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The effects of lipid location on non-invasive estimates of body composition using EM-SCAN technology

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Summary

We evaluated the effect of lipid location on body-composition estimation accuracy using electromagnetic scanning (EM-SCAN), a non-invasive [total body electrical conductivity (TOBEC)] method. Molds were constructed that simulated a 'general' small mammal, either 93% lean/7% lipid (control) or 82% lean/18% lipid (lipid-location groups). In the 18% lipid molds, we varied the location of the fat; simulating all the fat in the head, tail or midsection or simulating homogenous distribution. Comparisons were made between the EM-SCAN output of each lipid-location group, and multiple-regression techniques were performed to derive body-composition estimation equations for both lipid mass (M_L) and fat-free mass (M_{FF}). Device output varied significantly for all lipid-location groups even though all groups contained 18% body fat, showing a lipid-location effect on device output. Calibration equations derived for each lipid-location condition estimated both M_L and M_{FF} accurately, but an independent equation was required for each lipid-location condition. In situations where species significantly vary body fat content and location, for example during hibernation or reproductive periods, we suggest deriving a calibration equation that is more representative of the actual body composition to improve M_L and M_{FF} estimation accuracy using non-invasive EM-SCAN methods.

Key words: lipid, electromagnetic scanning (EM-SCAN), total body electrical conductivity (TOBEC), body composition.

Introduction

Small-mammal body composition (fat and lean) can be determined using chemical lipid extraction. Although this method is extremely accurate in determining body composition, euthanasia of the subject is necessary. However, in cases where the animal cannot be killed or where repeated measures of a subject over time are necessary, the need for non-invasive body-composition estimation exists. Technology utilizing total body electrical conductivity (TOBEC), such as EM-SCAN, is available and has been shown to be both precise and accurate in the estimation of fat-free mass (M_{FF}) and lipid mass (M_L) (Bell et al., 1994; Unangst and Wunder, 2001; Voltura and Wunder, 1998; Zuercher et al., 1997). The EM-SCAN device creates a 10 MHz electromagnetic (EM) field. The EM field is altered in proportion to electrolytes (divalent cations) present in the subject tissue (Harrison and Van Itallie, 1982). Because electrolytes are more prevalent in lean body tissue than in lipid tissue (in the ratio of 40:1; Presta et al., 1983), a greater disturbance of the EM field is caused by lean tissue than lipid tissue. The device measures the level of disturbance in the EM field and yields a unit-less value known as the EM value. This EM value is indicative of the conductivity of the subject. Using simple inverse-regression or multiple-regression techniques, calibration equations that incorporate morphological parameters and the EM value can be derived to estimate the actual M_L or M_{FF} of a sample derived from actual chemical extraction data for composition (Unangst and Wunder, 2001; Voltura, 1997; Voltura and Wunder, 1998; Wunder et al., 2000; Zuercher et al., 1999).

The accuracy and reliability of the body-composition estimates with EM-SCAN can be affected by hydration, body temperature, position and shape of the sample, and gastrointestinal tract contents if not properly controlled (Bachman, 1994; Bell et al., 1994; Voltura and Wunder, 1998; Walsberg, 1988; Zuercher et al., 1997). Estimation accuracy can also be improved by deriving species-specific equations (Unangst and Wunder, 2001) or specific-condition equations (Wunder et al., 2000). Comparisons of estimates for M_L and M_{FF} in species with very similar morphology (meadow vole *Microtus pennsylvanicus* and prairie vole *Microtus ochrogaster*) showed a threefold improvement in error estimates in equations derived for a particular species (Unangst and Wunder, 2001). These results suggest that each species might deposit fat differently or in different body locations, therefore affecting the EM-SCAN device output (Unangst and Wunder, 2001). Previously frozen specimens can also be analyzed accurately with EM-SCAN, with error estimates for lipid of 0.5 g, but this necessitates different calibration equations from live specimens (Wunder et al., 2000). Thus, we tested whether the location of lipid deposition can influence the disturbance of the EM field and the device output, even when

lipid mass is constant. In addition, we derived predictive equations for both lean and lipid masses for different lipid-location conditions and examined the estimation accuracy of these predictive equations.

