CO_2 on a Micromass VG Optima continuous flow mass spectrometer (Micromass UK Ltd., Manchester, UK) coupled to a gas injector (GV Instruments, Manchester, UK) at the Mass Spectrometry Isotope Facility at Colorado State University. The precision of these analyses was ± 0.17 (‰)

Table 1. The incorporation of ^{13}C into some tissues was best described by one-compartment models ($\Delta_{1\text{-}2}$ values negative and in bold), whereas that of others was best described by two-compartment models. Asterisks denote over-parameterized models with singular approximate Hessians

Tissue	One-compartment	AICc1	Two-compartment	AICc2	$\Delta_{1\text{-}2}$
Breath	$-14.6 - 11.9e^{\frac{-t}{0.9}}$	100.98	$-14.6 - 11.9(0.5e^{\frac{-t}{0.9}} + 0.5e^{\frac{-t}{0.9}})^*$	106.94	**−5.96**
Excreta	$-12.4 - 12.8e^{\frac{-t}{0.9}}$	67.48	$-11.8 - 13.4(0.9e^{\frac{-t}{0.8}} + 0.1e^{\frac{-t}{47.3}})$	69.43	**−1.95**
Plasma	$-13.0 - 10.2e^{\frac{-t}{4.7}}$	87.76	$-11.9 - 11.8(0.56e^{\frac{-t}{2.1}} + 0.44e^{\frac{-t}{19.3}})$	70.41	17.35
Intestine	$-12.7 - 10.2e^{\frac{-t}{7.4}}$	105.69	$-11.2 - 13.2(0.43e^{\frac{-t}{1.4}} + 0.57e^{\frac{-t}{25.0}})$	63.60	42
Liver	$-12.5 - 11.4e^{\frac{-t}{8.4}}$	83.116	$-11.3 - 13.4(0.44e^{\frac{-t}{2.5}} + 0.56e^{\frac{-t}{23.3}})$	39.89	43.23
Gizzard	$-11.3 - 11.7e^{\frac{-t}{15.7}}$	55.66	$-10.6 - 12.9(0.29e^{\frac{-t}{3.8}} + 0.71e^{\frac{-t}{27.1}})$	31.49	24.17
Heart	$-11.7 - 12.4e^{\frac{-t}{20.0}}$	64.93	$-11.4 - 13.3(0.14e^{\frac{-t}{2.0}} + 0.86e^{\frac{-t}{25.2}})$	59.18	5.75
Pectoralis	$-11.6 - 12.2e^{\frac{-t}{24.4}}$	99.84	$-10.6 - 14.1(0.2e^{\frac{-t}{3.2}} + 0.77e^{\frac{-t}{41.6}})$	95.68	4.16
Red blood cells	$-10.0 - 14.8e^{\frac{-t}{27.8}}$	86.16	$-10.0 - 14.8(0.50e^{\frac{-t}{27.8}} + 0.50e^{\frac{-t}{27.8}})^*$	92.12	**−5.96**
Bone collagen	$-13.0 - 14.8e^{\frac{-t}{29.5}}$	111.83	$-14.2 - 12.1(0.13e^{\frac{-t}{1.8}} + 0.87e^{\frac{-t}{31.8}})$	115.84	**−4.09**
Claw	$-8.6 - 14.7e^{\frac{-t}{85.2}}$	76.15	$-8.6 - 14.7(0.50e^{\frac{-t}{85.2}} + 0.50e^{\frac{-t}{85.2}})^*$	82.21	**−5.96**

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(SD). Our standard was CO_2 gas ($\delta^{13}C = -37.8‰$ Vienna Pee Dee Belemnite (VPDB). After breath samples had been collected, we obtained blood samples (≈50 μL) from the brachial vein using a 0.5 mL syringe with 30 gauge needles and transferred the samples to 50 μL capillary tubes. The samples were centrifuged for 3 min in a microhematocrit centrifuge to separate cells from plasma and then each was injected into separate 0.5 mL plastic microcentrifuge tubes. Red blood cells and plasma were dried to constant mass in an oven at 55°C and homogenized into a fine powder. After blood collection, the birds were sacrificed by CO_2 asphyxiation. Pectoralis muscle, heart, liver, gizzard, small intestine, a claw from the halux, and excreta material from the bottom of the cage were collected from each bird. The gastrointestinal tract was flushed with ultra-pure water to remove undigested food and excreta material. All tissues were dried in an oven at 55°C to constant mass. The tissues were then ground to a homogeneous mixture, placed in 2 mL scintillation vials, and soaked twice for 48 h in petroleum ether to remove lipids.[19] The samples were dried, homogenized into a fine powdered and weighed into 3 × 5 mm tin capsules (≈0.9 mg). The samples were analyzed with a Carlo-Erba NA1500 elemental analyzer (Milan, Italy) coupled to a VG Isochrom stable isotope ratio mass spectrometer (GV Instruments) at the Mass Spectrometry Isotope Facility at the University of Wyoming. The isotopic ratios in this paper are reported on a per mil (‰) basis relative to VPDB for carbon.

