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Non-invasive measure of body composition of snakes using dual-energy X-ray absorptiometry

Stephen M. Secor[a,*], Tim R. Nagy[b]

[a]*Department of Biology, University of Mississippi, P.O. Box 1848, University, MS 38677-1848, USA*
[b]*Department of Nutrition Sciences, University of Alabama at Birmingham, Birmingham, AL 35294-3360, USA*

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Abstract

Non-invasive techniques to measure body composition are critical for longitudinal studies of energetics and life histories and for investigating the link between body condition and physiology. Previous attempts to determine, non-invasively, the body composition of snakes have proven problematic. Therefore, we explored whether dual-energy X-ray absorptiometry (DXA) could be used to determine the body composition of snakes. We analyzed 20 adult diamondback water snakes (*Nerodia rhombifer*) with a DXA instrument and subsequently quantified their body composition by gravimetric and chemical extraction methods. Body composition components scaled with body mass with mass exponents between 0.88 and 1.53. DXA values for lean tissue mass, fat mass and total-body bone mineral mass were significantly correlated with observed masses of lean tissue, fat and ash from chemical analysis. Using regression models incorporating DXA values we predicted the fat-free tissue mass, lean tissue mass, fat mass, ash mass and total body water content for this sample of water snakes. A cross-validation procedure demonstrated that these models estimated fat-free tissue mass, lean tissue mass, fat mass, ash mass and total-body water content with respective errors of 2.2%, 2.3%, 16.0%, 6.6% and 3.5%. Compared to other non-invasive techniques, include body condition indices, total body electrical conductivity (TOBEC) and cyclopropane absorption, DXA can more easily and accurately be used to determine the body composition of snakes.

Keywords: Body composition; Dual-energy X-ray absorptiometry, DXA; Reptile; Snake

1. Introduction

Germane to studies of animal energetics, reproduction and life histories is knowledge of an individual's body composition, in particular the amount of stored body fat (Congdon et al., 1982). Body fat, due to its high energy content, is correlated with animal growth, activity, survivorship and reproductive success and thus overall fitness (Hadley, 1985). Animals commonly experience seasonal and even daily fluctuations in body composition as fat is deposited when food is available and the animal is eating and is catabolized when energy expenditure exceeds input such as during periods of fasting and reproduction (O'Connor, 1995). Therefore, it is highly desirable to be able to track temporal changes in body composition and to examine its link to energetics, fitness and physiology.

The determination of body composition can be made via two approaches, either directly by chem-

*Corresponding author. *Present address:* Department of Biological Sciences, Box 870344, University of Alabama, Tuscaloosa, AL 35487-0344, USA. Tel.: 1-205-348-1809; fax: 1-205-348-1786.
E-mail address: ssecor@biology.as.ua.edu (S.M. Secor).

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Table 1
Sex, snout–vent length (SVL), wet and dry body mass, total body water content, wet and dry mass of fat-free tissue, lean tissue and fat and ash mass of 20 diamondback water snakes (*N. rhombifer*) used to validate the application of dual-energy X-ray absorptiometry (DXA) to non-invasively measure body composition of snakes

Sex	SVL (cm)	Body mass (g)		Total body water content (g)	Fat-free tissue (g)		Lean tissue (g)		Fat (g)		Ash (g)
		wet	dry		wet	dry	wet	dry	wet	dry	
F	70.3	295.8	92.2	203.6	262.4	63.6	249.7	50.9	33.6	28.6	12.7
M	78.0	310.0	111.1	198.9	270.8	75.4	254.8	59.3	39.2	35.7	16.1
M	74.0	345.2	117.2	228.0	311.8	86.6	295.5	70.3	33.2	30.6	16.3
M	79.2	369.3	126.2	243.1	328.7	93.7	310.3	75.3	40.3	32.5	18.3
M	85.2	407.8	135.4	272.2	377.7	108.2	354.2	84.8	30.3	27.2	23.4
M	86.0	417.1	135.9	281.2	374.2	101.0	353.5	80.4	42.8	34.9	20.6
M	89.0	423.5	142.5	281.0	385.2	108.2	361.0	84.0	38.8	34.3	24.2
M	87.0	427.8	150.4	277.4	375.6	102.8	353.0	80.2	52.4	47.6	22.6
M	79.6	434.0	146.2	287.8	382.0	103.9	362.9	84.8	52.0	42.3	19.1
M	78.5	440.6	162.6	278.0	399.3	124.9	373.1	98.7	41.7	37.9	26.2
M	83.0	451.2	146.1	305.1	420.4	118.9	396.1	94.6	30.6	27.2	24.3
M	85.0	460.1	157.9	302.2	405.8	110.9	378.2	83.3	54.2	47.0	27.6
M	86.5	477.0	156.0	321.0	440.2	123.7	413.2	96.6	36.8	32.3	27.0
M	86.5	478.3	178.0	300.3	424.0	127.0	397.7	100.7	55.0	51.2	26.3
M	88.5	500.6	190.3	310.3	427.3	122.1	402.9	97.7	73.7	68.3	24.3
F	87.0	758.1	260.3	497.8	629.3	157.9	600.3	128.9	128.7	102.7	29.1
M	90.5	768.7	304.2	464.5	363.2	183.8	594.1	141.7	132.8	120.4	42.0
F	98.0	791.3	261.4	529.9	686.7	167.3	649.9	130.5	104.3	94.1	36.8
F	98.0	1024.0	413.6	610.4	759.1	202.9	754.6	162.5	228.9	210.7	40.5
F	111.0	1577.1	577.0	1000.1	1264.0	289.8	1192.6	218.3	313.0	287.2	71.5
Mean	86.0	557.9	198.2	359.6	479.8	128.6	452.4	101.2	78.1	69.6	27.5
Percentage of body mass (wet)			34.9±0.6%	65.1±0.6%	87.6±0.9%	24.0±0.6%	82.6±0.8%	19.0±0.5%	12.4±0.9%	10.9±0.9%	5.1±0.1%
Range			31.2–40.4%	59.6–68.8%	93.2–77.6%	18.4–28.3%	73.2–86.8%	13.8–22.4%	6.8–22.4%	6.0–20.6%	3.8–6.0%

