Chapter 12
Tissue-Specific Mass Changes During Fasting: The Protein Turnover Hypothesis

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12.1 Introduction

Facing a period without food intake is common among animals; fasting occurs in a variety of different contexts and it often varies in duration and in the extent to which metabolic and behavioral activity is suppressed during the fasting period (for review see McCue 2010). When the duration of time without food exceeds the time required for digestion and absorption of the previously ingested food, then the animal's biochemical requirements for subsequent activity and physiological processes have to be satisfied from body resources instead of food (McWilliams et al. 2004; McWilliams and Karasov 2005). The availability of resources stored prior to onset of the fasting period determines the tolerated length of the fasting period. Requirements are met by catabolizing body stores (phase II fasting, see Le Maho et al. 1981; Cherel et al. 1988; Robin et al. 1988); once body stores are depleted then animals must catabolize structural tissues to satisfy their continuous biochemical requirements and so avoid death (phase III of fasting). Scaling of metabolic rate and the pace of depletion of body stores adequately explains why heavier species may encounter longer fasting periods compared to lighter species (Kleiber 1932; Calder 1984; Bauchinger and McWilliams 2009), but cannot explain differential mass loss among similar sized animals.

Fasting periods may occur periodically and/or stochastically. Reoccurring patterns of fasting of rather similar length occurs in response to various kinds of rhythms (e.g., circadian, circannual, and tidal) and may often limit the availability of, or access to, nutritional resources (for reviews see Körtner and Geiser 2000;

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Piersma and Drent 2003; Piersma and van Gils 2010) (see also Bar and Volkoff, Chap. 6). Daily and seasonal movements of the earth and the moon determine sleep-awake rhythms of many animals as well as the timing, duration, and extent of seasonal hibernation and seasonal migration, each of which is associated with periodic fasting and refeeding periods. Less predictable, stochastic events, such as sand-, rain-, or snowstorms can make foraging virtually impossible for many animals and so result in fasting periods of less predictable durations (Secor and Diamond 1998, for review see Piersma and van Gils 2010) (see also Jenni-Eiermann and Jenni, Chap. 11).

Seasonal environmental changes often trigger animal movements including migrations to avoid unfavorable conditions at one area and/or anticipate favorable conditions at another (for reviews see Berthold 2001; Newton 2008). Animal movements over great distances may require sustained periods of locomotion that may limit foraging, especially if inhospitable areas have to be overcome (Biebach 1992; Butler et al. 1998; Biebach et al. 2000; Klaassen and Biebach 1994; Gill et al. 2009; Schmaljohann et al. 2007). These fasting periods may last for hours or as long as several days depending on the ecological barrier, and may be repeated several times with only relatively short rest periods at stopover sites before the next long-distance excursion (Butler et al. 1998; Biebach et al. 2000; Gill et al. 2009; Schmaljohann et al. 2007).

Extended fasting periods associated with migration require body stores that must be deposited before the onset of active migration. Large amounts of fat are deposited prior to take off, but protein storage also increases during the premigratory preparations (Fry et al. 1972; Marsh 1984; Piersma 1990; Biebach 1996; Bauchinger and Biebach 2005). Both, fat and protein tissues are subsequently catabolized during the fasting period with fat accounting for about 2/3 to 3/4 of the mass change of the migrating bird and proteinaceous tissue accounting for the remainder (Klaassen and Biebach 1994).

