Letters

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Extent of phenotypic flexibility during long-distance flight is determined by tissue-specific turnover rates: a new hypothesis

Ulf Bauchinger and Scott R. McWilliams

U. Bauchinger (ulf@etal.uri.edu), Dept Biologie II, Ludwigs-Maximilians-Universität München, Großhaderner Str. 2, 82152 Planegg-Martinsried, Germany. – S. R. McWilliams, Program in Wildlife and Conservation Biology, Dept Natural Resources Science, 105 Coastal Institute in Kingston, University of Rhode Island, Kingston, RI 02881, USA.

Phenotypic flexibility in organ size of migratory birds is typically explained in functional terms in accordance with the principal of economic design. However, proposed functional hypotheses do not adequately explain differences in phenotypic flexibility between organs during fasting and in-flight starvation. We show that the extent of phenotypic flexibility in organ mass in five species of migratory birds during actual migration or simulated in-flight starvation consistently ranked as follows from highest to lowest mass change: small intestine, liver, kidney, gizzard, heart, flight and leg muscle. This pattern of phenotypic flexibility in organ mass was not consistent with proposed functional hypotheses, and was almost completely explained by differences in tissue-specific turnover rate measured in vivo using nutrients differing in their isotopic values. Thus, the fundamental process of tissue-specific protein turnover determines extent of organ mass changes for birds during migration, this likely applies to other organisms during fasting, and no further functional explanation(s) for differences in the magnitude of phenotypic flexibility between organs is required.

Migratory birds evolved an adaptive suite of features to accomplish long-distance flights including extensive phenotypic flexibility in key components of the metabolic machinery required for efficient endurance exercise (Karasov 1996, Kersten and Visser 1996, Pigliucci 1996, Piersma and Lindström 1997, Piersma 2002, McWilliams and Karasov 2005). The ability of birds to rapidly and reversibly change the size of key organs (e.g., flight muscle, liver, intestine, heart) during flights and at stopovers between flights provides a classic example of the principle of economical design: the capacity of a physiological system is matched to the prevailing demand but can be modulated in response to changes in demand (Diamond and Hammond 1992, Hammond and Diamond 1997). Rapid reversible changes in physiological systems such as the digestive system provide examples of flexible norms of reaction (Stearns 1989, Travis 1994) or phenotypic flexibility in that they enable individuals to respond flexibly to changes in the environment (Piersma and Lindström 1997, Piersma 2002, Piersma and Drent 2003, Battley and Piersma 2005).

An especially remarkable aspect of this phenotypic flexibility is that organs within an individual differ quite dramatically in the extent of observed size change. Several functional hypotheses have been invoked to explain these intra-individual differences in extent of phenotypic flexibility between organs (for review see Piersma and Lindström 1997, Bauchinger and Biebach 1998, Jenni and Jenni-Eiermann 1998). For example, the use-hypertrophy/disuse-atrophy hypothesis proposes that digestive organs consistently decrease in size to a greater extent during fasting than other organs (e.g., heart, pectoral muscle) because they are expensive to maintain and are not used during fasting. Here we propose and test a novel, alternative hypothesis: tissue-specific turnover of protein determines extent of phenotypic mass changes in birds during migration. Unlike the use-disuse hypothesis and all other proposed functional hypotheses to date, the protein turnover hypothesis predicts that organs will differ in extent of phenotypic flexibility and that these differences between organs will not depend on environment (i.e., specific circumstances of use and disuse). We test this alternative hypothesis using measures of protein turnover in fasted birds.

Migration, hibernation, and survival during inclement conditions often require prolonged fasting, when animals must rely entirely on body stores to survive and when energy and nutrient metabolism must be minimized to maximally conserve limited stored resources. Our focus on the fasted condition allows us to estimate tissue-specific rate of protein catabolism while de novo protein synthesis is suspended. Vertebrate metabolism in the steady-state fed condition differs from that when fasting because rate of tissue synthesis and catabolism are balanced whilst feeding, whereas during fasting, tissue synthesis is negligible while tissue catabolism continues at relatively similar rates (Swick and Benevenga 1976, Waterlow 1999). The implication is that studies of fasted wild animals can reveal fundamental

differences in protein turnover of tissues that we can then relate to extent of phenotypic flexibility in these same organs. We evaluated the protein turnover hypothesis by analyzing the available data on extent of organ mass reduction in migrating birds during fasting or long-distance flight, and then relating this pattern of organ mass reduction to rate of protein turnover of the same organs as measured using stable isotope incorporation into tissues.

