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The effect of cold-induced increased metabolic rate on the rate of ^{13}C and ^{15}N incorporation in house sparrows (*Passer domesticus*)

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Abstract Animals with high metabolic rates are believed to have high rates of carbon and nitrogen isotopic incorporation. We hypothesized that (1) chronic exposure to cold, and hence an increase in metabolic rate, would increase the rate of isotopic incorporation of both ^{13}C and ^{15}N into red blood cells; and (2) that the rate of isotopic incorporation into red blood cells would be allometrically related to body mass. Two groups of sparrows were chronically exposed to either 5 or 22°C and switched from a ^{13}C-depleted C_3-plant diet to a more ^{13}C-enriched C_4-plant one. We used respirometry to estimate the resting metabolic rate ($\dot{V}_{O_2}$) of birds exposed chronically to our two experimental temperatures. The allometric relationship between the rate of ^{13}C incorporation into blood and body mass was determined from published data. The $\dot{V}_{O_2}$ of birds at 5°C was 1.9 times higher than that of birds at 22°C. Chronic exposure to a low temperature did not have an effect on the rate of isotopic incorporation of ^{15}N save for a very small effect on the incorporation of ^{13}C. The isotopic incorporation rate of ^{13}C was 1.5 times faster than that of ^{15}N. The fractional rate of ^{13}C incorporation into avian blood was allometrically related to body mass with an exponent similar to $-1/4$. We conclude that the relationship between metabolic rate and the rate of isotopic incorporation into an animal's tissues is indirect. It is probably mediated by protein turnover and thus more complex than previous studies have assumed.

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Introduction

Most diet reconstruction studies using stable isotope methods assume steady state. They assume that the isotopic composition of animal tissues are in equilibrium with diet. Because animals often change diets and because their tissues take time to incorporate the new diet's isotopic composition, the equilibrium assumption is not often satisfied. Tieszen et al. (1983) noted that isotope incorporation rates varied among tissues. This variation is very useful. Tissues with high incorporation rates (such as liver, and plasma proteins; Hobson and Clark 1992) track isotopic changes in diet closely, whereas tissues with low incorporation rates (such as red blood cells, muscle, and bone collagen) integrate an isotopic signature from a larger temporal window (Tieszen et al. 1983; Hobson and Clark 1992; Martínez del Rio and Wolf 2004).

Why do different tissues vary in their rates of isotopic incorporation? Because tissues with high protein turnover and synthesis also sometimes (albeit not always, see Lobley 2003) have high metabolic rates, Tieszen et al. (1983) hypothesized that tissues with high metabolic activity would also have high rates of isotopic incorporation. Tieszen et al.'s (1983) statement has come to be interpreted narrowly to mean that both organisms and tissues with high metabolic rate should have high rates of isotopic incorporation (Hobson and Clark 1992; Voigt et al. 2003). For example, Klaasen et al. (2004) measured incorporation of ^{13}C and ^{15}N into the blood of long-nosed bandicoots. They found very low levels of incorporation and speculated that these rates were low compared with those measured in other birds and mammals due to the typically low metabolic rate of marsupials (Klaassen et al. 2004). Voigt et al. (2003) investigated

the rate of carbon incorporation into the tissues of nectar-feeding bats. They found that the rate of carbon incorporation in these animals was the lowest reported for a vertebrate, which is surprising in view of the exceedingly high metabolic rates of these animals. Why did these bats have such low turnover rates in spite of a high metabolic rate? More generally, what is the relationship between metabolic rate and the rate of carbon and nitrogen isotopic incorporation? Answering this question is important because the notion that metabolic rate is a determinant of isotopic turnover is implicit in some isotopic incorporation studies (Hobson and Clark 1992; Voigt et al. 2003; Klaassen et al. 2004).