Calculation of total body water content, fat-free tissue mass, lean tissue mass, fat mass and ash mass are presented in the text.

Table 2
Parameters of allometric regressions of fat-free tissue mass, lean tissue mass, fat mass, ash mass and total body water content for 20 diamondback water snakes (*N. rhombifer*)

Dependent variable	Mass coefficient	Mass exponent	r^2	F	P
Fat-free tissue mass (FFM, g)	1.53±0.05	0.91±0.02	0.994	2932	<0.0001
Lean tissue mass (LTM, g)	1.43±0.04	0.91±0.02	0.994	3295	<0.0001
Fat wet mass (FM, g)	0.004±0.344	1.53±0.13	0.884	146	<0.0001
Ash mass (AM, g)	0.11±0.19	0.88±0.07	0.897	166	<0.0001
Total body water content (TBW, g)	0.87±0.05	0.95±0.02	0.993	2593	<0.0001

All variables were $\log_{10}$ transformed prior to regression analyses. Tissue and water mass can be predicted using the equation variable = a(body mass)^b, where a = the mass coefficient and b = mass exponent.

ical analysis of an animal's carcass or indirectly by non-destructive techniques (Speakman, 2001). The advantage of the former technique is greater accuracy in quantifying body components, while the clear disadvantage is loss of that animal for future study and that it probably would not be permissible for studies on rare or endangered animals. Non-destructive methods are favored if an animal's body composition is to be tracked over time or if it is to be compared with a future index of fitness. The down-side of these techniques is the variability in their precision and accuracy to estimate fat-free tissue and fat mass.

The current array of non-destructive techniques includes body condition indices, isotope dilution, total body electrical conductivity (TOBEC), lipid-soluble gas absorption and bioelectrical impedence analysis (BIA) (reviewed in chapters in Speakman, 2001). While each of these methods has been shown to predict, within acceptable accuracy, fat-free tissue mass and/or fat mass of target species, none have been documented to do so for snakes. A relatively recent addition to non-invasive methods of body composition analysis is dual-energy X-ray absorptiometry (DXA) (Nagy, 2001). DXA operates by scanning the body with two X-ray beams of different energy levels, and using the attenuation of the two beams to differentiate and quantify bone-free lean tissue mass (LTM), fat mass (FM), total-body bone mineral mass (TBBM) and total-bone mineral density (TBMD). Developed for research of human osteoporosis, DXA has since been used on non-human primates, livestock, dogs and small rodents (Nagy, 2001).

Encouraged by its success in measuring body composition of mammals, we set out to determine whether DXA could be used to determine fat-free mass (FFM), LTM, FM, ash mass (AM) and total-body water content (TBW) of snakes. We began this study by comparing DXA measurements of LTM, FM and TBBM of 20 diamondback water snakes (*Nerodia rhombifer*) with their LTM, FM and AM determined by chemical analysis. We subsequently found that regression models generated from DXA and body composition data could predict FFM, LTM, FM, AM and TBW with average relative differences from values based on chemical analysis on the order of 2.2%, 2.3%, 16.0%, 6.6% and 3.5%, respectively.

2. Materials and methods

2.1. Snakes

Diamondback water snakes inhabit a variety of aquatic habitats from large lakes and rivers to small ponds and streams throughout their range extending from western Texas and Oklahoma east to western Alabama and north into southern Iowa (Conant and Collins, 1991). The 20 water snakes used in this study were hand-captured from two adjacent ponds of a commercial channel catfish farm in Leflore County, Mississippi in early May 2000 under a collecting permit issued by the Mississippi Department of Wildlife, Fisheries and Parks. The sample included 15 male and five female water snakes ranging in snout–vent length from 70.3 to 111.0 cm (mean±1 S.E. = 86.0±2.0 cm) and in body mass from 296 to 1577 g (558±68 g) (Table 1). After capture, snakes were maintained in the laboratory for two weeks at 25 °C with water provided but no food to insure that they were postabsorptive prior to study. Following, snakes were euthanized by an intracardiac injection of sodium pentobarbital (Nembutal, 50 mg/kg) and their large intestines cleared of any feces and urate by palpation. Snakes were weighed and transported on ice to the University of Alabama at Birmingham for DXA analysis. Housing and experimental procedures were conducted with the approval of the University of Mississippi Institutional Animal Care and Use Committee.

