Carbon Turnover in Tissues of a Passerine Bird: Allometry, Isotopic Clocks, and Phenotypic Flexibility in Organ Size

Ulf Bauchinger[1,2,3,*]
Scott McWilliams[3]
[1]Department Biologie II, Ludwig-Maximilians-Universität München, Großhaderner Straße 2, 82152 Planegg-Martinsried, Germany; [2]Mitrani Department of Desert Ecology, Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, 84990 Midreshet Ben-Gurion, Israel; [3]Department of Natural Resources Science, University of Rhode Island, 105 Coastal Institute in Kingston, 1 Greenhouse Road, Kingston, Rhode Island 02881

Accepted 5/24/2009; Electronically Published 9/28/2009

ABSTRACT

Stable isotopes are an important tool for physiological and behavioral ecologists, although their usefulness depends on a thorough understanding of the dynamics of isotope incorporation into tissue(s) over time. In contrast to hair, claws, and feathers, most animal tissues continuously incorporate carbon (and other elements), and so carbon isotope values may change over time, depending on resource use and tissue-specific metabolic rates. Here we report the carbon turnover rate for 12 tissues from a passerine bird, the zebra finch (*Taeniopygia guttata*). We measured average carbon retention time (τ) for 8 d for small intestine; 10–13 d for gizzard, kidney, liver, pancreas, and proventriculus; 17–21 d for heart, brain, blood, and flight muscle; and 26–28 d for leg muscle and skin. We used these data, along with the few other published estimates, to confirm that the fractional rate of isotopic turnover for red blood cells, whole blood, liver, and leg muscle scales with body mass to approximately the $-1/4$ power. Our data also support several key assumptions of the "isotopic-clock" model, which uses differences in isotope value between tissues, along with estimates of turnover rate of these tissues, to predict time elapsed since a diet shift. Finally, we show that between-tissues differences in turnover rate largely, but not entirely, explain the extent of phenotypic flexibility in organs of garden warblers during their long-distance flight across the Sahara Desert during spring. More studies that measure tissue-specific protein synthesis, metabolic rate, and elemental turnover in many tissues from a variety of animals are needed.

* Corresponding author; e-mail: bauchinger@orn.mpg.de.

Physiological and Biochemical Zoology 82(6):787–797. 2009. 1522-2152/2009/8206-8168$15.00
DOI: 10.1086/605548

Introduction

Stable isotopes of carbon and other common elements can be used to study resource use, movement patterns, and the stoichiometry of natural biological processes because the landscape varies in isotopic composition and all organisms assimilate stable isotopes and incorporate them into their tissues (DeNiro and Epstein 1978; Hobson 1999; Martínez del Rio and Wolf 2005; West et al. 2006). Tissues such as hair and feathers incorporate elements during growth, and their isotopic values are relatively fixed in the part of the hair or feather that has completed growth (Hobson 1999; Ayliffe et al. 2004). Quantification of stable isotopes in such tissues can be used to address questions about the temporal and spatial distribution of animals or about food choices at scales ranging from weeks to months and from microhabitat to landscape. This is because the stable-isotope values reflect resources used in a limited time and at a certain place (for reviews, see Hobson 1999; Inger and Bearhop 2008).

Most animal tissues continuously incorporate carbon (and other elements), so their carbon isotope values may change over time, depending on resource use and tissue-specific metabolic rates (Podlesak et al. 2005; Phillips and Eldridge 2006). For example, about half the carbon atoms in the liver of the gerbil (*Meriones unguiculatus*) turn over within 6 d, whereas those in muscle turn over within 28 d (Tieszen et al. 1983). The carbon half-life ($C_{t1/2}$) of tissues in growing Japanese quail (*Coturnix japonica*) was 3 d for liver, 11 d for whole blood, and 173 d for bone collagen (Hobson and Clark 1992). Carleton and Martínez del Rio (2005) hypothesized that the fractional rate of isotopic turnover for a given tissue scales with body mass to approximately the $-1/4$ power, but their analysis was restricted to blood, which provided the best available data. Current estimates of turnover rates of metabolically active tissues in animals are restricted to a few tissues (e.g, blood, muscle, liver, collagen; but see Carleton and Martínez del Rio 2008) from relatively few species. We measured the carbon turnover rate for 12 different tissues from a small passerine bird, the zebra finch (*Taeniopygia guttata)*. We then used the data to (1) define the time window during which a given tissue indicates resource use, (2) evaluate the hypothesis that carbon turnover rate scales with body mass to approximately the $-1/4$ power, (3) evaluate several key assumptions and criteria of the "isotopic-clock" model of Phillips and Eldridge (2006), which uses differences in isotope value between tissues, along with estimates of turnover rate of these tissues, to predict time elapsed since a diet shift, and (4) evaluate the hypothesis that tissue-

specific turnover rate determines the pace of phenotypic flexibility in certain organs and hence its extent in time-constrained animals, such as birds during spring migration.

Material and Methods

Thirty adult zebra finches were kept in single-sex groups of four to six individuals per cage (45 cm × 90 cm × 45 cm) under a 12L : 12D regime at an air temperature of 31°C (±2°C). Birds were initially fed a diet of mixed seeds of C_4 plants (Blattner no. 1140103; $\delta^{13}C = -16.3‰ \pm 0.33‰$ [SD]) for at least 3 mo before being switched (hereafter "day 0") to a diet of C_3 seeds (Blattner no. 160505; $\delta^{13}C = -26.3‰ \pm 0.50‰$ [SD]). Both the C_4 and C_3 diets were enriched with minerals (2 g/kg seeds, Günter Enderie, Nektron-MSA lot 363850) and vitamins (Günter Enderie, Nektron-S lot 364636). Birds were offered food and water ad lib.

Sampling

Two to eight birds were killed for tissue sampling on the mornings of days 0, 1, 2, 4, 8, 16, 32, 56, 128, and 256. Sampling on day 0 was done immediately before the diet shift; thus, these tissues are from birds fed only the C_4 diet for at least 3 mo. Birds were weighed to ±0.1 g and decapitated, and then blood was collected from the carotid artery. Whole blood was centrifuged for 8 min (MIKRO-Centrifuge, LabTech) to separate blood cells from plasma. Pectoral and leg muscle, kidney, proventriculus, gizzard, small intestine, pancreas, liver, heart, brain, and 1-cm^2 sections of skin from over the pectoral muscle were excised within 10 min. All tissue samples were dried at 60°C to constant mass and then homogenized with a mortar and pestle. Fat was extracted from the homogenate of all tissues except blood cells for 24 h with a Soxhlet apparatus (Merck, petroleum benzene, boiling range 40°–60°C). Fat-free tissues and blood cells were analyzed for stable-isotope ratios with a Carlo-Erba NA 1500 Series II Elemental Analyzer attached to a continuous-flow isotope ratio micromass optima spectrometer (Podlesak and McWilliams 2006).

