TECHNICAL COMMENT

Measuring Fat Mass in Small Birds by Dual-Energy X-Ray Absorptiometry

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Introduction

In numerous studies, body condition of animals trapped in the wild has been expressed either as a function of body mass or as a ratio of body mass to some linear body measurement, such as snout-vent length in mammals, or simply by visual assessment of stored fat (Brown 1996). However, using body mass or a body mass : length ratio is problematic in that neither measure truly accounts for body composition and, depending on the source of additional body mass, be it water, fat, carbohydrates, or protein, this method may yield different, even spurious, results. Visual estimates of fat reserves are qualitative at best. Despite these limitations, biologists rely on "body condition," so measured, as a variable in many analyses, including animal decisions in game theory and other models (Elissa et al. 1999; Pasquet et al. 1999), which may lead to cascades of incorrect conclusions with serious theoretical and applied implications.

Different direct and nondestructive methods have been used to measure body condition. These methods include the ratio of total body water to body mass (Karasov and Pinshow 1998), total body electrical conductivity (TOBEC; Walsberg 1988), or bioelectrical impedance analysis (BIA; Nyboer 1991). Each method has its advantages and disadvantages, which are discussed in the associated references.

Recently, it has become possible to measure bone mineral density, fat mass, and lean body mass, and thus body composition, in small (10–50 g) live mammals using dual-energy x-ray absorptiometry (DXA) with acceptable accuracy and precision in mice (Nagy and Clair 2000) and rats (Libouban et al. 2002). Dual-energy scanning is based on the principle that in the diagnostic x-ray energy range, essentially all x-ray interactions are either through photoelectric absorption or Compton scattering, which have different energy dependence. These in turn have different dependence on atomic number and electron density. Thus, from measurements of the attenuation of x-ray beams of two different effective energies, it is possible to solve for Compton scattering and photoelectric absorption in the material being traversed. As different tissues have different compositions and therefore different attenuation properties, it is possible to obtain tissue signature information from the same two scans. Since there are only two types of attenuation mechanisms, unless the absorber has an absorption edge in the energy range being used, it is possible to separate the image into only two components such as bone and soft tissue, which have quite different average atomic numbers (Pietrobelli et al. 1996).

The only previous study using DXA for estimating body composition in birds that we are aware of is that of Mitchell et al. (1997). They used a Lunar DPX-L designed for humans and showed that DXA values of total body fat in chickens were a function of the scanning program, mode, and also the size of the bird. None of the scan modes provided accurate measurements of the fat content of chickens <2 kg in mass (Mitchell et al. 1997).

Here we report the first use of DXA to assess fat, lean, and total body masses in small migrating birds. Initially, we scanned 10 freshly dead birds and then used a second set of live birds to independently validate these measurements. In both cases we compared DXA values to chemical fat extraction. Precision was estimated by calculating the coefficient of variation (CV). We found that fat mass had the greatest CV for live birds (4.3%), while body mass had the lowest (0.17%) for feathered birds. Our results are in the range of the CV values reported in other studies and in accord with the general trend where CV values of body mass and lean mass are lower than CV values of fat mass. As in studies with mammals (Nagy and Clair 2000; Black et al. 2001; Lauten et al. 2001; Lukaski et al. 2001),

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Table 1: Coefficient of variation in percentage of DXA from duplicate or triplicate scans of body mass, lean mass, and fat mass

Variable	Feathered Birds ($n = 10$)	Plucked Birds ($n = 10$)	Live Birds ($n = 7$)	Feathered Birds ($n = 7$)	Plucked Birds ($n = 7$)
Body mass (g)	.17 ± .24 (0–.7)	.19 ± .25 (0–.5)	1.28 ± 1.80 (0–5.0)	.21 ± .28 (0–.4)	.24 ± .34 (0–.9)
Lean mass (g)	.26 ± .41 (0–1.2)	.18 ± .24 (0–.6)	1.87 ± 1.70 (0–4.8)	.47 ± .36 (0–.9)	.16 ± .27 (0–.6)
Fat mass (g)	.90 ± 1.21 (0–2.7)	.62 ± 1.31 (0–3.3)	4.92 ± 2.65 (0–6.1)	1.71 ± 1.61 (0–3.4)	2.06 ± 2.57 (0–4.9)

Note. Results are for 10 feathered and plucked dead birds (*Phylloscopus collybita, Luscinia svecica*, and *Calidris minuta*) and for seven live and freshly killed feathered and plucked birds (*L. svecica* and *Sylvia melanocephala*). Results are presented as mean ± SD, with the range shown in parentheses.