Statistical analyses

The isotopic incorporation data were fitted using a Marquardt non-linear fitting routine (NLIN code in Statistical Analysis Software®) (SAS, Cary, NC, USA) to either a one- or a two-compartment model using the following equations, respectively:

$$\delta^{13}C_{(t)} = \delta^{13}C_{(\infty)} - (\delta^{13}C_{(\infty)} - \delta^{13}C_{(0)})e^{-\frac{t}{\tau}} \quad (1)$$

$$\delta^{13}C_{(t)} = \delta^{13}C_{(\infty)} - (\delta^{13}C_{(\infty)} - \delta^{13}C_{(0)}) \times (pe^{-\frac{t}{\tau_1}} + (1-p)e^{-\frac{t}{\tau_2}}) \quad (2)$$

Equations (1) and (2) differ from those used in most isotopic incorporation studies in their use of the reciprocal of the fractional incorporation rate ($\tau = 1/k$, days) as a parameter to describe incorporation rate.[7,17,20] We chose to use this parameter for two reasons: (1) it has a clear intuitive interpretation as the average retention (or residence) time of ^{13}C for the one compartment model, and (2) the non-linear routine used in our analysis gave asymptotic standard error estimates.[18] In previous studies, such as those listed above, researchers estimated the fractional rate of incorporation ($k = 1/\tau$) and used it to estimate half-lives of an element in a tissue ($t_{1/2} = \tau \times \mathrm{Ln}(2) = \mathrm{Ln}(2)/k$). Although the non-linear algorithm always found a locally optimal one-compartment model, for several tissues the selected two-compartment model was an over-parameterized one-compartment model. In these cases, the algorithm selected $\tau_1 = \tau_2$ and $p = 0.5$, resulting in singular Hessians. To assess the weight of evidence in favor of a one- or a two-compartment model, we calculated the AIC corrected for small samples (AICc) for each of the models:

$$\mathrm{AICc} = \mathrm{n}[\mathrm{Log}(2\pi) + 1 + \mathrm{Log}(\mathrm{SSE}/\mathrm{n})] + 2\mathrm{K} + 2\mathrm{K}(\mathrm{K}+1)/(\mathrm{n}-\mathrm{K}-1), \quad (3)$$

where n equals the number of observations, K is the number of parameters in the model (4 and 6 for the one- and two-compartment models), and SSE is the error sum of squares. We chose the model with the lowest AICc value.[21] Burnham and Anderson[21] propose using the difference in AICc ($\Delta_{1\text{-}2} = \mathrm{AICc}_1 - \mathrm{AICc}_2$) as a measure of the plausibility of an alternative model. If $\Delta_{1\text{-}2}$ is negative model 1 has stronger support, whereas, if it is positive, model 2 has stronger support.[21] If the AICc revealed that the weight of evidence supported a one-compartment model, we used τ as an estimate of average retention time, whereas, if it supported a two-compartment model, we estimated the average retention time as:

$$\tau_{2-\mathrm{comps}} = p\tau_1 + (1-p)\tau_2 \quad (4)$$

We estimated the isotopic discrimination ($\Delta^{13}C$) as $\delta^{13}C\ (\infty)_{\mathrm{tissue}} - \delta^{13}C_{\mathrm{diet}}$. Following Martínez del Rio and Anderson-Sprecher,[18] we estimated the standard errors of $\tau_{2\text{-comps}}$ as $(s^2/\mathrm{n})^{1/2}$, where

$$s^2 = (\hat{\tau}_1 - \hat{\tau}_2 \quad \hat{p} \quad 1-\hat{p}) V \begin{pmatrix} \hat{\tau}_1 - \hat{\tau}_2 \\ \hat{p} \\ 1-\hat{p} \end{pmatrix} \quad (5)$$

and V is the variance matrix of the system estimated by the non-linear estimation procedure.

