Establishing elemental turnover in exercising birds using a wind tunnel: implications for stable isotope tracking of migrants

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Abstract: Stable isotope measurements are being used increasingly to track migratory wildlife, especially birds. This approach relies on the assumption that tissue isotopic values represent a known period of dietary integration and that such a period is long enough to provide information on previous geographic origin. To date, such measurements have been obtained by switching isotopic composition of diets of sedentary captive individuals. The assumption has been that such measurements of elemental turnover likely represent minimal estimates, since wild migratory birds undergo increased metabolism and exercise during migratory flights. We tested this assumption using isotopic manipulation of diet on captive Rosy Starling (*Sturnus roseus* (L., 1758)) conditioned for flight in a wind tunnel. We used four control (no exercise) and four experimental (exercised) birds. For both groups, diet was switched from primarily a C-3 content to a C-4 content and blood samples were taken throughout our experiment until day 53. Contrary to expectation, $\delta^{13}\text{C}$ values in blood did not follow an exponential model of growth to a plateau under the new diet. Instead, the best fit was a linear increase in $\delta^{13}\text{C}$ value of the blood cellular fraction following the switch (day 15) until day 50, after which no further isotopic change was noted. We found no difference between experimental and control groups in the rate of carbon turnover. Our results support the contention that metabolic costs of migratory flight in conditioned birds may not result in increases in carbon elemental turnover in tissues and that previous estimates of tissue isotopic turnover based on captive, nonexercised birds may be applied to wild birds.

Résumé : Les mesures d'isotopes stables sont de plus en plus utilisées pour suivre les migrations de la faune sauvage, particulièrement celles des oiseaux. Cette méthodologie présuppose que les valeurs isotopiques des tissus représentent une période connue d'intégration alimentaire et que cette période est suffisamment longue pour fournir des renseignements sur l'origine géographique antérieure. À ce jour, de telles mesures s'obtiennent par la modification de la composition isotopique du régime alimentaire d'individus sédentaires captifs. On présuppose donc que de telles mesures de remplacement des éléments représentent vraisemblablement des estimations minimales, car les oiseaux sauvages migrateurs connaissent une augmentation du métabolisme et de l'exercice durant leurs vols de migration. Nous vérifions cette présupposition par manipulation isotopique du régime alimentaire chez des étourneaux roselins (*Sturnus roseus* (L., 1758)) en captivité, mais conditionnés pour le vol dans un tunnel aérodynamique. Nous avons utilisé quatre oiseaux témoins (sans exercice) et quatre oiseaux expérimentaux (soumis à l'exercice). Chez les deux groupes, nous avons modifié le régime alimentaire d'un contenu principalement de type C-3 à un contenu C-4 et nous avons prélevé des échantillons de sang au cours de l'expérience jusqu'au jour 53. Contrairement à nos attentes, sous le nouveau régime, les valeurs de $\delta^{13}\text{C}$ ne suivent pas un modèle exponentiel de croissance pour atteindre ensuite un plateau. Au contraire, le meilleur ajustement est celui d'une augmentation linéaire de la valeur de $\delta^{13}\text{C}$ dans la fraction cellulaire du sang après le changement de régime (jour 15) jusqu'au jour 50, après quoi on n'observe plus de variation isotopique. Il n'y a pas de différence de taux de remplacement du carbone entre le groupe expérimental et le groupe témoin. Nos résultats appuient l'assertion que les coûts du vol de migration chez des oiseaux conditionnés peuvent ne pas entraîner d'augmentation du remplacement de l'élément carbone dans les tissus et que les estimations antérieures du remplacement des isotopes dans les tissus déterminées chez des oiseaux captifs non soumis à l'exercice peuvent être appliquées à des oiseaux sauvages.