Carbon Turnover

The carbon value measured for each organ over time was fitted to a one- and a two-compartment nonlinear model so that we could evaluate which model was better supported (Cerling et al. 2007; Carleton et al. 2008; Martínez del Rio and Anderson-Sprecher 2008; Martínez del Rio et al. 2009; Wolf et al. 2009). The one-compartment model was a standard first-order kinetic function: $y_t = y_\infty + ae^{-t/\tau}$, where y_t is the measured $\delta^{13}C$ of tissue in parts per thousand at time t; y_∞ is the estimated final $\delta^{13}C$ of tissue in parts per thousand after the switch from a C_3 to a C_4 diet; a is the estimated range in $\delta^{13}C$ in parts per thousand between diets; τ is the estimated average carbon retention time in days; and t is time in days since the diet switch (Martínez del Rio and Wolf 2005). The two-compartment model was similar to that recommended by Martínez del Rio and Anderson-Sprecher (2008) and used by Carleton et al. (2008): $y_t = y_\infty + a(p_1)e_1^{-t/\tau} + a(1 - p_2)e_2^{-t/\tau}$, where p is the fractional size of each "pool" (Cerling et al. 2007) or "phase" (Martínez del Rio and Anderson-Sprecher 2008). In our application of two-compartment models, p_1 and p_2 are used to describe the fractional size of pools 1 and 2, respectively, and τ_1 and τ_2 give the respective associated average carbon retention times. We estimated the average retention time for one-compartment models as $\tau_{\text{one-comp}}$ and that for two-compartment models as $\tau_{\text{two-comp}} = p\tau_1 + (1 - p)\tau_2$ (Carleton et al. 2008). The nonlinear regression algorithms always found locally optimal one- and two-compartment models for all tissues. However, brain and skin carbon had not reached an asymptote after 256 d, and this resulted in unreasonable estimates of final $\delta^{13}C$ of these tissues (y_∞) that were more negative than that of diet ($\delta^{13}C = -26.3‰ \pm 0.50‰$ [SD]). Thus, for these two tissues, we report parameter estimates from two-compartment models with these constraints: $y_\infty > -24.5‰$ and $a > 11.5‰$ (assuming ca. 1.5‰ discrimination from diet).

We compared the Akaike Information Criterion for small sample sizes (AIC_c) for one- and two-compartment models for each tissue. Burnham and Anderson (2002) use $\Delta AIC_c = AIC_i - AIC_{min}$ as an estimate of the information loss of a model i (AIC_i) compared with that of the estimated best model (AIC_{min}). The farther ΔAIC_c deviates from 0, the less supported is the respective model. In the range 0–2, both models have substantial support; in the range 4–7, model i has considerably less support; and models with values above 10 are not supported (Burnham and Anderson 2002). Accordingly, if $\Delta AIC_c \le 2$, we chose the simpler model (one-compartment model), following the principle of parsimony. In all other cases ($\Delta AIC_c > 2$), we chose the model with the higher level of support. We regressed average retention time estimates from one- and two-compartment models to compare turnover rate estimates between models.

We derived SEs for τ from the two-compartment models according to Martínez del Rio and Anderson-Sprecher (2008) and estimated the 95% confidence interval of the estimates as the critical value of the t distribution with $\alpha = 0.05$ multiplied by the SE of the estimate (Sokal and Rohlf 2000). We used t-tests with Bonferroni correction to determine whether the τ was significantly different between each pair of tissues. Carbon half-life for each tissue, as commonly used in previous studies, was estimated as $C_{t1/2} = \ln(2) \times \tau$. All statistical analyses were performed using SIGMAPLOT (ver. 10.0) and SYSTAT (ver. 12.0).

Results

The rate of carbon isotope incorporation into tissues with faster turnover rates (small intestine, gizzard, kidney, liver, pancreas, and proventriculus) was best described by two-compartment models (i.e., ΔAIC_c values > 2), whereas that of tissues with slower turnover rates (red blood cells, heart, brain, flight muscle, and leg muscle) was best described by one-compartment models (i.e., ΔAIC_c values ≤ 2; Table 1; Fig. A1). The average carbon retention time (τ) estimates for one- and

Table 1: Incorporation of ^{13}C into 12 tissues described by one-compartment and two-compartment models that were fitted for tissue-specific $\delta^{13}C$ values collected over 256 d after a diet shift

	One-Compartment Model[a] ($y_t = y_\infty + ae^{-t/\tau}_{\text{one-comp}}$)				Two-Compartment Model[b] ($y_t = y_\infty + a(p)e_1^{-t/\tau} + a(1-p)e_2^{-t/\tau}$)							
Tissue	Y_∞	a	$\tau_{\text{one-comp}}$	AIC_c1	Y_∞	a	p	τ_1	τ_2	$\tau_{\text{two-comp}}$	AIC_c2	Δ_{1-2}
Small intestine	−23.36	8.85	3.75	96.8	−23.86	9.60	.30	21.56	2.22	**8.01**	91.8	5.0
Gizzard	−22.53	9.20	7.59	98.9	−22.88	10.26	.62	15.13	1.65	**10.04**	88.3	10.6
Kidney	−23.53	9.35	9.19	89.8	−23.89	10.47	.68	16.32	1.56	**11.57**	74.8	15.0
Liver	−23.16	9.68	5.83	107.0	−23.93	11.10	.45	23.93	2.07	**11.93**	83.2	23.9
Pancreas	−23.21	9.48	8.00	107.7	−23.73	10.46	.49	21.10	3.19	**11.98**	102.8	4.9
Proventriculus	−22.90	9.74	7.37	100.5	−23.66	11.23	.50	2.28	24.58	**13.35**	71.7	28.8
Heart	−23.75	9.99	**17.24**	77.5	−23.83	10.60	.91	18.69	.23	17.04	76.9	.6
Brain	−22.66	9.48	**18.48**	117.2	−24.42	11.50	.34	172.70	11.51	65.53	116.7	.6
Red blood cells	−24.20	11.09	**19.31**	108.4	−24.48	11.42	.00	19.46	19.46	19.46	113.6	−5.2
Flight muscle	−23.63	9.43	**20.88**	92.9	−23.74	10.14	.86	24.09	1.28	20.96	92.6	.3
Leg muscle	−23.32	9.99	**26.04**	108.6	−23.47	11.02	.84	30.61	1.00	25.92	108.4	.3
Skin	−21.67	8.38	**28.09**	117.4	−23.74	11.50	.45	181.93	11.25	88.45	116.8	.6

Note. For each tissue, Δ_{1-2} (i.e., $\text{AIC}_c1 - \text{AIC}_c2$) was used to determine the model with the highest level of support (Burham and Anderson 2002) and the associated average retention time ($\tau_{\text{one-comp}}$ or $\tau_{\text{two-comp}}$) used for subsequent analyses (boldface). Here, y_t is the measured $\delta^{13}C$ value of the tissue at time t; t is the time in days; y_∞ is the estimated final $\delta^{13}C$ value; a is the estimated range in tissue $\delta^{13}C$ between diets; p_1 and p_2 are the respective fractional size of pools 1 and 2 for the two-compartment model; $\tau_{\text{one-comp}}$ is the estimated average carbon retention time for the one-compartment model; τ_1 and τ_2 are the respective estimated average carbon retention times for p_1 and p_2 of the two-compartment model; $\tau_{\text{two-comp}}$ is the average carbon retention time of the two-compartment model: $\tau_{\text{two-comp}} = p\tau_1 + (1-p)\tau_2$ (Carleton et al. 2008).

[a] Average retention time from the one-compartment model was used because $\Delta_{1-2} < 2$.

