Changes in Lean Mass and in Organs of Nutrient Assimilation in a Long-Distance Passerine Migrant at a Springtime Stopover Site

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ABSTRACT
The primary energy source for migration is fat, but nonfat body components can vary in concert with lipid stores in some migrants. The goals of this study were (1) to validate for a small Old World warbler (the blackcap, Sylvia atricapilla) non-destructive methods to measure lean and fat mass, (2) to quantify the relative contribution of lean mass to body-mass change of migrants, and (3) to ascertain what lean tissues might be involved. Using total-body electrical conductivity and dilution space of isotope-labeled water, we measured lean and fat mass with precision of 3%–4% and 10%–15%, respectively. In newly arrived migrants with apparently similar structural size (tarsus length), there was a significant positive correlation between lean mass and fat mass; 37% of each unit change was lean mass and 63% fat. Captive blackcaps, fed ad lib. for 7 d, gained body mass, with 40% being lean mass. When captures were fasted 1.5–3 d, both body mass and lean mass declined; lean mass accounted for 42% of body mass lost. In fasted birds, the masses of liver, stomach, and small intestine declined and accounted for 44% of the total lean mass decline, a disproportionate amount considering that these organs make up only 11% of a blackcap’s lean mass. In freshly captured blackcaps, organ masses were positively correlated with lean mass minus the organ masses, suggesting that these organs are a source of lean mass catalyzed by migrants. We conclude that migrants’ need for protein to rebuild lean mass during stopover could constrain diet selection and require increased foraging time, thus slowing mass gain and lengthening overall migration time. Also, stopover time may be lengthened if time is required to rebuild atrophied organs that are important in food digestion and assimilation.

Introduction
Migratory birds stop over at Negev or other desert sites for a variety of reasons, including the need to restore energy reserves or physiological imbalances, to avoid flying at certain times of day, and because of inclement weather or unfavorable winds (Bailey 1985; Biebach et al. 1986; Safrir and Lavee 1988). Studying the reasons that migrating birds break their flight is important for understanding the strategies of migration and for bird conservation. If a stopover site satisfies a critical need for a particular resource, for example, humans can better evaluate the impact of development schemes for the site on migratory birds or otherwise enhance that resource at the site. The birds’ requirements, and the resources available to satisfy them, determine the length of the stopover and thus influence a species’ overall migration speed and therefore its success. From theoretical models of optimal migration (Alerstam and Lindström 1990), it is apparent that the rate at which a species can add stores or restore a physiological deficit before the next flight is critical to migrating birds.

For these reasons, an understanding of the physiological bases for migratory stopover is of fundamental and practical importance. Early studies suggested that nonfat body components of migrating birds remain in homeostasis down to some lower limit of body mass, that fat is added and used without appreciable change in the largely nonfat tissue structure of the body, and that changes in body mass during stopover were due to fat deposition (Connell et al. 1960; Odum et al. 1964; Hicks 1967). However, more recent studies have demonstrated that the nonfat body components can vary in concert with lipid in some migrants (Marsh 1984; Gaunt et al. 1990; Piersma 1990; Jenni-Eiermann and Jenni 1991; Lindström and Piersma 1993). Hence, the assumption that all mass gain is fat will lead to overestimation of fat deposition and energy gain during stopover. Also, changes in nonfat body components may indicate that stopover sites are critical for providing resources aside from energy (water and protein, for instance). Carmi et al. (1992) suggested that stoppers might function importantly as sites where cross-desert migrants stop to replenish body water. Several of the above-mentioned studies hint at the use of body...
protein during migration (also Bairlein and Totzke 1992), which would necessitate replenishment of body protein during stopover. Indeed, Carpenter et al. (1993) proposed that such a requirement would explain the slow phase of mass gain in migrant hummingbirds and is an important constraint in hummingbirds' overall migration speed.

The aim of our study was to determine quantitatively what proportion of body-mass gains and losses of passerine migrants is lean mass and, in the event of notable changes in lean mass, to gain information on what tissues might be involved. We follow the terminology of van der Meer and Piersma (1994) in defining lean mass as the difference between body mass and fat mass. Our study species was the blackcap warbler (Sylvia atricapilla), one of the most widespread nocturnal migrants that breeds in the western and central Palaearctic and migrates to tropical Africa (Carré-Lindahl 1981). Many of our methods are necessarily indirect, because it is practically impossible to make repeated measurements on individual blackcaps before and after migratory flights and very difficult to make repeated measures on enough individuals during a stopover. Also, our study site was not one in which we could follow a synchronized population through mass changes and assume that differences between groups reflect what happens within an individual (cf. Lindström and Piersma 1993). However, van der Meer and Piersma (1994) have suggested a modeling approach to make inferences about utilization of fat and lean mass from data on body composition over a broad range of body masses of migrants. We have supplemented that approach with laboratory studies in which we simulated the mass gains and losses of migrants (Klaassen and Biebach 1994; Klaassen et al. 1997). In order to apply the modeling approach, it was ideal to make independent measures of lean and fat mass and to use nondestructive measures wherever possible. Therefore, our study also included calibration and validation procedures for determining lean and fat mass using total body electrical conductivity (TOBEC; Walsberg 1988; Castro et al. 1990; Roby 1991; Scott et al. 1991; Skagen et al. 1993; Lyons and Haig 1995) and the dilution of hydrogen (2H) isotope-labeled water (Child and Marshall 1970; Campbell and Leatherland 1980; Rumpfle et al. 1987; Chappell et al. 1993).

