Assessment of Body Composition of Rats by Bioimpedance Spectroscopy: Validation Against Dual-Energy X-Ray Absorptiometry

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Summary

Fat-free mass (FFM) and fat mass (FM) were determined in male and female out-bred Wistar strain rats by both dual-energy X-ray absorptiometry (DXA) and by bioimpedance spectroscopy (BIS). Data obtained by both methods were highly correlated and exhibited a small (4%) bias for FFM, with relatively small limits of agreement (approximately ± 10%), but differed to a much larger degree for FM (14 to 27% bias), with wide limits of agreement (± 50%). Inter-method correction equations are provided to allow conversion of data from one method to another, eliminating bias, but not altering the limits of agreement. Since both methods exhibited a high degree of precision of measurement, it is suggested that the poor agreement for measurement of fat mass is a reflection that fat mass is an indirectly-derived value, which includes unavoidable propagation of prediction errors associated with the primary measures. Both BIS and DXA provide rapid, minimally invasive and, in the case of BIS, portable techniques for body composition analysis. Their use for estimation of FFM may be recommended, but, for prediction of fat mass, exercise of caution would be prudent.

Introduction

Bioelectrical impedance analysis (BIA) has been used widely for the assessment of body composition in humans for the past two decades, since the seminal publication of *Lukaski et al. (1985)*. Surprisingly, it has found little application for measurement of body composition in laboratory animals. Hall et al. *(1989)* appear to be the first authors to report the use of BIA in rats, followed by Cornish and colleagues in 1992. These early studies were designed primarily to demonstrate the feasibility of the technique. It was not until 1993 that the method was used to provide body composition data as an outcome measure in a nutritional study *(Ilagan et al., 1993)*. Since that date, there have only been a few studies that have used the technique, notably Rutter et al. *(1998),* Narath et al. *(2001),* Skalicky et al. *(2001),* Cornish et al. *(2001),* Yokoi et al. *(2001)* and Konomi and Yokoi in 2005.

The technology of BIA has also advanced since 1985 and bioelectrical impedance spectroscopy (BIS) is now considered the method of choice *(Matthie, 2008; Jaffrin and Morel, 2008)*. In conventional BIA, a harmless AC electrical current at a single fixed frequency, typically 50 kHz, is applied to the body and the opposition to the flow of this electrical current, the impedance (Z), is measured. The measured impedance is related to the total body water volume (TBW) according to the following relationship:

$$V = p\frac{L^2}{Z}$$

where V is the volume (ml); L is the inter-electrode distance (cm), Z is the impedance (ohm) and ρ is the resistivity coefficient (ohm.cm) of tissue fluid (for detailed explanation *see Cornish et al., 1993;*

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Thomas et al., 1998). Z is a vector quantity comprising of inherent resistance of tissue fluids (R) and the opposition to current flow due to cell membranes, the reactance (Xc). Frequently, R is what is actually measured and substituted for Z in equation 1. Since the resistivity coefficient is unknown, equation 1 is solved by deriving empirically, using regression, the relationship between the impedance quotient (L^2/R) and volume. This relationship is then used as the prediction equation for TBW in all future studies. TBW volume is then transformed to a prediction of fat-free mass (FFM) by assuming a hydration factor of 72.3% for FFM *(Wang et al., 1999)*. Fat mass is then determined by difference with body weight.

In BIS, instead of a fixed single frequency current being used, current is applied over a range of frequencies, at each of which Z, R and Xc are measured. Using Cole modelling and graphical analysis *(Thomas et al., 1998)*, the resistances at zero (R_0) and infinite (R_∞) frequencies can be estimated. Theoretically *(Cornish et al., 1993)*, these are the optimal predictors of extracellular water (ECW) and TBW, respectively, since, at zero frequency, current can not penetrate cell membranes and thus, R_0 is the resistance of ECW alone, while at infinite frequency, current readily passes across the membrane and hence, R_∞ is the resistance of TBW. Body water volumes can then be predicted from R_0 and R_∞ by modifications of equation 1, based on mixture theory *(Ward et al., 1998)*. These equations, again, require empirical determination of resistivity coefficients for ECW and intracellular water (ICW).