[b] Average retention time from the two-compartment model was used because $\Delta_{1-2} > 2$.

two-compartment models were linearly related ($\tau_{\text{two-comp}} = 6.02[\pm 0.84] + 0.72[\pm 0.06] \times \tau_{\text{one-comp}}$); $r^2 = 0.95$; Fig. A2). Two-compartment models produced relatively higher estimates of τ for tissues with faster turnover rates than did one-compartment models, whereas estimates of τ for the one- and two-compartment models were similar for tissues with slower turnover rates.

Estimated average carbon retentions time for the 12 different tissues ranged from 8 d for small intestine to 10–13 d for gizzard, kidney, liver, pancreas, and proventriculus; 17–21 d for heart, brain, blood, and flight muscle; and 26–28 d for leg muscle and skin (Fig. 1). The carbon half-life ($C_{t1/2}$), or median residence time, ranged between 6 and 20 d for these same tissues (Table A1). Estimated $\delta^{13}C$ of tissues after the switch to the C_3 diet (y_∞) was, on average, −23.4‰ ± 0.71‰ (SD; range 21.7‰–24.2‰), which was, on average, 2.9‰ more positive than the isotope value for the C_3 diet ($\delta^{13}C = -26.3‰ \pm 0.50‰$ [SD]). The estimated range in tissue $\delta^{13}C$ between diets (a) was, on average, 10.1‰ ± 0.8‰ (SD), which was similar to the measured difference in $\delta^{13}C$ between diets of 10.0‰.

We used these estimates to describe the duration of time during which each tissue would reliably indicate resource use. In effect, 90% of the carbon ($2.3 \times \tau$) from the initial diet in small intestine and gizzard would be replaced within 19 and 23 d, respectively, whereas that in kidney, liver, and pancreas would be replaced within 27–28 d; that of proventriculus within 31 d; that in heart, brain, blood cells, and flight muscle within 40–48 d; and that in leg muscle and skin within 60–65 d. These differences between tissues in rate of carbon incorporation define the time window during which each tissue indicates resource use, and they result in predictable intertissue variation in $\delta^{13}C$ that can be used to estimate timing of diet shifts in free-living animals.

Discussion

Body Mass Dependence of Tissue Turnover Rate and the Time Window for Determining Resource Use

Using estimates of turnover rate to define the time window during which a given tissue reflects resource use has implications for understanding the ecology of migratory birds. Consider a hypothetical bird the size of a zebra finch (~15 g) with a migratory period of 24 d. Assume that during migration the bird spends, on average, one-eighth of its time in flight and seven-eighths of its time stopping over and refueling (Hedenström and Alerstam 1997), so its 24 d migration includes an initial 7-d premigratory fattening period and 3 d of flying (days 8, 16, and 24) interrupted by two 7-d stopovers to rest and refuel. A bird of this size flying for 72 h can cover 2,600–3,900 km at a ground speed of 10–15 m s^{-1} (Alerstam et al. 2007). Our data and predictive models indicate that when the bird arrives at the breeding area, about 50% of the carbon in the small intestine and gizzard originates from the final stopover site, more than 75% of the carbon in these tissues originates from the stopover sites, and about 50% of the carbon in flight muscle and blood originates from the two stopover sites used during migration, with the remaining carbon originating from the wintering site. This is why interpretation of resource use

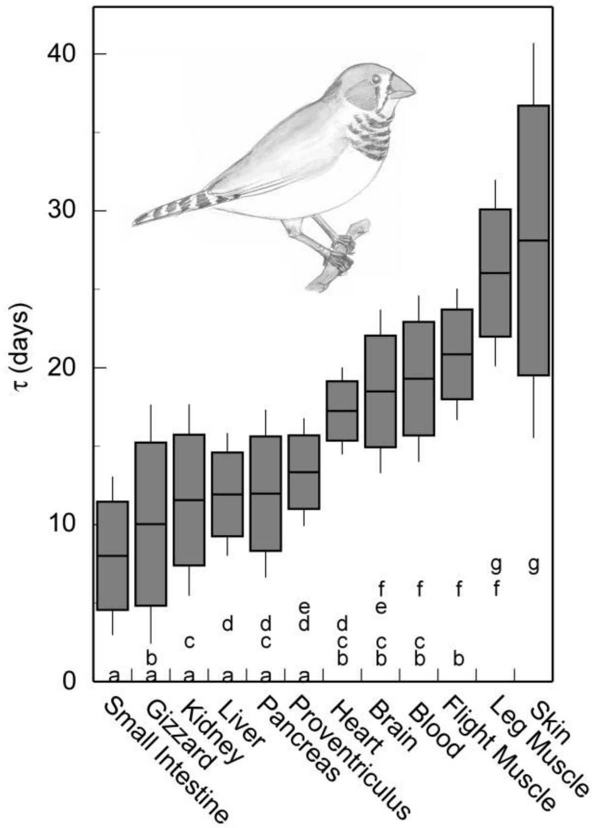

Figure 1. Average carbon retention time (τ) in days for 12 different organs in zebra finches. Identical letters indicate that organs were not significantly different (t-tests with Bonferroni correction). Box plots show 25% and 75% confidence intervals (CIs), error bars refer to 5% and 95% CIs.

over time in migrating birds is complicated when isotope values of different tissues are used (e.g., flight muscle, blood) sampled on breeding and wintering areas (e.g., Marra et al. 1998). In such cases, about half the carbon in flight muscle from migratory birds that recently arrived on breeding sites after a ca. 15–20 d migration (Norris et al. 2005) would have been acquired during migration, so knowledge of the isotope values of resources consumed during migration would be important.

We can use our estimates of turnover rate, along with other recently published estimates, to evaluate the hypothesis that the fractional rate of isotopic turnover (k) scales with body mass to approximately the $-1/4$ power (Carleton and Martínez del Rio 2005). If isotopic turnover rate of tissue(s) is predictable at a given body mass, then such allometric models would greatly broaden the sphere of applications. Carleton and Martínez del Rio (2005) determined that carbon turnover rate in blood (whole blood and red blood cells combined) of birds scaled with body mass to the $-1/4$ power. However, their allometric equation $\log(k) = -0.69 - 0.27\log(\text{body mass})$ predicts a fractional turnover rate in blood of 0.097 and a carbon half-life of 7.1 d for zebra finches, which is much faster that our measured value of 13.5 d. We derived separate allometric equations for whole blood and red blood cells, using the same data as Carleton and Martínez del Rio (2005) but additionally including more recent estimates of fractional turnover rates, including our own (Fig. 2*a*; Table A1). These two allometric equations, one for whole blood and the other for red blood cells, confirm that the fractional rate of isotopic turnover (k) scales with body mass to approximately the $-1/4$ power, and the separate allometric equations explain substantially more of the variance (>90%) than when whole blood and red blood cell data are combined ($r^2 = 0.59$; Carleton and Martínez del Rio 2005). The limited data for leg muscle provide further support for the $-1/4$-power scaling of tissue turnover rate, as does that for liver, although only when data for Japanese quail are excluded (Fig. 2*b*; Table A1). The two regressions for leg muscle and liver include all available data on turnover rate in these two tissues for birds and mammals, and the resulting $-1/4$-power scaling is heavily influenced by the inclusion of the very large alpaca in the data set.