RESULTS

During the 128 day experiment birds lost approximately 1.7% of their body mass (mean ± standard error (SE) = 0.37 ± 0.14 g; paired $t_{45} = -2.65$, $p = 0.01$). Incorporation of $\delta^{13}C$ into breath, excreta, blood cells, collagen, and claw was better described by one-compartment models (Table 1, Fig. 1). In contrast, the incorporation of $\delta^{13}C$ into plasma, small intestine, liver, gizzard, heart muscle, and pectoralis muscle was better described by two-compartment models (Table 1, Fig. 1). As predicted, splanchnic tissues (liver, intestine, gizzard, and heart; Fig. 2(A)) had higher rates of isotopic incorporation than structural tissues (pectoralis muscle and collagen; Fig. 2(A)). The average tissue retention times calculated from the one- (τ) and two-compartment ($\tau_{2\text{-comps}}$) models were tightly and linearly correlated ($r^2 = 0.98$, $p = 0.001$; Fig. 2(B)). The regression line relating τ and $\tau_{2\text{-comps}}$ had a slope that did not differ significantly from 1 (slope ± SE = 0.96 ± 0.05, $t_{10} = 0.87$, $p = 0.4$), but had an intercept (3.6 ± 1.4) that was significantly different from 0 ($t_{10} = 2.55$, $p = 0.03$; Fig. 2(B)). This result implies that the two-compartment models systematically estimated longer average retention times than the one-compartment models (Fig. 2(B)). The tissue to diet discrimination factor ($\Delta^{13}C = \delta^{13}C_{\mathrm{tissue}} - \delta^{13}C_{\mathrm{diet}}$) was positively correlated for one and two-compartment models ($r^2 = 0.6$, $p = 0.03$; Fig. 3). However, two-compartment models yielded consistently more positive $\Delta^{13}C$ values than one-compartment models (Fig. 3). The estimated difference in isotopic composition

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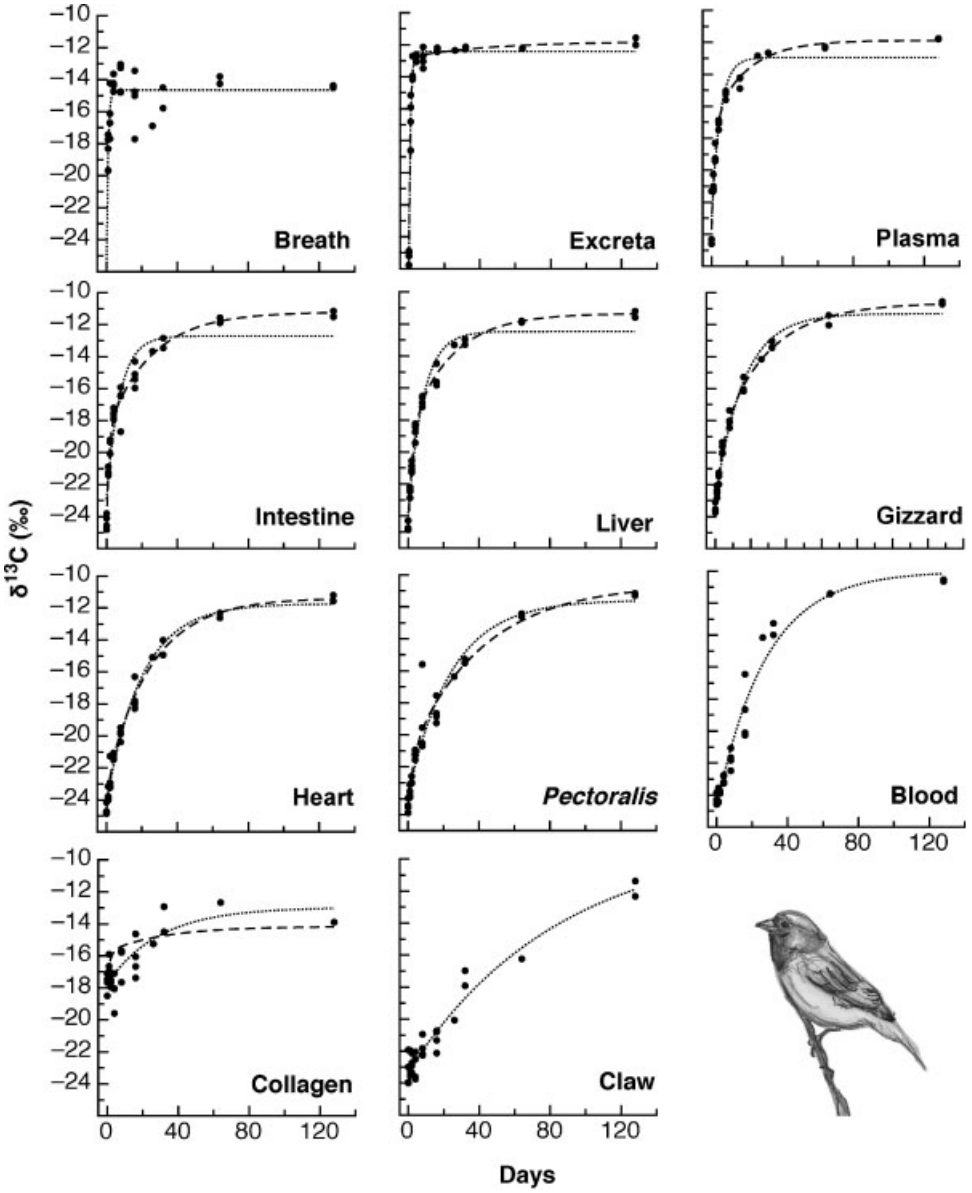

