TECHNICAL COMMENT

# Dual-Energy X-Ray Absorptiometry (DXA) Can Accurately and Nondestructively Measure the Body Composition of Small, Free-Living Rodents

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## ABSTRACT

Dual-energy x-ray absorptiometry (DXA) is a nondestructive technique that can potentially measure specific components of whole-body composition in free-living and lab-raised animals. Our aim was to test the ability of DXA to measure the composition of a common arvicoline rodent, the northern red-backed vole (*Clethrionomys rutilus*). We used a DXA apparatus to obtain measurements of fat mass (FM), lean mass (LM), bone mineral content, bone mineral density, and fat-free mass (FFM) in carcasses of free-living and lab-raised voles. We then used chemical carcass analysis to derive predictive algorithms for actual values of FM, total body water, total protein, total mineral, LM, and FFM. Unexplained error in the equations for all voles grouped collectively ranged from $R^2 = 0.82$ to $R^2 = 0.98$. The DXA FM measurement had the highest coefficient of variation, and it was higher for free-living voles than for lab-raised voles. However, FM can be determined by difference with excellent precision by using the FFM equation ($R^2 = 0.98$). We also derived corrective terms for passive integrated transponder–tagged animals. Thus, DXA is a nonlethal, nondestructive tool capable of precisely and accurately measuring many specific parameters of whole-body composition in small free-living and lab-raised rodents.

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## Introduction

Measurements of body composition are important because they characterize an animal's biological makeup and may indicate nutritional state or life-history stage. Composition may include the type and percentage of an animal's fuel reserves, the hydration state of an animal, or other measurements that describe portions of the animal's total chemical makeup. The body condition of an animal has traditionally referred to some measure of its fitness, either in a broad sense (e.g., measures of reproductive condition) or in a more specific sense (e.g., body fat percentage). Although these terms have, at times, been used interchangeably, we emphasize the whole biological composition in this study rather than the fitness or condition of an animal.

The body composition of rodents has been an important factor for clinical and dietary trials in the laboratory, but it is also important for studying the physiological ecology of small herbivores in varying environments. Composition has previously been measured by using an assortment of methods, but each has been problematic for small rodents. Body mass indices are generated by calculating a ratio of length to mass but can be nonrepeatable and inaccurate reflections of actual composition (Krebs and Singleton 1993; Schulte-Hostedde et al. 2001*a*). The negative correlation between body water and the amount of fat that some animals carry has also been used to estimate condition (Winstanley et al. 1998), but these relationships are not always consistent in small mammals (Schulte-Hostedde et al. 2001*a*). Total-body electrical conductivity (TOBEC) instruments have been used to measure the differences in electrical properties of lean tissue and body fat and can theoretically predict three body composition components: body water content, lean mass, and fat mass. However, these devices vary heavily in their precision and accuracy and, thus, their actual ability to measure the different parameters of body composition (Walsberg 1988; Castro et al. 1990; Zuercher et al. 1997; Frawley et al. 1999; Unangst and Wunder 2001). For instance, Zuercher et al. (1997) noted that two different TOBEC instruments produced poor fat estimates from lean mass predictions, and neither could be used to accurately measure changes in total body fat of individual voles. Additionally, TOBEC instruments require that animals be maintained at normal levels of hydration and body temperature (Walsberg 1988).

Chemical carcass analysis, or proximate analysis, is accurate

Table 1: Precision of DXA measurements

| DXA Measurement | Free-Living Animals ($n = 10$; %) | Lab-Raised Animals ($n = 12$; %) | Difference (%) | All Animals ($n = 22$; %) |
|---|---|---|---|---|
| $FM_{DXA}$ | 7.8 | 6.0 | 1.8 | 6.8 |
| $LM_{DXA}$ | 1.5 | 1.7 | −.2 | 1.6 |
| $BMC_{DXA}$ | 2.3 | 2.3 | .0 | 2.3 |
| $BMD_{DXA}$ | 3.4 | 3.7 | −.3 | 3.6 |

Note. Average percentage coefficients of variation (CVs) are listed for direct dual-energy x-ray absorptiometry (DXA) measurements of fat mass ($FM_{DXA}$), lean mass ($LM_{DXA}$), bone mineral content ($BMC_{DXA}$), and bone mineral density ($BMD_{DXA}$) in free-living and lab-raised voles. The $FM_{DXA}$ measurements were least precise, having the highest average CVs. The $LM_{DXA}$ and $BMC_{DXA}$ measurements, which together make up the sum of all DXA-derived fat-free mass components, had the lowest average CVs. The $BMC_{DXA}$ measurements were more precise than $BMD_{DXA}$ measurements, which depend on a calculation of bone mass and area ($g/cm^2$) and are more likely to be affected by animal repositioning. The only notable difference in magnitude of average CV between the two groups of voles was in the $FM_{DXA}$ measurement, which was higher in free-living voles than in lab-raised voles (difference of 1.8%). This suggests that DXA's lower limit of fat detection is being approached in leaner wild animals.

but lethal (Batzli and Pitelka 1971; Batzli and Esseks 1992; Zuercher et al. 1997; Schulte-Hostedde et al. 2001*a*, 2001*b*; Mata et al. 2006). It destroys the assessed tissues, thus preventing any further analyses, and it is difficult to justify for research on threatened or endangered species. However, technological advances in radiology and physiology have led to the formulation of a nondestructive, noninvasive method for determining body composition, known as dual-energy x-ray absorptiometry (DXA). DXA uses two x-rays, one of a high energy dose (70–90 keV) and one of a low energy dose (15–40 keV), to measure body composition. As photons emitted by the machine traverse a subject's tissues, physical interactions take place that reduce a beam's intensity (attenuation). These photons are either absorbed or scattered by interactions of Compton scattering and photoelectric effect (Pietrobelli et al. 1996). The differential attenuation of these x-rays through bone, lean tissue, and fat is quantified by the DXA apparatus and provides a unique system of measurement. When photons at two different energies pass through an absorber, the beam attenuation at the lower energy can be expressed as a ratio ($R$) of the beam attenuation at the higher energy.