Materials and methods

All laboratory work was completed at the Department of Biology of the United States Air Force Academy, CO, USA. We constructed specimens that were representative of small mammals in both body size and body mass. In forming our 'small mammal' models, we used 93% lean ground beef as our lean mass standard and animal fat (beef) for the lipid mass component. The shape and size of the specimen resulted from a plastic tubular mold (approximately 110 mm length × 24.5 mm diameter), in which we placed a predetermined amount of lean and lipid tissue in a specific arrangement, thus varying the lipid location and simulating head, tail, midsection or homogenous distribution (Fig. 1).

Experiment 1: lipid location effects on device output

In our first experiment, a total of 44 'small mammal' models were used. Groups consisted of one lean group (without additional fat; 93% lean) and four lipid-location groups (with additional fat, 82% lean), with fat either being added in the head, tail or midsection regions of the mold or being mixed homogenously throughout the mold. Each group contained 11 specimens, with each individual model weighing approximately 78 g and a pooled body fat of 7% in the lean group and 18% in each lipid-location group (later confirmed by Sohxlet ether extraction). To control for dehydration, each model was wrapped in plastic wrap and held in a freezer at –20°C. In preparation for EM-SCAN measures, all specimens remained in the freezer for 72 h and were then removed and placed in a cold room (at 7.4°C) for an additional 24 h to thaw. Each specimen, in turn, was removed from the cold room and allowed to warm to room temperature (23°C), weighed to the nearest 0.01 g (Ohaus E400D) and measured for length (to the nearest mm). Specimen temperature was measured with a digital thermocouple. Immediately upon warming to 23°C, the specimen was unwrapped to control dehydration and then centered on the EM-SCAN insertion platform for placement within the EM-SCAN SA-2 (EM-SCAN Inc., Springfield, IL, USA) chamber. Consistent with Unangst and Wunder (2001) and Voltura and Wunder (1998), we took seven readings for each specimen, omitted the highest and lowest value and averaged the remaining five values to calculate our EM_{avg} value. Comparisons of the EM_{avg} value between lipid-location groups were performed to evaluate the similarity of values across groups. Because absolute lipid mass was constant between groups, the EM-SCAN device output should not vary if lipid location has no effect on EM-field disturbance. After completion of EM-SCAN measures, each specimen was rewrapped and returned to the cold room. To control for temperature deviations of >4°C (Walsberg 1988), no specimen was subjected to room temperature for >10 min during the measurement procedure. Specimen temperature was reconfirmed immediately upon measurement completion using a digital thermocouple.

For the head and tail lipid-location groups, the same specimen was used for EM-SCAN measures, with the lipid introduced anteriorly to simulate the head and posteriorly to simulate the tail. We alternated the samples having the 'head' first or 'tail' first to reduce any effect of time for measurement. For example, the first specimen (no. 1) was scanned with the lipid-end inserted into the scanning chamber first (head), then turned around and scanned with the lipid-end inserted last (tail). Then, the next specimen (no. 2) was scanned lipid-end last (tail), then reversed and scanned lipid-end first (head). This alternating pattern was repeated for all 11 specimens in the head and tail lipid-location groups.

Statistical comparisons of the EM_{avg} value between groups were performed using analysis of variance (ANOVA). Because the head and tail lipid-location groups used the same specimens, a pair-wise *t*-test comparison was done. The level of significance in all statistical tests was set at $P=0.05$.