Here, we adopted a more general interpretation of Tieszen et al.'s (1983) hypothesis. We interpret high metabolic activity to mean a high rate of synthesis and catabolism, which is not the same as high "metabolic rate" that is narrowly defined by physiologists as the rate of energy consumption. Isotopic ecologists have often focused their analyses on protein or protein-rich tissues such as muscle, collagen, and blood (Gannes et al. 1997, 1998), and often measure the isotopic composition of lipid-free tissues. Thus, it is reasonable to assume that the rate of protein turnover, which is determined by the rates of protein synthesis and catabolism (Waterlow et al. 1978; Waterlow 1999), is the major determinant of the rate of isotopic incorporation into a tissue. Because tissues with high protein turnover and synthesis sometimes (albeit not always, see Lobley 2003) have high metabolic rates, it is sensible to hypothesize that animals with high metabolic rates should also have high rates of isotopic incorporation. Note, however, that the potential association between high metabolic rate and high rates of isotopic incorporation is indirect. It is mediated by the cost of protein turnover. Because protein turnover is not the only determinant of metabolic rate, this relationship can be uncoupled (Lobley 2003)

Our study tested two hypotheses: (1) We hypothesized that the rate of ^{13}C and ^{15}N incorporation into red blood cells would be higher in animals experiencing a chronic cold-induced increase in metabolic rate. (2) We hypothesized that among species, the fractional rate of isotopic incorporation would be allometrically dependent on body mass. More specifically, we expected the fractional rate of isotopic incorporation to scale with body mass to approximately the $-1/4$ power. This expectation is a consequence of both previous research on the scaling of whole body protein turnover with body mass and of a priori considerations. In vertebrates whole body protein turnover scales allometrically with body mass and the exponent of this relationship ranges from -0.15 to -0.35 (Houlihan et al. 1995). The fractional rate of protein turnover, and hence of isotopic incorporation, should be proportional to the ratio of the rate at which materials are incorporated into a tissue and the total size of the pool of the element in question (C or N) in the tissue. The rate of elemental incorporation into a tissue should be proportional to mass to the 3/4 power (West et al. 1997) and the size of the pool should be proportional to body mass (m_b). Thus, the rate of isotopic incorporation (k) should be proportional to m_b 3/4/ $m_b = m_b$ $-1/4$.

We tested hypothesis 1 by keeping house sparrows (*Passer domesticus*) at two different temperatures that led to two contrasting rates of oxygen consumption ($\dot{V}_{O_2}$). We measured the rate of isotopic incorporation of ^{13}C and ^{15}N into the birds' red blood cells after shifting them from a plant-based diet with C_3 composition to one with a C_4 composition. Because our experimental diets differed in both carbon and nitrogen isotopic composition, we examined the effect of temperature on the rate of incorporation of these two elements. We also examined whether the rate of ^{13}C incorporation differed from that of ^{15}N. We tested hypothesis 2 with data for eight species of birds. Isotopic ratios in this paper are reported on a per mil (‰) basis relative to the IAEA carbon isotope standard, Vienna Pee Dee Belemnite (VPDB) and the nitrogen isotope standard, atmospheric nitrogen (atmospheric N_2)

Methods

Thirty house sparrows ($m_b \pm SD = 25.17 \pm 1.40$) were captured with mist nets in Albany County in October 2002. They were housed in the University of Wyoming's Animal Care Facility at the Department of Zoology and Physiology. Birds were marked with unique combinations of color bands and randomly assigned into each of two experimental groups of 15 birds. Birds were housed in groups of five per cage (0.6 m×0.6 m×0.6 m) and placed in one of two temperature treatment groups, 22 and 5°C. Both groups were fed whole wheat ($\delta^{13}C = -25.46‰ \pm 0.07$ VPDB, $n = 5$, $\delta^{15}N = 4.3‰ \pm 0.09$ Air N_2, $n = 5$) for 60 days and were then switched to cracked corn ($\delta^{13}C = -11.28‰ \pm 0.06$ VPDB, $n = 5$, $\delta^{15}N = 1.46‰ \pm 0.26$ Air N_2, $n = 5$). Additionally, a mineral and vitamin supplement was mixed with the water (4 mgL^{-1}) to meet dietary needs ($\delta^{13}C = -25.54‰ \pm 0.23$ VPDB, $n = 5$, $\delta^{15}N = -1.22‰ \pm 0.07$ Air N_2, $n = 5$). Birds maintained relatively constant body mass throughout the experiment. The body masses of birds maintained at the two experimental temperatures did not differ significantly ($t = 0.10$, $p = 0.92$)