Every atomic element has a specific $R$ value and a characteristic mass attenuation coefficient ($\mu$) at high and low energies (Pietrobelli et al. 1996). A sigmoidal association between these $R$ values and their corresponding atomic numbers exists, with the main constituents of water and organic compounds (H, C, N, O) having small $R$ values, soft tissue minerals (Na, K, Cl) having large $R$ values, and elements primarily in bone mineral (Ca) having even larger $R$ values (see Pietrobelli et al. 1996). These $R$ values are unique and range from 1.2058 to 1.2289 for triglycerides and fatty acids and from 1.2906 to 2.9939 for many lean components (e.g., protein, glycogen, extracellular and intracellular water, soft tissue minerals) and bone mineral. The DXA method for estimating the three major components of composition (fat mass, lean mass, and bone) is to separate pixels into those with soft tissue only (fat + lean) and those containing soft tissue + bone mineral. Pixel separation, or point typing, is then accomplished by computing an $R$ value for each pixel in a total-body scan and setting an $R$ value threshold to distinguish between pixels that include bone mineral and those that consist of only soft tissue (Pietrobelli et al. 1996). Thus, the $R$ values obtained by DXA are used to identify the unknown components of body composition.

Initially developed as tools for clinical research (Peppler and Mazess 1981; Gotfredsen et al. 1986), DXA apparati have been used to study or develop treatments for osteoporosis (Grodum et al. 1995; Michaelsson et al. 1996), vertebral deformity (Ross et al. 1995), obesity (Carey et al. 1996; Hendel et al. 1996), idiopathic renal stone disease (Trinchieri et al. 1999), and a wide variety of other disorders and conditions in humans and lab mice (for a review, see Albanese et al. 2003). The application of DXA, however, has since broadened to the fields of agriculture, animal science, and veterinary science. It has been used to measure the body composition of sheep (Pouilles et al. 2000), pigs (Mitchell et al. 1998; Nielson et al. 2004), chickens (Mitchell et al. 1997; Swennen et al. 2004), dogs (Toll et al. 1994), and other domestic and agricultural animals. The success of these studies has led to recent applications of DXA in wildlife physiology. For example, DXA has been used to measure body composition in collared lemmings undergoing photoperiod-induced weight gain (Hunter and Nagy 2002), the effects of long-term dietary restriction on rhesus monkeys (Blanc et al. 2003), the effects of different diets on fat and lean mass changes in grizzly bears (Felicetti et al. 2003), and the body composition of passerine birds (Korine et al. 2004). Almost all of these studies have used colonized, lab-raised subjects (although some exceptions exist; e.g., Korine et al. 2004), and captive animals typically have body compositions very different from those of free-living animals. For instance, free-living brown lemmings (*Lemmus sibiricus*) and other arvicoline rodents often contain only 5% body fat, while lab-raised lemmings may reach 40% body fat (Batzli and Esseks 1992). In studies where DXA has been used on free-living terrestrial mammals, it appears to have usually been limited to measuring only changes in bone mineral content and density (Hiyaoka et al. 1994; Bjora et al. 2001; Dirrigl et al. 2004; Garriga et al. 2004 [with one exception to

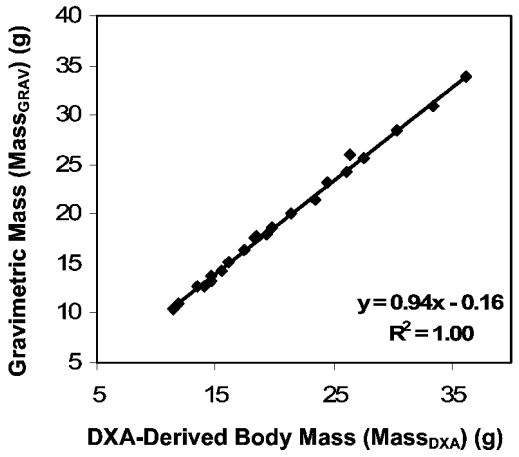



Figure 1. Gravimetric mass versus dual-energy x-ray absorptiometry (DXA)–derived body mass ($mass_{DXA}$). The $mass_{DXA}$ is the sum of the DXA-derived parameters of fat mass, lean mass, and bone mineral content. The DXA apparatus overestimates gravimetric body mass ($mass_{grav}$) slightly but with virtually no unexplained error ($R^2 = 1.00$).

our knowledge: Lehmer and Van Horne 2001]). This is presumably because DXA's bone measurements are known to have greater internal accuracy than its soft tissue measurements (Nagy and Clair 2000).

Previously, DXA apparati have been validated by proximate analysis for use on the noncranial region of lab-raised rodents. Brommage (2003) validated a machine for use with decapitated mice, and Nagy and Clair (2000) used the PIXImus DXA apparatus (GE Lunar) and software to digitally exclude head regions of full-bodied mice. Johnston et al. (2005) also excluded cranial and tail regions of obese and wild-type mice, Siberian hamsters, and bank voles to determine capabilities of the PIXImus2 for predicting fat mass. These validations involving lab-raised rodents are likely to be of most value to biomedical studies that focus on the effects of a given treatment on core body composition only. In physiological ecology and wildlife physiology studies, however, research concerns may lie more in the realm of whole-body composition and response to seasonal or environmental variables. For instance, the skull and brain add significant mass to the body, may change seasonally (Yaskin 1984; Zuercher et al. 1999), and should be included in any morphological measurements relative to body mass.

Unlike proximate analysis, DXA's nondestructive nature allows for the determination of body composition in live, immobilized animals during repeated-measures experiments, as well as in lethal-trap studies, for which the preservation of internal tissues and organs is desired. Most rodent-sized DXA apparati are, in fact, portable, nondestructive, inexpensive to maintain and operate, and time efficient (~3 min per scan on a sedated animal). Thus, a DXA instrument could be an ideal tool for nondestructively measuring the composition of small mammals in a broad array of field- and laboratory-based wildlife physiology studies if it could be validated for whole-bodied, leaner, free-living rodents with a high degree of precision and accuracy. The aim of our study was to use proximate analysis to validate the measurements of a DXA apparatus for use on whole free-living and lab-raised northern red-backed voles (*Clethrionomys rutilus*).

## Methods

Ten free-living northern red-backed voles (*Clethrionomys rutilus*) were collected from Chugach State Park in south-central Alaska and from forested areas around the University of Alaska Anchorage (UAA) campus and were frozen at −20°C. Twelve lab-raised *C. rutilus*, which were visibly fatter than the free-living voles, were donated from the University of Alaska Fairbanks (UAF) captive Bonanza Creek colony (Tavernier et al. 2004). These voles were killed, frozen at −20°C, and shipped on ice to the UAA campus. Procedures were approved by the UAA Institutional Animal Care and Use Committee (protocol 2005VanTe1).