Data

We compiled data on extent of tissue reduction in migratory birds from the literature that either included mass loss from before to after long flights in the wild, or after simulated migration by experimental fasting (Appendix 1). Currently available data for carbon turnover of tissues in birds are summarized in the lower part of the Appendix table. The rate of ^{13}C isotope incorporation (k) is presented for the Japanese quail *Coturnix japonica* (Hobson and Clark 1992), and for two passerine bird species, the house sparrow *Passer domesticus* (Carleton et al. 2008) and the zebra finch *Poephila guttata* (Bauchinger and McWilliams 2009). We correlate the ratio of the isotopic incorporation rate for each of the seven tissues (small intestine, liver, kidney, gizzard, heart, flight muscle and leg muscle) to that of the liver with the ratio of organ mass reduction of each of the seven tissues (small intestine, liver, kidney, gizzard, heart, flight muscle and leg muscle) to that of the liver (Fig. 1B).

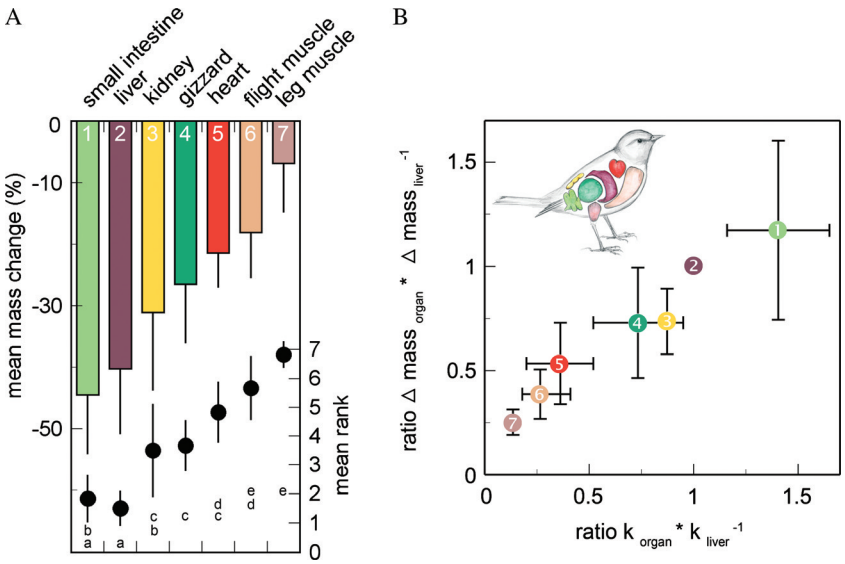

Figure 1. (A) Extent of mass loss and relative rank order of reduction for seven organs from five species of migratory birds, and (B) Correlation between tissue-specific carbon turnover and tissue-specific extent of mass reduction for seven organs from five species of migratory birds. (A) Mean percent mass change for each organ (bars) and mean rank order of reduction for each organ (circles) for five migratory bird species. Wild-caught birds had fasted during a long-distance flight whereas captive birds were fasted for periods as long as a migratory flight. Error bars give 95% CI. Nine published studies on five bird species report organ mass change in association with fasting during actual or simulated migratory flights for great knot *Calidris tenuirostris* (Battley et al. 2000, Battley et al. 2001), blackcaps *Sylvia atricapilla* (Karasov and Pinshow 1998, Karasov et al. 2001), garden warblers *Sylvia borin* (Hume and Biebach 1996, Schwilch et al. 2002, Bauchinger et al. 2005), pied flycatcher *Ficedula hypoleuca* (Schwilch et al. 2002) and willow warbler *Phyloscopus trochilus* (Schwilch et al. 2002; see also Appendix 1). Data sets with at least five different tissues were selected and the percentage of organ reduction to an initial mean organ mass (= 100%) was calculated for each tissue. The mean percentage mass reduction for each organ is presented in the bar graph of Fig. 1A ordered from highest to lowest mass change. Seven data sets on tissue reduction provided data to calculate the rank of organ reduction between seven tissues (see Appendix 1) from the tissues reduced the most (rank 1) to the tissue reduced the least (rank 7). The respective mean rank showed significant differences between the seven tissues and is presented with its 95% confidence interval in the lower part of the figure (ANOVA, $F_{6,35} = 26.5$, $p < 0.001$). Organs that do not share common letters are significantly different to each other based on post-hoc comparisons (Tukey HSD, $p < 0.05$). (B) Correlation between tissue-specific rate of carbon turnover and tissue-specific mass change for seven tissues. The ratios reported on each axis are calculated for each species and study (5 species for organ-specific mass changes and 3 species for organ-specific carbon turnover) and reveal that the extent to which a specific organ is reduced in mass compared to liver (y-axis) was related to the relative rate of carbon turnover (x-axis) (see Appendix 1for data used). For carbon turnover, k is the organ-specific fractional carbon turnover. Vertical error bars are 95% confidence intervals whereas horizontal error bars are the range. Use of ratios in this case is justified because both organ mass and carbon turnover are allometrically related to body mass (Carleton and Martinez del Rio. 2005, Bauchinger and McWilliams 2009). Colours for the organs refer to that of the sketched bird and organs in figure part (A). 1 = small intestine, 2 = liver, 3 = kidney, 4 = gizzard, 5 = heart, 6 = flight muscle and 7 = leg muscle. Data derived from truly migrating birds ($n_{studies} = 6$) together with those of birds subjected to simulated migration in the laboratory ($n_{studies} = 4$) show a highly significant correlation ($n_{tissues} = 7$, $r_p = 0.98$, $p < 0.001$). This correlation persists when analyzing only birds actively migrating ($n_{tissues} = 7$, $r_p = 0.91$, $p = 0.005$), or only fasted birds in the laboratory ($n_{tissues} = 7$, $r_p = 0.98$, $p < 0.001$), suggesting that the relative change between tissues is independent of migratory exercise performance. Calculation of tissue mass loss for birds during natural migration was entirely based on lean dry tissues, whereas that for birds during simulated migration in the laboratory was based on mass loss of lean wet, dry or wet whole tissue (see Appendix 1).

