The Veterinary Journal 212 (2016) 65–70

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Measuring body composition in dogs using multifrequency bioelectrical impedance analysis and dual energy X-ray absorptiometry

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ARTICLE INFO

Article history:
Accepted 11 April 2016

Keywords:
Dog
Bioelectrical impedance
DXA
Body composition
Fat-free mass
Obesity

ABSTRACT

Thirty-five healthy, neutered, mixed breed dogs were used to determine the ability of multifrequency bioelectrical impedance analysis (MFBIA) to predict accurately fat-free mass (FFM) in dogs using dual energy X-ray absorptiometry (DXA)-measured FFM as reference. A second aim was to compare MFBIA predictions with morphometric predictions.

MFBIA-based predictors provided an accurate measure of FFM, within 1.5% when compared to DXA-derived FFM, in normal weight dogs. FFM estimates were most highly correlated with DXA-measured FFM when the prediction equation included resistance quotient, bodyweight, and body condition score. At the population level, the inclusion of impedance as a predictor variable did not add substantially to the predictive power achieved with morphometric variables alone; in individual dogs, impedance predictors were more valuable than morphometric predictors. These results indicate that, following further validation, MFBIA could provide a useful tool in clinical practice to objectively measure FFM in canine patients and help improve compliance with prevention and treatment programs for obesity in dogs.

Introduction

Excess body fat is the most common nutritional disorder of dogs in Western countries, with an estimated prevalence of 33–44% or higher (German, 2006; Gossellin et al., 2007; Zoran, 2010; Laflamme, 2012). Obesity is known to induce insulin resistance, oxidative stress and a chronic, low-grade inflammatory state thought to contribute to the development of osteoarthritis and other diseases (Zoran, 2010; Laflamme, 2012), or osteoarthritis and reduced lifespan (Kealy et al., 2002). A moderately high fat diet has been shown to increase visceral fat two-fold in dogs, with minimal increases in bodyweight (BW; Kim et al., 2003) conducive to the development of insulin resistance; insulin resistance increases with adiposity, even if BW is stable. Prevention of obesity is more effective than its subsequent treatment and is best instituted while animals are just beginning to gain weight (Zoran, 2010; Laflamme, 2012), yet veterinarians often neglect to formally diagnose and discuss an increase in BW (Lund et al., 2005). Once obesity is established it is much more difficult to implement successful weight loss strategies (Gossellin et al., 2007; Zoran, 2010). For these reasons, some authors have stressed the importance of assessing adiposity per se rather than simply BW (Laflamme, 2012), as the latter does not necessarily reflect body fat content (Stanton et al., 1992).

Body fat can be accurately measured by various methods (Heymsfield et al., 2005; Gossellin et al., 2007), although many of these, e.g. computed tomography (Purushothaman et al., 2013), quantitative magnetic resonance imaging and dual-energy X-ray absorptiometry (DXA; Zanghi et al., 2013), require specialised equipment and/or general anaesthesia, and are not practical or available for many research and clinical applications. Body condition scoring (BCS), morphometric measurements, and bioelectrical impedance analysis (BIA) offer non-invasive, practical methods for estimating body composition. BCS, using a validated methodology, offers a semi-quantitative assessment that correlates well with percent body fat (Mawby et al., 2004; Shoveller et al., 2014), but is somewhat a subjective measure, relying on visual appraisal and palpation that requires some level of training for competency. BIA, by contrast, is an objective technique that measures the electrical resistance of body water (TBW) that relates directly to the fat-free mass (FFM) of the body (Stone et al., 2009; German et al., 2010).

This study aimed to evaluate the ability of multifrequency bioelectrical impedance analysis (MFBIA), BCS scoring and morphometric measures to predict FFM in dogs compared with FFM measured by DXA.

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http://dx.doi.org/10.1016/j.tvjl.2016.04.007
1090-0233/

Materials and methods

Dogs

Thirty-five neutered (18 male, 17 female) mixed breed research dogs were used. All dogs were clinically healthy based on history, physical examination findings, and recent haematological and biochemical blood analyses, and were determined to be free from internal parasites by faecal analysis after deworming. The dogs' ages were unknown but, based on physical characteristics and dentition, all were estimated to be >7 months of age. Bodyweights were 12.1–43.0 kg and BCS was 3–4.25 (average of two assessors) on a scale of 1–5 (1, emaciated; 5, obese; Table 1).