Recently, resistivity coefficients for the use of BIS in rats have been determined and the method validated for prediction of body fluid volumes in rats *(Ward et al., 2008)*. Resistivity coefficients were determined from impedance measurements, performed concurrently with measurements of water volumes by independent tracer-dilution methods, ^3H_2O dilution for TBW and Br dilution for ECW *(Schoeller, 1996)*, with ICW determined from the difference. The validity of these resistivity coefficients has been tested in a cross-validation study in rats of body composition predicted by BIS against carcass analysis *(Smith et al., 2008)*. Although, tracer dilution and carcass analysis are accepted reference methods for body composition assessment, the TBW space determined by ^3H_2O dilution differs from that estimated by techniques such as dual-energy X-ray absorptiometry (DXA).

Since DXA is used widely for body composition analysis in animals *(Nagy, 2001)*, it was necessary to determine whether prediction of body composition by BIS, based upon tracer dilution-derived estimates of ρ, is in agreement with that determined by DXA and, if necessary, to derive appropriate correction factors to facilitate comparison of data obtained by the two different methods.

Materials and Methods

Animals

Twenty-two male and 18 female out-bred, Wistar strain rats, ranging in weight from 119 g to 530 g, were obtained from the Biological Resources Facility of the University of Queensland. The rats were maintained in a dedicated animal holding facility under conditions of 12:12h light-dark cycle, at a temperature 22 ± 2°C. A standard laboratory rodent diet (Rat and Mouse pellets, Speciality Feeds, Glen Forrest, Western Australia) and water were provided *ad libitum.* The study received ethical approval from the University of Queensland Animal Ethics Committee and was conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes *(NHMRC, 2004).*

Procedures

The rats were weighed to the nearest g and anaesthetised with an intraperitoneal injection of sodium pentobarbitone solution (60 mg ml^{-1}) at a dose rate of 0.1 ml per 100 g body-weight. When fully anaesthetised, the rats were reweighed to the nearest 0.1 g. Bioimpedance measurements were performed, immediately followed by DXA analysis, over a period of approximately 30 min. The rats were maintained anaesthetised over this period by additional pentobarbitone administration if required. After all procedures had been completed, rats were euthanased,

without recovery from anaesthesia, by exsanguination via cardiac puncture.

Impedance measurements

Impedance measurements were performed using a Vet BIS1 impedance analyser (ImpediVet, ImpediMed Ltd., Brisbane, Australia) according to the manufacturer's instructions (*Smith et al., 2008*). Briefly, the animal was placed in the prone position on a non-conductive plastic surface. The limbs were abducted at approximately 90° to the body and the tail fully extended along the mid-line. Electrodes, fabricated from ½" 26G needles bent at right angles to the shaft 3 mm from the needle tip, were inserted approximately 3 mm under the skin. The inserted needle lay parallel to the skin surface. Four electrodes were required, two distal electrodes to form the current drive circuit and two proximal electrodes to form the measurement circuit. Insertion points were along the midline of the animals at the posterior edge of the occipital and pinnae openings, the sacral-caudal junction and base of the fur line at the tail. The electrode leads of the instrument were attached by the fitted crocodile clips to the shafts of the needles and three replicate measurements of the impedance were recorded. Measurement time was approximately 20 s and total time required for the procedure was approximately 1-2 min. A second set of measurements were obtained after repositioning to provide a measure of method precision. The distance between the insertion point of the two voltage sense (proximal) electrodes was measured (to nearest mm) with a flexible 8 mm wide plastic tape (BandagesPlus, Doral, USA). Data were uploaded to a PC and analysed, using software provided by the manufacturer (ImpediVet version 1.0.0.4 2007, ImpediMed Ltd., Brisbane, Australia), with default body composition parameters of: ρ_{ECW} = 289 and 328 ohm.cm for females and males respectively; ρ_{ICW} = 669 and 752 ohm.cm for females and males respectively; body density of 1.05 gml^{-1}; body proportion factor of 1 and a hydration factor of 0.732 mlg^{-1} FFM. Technical error of measurement reliability *(Dahlberg, 1940)* of duplicate measurements, with repositioning, was 4.4%, approximately twice that previously observed for multiple frequency BIA (as BIS was previously termed) in humans *(Ward et al., 1997)*.