Protein catabolism during fasting causes reduction in mass of internal organs. Much early research focused on the mass reduction of digestive organs (e.g., intestine, liver, and gizzard), which are obviously not in use during fasting (see also Lignot, Chap. 14). Other tissues including kidney and muscle were also shown to atrophy during fasting like that which occurs during migration (Hume and Biebach 1996; Bauchinger and Biebach 1998; Biebach 1998; Thouzeau et al. 1999) as well as during actual long-duration migrations (Biebach 1998; Bauchinger and Biebach 1998; Karasov and Pinshow 1998; Battley et al. 2000, 2001; Schwilch et al. 2002; Karasov et al. 2004; Bauchinger et al. 2005).We have shown that the extent of reduction in these organs seems to be organ-specific when considered for a single species (Bauchinger and McWilliams 2009) and across many species (Bauchinger and McWilliams 2010). The extent of mass reduction for a given tissue was consistent across all species studied to date and was in the following rank order from most to least reduced: small intestine, liver, kidney, heart, flight muscle, and leg muscle (Fig. 12.1, adapted from Bauchinger and McWilliams 2010).
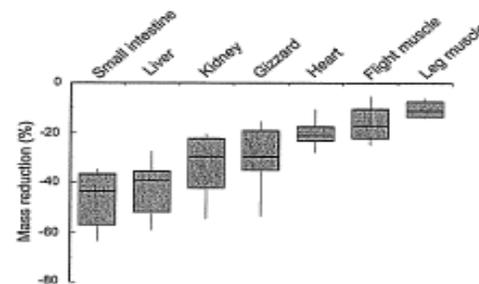
Fig. 12.1 Mass loss (as % of prefast condition) for seven organs in migratory birds sampled before and after a sustained period of flight, or sampled before and after fasting during a simulated migration (Figure adapted from Bauchinger and McWilliams 2010). Median mass change (box shows 20 and 75 percentiles, error bars gives 10 and 90 percentiles) for small intestine, liver, kidney, gizzard, heart, flight muscle, and leg muscle for great knot *Calidris tenuirostris* (Battley et al. 2000, 2001), blackcaps *Sylvia atricapilla* (Karasov et al. 2004), 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 *Phylloscopus trochilus* (Schwilch et al. 2002). Only studies that give mass data for at least five of the presented tissues were incorporated (see Bauchinger and McWilliams 2010 for details)

12.2 Hypotheses to Explain Differential Mass Loss

Several functional hypotheses have been proposed to explain why organs are changing in mass during migration, sustained flights, and other long-duration fasting periods (for reviews see Evans 1992; Biebach 1996; Piersma and Lindström 1997; Bauchinger and Biebach 1998; Jenni and Jenni-Eiermann 1998; Bauchinger and McWilliams 2009, 2010). Here we outline five functional hypotheses that have been proposed to explain phenotypic flexibility in organ size, in general, and the loss/gain of tissue protein during migration.

- Use-disuse hypothesis
- Functional allometry hypothesis
- Protein pool hypothesis
- Energy conservation hypothesis
- Protein turnover rate hypothesis

12.2.1 The Use-Disuse Hypothesis

The use-disuse hypothesis proposes that organs in use will hypertrophy whereas organs not in use will atrophy (Alexander and Goldspink 1977). Such changes are well documented for various animal models and humans: inactivity or

immobilization causes quick atrophy of muscles, whereas training increases mass of exercised muscle (Boonyarom and Inui 2006). Typically the 'training effect' requires considerable time to manifest itself. Studies of migratory waterfowl and waterbirds (e.g., geese, ducks, and grebes) provide support for this hypothesis (for review see Portugal et al. 2009). For example, eared grebes (*Podicepsnigricollis*) reduced flight muscle size and increased leg muscle size after arrival at a staging area and commencement of wing molt, flightlessness, and the increased necessity of swimming (Jehl 1997). Flight muscle size of grebes increased toward the end of wing molt when birds increased wing flapping activity (Piersma 1988; Jehl 1997); however, similar muscle mass changes occurred among waterfowl that did not increase their frequency of wing flapping (see Portugal et al. 2009 for review). Other results are inconsistent with the use–disuse hypothesis. For example, pre-migratory flight muscle mass increased without increased flight activity in a wader species suggesting endogenous control of muscle hypertrophy (Dietz et al. 1999). We can use the evidence portrayed in Fig. 12.1 to further test the use–disuse hypothesis.

Two lines of evidence can be used to evaluate the use–disuse hypothesis. First, a comparison of tissue reduction during fasting in birds during natural migration versus during simulated migration should reveal differences between tissues. Specifically, tissues such as flight muscle and heart that must be heavily used during flight should reduce less in mass than during simulated migration (i.e., when birds in cages were exposed to periods with and without food under light: dark cycles appropriate for stimulating migration state). In contrast to these predictions, Fig. 12.2 reveals quite consistent relative reductions in flight muscle, heart, and kidney during fasting for birds during natural and simulated migration. Second, the use–disuse hypothesis predicts that small intestine, gizzard, and leg muscle should reduce more in mass than flight muscle, heart, and kidney in birds during natural migration that are actively flying. Again, Fig. 12.2 provides little support for this prediction—in fact, small intestine was reduced the most and leg muscle reduced the least in birds during flight. Thus, despite the limited data available to date, the use–disuse hypothesis cannot explain the consistent pattern of organ mass loss observed during fasting in migratory birds (Figs. 12.1 and 12.2). This conclusion is consistent with others who have also found weak support for the use–disuse hypothesis in studies of geese (Portugal et al. 2009) and waders (Dietz et al. 2007) as outlined above.

