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AN EVALUATION OF WHOLE BODY POTASSIUM-40 CONTENT FOR ESTIMATING LEAN AND FAT MASS IN PIGEONS[1]

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Abstract. A severe limitation in studies of avian ecological energetics is the lack of an accurate, noninvasive technique for determining whole body fat storage in living birds. We explored a technique of assaying total body potassium as a predictor of lean mass (LM) and then derived fat mass (FM) by subtracting LM from total body mass. Body potassium (K), present in lean tissue but not in fat, was estimated noninvasively from naturally-occurring radioactive ^{40}K, which occurs as a fixed ratio to total body K. We assayed 29 pigeon (*Columba livia*) carcasses for ^{40}K and then measured LM from body composition analyses in which fat mass was extracted using petroleum ether. The ^{40}K results were regressed against LM using five different combinations of independent variables. Regression equations were tested by comparing predicted LM (and FM predictions by subtraction from body mass) to measured LM values obtained from a

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separate group of pigeons. Whole body assay of ^{40}K was not a useful predictor of LM in pigeons ($r^2 = 0.51$; mean absolute error was 14 ± 7%). Absolute errors increased with FM predictions (96 ± 50%). Adding body mass as an independent variable increased the r^2 to 0.97, but body mass alone explained 96% of the variability in LM.

Key words: body composition, Columba livia, *lean mass, lipids, pigeon, potassium-40 measurement.*

Many organisms must rely on internal energy reserves at different times during their lives because of the combination of two factors. First, the amount of external resources available to organisms can vary in time and space, and second, energy demands also can vary such that high allocation rates are required when external resource availability is low. Fats represent the primary internal energy reservoir for most organisms. One of the greatest hindrances to understanding the ecological energetics of animals is the lack of a noninvasive technique for determining whole body fat levels in individuals. A potential technique is the measurement of total potassium in the body.

Potassium occurs within an animal's lean tissue and not in fat (Coward et al. 1988). Thus, total potassium content can be used to estimate lean mass (LM; free of neutral lipids). Fat mass (FM) can then be derived by subtracting the lean mass estimate from body mass. Total body potassium (K) can be determined by whole-body counting an organism for naturally-occurring ^{40}K. The technique is possible because ^{40}K comprises a fixed percentage of total potassium in the body of animals (0.012% in humans; Westerterp-Plantenga et al. 1994), and ^{40}K emits readily detectable, characteristic gamma radiation.

Lean mass and/or FM of several species of large mammals have been estimated from ^{40}K counts (steers, Clark et al. 1976; cows, Belyea et al. 1978; pigs, Domermuth et al. 1976; humans, Westerterp-Plantenga et al. 1994). To our knowledge, the study reported herein is the first to evaluate the ^{40}K method for estimating LM and FM of adult birds.

METHODS

ANIMALS

We used 43 pigeons (*Columba livia*) ranging in body mass from 252 to 501 g and in fat mass from 16 to 94 g (5 to 22%). Pigeons were given water and food ad libitum for several weeks, euthanized with N_2, and frozen. Because animal size affects the attenuation of ^{40}K gamma rays emitted from an organism (Miller and Remenchik 1963), body mass, body length (from the anterior end of the keel to the cloaca), and body thickness (distance between the keel of the sternum and the back) were measured on the frozen carcasses to normalize ^{40}K estimates for the physical dimensions of the pigeons.

WHOLE BODY COUNTING

Each frozen pigeon was assayed for ^{40}K using a 15 × 15-cm diameter NaI detector (Bicron Inc., Newburg, Ohio) coupled to a multi-channel analyzer (EG&G, Oak Ridge, Tennessee). Total counts were determined within a 250 kiloelectron volt (keV) region of interest surrounding the 1.46 megaelectron volt (MeV) total absorption peak of ^{40}K. Contributions from background counts were regularly determined and subtracted from the gross counts.

Efficiency in detecting ^{40}K partially depends upon the size and number of NaI crystals used for the analysis; a larger total crystal area increases the probability of a ^{40}K gamma ray being detected. Our crystal was comparable in size to that used by other investigators who reported success with the technique (Roessler and Dunavant 1967, McNeill et al. 1991). Because the precision of detecting radioactivity can generally be increased merely by a longer analysis time, we also assayed each bird for 2 hr and 7 hr to determine the effect of assay times on estimates of LM.

STATISTICAL ESTIMATION OF LEAN AND FAT MASS

Lean masses of 29 birds, derived from body composition analyses as described in Gessaman et al. (1998), were regressed against five different combinations of independent variables: (1) ^{40}K counts, (2) body mass (wet weight), (3) ^{40}K counts and body mass, (4) body mass, body length and thickness, and (5) ^{40}K counts, body mass, body length and thickness. The distributions of body mass, ^{40}K counts, and lean mass were normalized by natural logarithm transformations, and regressions were performed on log-transformed data.