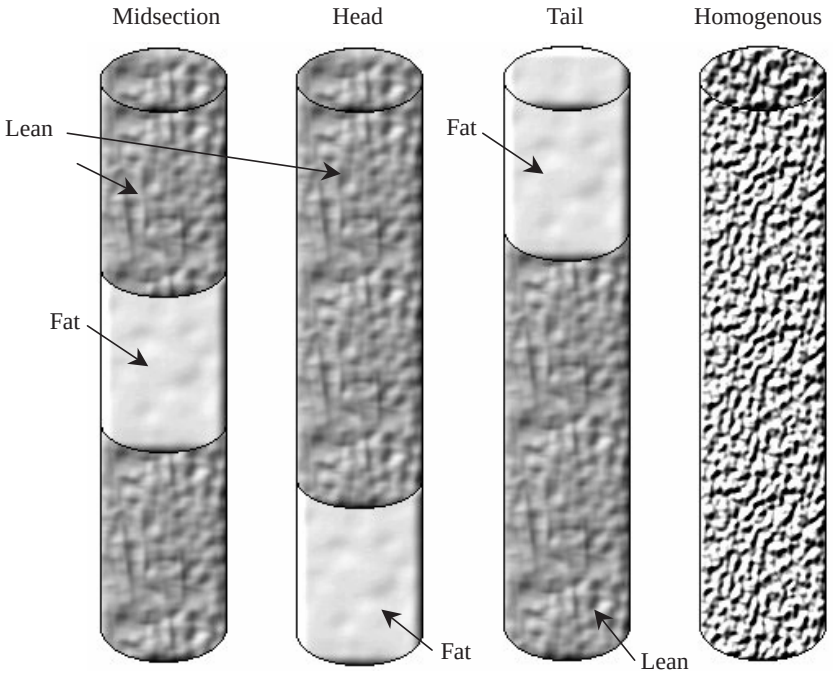

Fig. 1. Representation of 'small mammal' models, illustrating the lipid-location variation between head, tail, midsection or homogenous distribution in the 18% body fat lipid-location groups.

Experiment 2: calibration equation and estimate accuracy

In our second experiment, a total of 65 different 'small mammal' models was

Table 1. *Comparison of body mass, length and* EM_{avg} *values for lean and lipid-location groups from sample one (lipid-location effects on device output)*

	Lean	Head*	Tail*	Midsection	Homogenous
Mass (g)	78.13±0.03[a]	77.12±0.19[b]	77.12±0.19[b]	77.24±0.09[b]	76.90±0.32[b]
Length (mm)	104.1±0.67[a]	104.3±0.45[a]	104.3±0.45[a]	103.7±0.36[a]	104.0±0.40[a]
EM_{avg} value (unit-less)	49.7±0.4[a]	41.0±0.5[b]	43.9±0.8[c]	35.5±0.4[d]	24.3±0.8[e]

Different letters indicate significant difference between groups (per variable) (P<0.05, N=11). Values are means ± S.E.M.

*Head/tail EM comparison was calculated using a paired *t*-test (same specimens). All other comparisons were calculated using analysis of variance (ANOVA).

constructed. Groups consisted of one lean group (without additional fat; 93% lean; N=5) and four lipid-location groups (head, tail, midsection or homogenous distribution; N=20 in each group). Within each lipid-location group, five specimens with either 10%, 15%, 20% or 25% body fat, respectively, were made. As in experiment 1, the head and tail groups used identical specimens for EM-SCAN measures. Each model weighed approximately 80 g, with a pooled lipid mass of approximately 14 g (18% body fat) within each lipid-location group.

EM-SCAN procedures were identical to those previously described. Once measured, each specimen was dried in a convection-drying oven at 70°C until it reached constant mass. The specimens were then homogenized, and chemical lipid extractions were performed using a modified Soxhlet procedure at a contracted laboratory at the University of Western Ontario, USA. Estimation equations for M_{FF} and M_L by lipid-location group were completed using multiple-regression procedures (Unangst and Wunder, 2001; Voltura, 1997; Voltura and Wunder, 1998). To account for body-size effects, we incorporated a conductive index (*CI*; Fiorotto et al., 1987), defined as:

$$CI = (EM_{avg} \times L)^{0.5},$$

where L is body length. Regression models investigated body mass, body length, EM_{avg} value and *CI*, with the ten best models evaluated using an adjusted r^2-model-selection technique with the best-fit model selected parsimoniously (Unangst and Wunder, 2001). To determine the degree of accuracy for M_L and M_{FF} estimates in the best-fit model, we used cross-validation techniques that compared the actual values with the estimated values (Conway et al., 1994; Skagen and Knopf, 1993; Voltura and Wunder, 1998). Cross-validation predicts the average error of estimates by using the data set with one individual removed. A calibration equation is then generated using data from the remaining specimens and estimates the body composition for the removed individual. The estimate for that particular specimen is then compared with the actual (chemical analysis) M_L or M_{FF} of that particular individual. The absolute value of the difference (in g) between the actual and estimated value is the average error. This process was repeated with a different individual removed until all specimens were accounted. Thus, the average error for the estimation equation is the mean of the average error in all runs. By comparing the average error estimate for each lipid-location condition, an assessment of estimation accuracy between equations was possible.

Results

Lipid location effects on device output

The EM-SCAN output (EM_{avg}) varied significantly by lipid-location group (Table 1). In the 18% body fat lipid-location groups, EM_{avg} ranged from 24 to 44, with each group statistically different from one another, even though all groups had identical fat content. As expected, the lean group had the highest EM_{avg} (50) and was statistically different from each lipid group. There were no statistical differences between lipid-location groups with regard to body length or total body mass.

Calibration equation and estimate accuracy

Body composition and body length for each lipid-location group were not statistically different (Table 2). The EM-SCAN was able to estimate both M_L and M_{FF} very well, with r^2 values ranging from 0.80 to 0.87 for each body-composition component (Table 3). The average error in estimates for M_L was similar across lipid locations, averaging approximately 1.5 g or 9–13% lipid (Table 4). Error estimates for M_{FF} ranged from 1.3 g to 2.2 g, with a smaller percent error rate of approximately 3% (Table 4). This improved performance for

Table 2. *Comparison of morphological characters for each lipid-location group in sample two (calibration equation and estimate accuracy)*

	Head*	Tail*	Midsection	Homogenous
M_B	79.65±0.03	79.65±0.03	79.61±0.02	79.54±0.04
M_L	14.68±1.05	14.68±1.05	14.77±1.07	14.07±0.86
M_{FF}	64.97±1.04	64.97±1.04	64.85±1.07	65.46±0.88
L	105.6±0.2	105.6±0.2	105.5±0.2	105.5±0.3

L=body length in mm; M_B=body mass in g; M_L=lipid mass in g; M_{FF}=fat-free mass in g.

*Head/tail EM comparison was calculated using a paired *t*-test (same specimens). All other comparisons were calculated using analysis of variance (ANOVA).

No significant difference between groups (P<0.05, N=20). Values are means ± S.E.M.

Table 3. *Calibration equations for estimating lipid or fat-free mass in 'small mammal' models with varying fat location from experiment two (calibration equation and estimate accuracy)*

Condition	Equation*	r^2
Head	[1]M_L=56.54+1.18M–1.73CI	0.87
	[2]M_{FF}=(CI–46.10)/0.50	0.87
Tail	[1]M_L=–40.66+2.50M–1.82CI	0.80
	[2]M_{FF}=(CI–51.01)/0.43	0.80
Midsection	[1]M_L=505.27–4.99M–1.22CI	0.85
	[2]M_{FF}=(CI–32.22)/0.69	0.83
Homogenous	[1]M_L=8.74+0.70M–0.68CI	0.86
	[2]M_{FF}=(CI+8.86)/1.27	0.87

*M_L=lipid mass in g; M_{FF}=fat-free mass in g; M=wet mass in g; CI=conductive index, defined as (EM_{avg}×body length)$^{0.5}$.

[1]Multiple regression.

[2]Inverse regression two-stage.

M_{FF} was expected, because the pooled samples contained approximately 82% lean tissue.