Introduction

Important advances have been made over the last decade in the application of stable isotope analyses of consumer tissues to investigate dietary or trophic history and to track mi-

gratory organisms (Hobson 1999; Kelly 2000; Rubenstein and Hobson 2004). A fundamental assumption of these types of applications is that the target organism retains an isotopic signal for an appropriate temporal window of interest. For example, if an animal equilibrates with a food web at one

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location and then leaves that location and is captured as it arrives in a new isotopic landscape or “isoscape”, then the tissue of interest should have an elemental turnover rate that is slow enough to retain enough of the original location to be detectable (Hobson 2005). Marra et al. (1998) used this approach to link arrival date of American Redstarts (*Setophaga ruticilla* (L., 1758)) at their breeding locations in New Hampshire, USA, with the kind of habitats they occupied on the wintering grounds. That study assumed that these birds still retained an isotopic signal in their muscle tissue from the wintering grounds, potentially in the Caribbean, where birds may have departed as much as 2 weeks prior to capture on their breeding grounds.

To date, researchers have had to rely on the results of studies of captive animals that switch the isotopic signature of diets and monitor the uptake of the new dietary signal into various tissues (e.g., Tieszen et al. 1983; Hobson and Clark 1992a, 1992b, 1993; Pearson et al. 2003; Podlesak and McWilliams 2006). This approach has the drawback that turnover rates are usually based on sedentary, nonexercised individuals in captivity and have been considered an underestimate of the actual turnover rate to be expected in wild animals. This is especially the case for those undergoing the metabolic stress of extended exercises such as migration and long-distance movements. As suggested by Hobson (1999, 2005), one optimal way in which elemental turnover rates might be estimated for migrating birds is through the use of a wind tunnel. By switching the isotopic composition of the diet of birds trained to use a wind tunnel, elemental turnover rates comparable with those expected in actively migrating individuals can be estimated. We adopted this approach using a captive flock of Rosy Starling (*Sturnus roseus* (L., 1758)), a long-distance migratory species that breeds mainly east of the Black Sea and that travels south-east from their breeding range to spend the winter in northern India. Our objectives were to compare turnover rates of carbon in cellular fractions of blood of exercised and nonexercised individuals. We expected exercised birds to show faster elemental turnover in their blood compared with nonexercised birds.

Methods

Wind tunnel and diet switch

The experiments were conducted by E.Y. using the wind tunnel facilities of the Max Planck Institute for Ornithology in Seewiesen, Germany. From a flock of Rosy Starlings, which were hand-raised from the nestling stage in captivity and trained to fly in the wind tunnel, eight starlings were randomly selected. To control for the potential influence of sex, age, and moult on isotopic turnover rate, we used only male birds >1 year of age that had completed moult.

Prior to our experiment, starlings were held on a common standard C-3 diet for at least 2 years. This diet consisted of a mixture of eggs, dairy products, and a commercial cereal-based product supplemented with insects, soy oil, cattle heart, crustaceans, minerals, and vitamins (recipe available from the authors). The diverse nature of the diet precluded derivation of a mean $\delta^{13}\text{C}$ value. We assumed this diet labeled body tissues, particularly blood cells, of all birds with uniform isotopic values that reflect their diet. This was fur-

ther confirmed by measuring the blood isotopic signature of all birds 2 weeks prior to the switch to the C-4 diet and on the day of the diet switch. For wind tunnel experiments, not all birds respond immediately to experimental conditions by flying; prior to this experiment, birds were not exposed to flight in the wind tunnel for more than a year. So, 4 weeks before the diet switch, all birds were allowed to fly in the wind tunnel for at least 10–15 min-day⁻¹ at flight speeds of 8–14 m·s⁻¹. Thus, this short flight training prior to the diet switch was performed to expose the experimental groups to flight in the wind tunnel. To keep both groups to a similar condition before the start of the experiment (and without incorporating any ecological or physiological effect), the nonexercised groups were also exposed to short flight trainings in the wind tunnel. After this period, starlings were switched to a diet containing C-4 based corn seed, flakes, and flours homogenized in a gelatin matrix (recipe available from the authors: mean $\delta^{13}\text{C}$ = $-13.5\text{‰} \pm 1.7\text{‰}$, mean C:N = 6.1), and were supplied with vitamins and minerals. In addition, each day we provided each bird with 25 mealworms grown on a corn flour substrate (recipe available from the authors: mean $\delta^{13}\text{C}$ = $-15.0\text{‰} \pm 1.0\text{‰}$, mean C:N = 4.8). Both groups were fed with this diet for a total of 45 days. Thus, experimental and control groups were treated identically except for the provision of exercise to the experimental group.