Regression equations then were tested by comparing LM and fat mass predictions to values measured on a group of 14 pigeons, independent of the original 29 birds used to derive the equations. Predicted values of lean mass and fat mass were compared to actual values obtained from body composition analyses using *t*-tests for independent samples.

RESULTS

Potassium-40 was a poor predictor of LM in pigeons. Coefficients of determination (r^2) were 0.28 and 0.51 for 2- and 7-hr assays, respectively (Table 1). Adding body mass as an independent variable greatly improved the predictive ability; r^2 values increased from 0.51 to 0.97 (Table 1). However, body mass alone explained 96% of the variability in LM. Adding two additional morphological measurements to estimate LM (i.e., bird length and thickness) did not improve predictions ($r^2 = 0.97$, Table 1). Indeed, neither variable was significant within the multiple regression; P values for bird length and thickness were 0.56 and 0.36, respectively, for the 7-hr assay.

Measured LMs of 14 additional pigeons, determined from body composition analyses, were compared to LMs predicted from regression equations derived from the 29 birds. Comparisons were made for results obtained from ^{40}K assays, as well as ^{40}K in combination with bird mass. Coefficients of determination of measured versus predicted LM increased from 0.11 when ^{40}K was used alone, to 0.98 when body mass was added.

Surprisingly, there was no significant difference ($t_{13} = 0.13$, $P = 0.89$) between measured LM of the 14 birds (279 ± 47 g) and that predicted using regression equations derived from ^{40}K measurements (270 ± 31 g; Table 2). This suggests that ^{40}K could be used to reasonably estimate the mean LM among treatment

TABLE 1. Equations derived from regressing measured lean mass in 29 pigeons to their ^{40}K count rate (^{40}K), body mass (M), body thickness (D), and body length (L). Results of 2- and 7-hr ^{40}K assays are shown.

^{40}K assay duration (hr)	Regression equation	Parameters with $P < 0.05$ within the equation	r^2
2	6.49 ± 0.45 (^{40}K)	constant, ^{40}K	0.28
7	6.84 ± 0.66 (^{40}K)	constant, ^{40}K	0.51
2	1.24 ± 0.04 (^{40}K) + 0.78 (M)	constant, M	0.96
7	1.52 ± 0.09 (^{40}K) + 0.74 (M)	constant, ^{40}K, M[a]	0.97
2	1.25 ± 0.002 (D) + 0.6E-4 (L) + 0.03 (^{40}K) + 0.75 (M)	constant, M	0.96
7	1.64 ± 0.001 (D) + 0.0004 (L) + 0.09 (^{40}K) + 0.70 (M)	constant, ^{40}K, M[b]	0.97
7	1.07 ± 0.79 (M)	constant, M	0.96
7	1.13 ± 0.002 (D) + 0.0001 (L) + 0.76 (M)	constant, M	0.96

[a] ^{40}K's P value = 0.06.
[b] ^{40}K's P value = 0.08.

groups. Estimating means among treatment groups, however, may only be useful under limited experimental situations and is not as useful or powerful as being able to accurately estimate LM in the same individuals over time.

A more applicable test of the predictive ability of the ^{40}K technique is the deviation of predicted to observed values among individual animals. This deviation was estimated using absolute percent error and goodness-of-fit calculations, where: absolute percent error = [100 × (predicted value − observed value)/predicted value] and goodness-of-fit = [summation of each (predicted value − observed value)^2/sample size]. The closer the goodness-of-fit number is to zero, the better the prediction. Estimates of LM using ^{40}K had absolute errors of 14 ± 7% (Table 2), and a large goodness-of-fit value of 2,122. Addition of body mass to the regression reduced the absolute error associated with LM predictions to 2 ± 2%, and goodness-of-fit also improved substantially (47; Table 2).

Errors of predicting FM using equations derived from ^{40}K measurements were much larger (96 ± 50%) than those associated with LM predictions. Errors of predicting FM decreased to 16 ± 14% when body mass was added as an independent parameter (Table 2), and goodness-of-fit values decreased as they did with LM (from 2,122 to 47; Table 2).

DISCUSSION

Whole body assays of ^{40}K, when used alone, were not useful predictors of LM nor FM. Estimates of LM and FM from ^{40}K counts alone had absolute errors of 14 ± 7% and 96 ± 50%, respectively. Adding body mass greatly improved the accuracy of the prediction, but effectively nullified the contribution of ^{40}K values to the variation explained by linear regression. Ninety-six percent of the variation in LM was explained by body mass alone. When ^{40}K counts and body mass were used together to predict FM the absolute errors dropped to 16 ± 14%. Thus, our best estimates had errors too large for tracking FM changes within individual animals, and perhaps too large for comparing treatment means in many experimental situations.