The study was performed according to The University of Queensland Animal Ethics Committee's Policies and Guidelines and study protocols were approved by The University of Queensland Animal Ethics Committee SVS/307/04, approved 1st July 2004 and annually until 16th July 2007.

Bodyweight and body condition score

Bodyweight was measured (to the nearest 10 g using a veterinary scale [SK-Vet-150; Accuweigh]) and BCS was determined by two experienced research technicians following standardised assessment protocols (McGreevy et al., 2005). BCS was graded into 0.5 increments as proposed by Baldwin et al. (2010). BCS data were averaged for the two assessors; individual ratings did not vary more than 0.5 unit. BCS has been validated against DXA for assessment of body composition in dogs (Laflamme, 1997).

Morphometric measurement – length

In order to generate MFBIA-based prediction equations for body composition, a measurement of the current path is required. Since the precise path is unknown, a surrogate measurement is used, e.g. height in humans (Foster and Lukaski, 1996), or simple linear measurement between the sense electrodes in animals (Ward and Battersby, 2009). After dogs were sedated and placed in left lateral recumbency, body length to the nearest millimetre was measured from the middle of the right eye to the anus using a flexible tape measure.

Dual energy X-ray absorptiometry and multifrequency bioelectrical impedance analysis

Dogs were fasted for at least 20 h prior to DXA followed by MFBIA measurements, performed on the same day. Dogs were sedated with SC methadone (0.3 mg/kg; Methone Injection, 10 mg/mL, Ceva Animal Health) and acepromazine (0.03 mg/kg; ACP2, 2 mg/mL, Delvet) 30 min prior to anaesthesia with IV alfaxalone (1–2 mg/kg; Alfaxan CD-RTU, 10 mg/mL, Jurox).

Dogs were scanned (Hologic QDR-4500A) and scans were analysed using manufacturer's software (Hologic). Dogs were positioned in a standardised fashion, aided by gridlines on the scanner bed, in dorsal recumbency with the head extended, forelegs bent and taped away from the body and the hind legs extended. A single scan (2–3 min duration) was performed by an experienced DXA technician. Tissue quantification was achieved by measuring the differential attenuation by lean, fat and bone mineral of two X-ray beams of different energy levels to provide measurements of whole body lean mass, fat mass (FM) and bone mineral mass (Heymsfield et al., 2005). FFM was calculated as the sum of lean and bone mineral content (BMC). FFM determined by DXA was comparable with that determined from measurement of TBW by tracer dilution (Heymsfield et al., 2005).

Whole body impedance was measured using a tetrapolar multifrequency bioimpedance spectrometer (SFB7, ImpediVET®, ImpediMed), which measured resistance (R) and reactance (Xc) at 256 frequencies from 3 to 1000 kHz at a constant drive current of 200 μA. Ag-AgCl gel EKG-style (24 × 22 mm) skin electrodes (ImpediMed) were used. Hair at the electrode site was clipped closely to the skin and cleaned with an alcohol wipe. Based on preliminary reproducibility and reliability studies, the following electrode locations were used: voltage sense electrodes were placed cranially at the right stifle and right elbow with current drive electrodes 10 cm distal, similar to the protocol used in other studies (Scheltinga et al., 1991). Measurement time was <1 s and data (10 consecutive readings) were downloaded to a computer for analysis.

Multifrequency bioelectrical impedance analysis theory and data analysis

MFBIA data were uploaded to a computer and analysed (Bioimp software, version 4.15.0.0, ImpediMed). The software fitted the recorded resistance and reactance data to a semi-circular plot of resistance vs. reactance, after the Cole model of biological impedance (Thomas et al., 1998) that represents the body as a resistor representing the extracellular water (ECW), in parallel with a resistor representing intracellular water and a capacitor representing the cell membranes. According to this circuit model, the resistance measured at infinite frequency, or other high frequency (Kyle et al., 2004; McGree et al., 2007), is that of the overall conductive volume, i.e. TBW, while the resistance at zero frequency is that of the ECW (Cornish et al., 1993). The impedance at the frequency of maximal reactance, the characteristic frequency or *fc*, had special significance, since at *fc* current flow is dependent only on the resistances of the water compartments and not on membrane capacitance. Hence the impedance (Zc) at *fc* should also be an appropriate frequency from which to predict TBW (Cornish et al., 1996).