Material and Methods

Study Site

Upon migration from Africa to their Palearctic breeding sites, blackcaps cross the Sahara, with the most intensive migration into and through the eastern and western Mediterranean basin (Berthold 1988; Helbig 1994). We studied those birds that stop over at the northern edge of the Sahara ecological barrier in the Negev highlands of southern Israel. Our research site was the campus of Ben-Gurion University of the Negev at Midres-

het Ben-Gurion (30°52' N, 34°57' E, 475 m ASL): the campus includes gardens and greenery that attract the birds, where they feed on insects, fruit, and nectar (W. H. Karasov and B. Pinshow, personal observations).

Measurements on Freshly Captured Birds

In the last 2 wk of March 1995 and 1996, blackcaps were mist-netted in the park at Midrēshet Ben-Gurion between 0545 and 0800 hours. Birds captured early in the morning included individuals immediately following a nocturnal flight. We excluded any birds banded from a simultaneous study in the park. Birds were weighed twice to the nearest 0.1 g, and tarsus length was measured to within 0.1 mm. Each bird was injected with 60.4 ± 0.1 (SE; n = 17) mg 99.9% deuterated water (ICN Biomedicals), and then the TOBEC of each was measured twice in an EM-SCAN SA-3000 Small Animal Body Composition Analyzer with a Model 3044 detection chamber (Em-Scan, Inc., Springfield, Ill.). Appropriate permits were obtained from the Israel Nature Reserves Authority.

TOBEC Measurements

All TOBEC measurements were conducted in the laboratory at 22°C. Each measurement consisted of placing the unanesthetized bird in a custom-built chamber with a Velcro fastener to hold its position and geometry constant. The chamber was a clear Plexiglas cylinder (31 mm inner diameter) 18 cm long. It was covered with 8 cm of paper and tape at one end and had a movable, loose-fitting plunger at the other end. The plunger was removed, and a bird was inserted with keel pointed upward and head and bill parallel to the keel. The plunger was then inserted and secured with the Velcro fastener so that it pressed the bird snugly into the covered end of the chamber against a Plexiglas wall. The chamber was then repeatedly (4–6 times) placed in the EM-SCAN coil; each time the reading was recorded. The bird was then removed from the chamber. Either prior to this measurement or immediately following, at least three recordings were made on the empty chamber, and the bird's initial TOBEC value was calculated as the difference between the mean values for the chamber with and without the bird. Fifteen to 50 min later, the entire procedure was repeated for the second measurement.

It was most appropriate that the entire procedure be replicated, rather than simply repeatedly sliding the chamber into the EM-SCAN device, because the largest source of variation in TOBEC readings was positioning of the bird. We found that the TOBEC device gave repeatable readings on an individual bird only if extreme care were taken to be absolutely consistent in how the bird was placed in the chamber. The Velcro fastener was critical in achieving this. In 40 replicated (sometimes triplicate) measures on individual blackcaps in 1995, the coeffi-
cents of variation (CV = 100 x SD/mean) averaged 10% ± 2% (SE), though a few CVs ranged as high as 40%. When the second TOBEC value was regressed against the first (not shown), the y-intercept was not significant (P = 0.4). When the regression was forced through the origin, the slope (0.96 ± 0.03) was not significantly different from unity and the coefficient of determination (r²) was 0.97.

**Tissue Analyses**

Sixty minutes after injection with deuterium, and after completing the TOBEC measurements, a blood sample (110–170 µL) was taken from the brachial vein. Some birds were killed immediately by thoracic compression for subsequent carcass analysis, but others were transferred to the laboratory (below). Carcasses were plucked of feathers, and feather mass was calculated as the difference between carcass mass before and after plucking. We dissected out and weighed to the nearest milli-gram the excluding digesta, liver, and sometimes samples of depot fat to within 0.1 mg. The small intestine was dissected out, perfused with 1% NaCl solution to remove digesta, and then blotted dry and weighed. Then, tissues (excluding feathers) and digesta for each bird were pooled, freeze-dried, weighed again, and ground in a small coffee grinder. We refluxed 1-g subsamples (weighed after drying at 50°C for 5 h) with petroleum ether for 6 h (Dobush et al. 1985) in a Goldfisch apparatus to measure fat content. We then burned the insoluble residue at 550°C to determine ash content. We used as many replicates as was necessary to obtain a coefficient of variation less than 5%, although in most cases only two replicates were needed. Water content was determined by the difference between body mass at death and dry mass plus feathers. Body fat is the mass of extracted fat. Lean mass was defined as body mass minus body fat and thus includes material not extracted in either the feather mass and water mass.

Blood samples were microdistilled, and the water was analyzed for deuterium enrichment by infrared spectrophotometry at W. H. Karasov's laboratory (Karasov et al. 1988). We expected enrichments (F, atom %) of the water samples labeled with deuterium to be a function of the molar masses of deuterated water (20 g mol⁻¹), unlabeled water (18 g mol⁻¹), and the injection of 0.064 g of 99.9% enriched water into a deuterium space in the bird (S, g): E = 100 x [0.999(0.064 g/20) + 0.001(0.064 g/18) + (S/18)]. Rearranging for S gives: S = (5.4306/E) - 0.054306. The relation was checked in 18 trials with weighed amounts of water (range 5.1–20.1 g) that were injected with the same volume of deuterium as were birds. The relative error (100 x [predicted – measured]/measured) averaged 0.85% ± 0.63% (n = 18). In live birds, deuterium space exceeded measured water content (determined by freeze drying) by 6.8% ± 1.2% (μ = 29). Overestimation of body water due to distribution of labeled H in body spaces in addition to body water has been observed in many animals (e.g., Rumpel et al. 1987). Data were omitted from two birds whose deuterium spaces were greater than 79% of body mass, which is biologically unreasonable.

