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Estimating lipid and lean body mass in small passerine birds using TOBEC, external morphology and subcutaneous fat-scoring

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To assess regression models for lipid and lean body mass in small birds, we recorded live body mass ± 0.1 g, total body electrical conductivity (TOBEC; from "third generation" TOBEC machine EM-SCAN® SA-3000) or *E-Value*, visual fat score (VisFat), and seven body measurements for 52 migratory passerine birds of 13 species (5–40 g). We determined lipid and lean mass of each bird after petroleum-ether extraction of lipids. We obtained "*net*" *E-Value* (*NEV*) for each scanned bird by subtracting the *E-Value* of the empty bird-restraining tube, because these showed an inverse temperature dependence ($P < 0.005$). Leave-one-out cross validation was used to assess model selection and construct 95% confidence intervals. Although precision of TOBEC increased with bird size (CV of *NEV* vs. live mass: $r = -0.276$, $P = 0.002$) and it explained an increasing proportion of variation in lean mass moving from small- to medium- to large-bird classes of our data, it did no better than head length in single-variable prediction of lean or lipid mass and was included in five of the 14 multivariate models we developed. The best multiple regression to predict lean mass included live weight, VisFat, bill length, tarsus and *lnNEV* (adjusted $R^2 = 99.0\%$); however, the same model lacking only *lnNEV* yielded $aR^2 = 98.9\%$. A parallel to the above pair of models, but predicting lipid mass, yielded $aR^2 = 90.3\%$ and 90.0%, respectively. Subdividing the data by three size classes and three taxa (American redstart *Setophaga ruticilla*, ovenbird *Seiurus aurocapilla*, warblers), best-subset multiple-regression models predicted lean mass with aR^2 from 94.7 to 99.6% and lipid mass with aR^2 from 85.4 to 98.3%. Best models for the size- and species-groups included VisFat and zero to five body measurements, and most included live weight. *lnNEV* was included only in the models for ovenbird (lipid), warblers (lipid), all birds (both), and large birds (both). Actual lipid mass of all birds was more highly correlated with multiple-regression-predicted lipid mass ($r = 0.955$) than with visual subcutaneous fat-scoring ($r = 0.683$). These multiple-regression models predicting lipid content using live-bird measurements and visual fat score as independent variables represent more accurate and precise estimates of actual lipid content in small passerines than any previously published. They are particularly accurate for placing birds into percentage body-fat classes.

Determination of lipid content in wild birds is required in a variety of ecological studies. Many animals use lipid as their main energy reserve, since metabolic energy yield from lipid is more than twice that of carbohydrate (Hochachka 1973). Fat storage may be particularly important in vertebrates inhabiting seasonal or unpredictable environments and constitutes the main fuel for migration in birds (Blem 1990). An important determinant of individual condition and overwinter survival of songbirds is the amount of body fat they can store for metabolic heat production in times of cold stress (Lima 1986, Rogers and Smith 1993). It is possible that protein metabolism may also be employed as a mechanism to survive winter periods of food shortage (Lindström and Piersma 1993), and atrophy/hypertrophy of muscle tissue has been documented in birds during and preceding lengthy migrations, respectively (Piersma and Jukema 1990).

Maintenance of body mass is a strong predictor of annual survival (Johnson et al. 2006) and wintering condition (Studds and Marra 2005) in American redstart *Setophaga ruticilla*, but it is not known which mass components are important. Rogers (2005) recently reviewed models of adaptive body mass (selective agents are food-limitation and predation) in wintering Neotropical migrant birds and pointed out the conflicting predictions they make about changes in mass components (fat vs. lean mass) with changes in habitat quality. Rogers (1991, 2003, 2005) also argued that visual fat scoring is the only reliable non-invasive means of estimating body fat in small birds. Thus, it is important to determine the degree to which body-mass changes associated with migration and winter are the result of lipid metabolism or represent changes in fat-free body mass and to develop reliable non-invasive techniques for estimating body-mass components.