12.2.2 The Functional Allometry Hypothesis

The functional allometry hypothesis proposes that flight muscle mass is adjusted to optimize the power requirements of flying given changes in whole-body mass during migration of birds. Fat stores provide more than 90% of the energy required for sustained flapping flight, and there is a corresponding need to increase flight muscle mass to power flight of the fat-loaded bird at take off (Pennycuick 1978, 2008).
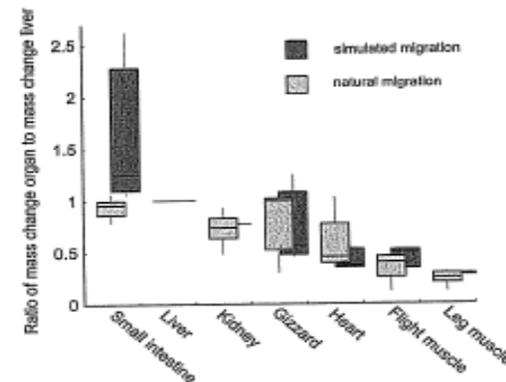
Fig. 12.2 Ratio of organ mass change to liver mass change for seven tissues. For each of the data sets introduced in Fig. 12.1 all organ mass changes were related to that of the liver, and the median ratio for each organ and all data sets was plotted for the same tissues as in Fig. 12.1 (box shows 20 and 75 percentiles, error bars gives 10 and 90 percentiles). Data for natural migrating birds are depicted in *light gray*, data for birds subjected to simulated migration by food deprivation in the laboratory (for method see Gwinner et al. 1985; Battley et al. 2001) in *dark gray*. Liver data must equal 1.0 by definition for both natural and simulated migration

During long-duration flights, these fat stores are depleted, whole-body mass decreases, and there is a corresponding decrease in power requirements and hence flight muscle mass over the course of the flight (Bauchinger and Biebach 2001; Pennycuick and Battley 2003). The functional allometry hypothesis was originally formulated to explain the increase in flight muscle during premigratory fattening (Evans 1969; Fry et al. 1972; Marsh 1984); increased fat deposition increased power requirements and this was provided by increased flight muscle mass. The tight link between the power requirements of flight and the mass of flight muscle has been confirmed on many occasions (e.g., Lindtsröm et al. 2000; Bauchinger and Biebach 2001). However, the aforementioned example of eared grebes poses challenges to such proposed relationships: recall that birds reduced flight muscle mass while gaining body mass upon arrival at their staging ground (Jehl 1997). Similarly, flight muscle mass of warblers during migration was not always tightly related to their body mass especially when their stage of migration was considered (Bauchinger and Biebach 2005; Barboutis et al. 2011).

The functional allometry hypothesis was postulated to explain mass changes of the flight muscle relative to body mass, but it can be expanded to apply also to other skeletal muscles (e.g., leg muscle) that provide posture and mobility (Bauchinger and Biebach 2005). In fact analysis of tissue mass in warblers during migration suggests a more uniform relationship between leg muscle and body mass
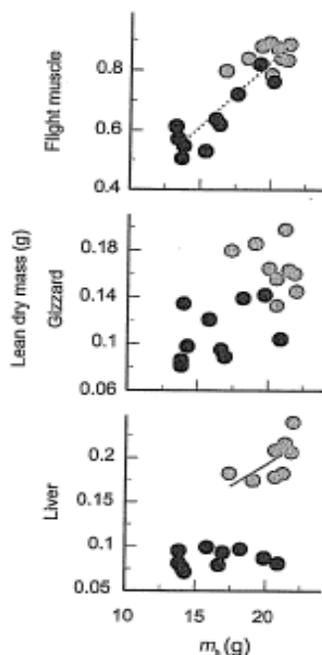
Fig. 12.3 Relationship between lean dry mass of three tissues (flight muscle, gizzard, and liver) and body mass (m_b). Body mass always represents the mass of the body minus the mass of the respective organ (i.e., corrected body mass). *Top graph* shows a significant relationship between flight muscle and body mass ($p < 0.001$); small intestine ($p = 0.041$), and heart ($p = 0.008$) also reveal a significant relationship, but are not shown. *Middle graph* shows that gizzard mass is not significantly related to body mass ($p = 0.54$); leg muscle ($p = 0.11$), and kidney ($p = 0.79$) also revealed a nonsignificant relationship, but are not presented here. *Bottom graph* shows liver mass was significantly related to body mass ($p = 0.046$) for birds in Ethiopia (*gray circles*) but not so for birds in Egypt (*black circles*). We fitted a model with the factors body mass, sampling site, and the interaction of both; nonsignificant interactions were then removed and the final model was recalculated