On days 0, 2, 4, 8, 19, 24, 32, 40, 48, and 56 after switching to the corn diet, we obtained a blood sample (≈50 µl) from the brachial vein using a 0.5-ml syringe with 30-gauge needles. After centrifugation (3 min in a microhematocit centrifuge), we separated cells from plasma. Red blood cells were dried to constant mass in an oven at 50°C and homogenized into a fine powder. Samples were loaded into tin capsules (≈0.25 mg for $\delta^{15}N$ and 0.15 mg for δ ^{13}C analyses). Samples were analyzed for $^{13}C/^{12}C$ and ^{15}N /^{14}N ratios with a Carlo-Erba NA1500 elemental analyzer (Milan, Italy) coupled

to a VG Isochrom stable isotope ratio mass spectrometer (GV Instruments, Manchester, UK) at the Mass Spectrometry Isotope Facility at Colorado State University.

Respirometry

Upon completion of the isotopic incorporation experiment, six sparrows from each treatment were placed individually in 3-l glass chambers connected to a flow-through respirometry system. Each chamber was inside a constant environment chamber set at 5°C ± 1°C or 22°C ± 1°C. Each bird was fasted overnight and then placed into the chamber within 15 min of the lights coming on in the aviary. The chamber was opaque and hence metabolic rates were measured in the dark. Birds acclimated at 5°C were held at 5°C in the chamber until a constant $\dot{V}_{O_2}$ was maintained and the temperature of the environmental chamber was then increased to 22°C. Measurements continued until a constant $\dot{V}_{O_2}$ was again established. The same procedure was repeated for birds acclimated at 22°C except that initial chamber temperatures were 22°C and then decreased to 5°C. Air was pulled through the system at 350 ml min^{-1} by a pump. Fresh air was drawn through bev-a-line IV tubing (used for the entire respirometry setup) from outside the constant environment chamber, through soda lime (to absorb CO_2), then through Drierite (self-indicating anhydrous CaSO4: 10–20 mesh; to absorb water vapor), and then into an S-3A/I Applied Electrochemistry O_2 analyzer. A control chamber was run during each experiment to measure the $\%O_2$ flowing through a chamber without a bird. This value was then compared to the values measured for each chamber containing a bird. Instantaneous O_2 percentage values were recorded automatically on a computer (Sable Systems, Henderson, NV, USA) six times min^{-1} with a 10-min lag between chambers. Metabolic rate, $\dot{V}_{O_2}$, was calculated (in ml O_2 g^{-1} h^{-1}) as:

$$\dot{V}_{O_2} = \frac{(\dot{V} \times 60) \times (F_iO_2 - F_eO_2)}{1 - F_iO_2}, \quad (1)$$

where flow rate ($\dot{V}$) is in ml air min^{-1}, F_iO_2 is the fractional concentration of O_2 in the excurrent air from the control chamber, and F_eO_2 is the fractional concentration of O_2 in the excurrent air of the chamber containing a bird (Lotz et al. 2003). Values were then divided by body mass averaged between the start and end of each experimental run to yield mass specific values (ml O_2 g^{-1} h^{-1}).