DXA values of plucked birds in both experiments significantly overestimated body mass, fat mass, and lean mass for at least three possible reasons: (1) the presence of feathers; (2) the DXA device was calibrated against a Lucite phantom designed according to rat tissue densities; and (3) petroleum ether, the fat solvent used in this study, extracts mainly triglycerides, which comprise about 84% of total lipids. Using DXA-measured data and the chemical and the gravimetric analyses for fat and lean body masses, we derived a predictive equation that takes feather mass into account to more accurately predict body fat mass in small birds. We found no significant difference between observed and predicted chemical fat mass ($t = -0.42$, $P > 0.19$).

Material and Methods

We used a Lunar PIXImus 2 (software version 1.4) to estimate fat mass in small migrant passerine and shore birds. The PIXImus 2 is a fully integrated densitometer designed to estimate bone mineral density and body composition in small mammals. First, we measured the fat mass of fresh carcasses of migrants that met accidental deaths, which were collected at the International Birding and Research Centre, Eilat, Israel, during the spring migration. Second, we made measurements on live birds.

Experiment 1: Dead Birds

We made measurements on three chiffchaffs *Phylloscopus collybita* (~7 g), three blue throats *Luscinia svecica* (~13 g), and four little stints *Calidris minuta* (~22 g). Initially, we precisely followed the manufacturer's calibration instructions, using the PIXImus QC mouse phantom (bone mineral density = 0.0635 g/cm^2; fat = 11.4%) for its field calibration. Thereafter we did daily quality control checks with the phantom. Each bird was placed on its ventral surface on the positioning tray and scanned at least twice. The length of some of the birds, all *C. minuta* and one *L. svecica*, exceeded the positioning tray length (85 mm). In these cases, the torso was maintained within the imaging area, while the tarsometatarsi and feet were outside the imaging area. Birds that were scanned without their tarsometatarsi and feet were also weighed and chemically analyzed for fat content without these parts.

Immediately after the initial scans, we carefully plucked the birds of every possible feather, rescanned each twice, and prepared the carcass for chemical fat extraction. We collected and weighed all the feathers.

The freeze-dried carcasses were weighed, ground in a household coffee mill into fine uniform particles, and weighed again and stored at −20°C. Fat mass (m_f) was measured according to Bigogno (1999). In brief, fat was extracted from duplicate, 1-g samples of the carcass, each in a Teflon tube containing 14 mL of petrol-ether. The tubes were than incubated for 1 h at 35°C, and thereafter the mixture was centrifuged for 10 min at 3,500 rpm. The supernatant was decanted to a preweighed 50-mL boiling flask, and the pellet was reextracted with another 14 mL of petrol-ether. This procedure was repeated three times. Each time, we vaporized the petrol-ether from the Teflon tubes using a Buchi Rotavapor (R-114) in a water bath at 35°C. Finally, fat mass was calculated as the difference between the mass of the fat-containing flask and the flask's initial mass.

Body mass (m_b) was measured by DXA and by gravimetry, m_f was measured by DXA and by chemical analysis, and lean mass (m_l) was calculated by subtracting m_f from m_b. We used the same balance for all the measurements made in this study, except when small samples were weighed in the laboratory; there we used an analytical balance (±0.0001 g; Precisa 40SM-200A).

Experiment 2: Live Birds

We captured six blue throats (~15 g) and one Sardinian warbler *Sylvia melanocephala* (~8 g) in Rybachy-type funnel traps, mist nets, and wire-floored bow net traps at the International Birding and Research Centre during the spring migration and scanned them, as described above, within 1 h of capture. To immobilize the birds during the scanning procedure, we covered their eyes with a small mask made of soft black cloth. The eyes, beak, and the mask were excluded from the DXA measurement. On completion of DXA measurements, we killed the birds by cervical dislocation and stored them on ice. We transferred the carcasses to the laboratory and made exactly the same measurements on them as described above for feathered and plucked freshly dead birds and did the same statistical analyses. We received permission to kill the birds according to the guidelines of the Israeli Nature and National Parks Protection Authority (permit 2002/14028).