### *DXA Analysis*

Voles were individually thawed to room temperature and weighed on a laboratory balance. Fat mass ($FM_{DXA}$), lean mass ($LM_{DXA}$), bone mineral content ($BMC_{DXA}$), and bone mineral density ($BMD_{DXA}$) were measured by using a PIXImus2 DXA apparatus (GE Lunar) and accompanying Lunar PIXImus2 2.00 software. Quality control statistics of the apparatus were set to allow no more than 0.2% error during calibration with a quality control phantom standard (BMD = 0.0630 g/cm$^2$, %fat = 9.5%). Voles were placed dorsal side up on the scanning platform of the apparatus. Appendages were stretched out to the corners of the rectangular platform, and the tail was curled under the hind leg. Larger voles had to be positioned diagonally on the platform so that their entire carcass could be scanned and analyzed by the machine. All animals were scanned at least

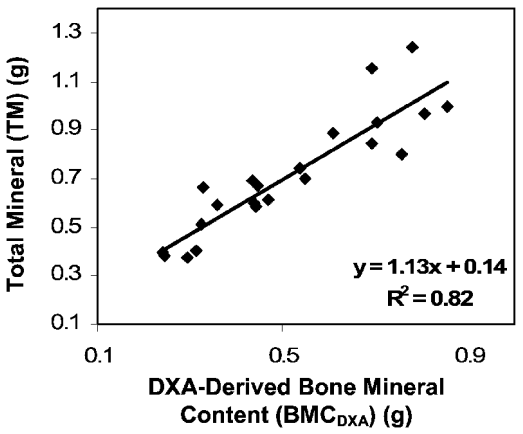
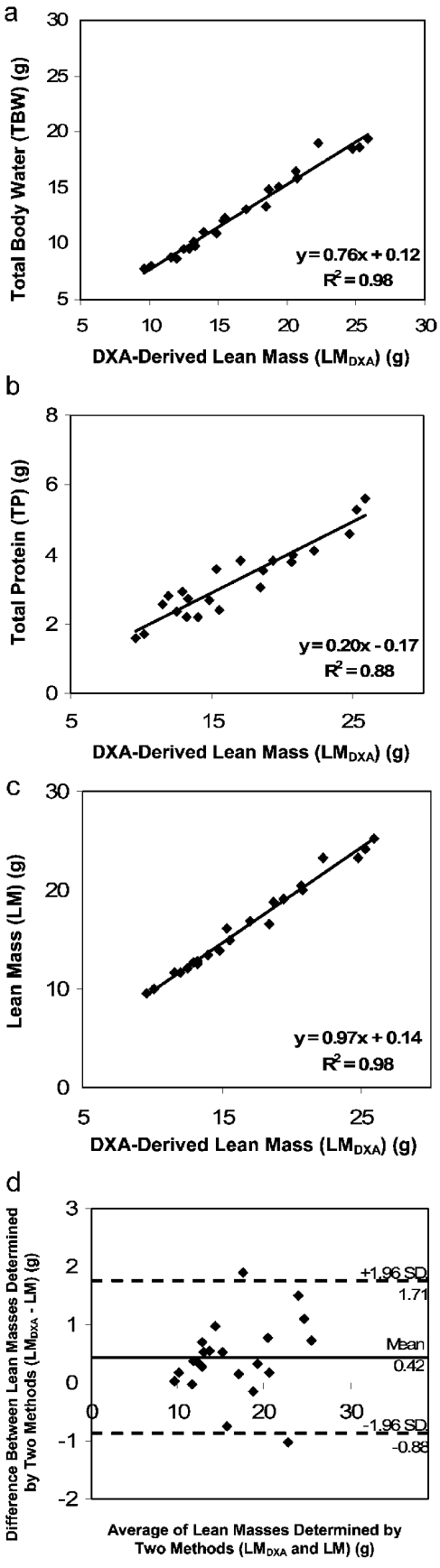



Figure 2. Total mineral (TM) versus dual-energy x-ray absorptiometry (DXA)–derived bone mineral content ($BMC_{DXA}$). DXA predicts TM with a good amount of error explained by the resulting equation. The internal accuracy of the Lunar PIXImus $BMC_{DXA}$ measurement has already been affirmed (Nagy and Clair 2000).




five times, with repositioning of the carcass between each measurement.

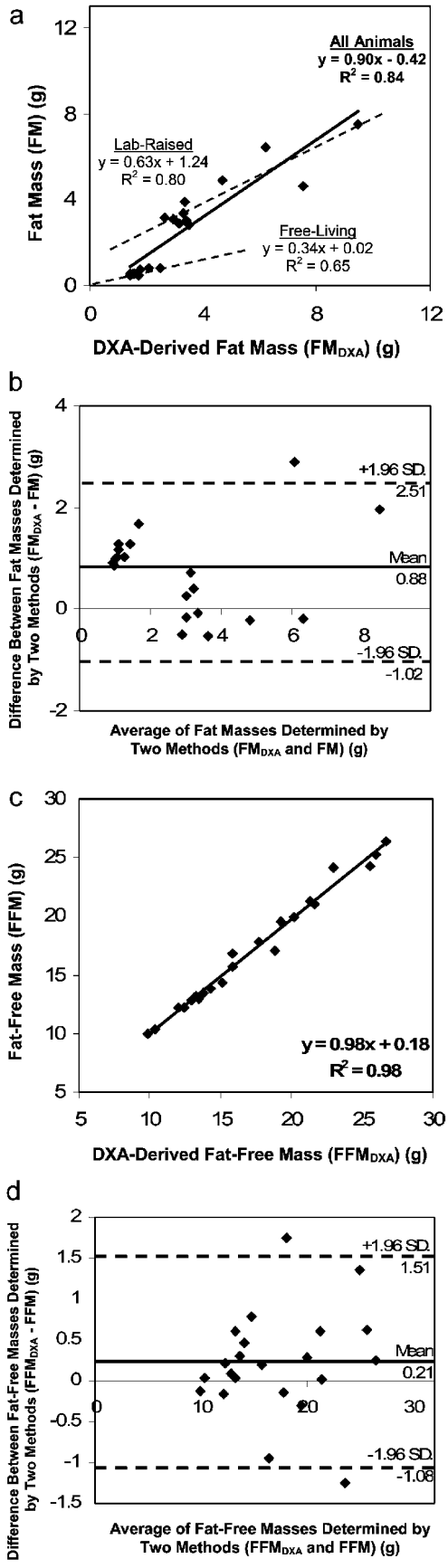
### *Chemical Carcass Analysis*

After DXA scans, whole vole carcasses were reweighed and subsequently homogenized in beakers by using a Kinematica homogenizer. Homogenate was dried in an oven at 60°C to a constant mass to determine total body water (TBW) content. For three of the voles, subsamples of homogenate were separated, dried in triplicate, and averaged to ensure precision in the drying technique. The total mineral (TM) content of the dried homogenate was determined by weighing triplicate samples of homogenate after ashing in a muffle furnace at 500°C for more than 8 h. Nonbone mineral content (NBMC) was determined by calculating the difference between the TM value and the PIXImus2 DXA $BMC_{DXA}$ value ($NBMC = TM - BMC_{DXA}$).