**Studies on Captive Birds**

Birds were retained in the laboratory so that analyses could be made while they gained or lost body mass. The birds were placed in cages (26.5 cm width × 17.5 cm depth × 26.5 cm height) with a perch and containers for water and food. All the birds were initially provided mealworms and a fruit mash made of bananas, protein (soy protein), vitamins, and minerals (Denslow et al. 1987) ad lib. and then gradually transferred to a diet of ad lib. fruit mash and 15 mealworms d⁻¹. These foods were chosen because the warblers are omnivorous during migration (Cramp 1992), taking insects, fruit, and nectar at Midreshet Ben-Gurion (W. H. Karasov and B. Pinskiow, personal observations). Cages were cleaned daily and checked three times per day to ensure that food and water were adequate.

Fasting experiments were begun after 7 d of habituation in captivity. In 1995, four birds were fasted for 3 d, beginning at 1100 hours, and then killed in order to provide calibration data on birds with very low fat contents. They were allowed free access to water. In these birds, TOBEC measurements were made daily during the fast.

The energy spent in a 3-d fast is probably similar to that used in an 8-h migratory flight (see "Discussion" below). In 1996, six birds were fasted for 1.5 d, with free access to water, beginning at 1800 hours. This is a length of time equivalent to that taken by a bird that begins a migratory flight in the evening, lands the next day at a site with no food, and flies on the following night to a site with food (Bairlein 1985; Bieblich et al. 1986). In these birds, TOBEC measurements were made twice daily, at 2100 hours and 0800 hours. Two birds in 1995 and four birds in 1996 were held concurrently with fasted birds for similar amounts of time but were fed ad lib. and were subject to TOBEC measurements according to the schedule of the fasted birds and then killed.

**Data Analysis**

**Calibration and Validation of Body-Composition Measures.** We used about half of the body-composition data to create a calibration curve for estimating lean mass and fat mass from TOBEC readings and deuterium space, respectively, and used the other portion of the data, all from wild-caught birds, as an independent validation set. The calibration set was composed of blackcaps maintained either fed or fasted in the laboratory. The largest blackcap captured wild was also added to this group so that the values for the calibration group would
span the range of values for the validation group. As indices of the precision of the predictive equations we present the r² values and the standard estimates of the mean (square root of the mean square error) from the least squares linear regression of lean or fat mass measured by chemical extraction on TOBEC reading or deuterium space. To determine the absolute and relative errors associated with our estimations of lean mass and fat mass, independent variables from the validation group were placed in predictive equations derived from the calibrating group, and the absolute difference between predicted values and actual values was determined (absolute error = |predicted – measured|) as well as the relative error (100 × |predicted – measured|/measured).

A first-order model using the TOBEC value (T) was used to predict lean mass (L): L = a + bT. Tarsus length was not included in the model because in both years of the study, it was not significantly correlated with lean mass in field-captured blackcaps (F₁,₁₀ = 0.001, P > 0.9). To rule out the possibility that the addition of fuel stores containing lean and fat mass would override structural size effects in the sample, we also tested whether tarsus length correlated to lean mass among subsets of the birds with only small fat loads (and, hence, small fuel stores), but there was still no significant correlation between tarsus length and lean mass (all P’s > 0.15). Sex also was not included in the model for predicting lean mass, because among migrant blackcaps in Israel, there is no significant difference in body mass or fat content between sexes (Izaki and Maïtav 1998).

The calibration curve for predicting body fat from deuterium space was derived by fitting data to the equation of Campbell and Leatherland (1980): F = (M – W – (W/C)) / (1 – (C/D)), where F is dry fat (g), M is body mass (g), W is the deuterium space (a proxy for water space; g), C is the ratio of deuterated-water mass to dry fat mass, and D is the ratio of deuterated-water mass to fat-free dry mass.

Statistical Analyses. Values are given as mean ± 1 SE (n = number of birds, unless otherwise indicated). ANOVA, using the general linear model in SYSTAT (Wilkinson 1992), was used to test for differences between discrete groups (e.g., by year, fasting period). Subsequent Tukey post hoc comparisons among grouping factors were made on the adjusted least-squares means from the ANOVA, as described in SYSTAT. When multiple measures were made on the same individuals, repeated-measures ANOVA was done.

Model I regression was used when the independent variable was not subject to error (e.g., fasting time) or, in the case of calibrations, when the regression line was fitted for purposes of prediction (Sokal and Rohlf 1981, p. 549). When using model I regression, differences in regression lines between discrete groups were tested by ANCOVA with the general linear model in SYSTAT (Wilkinson 1992). Interactions between grouping factors and the covariate were tested and removed if nonsignificant.

We used reduced major axis regression (model II regression) to determine relationships between body components and either fat mass or body mass minus the body component, because both x- and y-variables were subject to error, and in this case the estimated functional relation between the two would be biased if determined by model I regression (Sokal and Rohlf 1981, p. 549). We used body mass minus the body components, rather than body mass itself, to avoid using p-values that are part of the x-values, though this procedure did not change any conclusions. When using model II regression, we tested for differences in regression lines between discrete groups by performing a t-test on each group’s residuals.

In the course of model derivation by regression, data and plots of residuals were inspected. We decided that data would be considered outliers and removed if tests showed both significant deviation from normality (P < 0.05 in Kolmogorov–Smirnov test with the Lilliefors option in SYSTAT) and if values for Cook’s distance, a measure of the influence of each sample observation on the coefficient estimates, approached or exceeded 1 (Glantz and Slinker 1990). We thus removed one such datum from our analyses. Throughout, two-tailed tests were used, and a P-value < 0.05 was considered significant.

Results

Calibration and Validation

The TOBEC score was positively correlated with lean mass (F₁,₁₀ = 3.1, P < 0.001; Fig. 1a) and differed by year in intercept (F₁,₁₀ = 17.1, P < 0.001) but not in slope (F₁,₁₀ = 1.18, P = 0.29). The difference by year was almost certainly a machine (not operator) effect. Between the two field seasons, the machine had been dropped and was sent to the manufacturer for repair. Accordingly, separate calibration equations for each year were developed (below). There was no apparent difference in the relation between TOBEC score and lean mass between fed or fasted laboratory blackcaps and those measured immediately upon capture (hereon called field-caught) (tested in 1995; F₁,₁₀ = 0.69, P = 0.52).