Table 2: Least squares linear regression equations of the relationships between total body, lean, and fat masses

	n	r^2	F	P
(1) $m_{b,DXAF} = 1.1m_{b,F} + .73$	10	.996	2,070	<.001
(2) $m_{b,DXAP} = 1.006m_{b,P} + 1.6$	10	.98	441	<.001
(3) $m_{f,DXAF} = 1.07m_f + 1.3$	10	.84	41.1	<.001
(4) $m_{f,DXAP} = .74m_f + 1.2$	10	.89	65.2	<.001
(5) $m_{l,DXAF} = 1.05m_{l,f} + .24$	10	.99	1,080	<.001
(6) $m_{l,DXAP} = .99m_{l,P} + 1.16$	10	.98	250	<.001
(7) $m_{b,DXAL} = 1.03m_{b,L} + .57$	10	.97	167	<.0001
(8) $m_{b,DXAF} = 1.21m_{b,F} + .08$	10	.995	944	<.0001
(9) $m_{b,DXAP} = 1.19m_{b,P} - .41$	7	.99	443	<.0001
(10) $m_{f,DXAL} = 1.19m_f - 1.81$	7	.61	7.76	<.04
(11) $m_{f,DXAF} = .76m_f - 1.80$	7	.76	14.69	<.01
(12) $m_{f,DXAP} = .58m_f - 1.12$	7	.71	12.36	<.02
(13) $m_{l,DXAL} = 1.06m_{l,L} - 1.59$	7	.975	41.53	<.0001
(14) $m_{l,DXAF} = 1.25m_{l,F} - 2.0$	7	.996	1,414	<.0001
(15) $m_{l,DXAP} = 1.24m_{l,P} - 1.81$	7	.985	246	<.0001

Note. As measured by gravimetry and by chemical analysis in dead birds in experiment 1 (Eqq. [1]–[6]) and in experiment 2 (Eqq. [7]–[15]). $m_{b,DXAF}$ = DXA-measured body mass in feathered birds; $m_{b,DXAP}$ = DXA-measured body mass in plucked birds; $m_{f,DXAF}$ = DXA-measured fat mass in feathered birds; $m_{f,DXAP}$ = DXA values of fat mass in plucked birds; $m_{l,DXAF}$ = DXA-measured lean mass in feathered birds; $m_{l,DXAP}$ = DXA-measured lean mass in plucked birds; $m_{b,DXAL}$ = DXA-measured body mass in live birds; $m_{f,DXAL}$ = DXA-measured fat mass in live birds; $m_{l,DXAL}$ = DXA-measured lean mass in feathered birds; $m_{b,F}$ = gravimetrically measured body mass in feathered birds; $m_{b,P}$ = gravimetrically measured body mass in plucked birds; m_f = fat mass measured by chemical analysis; $m_{l,F}$ = lean mass in feathered birds calculated by subtracting chemically analyzed fat mass from body mass of feathered birds; $m_{l,P}$ = lean mass in plucked birds calculated by subtracting chemically analyzed fat mass from body mass of plucked birds; $m_{b,L}$ = gravimetrically measured body mass of live birds; $m_{l,L}$ = lean mass of live birds calculated by subtracting chemically analyzed fat mass from body mass of live birds.

Statistics

We calculated the CV from the DXA measures for each bird to determine the precision of its use in measuring m_b, m_l, and m_f. To assess the accuracy of the technique, we compared DXA and chemical-extraction data from experiment 1 on dead birds with paired *t*-tests, while DXA data from live birds in experiment 2 were compared by one-way ANOVA and paired *t*-tests, where appropriate. The m_b, m_f, and m_l from the DXA measurements, chemical extraction, and gravimetry were analyzed by least squares linear regression. To relate the DXA data and the chemical and the gravimetric data, we used the following indices of accuracy: (1) absolute error (residual) = (predicted − measured), (2) relative error = (100 × [predicted − measured]/measured), and (3) the standard error (SE) of the estimate.

We generated a predictive linear regression equation relating adjusted fat mass values from DXA to fat mass values from the chemical analysis in experiment 2. Adjusted fat mass takes into account feather mass and differences in fat mass measured by DXA in live feathered and dead feathered and plucked birds. We also determined the relative errors associated with estimates of fat mass from chemical analysis values obtained for dead birds in experiment 1. We followed the methodology of Mayer and Butler (1993) for statistical validation of predicted versus observed data, using parametric paired *t*-tests to compare means and linear regression analysis to test for differences in intercepts and slopes. In the analysis, we used only duplicate scans that were different by less than ±0.2 g fat. We used two-tailed tests throughout, and a P value <0.05 was considered significant.