We determined the total protein (TP) content of each vole by measuring the nitrogen content of dried homogenate in triplicate with a carbon, nitrogen, hydrogen (CNH) spectrophotometer (Leco) at the UAF Agricultural and Forestry Experimental Research Station (Palmer, AK) and multiplying by the standard nitrogen-protein conversion factor of 6.25 for animal protein (Jones [1931] 1941; Jones et al. 1942; FAO/WHO 1973; FAO 2003).

We used an accelerated solvent extractor (ASE; Dionex) with a mixture of 65%/35% chloroform/methanol ($CHCl_3$/MeOH) solvent and followed the methods of Dodds et al. (2004) to chemically determine FM. Solvent type has been known to influence lipid recovery (Giergielewicz-Mozajska et al. 2001; Dodds et al. 2004), and $CHCl_3$/MeOH was chosen because it is strongly polar and a very effective and consistent extractor of lipid (Dodds et al. 2004). Accelerated solvent extraction is more effective than traditional methods of total body lipid extraction, such as soxhlet, because the solubility of analytes and diffusion rates are increased, strong interactions between analytes and matrix components are weakened or disrupted, and viscosity and surface tension of solvents are decreased (Giergielewicz-Mozajska et al. 2001). Each of these allows for a more accurate recovery of total lipid.

Figure 3. *a*, Total body water (TBW) versus dual-energy x-ray absorptiometry (DXA)–derived lean mass ($LM_{DXA}$); *b*, total protein (TP) versus $LM_{DXA}$; *c*, lean mass (LM) versus $LM_{DXA}$; *d*, Bland-Altman plot comparing two methods for determining lean mass. Water is the major component of both LM and fat-free mass (FFM), and the strong relationship between TBW and $LM_{DXA}$ ($R^2 = 0.98$; *a*) is tightly linked to the predictive equations for LM (*c*) and FFM (Fig. 4*b*). *d* compares the two methods used in determining lean mass (DXA analysis and chemical analysis), in which the difference is plotted against the average of the two methods to show sufficient agreement between them (Bland and Altman 1986). The solid line is the mean difference, and the dashed lines represent 2 SD from the mean difference (95% limits of agreement).




The extractor injected solvent into heated (100°C) metal cells that contained subsamples of dried homogenate (100–500 mg) and hydromatrix material. The extract was filtered into collection vials, mixed with 0.9% NaCl solution, and passed through a sodium sulfate matrix with chloroform rinse. Samples were analyzed in duplicate or triplicate and averaged. Data from one free-living vole and one lab-raised vole were excluded from lipid recovery analysis because we were unable to obtain multiple values for these animals.

Our equipment and techniques were used to measure the total lipid recovery of a standard reference material against its certified National Institute of Standards and Technologies (NIST) value (Dodds et al. 2004). Sample lipid recovery values exceeded the NIST value by 3.3%, and we corrected for this overestimate by multiplying all of our percent lipid recovery values by a factor of 0.967.

The percent lipid recovery from samples of dried free-living vole homogenate averaged 15.5% ± 1.6% (as a result of the absence of water), which was within the percent lipid recovery range of 2%–20% that had been validated for our equipment and techniques (Dodds et al. 2004). Percent lipid recovery from dried lab-raised vole homogenate, however, was much higher, reaching up to 53.4% ± 3.6%, and was well outside the validated range of the extractor.

In the free-living voles, the sum of all chemically analyzed components (TBW + TP + TM + FM) equaled 98.5% ± 0.3% of the total gravimetric body mass ($mass_{grav}$). The small

Figure 4. *a*, Fat mass (FM) versus dual-energy x-ray absorptiometry (DXA)–derived fat mass ($FM_{DXA}$) in free-living voles, in lab-raised voles, and in all voles grouped collectively; *b*, Bland-Altman plot comparing two methods for determining fat mass; *c*, fat-free mass (FFM) versus DXA-derived FFM ($FFM_{DXA}$) in all voles; *d*, Bland-Altman plot comparing two methods for determining FFM. Predictive equations for FM explained a fair amount of error in free-living voles (*lower dashed line*; $R^2 = 0.65$) and a good amount of error in both lab-raised voles (*upper dashed line*; $R^2 = 0.80$) and all voles grouped collectively (*solid line*; $R^2 = 0.84$). DXA appears to be approaching its lower limit of fat detection capabilities in leaner free-living animals, as evidenced by the lower $R^2$ value and by the reduced precision of the $FM_{DXA}$ measurement in free-living voles when compared to lab-raised voles (Table 1). These factors appear to contribute to the formation of two distinct lines, although the one general regression line for all voles and the resulting predictive equation still yields a sufficient determination of FM. A better predictor of percent body fat can be determined using the FFM parameter (*c*; Table 2). The equations in *a*, however, are still sufficient for use both in fatter lab-raised individuals and in instances when the lean mass of free-living animals may have been compromised (e.g., animals are dehydrated, have had blood drawn, or have had tissues or organs extracted for other purposes). *c* describes the excellent relationship between FFM and $FFM_{DXA}$ ($R^2 = 0.98$) that could be used to determine FM by difference ($FM_{diff} = mass_{grav} - FFM$). The two methods used in determining FM and FFM (DXA analysis and chemical analysis) are compared in *b* and *d*, respectively. In each graph, the difference is plotted against the average of the two methods to show sufficient agreement between them (Bland and Altman 1986). The solid line is the mean difference, and the dashed lines represent 2 SD from the mean difference (95% limits of agreement).