If elemental turnover rate for most or all tissues scales with body mass to approximately the $-1/4$ power—as we have shown for whole blood, red blood cells, liver, and leg muscle—then calculating standardized turnover rates (e.g., turnover rate of tissue[s] relative to that of liver, a tissue with a fast turnover rate) provides a useful way to compare turnover rates across species. Sponheimer et al. (2006) measured $C_{t1/2}$ for muscle and liver of alpacas and compared their results with those for other species by calculating a ratio of $C_{t1/2}$ in muscle to that in liver to control for interspecific differences in tissue turnover rate. We used more recently published data on mice (Arneson et al. 2006; MacAvoy et al. 2006; Carleton et al. 2008), as well as our own data, to extend this comparison (Table A1). In general, muscle turnover rate was consistently one-fourth to one-fifth that of liver for a variety of species, which suggests broad interspecific similarities in relative turnover rate of certain tissues. We caution that the use of such ratios in physiology rests on many untested assumptions (Packard and Boardman 1999). We have provided some support for one of the most important of these assumptions: tissue-specific turnover rates (i.e., whole blood, red blood cells) scaled consistently with body mass to approximately the $-1/4$ power (Fig. 2). Clearly, more studies such as ours are needed on species of different body sizes to provide further confirmation that these allometric scalings hold across many other tissues.

Metabolic Rate, Protein Synthesis, and Carbon Turnover Rate

Several mechanistic explanations have been proposed to explain the allometry of carbon turnover across species. One popular hypothesis states that larger animals have higher metabolic rates (scaling is approximately 3/4 power; Kleiber 1932, 1961; Brown et al. 2004) and that therefore tissues from larger animals have higher metabolic and carbon turnover rates (reviewed by Carleton and Martínez del Rio 2005; MacAvoy et al. 2006). However, several lines of evidence suggest that whole-animal and tissue-specific metabolic rates do not directly relate to carbon turnover rates: (1) the relationship between the whole-animal metabolic

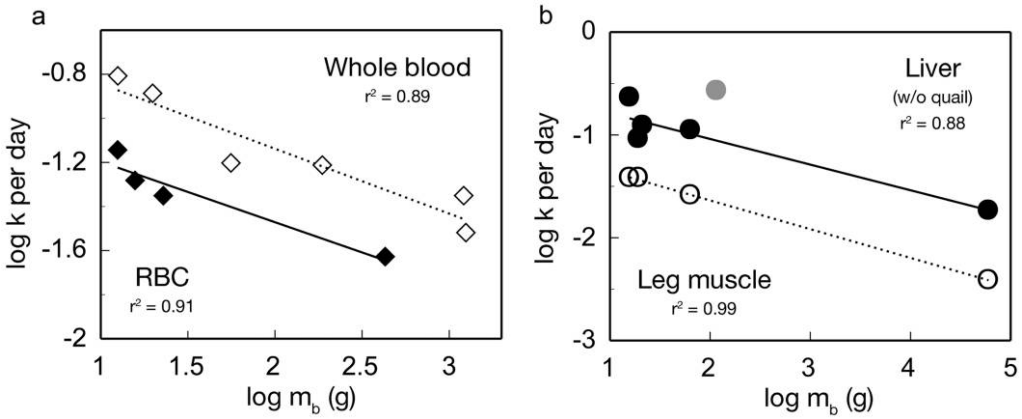

Figure 2. Fractional rates of carbon incorporation (k) into (a) whole blood (*open diamonds*) and red blood cells (RBC; *filled diamonds*) in birds and (b) liver (*filled circles*) and leg muscle (*open circles*) in birds and mammals plotted against body mass. All data are log transformed. Linear regressions ($\pm$SE) for whole blood: $y = -0.29(\pm 0.05) \times x - 0.55(\pm 0.12)$; RBC: $y = -0.27(\pm 0.06) \times x - 0.88(\pm 0.10)$; liver: $y = -0.50(\pm 0.05) \times x - 0.54(\pm 0.13)$; leg muscle: $y = -0.28(\pm 0.01) \times x - 1.08(\pm 0.01)$. r^2 values are given in each panel for each linear regression.

rate and the turnover rate of a given tissue is often weak (Carleton and Martínez del Rio 2005; but see MacAvoy et al. 2006); (2) many animals with relatively high metabolic rates (e.g., bats) or relatively low metabolic rates (e.g., marsupials) do not have relatively high or low turnover rates, respectively (Voigt et al. 2003; but see Hobson and Clark 1992; Klaassen et al. 2004; Mirón et al. 2006); and (3) the allometric scaling of tissue-specific metabolic rates is often different from that of the whole-animal metabolic rate (Wang et al. 2001). This has led to alternative hypotheses that propose that carbon turnover rates are more closely associated with protein turnover.

Tieszen et al. (1983) proposed that the carbon turnover rates of tissues were more directly related to their metabolic activity, which is determined by some combination of protein turnover and metabolic rate, depending on the state of the animal. For example, most of the energy consumed by vertebrates during growth has been associated with protein synthesis (Bayne and Hawkins 1997), whereas protein synthesis accounted for <25% of energy consumed by nongrowing endothermic animals in a steady state (Welle and Nair 1990; Lobley 2003). Carleton and Martínez del Rio (2005) argued that because carbon turnover is typically measured in high-protein tissues (e.g., muscle, blood, cartilage) that have had their lipids removed, tissue-specific carbon turnover rates were more likely directly related to rates of protein turnover and may be only roughly related to metabolic rate. In support of this hypothesis, Houlihan et al. (1995) showed that whole-body protein turnover in vertebrates scaled with body mass to approximately the $-1/4$ power (range: -0.15 to -0.35). Protein synthesis in liver and leg muscle accounted for 23.3% and 4.8%, respectively, of total protein synthesis in pigs, whereas liver accounted for 67.5% and leg muscle for 13.0% of total protein synthesis in rats (Garlick et al. 1975). Thus, 4.9 and 5.2 times as much protein was synthesized in the liver than in the muscles in pigs and rats, respectively, similar to the estimates of relative differences in carbon turnover in these same tissues from other mammals (Sponheimer et al. 2006).

The crucial point is that protein and energy turnover rates are not necessarily tightly coupled (Lobley 2003), so rates of isotope turnover may not simply increase with whole-animal metabolic rate and are more likely directly related to rates of protein synthesis (Carleton and Martínez del Rio 2005). Two recent studies predicted that increased metabolic rate, associated with cold acclimation (Carleton and Martínez del Rio 2005) and exercise (Hobson and Yohannes 2007), would increase isotope turnover rate, but they found little or no support for this hypothesis. Testing these hypotheses requires measurements of tissue-specific protein synthesis, metabolic rate, and elemental turnover in a variety of animals.

Intertissue Differences in Turnover Rate Estimate Timing of Shifts in Resource Use

Phillips and Eldridge (2006) presented a quantitative model that used differences in isotope value between tissues, along with estimates of turnover rate in these tissues, to predict time elapsed since a diet shift. They concluded that accurate estimates of the timing of diet shifts requires that the turnover rates of selected tissues be known and be sufficiently different and that the tissue sampling must occur while carbon (or other elements) from the initial diet is still readily apparent in selected tissues. Our results provide the most complete analysis to date that defines both the necessary time window for sampling a given tissue and an evaluation of which tissues have turnover rates sufficiently different to be used for predicting timing of diet shifts. Our results also support a key assumption of these models, namely, that the change in isotope value after the diet switch is similar for all tissues (i.e., tissues were on average 2.9‰ more positive than the isotope value for the final diet).