Results

Dead Birds

The mean CV for DXA scans in individual feathered and plucked dead birds ranged from a low of 0.17% for body mass to a high of 0.9% for fat mass in feathered birds and ranged from a low of 0.18% for lean mass to a high of 0.6% for fat mass in plucked birds (Table 1). DXA values of m_b in both feathered ($m_{b,DXAF}$) and plucked ($m_{b,DXAP}$) birds were significantly correlated with values of body mass measured with the analytical balance in feathered birds ($m_{b,F}$) and in plucked birds ($m_{b,P}$; Eqq. [1], [2] in Table 2).

DXA values of m_f of feathered ($m_{f,DXAF}$) and plucked ($m_{f,DXAP}$) birds were both significantly correlated with values obtained by chemical extraction (m_f; Eqq. [3], [4] in Table 2). DXA values for m_l for feathered ($m_{l,DXAF}$) and plucked ($m_{l,DXAP}$) birds were both significantly correlated with lean mass, which was calculated by subtracting chemically analyzed fat mass from body mass of plucked feathered birds ($m_{l,F}$) and from body mass of plucked birds ($m_{l,P}$; Eqq. [5], [6] in Table 2).

Body mass, fat mass, and lean mass estimated by DXA in feathered and plucked birds were significantly different ($t = 5.28$, $P < 0.001$; $t = 4.78$, $P < 0.001$; $t = 3.35$, $P < 0.01$, respectively; Table 3). Body mass, fat mass, and lean mass estimated by DXA in plucked birds were significantly higher than when measured by chemical analysis or gravimetry ($t = 0.02$, $P < 0.001$; $t = -4.36$, $P < 0.002$; $t = -4.57$, $P < 0.002$, respectively; Table 3). Fat mass of plucked birds was estimated more accurately than in feathered birds, and body mass and lean mass of feathered birds were estimated more accurately than in plucked birds (Table 4).

Live Birds

The mean CV for DXA scans in feathered and plucked live birds ranged from a low of 1.27% for body mass to a high of 4.29% for fat mass. In the same birds, after they were freshly killed, CV for mean body mass ranged from 0.21% to 1.71%

Table 3: Comparison between fat mass, lean mass, and body mass

Variable	DXA, Feathered Birds ($n = 10$)	DXA, Plucked Birds ($n = 10$)	Chemical Analysis and Gravimetric Measurements of Plucked Birds ($n = 10$)
Body mass (g)	17.9* ± 6.7 (9.1–26.1)	16.0** ± 5.7 (9–23.7)	14.2 ± 5.6 (6.8–21.5)
Fat mass (g)	3.7* ± 1.4 (2.4–6.9)	2.9** ± .96 (2.0–4.8)	2.3 ± 1.2 (.6–3.4)
Lean mass (g)	14.2* ± 6.1 (6.1–21.8)	13.1** ± 5.2 (6.0–21.2)	12.1 ± 5.8 (4.5–18.6)

Note. Results are for 10 feathered and plucked birds (*Phylloscopus collybita, Luscinia svecica*, and *Calidris minuta*) measured by dual energy x-ray absorptiometry (DXA) and by chemical fat extraction method and gravimetric analysis. Results are presented as mean ± SD, with the range in parentheses. Comparisons were made by paired *t*-tests.

* Significant differences between DXA values of feathered and plucked birds.

** Significant differences between DXA values of plucked birds and chemical and gravimetric analysis.

for fat mass in feathered birds and ranged from a low of 0.16% for lean mass to a high of 2.06% for fat mass in plucked birds (Table 1). CV for body mass and lean mass were lower than for fat mass, and these values were always lower in dead feathered and plucked birds than in live birds.

As in dead birds, DXA values for body mass of intact live birds ($m_{b,\,DXAL}$) were significantly correlated with their body mass ($m_{b,\,L}$) measured by gravimetry at the field site (Eq. [7] in Table 2). Similarly, the DXA measures of mass in the same birds, freshly killed, and either still feathered ($m_{b,\,DXAF}$) or plucked ($m_{b,\,DXAP}$), were also significantly correlated with these masses measured by gravimetry in the laboratory ($m_{b,\,F}$ or $m_{b,\,P}$; Eqq. [8], [9] in Table 2). DXA values for fat mass of live birds ($m_{f,\,DXAL}$) and in the same birds, freshly killed ($m_{f,\,DXAF}$) and plucked ($m_{f,\,DXAP}$), were significantly correlated with values obtained by chemical analysis (m_f; Fig. 1; Eqq. [10]–[12] in Table 2). Because DXA measures of body mass and fat mass were significantly correlated with values obtained by chemical extraction and gravimetry, calculated lean mass for live ($m_{l,\,DXAL}$), feathered ($m_{l,\,DXAF}$), and plucked ($m_{l,\,DXAP}$) birds were autocorrelated with calculated values of the chemical analysis and the gravimetry measurements ($m_{l,\,L,\,F,\,P}$; Eqq. [13]–[15] in Table 2).