2.2. DXA measurements

We analyzed each snake using a Lunar Prodigy DXA scanner (GE-Lunar, Madison, WI) manufactured for human scanning. Snakes were coiled in an oval shape such that there was no overlap of body coils and scanned twice without repositioning. Each scan was analyzed using the manufacturer's software (version 6.10), which provided a measure of LTM, FM and TBBM. For analysis, we averaged for each snake the two measures of each variable, with intraindividual coefficient of variation for the two scans equaling $0.6 \pm 0.1\%$ for LTM, $9.2 \pm 2.3\%$ for FM and $1.0 \pm 0.2\%$ for TBBM.

2.3. Chemical body composition analysis

Following DXA, we dissected each snake and weighed the wet mass of each organ, the discernible fat bodies and remaining carcass (combined bone, muscle and skin). Organs, fat and carcasses were dried to a constant mass at 60 °C and reweighed. Each snake's dried organs and carcass were combined and ground to a fine powder. We measured fat (non-polar lipids) content of a 7-g subsample of the ground homogenate (which lacked the abdominal fat bodies) of each snake by extraction with petroleum ether in a Soxhlet apparatus (Dobush et al., 1985). A second 2-g subsample of the homogenate was placed into a muffle furnace (600 °C for a minimum of 8 h) to determine ash content. We calculated for each snake its total body dry mass, TBW, FFM, LTM, FM and AM. Body dry mass was calculated as the sum of dried fat, organs and carcass mass. Total body water content was determined by subtracting body dry mass from body wet mass. We calculated total FM by summing the mass of dissected fat bodies and the estimated wet mass of fat within the organs and remaining carcass; the latter determined from the proportion of fat in the dried subsample multiplied by the combined dry mass of the organs and carcass. Fat-free mass was calculated as body mass minus FM. Total AM was determined as the proportion of ash in the dried subsample multiplied by the combined organ and carcass dry mass. We calculated LTM as FFM minus AM.

2.4. Data analysis

Because of the significant difference ($P = 0.002$) in body mass between male and female water snakes of this study, we used analysis of covariance (ANCOVA, body mass as the covariate) to determine whether there were any significant differences in body composition with respect to sex. We next identified the allometric relationship of FFM, LTM, FM, AM and TBW for water snakes by analyzing each variable against body mass using least-square regression. All masses were $\log_{10}$ transformed prior to analysis. We tested for the correlation between DXA values of LTM, FM and TBBM and those values determined from body composition analysis (TBBM was compared with AM). Single and multivariate regression models were next constructed to predict FFM, LTM, FM, AM and TBW from DXA readings (entered as independent variables) and measurements made from chemical body composition analyses (entered as dependent variables).

To illustrate the predictive power of our equations to estimate body composition, we employed a cross-validation ('jack-knife') procedure to the data (Sokal and Rohlf, 1995). For each statistical analysis, we removed one snake from the data set and generated equations based on the data from the other 19 snakes. DXA values from the one removed snake were entered into the equations to generate predicted measures of that snake's FFM, LTM, FM, AM and TBW. This was done in turn for all 20 water snakes, allowing measured FFM, LTM, FM, AM and TBW of each snake to be compared with predicted values derived independently.

We report the results of our statistical analyses in terms of their F, r, r^2 and P values. The level of statistical significance was designated as $P < 0.05$ and mean values are presented as means ± 1 S.E.

3. Results

3.1. Body composition

For this sample of 20 water snakes, body wet mass was comprised of $87.6 \pm 0.9\%$ FFM and $12.4 \pm 0.9\%$ FM (Table 1). Females, which were larger (889 ± 209 g), possessed a significantly ($P = 0.003$) larger percentage of their body mass as fat ($16.7 \pm 2.0\%$) and a reciprocal smaller percentage as fat-free tissue than males (447 ± 27 g, $10.9 \pm 0.7\%$ fat). The easily dissected fat pads (located ventral to the intestines) constituted $89.5 \pm 1.0\%$ of total fat; the rest was extracted

from the organs and carcass. Water content of snakes averaged 65.1±0.6% of body mass and did not significantly differ between males and females. Of body dry mass, 54.6±1.6% was lean tissue, 30.9±2.0% was fat and 14.5±0.5% was ash. Ash mass as a percentage of body lean dry mass did not differ between male and female water snakes. Wet FFM, LTM and FM and AM and TBW scaled with body mass with exponents ranging from 0.88 to 1.53 (Table 2). The scaling mass exponent of FM was significantly ($P<0.001$) greater than that of FFM and LTM due to larger snakes (females) possessing a relatively greater amount of fat (thereby increasing its scaling slope) and a relatively smaller amount of FFM and LTM (thereby decreasing their scaling slopes).