Condition indices and multiple regression of external body measurements, combined with fat scoring, have shown some promise of estimating total body fat (Conway et al. 1994, Brown 1996, DeLong and Gessaman 2001), but the search for a more precise and accurate non-invasive method continues. Total body electrical conductivity

(TOBEC) is a nondestructive means to estimate lean mass and therefore total lipid mass of live birds, the underlying principles of which were reviewed by Walsberg (1988), Brown (1996), and Scott et al. (2001). The conclusions of most studies evaluating TOBEC have been that the technique is of some predictive value when included with various metric body indices in a multiple regression (Castro et al. 1990, Skagen et al. 1993, Spengler et al. 1995, Burger 1997, Golet and Irons 1999). Most of these studies involved birds much heavier than 20 g live mass, and all employed earlier model EM-SCAN machines. Several studies have concluded that accuracy increases with increasing subject size (e.g. Asch and Roby 1995). These studies also were restricted to either single species or a few related species. We sought an accurate, non-destructive method to estimate lipid content of various wintering Neotropical migrant species, especially of warblers <25 g. A third-generation EM-SCAN SA-3000 (EM-SCAN 1993) TOBEC machine with 3044 detection chamber (44 mm diameter, or the smallest available) was touted by its manufacturer as specially designed for accuracy with very small subjects (<30 g).

By building and testing multiple-regression models incorporating as predictor variables detailed visual fat-scores, live mass, body measurements, and TOBEC readings for 52 songbirds (5–40 g) of 13 species, we here identify models that most accurately and reliably predict lean and lipid mass – as verified by ether extraction – in a series of small passerines. By using the best subsets of the above predictive variables, we provide prediction equations for body fat that are more precise and accurate than any previously published, particularly for small songbirds (cf. Conway et al. 1994, Meijer et al. 1994, Asch and Roby 1995, Spengler et al. 1995, Burger 1997, DeLong and Gessaman 2001), and this holds true for equations including and not including TOBEC.

Methods

Birds were live-captured at four localities in southeastern U.S. during migration (Sept–Oct 1994 and March–April 1995) and at a site in northeastern U.S. during the summer breeding season, in order to maximize the range of body conditions (see Scott et al. 2001), especially lipid content, of the subject animals. The 52 live-captured individuals were partitioned arbitrarily into three size classes: *Small* (5.0–9.9 g live mass; $n = 18$) included blue-gray gnatcatcher (BGGN) *Polioptila caerulea* (4), prairie warbler (PRAW) *Dendroica discolor* (3), American redstart (AMRE) *Setophaga ruticilla* (7), black-throated blue warbler (BTBW) *Dendroica caerulescens* (3) and hooded warbler (HOWA) *Wilsonia citrina* (1). *Medium* (10.0–21.9 g; $n = 22$) included hooded warbler (2), worm-eating warbler (WEWA) *Helmitheros vermiveros* (3) prothonotary warbler (PROW) *Protonotaria citrea* (2), northern waterthrush (NOWA) *Seiurus noveboracensis* and (4) ovenbird (OVEN) *S. aurocapillus* (11). *Large* (22.0–40 g; $n = 12$) included red-eyed vireo (REVI) *Vireo olivaceus* (4), veery (VEER) *Catharus fuscescens* (2), gray-cheeked thrush (GCTH) *Catharus minimus* (1) and gray catbird (GRCA) *Dumetella carolinensis* (5) (cf appendix 2).

Cardboard tubes of two sizes were constructed to restrain individuals for weighing on a top-loading digital balance (± 0.1 g; LivWt) and placing in the EM-SCAN; each bird was confined in the smallest tube possible and scanned at least five times, and the average recorded as *E-Value*. The cardboard restraint and plastic sleeve into which it and the bird were later placed were separately scanned at the time of each bird-scan, and the average reading for these empty tubes was subtracted from average *E-Value* to obtain *net E-Value (NEV)*. Time and temperature were recorded at each scanning, since Robin et al. (2002) reported that *E-Values* varied inversely with subject temperature, likely due to increasing relative humidity with decreasing temperatures, which increased condensation onto the "inert" restraining tubes. We tested this temperature relationship and then factored it out of subsequent analyses. For each bird we recorded in mm (see Pyle et al. 1987): wing chord (Wing) with a wing ruler, and tarsus length (Tars), tail length (TL), head length (Head), bill = culmen length (BL), bill width (BW), and bill depth (BD), with a dial caliper (± 0.1 mm). Visual subcutaneous fat score (VisFat) was assigned using the technique of Kaiser (1993). All scanning, measuring, weighing and fat-scoring was done by one of us (BJB) after practicing these procedures on several hundred birds in the months preceding the sampling for this study.