In experiment 2, m_f of feathered, dead birds was estimated more accurately than m_f of live or dead plucked birds (Table 4). Also in experiment 2, m_b and m_l of feathered dead birds

Table 4: Error indices of a comparison between DXA-measured variables (dependent variables) and the same variables measured by gravimetric or chemical fat extraction analysis (independent variables)

	Dependent Variables (g)	Independent Variables (g)	SE of Estimate (g)	Mean Absolute Error (g)	Mean Relative Error (%)
Experiment 1:					
Equation 1	($m_{b,\,DXAF}$)	($m_{b,\,F}$)	.44	∝0 ± .42 (−.77 to .53)	.05 ± 2.35 (−4.27 to 2.39)
Equation 2	($m_{b,\,DXAP}$)	($m_{b,\,P}$)	.80	∝0 ± .76 (−1.79 to 1.01)	−.24 ± 4.01 (−8.98 to 5.21)
Equation 3	($m_{f,\,DXAF}$)	(m_f)	.61	∝0 ± .57 (−.56 to .98)	−1.60 ± 18.38 (−16.42 to 32.28)
Equation 4	($m_{f,\,DXAP}$)	(m_f)	.34	∝0 ± .31 (−.54 to .32)	−.87 ± 14.02 (−26.25 to 19.23)
Equation 5	($m_{l,\,DXAF}$)	($m_{l,\,F}$)	.55	∝0 ± .52 (−.97 to .42)	.36 ± 4.39 (−9.03 to 4.98)
Equation 6	($m_{l,\,DXAP}$)	($m_{l,\,P}$)	.8	∝0 ± .57 (−.99 to 1.23)	−.10 ± 4.45 (−8.89 to 8.67)
Experiment 2:					
Equation 7	($m_{b,\,DXAL}$)	($m_{b,\,L}$)	.51	∝0 ± .48 (− 1.06 to .34)	−.03 ± 3.20 (−7.67 to 2.24)
Equation 8	($m_{b,\,DXAF}$)	($m_{b,\,F}$)	.27	∝0 ± .24 (−1.06 to .34)	−.06 ± 1.37 (−7.67 to 2.24)
Equation 9	($m_{b,\,DXAP}$)	($m_{b,\,P}$)	.37	∝0 ± .34 (−.40 to .55)	−.04 ± 2.01 (−2.81 to 3.44)
Equation 10	($m_{f,\,DXAL}$)	(m_f)	.18	∝0 ± .57 (−.56 to .98)	−.56 ± 8.30 (−14.28 to 6.33)
Equation 11	($m_{f,\,DXAF}$)	(m_f)	.14	∝0 ± .17 (−.11 to .19)	−.32 ± 6.15 (−10.26 to 8.33)
Equation 12	($m_{f,\,DXAP}$)	(m_f)	.12	∝0 ± .11 (−.07 to .23)	−.55 ± 7.47 (−6.38 to 14.37)
Equation 13	($m_{l,\,DXAL}$)	($m_{l,\,L}$)	.46	∝0 ± .39 (−.80 to .47)	−.01 ± 3.10 (−6.65 to 3.06)
Equation 14	($m_{l,\,DXAF}$)	($m_{l,\,F}$)	.20	∝0 ± .17 (−.16 to .31)	−.03 ± 1.11 (−1.0 to 1.94)
Equation 15	($m_{l,\,DXAP}$)	($m_{l,\,P}$)	.47	∝0 ± .38 (−.53 to .60)	−.11 ± 2.69 (−4.16 to 4.13)

Note. Results are for 10 dead feathered and plucked birds (*Phylloscopus collybita, Luscinia svecica*, and *Calidris minuta*) and for seven live and then freshly killed feathered and plucked birds (*L. svecica* and *Sylvia melanocephala*). Equation numbers refer to the appropriate equations in Table 2. Results are presented as mean ± SD, with the range in parentheses. As the absolute mean, errors were all $<1 \times 10^{-5}$; we have listed them as ∝0. Abbreviations as in Table 2.