Table 2: Predictive algorithms for body composition parameters based on DXA measurements

| Predictive Equation | $n$ | $R^2$ | SEE (g) |
|---|---|---|---|
| $Mass_{grav} = .94(mass_{DXA}) - .16$ | 22 | 1.00 | .41 |
| $TM = 1.13(BMC_{DXA}) + .14$ | 22 | .82 | .11 |
| $TBW = .76(LM_{DXA}) + .12$ | 22 | .98 | .60 |
| $TP = .20(LM_{DXA}) - .17$ | 22 | .88 | .38 |
| $LM = .97(LM_{DXA}) + .14$ | 22 | .98 | .64 |
| $FM = .90(FM_{DXA}) - .42$ | 20 | .84 | .88 |
| $FM_{free\text{-}living} = .34(FM_{DXA}) + .02$ | 9 | .65 | .09 |
| $FM_{lab\text{-}raised} = .63(FM_{DXA}) + 1.24$ | 11 | .80 | .75 |
| $FFM = .97(FFM_{DXA}) + .13$ | 22 | .98 | .65 |
| $Fat_{diff} = mass_{grav} - FFM$ | | | |

Note. Equations and standard errors of the estimate (SEEs) are listed for actual values of gravimetric body mass ($mass_{grav}$), total mineral (TM), total body water (TBW), total protein (TP), lean mass (LM), fat mass (FM), and fat-free mass (FFM) in free-living and lab-raised voles (*Clethrionomys rutilus*). The algorithms use only dual-energy x-ray absorptiometry (DXA)–derived measurements of fat mass ($FM_{DXA}$), lean mass ($LM_{DXA}$), bone mineral content ($BMC_{DXA}$), body mass ($mass_{DXA} = FM_{DXA} + LM_{DXA} + BMC_{DXA}$), and fat-free mass ($FFM_{DXA} = LM_{DXA} + BMC_{DXA}$). The FM equations have higher amounts of unexplained error and varying results for free-living versus lab-raised voles, as opposed to the FFM equation, which shows very little error and is consistent for all groups of animals (Fig. 4*c*). This is consistent with the result that the $FM_{DXA}$ measurement is less precise than the $LM_{DXA}$ and $BMC_{DXA}$ measurements, which together comprise the $FFM_{DXA}$ measurement (Table 1). Percent fat is most accurately determined using the FFM parameter to determine percent fat by difference ($fat_{diff} = 100\% - \%FFM$).

amount of residual mass (approximately 1.5%) was made up of the nonanalyzed components—that is, all other dry, fat-free, ash-free, nonprotein mass (e.g., glucose, glycogen, sugars, vitamins, DNA, RNA, fiber components, etc.). Although not measured chemically in our study, these components, collectively termed "residual lean mass" ($LM_{resid}$), are measured by DXA and are included in the $LM_{DXA}$ measurement (Pietrobelli et al. 1996).

Because the sum of all chemically analyzed components in the lab-raised vole homogenate equaled 103.1% ± 1.4% of $mass_{grav}$, we concluded that the lipid recovery technique of Dodds et al. (2004) overestimates actual lipid content and introduces greater variation in these heavily concentrated samples. To determine the fat-free mass (FFM) of these lab-raised animals, we added the average $LM_{resid}$ value calculated for free-living voles (0.207 g) to the sum of each lab-raised vole's FFM components that were chemically analyzed (TBW + TP + TM). The actual average $LM_{resid}$ value should be quite similar in the two groups because a heavy increase in percent body fat does not constitute a proportional or heavy increase in $LM_{resid}$. We were then able to determine each lab-raised vole's actual fat content by calculating the difference between the chemically derived FFM and the $mass_{grav}$. For both free-living and lab-raised voles, fat content was measured both by a DXA scan and by another analytical process. Although the lipid content of the lab-raised vole homogenate could not be determined by direct chemical extraction because it was well outside the validated range for ASE, greater than 99% of the FFM was determined chemically in order to calculate an actual value of fat by difference for lab-raised voles.

We defined the total LM as the sum of the TBW, TP, and $LM_{resid}$ components. We do not include bone mass in the LM measurement (although some authors do) because the PIXImus 2.00 software does not include bone mass in its $LM_{DXA}$ measurement. The "percent fat" value displayed by the PIXImus2 is actually a measure of fat as a percentage of all soft tissue and not as a percentage of total body mass that includes bone. We defined FFM as the sum of total LM and bone mineral and defined $FFM_{DXA}$ as the sum of all DXA-derived FFM components displayed in the PIXImus2 output screen ($LM_{DXA} + BMC_{DXA}$).

We also evaluated the effect of passive integrated transponder (PIT) tags on direct DXA measurements. Free-living voles ($n = 38$) that had been injected with subcutaneous tags (Destron Technologies, TX1440L10S-CD81740) between their scapulae as part of a different study were recaptured in Chugach State Park, Alaska, killed with halothane, and frozen at −20°C. We thawed these voles to room temperature and scanned animals on the DXA platform. Tags were then removed by applying physical pressure and forcing them through the skin. Animals were reset on the platform with minimal repositioning and scanned a second time.

We used SPSS statistical software (ver. 14.0) to analyze our data. Regression analysis was used to derive relationships between the body composition parameters measured chemically by proximate analysis and those measured by DXA. Simple linear regressions were used in all but one of our statistical comparisons (FM). A stepwise multiple regression was employed only in our determination of actual FM, and we used $FM_{DXA}$ and $LM_{DXA}$ as independent input variables (Nagy and Clair 2000). The selected model of best fit, however, used only the $FM_{DXA}$ variable and not the LM variable (Fig. 4*a*), so we have also reported this as a simple linear regression. Bland-Altman graphs were used where appropriate to show agreement between established chemical techniques and DXA measurements of the same parameter (Bland and Altman 1986). To determine the effect of PIT tags on free-living voles, paired *t*-tests were used to determine the mean differences between DXA values recorded in each of the two scans (tagged vs. untagged).

## Results

We tested the precision of each of the four DXA measurements directly recorded by the PIXImus2 2.00 software ($FM_{DXA}$, $LM_{DXA}$, $BMC_{DXA}$, and $BMD_{DXA}$) by calculating a coefficient of variation (CV) for each parameter in each animal that was measured by DXA, with repositioning of the carcass between scans. The CVs were averaged for three groups: the lean free-living voles, the fatter lab-raised voles, and all voles grouped collectively (Table 1). For all voles grouped collectively, the $FM_{DXA}$ measurement had the highest average CV (6.8%). The

Table 3: Effect of PIT tags on DXA measurements

| DXA Measurement | $Mean_{tagged}$ | $Mean_{untagged}$ | Paired Mean Difference | *P* Value | Corrective Term |
|---|---|---|---|---|---|
| FM (g) | 2.5 ± .15 | 2.4 ± .15 | .1 | .038 | −.1 |
| LM (g) | 17.9 ± .81 | 17.7 ± .81 | .1 | .046 | −.1 |
| BMC (g) | .624 ± .034 | .550 ± .034 | .074 | <.001 | −.074 |
| BMD ($g/cm^2$) | .0699 ± .0014 | .0609 ± .0018 | .0090 | <.001 | −.0090 |