DXA measurements

DXA measurements were performed using a Norland XR36 DXA instrument (Norland Corp., Fort Atkinson, USA). DXA scans were analysed using the manufacturer's recommended software for use in laboratory animals (Small Subject Analysis Software, version 2.5.3/1.3.1, Norland Corp., Fort Atkinson, USA). Rats were placed on the scanning bed in the prone position, with the legs slightly abducted and the tail looped back to lie alongside the rear legs and body. Start and finish positions for scanning were approximately 5 mm distal to the nose and outermost curve of the tail, respectively, with soft tissue baseline point on the abdomen approximately 10 mm from the spine, as recommended by the manufacturer. Scan conditions were resolution of 1.5 x 15 mm and scan speed of 60 mm s^{-1}. Depending upon the size of the animal, scan times were between 12 and 16 min. In a sub-set of 9 females and 18 male animals, duplicate scans were performed, after repositioning, to assess scan reproducibility. The precision error of FFM for replicate measurements, with repositioning, (*Baim et al., 2005*) was 3.2%.

Data analysis

Data are presented as means ± standard deviation (SD). Comparison of group data, where appropriate, was performed by paired *t*-test. Agreement between the methods, for the measurement of fat-free and fat mass, was performed by concordance correlation analysis *(Lin, 1989)* and the limits of agreement procedure *(Bland and Altman, 1986).* Statistical significance was set at $P < 0.05$. Statistical analysis was performed using MedCalc (version 9.6.4.0, Gent, Belgium).

Results

Body composition characteristics of the rats are presented in Table 1. The ranges of body weights were

Table 1. Body composition parameters of rats measured by bioelectrical impedance spectroscopy (BIS) and by dual energy X-ray absorptiometry (DXA). Data presented as mean ± SD (range).

Parameter	Sex	
	Male	Female
Number	22	18
Body weight - scale (g)	[c]271.0 ± 113.5 (529.3 − 119.1)	320.1 ± 58.2 (514.0 − 248.0)
Body weight - DXA (g)	[d]268.2 ± 113.0 (527.1 − 117.6)	317.1 ± 57.6 (509.7 − 247.0)
Scale weight : DXA weight (%)	101.0 ± 0.3 (101.5 − 100.4)	101.0 ± 0.5 (102.7 − 100.0)
Inter-electrode distance (mm)	16.6 ± 2.1 (20.5 − 12.4)	17.0 ± 0.8 (18.5 − 15.1)
[1]R_0 (ohm)	438.0 ± 68.7 (557.5 − 318.6)	440.4 ± 43.6 (570.6 − 388.3)
R_∞ (ohm)	192.3 ± 25.9 (230.1 − 140.8)	183.3 ± 22.2 (217.7 − 135.5)
Ri (ohm)	354.5 ± 72.5 (546.4 − 252.2)	337.0 ± 71.1 (482.3 − 207.8)
TBW-BIS (g)	[a]158.5 ± 65.4 (322.0 − 77.7)	170.8 ± 16.3 (209.5 − 138.2)
TBW-DXA (g)	[b]165.1 ± 68.9 (337.8 − 83.6)	176.2 ± 18.5 (205.0 − 139.1)
ECW-BIS (g)	62.8 ± 27.4 (127.0 − 28.1)	63.9 ± 8.1 (85.4 − 49.5)
ICW-BIS (g)	95.7 ± 38.7 (194.9 − 49.5)	106.9 ± 12.0 (129.7 − 81.7)
FFM-BIS (g)	[a]216.6 ± 89.3 (439.8 − 106.1)	233.3 ± 22.2 (286.3 − 188.8)
FFM-DXA (g)	[b]225.5 ± 94.1 (461.5 − 114.2)	240.8 ± 25.3 (280.1 − 190.0)
FM-BIS (g)	[c]54.4 ± 28.2 (121.3 − 13.0)	[a]86.8 ± 43.8 (227.7 − 24.6)
FM-DXA (g)	[d]42.7 ± 23.7 (88.0 − 3.4)	[b]76.3 ± 50.9 (245.7 − 32.6)
BMC (g)	6.9 ± 3.6 (14.4 − 1.9)	10.8 ± 2.1 (15.7 − 7.6)

[1]For explanations of abbreviations see text. Superscripts indicate significantly different (pair *t*-test): a *versus* b, $P < 0.015$; c *versus* d, $P < 0.001$.