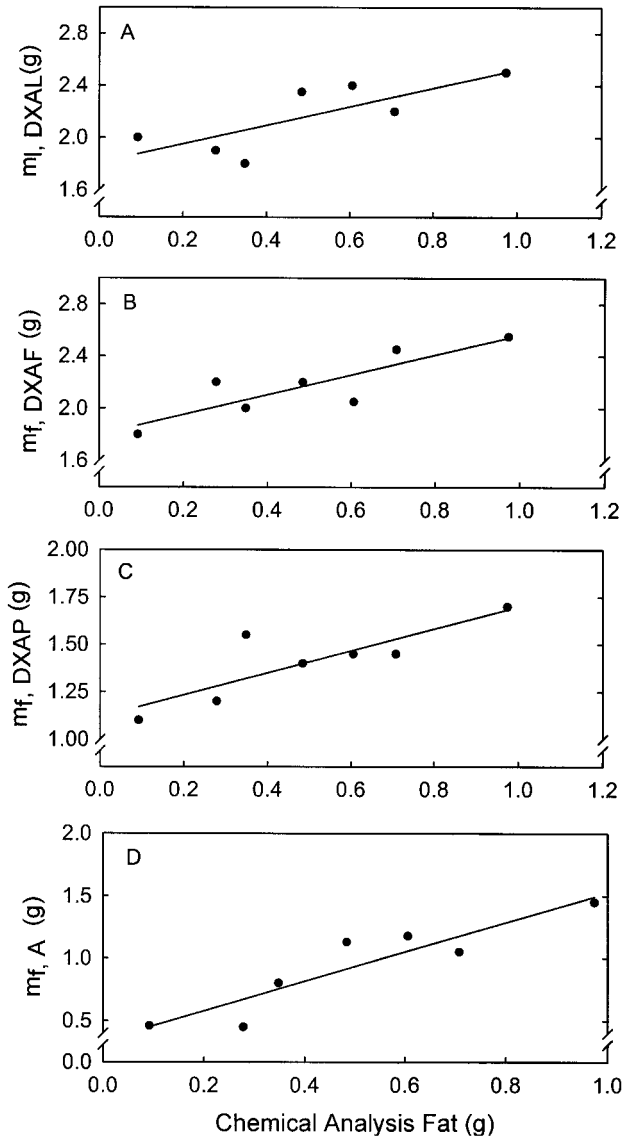

Figure 1. Relationships between fat mass in live (*A*), feathered (*B*), and plucked (*C*) birds and adjusted fat mass (*D*) versus fat mass measured by chemical extraction in six blue throats *Luscinia svecica* and one Sardinian warbler *Sylvia melanocephala*. $m_{l,\ DXAL}$ is DXA-measured fat mass in live birds; $m_{f,\ DXAF}$ is DXA-measured fat mass in dead feathered birds; $m_{p,\ DXAP}$ is DXA-measured fat mass in plucked birds; $m_{f,\ A}$ adjusted fat values (see "Discussion").

were estimated more accurately than these masses for live and plucked birds (Table 4). One-way ANOVA revealed that the m_f of dead plucked birds, estimated by DXA, was significantly lower than the fat masses of both live and dead feathered birds ($F = 20.92$, $P < 0.001$; Table 5). As in experiment 1 on dead birds, body mass, fat mass, and lean mass estimated by DXA in dead, plucked birds in experiment 2 were significantly higher than the values from chemical analysis and gravimetric measurements ($t = 8.65$, $P < 0.001$; $t = -14.66$, $P < 0.001$; $t = -4.07$, $P < 0.007$, respectively; Table 5).

Discussion

In this study, we validated the use of DXA to determine body composition in birds, and we examined its precision and accuracy with respect to gravimetry and a chemical fat extraction. We found that fat mass had the greatest CV (4.3% for live birds; Table 1), while body mass had the lowest (0.17% for feathered birds; Table 1). Although there are no previous reports in the literature of the precision of body composition measurement by DXA in small birds, there are reports on small mammals. For example, Rose et al. (1998) determined the CV for total body bone mineral density (TBBD), lean mass, and fat mass, which were 2.5%, 1.1%, and 11%, respectively, in a single rat measured with a human DXA machine with small-animal software installed. Bertin et al. (1998) determined the CV for TBBD, lean total mass, and fat mass (1.7%, 1.1%, and 4.7%, respectively). Lukaski et al. (2001) reported a CV of <1% for body mass and CV values ranging from 2.6% to 8.9% for fat mass in 49 male rats using a Hologic QDR 2000W instrument and the manufacturer's small-animal module software. Nagy and Clair (2000) reported the CV for TBBD, body mass, lean total mass, and fat mass (0.84%, 1.6%, 2.2%, and 0.86%, respectively) in 25 mice measured with a Lunar PIXImus1. Hunter and Nagy (2002) reported CV values ranging from 4.14% for fat mass to 1.63% for lean mass in 34 female collared lemmings (*Dicrostonyx groenlandicus*) using a Norland pDEXA Sabre densitometer. Our results are in the range of the CV values reported in these studies and in accord with the general trend where CV values of body mass and lean mass are lower than CV values of fat mass.