3.2. DXA evaluation of body composition

DXA measures of LTM, FM and TBBM were highly correlated (P values <0.0001, r values ranging between 0.85 and 0.99) with respected masses determined from chemical body composition analysis (Fig. 1). Single and multivariate regression models constructed to predict FFM, LTM, FM, AM and TBW from DXA values are presented in Table 3. We found that the best predictive equations (r^2 values $=0.983–0.998$) for FFM, LTM, FM and TBW include DXA values for LTM and FM, whereas for ash, mass was best predicted using only the DXA measure of TBBM. The predicted values based on these equations differed from actual measures by an average of 7.1 g (1.6%), 7.4 g (1.7%), 7.5 g (14.1%), 1.6 g (6.2%) and 10.3 g (2.9%), respectively, for FFM, LTM, FM, AM and TBW. As an independent evaluation of these predictive models, the cross-validation procedures demonstrated that FFM, LTM, FM, AM and TBW of this sample of water snakes can be predicted from DXA results with average errors of 2.2%, 2.3%, 16.0%, 6.6% and 3.5%, respectively, (Fig. 2). For each body component, predicted values did not differ significantly (paired t-test, P values >0.7) from those observed.

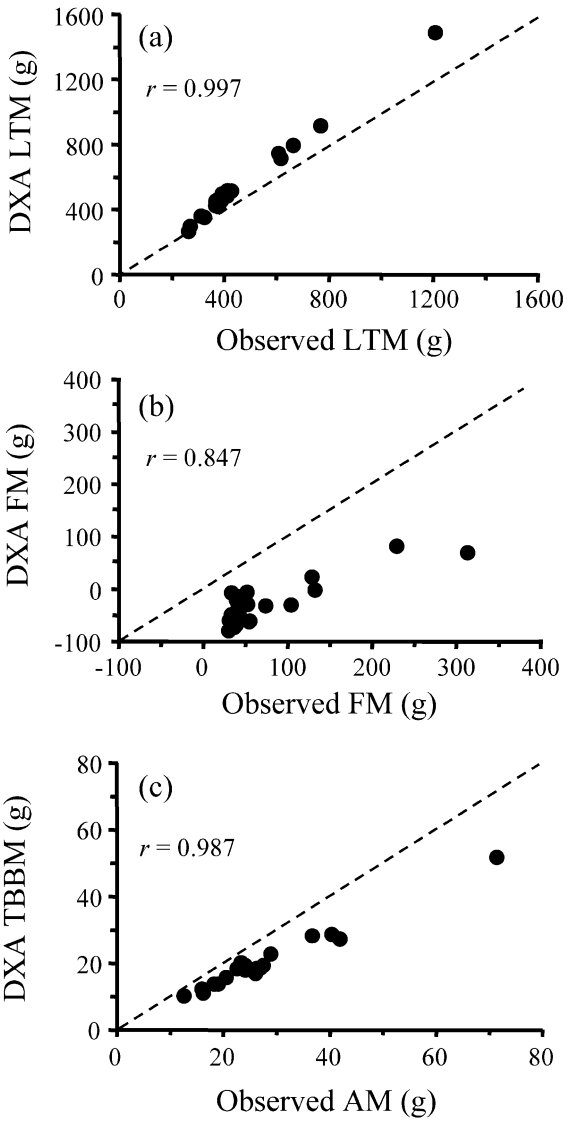

Fig. 1. DXA measures of lean tissue mass (LTM), fat mass (FM) and total-body bone mineral mass (TBBM) plotted against observed (a) LTM, (b) FM and (c) ash mass (AM) determined from chemical body composition analysis of 20 diamondback water snakes (*N. rhombifer*). For each body component, mass calculated by DXA was significantly correlated with that determined from chemical analysis. The dashed line represents the line of identity between DXA and observed body composition values.

4. Discussion

Accurate non-invasive techniques to determine body composition are certainly preferred over destructive methods requiring euthanasia and are essential for longitudinal studies using the same individuals and/or in studies involving expensive, rare or endangered species. While non-invasive techniques have been successfully employed to estimate (within an acceptable degree of accuracy) animal lean tissue and fat masses (reviewed in Speakman, 2001), none have been documented (although tried) to accurately predict the fat-free

Table 3
Regression models for predicting fat-free tissue mass, lean tissue mass, fat mass, ash mass and total body water content from dual-energy X-ray absorptiometry (DXA) measures of lean tissue mass (LTM), fat mass (FM) and total-body bone mass (TBBM) for diamondback water snakes (*N. rhombifer*)