We found substantial differences in average carbon retention

time among 12 different metabolically active tissues of zebra finches (vs. tissues, such as feathers, that incorporate no new carbon once growth is complete; Fig. 1). Carbon turnover rates also differed between metabolically active tissues in yellow-rumped warblers (*Dendroica coronata*; Podlesak et al. 2005), American crows (*Corvus brachyrhynchos*; Hobson and Clark 1993), mice (*Mus musculus*; MacAvoy et al. 2005; Arneson et al. 2006), alpacas (*Lama pacos*; Sponheimer et al. 2006), gerbils (*Meriones unguiculatus*; Tieszen et al. 1983), and quails (*Coturnix japonica*; Hobson and Clark 1992). Our results, along with these others, suggest that only a few different tissues must be sampled from an individual to estimate timing of diet shifts within a specified time period. In general, the time window during which diet shifts can be accurately detected is determined by the sampled tissue with the slowest turnover rate. Diet shifts must have occurred relatively recently, ideally within five half-lives of the slowest-turnover tissue, but at least as long ago as 0.1–0.3 half-lives of the slowest-turnover tissue (Phillips and Eldridge 2006). These criteria are required so that the slowest-turnover tissue has had enough time to incorporate carbon from the new diet but not so much time that both sampled tissues have replaced all carbon from the old diet with carbon from the new diet and thus have the same carbon value. For example, in birds the size of zebra finches, sampling tissues with very rapid turnover rates (e.g., small intestine or plasma) and tissues with moderately slow turnover rates (e.g., red blood cells or flight muscle) would allow estimating diet shifts of finches that occurred 2–65 d ago. If we also sampled tissues with very slow turnover rates (e.g., leg muscle or skin) and very rapid turnover rates (e.g., breath; Podlesak et al. 2005; Voigt et al. 2008), then we could estimate diet shifts of finches that occurred 1–100 d ago.

$C_{t1/2}$ and Phenotypic Flexibility in Organ Size of Migrating Birds

If the protein turnover rate of tissues largely determines their carbon turnover rates, as hypothesized, then this has important implications for our understanding of phenotypic flexibility in the organs of migratory birds. Studies on migrating birds have revealed reversible dynamic changes in organ sizes, with a decrease during flights and an increase during subsequent stopovers or premigratory preparation (Piersma and Lindström 1997; Piersma and Drent 2003; Bauchinger et al. 2005; McWilliams and Karasov 2005). This phenotypic flexibility may be an adaptive means to conserve energy and nutrients during migration (Piersma and Drent 2003). In general, digestive organs, including the liver, change substantially more than skeletal muscle (Piersma 1998; Piersma and Gill 1998; Piersma et al. 1999; Starck 1999*a*, 1999*b*; van Gils et al. 2005), especially during long flights (Åkesson et al. 1992; Biebach 1998; Battley et al. 2000; Schwilch et al. 2002; Bauchinger et al. 2005) or during experimental food deprivation (Hume and Biebach 1996; Biebach 1998; Karasov and Pinshow 1998; Battley et al. 2001; Karasov et al. 2004).

Several researchers have hypothesized that between-tissues variation in phenotypic flexibility is related to the functional significance of the specific organs (e.g., McWilliams and Karasov 2001; Piersma and Drent 2003). For example, during long-distance flight, birds may significantly reduce their digestive organs because they do not eat during flight, and the loss of these high–metabolic rate tissues reduces energy expenditure during flight.

We propose an alternative, nonfunctional hypothesis to explain phenotypic flexibility in the organs of such animals: tissue-specific turnover rate determines the pace of phenotypic flexibility in certain organs and hence its extent in fasting animals, such as birds during long-distance migratory flights. Rates of tissue synthesis and degradation determine tissue turnover rates, so that when animals do not eat, the rate of tissue synthesis declines while the rate of tissue degradation remains rather unaffected (Swick and Benevenga 1976). We can use our data on the relative differences in turnover rates between tissues, along with selected data on phenotypic flexibility of organs in migratory birds, to evaluate this alternative hypothesis.

If our estimates of tissue-specific turnover rates are generally applicable to migratory birds, then the hypothesis predicts that the extent of phenotypic flexibility in the organs of free-living birds during migration relates to tissue-specific turnover rates.

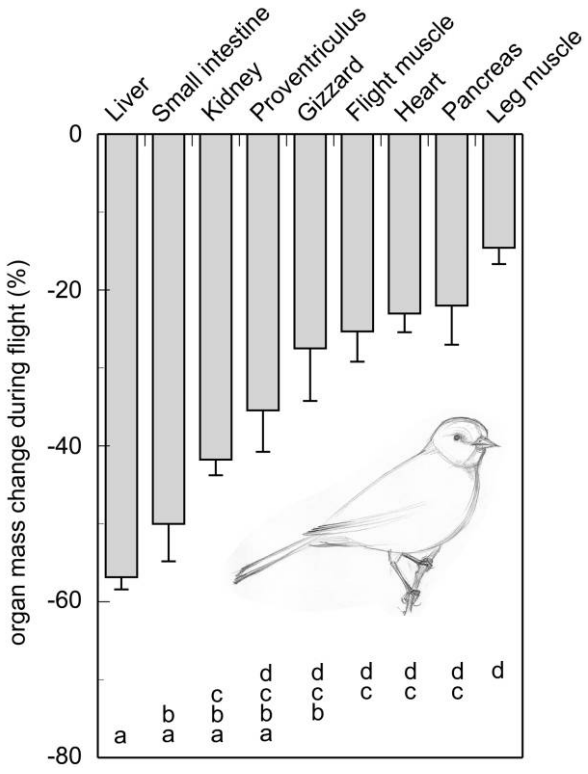

Figure 3. Percentage of organ mass change (± 1 SE) during flight across the Sahara Desert for garden warblers. Nine warblers were sampled in Ethiopia before their flight, and 10 warblers were sampled in Egypt immediately after completion of their flight (from Bauchinger et al. 2005). Identical letters indicate that organs were not significantly different in post hoc Scheffé tests (one-way ANOVA: $F = 13.8$, $P < 0.001$).

We used data on phenotypic flexibility of organs in garden warblers (*Sylvia borin*) during their long-distance flight across the Sahara Desert during spring (Bauchinger et al. 2005) because most of the same organs were studied as in our study and because these are the best available data on changes in a variety of organs for a free-living songbird during spring migration, when time constraints are likely. In general, those tissues with the fastest turnover rates (e.g., small intestine, kidney, and liver) changed the most in garden warblers during migration, whereas those tissues with the slowest turnover rates (e.g., leg and flight muscle) were among the organs that changed the least (Fig. 3). However, some tissues with relatively rapid turnover rates, such as gizzard and pancreas, changed relatively less in garden warblers. Thus, between-tissues differences in turnover rate largely, but not completely, explained the extent of phenotypic flexibility in organs of garden warblers. The same pattern of significantly greater reductions in the mass of organs with typically rapid turnover rates (small intestine, kidney, and liver), compared with those with slow turnover rates (flight and leg muscles) holds true for several migratory bird species during in-flight starvation (Battley et al. 2000, 2001) or food deprivation (Hume and Biebach 1996; Karasov and Pinshow 1998; Battley et al. 2001). If tissue-specific turnover rate determines the magnitude of phenotypic flexibility in certain organs, then we suggest that it provides a useful null model for testing alternative hypotheses about the extent of observed phenotypic flexibility in organs of wild vertebrates. In short, functional explanations for observed phenotypic flexibility need not be invoked unless organs change unusually more or less than predicted from their turnover rates. This mechanism may also apply to other situations in which phenotypic flexibility in organ mass is observed, including when animals hibernate, fast, or thermoregulate in extreme environments.