than for the flight muscle and body mass. We used data for garden warblers sampled before and after the migration across the Sahara desert in spring 1998 (Bauchinger et al. 2005) to test for such a causal relationship. We confirmed a significant, positive allometric relationship for flight muscle, small intestine, and heart. However, three tissues, gizzard, kidney, and leg muscle were not positively related to body mass (Fig. 12.3). Liver mass of birds sampled before the migration across the Sahara was significantly related to body mass, whereas liver mass of birds sampled immediately after completion of the migration across the Sahara was not related to body mass (Fig. 12.3). We conclude that the functional allometry hypothesis does not fully explain the observed organ mass changes in migratory songbirds.

12.2.3   The Protein Pool Hypothesis

The protein pool hypothesis has been proposed in several different but related forms. In general, the protein pool hypothesis assumes that protein catabolism simultaneously uses amino acids from a variety of different tissues either irrespective of protein pool size (so equal use of amino acids from all tissues) or in rough proportion to the mass of the tissue and hence the size of the protein pool.

Tissue protein serves as a pool of amino acids for repair mechanisms (Piersma 1990), gluconeogenesis to meet the energy requirements of the brain (Jenni-Eiermann and Jenni 1991), and the perpetuation of the the β-oxidation pathway (Jenni-Eiermann and Jenni 1991). Protein stores may also serve as an energy source when fat stores are depleted. Estimation of the contribution of energy derived from protein catabolism to the overall energy requirements ranges 'only' around 5% (Klaassen and Biebach 1994; Jenni and Jenni-Eiermann 1998). Protein is not considered to be the main energy store (Dohm 1986) and its catabolism for energy production is not efficient (Evans 1992), although protein can be the final energy source when birds run out of fat stores during flight (Jenni et al. 2000). Protein catabolism also provides metabolic water during periods without food or free water (Klaassen 1996, see as well Bauchinger and Biebach 1998; Jenni and Jenni-Eiermann 1998; Gerson and Guglielmo 2011) (see also Jenni-Eierman and Jenni, Chap. 11). Finally, protein catabolism may produce antioxidants that counter balance free radical production (Klaassen et al. 2000) (see also Champagne et al., Chap. 19), which is, for example, increased due to lipid per-oxidation during flight or heavy muscular workload (McWilliams et al. 2011). Thus, tissue protein has many important functions and organ reduction may either liberate specific proteins or amino acids for these many uses (but see McCue et al., Chap. 8).

We can test the protein pool hypothesis by determining whether fasting results in a uniform decrease in tissue protein across all tissues, or a decrease in tissue protein in proportion to the size of the protein pool in that tissue. In fact, not all tissues were reduced to a similar extent during fasting (Fig. 12.1) or relative to the size of the tissue (Bauchinger and McWilliams 2009, 2010). Thus, the protein pool hypothesis cannot explain the observed pattern of reduction in tissue mass of fasted birds.

12.2.4 The Energy Conservation Hypothesis

The energy conservation hypothesis proposes energy savings due to organ reduction. Small intestine is considered to be the most costly tissue in the verte- brate body to be maintained (Stevens and Hume 2004). The observed reduction of small intestine during simulated migration (Hume and Biebach 1996) was there- fore considered to reduce the costs required for maintaining an organ that is of no use during in-flight starvation during migration. The reduction in organ mass that reduces maintenance costs also saves weight and thus reduces flight costs (Hume and Biebach 1996; Biebach and Bauchinger 2003).