Dependent variable (g)	Model	r^2	Regression model		Cross-validation	
			difference (g)	difference (%)	difference (g)	difference (%)
Fat-free tissue mass	$=0.84\text{DXA}_{\text{LTM}}-7.06$	0.996	11.8 ± 1.8	3.0 ± 0.6	12.9 ± 1.9	3.3 ± 0.6
Fat-free tissue mass	$=0.80\text{DXA}_{\text{LTM}}+0.36\text{DXA}_{\text{FM}}+24.2$	0.998	7.1 ± 1.0	1.6 ± 0.2	9.8 ± 2.0	2.2 ± 0.5
Lean tissue mass	$=0.79\text{DXA}_{\text{LTM}}-7.92$	0.995	12.6 ± 2.0	3.3 ± 0.6	13.6 ± 2.2	3.6 ± 0.7
Lean tissue mass	$=0.75\text{DXA}_{\text{LTM}}+0.39\text{DXA}_{\text{FM}}+26.2$	0.998	7.4 ± 1.2	1.7 ± 0.3	9.6 ± 1.4	2.3 ± 0.4
Fat mass	$=1.43\text{DXA}_{\text{FM}}+115$	0.700	29.8 ± 5.5	56.7 ± 11.9	33.9 ± 7.2	61.8 ± 12.6
Fat mass	$=0.19\text{DXA}_{\text{LTM}}+0.64\text{DXA}_{\text{FM}}-14.0$	0.983	7.5 ± 1.0	14.1 ± 2.1	8.8 ± 1.3	16.0 ± 2.3
Ash mass	$=1.40\text{DXA}_{\text{TBBM}}-0.09$	0.973	1.6 ± 0.3	6.2 ± 1.0	1.7 ± 0.3	6.6 ± 1.0
Total body water	$=0.68\text{DXA}_{\text{LTM}}-32.2$	0.989	15.7 ± 2.3	5.2 ± 1.0	17.8 ± 2.6	5.7 ± 1.1
Total body water	$=0.63\text{DXA}_{\text{LTM}}+0.43\text{DXA}_{\text{FM}}+4.98$	0.994	10.3 ± 1.8	2.9 ± 0.5	13.1 ± 2.5	3.5 ± 0.6

Equations were developed from body composition and DXA measurements of 20 adult water snakes. Presented for each equation are the absolute differences (g) and relative differences (%) between the model's predicted values and those observed from body composition analysis and those differences between observed measures and predicted values generated from the cross-validation procedure as explained in the text.

tissue and fat masses of snakes. This study was initiated to determine whether dual-energy X-ray absorptiometry (DXA), a non-destructive method that accurately measures bone-free lean tissue mass (LTM), fat mass (FM), total-body bone mineral mass (TBBM) and total-bone mineral density (TBMD) of humans and other mammals, could be used to determine the body composition of snakes. We found for 20 diamondback water snakes, that DXA and chemical body composition measures of LTM, FM and AM (TBBM) were significantly correlated. Single and multivariate regression equations using DXA values were able to predict the FFM, LTM, FM, AM and total body water content (TBW) with an average error of 2.2%, 2.3%, 16.0%, 6.6% and 3.5%, respectively. In the following discussion we shall review the application of three other non-destructive methods to calculate body composition of snakes and the practicality of using DXA to determine body composition of snakes and other vertebrates.

4.1. Application of other non-destructive methods

Of the non-destructive methods for calculating body composition, body condition indices have had the longest history of use and are by far the least technically challenging (Hayes and Shonkwiler, 2001). A condition index is derived as a direct measure, ratio or residual of body mass and some measure of structural size or linear length. The basic premise of a body condition index is that for a given length, a heavier animal possesses more fat, therefore, the index is correlated with FM and reciprocally with FFM (or LTM). If validated from a destructive analysis of FFM and FM, such an index can be used to estimate FFM and FM from convenient measures of mass and size. To address whether body condition indices could be used to accurately assess FFM and FM of our 20 water snakes, we calculated for each snake the following seven indices; mass, log mass, mass residual (from mass plotted against SVL), log mass residual (from log mass plotted against log SVL), mass/SVL, $\log_{10}$ mass/$\log_{10}$ SVL and $(\text{mass}/\text{SVL})^2$ (Hayes and Shonkwiler, 2001).

We found each index to significantly correlate (r values >0.43) with both FFM and FM (Table 4). We next subjected each index, together with SVL (or $\log_{10}$ SVL) as a second independent variable, with measured FFM and FM (as the dependent variable) to multivariate regression analyses in order to construct predicted equations for FFM and FM (Table 4). Cross-validation analyses revealed that each index could predict FFM and FM of water snakes with respective mean errors ranging from 3 to 10% and 20 to 41% (Table 4). For each index, differences between measured and predicted FFM and FM were significantly (paired t-test, P values <0.05) greater than those differences between measured and predicted LTM and FM generated from DXA-based models.