The low correlation between LM and ^{40}K counts that we observed can possibly be explained by one, or both, of the following: (1) the basic assumption that K is found solely in lean tissues of birds is incorrect, or (2) the ^{40}K content of our birds was too low to be

TABLE 2. Comparisons of grams of fat mass (measured by fat extraction) and lean mass (measured as total body mass − fat mass) from 14 pigeons to values predicted from regression equations presented in Table 1. Data are based on a 7-hr assay of ^{40}K. Independent parameters are coded as ^{40}K = potassium-40 counts, M = total body mass, D = body depth, and L = body length. See text for definition of absolute percent error and goodness-of-fit.

Body compartment	Method of determination	Variables used to predict compartment	Mass (g) (mean ± SD)	Absolute % error (mean ± SD)	Goodness-of-fit
Lean mass	Measured	—	279 ± 47	—	—
	Predicted	^{40}K	270 ± 31	14 ± 7	2,122
		^{40}K + M	280 ± 44	2 ± 2	47
		^{40}K + M + D + L	281 ± 44	2 ± 2	56
		M	284 ± 47	3 ± 2	71
		M + D + L	285 ± 46	3 ± 2	80
Fat mass	Measured	—	45 ± 21	—	—
	Predicted	^{40}K	53 ± 64	96 ± 50	2,122
		^{40}K + M	43 ± 23	16 ± 14	47
		^{40}K + M + D + L	42 ± 24	17 ± 17	56
		M	39 ± 20	18 ± 13	71
		M + D + L	38 ± 21	20 ± 14	80

accurately counted with our analytical system. We tested the first possibility by measuring K with atomic absorption spectroscopy in extracted fat and lean tissue of a pigeon. Extracted fat contained 0.25 mg of K g^{-1}, and lean tissue had 2.6 mg of K g^{-1} wet weight. Because on average a pigeon contained 30 g of fat and 250 g of lean tissue, only 7.5 mg of K would be present in its body fat (30 g fat $\times$ 0.25 mg of K g^{-1}), whereas lean tissue contained 650 mg of K (250 g lean $\times$ 2.6 mg of K g^{-1}). Therefore, only about 1% of total body K was present in the pigeon's fat. Although the basic assumption that K is not present in fat is not totally supported by our data, the low levels of K detected in fat cannot account for the poor correlation observed between ^{40}K count and LM.

The small body size of pigeons, and thus their low level of ^{40}K, seems to be the major cause for the low correlations between ^{40}K counts and LM. Because the lean tissue of our pigeons contained 2.6 mg of K g^{-1} (essentially the same as found in the lean mass of humans; Behnke and Wilmore 1974), a 500 g pigeon with a body fat of 10% would only have about 1 g of total body K. Comparable calculations for a 70 kg man (15% body fat) would indicate about 150 g of total body K. Potassium-40 whole-body counts have been relatively successful in predicting LM of mammals much larger than pigeons; however, researchers have had limited success with this method on animals weighing < 5 kg (i.e., < 10 g of total body K). Schmidt et al. (1974) found that piglets weighing 1.2 kg were too small to obtain ^{40}K results with the limitations of their equipment; the smallest pigs they counted successfully weighed 5.4 kg. Clay et al. (1979) were unsuccessful as well in predicting FM of 1- to 77-day-old Wood Ducks (*Aix sponsa*) from ^{40}K counts.

A whole-body liquid, rather than a solid, scintillation counter, may be the instrument of choice for animals with low total body K, such as the one used to measure total body K (100 mEq; 3.9 g) with an accuracy of 8% in one hour in a potassium-deficient child weighing 5 kg (Garrow 1965). Liquid scintillometers detect gamma rays emitted from the body more efficiently because they literally surround the subject, greatly increasing the efficiency of detecting ^{40}K emissions. With a solid crystal, such as used in our study, only the gamma rays emitted in the direction of the crystal are detected. We had hoped that the use of large, efficient crystals and long assay times would overcome the small-body size limitation of the ^{40}K method, however, we were incorrect. Further technological development may be required before ^{40}K whole-body counts can be used to accurately predict LM of animals substantially smaller than 5 kg.

More fundamentally, inaccuracy is inherent in any method that subtracts LM from total body mass to derive FM because FM typically constitutes a relatively small portion of the total mass of an organism; thus small errors in LM estimates result in much larger errors in FM estimates.

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