Following examination, birds were immediately euthanized by asphyxiation in chloroform and frozen. After thawing, ingesta, including esophageal contents, were removed and weighed wet. Carcasses minus ingesta but otherwise intact, including feathers, were cut into small pieces and placed on metal pans to dry to a constant weight in an 80° C oven. Dried carcasses were then powderized in an electric coffee grinder and mixed by hand to ensure homogeneity. The homogenate for each bird was placed in one or more cellulose thimbles (pre-dried to constant weight) then again dried to constant weight; this "pre-extraction weight" (± 0.001 g) was subtracted from live weight minus ingesta to calculate water weight. Lipids were then extracted in petroleum ether (Soxhlet) using procedures outlined in Dobush et al. (1985). Following lipid extraction, the homogenate in thimbles was oven-dried to constant weight one final time and weighed ± 0.001 g. This final weight (net, minus thimble weight) constituted dry lean mass, and the difference between it and the pre-extraction dry weight constituted the total lipid mass. Lean mass reported herein is live weight minus lipid mass (ingesta were included in lean mass for the same reasons as given by Skagen et al. 1993).

MINITAB Release 11.21 (1996) was used for regression and other statistical analyses. The "best subsets" program was used for independent-variable selection in multiple-regression models with lean mass or lipid mass as the dependent variable. For models with varying numbers of predictors, the model with the largest coefficient of determination, or adjusted R^2 (aR^2) – equivalent to the smallest mean square error – was chosen as best. In cases of ties (≤ 0.1), the model with the lowest (if > 0.1 lower) Mallow's Cp (equivalent to an Akaike estimator of prediction error for the general linear model; Burman and Nolan 1995) was chosen as the more precise; where $CP = (SSEp/MSEm) - (n - 2p)$; n = sample size; p = number of parameters in the model; $SSEp$ = sum of squares error for

the best model with p predictors, and MSEm = mean square error for the model with all m predictors (Minitab 1996). Adjusted R^2s are lower than the raw R^2s typically reported, sometimes by $\geq 5\%$, and the model that yields the highest raw R^2 does not always yield the highest aR^2.

Because we were particularly interested in estimating lipid and lean mass in small warblers, we generated and evaluated separate prediction equations for the subsamples of small birds (<10 g) and of warblers (6.5–21.5 g; n = 36). For completeness, we did the same within the other two size classes. Because prediction of lean or lipid mass from TOBEC regressions is reportedly more accurate when separating even closely related species compared to pooling (Unangst and Wunder 2001), we recalculated single-species best-subset regressions and estimation errors for ovenbird and American redstart, for which we had sufficiently large individual samples. We estimated generalization error for all regressions by the resampling technique leave-one-out cross validation (LOOCV; Dudoit and Van der Laan 2003) for each of the seven data subsets and for each of the two dependent variables by running the model-selection routine n additional times. This generated n estimates of aR^2 and Cp, from which we constructed 95% confidence intervals.

Results

Actual fat-free mass ("Lean") of the 52 birds ranged from 4.8 to 33 g (mean ± SD: 14.25 ± 7.36 g; all raw data from field and lab, plus predicted Lean and Lipid, can be found in Appendix 2). Actual lipid mass ranged from 1.7% to 35% of total live mass (12.0 ± 9.59%, or 2.15 ± 2.39 g actual lipid mass). Visual fat scores ranged from 0 to 8 (2.66 ± 2.74); in our sample we found few cases of intermediate rank (subclasses) for main classes 4–7 (main class 8 has no subclasses; Kaiser 1993). Therefore, our fat-scoring technique effectively offered 21 possible fat classes (ranging from 0.0–8.0), rather than Kaiser's 31. Digestive lumen (food) contents ranged from 0.5% to 4.5% of live mass (1.87 ± 0.88%), and water content ranged from 42.5% to 76.5% (60.47 ± 7.81%).