We randomly assigned individual birds to two groups of four individuals each. Exercised birds (experimental group) were switched to the ¹³C-enriched C-4 diet described above and were allowed to fly daily in the wind tunnel for several hours (see below). Exercised birds were housed in individual aviaries (ca. 1 m × 2 m × 2 m) next to the wind tunnel. Unlike the boxes for the control groups, the aviaries were supplied with perches and food was kept on the floor of the aviaries. Birds could fly between the perch and the food on the floor to get food. The closed circuit of the wind tunnel has a flight chamber 2 m × 1.2 m. The air speed was set at 11 m·s⁻¹ with an accuracy of 0.1 m·s⁻¹. Air speeds were recorded with air density variation (that determines wing and body force of the birds; Pennycuik et al. 1997) taken into consideration. Nonexercised birds (control group) were switched to the same ¹³C-enriched diet as the experimental group but were kept in individual boxes in the institute and never allowed to fly after the diet switch to mimic previous studies examining elemental turnover rates in blood using stable isotopic tracers.

Changing environmental conditions (moving birds from aviaries to boxes) and a sudden diet switch could cause a stressful condition in birds and influence the tissue elemental turnover rate. Thus, control birds were transferred to boxes 3 days prior to the diet switch, while the experimental groups continued to fly in the wind tunnel. To get the gross food intake over a day, remaining food in cages was weighed using an electronic balance (to nearest 0.01 g) and subtracted from that given the previous day. For both groups, the light cycle was maintained according to their natural photoperiod condition in the wild. Aviaries and box temperatures were kept at approximately 17–21 °C, while the average temperature in the wind tunnel was 14.6 °C.

Body mass and avian samples

Prior to taking blood samples, birds were weighed to the

nearest 0.01 g using an electronic balance. Blood sampling was conducted 2 weeks before the diet switch, on the day of the diet switch (day 15) before feeding, and then, on average, every 3.7 days throughout our experiment until day 53. Approximately 150 μL of blood was sampled from all birds via puncture of the brachial vein. Blood samples were centrifuged to separate the plasma, stored at -25°C , and later freeze-dried. We were interested in turnover rates in the cellular fraction of blood, since plasma responds rapidly to a diet switch (Hobson and Clark 1992a) and the slower cellular fraction was considered most useful for migratory bird applications where researchers require information on time periods extending to several weeks. The experiments were conducted following the guidelines and official permits of animal care for the Regierung von Oberbayern (permit license No. 209.1/211-2531.2-14/05), Germany.

Stable isotope analysis

Isotopic analyses were performed at the Department of Soil Science, University of Saskatchewan, Saskatoon. The procedures followed those described by Hobson and Bairlein (2003). Assays were performed using a 1 mg homogenized tissue material combusted at 1200°C in a Robo-Prep elemental analyzer and were analysed using a Europa 20:20 continuous flow isotope ratio mass spectrometer. A measurement precision (SD) of $\pm 0.1\text{‰}$ was estimated using replicate analyses of an egg albumen standard run during batches of unknowns.

Statistical analysis

For each bird, the (cellular) blood isotope data uptake curves were generated using SigmaPlot[®] version 10 (Systat Software Inc. 2002) and the mean turnover rates were calculated. Statistical models that best described isotopic patterns of change were tested. All statistical tests were performed with SPSS[®] version 12.0.1 (SPSS Inc. 2004).