Figure 1. Incorporation of ^{13}C into excreta, plasma, intestine, liver, gizzard, heart, pectoralis, and collagen was described well by both one- (dotted line) and two-compartment (dashed line) models. However, we were unable to fit the incorporation data for breath, blood, and claw tissues using the two-compartment model.

between tissues and diet ($\Delta^{13}C$) was smaller when estimated by the two- than by the one-compartment model.

DISCUSSION

We hypothesized that because isotopic incorporation rates are correlated with tissue-specific rates of protein turnover, the rate of isotopic incorporation for splanchnic tissues should be faster than that of structural tissues.[9–12] Our data set confirmed this prediction. In addition, we were interested in testing the application of multi-compartment models to investigate isotopic incorporation rates. AICc revealed that data did not support a single type of model in all cases. One-compartment models were better supported in some cases (breath, excreta, blood cells, bone collagen, and claw) whereas two-compartment models were better supported in others (plasma, intestine, liver, gizzard, heart muscle, and pectoralis). The two-compartment models estimated average retention times that were consistently higher than those estimated by the one-compartment models. Here, we will discuss (1) the relationship between protein turnover and isotopic incorporation, (2) whether we should use one- or multi-compartment models, and (3) what the ecological implications are for the use of multi-compartment models.

Protein turnover and isotopic incorporation rate

In all animals studied, the rate of protein synthesis is higher in splanchnic than in structural tissues.[13] Waterlow[13] ranked the protein turnover of the following tissues from 'fastest' to 'slowest': intestine > liver > heart > skeletal muscle >bone collagen. Note that this ranking is the same as that of the rate of ^{13}C incorporation in house sparrow tissues (Fig. 3).[14] This