Statistical analyses

We estimated k, the fractional rate of isotopic incorporation, using a non-linear fitting procedure (JMP) for each individual bird using the equation

$$\delta X(t) = \delta X(\infty) + (\delta X(0) - \delta X(\infty))e^{-kt}, \quad (2)$$

where $\delta X(t)$ is the isotopic composition at time t, $\delta X(0)$ the initial isotopic composition, $\delta X(\infty)$ the asymptotic equilibrium isotopic composition, and k is the fractional rate of isotope incorporation (O'Brien et al. 2000; Martínez del Rio and Wolf 2004). We compared the fractional rate of elemental (C and N) incorporation for birds exposed to the two temperatures using unpaired t-tests. We used repeated measures analysis of variance to compare (1) the effect of temperature on $\dot{V}_{O_2}$ for birds chronically exposed to 5 and 22°C, and (2) the difference in the fractional rate of incorporation between C and N. Because both the fractional rate of elemental incorporation (k) and body mass in our allometric analysis are measured with error, we used both ordinary least squares (OLS) and reduced major axis (RMA) regression to analyze allometric data (Sokal and Rohlf 1995). To test the expectation that the slope of the allometric relationship between k and body mass would be either significantly different or not from $-1/4$, we constructed 95% confidence intervals for the estimated slopes and determined whether $-1/4$ was included within them (this approach is equivalent to conducting a single sample t-test).

Results

Respirometry

There were no differences in $\dot{V}_{O_2}$ between birds maintained for 56 days at either 5°C or 22°C (RMANOVA,

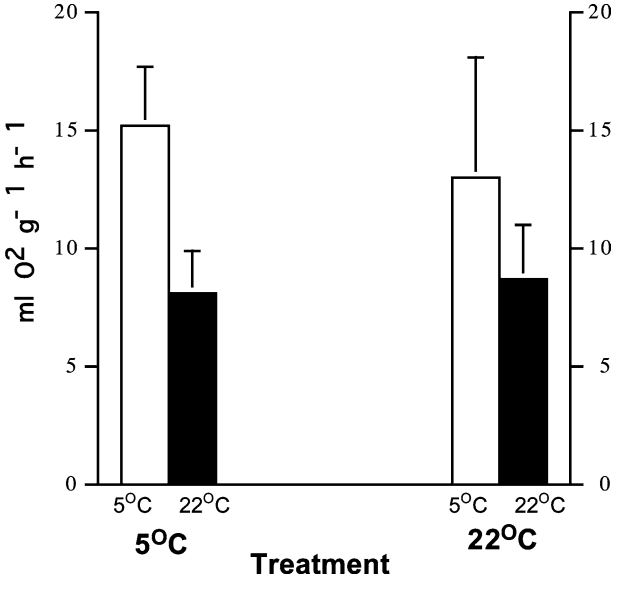

Fig. 1 The $\dot{V}_{O_2}$ (mlO_2g^{-1} h^{-1}) of birds exposed at rest to 5 and 22°C was not significantly different between birds exposed chronically for 56 days at either 5 or 22°C; however $\dot{V}_{O_2}$ was ≈1.9 times higher at 5°C than at 22°C

$F_{1,26} = 0.77$, $p = 0.40$). However, birds measured at 5°C had $\dot{V}_{O_2}$ that was approximately 1.9 times higher than that measured on birds measured at 22°C (Fig. 1; $F_{1,26} = 12.45$, $p = 0.0001$).

Isotopic incorporation

The incorporation of ^{13}C and ^{15}N into blood cells as a function of time is adequately described by Eq. 2 (Fig. 2.). There were no statistically significant differences in the fractional incorporation rate (k, day^{-1}) between birds at 5 and 22°C for either carbon or nitrogen (Table 1). The half life of ^{13}C (defined as $t_{1/2} = \mathrm{Ln}(2)/k$) was significantly higher for birds exposed to 5°C than that of birds at 22°C (Table 1). There were no significant effects of temperature on the rate of ^{15}N incorporation. The incorporation of ^{13}C was approximately 1.5 times faster than that of ^{15}N (paired $t = 7.28$, $p = 0.0001$, $n = 26$, Table 1). Temperature had no effect on the diet to tissue discrimination factor of either carbon or nitrogen ($\Delta\,^{13}C_{\text{diet-tissue}} = \delta^{13}C_{\text{diet}} - \delta^{13}C_{\text{tissue}}$ and $\delta^{15}N_{\text{diet-tissue}} = \delta^{15}N_{\text{diet}} - \delta^{15}N_{\text{tissue}}$, respectively, Table 1).