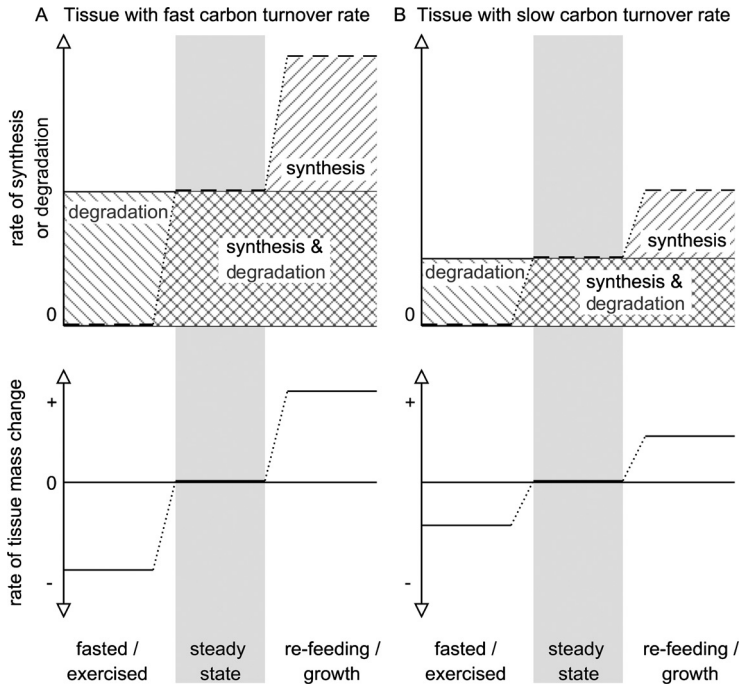

Figure 2. Schematic illustration of the processes of tissue synthesis and breakdown, and for the rate of tissue mass change under various conditions. Conceptual model of the two processes controlling tissue turnover, synthesis and degradation, shown for three different physiological states and the respective effect on tissue mass change for a hypothetical tissue with fast turnover rate (A), and for a hypothetical tissue with slow turnover rate (B). Grey areas depict the steady state during which no tissue mass change occurs and synthesis equals degradation. Measurements of carbon turnover typically are performed under such maintenance conditions. During fasting as, for example, during in-flight starvation, de novo synthesis of protein can not occur. The rate of degradation and a hypothetical portion of specific tissue synthesis due to resource allocation from other tissues (upper panels) define the rate of tissue mass loss over time (lower panels). Resource reallocation during starvation may affect tissue isotope value although this seems limited in our study. During resumption of feeding after fasting or during growth, the higher rate of tissue synthesis determines, in concert with the rate of degradation (upper panels), the rate of tissue mass gain (lower panels). Dotted lines between the stages indicate that the time frame of the transition between stages is not known. The order of the stages is hypothetical. Whether or not tissue degradation continues unchanged during the three stages is not known, but amplitude of tissue syntheses is considered to be much greater than that of degradation (Swick and Benevenga 1976, Waterlow 1999).

Results and Discussion

We found that the extent of organ mass changes for long-distance migratory birds differed by organ, from at least 40% in the liver and small intestine to less than 20% for skeletal muscles with intermediate values for kidney, gizzard and heart (Fig. 1A). Ranking the organ reduction in different species from literature studies revealed a common pattern across migratory bird species ($p < 0.001$, Fig. 1A) suggesting an underlying common mechanism. Previously proposed functional hypotheses for why protein tissue is catabolized during migratory flight (Piersma and Lindström 1997, Bauchinger and Biebach 1998, Jenni and Jenni-Eiermann 1998) do not offer adequate explanations for more pronounced reductions in some organs than others or why the order of changes were consistent across species and studies. For example, the use-hypertrophy/disuse atrophy hypothesis (Alexander and Goldspink 