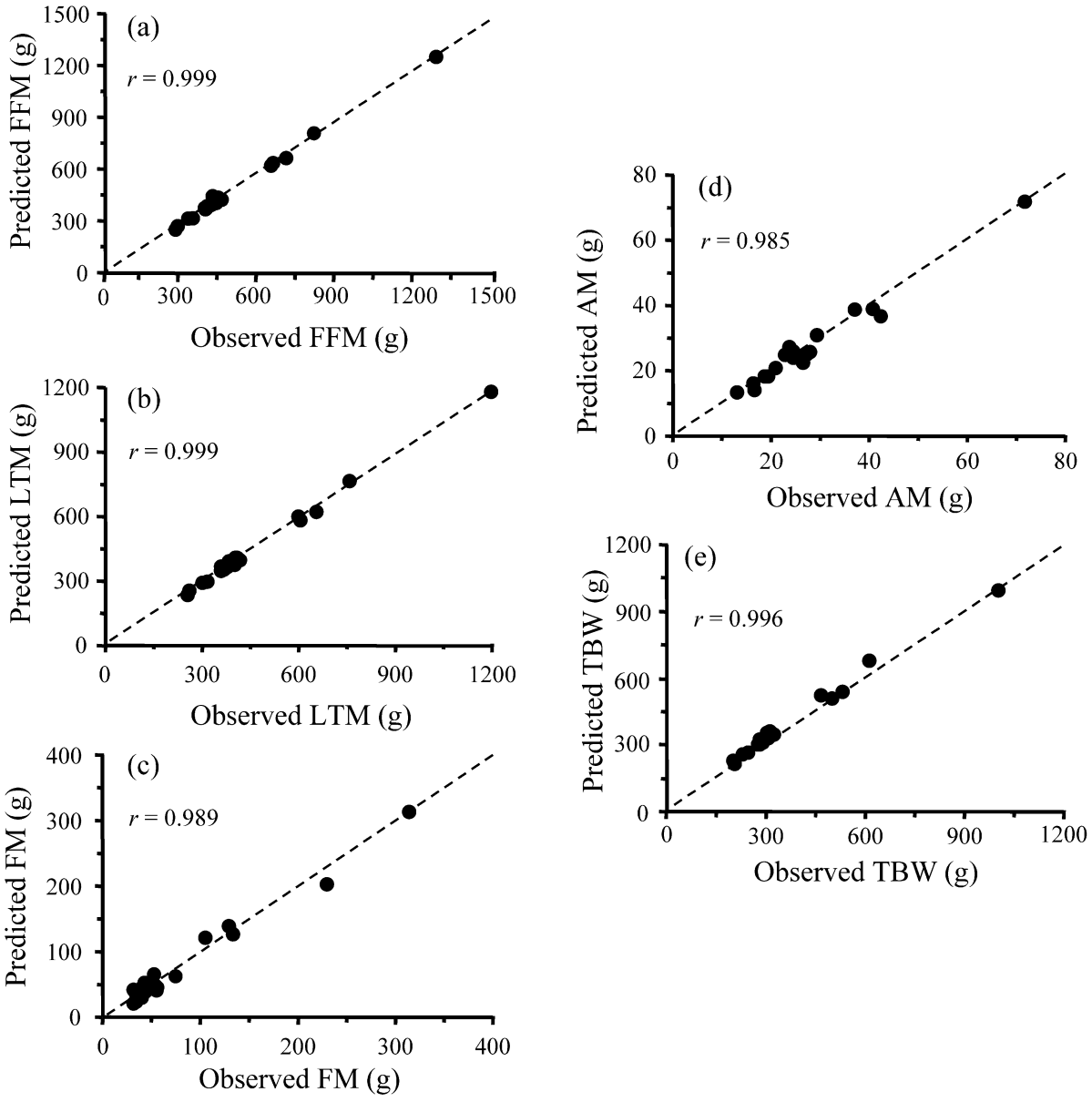

Fig. 2. Model-based predicted mass of fat-free tissue (FFM) lean tissue (LTM), fat (FM), ash (AM) and total body water content (TBW) plotted against observed (a) FFM (b) LTM, (c) FM, (d) AM and (e) TBW determined from chemical body composition analysis for 20 diamondback water snakes (*N. rhombifer*). Predicted values are the result of a cross-validation procedure using DXA-based regression models (explained in the text). The dashed line represents the line of identity between predicted and observed body composition values.

Therefore, DXA provided greater predicted power for estimating LTM (or FFM) and FM than traditional body condition indices. Similarly, Weatherhead and Brown (1996) found that a body condition index based on logged mass residuals was relatively unreliable in predicting FM of northern water snakes (*N. sipedon*). The use of ratio-based indices is also highly cautioned due to the dependency of the ratio with body size (Hayes and Shonkwiler, 2001).