Data from 10 laboratory-held (captive) blackcaps in 1995 (fed ad lib. or fasted for 3 d; Table 1) plus one field-caught bird were used to generate the calibration equation for 1995 (Table 2), and data from nine field-caught blackcaps in 1995 were used in the cross-validation (Table 2). The average error was about 1 g, or about 7% of the lean mass measured by chemical extraction. A calibration equation using all the data for 1995 is also shown in Table 2, as is the separate calibration equation for 1996. Precision, as measured by the SE of the estimate of lean mass, was better in 1996, probably due to increased operator experience.
Data from eight blackcaps in the 1995 calibration group were used to generate the predictive relation for estimating body fat from deuterium space. When the data were first fit to the calibration equation, the value for the ratio of deuterated-water mass to dry fat mass was negative (i.e., not biologically reasonable; mean ± SE = -0.36 ± 0.24), and its confidence interval included zero. A very low value for this ratio is consistent with our empirical finding of very low water content of depot fat, 0.17 ± 0.07 g water g⁻¹ dry fat (n = 4), and using that value in the calibration equation yielded a slightly higher standard error of the estimate (0.40) than when the ratio of deuterated-water mass to dry fat mass was unspecified (SE of estimate = 0.34) and a slightly lower r² (0.95 vs. 0.96). Setting the ratio of deuterated-water mass to dry fat mass equal to zero in the calibration equation yields a simple model: 

\[ F = (M - S - ([S]/C)) \]

where F is dry fat (g), M is body mass (g), S is the deuterium space (g), and C is the ratio of deuterated-water mass to fat-free dry mass. Nonlinear curve fitting of data from the eight calibration blackcaps to this simple model yielded a value for the ratio of deuterated-water mass to fat-free dry mass of 2.35 ± 0.07, an r² of 0.96, and SE of the estimate of 0.35 g. Consequently, this value of the ratio of deuterated-water mass to fat-free dry mass was used in the predictive relation for estimating fat mass in 17 field-captured blackcaps in 1995 (Fig. 2). The average error was about 0.4 g of fat, or about 16% of the fat mass measured by chemical extraction (Table 2). A comparable analysis using the data from 10 blackcaps analyzed in 1996 gave very similar results (Table 2); the fitted value of the ratio of deuterated-water mass to dry fat mass in the calibration equation was not significant, and when the data were fitted to the simpler model, the value of the ratio of deuterated-water mass to fat-free dry mass was nearly identical to that found previously (2.38 ± 0.04), the r² value was very high (0.98), and the SE of the estimate was very low (0.04 g).

Thus, we found that we could accurately determine lean mass using the TOBEC score with a precision to within ±1 g lean mass, and we could independently and accurately determine fat mass using deuterium dilution with a precision to within ±0.4 g.

**Lean and Fat Mass in Field-Caught Birds**

To determine the lean-mass proportion of body-mass gains and losses in field-caught migratory blackcaps, we regressed lean mass on fat mass, each measured by a different technique (Fig. 3). This approach is analogous to model 2 in van der Meer and Piersma (1994). If migrating blackcaps have uniform structural size and use and replace only fat, then there will be no correlation (zero slope), but if lean mass as well as fat is used and replaced, we expect a positive slope. The composition of body mass gained or lost (grams of lean mass gained or lost
Table 1: Characteristics of blackcap warblers (Sylvia atricapilla) maintained in the laboratory fasted or fed ad lib., or taken immediately from the field (field-caught).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Laboratory, Fed ad lib.</th>
<th>Laboratory, Fasted 1–1.5 d</th>
<th>Laboratory, Fasted 2.5–3 d</th>
<th>Field-Caught</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>23</td>
<td>$F_{3,21} = .6$</td>
<td>.7</td>
</tr>
<tr>
<td>Tarsus (mm)</td>
<td>20.3 ± .2 (19.7–21.0)</td>
<td>20.8 ± .4 (19.9–22.1)</td>
<td>20.6 ± .3 (19.4–21.4)</td>
<td>20.8 ± .1 (19.6–22.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass (g)</td>
<td>18.2 ± .6 (16.4–20.3)</td>
<td>16.9 ± .6a (14.7–18.6)</td>
<td>13.9 ± .4b (12.5–14.5)</td>
<td>17.3 ± .5c (12.7–22.6)</td>
<td>$F_{3,21} = 5.5$</td>
<td>.003</td>
</tr>
<tr>
<td>Feather mass (g)</td>
<td>1.39 ± .15 (1.04–2.11)</td>
<td>1.33 ± .14 (1.06–1.97)</td>
<td>1.16 ± .03 (1.09–1.23)</td>
<td>1.26 ± .04 (.98–2.04)</td>
<td>$F_{3,21} = 0.4$</td>
<td>.4</td>
</tr>
<tr>
<td>Non-ether-extractable mass (g)</td>
<td>3.85 ± .11a (3.60–4.38)</td>
<td>3.40 ± .12ac (2.92–3.77)</td>
<td>3.23 ± .10bc (2.96–3.47)</td>
<td>3.55 ± .09ac (2.61–4.33)</td>
<td>$F_{3,21} = 3.3$</td>
<td>.032</td>
</tr>
<tr>
<td>Ether-extractable mass (g)</td>
<td>2.85 ± .52a (1.64–4.86)</td>
<td>2.56 ± .55ac (1.18–3.79)</td>
<td>.47 ± .15a (1.17–.89)</td>
<td>2.87 ± .36a (1.75–3.55)</td>
<td>$F_{3,21} = 3.87$</td>
<td>.018</td>
</tr>
<tr>
<td>Ash (g)</td>
<td>.583 ± .01a (.35–.621)</td>
<td>.540 ± .018 (.493–.602)</td>
<td>.556 ± .015 (.51–.585)</td>
<td>.535 ± .014 (.375–.639)</td>
<td>$F_{3,21} = 1.31$</td>
<td>.28</td>
</tr>
<tr>
<td>Water (g)</td>
<td>9.7 ± .2 (8.8–10.6)</td>
<td>9.1 ± .4 (8.2–10.2)</td>
<td>9.0 ± .2 (8.1–9.5)</td>
<td>9.7 ± .2 (7.9–11.8)</td>
<td>$F_{3,21} = 1.2$</td>
<td>.3</td>
</tr>
</tbody>
</table>