1977) predicts that mass of organs associated with exercise (e.g. pectoral muscle) should remain constant or increase amongst actively migrating birds but not amongst birds during a simulated migration (fasted but not flying, see as well Portugal et al. 2009). Organs associated with digestion (e.g., small intestine, gizzard) should similarly decrease in mass amongst fasted birds. Organ size is hypothesized to be positively related to body mass as shown for flight muscle in a wader species during various experimental manipulations including flight (Lindstöm et al. 2000, see as well Dietz et al. 2007). However, organ size was not consistently positively associated with body mass for a passerine sampled during various stages of migration (Bauchinger and Biebach 2005). The protein-pool hypothesis predicts that all tissue protein is catabolized at similar rates to create a protein pool to act as a resource for repair mechanisms (Piersma 1990), water (Klaassen 1996), antioxidants (Klaassen et al. 2000), intermediates for fatty acid breakdown (Jenni-Eiermann and Jenni 1991), or precursors for gluconeogenesis (Jenni-Eiermann and Jenni 1991). None of these predictions match the observed pattern of tissue reduction (Fig. 1A).

Recent advances in stable isotope studies enable in vivo measures of rate of isotopic incorporation of carbon into the same organs that varied in their extent of phenotypic flexibility during fasting. Carbon incorporation was most rapid in small intestine and liver, intermediate in kidney, gizzard and heart and slowest in flight and leg muscle (Appendix; Hobson and Clark 1992, Carleton et al. 2008, Bauchinger and McWilliams 2009, Bauchinger et al. 2010). Given that fat was removed from tissues prior to stable isotope analysis (Post et al. 2007), and carbohydrates are rare in avian tissues (Marsh 1984), carbon turnover of

tissues as measured using stable isotopes provides an accurate indirect measure of protein turnover (Carleton and Martinez del Rio 2005, Bauchinger and McWilliams 2009, Martinez del Rio et al. 2009, Wolf et al. 2009). The highly significant correlation between extent of phenotypic tissue mass loss and protein turnover rate for small intestine, liver, gizzard, kidney, heart, skeletal muscle and leg muscle explained more than 95% of the variation (Fig. 1B) and persisted with or without birds that were actively migrating or birds that were fasted for periods as long as a migratory flight. Thus, when metabolism is primarily catabolic (as during fasting or sustained exercise) the restrictions on protein turnover determine the relative mass loss of organs.