TOBEC scans of empty bird-restraining cardboard tubes within the plastic carrier gave nonzero readings, which were inversely temperature dependent: for the small tube and carrier (n = 62), r = −0.497 (P < 0.0005); and for the medium tube and carrier (n = 91, r = −0.571, P < 0.001). The machine ceased to function at all above ambient temperature 33–34° C.

Relationship of TOBEC to lean mass

Fat-free mass (Lean) regressed on *E-Value* resulted in an adjusted R^2 (aR^2) = 82.5%, whereas Lean regressed on *NEV* resulted in aR^2 = 88.1%. Since the regression was best with *lnNEV* (aR^2 = 92.3%), *lnNEV* was used to represent TOBEC data in all subsequent multiple regressions. By comparison, Lean regressed on the single morphometric variable, head length, resulted in aR^2 = 93.3%.

TOBEC was more accurate with larger birds, predicting more variation in Lean as body size increased among the three classes (Table 1). TOBEC explained little to none of

Table 1. Summary of precision and accuracy of best-subsets multiple-regression prediction equations for lean and lipid mass in selected subsets of the sample of 52 songbirds (equations given in Appendix 1).

Species/group	Prediction of:	Percentage variation TOBEC expl.[1]	Predictors in best model:	Adj. $R^{2\alpha}$	Lower C.I. $aR^{2\beta}$	X ± SD % error[3]	Max. percent error[3]	Prop. Runs best model[β]
All birds (n = 52)	Lean mass	92.1*	LivWt, VisFat, BL, Tars, *lnNEV*	99.0 (*98.9*)	98.95	−2.85 ± 6.69	16.8	43/52
All birds	Lipid mass	27.0	LivWt, VisFat, BL, Tars, *lnNEV*	90.4 (*90.0*)	90.25	2.8 ± 145.9	424.9	52/52
Large birds (n = 12)	Lean mass	93.3*	LivWt, VisFat, BL, BD, BW, Wing, Tail, *lnNEV*	99.4 (*98.7*)	99.13	−0.048 ± 2.54	6.18	6/12
Large birds	Lipid mass	22.0	LivWt, VisFat, BL, BD, BW, Wing, Tail, *lnNEV*	95.4 (*90.3*)	93.58	−0.23 ± 7.23	15.24	8/12
Medium birds (n = 22)	Lean mass	76.7*	LivWt, VisFat, Tars, Tail	98.5	98.45	−0.111 ± 2.86	7.04	17/22
Medium birds	Lipid mass	0.0	VisFat, Tars, Tail	95.3	94.98	−9.1 ± 72.1	195.1	21/22
Small birds (n = 18)	Lean mass	29.1	LivWt, VisFat, BD, Tail, Head	96.6	95.54	0.2 ± 2.94	5.74	11/18
Small birds	Lipid mass	0.0	LivWt, VisFat, BD, Tail, Head	85.4	83.96	9.6 ± 61.1	137.4	9/18
Warblers (n = 36)	Lean mass	81.3*	LivWt, VisFat, BL, BD, Tail	98.5	98.47	0.054 ± 5.2	15.0	30/36
Warblers	Lipid mass	0.0	LivWt, VisFat, BL, BD, Tail	87.0	86.78	−2.4 ± 101.3	313.0	26/36
Ovenbird (n = 11)	Lean mass	20.3	LivWt, VisFat, Tars, Head	99.6	99.48	0.093 ± 0.497	0.88	8/11
Ovenbird	Lipid mass	0.0	LivWt, VisFat, Wing, Tail, Head, *lnNEV*	99.3 (*96.2*)	95.45	−0.1 ± 5.97	10.8	3/11
Am. redstart (n = 7)	Lean mass	0.0	LivWt, VisFat	94.7	88.93	0.14 ± 1.84	2.76	4/7
Am. redstart	Lipid mass	0.0	VisFat, Head	95.4	93.41	3.8 ± 32.0	66.9	1/7