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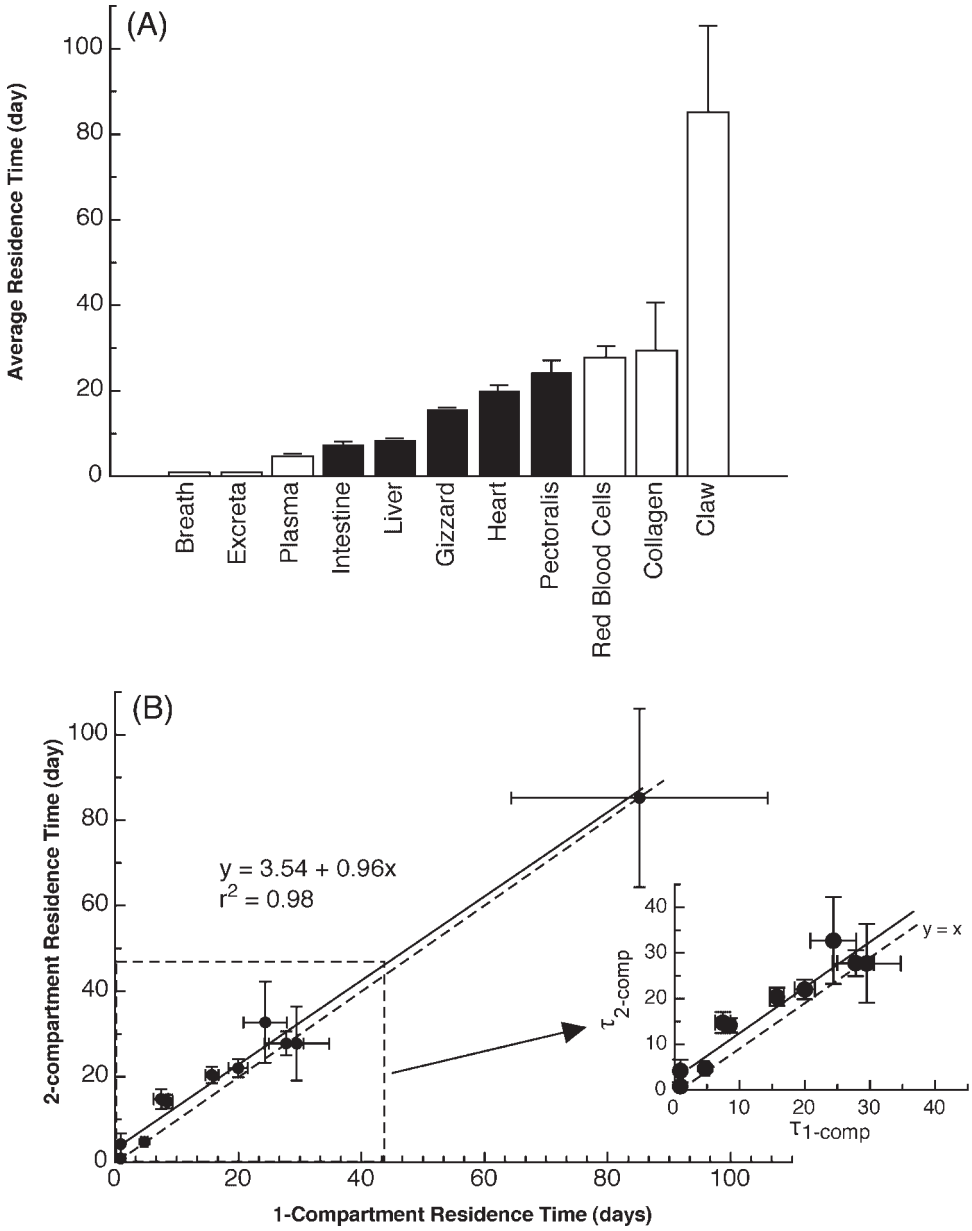

Figure 2. ^{13}C residence times were shorter for splanchnic tissues (intestine and liver) than for structural (pectoralis and collagen) tissues (A). Respired CO_2 collected from fasted sparrows had the shortest residence times presumably indicating that birds rely heavily on endogenous energy reserves or do not have large reserves that are rapidly replaced. Interestingly, gizzard and heart had intermediate $\delta^{13}C$ residence times between splanchnic and structural tissues (A). White bars indicate AIC support for one-compartment models. Black bars indicate AIC support for two-compartment models (A). There was a positive linear relationship (near 1:1) between one- and two-compartment residence times (B). However, two-compartment residence times were slightly higher than residence times calculated from one-compartment models (Inset B).

result supports the hypothesis of Carleton and Martínez del Rio[7] of a relationship between protein turnover incorporation and isotopic incorporation rate, and suggests that we might be able to predict isotopic incorporation from protein turnover – and vice versa. Note that this prediction applies only to proteinaceous tissues after the lipids in them have been removed.[19] The notion that isotopic turnover is related to the metabolic activity of the tissue, construed narrowly as $\dot{V}O_2$, is still widely cited.[4–6,22] The results of our study suggest that the hypotheses of Tiezen *et al.*[1] should be refined and that metabolic activity should be construed narrowly as the rate of macromolecular synthesis and catabolism, which might or might not be positively correlated with $\dot{V}O_2$.[23]

Carleton *et al.*[15] interpreted the rate of incorporation of ^{13}C into exhaled CO_2 as an estimate of the turnover of endogenous reserves. The incorporation of the ^{13}C composition of diet into the breath of fasted sparrows was surprisingly fast (average retention time ≈0.9 days), indicating rapid turnover