Precision in DXA measurements is affected by the positioning of the animal subject, by the software used, and by the ability of the operator to define the same region of interest (Grier et al. 1996; Nagy and Clair 2000) each time a scan is made. In this study, we did not anesthetize the birds but instead used an unpublished technique for "calming" them. It is possible that this technique is less effective at immobilizing animals than anesthesia and that therefore minor movements occurred during the scanning procedure that caused the observed differences between scans. Other possible sources of error lie in the hardware and in the software that controls it. Each day, we performed the required quality control test using the manufacturer-supplied mouse phantom. Over the course of the measurements ($n = 17$), total bone mineral density (TBMD) and "percent fat" of the phantom had CVs of 0.4% (i.e., 0.0635 ± 0.0002% fat) and 6.9% (12.1 ± 0.83), respectively. If such variation for percent fat mass is linearly extrapolated to a bird carcass of 14.6 g (Table 5), the estimated standard deviation is 0.059 g for fat mass and lean mass, which translates into a CV of 0.34 and 0.25 for fat mass and lean mass, respectively.

Table 5: Comparison between total fat mass (m_f), lean mass (m_l), and body mass (m_b)

Variable	DXA, Live Birds ($n = 7$)	DXA, Feathered Birds ($n = 7$)	DXA, Plucked Birds ($n = 7$)	Chemical and Gravimetric Measurements of Plucked Birds
Body mass (g)	14.8 ± 2.8 (9.1–17.8)	16.8 ± 3.3 (9.5–19.6)	14.6* ± 3.2 (7.7–16.5)	12.5 ± 2.7 (6.8–15.0)
Fat mass (g)	2.2[a] ± .3 (1.8–2.5)	2.2[a] ± .3 (2.0–2.8)	1.4[b,*] ± .2 (1.1–1.6)	.5 ± .3 (.09–.1)
Lean mass (g)	12.6 ± 2.7 (7.1–15.4)	14.6 ± 3.1 (7.7–16.3)	13.2* ± 3.0 (6.6–15.7)	12.0 ± 2.4 (6.7–14.0)

Note. Results are for seven live and then freshly killed feathered and plucked birds (*Luscinia svecica* and *Sylvia melanocephala*) measured by dual x-ray absorptiometry (DXA) and by chemical and gravimetric analysis. Results are presented as mean ± SD, with the range in parentheses. Different letters within a row indicate significant differences ($P < 0.05$) between DXA values (one-way ANOVA).

* Significant differences ($P < 0.05$) between DXA values and chemical and gravimetric analysis for plucked birds (paired *t*-tests).

We tested the accuracy of the Lunar PIXImus2 by comparing the DXA results with those obtained by gravimetry and chemical analysis. DXA values of plucked birds in both experiments significantly overestimated body mass, fat mass, and lean mass (Tables 3, 5). There are several possible explanations for this overestimation. First, and most obviously, the Lunar PIXImus2 was calibrated against a Lucite phantom designed according to rat bone density, which may lead to systematic errors when used to measure densities in other small animals (Nagy and Clair 2000). Second, the orientation of the animal in the path of the x-ray beam is important because DXA can discriminate only two tissues in any single pixel (fat mass and lean mass or bone and nonbone soft tissue). We should point out that to fit the mice they studied on their DXA's scanning stage, Nagy and Clair (2000) removed the heads, although the manufacturer's calibration included the head. In this study, we included the heads of the birds; however, in the case of larger birds with body masses within the range of the PIXImus, we assume that the head may be removed because, in birds, it contains little fat relative to the rest of the body.