[1]Represents adjusted R^2 for a simple linear regression with *lnNEV* as the only predictor variable.
*significant at P < 0.05.
[α]aR^2 values in parentheses are given, in 5 cases, for alternative models without *lnNEV*.
[β]Lower limit of 95% confidence interval for adjusted R^2 from LOOCV of "best" model (all P < 0.001); proportion of n runs of variable-selection routine from LOOCV where "best" model was chosen.
[3]Errors are expressed as percentages of actual lean mass and actual lipid mass, respectively, all P < 0.001.

the variation in lean mass among individuals of a single species, especially of small to medium sized songbirds (cf. OVEN, AMRE), but it did explain a significant portion of the variation for the smaller-bodied mixed-species group of warblers (Table 1). TOBEC was also more precise with larger birds, with a significant inverse relationship ($r = -0.276$; $P = 0.002$) between live mass and CV of *NEV*. TOBEC alone had no predictive value for lipid mass for any subset of the data (Table 1).

Multiple regressions including all species

Including all 52 birds, the best multiple regression predicting Lean included LivWt, VisFat, BL, Tars and *lnNEV* and had an $aR^2 = 99.0\%$ ($F = 981.88$; $P < 0.001$; Table 1). The best multiple regression for Lean, but without *lnNEV*, had just as good a fit ($aR^2 = 98.9\%$; $F = 1190.30$; $P < 0.001$) and included the same non-TOBEC predictors (regression equations for these and all subsequent best-subset models are in Appendix 1). Results of LOOCV resampling for all models (summarized in Table 1 and detailed in Appendix 2) show that $\geq$half the n runs chose the same best model as with the entire data set in 12 of 14 predictions, and in the other two, iteration of the single best model produced lower 95% CI for $aR^2 > 90\%$. All LOOCV-derived lower 95% CIs for aR^2 for "best model" were $> 90\%$ except for small-birds Lipid, warblers Lipid, and AMRE Lean (Table 1).

The same set of four predictors as above also was the best linear combination to estimate Lipid ($aR^2 = 90.4\%$; $F = 116.18$; $P < 0.001$; Table 1). The relative error in predicting lipid mass with this model was 0.004 ± 0.728 g ($2.8 \pm 145.9\%$ of the actual lipid mass), with the greatest absolute error being 1.439 g, and the greatest percentage error being 424.9% of the actual lipid mass (Table 1; latter was a 9.2-g HOWA with 0.224 g of actual lipid). This regression-estimated lipid mass correlated more highly with actual lipid mass ($r = 0.955$; $P < 0.0001$) than did VisFat ($r = 0.683$; $P < 0.001$). Removing *lnNEV* from the model yielded a slightly lower coefficient of determination ($aR^2 = 90.0\%$) but also slightly lower average error, identical maximum absolute error and lower percentage errors.

Multiple regressions for single size class

The best-subsets model predicting Lean for small birds (<10 g), with $aR^2 = 96.6\%$ ($F = 96.52$; $P < 0.001$), included LivWt, VisFat, BD, Tail, and Head (Table 1). The error in predicting lean mass was 0.0097 ± 0.2114 g ($0.2 \pm 2.94\%$ of actual lean mass), with the greatest absolute error being 0.3786 g and the greatest percentage error being 5.74% of lean mass (Table 1). Forcing *lnNEV* into the above model did not improve the fit.

Best-subsets regression estimating Lipid for this same small-bird group included the same five predictor variables and yielded $aR^2 = 85.4\%$ ($F = 20.8$; $P < 0.001$; Table 1). The relative error in using this equation to estimate body fat, compared to extracted lipid mass, was -0.0047 ± 0.228 g ($9.6 \pm 61.1\%$), with the greatest absolute error being 0.396 g and the greatest percentage error being 137.4% of actual lipid mass (Table 1). The correlation between estimated lipid mass (by above equation) and actual lipid mass for the sample of small birds was $r = 0.938$ ($P < 0.0001$). Adding *lnNEV* to the above predictors yielded a very similar model with nearly identical coefficient of determination and absolute and percentage error rates.