Note. Values are means ± SE (range in parentheses). Ash and non-ether-extractable dry mass are for whole body minus feathers. Fasted birds were fed ad lib. for 7 d before their fast. F- and P-values are for one-factor ANOVA. Within a row where ANOVA P-values < .05, values that do not share the same superscript are significantly different from each other (P < .05).

Table 2: Calibration and validation of the use of TOBEC and deuterium space to determine lean mass and fat mass, respectively, for blackcaps (Sylvia atricapilla)

<table>
<thead>
<tr>
<th>Calibration Group</th>
<th>Correlation Statistics</th>
<th>Validation Group</th>
<th>Cross-Validation Statistics</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year</td>
<td>Model</td>
<td>Data Set</td>
<td>n</td>
</tr>
<tr>
<td>1995  ......</td>
<td>$L = 12.07 \pm .027 (\pm .008)^T$</td>
<td>Calibration</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>$L = 12.30 \pm .021 (\pm .006)^T$</td>
<td>All</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>$F = M - S - {5[2.35 (\pm .07)]}$</td>
<td>Calibration</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>$F = M - S - {5[2.46 (\pm .07)]}$</td>
<td>All</td>
<td>23</td>
</tr>
<tr>
<td>1996  ......</td>
<td>$L = 9.237 \pm .024 (\pm .005)^T$</td>
<td>All</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>$F = M - S - {5[2.38 (\pm .04)]}$</td>
<td>All</td>
<td>10</td>
</tr>
</tbody>
</table>

Note. T is the TOBEC value, S is deuterium space, L is lean mass, and F is fat mass. Body mass (M) is included in the predictive model for F. The data used for the validation statistics are from field-caught blackcaps and are an independent set from the calibration data set used to generate the predictive models. Values for errors in the cross-validation statistics are means ± SE (range in parentheses) from least squares linear regression for lean mass or nonlinear curve fitting for fat mass.
per gram of fat gained or lost) is derived from the slope of the relationship. We assumed no difference in structural size, because our single external size measure, tarsus length, proved nonsignificant in stepwise regression ($F = 1.17$, $P = 0.29$). Lean mass and fat mass were positively correlated by reduced major axis regression ($r = 0.39$, $P < 0.001$), with no significant difference by year ($r = 1.88$, $P = 0.067$). The slope, $0.58 \pm 0.08$ g lean mass g$^{-1}$ fat, indicated that for a change in body mass of 1 g, 37% was lean mass (100 $\times$ 0.58 g lean/1 g fat + 0.58 g lean) and 63% was fat mass.

**Change in Lean Mass during Refeeding**

Another approach to gain information on body-composition changes was to monitor mass trajectories of wild-caught birds. We captured nine birds, measured their body mass and lean mass (by TOBEC), and then placed them in the laboratory and fed them ad lib. quantities of the fruit mash and insect diet. Mean body mass increased by up to 5 g in some individuals and leveled off after a week (Fig. 4), at which point lean mass was measured again by TOBEC. The trajectory of body mass was steeper than that of lean mass (Fig. 4). This was expected, and the difference was body fat gained. By reduced major axis regression, the change in lean mass was significantly correlated with change in body mass minus lean mass, with a slope equal to $0.676 \pm 0.022$ (s.e. = 2.96, $P < 0.025$) and a nonsignificant intercept ($-0.02 \pm 0.4$; Fig. 5). The slope indicated that for a change in body mass of 1 g, 40% was lean mass ($100 \times 0.676$ g lean/1 g fat + 0.676 g lean)) and 60% was fat mass.

**Change in Lean Mass during Laboratory Fast**

Because the migratory flight over the Sahara entails an extended period of fasting for many of the migrants, we simulated the fasting period in the laboratory. One of the birds in the 3-d fast experiment in 1995 died at 2.5 d, and one of the birds in the 1.5-d fast experiment in 1996 died at 1 d; these data were included in analyses.

Because birds that fasted for 3 d (in 1995) had lower dry lean masses than those fed ad lib. (Table 1), they apparently catabolized lean mass as well as fat. But the apparent decline probably included mass lost from the emptying of the gastrointestinal tract, because birds measured 3–4 h after food was removed exhibited declines in body mass ($-0.8 \pm 0.2$ g wet mass, $n = 7$) and lean mass ($-0.5 \pm 0.1$ g wet mass). Thus, we repeatedly measured lean mass (with TOBEC) during the fast beginning 3–5 h after removal of food (Fig. 6a).
Figure 4. Changes in body mass (circles) and lean mass (squares) determined from TOBEC in nine blackcaps (Sylvia atricapilla) captured in 1996 (on day 0) and fed ad lib. in the laboratory for 7 d. The dashed line connects the values at days 0 and 7 so that slopes can be compared visually.