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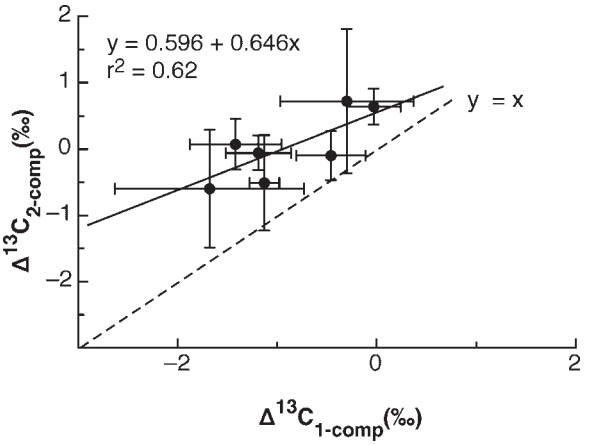

Figure 3. The discrimination factors ($\Delta^{13}C = \delta^{13}C_{tissue} - \delta^{13}C_{diet}$) estimated by one- and two-compartment models were positively correlated ($y = 0.6 + 0.7x$, $r^2 = 0.62$, $p = 0.03$). One-compartment models estimated a more negative discrimination factor than two-compartment models.

of storage endogenous reserves. Carleton *et al.*[15] and Voigt and Speakman[24] reported slightly longer average residence times of carbon in the stored endogenous reserves of a hummingbird (*Selasphorus platycercus*) and a nectar-feeding bat (*Glossophaga soricina*). This result is perplexing given two observations: (1) Carleton and Martínez del Rio[7] reported that incorporation rates decrease allometrically with body mass, and (2) sparrows are an order of magnitude heavier than the hummingbird and about twice as heavy as the bat. Because the fractional rate of isotopic incorporation in a tissue is determined by the ratio of the input rate and the size of the elemental pool in the tissue,[7] we hypothesize that the endogenous reserves of both the hummingbird and the bat were larger than those of the sparrows. To our knowledge, the rate of incorporation of ^{13}C into the reserves of house sparrows is the fastest ever recorded in a vertebrate.[24] However, this dubious world record is likely to simply be the result of small exogenous reserves.

Should we use one- or multi-compartment models, and does it matter?

Our data supported one-compartment models in some tissues, and two-compartment models in others (Table 1). Perhaps more importantly, the inferences drawn from the two types of models differed. Two-compartment models consistently estimated a smaller difference in isotopic composition between tissues and diet (Fig. 3). Many problems in isotopic ecology demand the estimation of the contribution of different dietary sources to the tissues of an animal.[25–28] Discrimination factors derived from isotopic incorporation studies are sometimes used as ingredients of these mixing models.[29] Because the output of mixing models is sensitive to the values of discrimination factors, ecologists must make sure that they use the incorporation model that is better supported by the data and that therefore estimates discrimination factors with the least error and bias.

Although there was a positive linear relationship between the average retention times estimated by two- and one-compartment models, and although the slope of this relationship was approximately 1, its intercept was significantly positive and approximately equal to 3.5 days (Fig. 3). This result indicates that two-compartment models estimated consistently longer average retention times than one-compartment models. Hence, the fractional difference between the average retention times estimated by two- and one-compartment models in our study seems to decrease with the length of time that the ^{13}C stays in the tissue (Fig. 3). Thus, choosing a two- rather than a one-compartment model will lead to biologically significant differences when the models are applied to tissues with rapid turnover (such as plasma, liver, and intestine), but these differences appear to be less important when the models are applied to tissues with slower turnover. The support of our data for one-compartment over two-compartment models in some tissues must be interpreted cautiously. The approach that we have chosen to assess the evidence for one- or two-compartment models is conservative. It tends to favor the simpler, one-compartment model, if the data set has significant error.[30] Thus, the choice of one-compartment over two compartment models in noisy data sets (such as those for breath and collagen, Fig. 2) should be viewed as tentative.