Allometry of fractional turnover

The available comparative data on the fractional rate of ^{13}C incorporation into avian blood (k) are summarized in Table 2. The logarithm of the fractional rate of carbon incorporation decreased linearly with the logarithm of body mass (Fig. 3). The slopes of the log–log regression estimated by ordinary least squares and reduced major axis always included 0.25 within their 95% confidence intervals (Table 3).

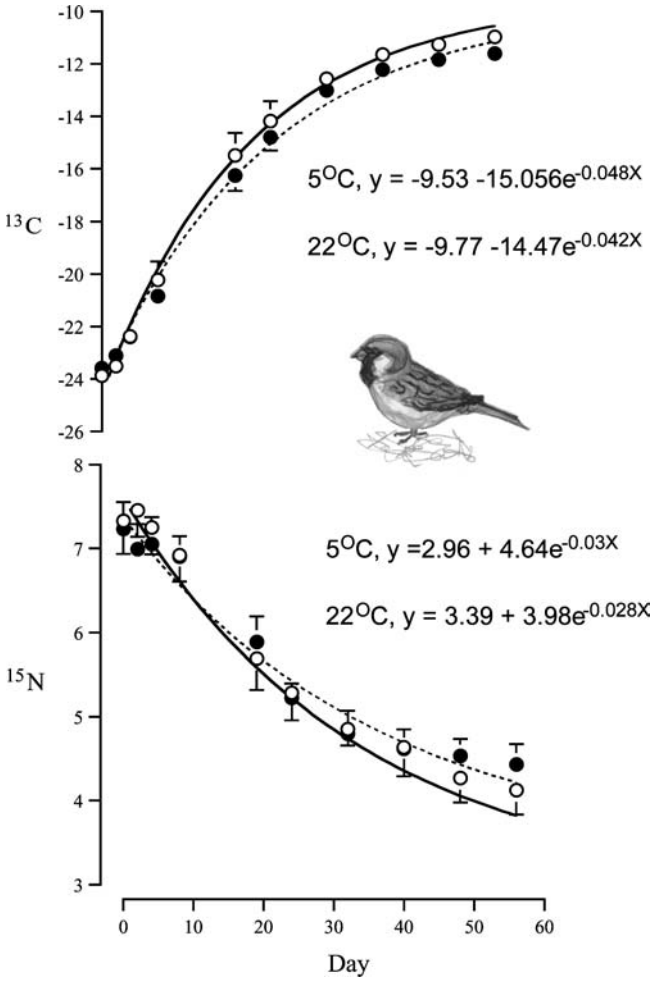

Fig. 2 The incorporation of ^{13}C and ^{15}N into red blood cells was well described by the model $\delta X(t) = \delta X(\infty) + (\delta X(0) - \delta X(\infty))e^{-kt}$. We estimated the value of $\delta X(\infty)$ and $(\delta X(0) - \delta X(\infty))$, where X is either ^{13}C or ^{15}N, for each individual bird and used the averages in Table 1 to draft the curves in this figure. These curves explained over 97% of the variation in the average isotopic composition observed ($r^2 > 0.97$). The isotopic incorporation of nitrogen was not significantly different between birds exposed to 5°C (*open points*) and 22°C (*closed points*, Table 1) and slightly significant for carbon. However, the isotopic incorporation rate was ≈1.5 times faster for carbon than for nitrogen (Table 1). Points are means ± SD