Note. The effect of subcutaneous passive integrated transponder (PIT) tags on direct dual-energy x-ray absorptiometry (DXA) measurements of fat mass (FM), lean mass (LM), bone mineral content (BMC), and bone mineral density (BMD) in free-living voles is shown. Means and standard errors of the mean are reported. A paired *t*-test was used to compare the DXA measurements of voles ($n = 38$) that were scanned while tagged and then while untagged with slight repositioning between scans. PIT tags caused a significant ($P < 0.05$) mass increase in all DXA parameters, but the corrective terms can be applied to obtain safe, reliable measurements in tagged, sedated voles. To acquire an accurate measurement for each parameter, DXA values obtained from tagged animals must first be corrected using these terms before any of the predictive equations in Table 2 are used.

$LM_{DXA}$ measurement, which comprises most of the $FFM_{DXA}$ measurement, had the lowest average CV (1.6%). The CV for the $BMC_{DXA}$ measurement was next lowest (2.3%), followed closely by the $BMD_{DXA}$ measurement (3.6%), which the machine calculates by dividing the $BMC_{DXA}$ value (g) by the measured bone area ($cm^2$) to determine bone density ($g/cm^2$).

We grouped all voles collectively in each of our regression analyses. To test whether DXA estimates total body mass accurately, we derived a relationship between the $mass_{grav}$ and the DXA-derived body mass ($mass_{DXA}$), that is, the sum of all DXA-derived body composition components displayed in the output screen ($FM_{DXA} + LM_{DXA} + BMC_{DXA}$). The DXA apparatus overestimated body mass slightly but consistently, and the predictive equation contained virtually no unexplained error (Fig. 1).

For all voles grouped collectively, the DXA apparatus predicted the tested components of body composition with $R^2$ values of 0.82 (TM), 0.88 (TP), 0.98 (TBW, LM, FFM), and 1.00 ($mass_{grav}$; Figs. 1–4). DXA values agreed closely with the corresponding chemical values of the same parameter (Fig. 3*d*; Fig. 4*b*, 4*d*). Predictive equations and reported errors for all tested components of body composition are listed in Table 2. Separate regression lines and predictive equations were added to the FM graph to show the difference in results between the free-living and the lab-raised voles (Fig. 4*a*) only because of an apparent difference in magnitude of average CV and because we were interested in the instrument's ability to directly measure FM specifically in lean free-living voles.

Finally, standard subcutaneous rodent PIT tags caused a significant ($P < 0.05$) increase in each of the four DXA parameters directly recorded by the PIXImus2 2.00 software (Table 3), and we derived corrective terms to allow for safe, accurate measurements of sedated animals without the need for tag removal. To acquire an accurate measurement of each parameter in a tagged animal, DXA values should first be corrected using these terms before any of the predictive equations are used.

## Discussion

The results of our study are consistent with a prior validation of the same apparatus on decapitated lab mice in which CVs were higher in the $FM_{DXA}$ measurement than in the other DXA measurements and in which FM was overestimated by DXA (Nagy and Clair 2000). Lean mass and total body mass were also slightly overestimated by DXA in our study. In comparing the precision of DXA's measurements between free-living and lab-raised vole groups, the only difference of any noticeable magnitude in average CV was in the $FM_{DXA}$ measurement, with the difference between groups equaling 1.8%. Absolute values of the difference between average CVs in free-living and lab-raised voles were only 0.2%, 0.0%, and 0.3% for $LM_{DXA}$, $BMC_{DXA}$, and $BMD_{DXA}$, respectively (Table 1). This suggests that DXA's capability to detect and measure fat levels by the $FM_{DXA}$ measurement alone approaches its lower limit of reliability for very lean free-living animals. In general, there seems to be some loss in precision in the $FM_{DXA}$ measurement as fat levels diminish. This result is consistent with results of a prior study in which both obese and leaner wild-type rodents scanned on two similar PIXImus2 DXA machines (with head and tail regions excluded) yielded the same $FM_{DXA}$ readings for obese animals but not for leaner wild-type animals (Johnston et al. 2005). They concluded that DXA apparati using the same software could use the same corrective equation to accurately predict FM for obese mutants but not for lean wild-type animals. Additionally, small systematic errors in DXA soft-tissue analysis have been known to arise with variation in fluid balance (Pietrobelli et al. 1998), which could potentially affect free-living voles more than lab-raised voles.

In our study, predictive equations for FFM contained very little unexplained error ($FFM = 0.97(FFM_{DXA}) + 0.13$; $R^2 = 0.98$; standard error of the estimate [SEE] = 0.65; Fig. 4*d*). This is largely due to the machine's excellent ability to predict TBW via the lean mass measurement ($TBW = 0.76(LM_{DXA}) + 0.12$; $R^2 = 0.98$; SEE = 0.60; Fig. 3*a*). In general, water comprises approximately 70% of a free-living vole's total body mass, more than 75% of its FFM, and almost 80% of its lean mass. Hence, the precision of the $LM_{DXA}$ measurement (Table 1) and the accuracy with which it predicts TBW (Fig. 3*a*) are very important contributors to the machine's ability to predict LM and FFM with very little unexplained error ($R^2 = 0.98$ for both;

Figs. 3*c*, 4*c*, respectively). Percent body fat can therefore be predicted by DXA with tremendous accuracy for all voles by using the $mass_{grav}$ and $FFM_{DXA}$ values to calculate FM by difference. However, caution must be used to ensure that trapped animals do not dehydrate and are weighed both at the time of capture and at the time of DXA analysis. Drawing blood or removing tissues from an animal will also affect FFM. In such cases, it may be best to use the $FM_{DXA}$ equation because the $FM_{DXA}$ measurement is not dependent on LM values and retains slightly reduced yet satisfactory precision and accuracy. In instances where voles are collected from different seasons, body mass and composition are likely to vary. In such instances, the relative body fat levels of pregnant or lactating females, very large individuals, or even very small individuals could resemble the percentage body fat of the leanest lab-raised voles. The $FM_{DXA}$ equation for free-living or all voles could be used if there were high numbers of gestating, lactating, or relatively fatter voles or if there were high variability in the body composition, season, or latitude at which animals were trapped (Fig. 4*a*). In any instance, the equation selected should be used consistently throughout comparisons of free-living animals.