Acknowledgments

J. Keil processed tissue samples. J. M. Starck provided regular constructive input and facilities at the Ludwig-Maximilians-Universität. K. Winiarski and J. Keil ran the continuous-flow isotope ratio micromass optima spectrometer under the guidance of R. McKinney at the Atlantic Ecology Division of the U.S. Environmental Protection Agency. C. Böhm provided the bird sketches. B. Pinshow provided valuable input to the manuscript. Carlos Martínez del Rio and two anonymous reviewers provided productive comments that helped to improve the manuscript. This project was funded by National Science Foundation grant IBN-9984920 (to S.M.) and U.S. Department of Agriculture grant 538748 (to S.M.). This is contribution 5215 from the University of Rhode Island Agricultural Experiment Station.

Appendix

Supplementary Figures and Table

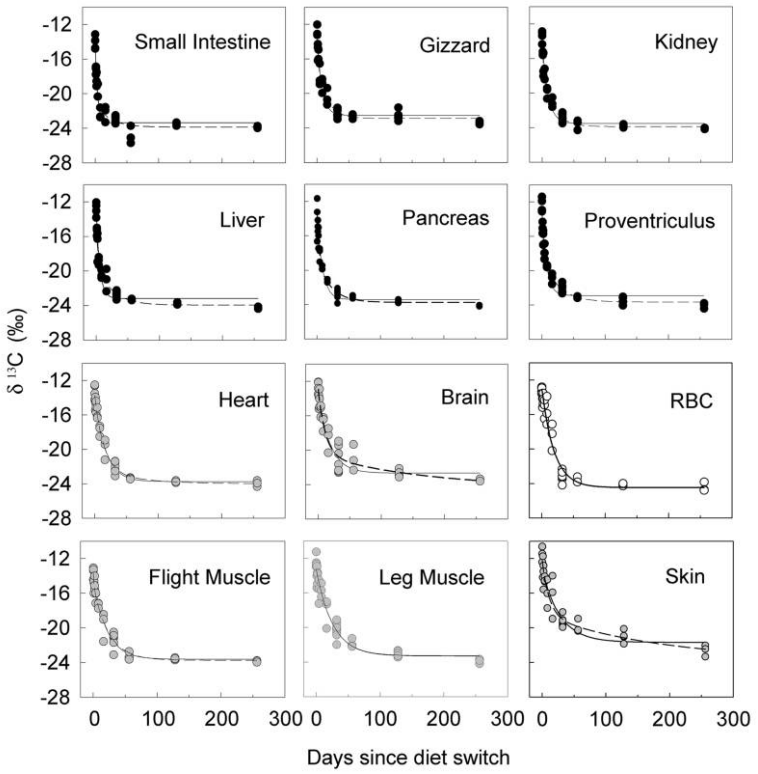

Figure A1. Incorporation of ^{13}C into small intestine, gizzard, kidney, liver, pancreas, proventriculus, heart, brain, red blood cells, flight muscle, leg muscle, and skin sampled over 256 d after a diet shift. Order of the tissues corresponds to increasing mean residence time (τ). For all tissues, curves were fitted and plotted for both a one-compartment model (*solid line*) and a two-compartment model (*dashed line*; see Table 1 for parameter estimates and model algorithms). Data for tissues presented as black circles are best described by a two-compartment model, data presented as white circles are best described by a one-compartment model, and data presented as gray circles are substantially supported by both one- and two-compartment models.

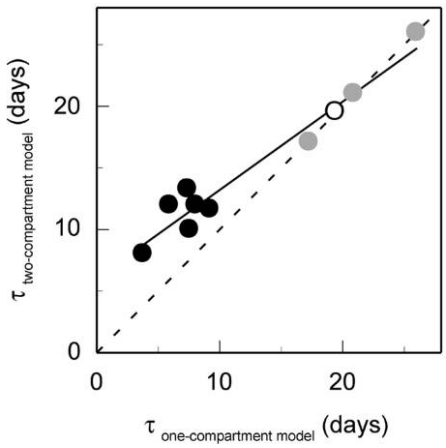

Figure A2. Average ^{13}C retention time (τ) for 10 tissues as estimated from one- and two-compartment models. Dashed line indicates $y = x$ line. The solid line is the best-fit regression line ($y = 6.02[\pm 0.84] + 0.72[\pm 0.06] \times x$; $r^2 = 0.95$, $n = 10$). Brain and skin were excluded from this analysis because average residence time estimated from the two-compartment model was unusually high compared with that from the one-compartment model (Table 1). Regression equation for all 12 tissues, as estimated from one- and two-compartment models: $y = -4.64(\pm 11.24) + 2.09(\pm 0.69) \times x$; $r^2 = 0.48$.

Table A1: Carbon half-life ($C_{t1/2} = \ln(2)/k$) of certain tissues for zebra finches and other vertebrates

	Birds[a]				Mammals[b]			
Tissue Type	Zebra Finch (.016 kg)	House Sparrow (.022 kg)	Yellow-Rumped Warbler (.012 kg)	Japanese Quail (.115 kg)	Mouse, 28 d (.019 kg)	Mouse, 172 d (.019 kg)	Gerbil (.065 kg)	Alpaca (60 kg)
Small intestine	5.6	10.3	…	…	…	…	…	…
Gizzard	7.0	14.1	…	…	…	…	…	…
Kidney	8.0	…	…	…	4.6	…	…	…
Liver	8.3	9.8	…	2.6	5.0	7.7	6.4	37.3
Pancreas	8.3	…	…	…	…	…	…	…
Proventriculus	9.3	…	…	…	…	…	…	…
Heart	12.0	15.2	…	…	13.9	…	…	…
Brain	12.8	…	…	…	17.8	…	28.2	…
Blood cells[c]	13.4	19.3	10.9	11.4	20.4	19.8	…	…
Flight muscle	14.5	23.5	…	12.4	…	…	…	…
Leg muscle	18.1	…	…	…	23.1	18.2	27.6	178.7
Skin	19.5	…	…	…	…	…	…	…
Plasma	…	6.7	1.0	…	…	…	…	…
Bone collagen	…	20.4	…	173.3	…	…	…	…

Note. All studies that estimated $C_{t1/2}$ for more than one tissue were considered. Body mass (kg; body mass for all species except alpaca according to MacAvoy et al. 2005); body mass of alpaca from http://animaldiversity.ummz.umich.edu) for each species is given in parentheses.

[a] Turnover rates ($C_{t1/2}$) of the birds were measured over 256 d for zebra finch (*Taeniopygia guttata*; this study), 128 d for house sparrow (*Passer domesticus*; Carleton et al. 2008), 15 d for yellow-rumped warblers (*Dendroica coronata*; Podlesak et al. 2005), and 212 d for quail (*Coturnix japonica*; Hobson and Clark 1992).

[b] Turnover rates ($C_{t1/2}$) of the mammals were measured over 28 d (left column) and 172 d (right column) for mouse (*Mus musculus*; Arneson et al. 2006), 155 d for gerbil (*Meriones unguiculatus*; Tieszen et al. 1983), and 35 d for alpaca (*Lama pacos*; Sponheimer et al. 2006).