Total body electrical conductivity (TOBEC) represents the sum of electrical conductivity of an individual's lean tissue (lipid conductivity is $<5\%$ of lean tissue) and when matched with a

Table 4
Regression models for predicting fat-free tissue mass and fat mass generated from seven different body condition indices and snout–vent length (SVL) for diamondback water snakes (*N. rhombifer*)

	Regression model	r	r^2	Regression model		Cross validation	
				difference (g)	difference (%)	difference (g)	difference (%)
Fat-free tissue mass (g)	$=0.72\text{mass}+1.51\text{SVL}-52.6$	0.988	0.996	16.8 ± 2.7	2.3 ± 0.4	12.9 ± 2.8	2.7 ± 0.5
	$=1242(\log_{10}\text{mass})-8.87(\log_{10}\text{SVL})-2862$	0.971	0.936	37.4 ± 8.9	7.6 ± 1.6	50.0 ± 15.3	9.6 ± 2.2
	$=0.73(\text{mass residual})+23.0\text{SVL}-150$	0.434	0.996	10.4 ± 2.2	2.3 ± 0.4	12.9 ± 2.8	2.7 ± 0.5
	$=1149(\log_{10}\text{mass residual})+22.6\text{SVL}-1466$	0.430	0.962	29.7 ± 6.7	6.0 ± 1.1	40.2 ± 12.3	7.6 ± 1.6
	$=73.8(\text{mass}/\text{SVL})+5.47\text{SVL}-454$	0.988	0.987	17.0 ± 4.0	3.2 ± 0.5	23.7 ± 7.4	4.1 ± 0.8
	$=4.09(\text{mass}/\text{SVL})^2+6.37\text{SVL}-254$	0.986	0.991	16.8 ± 2.7	3.8 ± 0.7	21.1 ± 3.7	4.5 ± 0.8
	$=2212(\log_{10}\text{mass}/\log_{10}\text{SVL})+10.3\text{SVL}-3498$	0.943	0.940	37.7 ± 8.2	8.0 ± 1.6	50.3 ± 14.9	9.8 ± 2.1
Fat mass (g)	$=0.28\text{mass}+1.51\text{SVL}+52.6$	0.977	0.957	10.4 ± 2.2	18.8 ± 4.3	12.9 ± 2.8	21.5 ± 4.5
	$=460(\log_{10}\text{mass})-348(\log_{10}\text{SVL})-474$	0.936	0.871	19.8 ± 3.3	35.0 ± 7.5	24.8 ± 4.8	41.4 ± 9.0
	$=0.28(\text{mass residual})+6.77\text{SVL}-504$	0.523	0.957	10.4 ± 2.2	18.8 ± 4.3	12.9 ± 2.8	21.5 ± 4.5
	$=433(\log_{10}\text{mass residual})-6.63\text{SVL}-492$	0.499	0.897	17.5 ± 3.0	31.3 ± 6.9	21.8 ± 4.2	36.6 ± 7.9
	$=28.6(\text{mass}/\text{SVL})-0.022\text{SVL}-99.4$	0.977	0.948	12.5 ± 2.0	23.1 ± 5.0	15.0 ± 2.5	26.6 ± 5.5
	$=1.59(\text{mass}/\text{SVL})^2+0.33\text{SVL}-22.0$	0.979	0.954	10.2 ± 2.4	17.0 ± 4.0	14.2 ± 4.3	19.9 ± 4.3
	$=833(\log_{10}\text{mass}/\log_{10}\text{SVL})+1.98\text{SVL}-1257$	0.927	0.867	19.8 ± 3.5	35.8 ± 8.0	24.7 ± 5.1	41.2 ± 8.9

Equations were generated from body composition measurements of fat-free tissue mass and fat mass (dependent variables) and body condition indices (explained in the text) and SVL entered as independent variables for 20 adult water snakes. Presented for each equation are the correlation coefficient (r) for the relationship between the index and the dependent variable, the (r^2) of the model and the absolute differences (g) and relative differences (%) between the model's predicted values and those observed from body composition analysis and those differences between observed measures and predicted values generated from the cross-validation procedure as explained in the text.

destructive validation study can be used to estimate an individual's FFM (Scott et al., 2001). Fat mass is then derived indirectly by subtracting FFM from body mass. Studies using TOBEC, largely on small mammals and birds, have found the technique to be relatively reliable at predicting FFM, but less effective at predicting FM (Scott et al., 2001). Since body mass of fat is much less than that of fat-free tissue, small errors in FFM is translated into much larger errors in calculated FM (Fischer et al., 1996). For the lizard, *Sceloporus undulatus*, regression equations including TOBEC readings and body mass as independent variables generated predicted measures of fat-free dry mass and FM that differed on average by 5.8% and 30.3% from those determined from body composition analyses (Angilletta, 1999). Although a relatively simple technique, TOBEC is sensitive to body size, shape and temperature, hydration state, distribution of lean tissue, movement and positioning of the animal within the scanning chamber and laboratory vs. field application (Scott et al., 2001; Wirsing et al., 2002). Problematic for its use on snakes, is that they need to be coiled in order to fit within the scanning chamber and any differences in coiling pattern or the amount of overlap of body coils adds additional variation to the TOBEC readings (Secor and Frank, unpublished observations). These potential problems combined with a relatively low body fat content probably explains the observed 100% error in predicted body fat of copperheads (*Agkistrodon contortrix*) using TOBEC (Hill and Beaupre, unpublished observations).