The LM in free-living and lab-raised voles can be predicted with excellent accuracy ($R^2 = 0.98$) using DXA. Although LM is comprised mostly of water, it is important to keep in mind that TP and $LM_{resid}$ are also contributing factors. The TP of voles comprises a much smaller portion of the LM, and a slightly more reliable and independent measurement of TP could be obtained by nonlethal means. By using deuterium dilution to measure the TBW of a scanned animal and by continuing to assume that changes in $LM_{resid}$ are negligible, we could measure TP independently by using $TP = LM - TBW - LM_{resid}$, where LM and TBW are derived from the predictive equations in Table 2 and $LM_{resid}$ is constant (0.207 g in the case of *Clethrionomys rutilus*). Estimates of TP using the derived DXA algorithm (Fig. 3*b*) are therefore highly reliable under the assumption that %TBW is constant in all study animals and less reliable when not measured independently.

Subcutaneous PIT tags embedded in recaptured animals caused a significant but predictable increase in all parameters reported by the DXA software (Table 3). Field studies using portable DXA machines and the same type/size tag should use these correction factors to avoid including the effect of tags in the data and thus elevating the level of actual body composition values in immobilized animals. If other types or sizes of PIT tags are used, a DXA scan before and after tagging could be used to define similar correction terms. The digital cropping function available on the Lunar PIXImus2 DXA apparatus might also be able to exclude both embedded and external tags without affecting measurements.

Unfortunately, the Lunar PIXImus2 DXA machine used in these experiments is no longer being manufactured, and there is, at present, no portable equivalent available on the market. Therefore, a used or remanufactured Lunar machine would have to be purchased for field studies that require a portable machine. Alternatively, animals could be brought to a facility where a stationary DXA machine is available, although the feeding, handling, and transport of the study animals might change their body composition.

The PIXImus2 DXA apparatus can be used to accurately determine values of several body composition parameters in free-living and lab-raised voles. It can provide reliable data while preserving population numbers, even in recapture studies where animals are PIT tagged. It is uncertain whether a DXA apparatus could accurately determine body composition in animals smaller than 10 g, such as shrews, because their bones and fat deposits are very small and may not be detected by DXA. The derived equations in this study should, however, be applicable to all small free-living and lab-raised rodents of 10–35 g in size, encompassing species of *Clethrionomys*, *Microtus*, *Mus*, *Peromyscus*, and others. For these rodents, DXA represents a nonlethal, nondestructive tool that is capable of measuring many specific parameters of whole-body composition in small free-living and lab-raised individuals with excellent precision and accuracy.

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## Literature Cited

Albanese C.V., E. Diessel, and H.K. Genant. 2003. Clinical applications of body composition measurements using DXA. J Clin Densitom 6:75–85.

Batzli G.O. and E. Esseks. 1992. Body fat as an indicator of nutritional condition for the brown lemming. J Mammal 73: 431–439.

Batzli G.O. and F.A. Pitelka. 1971. Condition and diet of cycling populations of the California vole, *Microtus californicus*. J Mammal 52:141–163.

Bjora R., J.A. Falch, H. Staaland, L. Nordsletten, and E. Gjengedal. 2001. Osteoporosis in the Norwegian moose. Bone 29: 70–73.

Blanc S., D. Schoeller, J. Kemnitz, R. Weindruch, R. Colman, W. Newton, K. Wink, S. Baum, and J. Ramsey. 2003. Energy expenditure of rhesus monkeys subjected to 11 years of dietary restriction. J Clin Endocrinol Metab 88:16–23.

Bland J.M. and D.G. Altman. 1986. Statistical methods for as-

sessing agreement between two methods of clinical measurement. Lancet 12:307–310.

Brommage R. 2003. Validation and calibration of DEXA body composition in mice. Am J Physiol 285:E454–E459.

Carey D.G., A.B. Jenkins, L.V. Campbell, J. Freund, and D.J. Chisholm. 1996. Abdominal fat and insulin resistance in normal and overweight women: direct measurements reveal a strong relationship in subjects at both high and low risk of NIDDM. Diabetes 45:633–638.

Castro G., B.A. Wunder, and F.L. Knopf. 1990. Total body electrical conductivity (TOBEC) to estimate body fat of free-living birds. Condor 92:496–499.

Dirrigl F.J., G.P. Dalsky, and S.E. Warner. 2004. Dual-energy x-ray absorptiometry of birds: an examination of excised skeletal specimens. J Vet Med A 51:313–319.

Dodds E.D., M.R. McCoy, A. Geldenhuys, L.D. Rea, and J.M. Kennish. 2004. Microscale recovery of total lipids from fish tissue by accelerated solvent extraction. J Am Oil Chem Soc 81:835–840.

FAO (Food and Agriculture Organization). 2003. Food Energy: Methods of Analysis and Conversion Factors: Report of a Technical Workshop. FAO Food and Nutrition Paper 77. FAO, Rome.

FAO/WHO Expert Group. 1973. Energy and Protein Requirements. FAO Nutrition Meetings Report Series 52. FAO/WHO, Rome.

Felicetti L.A., C.T. Robbins, and L.A. Shipley. 2003. Dietary protein content alters energy expenditure and composition of the mass gain in grizzly bears (*Ursus arctos horribilis*). Physiol Biochem Zool 76:256–261.

Frawley D.J., D.A. Osborne, H.P. Weeks Jr., L.W. Burger Jr., and T.V. Dailey. 1999. Use of total body electrical conductivity to predict norther bobwhite lipid mass. J Wildl Manag 63:695–704.

Garriga R.M., A.W. Sainsbury, and A.E. Goodship. 2004. Bone assessment of free-living ground squirrels (*Sciurus vulgaris*) from the United Kingdom. J Wildl Dis 40:515–522.

Giergielewicz-Mozajska H., L. Dabrowski, and J. Namiesnik. 2001. Accelerated solvent extraction (ASE) in the analysis of environmental solid samples: some aspects of theory and practice. Crit Rev Anal Chem 31:149–165.

Gotfredsen A., J. Jensen, J. Borg, and C. Christiansen. 1986. Measurement of lean body mass and total body fat using dual photon absorptiometry. Metabolism 35:88–93.

Grodum E., J. Gram, K. Brixen, and J. Bollerslev. 1995. Autosomal dominant osteoporosis: bone mineral measurements of the entire skeleton of adults in two different subtypes. Bone 16:431–434.