Testing this hypothesis requires knowing the organ-specific metabolic costs and the size of the respective organ within the birds body. Unfortunately, specific metabolic rates for bird organs are largely unexplored. We are aware of only one investigation that reports organ-specific metabolic rates for liver and flight muscle in two species, Dunlins (*Calidris alpina*) and European starlings (*Sturnus vulgaris*, Scott and Evans 1992). It appears that oxygen uptake for liver tissue is consistently 4 times higher compared to flight muscle.

In terms of relative organ mass, we collected data on liver and flight muscle mass for three passerine bird species (Schwilch et al. 2002; Bauchinger et al. 2005) and one wader species (Battley et al. 2001). Flight muscle was on average 5.7 times heavier than liver (range 4.1–7.0), small intestine was similar in mass to liver (the ratio was on average, 0.9, range 0.7–1.1), leg muscle was on average 1.4 times heavier than liver (range 0.8–1.9), gizzard was slightly lighter than liver (the ratio was on average 0.7, range 0.6–0.8), and kidney and heart were much lighter than liver (the ratios were 0.3 and 0.2, respectively; range 0.2–0.4 for kidney, 0.2–0.3 for heart). Thus the relative size of the organs within a bird ranks from highest to lowest: flight muscle, leg muscle, liver, small intestine, gizzard, kidney to heart. And this rank order is not the same as the relative reduction in tissue mass during fasting (Fig. 12.1). We conclude that the energy conservation hypothesis does not fully explain the observed organ mass changes in migratory songbirds, although more information about tissue-specific metabolic rates are needed to more adequately test this hypothesis.

12.2.5 The Protein Turnover Rate Hypothesis

The protein turnover rate hypothesis proposes that organs with the fastest rates of protein turnover will reduce the most during fasting and that organs with the slowest rates of protein turnover will reduce the least during fasting (Bauchinger and McWilliams 2009, 2010). This hypothesis capitalizes on the now available measurements for carbon turnover in specific tissues that can be used as proxy for protein turnover. Rate of isotopic incorporation differs between organs, both in mammals (Tieszen et al. 1983; Arneson et al. 2006; Sponheimer et al. 2006) and birds (Hobson and Clark 1992; Carleton et al. 2008; Bauchinger and

Fig. 12.4 Ratio of organ mass change to liver mass change (*upper graph*) and the ratio of organ rate of isotopic incorporation (k$_{organ}$) to liver rate of isotopic incorporation (k$_{liver}$; *lower graph*) for seven tissues. Liver values equal 1.0 by definition. Median ratio of mass change (box shows 20 and 75 percentiles, error bars gives 10 and 90 percentiles) is shown for small intestine, liver, kidney, gizzard, heart, flight muscle, and leg muscle. Data for ratio of organ mass change to liver mass change (*upper graph*) from Hume and Biebach 1996; Karasov and Pinshow 1998; Battley et al. 2000, 2001; Schwilch et al. 2002; Karasov et al. 2004 and Bauchinger et al. 2005; data for ratio of organ k to liver k (*lower graph*) from Hobson and Clark 1992; Carleton et al. 2008 and Bauchinger and McWilliams 2009, 2010)
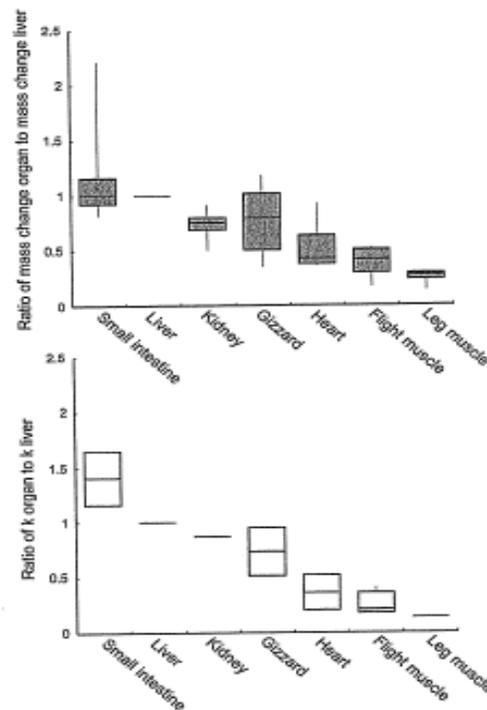
McWilliams 2009), with fast turnover rates for small intestine and liver and slow turnover rates for skeletal muscles. We can test the protein turnover rate hypothesis by determining the extent to which differences in reduction of mass between tissues during fasting corresponds with that of tissue turnover rate.