Optimal multiple-regression equations for the medium and large size classes, predicting both Lean and Lipid, included between three and eight independent variables – generally more, and including *lnNEV* for the large-bird category – and generally had higher aR^2 (Table 1). For the sub-sample of warblers ($n = 36$), *lnNEV* explained somewhat more of the variation in Lean than for the medium size class (Table 1), and best regression models for Lean and Lipid produced similar and slightly lower aR^2, respectively, compared to the medium size class (Table 1). The average and maximum absolute percentage errors of estimated lipid generated from warbler regression models predicting Lipid were similar to those for medium birds (Table 1). Mean error was -0.012 ± 5.16 g of actual lipid mass; maximum error was 1.23 g of actual lipid mass.

Single-species regressions

For ovenbird ($n = 11$), the best model for Lean included LivWt, VisFat, Tars, and Head as predictors ($aR^2 = 99.6\%$; $F = 589.6$; $P < 0.001$) and had the lowest mean ($<0.1\%$) and maximum ($<1\%$) percentage errors of any regression in our study (Table 1). Average absolute error was 0.001 ± 0.005 g, with greatest absolute error $= 0.149$ g. Adding *lnNEV* as a predictor to this model yielded $aR^2 = 99.5\%$ ($F = 391.51$; $P < 0.001$); errors were nearly identical to or slightly greater than those for the model without TOBEC, with 0.0167 ± 0.0884 g average and 0.148 g maximum absolute error, and $0.098 \pm 0.497\%$ average and 1.486% maximum percentage errors. Therefore, the non-TOBEC model was more efficient.

Predicting Lipid from ovenbird data, the best model without TOBEC included LivWt, VisFat, BW, Wing, Tars, Tail and Head as predictors and yielded $aR^2 = 98.3\%$ ($F = 85.07$; $P = 0.002$). The error in predicting actual lipid mass was -0.014 ± 0.049 g ($-1.36 \pm 12.8\%$ of actual lipid mass), with the largest absolute error being 0.104 g and the largest percentage error being 30.9% of actual lipid mass (in the latter case, this error was 0.53% of live mass of a bird that was 1.7% lipid and scored a 0). Adding *lnNEV* to the above model to predict lipid mass increased both accuracy and precision, yielding $aR^2 = 99.3\%$ ($F = 173.24$; $P = 0.006$) for this better model. The error in predicting actual lipid mass was -0.003 ± 0.026 g, with the largest absolute error being 0.055 g, and with lower percentage errors (Table 1).

For American redstart ($n = 7$), the model including only LivWt and VisFat as predictors of Lean yielded $aR^2 = 94.7\%$ ($F = 54.88$; $P = 0.001$); adding *lnNEV* decreased aR^2 to 93.6% ($F = 30.04$; $P = 0.01$). With the 2-predictor model, error in predicting Lean was -0.0119 ± 0.1485 g, and the largest absolute error was 0.212 g (% errors in Table 1). The model including *lnNEV* yielded similar but slightly higher errors and so was not chosen.

Predicting Lipid directly for American redstarts, the best model included only VisFat and Head as predictors and yielded $aR^2 = 95.4\%$ ($F = 63.59$; $P < 0.001$). Error in

predicting lipid mass was -0.0083 ± 0.141 g ($3.8 \pm 32.0\%$ of actual lipid mass, or $0.111 \pm 1.78\%$ of live mass), with the largest absolute error being 0.202 g (this individual was 18.5% fat but predicted to be 16.1% fat, and scored a 2.5) and the largest percentage error being 66.9% of the actual lipid mass (the latter individual was 3.2% fat but predicted to be 5.35% fat, and scored a 0). Adding *lnNEV* to the above model to predict lipid mass yielded $aR^2 = 94.0\%$ ($F = 32.07$; $P = 0.009$), with similar to slightly higher absolute and percentage errors.