The soft tissue within a pixel that contains bone must be interpolated (Pietrobelli et al. 1996). For soft tissue above or below the spine in the abdominal region of rats, the interpolation process is based on the fat percentage of the adjacent tissues; however, in the limbs, for instance, there may not be adequate soft tissue to make the interpolation. In the latter case, the soft tissue composition is reconstructed by applying the average percentage body fat for the entire animal (Nagy and Clair 2000); fat will be overestimated if this percentage is greater than the percentage fat of the limb. For example, Speakman et al. (2001) showed that the DXA unit that they used, a Hologic QDR-1000 W pencil-beam system, had problems identifying fat in skeletal muscle tissue in dogs and cats. They suggested two explanations: (1) that the error appears to be related to lean tissue hydration and (2) that skeletal muscle is generally located close to areas of bone and hence the error may be linked to errors in the interpolation process by which soft tissue composition is reconstructed for those pixels in which bone is also detected (Speakman et al. 2001). Third, we used petroleum ether as our extraction solvent. Petroleum ether extracts mainly triglycerides, which comprise approximately 84% of the total lipids (Bondi 1987), whereas DXA estimates total lipids.

Overestimation of fat mass by DXA was more pronounced in live birds than in dead ones, 280% compared with 126%, respectively. The main difference between these experiments was the "condition" of the birds. In experiment 1, the birds we analyzed were in the midst of migration and had a large range of fat masses (0.6–3.4 g), while the birds that we caught in experiment 2 were wintering birds, with a much smaller range of fat masses (0.09–0.1 g). This may indicate that the PIXImus2 is less accurate at low fat mass than at high, resulting in erroneous results.

The slopes and intercepts of the regression of DXA values on chemically obtained values were always lower for the plucked than for the feathered birds, live or dead (Eqq. [3], [4], [10]–[12] in Table 2), indicating that DXA mistakes feathers for fat. Although the quantity of fat in feathers is small (1%–2% in various species; Murphy 1996), apparently due to their relatively low density the feathers are perceived by DXA as "fat," and a correction for this needs to be made. We therefore suggest that the DXA device used should be calibrated for each species studied in order to take the feathers into account while chemical extraction for each species is not necessary. Birds should be scanned both feathered and plucked; the feathers should also be weighed. Once the mass of the feathers is known and their "fat content" is also known, we suggest the following transformation, which takes into account feather mass and differences in fat mass measured by DXA in live feathered and dead feathered and plucked birds:

$$\text{Adjusted fat value } (m_{f,A}) = m_{f,\,DXAL} - \text{mean of } [(m_{f,\,DXAL}/m_{f,\,DXAP}) + (m_{f,\,DXAF}/m_{f,\,DXAP})/\text{feather mass}].$$

We regressed the adjusted fat masses calculated for live birds against their m_f measured by chemical analysis, which yields

$$m_{f,\,A} = 1.19m_f + 0.34,$$

where $n = 7$, $r^2 = 0.85$, $F = 27.6$, $P < 0.003$, and SE = 0.16.

We then rearranged this equation, making m_f the dependent variable, and recalculated the chemical analysis values obtained for dead birds in experiment 1. We calculated the mean relative error, excluding two values that were clear outliers (one *Luscinia svecica* and one *Calidris minuta*, with relative error values of 140% and 180%, respectively), to be 0.25% ± 6.27% (−8.9% to +8.4%).

The predictions of our model fit the data set derived in experiment 1 closely. Using a paired *t*-test, the hypothesis that there is no difference between observed and predicted chemical fat mass was not rejected ($t = -1.42$, $P > 0.19$). The regression slope was significantly different from 0 but not significantly different from 1 ($t = 5.26$, $P > 0.003$), and the intercept was not different from 0 ($t = 2.76$, $P < 0.04$). Thus, because of the close relationship between DXA-measured data and the chemical and the gravimetric analysis for body, fat, and lean masses, predictive equations may be derived to predict more accurately body fat mass in small birds.

In conclusion, the main advantages of DXA are that it is not destructive, scanning time is relatively short, animal handling time is short, and it is extremely easy to use. It can be safely used in long-term studies, for example, changes in body composition in animals undergoing photoperiod-induced mass gain and loss (Hunter and Nagy 2002), or body composition in migrating birds during the migration period, or at stopover sites. In addition, the technique also measures TBMD and TBBM. The disadvantages of DXA are the initial expense and the need for the subject to be motionless during the scanning procedure. In addition, it tends to overestimate fat mass compared with chemical analysis, but this can be corrected for. Our results also show that DXA is a reasonably accurate method to noninvasively determine fat and lean masses of migrating birds. With the appropriate predictive equations and with new model phantoms designed for specific taxa, the DXA will prove a useful tool for measuring body condition in small endothermic animals.

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