Discussion

Our data show that the location of lipid deposition affects the output of EM-SCAN. In comparing the four lipid-location groups from experiment 1, each with 18% body fat, one would expect similar EM_{avg} values if lipid location did not influence the device output. However, each lipid-location group had a significantly different value (Table 1). Because body mass and length did not differ by group, these results show that lipid location influences EM output and should be considered when using EM-SCAN. This would be most applicable in species that significantly vary white and brown adipose tissue seasonally, e.g. hibernators, and in a species' preparation for reproductive efforts. In addition, adiposity research investigating diet-inducted obesity, body regulation and leptin (also termed OB protein) in laboratory animals such as mice and rats may encounter such body-composition changes. Explanations for output differences remain unknown.

In mammalian hibernators, energy reserves are often met by stored energy in the form of body fat. Increases in body mass exceeding 30% (primarily fat) are common in pre-hibernating mammals (Lyman et al., 1982), as documented for bears (Hilderbrand et al., 2000), bats (Kunz et al., 1998; Serra-Cobo et al., 2000) and other small mammals (Arnold, 1993; Buck and Barnes, 1999; Lehmer and Van Horne, 2001; Pulawa and Florant, 2000). In hibernators and many non-hibernators, brown adipose tissue (BAT) increases are found primarily in the cervical, interscapular and thoracic regions to provide energy via non-shivering thermogenesis (Hayward and Lyman, 1967; Lyman et al., 1982; Nedergard et al., 1993; Smalley and Dryer, 1967; Trayhurn, 1993). Increased body fat is also found in many mammalian, avian and reptilian species prior to reproduction to meet gestation and lactation demands (Bronson, 1989; Meier and Burns, 1976). Finally, studies involving rodent obesity models differ widely in the type and extent of obesity and warrant such consideration for estimation-model specificity (Tschop and Heiman, 2001).

As demonstrated in several studies (Castro et al., 1992; Unangst and Wunder, 2001; Voltura, 1997; Voltura and Wunder, 1998; Wunder et al., 2000), the EM-SCAN estimates M_{FF} accurately and performs well in estimates of M_L in relatively fatter individuals. Our data from experiment 2 clearly show that the EM-SCAN allows good estimation of body composition in relatively fat specimens (18% lipid) even when lipid-deposition locations vary (Table 3). However, a specific calibration equation for each lipid location was necessary to achieve a higher degree of estimation accuracy (Tables 3, 4). In our design, the pooled relative body fat used in equation derivation was confirmed by chemical lipid extraction to be approximately 18%; thus, the accuracy in error estimates was improved over conditions with very lean individuals (Voltura and Wunder, 1998). Estimate errors of approximately 1.5 g represent a 10% error as a percentage of the total body lipid. Because lean mass averaged 82% of the total body mass, the 2 g error in M_{FF} estimates equates to a 3% error rate of total body lean tissue.

Thus, we suggest that body composition can be estimated most accurately in species that vary lipid location and amounts by deriving body-composition estimation equations specific to the expected condition. In hibernators, where significant seasonal changes in both white and brown fat amounts and deposition in specific locations occur, using an estimation equation derived during lean body-composition periods may

Table 4. *Average error estimates (from cross validation) for estimation models estimating fat-free or lipid content on 'small mammal' models with varying lipid location from experiment two (calibration equation and estimate accuracy)*

	Location of fat			
Parameter	Head	Tail	Midsection	Homogenous
Average error (M_L)	1.52±0.22	1.96±0.25	1.65±0.26	1.31±0.17
[1]Average error (% lipid)	10	13	11	9
Average error (M_{FF})	1.64±0.24	2.24±0.27	1.84±0.31	1.26±0.23
[1]Average error (% fat-free)	2.5	3.4	2.8	1.9

[1]Average error (%)=average error (g)/M_{FF} or M_L.

Values are means ± S.E.M. N=20 for each group.

not be appropriate. The significant preparatory fat deposition associated with reproduction in some mammalian, avian and reptilian species is another possible condition where different estimation equations may be warranted. Overall, derivation of body-composition estimation equations most representative of the physiological state of the specimen will improve both the reliability and accuracy of body-composition estimates using non-invasive methods such as EM-SCAN.

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