The strikingly similar pattern of organ mass changes and rate of tissue-specific turnover rate within migratory species (Fig. 1B) suggests a common physiological basis. Synthesis and degradation of tissue is a continuous process assuring the maintenance of the respective tissue. Under maintenance conditions, synthesis equals degradation (Fig. 2) and the measured carbon turnover (Carleton et al. 2005, Bauchinger and McWilliams 2009) must be a product of both processes (Martinez del Rio et al. 2009, Wolf et al. 2009). During migratory flight, birds do not eat for many hours or even days so that de novo tissue synthesis stops, and any new requirements must be satisfied by resource reallocation. We propose that tissue-specific rate of degradation, which is relatively invariant to food intake, determines the pace of organ mass reduction in migrating birds, and that tissue-specific rate of carbon turnover accurately estimates tissue-specific rate of degradation when birds are fasting. Therefore, between-tissue differences in protein turnover rate explain the magnitude of phenotypic organ mass changes observed in migratory birds and require no further functional explanation.

Our focus on protein turnover in fasted birds made it possible to isolate the process of protein degradation and reveal its relationship to extent of phenotypic flexibility. These results have several implications. Fasting is common among vertebrates, some of which endure prolonged periods without feeding and suffer substantial body and tissue mass loss, e.g. penguins during incubation and chick-rearing, sit-and-wait foraging animals like pythons, or hibernating mammals (Secor and Diamond 1998, Cherel and Groscolas 1999, Hume et al. 2002). The predicted link between tissue turnover rate and phenotypic mass changes during fasting likely applies in all these cases, although tissue-specific turnover rates are not yet known for heterothermic animals or in animals during torpor and hibernation.

We suggest that this mechanistic link between tissue turnover rate and phenotypic mass changes also applies to anabolic situations in which animals require rebuilding of tissue at maximum rate, such as during migratory stopover. If synthesis of all tissues is higher compared to degradation by a fixed percentage, then tissue specific turnover rates define the maximum rate of mass gain per tissue and thus, for example, may set the minimum time a bird must spend at stopover sites during migration. In the anabolic state tissue-specific turnover rates and more specifically the difference between tissue synthesis and tissue degradation (see Figure 1, re-feeding or growth condition) define the maximum rate of mass gain for each tissue. Increase in tissue mass due to diet shifts and change in size of prey items (Dekinga et al. 2001, van Gils et al. 2006), stopover (Piersma 1998, Piersma et al. 1999, Landys-Ciannelli et al. 2003, Bauchinger et al. 2005), or following increased perceived predation risk (van den Hout et al. 2006) may thus only occur at a tissue-specific maximum rate or slower. Two obvious differences between the catabolic state of in-flight starvation and the anabolic state of, for example, stopover exemplify the added complexity in demonstrating the link between tissue turnover and phenotypic mass gain: (1) compared to catabolic situations where tissue mass decreases, in the anabolic state the increase in tissue mass will arrest once a suitable and context-specific organ size is reached, while other tissues may still continue to re-grow (see for example the temporal mass changes in birds during staging, Piersma et al. 1999, Jehl 1997); (2) extent of mass increase will be strongly associated with tissue-specific demands which can vary with environmental conditions, whereas during catabolism the decrease seems strictly related to the fundamental process of turnover and less variable in extent. This variation in extent of mass change during catabolism is especially evident in spring-staging grebes which decrease flight muscle mass while other tissues increase during re-fuelling (Gaunt et al. 1990, Jehl 1997).

In conclusion, rapid and reversible changes in organ size are consistent with the principle of economic design and enable animals to flexibly and efficiently respond to environmental change. We have shown that the magnitude of organ flexibility among several species of migratory birds is directly related to tissue-specific protein turnover. This functional link between extent of phenotypic flexibility and protein turnover rate suggests that the evolution of rapid protein turnover of certain organs is a fundamental part of the adaptive suite of features associated with migration and likely other mostly catabolic conditions.