During the 1.5-d fasting experiment in 1996, blackcaps’ lean mass declined (−0.7 ± 0.1 g; n = 5; repeated-measures ANOVA, F1,4 = 7.73, P < 0.004), as did total body mass (−2.5 ± 0.2 g; F1,4 = 75.6, P < 0.001; Fig. 6a). Four blackcaps fed ad lib. and monitored simultaneously with the fasted birds exhibited little change in total body mass (−0.6 ± 0.24 g; repeated measures F1,4 = 3.83, P = 0.051) and no significant change in lean mass (−0.3 ± 0.3 g; F1,4 = 0.8, P = 0.5). During the 3-d fasting experiment in 1995, blackcaps’ lean mass declined (−1.6 ± 0.3 g; n = 3) as did body mass (−4.5 ± 0.2 g). The change in lean mass was significantly correlated with change in body mass minus lean mass (t4 = 3.994, P < 0.025), with a slope of 0.73 ± 0.24 and a nonsignificant intercept (0.592 ± 0.559). The slope indicated that for a change in body mass of 1 g, 42% was lean mass (100 × 0.73 g lean/1 g fat + 0.73 g lean) and 58% was fat mass.

We could discern no difference in the rates of body-mass and lean-mass loss in fasting blackcaps over the course of the fasting period in either 1995 or 1996 (Fig. 6b), with the exception of the very high rate in the 3–5 h immediately after food was removed (not shown), though the data were few and the power to detect such a difference was consequently low. During the 3 d of fasting in 1995, there was no significant difference in the mass lost between the first and second days (F1,4 = 3.5, P = 0.16). During the 1.5-d fasting experiment in 1996, the rate of mass loss of fasting blackcaps during the first night (−0.72 ± 0.21 g in 11 h, n = 6) was not significantly different from that of the second night (−0.59 ± 0.15 g, n = 5; repeated-measures ANOVA, F1,4 = 0.7, P > 0.4). The rate of body-mass decline during the first day of fast in 1995 (−1.7 ± 0.3 g d⁻¹) did not differ significantly from that in 1996 (−1.9 ± 0.3 g d⁻¹; F1,7 = 0.1, P > 0.8).

Organ Masses

The correlation of lean mass and organ mass in the field—

Figure 5. Relationship between the change in lean mass (determined from TOBEC) and the change in body mass minus lean mass in two groups of blackcaps (Sylvia atricapilla). One group (filled circles, solid line; same birds as in Fig. 4) were brought immediately from the field and fed ad lib. in the laboratory for 7 d. The second group (open circles, dashed line) were first habituated to the laboratory and fed ad lib. for 7 d and then either fasted for 1.5–3 d or fed ad lib. The lines are reduced major axis regression fits of the data for the two groups, which did not differ significantly (see "Changes in Body Composition of Migrant Blackcaps" in "Discussion").
caught blackcaps was consistent with the idea that the source of some of the lean mass catabolized by migrants was in these organs. More direct evidence comes from the progressive decline in the masses of stomach, small intestine, and liver during the laboratory fasting study (Table 3). Organ masses were compared among four groups of blackcaps: birds fed ad lib., birds fasted 1–1.5 d, birds fasted 2.5–3 d (one bird that was captured but never ate for 2.4 d was included in this group), and field-caught birds. ANOVA indicated significant differences among the groups in the masses of stomach ($F_{1,31} = 5.76, P = 0.003$), small intestine ($F_{1,31} = 6.91, P = 0.001$), and liver ($F_{1,31} = 10.3, P < 0.001$) and that liver mass differed according to year ($F_{1,31} = 16.9, P < 0.001$). The field-caught birds had stomachs similar in mass to ad lib.–fed birds but intermediate-sized small intestines. The difference in the mean mass of the three organs between ad lib.–fed birds (1.81 ± 0.11 g, n = 6) and those fasted for 2.5–3 d (1.11 ± 0.7 g, n = 4), indicated that 44% of the total lean-mass decline during fasting (from above, 1.6 g) was mass of the gastrointestinal organs and liver.

**Discussion**

We validated the use of two nondestructive methods for determining the body composition of blackcaps and then used...
Table 3: Organ masses of blackcaps (Sylvia atricapilla) freshly captured (field) or held in the laboratory and fed or fasted for two different lengths of time

<table>
<thead>
<tr>
<th>Group</th>
<th>Stomach Mass (g)</th>
<th>Small Intestine Mass (g)</th>
<th>Liver Mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad lib.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fed</td>
<td>0.41 ± 0.04ab</td>
<td>0.77 ± 0.05a</td>
<td>0.67 ± 0.04a</td>
</tr>
<tr>
<td></td>
<td>(6)</td>
<td>(6)</td>
<td>(6)</td>
</tr>
<tr>
<td>Fasted</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–1.5 d</td>
<td>0.37 ± 0.04a</td>
<td>0.61 ± 0.06ab</td>
<td>0.58 ± 0.05a</td>
</tr>
<tr>
<td></td>
<td>(6)</td>
<td>(6)</td>
<td>(6)</td>
</tr>
<tr>
<td>Fasted</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5–3 d</td>
<td>0.33 ± 0.04ab</td>
<td>0.37 ± 0.09ab</td>
<td>0.31 ± 0.05b</td>
</tr>
<tr>
<td></td>
<td>(5)</td>
<td>(5)</td>
<td>(5)</td>
</tr>
<tr>
<td>Field-caught</td>
<td>0.48 ± 0.02ab</td>
<td>0.58 ± 0.03cd</td>
<td>0.56 ± 0.03a</td>
</tr>
<tr>
<td></td>
<td>(23)</td>
<td>(20)</td>
<td>(21)</td>
</tr>
</tbody>
</table>

Note: Values are adjusted least square means ± SE (in parentheses, n = number of birds) from the ANOVA using year and group as factors. Group was significant for all three organs (P < .001), but year was significant only for the liver (F = 16.9, P < .001). Values within a column with different superscripts are significantly different (P < .05). The masses and body composition of these birds are reported in Table 1 above.

them with both field-caught birds and those held in the laboratory under different feeding regimes. In the following sections, we discuss the methods and their application and make inferences about the catabolism and anabolism of body tissues in migratory birds.