Best estimates of lipid mass and percentage body fat

Using the most targeted of the six pairs of regression equations (lipid-mass prediction equations for each of the six species- or body-size-specific models) for each bird in our sample, and assigning observed and predicted percentage lipid (lipid mass/LivWt $\times 100$) to eight 5%-interval classes (0.0–4.9%, 5.0–9.9%, ... 35.0–39.9%), 42 of the 52 birds were predicted to have the same category of percentage lipid as they actually had (Appendix 2). The other 10 all deviated by only one 5% interval (six predicted to be one interval higher and four one interval lower); six of these were in the small-bird category (3 BGGN, 2 AMRE, 1 PRAW), three medium (2 WEWA, 1 NOWA), and one (VEER) large (Appendix 2).

Discussion

Many factors have been found to affect the reliability of TOBEC readings from the EM-SCAN device (reviewed by Scott et al. 2001), including absolute subject size (Asch and Roby 1995), size of the subject relative to the chamber (Golet and Irons 1999), species of the subject animal (Unangst and Wunder 2001), body temperature of the subject (Robin et al. 2002, but see Bachman and Widemo 1999), movement of the subject within the chamber, the presence of metal leg bands (Skagen et al. 1993, but see Castro et al. 1990, Roby 1991), individual hydration state (Roby 1991, Walsberg 1988), position of the animal in the chamber (Walsberg 1988, Castro et al. 1990, Roby 1991), contents of the gastrointestinal tract (Voltura and Wunder 1998) and even salinity of water adhered to feathers (Bachman and Widemo 1999; this presumably would mostly affect TOBEC readings in aquatic species, such as shorebirds and waders). To reduce the effect of some of these potentially confounding variables, we immobilized our subjects with restraining tubes, recorded at least five different *E-Values* only after the readings appeared stable, and subtracted average *E-Values* of the empty tubes, scanned immediately before each subject was scanned in them. At the time of our use of the device in the field (1994) ambient-temperature dependency of EM-SCAN SA-3000 readings was an unanticipated problem that required such "taring" to generate *net E-Values*, without which the readings would have been less meaningful.

When combined with live weight, visible subcutaneous fat score and body proportions, TOBEC explained some additional variance in the prediction of lean mass, especially for birds heavier than 10 or 20 g. However, a multivariate linear equation including just live weight, fat score, bill length, and tarsus length explained 99% of the variation in lean mass across this 5–40 g range of passerines and may be particularly accurate in estimating lipid mass in birds >10 g. The extra expense and logistical problems of using TOBEC in the field for noninvasive lipid mass determination may be deemed undesirable for the small increase in predictive power.

Although TOBEC provides readings that correlate highly with lean mass, because of the greater relative error of predicting the smaller lipid mass by regression on TOBEC (Morton et al. 1991), it has been proposed that multiple-regression models incorporating live body mass be used to estimate lipid mass more precisely (Skagen et al. 1993, Brown 1996); this recommendation is confirmed by most of our regressions. All of our regression models predicting lean mass for groups ranging from single species to size-classes to all birds were highly significant and had average percentage errors of less than 5% and maximum percentage errors of less than 20%. Predicting lipid mass directly produced substantially greater percentage error (see Brown 1996). Average and maximum percentage errors were smaller for the single-species models (see Unangst and Wunder 2001) – impressively so for ovenbird – and surprisingly were reasonably small for the smallest size-class of birds in our sample (5.0 g to 9.9 g live mass). Our most significant result was that direct lipid mass estimation for the entire mixed-species sample of 52 birds achieved $\geq 90\%$ aR^2, with or without TOBEC, that mean percentage error was $<3\%$ (comparable to single-species models; Table 1) and that LOOCV of model selection on this data set had 100% repeatability. This equation's surprising usefulness is partly explained by the relatively great variation across our sample of lipid mass ($CV = 111\%$, lean mass 52%, see Scott et al. 2001). Moreover, maximum percentage errors cited for the direct lipid-estimation equations (Table 1) are misleadingly high, because these apply to individuals with relatively little body fat. Predicted fat of 1 g represents a 100% error if actual lipid content is 0.5 g, yet the absolute error is still small. If estimated lipid is translated into categories based on percentage body fat, this still represents a usefully accurate estimate, given that actual lipid content in our sample of songbirds ranged from 1.7% to 35% (Appendix 2).