Results

Turnover rates

Cellular blood isotopic signatures for all birds 2 weeks before the diet switch were not different than those at the time of the diet switch (paired t test: $t = -0.17$, $P = 0.87$; bird 7 not available for day 0). Thus, blood tissue $\delta^{13}\text{C}$ values were in equilibrium with dietary values at the start. Consistent with the diet switch, blood $\delta^{13}\text{C}$ values shifted towards more enriched values over the course of the experiment (Fig. 1). Based on previous isotopic dietary shift experiments to determine tissue elemental turnover rates, we expected uptake curves to approximate an exponential model (e.g., Evans-Ogden et al. 2004; Hobson and Bairlein 2003). We examined each response curve and determined the best curve fit using SigmaPlot[®] version 10 (Systat Software Inc. 2002). However, with the exception of bird 6 (nonexercised) that showed a sigmoid response ($r^2 = 0.85$ vs. 0.70), the best fit for all birds corresponded to a simple linear regression (Fig. 1) between day of the diet switch (day 15) and day 50. Excluding bird 6, curves had r^2 values from 0.87 (bird 7) to 0.99 (bird 1). Therefore, the best fit for the model conformed to the equation $f(t) = m \cdot t + b$, where m is the change in the tissue isotope (tissue fractional turnover

rate) associated with the change in the diet, b is the initial tissue isotopic value predicted by the equation, and t is the time since the diet switch. There was no significant difference between rate of turnover (m) for control ($0.193\text{‰} \cdot \text{day}^{-1}$ (mean $\pm$ SD), $n = 4$) and experimental ($0.204\text{‰} \cdot \text{day}^{-1} \pm 0.01\text{‰} \cdot \text{day}^{-1}$, $n = 4$) birds by inspection of Fig. 1 or by a Mann–Whitney U test ($U = 5.5$, $P = 0.48$).

Flight, food, and body mass

At the end of the experiment, on an individual basis birds 1, 2, 3, and 4 of the exercised groups had covered a total distance of 1305, 1297, 1030, and 865 km, respectively. We were unable to maintain experimental birds at high levels of exercise; however, on average, birds 1, 2, 3, and 4 had been exercised for 67.01 , 56.09 , 46.5 , and $35.4 \text{ min} \cdot \text{day}^{-1}$ for 34 days. Body masses of individuals at the start and end of the experiment were significantly different (start: $67.3 \pm 2.10 \text{ g}$, $n = 8$; end: $78.5 \pm 3.32 \text{ g}$, $n = 8$; Wilcoxon's signed ranks: $Z = -2.380$, $P = 0.02$). In both groups, mean body mass increased significantly during the course of the experiment. Exercised birds gained, on average, 15.24% (range $6.81\%–33.06\%$) of their initial body mass during the course of the experiment, while nonexercised birds increased, on average, 5.57% (range $0.30\%–5.30\%$). However, the increase in body mass recorded in the exercised birds was not significantly different than those recorded for the nonexercised birds (Student t test: $t = 1.701$, $\text{df} = 6$, $P = 0.14$). Exercised birds consumed $19.01 \pm 0.58 \text{ g}$ of the processed food per day, while nonexercised birds consumed $16.47 \pm 0.43 \text{ g}$ of the food. Both groups had, on average, a maximum daily food intake of 27 g .

Discussion

Several studies have demonstrated that tissue stable isotope turnover rates are related to tissue metabolic rate (e.g., Tieszen et al. 1983; Hobson and Clark 1992a; Pearson et al. 2003). However, direct quantification of the extent to which metabolic rate may influence blood tissue elemental turnover rate is difficult. At least to the degree of exercise attained here, our results support the contention that metabolic costs of exercise in conditioned birds may be small enough to not result in increased elemental turnover differences in blood cells.

Our study simulated the situation of daily migratory flights punctuated by stopover where birds were able to feed and drink. Although the duration of flight was likely less than that typically experienced by migrating birds, the level of exercise was substantial compared with those experienced by the controls. Based on allometric equations, the basal metabolic energy of our Rosy Starlings, with an average body mass of 68 g , was between 0.5 and $0.7 \text{ W} \cdot \text{day}^{-1}$ (Gavrilov and Donlik 1985; McKechnie and Wolf 2004; Engel 2005). Using metabolic chambers and doubly labelled water in thermoneutral conditions, Engel (2005) estimated an average resting metabolic rate and wind-tunnel flight cost for Rosy Starlings of 1.4 and 8.2 W , respectively, which is considerably greater than the basal metabolic energy calculated above. In a similar experiment to ours on Rosy Starlings that flew about 3 h daily, C.A. Schmidt-Wellenburg (unpublished data) determined an