[c] Red blood cells for zebra finch, house sparrow, and yellow-rumped warbler; whole blood (red blood cells and plasma) for all other species.

Literature Cited

Åkesson S., L. Karlsson, J. Pettersson, and G. Walinder. 1992. Body composition and migration strategies: a comparison between robins (*Erithacus rubecula*) from two stop-over sites in Sweden. Vogelwarte 36:188–195.

Alerstam T., M. Rosen, J. Bäckman, P.G.P. Ericson, and O. Hellgren. 2007. Flight speeds among bird species: allometric and phylogenetic effects. PLoS Biol 5:e197, doi:10.1371/journal.pbio.0050197.

Arneson L.S., S.E. MacAvoy, and E. Basset. 2006. Metabolic protein replacement drives tissue turnover in adult mice. Can J Zool 84:992–1002.

Ayliffe L.K., T.E. Cerling, T. Robinson, A. West, M. Sponheimer, B. Passey, J. Hammer, B. Roeder, M.D. Dearing, and J.R. Ehleringer. 2004. Turnover of carbon isotopes in tail hair and breath CO_2 of horses fed an isotopically varied diet. Oecologia 139:11–22.

Battley P.F., M.W. Dietz, T. Piersma, A. Dekinga, S.X. Tang, and K. Hulsman. 2001. Is long-distance bird flight equivalent to a high-energy fast? body composition changes in freely migrating and captive fasting great knots. Physiol Biochem Zool 74:435–449.

Battley P.F., T. Piersma, M.W. Dietz, S.X. Tang, A. Dekinga, and K. Hulsman. 2000. Empirical evidence for differential organ reductions during trans-oceanic bird flight. Proc R Soc B 267:191–195.

Bauchinger U., A. Wohlmann, and H. Biebach. 2005. Flexible remodeling of organ size during spring migration of the garden warbler (*Sylvia borin*). Zoology (Jena) 108:97–106.

Bayne B.L. and A.J.S. Hawkins. 1997. Protein metabolism, the costs of growth, and genomic heterozygosity: experiments with the mussel *Mytilus galloprovincialis* Lmk. Physiol Zool 70:391–402.

Biebach H. 1998. Phenotypic organ flexibility in garden warblers *Sylvia borin* during long-distance migration. J Avian Biol 29:529–535.

Brown J.H., J.F. Gillooly, A.P. Allen, V.M. Savage, and G.B. West. 2004. Toward a metabolic theory of ecology. Ecology 85: 1771–1789.

Burnham K.P. and D.R. Anderson. 2002. Model Selection and Multimodel Inference: A Practical Information Theoretic Approach. Springer, New York.

Carleton S.A., L. Kelly, R. Anderson-Sprecher, and C. Martínez del Rio. 2008. Should we use one-, or multi-compartment models to describe ^{13}C incorporation into animal tissues? Rapid Commun Mass Spectrom 22:3008–3014.

Carleton S.A. and C. Martínez del Rio. 2005. The effect of cold-induced increased metabolic rate on the rate of ^{13}C and ^{15}N incorporation in house sparrows (*Passer domesticus*). Oecologia 144:226–232.

Cerling T.E., L.K. Ayliffe, M.D. Dearing, J.R. Ehleringer, B.H. Passey, D.W. Podlesak, A.-M. Torregrossa, and A.G. West. 2007. Determining biological tissue turnover using stable isotopes: the reaction progress variable. Oecologia 151:175–189.

DeNiro M.J. and S. Epstein. 1978. Influence of diet on distribution of carbon isotopes in animals. Geochim Cosmochim Acta 42:495–506.

Garlick P.J., D.J. Millward, W.P.T. James, and J.C. Waterlow. 1975. The effect of protein deprivation and starvation on the rate of protein synthesis in tissues of the rat. Biochim Biophys Acta 414:71–84.

Hedenström A. and T. Alerstam. 1997. Optimum fuel loads in migratory birds: distinguishing between time and energy minimization. J Theor Biol 189:227–234.

Hobson K.A. 1999. Tracing origins and migration of wildlife using stable isotopes: a review. Oecologia 120:314–326.

Hobson K.A. and R.G. Clark. 1992. Assessing avian diets using stable isotopes. I. Turnover of ^{13}C in tissues. Condor 94:181–188.

———. 1993. Turnover of ^{13}C in cellular and plasma fractions of blood: implications for nondestructive sampling in avian dietary studies. Auk 110:638–641.

Hobson K.A. and E. Yohannes. 2007. Establishing elemental turnover in exercising birds using a wind tunnel: implications for stable isotope tracking of migrants. Can J Zool 85:703–708.

Houlihan D.F., C.G. Carter, and I.D. McCarthy. 1995. Protein turnover in animals. Pp. 1–32 in P.J. Walsh and P. Wright, eds. Nitrogen Metabolism and Excretion. CRC, Boca Raton, FL.

Hume I.D. and H. Biebach. 1996. Digestive tract function in the long-distance migratory garden warbler, *Sylvia borin*. J Comp Physiol B 166:388–395.

Inger R. and S. Bearhop. 2008. Applications of stable isotope analyses to avian ecology. Ibis 150:447–461, doi: 10.1111/j.1474-919X.2008.00839.x.

Karasov W.H. and B. Pinshow. 1998. Changes in lean mass and in organs of nutrient assimilation in a long-distance passerine migrant at a springtime stopover site. Physiol Zool 71:435–448.

Karasov W.H., B. Pinshow, J.M. Starck, and D. Afik. 2004. Anatomical and histological changes in the alimentary tract of migrating blackcaps (*Sylvia atricapilla*): a comparison among fed, fasted, food-restricted, and refed birds. Physiol Biochem Zool 77:149–160.

Klaassen M., M. Thums, and I.D. Hume. 2004. Effects of diet change on carbon and nitrogen stable-isotope ratios in blood cells and plasma of the long-nosed bandicoot (*Perameles nasuta*). Aust J Zool 52:635–647.

Kleiber M. 1932. Body size and metabolism. Hilgardia 6:315–353.

———. 1961. The Fire of Life: An Introduction to Animal Energetics. Wiley, New York.

Lobley G.E. 2003. Protein turnover: what does it mean for animal production? Can J Anim Sci 83:327–340.

MacAvoy S.E., L.S. Arneson, and E. Bassett. 2006. Correlation of metabolism with tissue carbon and nitrogen turnover rate in small mammals. Oecologia 150:190–201.

MacAvoy S.E., S.A. Macko, and L.S. Arneson. 2005. Growth versus metabolic tissue replacement in mouse tissues determined by stable carbon and nitrogen isotope analysis. Can J Zool 83:631–641.

Marra P.P., K.A. Hobson, and R.T. Holmes. 1998. Linking winter and summer events in a migratory bird by using stable-carbon isotopes. Science 282:1884–1886.

Martínez del Rio C. and R. Anderson-Sprecher. 2008. Beyond the reaction progress variable: the meaning and significance of isotopic incorporation data. Oecologia 156:765–772.

Martínez del Rio C. and B.O. Wolf. 2005. Mass-balance models for animal isotopic ecology. Pp. 141–174 in J.M. Starck and T. Wang, eds. Physiological and Ecological Adaptations to Feeding in Vertebrates. Science, Enfield, NH.