Estimation of Lean Mass and Fat Mass

Measurement of body composition based on water content can be accurate and precise (Child and Marshall 1970; Conway et al. 1994), and if isotopes of water are used, it has the virtues of being noninvasive and fairly quick to use in the field. It takes only a few seconds to inject carefully a measured amount of deuterium and less than a minute to take a blood sample. During the 1-h equilibration period (for small birds), other work can be performed. We found measurement of lean mass by deuterium dilution to be sufficiently precise to permit estimation of fat mass, essentially by difference from total body mass. Dehydration, which might be expected in some migrants, would lead to underestimation of lean mass, and hence overestimation of fat, if the original calibration equation was developed using hydrated birds. The error may be relatively small, and in any event was not apparent in our analysis, in which we measured fat mass in field-caught blackcaps using a calibration equation based on at least eight captive birds, all supplied with water ad lib. Lean mass estimated from water content includes any digesta in the gastrointestinal tract.

The virtues of TOBEC measures are that they are noninvasive and nondestructive and can be made repeatedly. We found measurement of lean mass by TOBEC to be less precise than by deuterium dilution. Nonetheless, it proved useful in two ways. First, the daily measures of lean mass that we made would have been difficult with isotopes of water, due to carryover effects, and would require daily blood sampling that is possibly harmful to small birds. Second, in order to determine the lean-mass proportion of body-mass gains and losses in field-caught migratory blackcaps, we planned to regress lean mass on fat mass, each measured by a different technique, and use the slope. If only one component is measured (e.g., lipid content by chemical extraction) and the other estimated by subtraction (e.g., lean mass by subtraction of lipid from total body mass), then the measurements are not independent, and measurement errors will lead to a flattening of the slope when the two components are regressed against each other.

At the outset, it is not obvious that the interindividual calibration curve from TOBEC was valid for the repeated intra-individual measurements we made during the fasting or feeding studies. But a comparison of our findings during the fasting study using TOBEC with those from chemical extraction validates it. Using TOBEC, we concluded that for a change in body mass of 1 g, 42% was lean mass (see “Change in Lean Mass during Laboratory Fast” in “Results,” above). For comparison, blackcaps that fasted 2.5–3 d had declines in body mass of 4.3 g (18.2–13.9 g) and in lean mass of 1.92 g (18.2 – 2.85) – [13.9 – 0.47]; Table 1), which yields a similar fractional lean mass loss of 45% (100 x [1.92/4.3]), or an even closer value of 41% if we discount the body- and lean-mass changes for apparent material in the gastrointestinal tract (see “Change in Lean Mass during Laboratory Fast” in “Results,” above).

Changes in Body Composition of Migrant Blackcaps

All three of our approaches, regression analysis of lean and fat mass of field-caught blackcaps and feeding and fasting experiments with captives, indicated that migrant blackcaps catabolized and anabolized lean mass in concert with fat mass. Quantitative estimates of the proportional change in body mass that was lean mass varied between 37% and 42%, depending on the particular approach. For comparison, the proportional change in body mass that is lean mass has been estimated for other migrant passerines by other methods: 27% (Klaassen and Biebach 1994) and 40% (Biebach 1990) in garden warblers (Sylvia borin), 18% in thrush nightingales (Luscinia luscinia; Klaassen et al. 1997), and 23% in willow warblers (Phylloscopus trochilus; Biebach 1990).

A virtue of determining the lean-mass proportion of body-
mass gains and losses in field-captured migratory blackcaps from the slope of lean mass on fat mass (Fig. 3) is ecological realism. The field-based approach may incorporate phenomena that do not occur in the laboratory setting. But interpretation of plots such as Figure 3 is conceptually complicated (Van der Meer and Piersma 1994), and it can be confounded by differences in structural size that may be difficult to quantify and control for (Lindström and Piersma 1993). We used tarsus length as a measure of structural size and found that it explained a significant amount of the variation in lean mass, but perhaps we chose a poor measure. Thus, the laboratory-based approaches proved very important for confirming our conclusion from field-caught birds that for every gram of body mass gained or lost by migrant blackcaps, at least a third was lean mass.

The data on mass gains by captive blackcaps fed ad lib. for 1 wk in the laboratory seem especially important in this regard. Forty percent of the mass gained was apparently composed of lean mass. This value would not reflect the situation in the wild if birds replenished lean mass sooner than fat like rats do (Cherel and Le Maho 1991) and if we had not provided enough time in the laboratory. Our measurements were too infrequent to evaluate the first possibility, but after 1 wk feeding in the laboratory, the birds reach a plateau in body mass (Fig. 4). Therefore, we concluded that free-living migrants that gain mass during stopover may add lean-mass equivalent to at least a third of their body-mass gain.

Interestingly, birds that lose mass during a fast may lose an equivalent proportion as lean mass. This is apparent from the similar slopes for data from the feeding and fasting experiments plotted together in Figure 5 (residuals did not differ by group; t = 1.35, P = 0.2). Of the three approaches we used for estimating the composition of body-mass changes of migrants, this one is probably the least ecologically sound because of the possible differences in catabolic processes. Catabolism of body tissue by migrants results in part from very high metabolism associated with flight, a feature not duplicated in the laboratory where the catabolism was achieved by a sustained resting fast. Energetically, the total effect might be similar, though the intensity differs. A bird flying for 10 h metabolizing energy at a rate of at least 10 times the resting rate expends as much energy as a resting bird fasting for 80–100 h and metabolizing at 1–1.25 times its basal metabolic expenditure. Though the total energy metabolized might be similar, the breakdown of body protein might be quite different in a flying versus resting bird.