generally similar for both male and female animals, although the females were generally heavier, due, in large part, to a greater fat mass. Body weight, measured by electronic scale, averaged 1% higher than that determined by DXA. This difference, although small, was significant ($P < 0.001$). Impedance data were similar for both male and female rats. Predicted TBW was, on average, slightly, but significantly less (3.9%, $P < 0.01$), than that measured by DXA in males and only 3.1% less in females (not significantly different). Correspondingly, the derived FFM values differed similarly. The differences in measured fat masses between methods were greater, 21%, $P < 0.001$ and 12%, $P < 0.01$ for males and females respectively. This reflects propagation of the observed difference in FFM and scale *versus* DXA-weight, since, for BIS estimation, FM is derived by difference between predicted FFM and measured body weight.

The two methods were highly correlated when measuring FFM in both males ($r = 0.958$) and females ($r = 0.954$), with relatively small biases (approximately 4%) and limits of agreement (approximately ± 12%) (Table 2 and Figs 1a & 2a). The methods were less well correlated for the measurement of fat mass, especially in females, with large biases and limits of agreement (Table 2 and Figs 1b and 2b).

Regression equations, describing the relationship for the prediction of from BIS and that measured by DXA, were not significantly different for males and females *(Zar, 1999)*. The combined regression equation was $FFM_{DXA} = 0.927 \times FFM_{BIS} + 8.607$ ($r^2 = 0.949$; $P < 0.0001$; SEE = 15.870 g). Similarly for fat mass, the equation was $FM_{DXA} = 0.874 \times FM_{BIS} + 18.444$ ($r^2 = 0.723$; $P < 0.001$; SEE = 21.312 g). These equations may be used to inter-convert measurements between methods.

Discussion

There are many areas of biomedical research, such as pharmacological or nutritional trials, where it would be valuable to be able to measure body composition before, during and after intervention. Typically, it has been necessary in such studies to use large numbers of animals, with groups of animals being killed at different time-points and body composition determined by carcass analysis (*Reynolds and Kunz, 2001*). This approach, however, has the disadvantages of increasing error, owing to inter-animal variation across the samples of animals, necessitating an increase in the number of animals used to achieve the required statistical power. The use of large numbers of animals is difficult to justify on ethical grounds. Alternatively, non-destructive techniques for body composition analysis, such as tracer dilution, imaging methods (DXA) and now

Table 2. Correlation and limits of agreement between fat mass and fat-free mass predicted by bioelectrical impedance spectroscopy (BIS) and measured by dual energy X-ray absorptiometry (DXA), respectively.

Comparison	Male					Female				
	[1]r_c	[2]SEE (g)	[3]Bias (g)	Limits (g)	[3]P	r_c	SEE (g)	Bias (g)	Limits (g)	P
Fat mass (g)	0.814	12.5	-11.7 (27.4%)	-35.9 to 12.4 (-84.0 to 29.2%)	0.0004	0.922	13.9	-10.5 (13.7%)	-42.8 to 21.9 (-56.1 to 28.7%)	0.015
Fat-free mass (g)	0.996	10.9	8.9 (4.0%)	-14.6.9 to 32.5 (-6.5 to 14.4%)	0.0002	0.994	15.1	7.4 (3.1%)	-25.8 to 40.5 (-10.7 to 16.8%)	0.076

[1]Concordance correlation; [2]standard error of the estimate; [3]DXA – BIS; [4]statistical significance of paired comparison (DXA *versus* BIS).

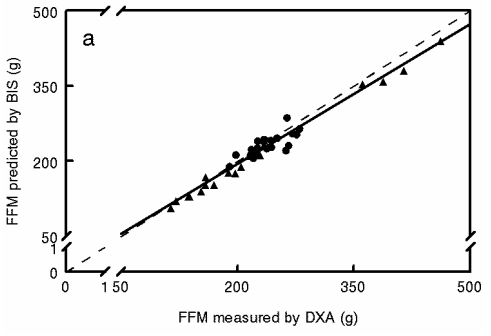

Figure 1. a. Correlation of fat-free mass measured by DXA with that predicted by BIS.
- • Data point for female rats
- ▲ Data point for male rats
- --- Line of identity
- — Correlation line