Hendel H.W., A. Gotfredsen, I. Anderson, L. Hojgaard, and J. Hilsted. 1996. Body composition during weight loss in obese patients estimated by dual energy x-ray absorptiometry and by total body potassium. Int J Obes Relat Metab Dis 20: 1111–1119.

Hiyaoka A., T. Yoshida, F. Cho, and Y. Yoshikawa. 1994. Age-related changes in bone-mineral density, mean width and area of the lumbar vertebrae in male African green monkeys (*Cercopithecus auethiops*). Exp Anim 43:235–241.

Hunter H.L. and T.R. Nagy. 2002. Body composition in a seasonal model of obesity: longitudinal measures and validation of DXA. Obes Res 10:1180–1187.

Johnston S.L., W.K. Peacock, L.M. Bell, M. Lonchampt, and J.R. Speakman. 2005. PIXImus DXA with different software needs individual calibration to accurately predict fat mass. Obes Res 13:1558–1565.

Jones D.B. (1931) 1941. Factors for Convening Percentages of Nitrogen in Foods and Feeds into Percentages of Proteins. USDA Circular 183. USDA, Washington, DC.

Jones D.B., V.E. Munsey, and L.E. Walker. 1942. Report of committee on protein factors. J Assoc Agric Chem 25:118–120.

Korine C., S. Daniel, I.G. van Tets, R. Yosef, and B. Pinshow. 2004. Measuring fat mass in small birds by dual-energy x-ray absorptiometry. Physiol Biochem Zool 77:522–529.

Krebs C.J. and G.R. Singleton. 1993. Indices of condition for small mammals. Aust J Zool 41:317–323.

Lehmer E.M. and B. Van Horne. 2001. Seasonal changes in lipids, diet, and body composition of free-ranging black-tailed prairie dogs (*Cynomys ludovicianus*). Can J Zool 79: 955–965.

Mata A.J., M. Caloin, J.-P. Robin, and Y.L. Maho. 2006. Reliability in estimates of body composition of birds: oxygen-18 versus deuterium dilution. Physiol Biochem Zool 79:202–209.

Michaelsson K., R. Bergstrom, H. Mallmin, L. Holmberg, A. Wolk, and S. Ljunghall. 1996. Screening for osteopenia and osteoporosis: selection by body composition. Osteopor Int 6:120–126.

Mitchell A.D., R.W. Rosebrough, and J.M. Conway. 1997. Body composition analysis of chickens by dual energy x-ray absorptiometry. Poult Sci 76:1746–1752.

Mitchell A.D., A.M. Scholz, and J.M. Conway. 1998. Body composition analysis of small pigs by dual-energy x-ray absorptiometry. J Anim Sci 76:2392–2398.

Nagy T.R. and A.-L. Clair. 2000. Precision and accuracy of dual-energy x-ray absorptiometry for determining in vivo body composition of mice. Obes Res 8:392–398.

Nielson D.H., F.J. McEvoy, H.L. Poulsen, M.T. Madsen, L.E. Buelund, and E. Svalastoga. 2004. Dual-energy x-ray absorptiometry of the pig: protocol development and evaluation. Meat Sci 68:235–241.

Peppler W.W. and R.B. Mazess. 1981. Total body bone mineral and lean body mass by dual-photon absorptiometry. Calcif Tissue Int 33:353–359.

Pietrobelli A., C. Formica, Z. Wang, and A.B. Heymsfield. 1996. Dual-energy x-ray absorptiometry body composition model: review of physical concepts. Am J Physiol 271:E941–E951.

Pietrobelli A., Z. Wang, C. Formica, and A.B. Heymsfield. 1998. Dual-energy x-ray absorptiometry: fat estimation errors due to variation in soft tissue hydration. Am J Physiol 74:E808–E816.

Pouilles J.M., P. Collard, F. Tremollieres, P. Frayssinet, J.J. Railhac, J.P. Cahuzac, A. Autefage, and C. Ribot. 2000. Accuracy and precision of *in vivo* bone mineral measurements in sheep using dual-energy x-ray absorptiometry. Calcif Tissue Int 66: 70–73.

Ross P., C. Huang, J. Davis, K. Imose, J. Yates, J. Vogel, and R. Wasnich. 1995. Predicting vertebral deformity using bone densitometry at various skeletal sites and calcaneous ultrasound. Bone 16:325–332.

Schulte-Hostedde A.I., J.S. Millar, and G.J. Hickling. 2001*a*. Evaluating body condition in small mammals. Can J Zool 79:1021–1029.

———. 2001*b*. Sexual dimorphism in body composition of small mammals. Can J Zool 79:1016–1020.

Swennen Q., G.P.J. Janssens, R. Geers, E. Decuypere, and J. Buyse. 2004. Validation of dual-energy x-ray absorptiometry for determining in vivo body composition of chickens. Poult Sci 83:1348–1357.

Tavernier R.J., A.L. Largen, and A. Bult-Ito. 2004. Circadian organization of a subarctic rodent, the northern red-backed vole (*Clethrionomys rutilus*). J Biol Rhythms 19:238–247.

Toll P.W., K.L. Gross, S.A. Berryhill, and D.E. Jewell. 1994. Usefulness of dual energy x-ray absorptiometry for body composition measurement in adult dogs. J Nutr 21:2601S–2603S.

Trinchieri A., R. Nespoli, F. Ostini, F. Rovera, and A. Curro. 1999. Bone mineral content in calcium renal stone formers. Scanning Microsc 13:2–3, 281–289.

Unangst E.T., Jr., and B.A. Wunder. 2001. Need for species-specific models for body composition estimates of small mammals using EM-SCAN. J Mammal 82:527–534.

Walsberg G.E. 1988. Evaluation of a non-destructive method for determining fat stores in small birds and mammals. Physiol Zool 61:153–159.

Winstanley R.K., G. Saunders, and W.A. Buttemer. 1998. Indices for predicting total body fat in red foxes in Australia. J Wildl Manag 62:1307–1312.

Yaskin V.A. 1984. Seasonal changes in brain morphology in small mammals. Pp. 183–191 in J.F. Merritt, ed. Winter Ecology of Small Mammals. Carnegie Mellon University Press, Pittsburgh.

Zuercher G.L., D.D. Roby, and E.A. Rexstad. 1997. Validation of two new total body electrical conductivity (TOBEC) instruments for estimating body composition of live northern red-backed voles *Clethrionomys rutilus*. Acta Theriol 42:387–397.

———. 1999. Seasonal changes in body mass, composition, and organs of northern red-backed voles in interior Alaska. J Mammal 80:443–459.