Martínez del Rio C., N. Wolf, S.A. Carleton, and L.Z. Gannes. 2009. Isotopic ecology ten years after a call for more laboratory experiments. Biol Rev 84:91–111, doi:10.1111/j.1469-185X.2008.00064.x.

McWilliams S.R. and W.H. Karasov. 2001. Phenotypic flexibility in digestive system structure and function in migratory birds and its ecological significance. Comp Biochem Physiol A 128: 577–591.

———. 2005. Migration takes gut: digestive physiology of migratory birds and its ecological significance. Pp. 67–78 in R. Greenberg and P.P. Marra, eds. Birds of Two Worlds: The Ecology and Evolution of Migration. Johns Hopkins University Press, Baltimore.

Mirón L., L.G. Herrera, N. Ramírez, and K.A. Hobson. 2006. Effect of diet quality on carbon and nitrogen turnover and isotopic discrimination in blood of a New World nectarivorous bat. J Exp Biol 209:541–548.

Norris D.R., P.P. Marra, T.K. Kyser, and L.M. Ratcliffe. 2005. Tracking habitat use of a long-distance migratory bird, the American redstart *Setophaga ruticilla*, using stable-carbon isotopes in cellular blood. J Avian Biol 36:164–170.

Packard G.C. and T.J. Boardman. 1999. The use of percentages and size-specific indices to normalize physiological data for variation in body size: wasted time, wasted effort? Comp Biochem Physiol A 122:37–44.

Phillips D.L. and P.M. Eldridge. 2006. Estimating the timing of diet shifts using stable isotopes. Oecologia 147:195–203.

Piersma T. 1998. Phenotypic flexibility during migration: optimization of organ size contingent on the risks and rewards of fueling and flight? J Avian Biol 29:511–520.

Piersma T. and J. Drent. 2003. Phenotypic flexibility and the evolution of organismal design. Trends Ecol Evol 18:228–233.

Piersma T. and R.E. Gill. 1998. Guts don't fly: small digestive organs in obese bar-tailed godwits. Auk 115:196–203.

Piersma T., G.A. Gudmundsson, and K. Lilliendahl. 1999. Rapid changes in the size of different functional organ and muscle groups during refueling in a long-distance migrating shorebird. Physiol Biochem Zool 72:405–415.

Piersma T. and A. Lindström. 1997. Rapid reversible changes in organ size as a component of adaptive behaviour. Trends Ecol Evol 12:134–138.

Podlesak D.W. and S.R. McWilliams. 2006. Metabolic routing of dietary nutrients in birds: effects of diet quality and macronutrient composition revealed using stable isotopes. Physiol Biochem Zool 79:534–549.

Podlesak D.W., S.R. McWilliams, and K.A. Hatch. 2005. Stable isotopes in breath, blood, feces and feathers can indicate intra-individual changes in the diet of migratory songbirds. Oecologia 142:501–510.

Schwilch R., A. Grattarola, F. Spina, and L. Jenni. 2002. Protein loss during long-distance migratory flight in passerine birds: adaptation or constraint. J Exp Biol 205:687–695.

Sokal R.R. and F.J. Rohlf. 2000. Biometry. 3rd ed. Freeman, New York.

Sponheimer M., T.F. Robinson, T.E. Cerling, L. Tegland, B.L. Roeder, L. Ayliffe, M.D. Dearing, and J.R. Ehleringer. 2006. Turnover of stable carbon isotopes in the muscle, liver, and breath CO_2 of alpacas (*Lama pacos*). Rapid Commun Mass Spectrom 20:1395–1399.

Starck J.M. 1999*a*. Phenotypic flexibility of the avian gizzard: rapid, reversible and repeated chances of organ size in response to changes in dietary fibre content. J Exp Biol 202: 3171–3179.

———. 1999*b*. Structural flexibility of the gastro-intestinal tract of vertebrates: implications for evolutionary morphology. Zool Anz 238:87–101.

Swick R.W. and N.J. Benevenga. 1976. Labile protein reserves and protein turnover. J Dairy Sci 60:505–515.

Tieszen L.L., T.W. Boutton, K.G. Tesdahl, and N.A. Slade. 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: implications for $\delta^{13}C$ analysis of diet. Oecologia 57:32–37.

van Gils J.A., P.F. Battley, T. Piersma, and R. Drent. 2005. Reinterpretation of gizzard sizes of red knots world-wide emphasises overriding importance of prey quality at migratory stopover sites. Proc R Soc B 272:2609–2618.

Voigt C.C., L. Baier, J.R. Speakman, and B.M. Siemers. 2008. Stable carbon isotopes in exhaled breath as tracers for dietary information in birds and mammals. J Exp Biol 211:2233–2238.

Voigt C.C., F. Matt, R. Michener, and T.H. Kunz. 2003. Low turnover rates of carbon isotopes in tissues of two nectar-feeding bats. J Exp Biol 206:1419–1427.

Wang Z.M., T.P. O'Connor, S. Heshka, and S.B. Heymsfield. 2001. The reconstruction of Kleiber's law at the organ-tissue level. J Nutr 131:2967–2970.

Welle S. and K.S. Nair. 1990. Relationship of resting metabolic rate to body composition and protein turnover. Am J Physiol 258:E990–E998.

West J.B., G.J. Bowen, T.E. Cerling, and J.R. Ehleringer. 2006. Stable isotopes as one of nature's ecological recorders. Trends Ecol Evol 21:408–414.

Wolf N., Carleton S.A., and C. Martínez del Rio. 2009. Ten years of experimental animal isotopic ecology. Funct Ecol 23:17–26.

ERRATUM

Carbon Turnover in Tissues of a Passerine Bird: Allometry, Isotopic Clocks, and Phenotypic Flexibility in Organ Size

Ulf Bauchinger
Scott R. McWilliams

In "Carbon Turnover in Tissues of a Passerine Bird: Allometry, Isotopic Clocks, and Phenotypic Flexibility in Organ Size" (*Physiological and Biochemical Zoology* 82:787–797), Figure 2 reported allometric equations for four tissues (whole blood, red blood cells [RBC], liver, and leg muscle) that allowed prediction of fractional rates of carbon incorporation ($\log k$) given measured body mass ($\log m_b$). Unfortunately, the slope for the liver tissue in the published figure legend was erroneous because of a missing number and exclusion of one species from the original data set. In addition, the constant for RBC presented in the original article refers to an older calculation based on birds and mammals combined, but in the final paper only bird data were used for RBC. Table 1 summarizes the corrected parameter estimates (in bold) to four significant digits from the regression analyses for the liver and RBC along with the other correct parameter estimates for the other tissues.

Table 1: Corrected parameter estimates

Tissue	Slope (±SE)	Intercept (±SE)	Allometric Equation
Whole blood	−.2945 (±.0519)	−.5490 (±.1166)	$y = -.2945 \times x - .5490$
RBC	−.2744 (±.0595)	**−.9226 (±.1004)**	$\boldsymbol{y = -.2744 \times x - .9226}$
Liver	**−.2758 (±.0676)**	**−.4224 (±.1684)**	$\boldsymbol{y = -.2758 \times x - .4223}$
Leg muscle	−.2793 (±.0055)	−1.0788 (±.0149)	$y = -.2793 \times x - 1.0788$

Physiological and Biochemical Zoology 83(6):1032. 2010. 1522-2152/2010/8306-0126$15.00.
DOI: 10.1086/657659