Studies with geese and penguins indicate that rates of mass loss and the composition of the mass loss, vary with duration of fasting (Le Maho et al. 1981; Le Maho 1983; Groscolas 1986; Cherel et al. 1988; Boisnault et al. 1992). The authors of these studies define three phases in a fast. In phase I, body-mass loss is rapid due to carbohydrate, protein, and fat catabolism. In phase II, body-mass loss is slower and is composed mainly of fat, with protein catabolism minimized; and in phase III, body-mass loss accelerates due to increased protein catabolism as body fat becomes exhausted. We could not discern a significant change in rate of body-mass loss or lean-mass loss with fast duration (Fig. 6), though our sample size was small and thus the power to detect such a change was low. As far as we can see, fat and lean mass were lost in concert. Other studies with fasting small passerines also failed to discriminate either a marked change in rate of mass loss during fasting (Swain 1992) or in the protein content of the catabolized tissue (Klaassen et al. 1997). Whether the differences between these studies with passerines and those with geese and penguins relate to a body size or phylogeny effect is unknown.

The loss and gain of lean mass by passerines during migration has at least two ecological implications at their stopover sites. First, if birds must add lean mass prior to resuming their migratory sojourn, then their need for protein becomes a possible constraint in their diet selection. For example, nectar and low-protein fruits might provide enough energy for fat deposition but might not provide enough protein for lean-mass anabolism. Feeding on insects or particular higher protein fruits might be necessary for migrants. To the extent that it takes longer for birds to search for particular foods to satisfy specific nutrient needs, the stopover time might be increased, lengthening the overall migration time. Evaluation of this possibility requires knowledge of the foods available at stopover sites and their nutritional value.

Second, lean mass is metabolically active tissue, and its deposition will increase energy expenditure. This could represent a metabolic cost associated with an increase in fuel load and could influence the optimal departure fuel load in time-minimizing migrants (Klaassen and Lindström 1996).

Catabolism of Specific Organs

What body tissues are involved in the apparent catabolism and anabolism of lean mass in migratory passerines? We were particularly interested in whether the gastrointestinal tract and liver might be catabolized, because this could then compromise a migrant’s ability to digest, absorb, and process food rapidly during its stopovers to refuel. Also, birds fast during long flights and in some cases do not eat during stopover. Studies in rodents and chickens have shown that fasting decreases intestinal enterocyte number and villous height, and this is reflected in lower intestinal mass and nutrient absorption rate (reviewed in Karasov (1988)). Hume and Biebach (1996) showed that fasting garden warblers during the migratory phase have reduced small intestine mass.

Based on this information, we expected to see the following three patterns in our data: (1) a positive correlation between gastrointestinal tract and liver mass and lean mass in field-captured blackcaps; (2) lower stomach, intestinal, and liver mass
in captive fasted versus fed birds; and (3) that freshly caught migrants will be intermediate between these two groups, some will have been fasting and some will be recovering by feeding or even be hyperphagic. Observations with field-caught blackcaps were consistent with the pattern 1 (Fig. 7), though interpretation can be confounded by differences in structural size. Again, we used tarsus length as a measure of structural size and found that it explained an insignifiant amount of the variation in gut mass, but, as pointed out above, perhaps we chose a poor measure. Thus, the experiments with captives provided an important confirmation, and analyses of their organ masses were consistent with patterns 2 and 3 (Table 3).

Apparently, decline in the gastrointestinal organs and liver accounted disproportionately for decline in lean mass. Though these organs made up about 11% of the lean mass, 44% of the decline in lean mass during a 3-d fast was represented by decline in mass of these organs. The slope of the reduced major axis regression in Figure 7 (0.31 g organs g⁻¹ lean mass minus the organs) also indicates disproportionate catabolism of gastrointestinal organs and liver in field-caught blackcaps.

The time course for intestinal changes with fasting and refeeding has not been studied in birds, but laboratory mice fasted for only 1 d reduce small intestine mass by 30% (Diamont and Karasov 1984), and reductions in intestine length, mass, and nutrient absorption have been observed in rats after 2 d of fasting (Karasov and Diamond 1983). Considering that the half-life for replacing enterocytes is at least a day in small birds (Immondi and Bird 1966; Smith and Peacock 1989), it may take several days to rebuild completely an intestine atrophied by fasting. Conceivably, during such a recovery interval, hyperphagia might not be possible, and this could be the basis for the absence of immediate body-mass gain in some migratory birds at stopover sites (Langslow 1976; Kaassen and Biebach 1994), including blackcaps at Midreetheh Ben-Gurion (Gannes 1997).

Summary

Our primary goals were to validate methods to measure lean mass and fat in migrants and to quantify how much of the body-mass change of migrants might be lean mass. We found that, using TOBEC and deuterium dilution, we could accurately measure lean mass with a precision of a few percent and fat mass with a precision of between 10% and 15% By three different approaches, we found that for every gram of body mass gained or lost by a spring migrant, 37%-42% of the change was in the lean-mass component. Further, we found that a disproportionate amount of the lean mass lost was in the mass of the stomach, small intestine, and liver, important organs for processing foods during a stopover. The ecological implications are that migrants' need during stopover for protein to rebuild lean mass could constrain diet selection and require increased foraging time, thus slowing mass gain and lengthening overall migration time. Also, stopover time may be lengthened if time is required to rebuild atrophied liver and gastrointestinal organs important in food digestion and assimilation. Alternatively, atrophy of these organs offers a possible benefit in reduced wing-loading and hence gains in flight distance and reduction in the required number of stopovers.

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