Discussion

We found that a cold-induced doubling in resting metabolism had no effect on the rate of ^{15}N incorporation into red blood cells and had a very small effect on the rate of ^{13}C incorporation. Most birds do not vary field metabolic rate more than twofold under field conditions (Dawson and O'Connor 1996). Thus, the change elicited by our experimental treatment is ecologically realistic. We also found that the fractional rate of carbon incorporation was higher than that of nitrogen. Finally, we found that in birds the fractional rate of carbon incorporation was allometrically related with body mass, with an exponent close to $-1/4$. Our first result casts doubt on the oft-cited assumption that claims a correlation between metabolic rate and the rate of isotopic incorporation into an animal's tissues. Here, we first consider why metabolic rate and the rate of isotopic incorporation into an animal's tissues can be uncoupled. Then, we propose a hypothesis to explain the differences in isotope incorporation between ^{13}C and ^{15}N. Finally, we discuss the possible causes and implications of an allometric relationship between the rate of elemental incorporation and body mass.

Does a cold-induced increase in metabolic rate elicit an increase in the rate of elemental incorporation into an animal's tissues?

Although temperature had no effect on the incorporation of N, some may quibble that it did have an effect on the k of carbon if we adopt $\alpha = 0.07$ as our level of significance. Furthermore, temperature did have a statistically significant effect on the half-life of ^{13}C. We

Table 1 Temperature had no significant effect on the fractional rate of ^{13}C and ^{15}N incorporation (k) into the red blood cells of house sparrows

	$\delta^{13}C$				$\delta^{15}N$			
	$\delta^{13}C$ (∞)	k	Half-life	$\Delta^{13}C_{2(\text{tissue-diet2})}$	$\delta^{15}N$ (∞)	k	Half-life	$\Delta^{15}N_{2(\text{tissue-diet2})}$
5°C	−9.53 ± 0.6	0.048 ± .009	14.74 ± 2.37	1.75 ± 0.59	2.96 ± 1.21	0.03 ± 0.009	23.36 ± 7.12	1.80 ± 0.65
22°C	−9.77 ± 0.39	0.042 ± 0.0046	16.53 ± 1.78	1.51 ± 0.39	3.4 ± 0.67	0.029 ± 0.012	24.55 ± 7.41	1.99 ± 0.47
t	1.447	1.916	2.19	1.22	0.572	0.573	0.409	0.47
p	0.615	0.0679	0.039	0.24	0.252	0.803	0.69	0.64

The discrimination factor between this tissue and diet for ^{13}C and ^{15}N was also independent of temperature. Note that the fractional rate of isotopic incorporation of ^{13}C was approximately 1.5 times higher than that of ^{15}N. Values are means ± SD for 13 individuals per temperature.

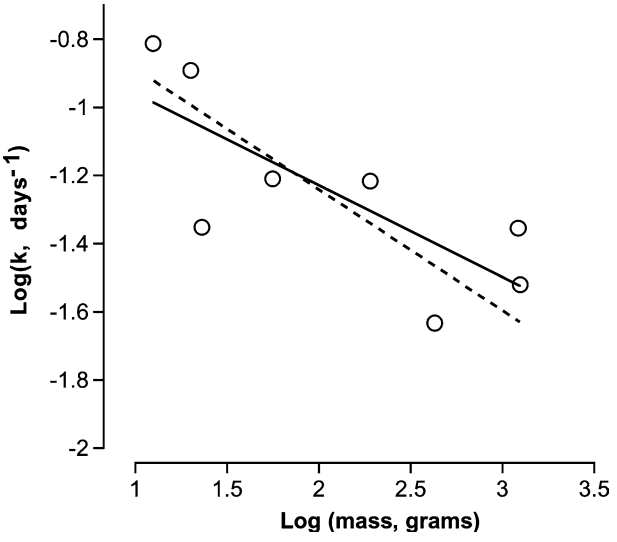

Fig. 3 The rate of ^{13}C incorporation (k) into avian blood depended allometrically on body mass. The *dashed line* ($\log(k) = -0.52 - 0.35 \log(m_b)$, $r^2 = 0.59$) represents the allometric relationship estimated by reduced major axis. The solid line represents that estimated by ordinary least squares ($\log(k) = -0.69 - 0.27 \log(m_b)$, $r^2 = 0.59$). Note that the slopes estimated by both of these values are similar to that predicted by allometric considerations (i.e. 0.25)