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Appendix 1. Table provides data used in Figure 1A and B

Mass reduction (%) of tissues in birds during active migration (in-flight starvation) or during simulated migration (fasted in laboratory), and the rate of carbon incorporation (k, in $days^{-1}$) into certain organs or tissues in birds. We included only those data sets with measurements of mass change for at least three different tissues. All available data for isotopic incorporation in birds are shown. Graphs in Figure 1 comprise all available data listed, apart from the data for the non-migratory barn owl. Data points for rank order in Figure 1A are based only on citations for which data of all seven tissues (small intestine, liver, gizzard, kidney, heart, flight and leg muscle) are available and aerial feeding can be excluded (great knot; Battley et al. 2000, Battley et al. 2001; garden warbler Hume and Biebach 1996, Schwilch et al. 2002, Bauchinger et al. 2005); pied flycatcher (Schwilch et al. 2002) and willow warbler (Schwilch et al. 2002)).

	Small intestine	Liver	Gizzard	Kidney	Heart	Flight muscle	Leg muscle	Sampling	Ref.
Percent reduction during natural migration									
Calidris tenuirostris	64	60	61	56	23	24	14	before and after a flight of 5400 km across the Pacific	Battley et al. 2000[(1)]
Sylvia borin	34	35	36	28	30	4	11	during migration over continental Europe in	Schwilch et al. 2002[(1)]
Hirundo rustica[#]	54	52	20	19	32	35	+15	autumn and after a flight across the Sahara and the	Schwilch et al. 2002[(1)]
Ficedula hypoleuca	36	46	14	22	21	14	5	Mediterranean Sea in spring	Schwilch et al. 2002[(1)]
Phylloscopus trochilus	37	38	29	20	20	15	8		Schwilch et al. 2002[(1)]
Sylvia borin	51	57	34	42	23	25	14	before and after flight across the Sahara during spring migration	Bauchinger et al. 2005[(2)]
Percent reduction during simulated migration									
Calidris tenuirostris	42	40	18	31	15	20	11	before and after ca 2 week fast	Battley et al. 2001[(1)]
Sylvia borin	63	24	21	30	8	7	7	before and after a 2 day fast	Hume and Biebach 1996[(3)]
Sylvia atricapilla	52	54	20					before and after a 2.5–3 day fast	Karasov and Pinshow 1998[(4)]
Sylvia atricapilla	45	36	20		21	19		before and after a 1–2 day fast	Karasov et al. 2004[(5)]
Tyto alba[##]	(43)*	61	(43)*		22	34	16	before and after a fast of 2.7 – 7.7 days	Thouzeau et al. 1999[(6)]
Rate of isotopic incorporation (k, in $days^{-1}$)									
Passer domesticus **	0.330	0.200	0.103		0.104	0.082		Sampling over 128 days after diet shift	Carleton et al. 2008
Poephila gutatta	0.330	0.284	0.270	0.248	0.058	0.051	0.038	Sampling over 256 days after diet shift	Bauchinger and McWilliams 2009
Coturnix japonica		0.272				0.056		Sampling over 212 days after diet shift	Hobson and Clark 1992

[#] not included in analysis because aerial feeding during migration can not be excluded.
[##] non-migratory species, not included in analysis.
* data only available for total digestive tract.
** we estimated *k* as $1/\tau$ for tissues best described by one-compartment model and as $k_{\text{two comp}} = pk_1 + (1-p)k_2$ for tissues best described by two-compartment model, where k_1 is the carbon incorporation rate ($1/\tau_1$) for $pool_1$ with pool size p and k_2 is the carbon incorporation ($1/\tau_2$) rate for $pool_2$ with pool size $1 - p$.
– Mass changes are based on [(1)] lean dry tissue, [(2)] lean dry tissue, with percentage of fat quantified for pooled data of digestive tract (proventriculus, gizzard, small intestine and colon) and subtracted from dry mass of gizzard and small intestine, [(3)] dry whole tissue, [(4)] lean wet tissue, [(5)] wet whole tissue for small intestine and pectoral muscle, dry whole tissue for heart, liver and gizzard, [(6)] protein estimation.