For the purposes of comparative studies examining relative amount of body fat in small songbirds, we feel that these best multiple regression models – all of them including visual subcutaneous fat score, most of them including live mass, plus certain morphometric values, and a few including TOBEC scores (primarily the large-birds category, but our results for ovenbird suggest TOBEC's value for medium to large songbirds if generating single-species prediction equations) – provide a more accurate estimate of body fat than visual fat scoring alone. For the latter we recommend using either Kaiser's (1993) technique or that of Ralph et al. (1993), with its eight

classes. This estimated lipid mass correlates better with actual lipid mass than fat scoring alone. For studies of the same or similar-sized species of small passerines, these equations enable estimation of actual percentage body fat and not just uncalibrated "fat class" and are particularly accurate in assigning individual birds to percentage-fat classes (Appendix 2). Timing of the study may be a consideration; different mass components may have different selective values at different seasons, leading to seasonally variable relationships among some of the variables used in our models (e.g., flight is critical to migration but less so in winter or breeding seasons). We tried to maximize this variability by selecting subjects from spring and fall migration as well as summer breeding sites, and in so doing we achieved highly precise estimates of actual body composition for the overall sample.

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Appendix 1

Best regression models for lean mass[1]	
ALLBIRDS	Lean = −4.15 +0.668 LivWt −0.495 VisFat +0.333 BL +0.122 Tars +0.991 *lnNEV*
	Lean = −2.41+0.724 LivWt −1.03 VisFat+0.344 BL+0.147 Tars
SMALL	Lean = −1.48+0.862 LivWt −0.511 VisFat −0.692 BD −0.0302 Tail+0.207 Head
AMRE	Lean = −1.28+1.11 LivWt −0.362 VisFat
OVEN	Lean = −3.41+0.912 LivWt −1.65 VisFat −0.195 Tars+0.262 Head
MED	Lean =12.9+1.01 LivWt −0.79 VisFat −0.459 Tars −0.0636 Tail
LARGE	Lean = −32.4+0.470 LivWt −0.92 VisFat −2.30 BL+2.63 BW+7.39 BD −0.212 Wing −0.104 Tail+12.3 *lnNEV*
	Lean = −9.00+0.734 LivWt −0.835 VisFat −0.435 BL+2.08 BW+2.39 BD −0.0253 Wing+0.0136 Tail
Warblers	Lean =3.28+0.856 LivWt −0.933 VisFat −0.328 BL+1.30 BD −0.0696 Tail
Best regression models for lipid mass[2]	
ALLBIRDS	Lipid =4.15+0.332 LivWt+0.495 VisFat −0.333 BL −0.122 Tars −0.991 *lnNEV*
	Lipid =2.41+0.276 LivWt+1.03 VisFat −0.344 BL −0.147 Tars
SMALL	Lipid =1.48+0.138 LivWt+0.511 VisFat+0.692 BD+0.0302 Tail −0.207 Head
AMRE	Lipid =5.65+0.324 VisFat −0.189 Head
OVEN	Lipid =5.76+0.187 LivWt+0.792 VisFat −0.0944 Wing+0.0817 Tail −0.0709 Head −1.00 *lnNEV*
	Lipid =3.79+0.112 LivWt +0.749 VisFat −0.0444 Wing +0.0552 Tail −0.145 Head
MED	Lipid = −12.7+0.785 VisFat+0.444 Tars+0.0628 Tail
LARGE	Lipid =32.4+0.530 LivWt+0.92 VisFat+2.30 BL −2.63 BW −7.39 BD+0.212 Wing+0.104 Tail −12.3 *lnNEV*
	Lipid =9.00+0.266 LivWt+0.835 VisFat+0.435 BL −2.08 BW −2.39 BD+0.0253 Wing −0.0136 Tail
Warblers	Lipid = −3.28+0.144 LivWt+0.933 VisFat+0.328 BL −1.30 BD+0.0696 Tail

[1,2]Notes: if using the 5-rank (0–4) fat-scoring method of Pyle et al. (1987), one must double the coefficient for VisFat in any of the above equations. For each of those 5 models where *lnNEV* was chosen as a predictor variable by best subsets, an alternative regression equation is given immediately following, without *lnNEV* (see Table 1 for aR^2s).

Appendix 2

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