might then conclude that a cold-induced increase in metabolic rate has a significant effect on the rate of ^{13}C incorporation. The merit of this argument can be best evaluated by considering the magnitude of the effect of temperature on the k and $t_{1/2}$ of carbon. The effect of temperature on the k and $t_{1/2}$ of carbon were 0.006 day^{-1} (≈13%) and 1.8 days (≈11%), respectively. Although temperature had a significant effect on $t_{1/2}$, we do not consider this effect biologically significant. Given that our two experimental temperatures elicited a doubling in metabolic rate, we can conclude that a cold-induced increase in metabolic rate had no effect on ^{15}N incorporation and only a very small effect on the rate of ^{13}C incorporation into red blood cells. Metabolic rate and the rate of isotopic incorporation can be uncoupled. As the results described here, and Voigt et al.'s (2003) study on bats illustrates, high metabolic rate does not necessarily imply a high rate of C or N incorporation into an animal's tissues. The relationship between the rate of isotopic incorporation and metabolic rate is not as straightforward as the early isotopic incorporation studies envisioned and as more recent ones have tacitly assumed.

In the introduction we argued that the rate of isotopic incorporation should be correlated with the rate of protein turnover. Because protein turnover has been relatively well studied, we can use what is known about it to inform our understanding of isotopic incorporation. It is of significance (and a little ironic) that two decades ago it was fashionable to correlate metabolic rate with the rate of protein synthesis and degradation (Lobley 1988; Houlihan et al. 1995). A large number of correlative studies yielded two major conclusions: first, protein synthesis is often positively correlated with $\dot{V}_{O_2}$ in rapidly growing animals (e.g. Marsh et al. 2000) and can account for up to 60% of total respiratory energy expenditures in growing animals (Bayne and Hawkins

Table 2 Isotopic incorporation rates, half-lives for carbon reported in the literature for avian whole and red blood cells

Species		Mass (g)	Half-life	k	Reference
Yellow-rumped Warbler	*Dendroica coronata*	12.5	4.5	0.1540	Pearson et al. 2003
Garden Warbler	*Sylvia borin*	20	5.4	0.1284	Hobson and Bairlein 2003
House sparrow	*Passer domesticus*	23	15.6	0.0444	Current study
Dunlin	*Calidris alpina pacifica*	56	11.23	0.0617	Ogden et al. 2004
Japanese Quail	*Coturnix japonica*	190	11.4	0.0608	Hobson and Clark 1992
American Crow	*Corvus brachyrhynchos*	428	29.8	0.0233	Hobson and clark 1993
Great Skua	*Catharacta skua*	1218	15.7	0.0441	Bearhop et al. 2002
Canvasback	*Aythya valisineria*	1248	23	0.0301	Haramis et al. 2001

Data in this table were used to draft Fig. 3

Table 3 Slopes, intercepts, and r^2 of log-log regressions between body mass and fractional rate of ^{13}C incorporation into avian blood estimated by ordinary least squares (OLS) and reduced major axis (RMA)

Method	Slope ± SE	Intercept ± SE	r^2
OLS	-0.27 ± 0.09	-0.69 ± 0.20	0.58
RMA	-0.35 ± 0.09	-0.52 ± 0.20	0.59

The 95% confidence intervals of the slopes estimated by these two methods included the value expected from allometric considerations (−0.25)