The gas dilution method calculates an individual's fat mass directly as a product of the amount of absorbed lipid-soluble gas, usually cyclopropane and the gas' lipid solubility coefficient (Henen, 2001). Validated for laboratory rats (*Rattus norvegicus*), red-eared sliders (*Trachemys scripta elegans*) and rock doves (*Columbia livia*) this method has the advantage of potential greater accuracy in predicting FM (mean errors of 5.6–11% for these three studies), but the disadvantage of being complex, time consuming and technically challenging (Lesser et al., 1952; Henen, 1991; Gessaman et al., 1998). Inherent to this method is a greater number of potential sources of error, stemming from its many measured and calculated variables, including animal volume and temperature, chamber gas volume, pressure and temperature and cyclopropane volume, pressure, temperature and analysis. In an attempt to validate this method for sidewinder rattlesnakes (*Crotalus cerastes*) of 80–100 g, cyclopropane absorption varied as much as 100% and did not correlate with the 50% variation in FM (Secor and Mock, unpublished observations.). The difficulties of this procedure have undoubtedly limited its use and precluded it from consideration for most studies (Henen, 2001).

4.2. Practicality of DXA for body composition analysis

To date, body condition indices, TOBEC and cyclopropane absorption have not proven to be reliable methods to predict FFM (or LTM) and FM of snakes. In contrast, we found that DXA can provide a rapid, user-friendly and relatively accurate means to predict the FFM, LTM, FM, AM and TBW of snakes. Unlike other non-invasive methods that only measures fat-free or fat mass, DXA measures three compartments of the body; LTM, FM and TBBM. In this study, we compared DXA TBBM with residual AM, assuming that the majority of ash originated from bone. Understandably, bone contains organic material and salts, which would be not be included in the residual ash and inorganic material present in non-bone tissues (i.e. skin) would contribute to the ash. We predicted the dried skeletal mass of each snake using the regression equation, $\log_{10}$ skeletal mass $= 1.007 \log_{10}$ body mass $- 1.202$ ($r^2 = 0.975$), constructed from skeletal and body masses of 171 diamondback water snakes (7.5–1871 g, Secor, unpublished data). Ash mass and DXA TBBM averaged $22.7 \pm 2.2\%$ and $44.6 \pm 1.7\%$ less than predicted dried skeletal mass, respectively, for our 20 water snakes.

The most significant advantage of DXA is the accuracy that the technique can measure the components of body composition. DXA has already proven to be as, or more, accurate than any other non-invasive method. For small mammals, DXA-based models can estimate LTM and FM with errors of only 1–5% (Nagy and Clair, 2000; Hunter and Nagy, 2002). Although the human DXA machine that we used to scan snakes was not designed to analyze or calculate the body composition of reptiles, its values when applied to regression models could accurately predict tissue mass. We attempted to improve upon these models by incorporating additional independent variables into the regression equations. We found that the

addition of mass, SVL and sex resulted in only a minor improvement in the model's ability to predict LTM (error of 1.7% reduced to 1.6%) and FM (error of 14.1% reduced to 12.7%).

As for any method, DXA does have its drawbacks. First, as for any non-invasive technique, a validation study requiring the destructive measure of body composition must be undertaken to generate predictive models. Second, animals need to be immobile during the scan, which may require sedation by anesthesia. In this study, we elected to scan dead rather than anesthetized snakes for convenience, though we do acknowledge that death may have a potential impact on DXA measurements. In a pilot trial, we were able to scan an unsedated Burmese pythons (*Python molurus*) that lied still and coiled, within an open plastic container. Third, DXA machines are relatively expensive; compared to $10 000 for a TOBEC scanner (EM-SCAN, Springfield, IL) and $5000 for a gas chromatograph for cyclopropane analysis, a human-version DXA machine runs between $35 000 and $100 000. Smaller DXA units that are designed for rodents, but can also scan small birds and reptiles (<100 g), are approximately $45 000. And fourth, because the machines are designed for scanning humans (or small mammals), the computational programs will undoubtedly provide erroneous measures of LTM and FM from the scans of non-mammalian species. As illustrated in Fig. 1, DXA-computed LTM averaged 28% greater than actual LTM, and DXA FM were negative for all but three snakes.

On a positive note, DXA has become one of the most widely employed methods for determining body composition of humans. Hence, the number of DXA machines is increasing steadily in hospitals, clinics, medical research institutions and physician offices, therefore, increasing the opportunity for their use in animal research. Also, companies aware of their utility in animal research are manufacturing less expensive, desk-top models that when coupled with the appropriate software shall be able to analyze the body composition of a wide variety of animals. In addition, to small mammals, DXA is currently being used to analyze the body composition of small birds (Korine et al., 2002). Representing the next generation in analyzing body composition, DXA enables researchers to accurately track seasonal and ontogenetic changes in lean tissue, fat and bone mass for individual animals and to correlate those findings with fitness and/or physiological parameters.

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