Fig. 1. Patterns of change in the stable carbon isotope ratios in blood of experimental (exercised) and control (nonexercised) Rosy Starlings (*Sturnus roseus*) following a diet switch on day 15.

average daily energy expenditure of 55% more than that of nonexercised birds. The flight duration in our birds varied between 0.5 and 3 h·day⁻¹. Thus, while we did not examine energetic budgets in our birds, we expect energetic consequences of the exercise performed to be substantial. That we did not see a commensurate increase in elemental turnover rate in blood suggests either moderate exercise associated with our simulated migratory flights were low or the consequences of migration may be overrated in terms of actual cell turnover.

During sustained flight, birds fuel their energy expenditures primarily through mobilization of fat stores and typically only small amounts of protein are involved (Jenni and Jenni-Eiermann 1998). Under such conditions, we expect the isotopic consequences of migratory flight to be less severe (i.e., isotopic signals in blood would represent source signatures for longer periods than if flight was fueled by proteins). However, in wild birds, there are cases where significant amounts of muscle protein are catabolized during long-distance endurance flight and periods of fasting (Lindstrom et al. 2000; Bauchinger and Biebach 2001). Such extreme conditions are mimicked by exercising birds for a longer duration. An unexpected result from our study was the linear uptake of the new dietary signal, since previous studies on captive birds conformed more to exponential models. We have no clear explanation for this result but note that it is similar to the results found by Pearson et al. (2003) in their study of captive Yellow-rumped Warbler (*Dendroica coronata* (L., 1766)) and Voigt et al. (2003) in their study of bats. Our birds were conditioned to spring photoperiod and individuals started singing during the experiment. It is possible that such pre-migratory conditions lead to physiological changes that correspond with slower assimilation of nutrients but rapid accumulation of nutrients in preparation for migration. Birds gained mass on their new diets and exercise regimes and it is possible that those mass increases influenced the nature of the uptake curves. However, isotopic uptake curves accounting for individual growth are also expected to show an exponential change following a diet switch (MacAvoy et al. 2005).

While the C4 diet was not as diverse as the control C3 diet, it was nutritious and both exercised and nonexercised birds gained mass following the diet switch. So, we do not think that the composition of the new diet necessarily influenced the shape of the blood $\delta^{13}\text{C}$ uptake curve. However, if dietary assimilation changed between control and experimental diet, with poorer assimilation occurring following the diet switch, then this could have resulted in a slower (i.e., linear) uptake curve. We have some anecdotal evidence to support this contention. We measured the C:N ratio of diet and fecal material before and after the diet switch. Whereas the C:N ratio of the two diets were similar, the C:N value of the fecal material of all birds except bird 6 increased following the diet switch. This suggests poorer assimilation of carbon following the diet switch. Bird 6 showed the opposite effect and was the only bird showing a more exponential uptake curve pattern. Similarly, if carbon liberated from body fat or muscle became available for blood cell synthesis, then this might slow the apparent uptake rate following the diet switch. Whatever the explana-

tion for the linear nature of the uptake curves, the important finding of our study was the lack of a difference in the rate of elemental turnover between exercised and nonexercised birds. However, we suggest the shape of the uptake curve in diet-switch experiments, especially of those involving birds undergoing pre-migratory physiological adjustments, requires further study.

Our findings suggest isotopic measurement of blood in wild, migratory species can be used to investigate previous isoscapes used by individuals and previous findings for captive, unexercised birds yield a reasonable approximation to this temporal window of integration, at least for whole blood (reviewed by Evans-Ogden et al. 2004). In the case of our starlings, this period was at least up to about 25 days. However, future studies should attempt to better approximate real migration by exercising birds for longer periods (flights of more than 6 h·day⁻¹ are required to approximate endurance flight) and mimic stopover scenarios that involve periods of fasting and hyperphagia. Nonetheless, as shown by Nagy (1987), metabolic rates of captive animals could vary substantially from those of free-living animals. Caution should be taken when considering relationships and rates determined from captive studies, as they may not accurately and precisely be applicable for animals in the field.

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