CONCLUSIONS

In conclusion, although the vast majority of the published isotopic incorporation studies have relied on one-compartment models, our study indicates that sometimes these models might not be the ones best supported by data. It also suggests that using models with two (or more, when appropriate) compartments can lead to differences in the estimation of the parameters that ecologists are interested in.[31] Our study suggests that a re-analysis of isotopic incorporation studies following the guidelines of Cerling *et al.*[17] and Martínez del Rio and Anderson-Sprecher[18] should be an important priority in isotopic ecology. Independently of the type of model used, our study also revealed large inter-tissue differences in the average time that a tissue retains isotopes. For example, by analyzing the isotopic composition of breath, plasma proteins, blood cells, and claws an ecologist can resolve the isotopic composition of diets incorporated at time scales that range from less than 1 day to over 80 days. We emphasize that all these samples can be gathered relatively non-invasively. The variation in isotopic incorporation among tissues revealed by our study suggests that, by analyzing several tissues, ecologists can infer the breadth of resources used by an individual, and determine the contribution of intra- and inter-individual variation to the spectrum of resources used by apopulation.[14,32]

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REFERENCES

1. Tieszen LL, Boutton TW, Tesdahl KG, Slade NA. *Oecologia* 1983; **57**: 32.
2. Hobson KA, Clark RG. *Condor* 1994; **94**: 181.
3. Martínez delRio C, Wolf BO. *Physiological and Ecological Adaptation to Feeding Invertebrates*. Science Publishers: New Hampshire, 2005.
4. Klaasen M, Thums M, Hume ID. *Aust. J. Zool.* 2004; **52**: 635.
5. Paulet Y, Lorrain A, Richard J, Pouvreau S. *Org. Geochem.* 2006; **37**: 1359.
6. Vollaire Y, Banas D, Thomas M, Roche H. *Comp. Biochem. Physiol., Part A* 2007; **148**: 504.
7. Carleton SA, Martínez del Rio C. *Oecologia* 2005; **144**: 226.
8. Hobson KA, Bairlein R. *Can. J. Zool.* 2003; **81**: 1630.
9. Schoenheimer R, Rather S, Rittenberg D. *J. Biol. Chem.* 1939; **130**: 703.
10. Ratner S, Rittenber D, Keston S. *J. Biol. Chem.* 1940; **134**: 665.
11. Shemin D, Rittenberg D. *J. Biol. Chem.* 1944; **153**: 401.
12. Buchanan DL. *Arch. Biochem. Biophys.* 1961; **94**: 500.
13. Waterlow JC. *Protein Turnover*. CAB International: Cambridge, Massachusetts, 2006.
14. Dalerum F, Angerbjörn A. *Oecologia* 2005; **144**: 647.
15. Carleton SA, Hartman-Bakken B, Martínez del Rio C. *J. Exp. Biol.* 2006; **209**: 2622.
16. Sponheimer M, Robinson TF, Cerling TE, Tegland L, Roeder BL, Ayliffe L, Dearing MD, Ehleringer JR. *Rapid Commun. Mass Spectrom.* 2006; **20**: 1395.
17. Cerling TE, Ayliffe LK, Dearing MD, Ehleringer JR, Passey BH, Podlesack DW, Torregrossa A, West AG. *Oecologia* 2007; **151**: 175.
18. Martínez delRio C, Anderson-Sprecher R. *Oecologia* 2008; in press.
19. Post D, Layman CA, Arrington DA, Takimoto G, Quakttrochi J, Montaña CG. *Oecologia* 2007; **152**: 179.
20. Mirón LLM, Herrera LGM, Ramírez NP, Hobson KA. *J. Exp. Biol.* 2006; **209**: 541.
21. Burnham KP, Anderson DR. *Model Selection and Multi-model Inference: A Practical Information-Theoretic Approach*. Springer: New York, 2002.
22. MacAvoy SE, Macko SA, Arneson LS. *Can. J. Zool.* 2005; **83**: 631.
23. Marsh AG, Maxson RE Jr, Manahan DT. *Science* 2001; **291**: 1950.
24. Voigt CC, Speakman JR. *Funct. Ecol.* 2007; **21**: 921.
25. Phillips DL. *Oecologia* 2001; **127**: 166.
26. Phillips DL, Gregg JW. *Oecologia* 2001; **126**: 171.
27. Phillips DL, Koch PL. *Oecologia* 2002; **130**: 114.
28. Phillips DL, Eldridge PM. *Oecologia* 2006; **147**: 195.
29. Caut S, Angulo E, Courchamp F. *Can. J. Zool.* 2008; **86**: 438.
30. Myhill J. *Biophys. J.* 1967; **7**: 903.
31. Ayliffe LK, Cerling TE, Robinson T, West AG, Sponheimer M, Passey BH, Hammer J, Roeder B, Dearing MD, Ehleringer JR. *Oecologia* 2004; **139**: 1432.
32. Newsome S, Martínez del Rio C, Phillips DL, Bearhop S. *Frontiers Ecol. Environ.* 2007; **5**: 429.

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