TBW volume was related to impedance or resistance and length according to the following equation:

$$TBW = \rho \frac{L^2}{Z(R)}$$

where TBW is the volume of TBW, ρ is the specific resistivity of TBW (ohm.cm), L is conductive length (cm) and Z or R is impedance or resistance (ohm), respectively (Thomas et al., 1998). FFM was readily obtained from TBW by dividing by the hydration constant of FFM, assumed to be 0.732 (Schoeller, 1996); consequently FFM could be substituted for TBW in the above equation.

Statistical analysis

Descriptive data are presented as mean ± standard deviation (SD) with group differences assessed using Student's *t* test following normality testing (D'Agostino–Pearson test; MedCalc, version 12.7.0, MedCalc Software). Body composition prediction equations were produced using multiple linear regression techniques (Zar, 1999), using a backward stepwise method (MedCalc, version 12.7.0, MedCalc Software); FFM by DXA was the dependent variable. Independent variables examined were sex, BW, BCS, length (L), and the impedance indices: R_{50} index (L^2/R_{50}), R_{500} index (L^2/R_{500}), R_∞ index (L^2/R_∞), Z_c index (L^2/Z_c). The coefficient of determination adjusted for multiple independent variables (r^2 adjusted) and the root mean square error (RMSE) were determined with alpha level of significance set at 0.05.

A 'split-group' cross-validation procedure was used in which prediction equations were generated in a random, selected by sex, 'prediction' group (12 males and 11 females). These equations were then used to predict FFM in the remaining one-third of the population (six females and six males), the 'validation' group. The validation and prediction groups were not significantly different ($P > 0.05$) in any characteristics. Predicted FFM was compared to that measured by DXA using the concordance correlation coefficient, r_c (Lin, 1989), and Pearson correlation coefficient, r_p (Zar, 1999), and agreement between the two methods was assessed using limits of agreement (LOA) analysis (Bland and Altman, 1986).

Table 1
Mean ± standard deviation data for enrolled dogs.

Parameter	Neutered males	Neutered females	All
n	18	17	35
Scale weight (kg)	24.2 ± 7.5	19.1 ± 4.4[b]	21.6 ± 6.8
Length (cm)	80.4 ± 8.8	74.1 ± 7.5	77.4 ± 8.6
		Body composition	
BCS	3.2 ± 0.37	3.2 ± 0.38	3.2 ± 0.4
Lean mass (kg)	18.5 ± 5.0[a]	14.8 ± 3.4[b]	16.8 ± 4.6
Fat mass (kg)	4.5 ± 2.3	3.4 ± 1.5	3.9 ± 2.0
Bone mineral content (g)	702.9 ± 199.9[a]	525.8 ± 129.1[c]	616.8 ± 189.4
Fat-free mass (kg)	19.2 ± 5.2[a]	15.4 ± 3.5[b]	17.3 ± 4.8
DXA weight (kg)	23.5 ± 7.2	18.8 ± 4.8[b]	21.3 ± 6.5
DXA weight (% scale weight)	98.4 ± 0.9	98.4 ± 1.2	98.4 ± 1.1
		Whole body impedance	
R_{50} (ohm)	136.3 ± 13.6	138.3 ± 10.6	137.3 ± 12.1
R_{500} (ohm)	101.7 ± 10.8	102.3 ± 8.0	102.0 ± 9.4
R_∞ (ohm)	94.9 ± 10.3	95.7 ± 7.5	95.3 ± 8.9
Zc (ohm)	140.2 ± 15.2	140.4 ± 12.2	140.3 ± 13.6

BCS, body condition score; DXA, dual energy X-ray absorptiometry; R, resistance.
[a] Neutered males, $P < 0.05$.
[b] Neutered females, $P < 0.05$.
[c] Neutered females, $P < 0.001$.

Results

Dogs enrolled in this study (Table 1) ranged from small to large breed crosses of varying lengths (61–98 cm) and weights (12.1–43 kg). BCSs, however, were more uniform, with 89% of dogs in ideal condition (BCS 3), 11% classified as overweight (BCS 4) and none classified as obese or underweight (Table 1). Male animals were significantly heavier and had significantly greater lean ($P < 0.05$), BMC ($P < 0.001$) and FFM ($P < 0.05$) than female animals. FM was 30% greater in males than females, although this difference was not significant ($P = 0.124$), reflecting the difference in BW. When expressed