1997). However, and second, the fraction of total respiratory energy expenditure that is accounted for by protein turnover in non-growing adults is much lower (≈20% of basal metabolic rate in endotherms, Lobley 2003), and its relative contribution to daily metabolic rate depends on the energy allocated to other functions (Welle and Nair 1990). The relationship between respiratory energy expenditures and protein turnover is obscured by other energy demands such as thermoregulation and activity (Waterlow 1968; Houlihan et al. 1995). Research on the relationship between metabolic rate and protein turnover holds an important lesson for studies on the factors that determine the rates of ^{13}C and ^{15}N incorporation into an animal's tissues (Ponsard and Averbuch 1999; Williams et al. 2001; Phillips and Koch 2002; Post 2002; Lobley et al. 2003; Pearson et al. 2003). We should expect the metabolic rate to be only weakly related to the rate of ^{13}C and ^{15}N incorporation in non-growing animals.

Why does the rate of carbon incorporation exceed that of nitrogen?

There are good reasons to expect differences in the incorporation rate of ^{13}C and ^{15}N. To understand these reasons, we must briefly consider the sources of C and N in protein. Dietary carbon can be incorporated into protein by two pathways: (1) from dietary amino acids, and (2) from the incorporation of dispensable amino acids synthesized from non-nitrogenated dietary sources (e.g. the modified carbon skeletons of carbohydrates). These carbon skeletons can be aminated with reused non-specific endogenous nitrogen (sensu Brody 1999). The fate of the N-containing amino group of oxidized amino acids is not only "waste" nitrogen (i.e. ammonia, urea, and/or uric acid). It can be used to synthesize dispensable ("non-essential") amino acids from carbon skeletons derived from non-protein dietary sources (Brody 1999, Hiramatzu et al. 1994). This is not the case for indispensable ("essential") amino acids (Reeds 2000). We hypothesize that the contribution of endogenous nitrogen to the synthesis of dispensable amino acids reduces the incorporation of dietary nitrogen into proteins relative to the incorporation of dietary carbon. Furthermore, we hypothesize that this effect is more marked when animals feed on protein-poor diets that favor nitrogen conservation such as seed-eating birds like house sparrows. These animals are more likely to reuse nitrogen from catabolized amino acids to synthesize dispensable amino acids than animals with higher nitrogen intakes such as carnivores.

This hypothesis is currently difficult to test because very few studies have measured the rate of ^{13}C and ^{15}N incorporation concurrently (Haramis et al. 2001; Bearhop et al. 2002; Pearson et al. 2003; Hobson and Bairlein 2003; and Evans-Ogden et al. 2004). All but one of these studies documented similar incorporation rates for carbon and nitrogen. The exception was Hobson and Bairlein's (2003) study on a fruit-eating bird (*Sylvia borin*). As predicted, Hobson and Bairlein (2003) found that the rate of carbon incorporation was higher (by a factor of 2, as was in this study) than that of nitrogen. It is worth noting that the disparity between the rate of ^{13}C- and ^{15}N-incorporation rates was found in a species in which nitrogen conservation should be expected (McWhorter et al. 2003).

The allometry of isotopic/elemental incorporation

As predicted, the rate of ^{13}C incorporation varied allometrically with body mass. In addition, the exponent of this relationship was close to that predicted by "quarter power" theories (Brown et al. 2000), and was consistent with the findings of previous studies on the scaling of protein turnover (Houlihan et al. 1995). This allometric relationship relating k and m_b is useful for two reasons: (1) It can be used to make educated guesses on the value of k (and hence $t_{1/2}$) from estimates of body mass, and (2) It can be used in comparative analyses. The variation in k probably has biological significance. McWhorter et al. (2003) have speculated that vertebrates with low nitrogen intake, such as fruit- and nectar- feeding birds and bats, have low rates of protein turnover. Thus, we hypothesize that the rates of ^{13}C and ^{15}N incorporation in these species must be low as well. Testing this comparative hypothesis requires a larger species sample and incorporating the dependence of k on body mass.

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