Although rate of isotopic incorporation for organs are only available for three bird species, namely zebra finch, house sparrow, and Japanese quail (see Bauch- inger and McWilliams 2010 for review), the rate of isotopic incorporation is allometrically related to body mass. This allows us to standardize the isotopic incorporation for a given tissue by dividing with that of liver for the same species (Bauchinger and McWilliams 2010). Figure 12.4 summarizes the ratio of mass change for an organ to that of the liver (upper panel), and the ratio of isotopic incorporation for a given tissue to that of the liver (lower panel). As predicted by

the protein turnover rate hypothesis, small intestine and liver were reduced the most and had the highest rate of isotopic incorporation, flight muscle and leg muscle were reduced the least and had the lowest rate of isotopic incorporation, while kidney, gizzard, and heart were intermediate (see as well Bauchinger and McWilliams 2010). The clear match between the two measurements is even more remarkable considering the limited data for organ reductions (nine data sets out of six studies: Hume and Biebach 1996; Karasov and Pinshow 1998; Battley et al. 2000, 2001; Schwilch et al. 2002; Bauchinger et al. 2005) and the limited data for isotopic incorporation (three studies: Hobson and Clark 1992; Carleton et al. 2008; Bauchinger and McWilliams 2009). Furthermore, the tight relationship between isotopic rate of incorporation and organ mass reduction remains highly significant if only data for organ mass are analyzed that were either collected from naturally migrating birds, or from simulated migration experiments that were conducted in the laboratory (Bauchinger and McWilliams 2010).

12.3 Conclusion

Analysis of the available data on organ mass changes during natural or simulated migration revealed that some organs were more reduced than others and that the observed pattern was statistically significant (Bauchinger and McWilliams 2010). Small intestine and liver were the organs that reduced the most, flight muscle and leg muscle reduced the least, and organs like kidney, gizzard, and heart ranged intermediate (Fig. 12.1). We reviewed five hypotheses proposed to explain the observed patterns. We rejected three of the five hypotheses (the use-disuse hypothesis, the functional allometry hypothesis, and the protein pool hypothesis). At present, adequately testing the energy conservation hypothesis requires better data on organ-specific metabolic rates. The best available data (though still limited) support the protein turnover rate hypothesis: the rate of protein turnover determines the rate of organ mass reduction observed in birds during migration.

It seems very plausible that the energy conservation hypothesis, at least part of it, namely the reduction of organ maintenance costs may actually be linked to the protein turnover rate hypothesis. The continuous process of protein turnover requires energy for both protein synthesis and protein degradation, and thus the rate of protein turnover of an organ is likely positively related to the potential metabolic costs (Krebs 1950; Martin and Fuhrman 1955; Scott and Evans 1992) that can be saved if organs are reduced. It is therefore not surprising that the most costly organ to maintain, the small intestine (Stevens and Hume 2004), is the organ with the highest rate of isotopic incorporation and therefore protein turnover.

We would like to point out that the support for the protein turnover rate hypothesis can help to explain the rate of organ reductions between organs, but does not address what the degraded protein from the respective organs is used for. Birds may very well use the protein to liberate water bound to the protein (Gerson and Guglielmo 2011), to supply necessary intermediates for fatty acid metabolism

(Jenni-Eiermann and Jenni 1991), or to supply protein for repair mechanisms (Piersma 1990) or as antioxidants (Klaassen et al. 2000).

We have shown that the magnitude of organ flexibility among several species of migratory birds is directly related to tissue-specific protein turnover. We propose that tissue-specific rate of protein turnover determines the pace of organ mass reduction in migrating birds and hence the magnitude of phenotypic organ mass changes observed in migratory birds. As such, no further functional explanation is needed to explain phenotypic flexibility in organ size of migratory birds unless there are detectable differences in organ mass change beyond that predicted by the protein turnover hypothesis.

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