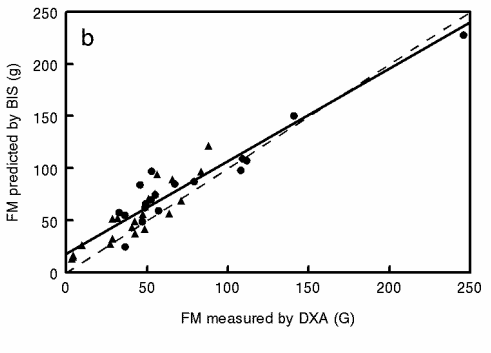

Figure 1. b. Correlation of fat mass measured by DXA with that predicted by BIS.
- • Data point for female rats
- ▲ Data point for male rats
- --- Line of identity
- — Correlation line

BIS, are appealing alternatives. The present study demonstrated a high degree of correlation between the latter two methods, with acceptable limits of agreement for the estimation of fat-free mass, but not of fat mass. BIS underestimated FFM by approximately 4% compared to DXA. Since the two methods are so highly correlated, correction equations may be used to account for this bias. This will not, however, alter the limits of agreement.

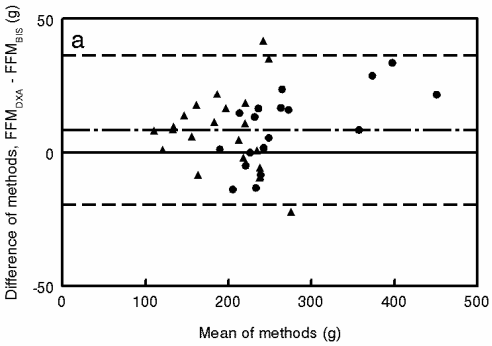

Figure 2. a. Limits of agreement for fat-free mass measured by DXA and predicted by BIS.
- • Data point for female rats
- ▲ Data point for male rats
- --- ± 2SD
- -•- Mean of differences between methods (bias)

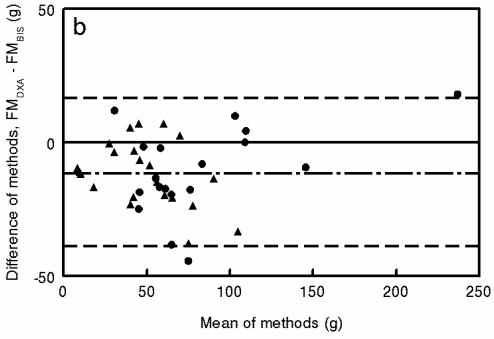

Figure 2. b. Limits of agreement for mass measured by DXA and predicted by BIS.
- • Data point for female rats
- ▲ Data point for male rats
- --- ± 2SD
- -•- Mean of differences between methods (bias)

The present study does not provide information as to which of the methods is the most accurate, i.e. the ability to determine the true value for FM or FFM. The novelty of the use of BIS, or indeed impedance techniques in general, in laboratory animals means few publications are available that attest to its accuracy. *Cornish et al (1992)* reported a standard error of the estimation of TBW by BIS (then termed

multiple frequency BIA) of 6.5% and noted that this was comparable to the uncertainty seen in studies in humans and to that observed in dilution studies. No cross-validation was undertaken. In a later cross-validation study (*Rutter et al., 1998)*, accuracy of prediction of TBW of 4-5% was claimed, although this declined to about 10% in overweight or obese rats. Notably, they reported a bias of 11% and limits of agreement with TBW, determined by dilution, approximately twice (≈25%) that observed here for between FFM predicted by BIS and measured by DXA. Most recently, Smith et al. (*2008*) reported excellent correlations (r^2 = 0.963 and 0.989 for FM and FFM respectively) between prediction by BIS compared with carcass analysis. These authors also observed underestimation of FFM and overestimation of FM by BIS, as observed in the present study. The magnitude of the differences was also proportionately larger for FM than FFM, again as noted here. This observation, coupled with the lower correlation, is further suggestive that BIS predicts FM less well than FFM.

There is a far more extensive literature concerning the measurement of body composition in small animals by DXA. Generally, studies have found that DXA estimates of FM in rats and mice are inaccurate *(Jebb et al., 1996; Rose et al., 1998; Nagy and Clair, 2000; Lukaski et al., 2001; Johnston et al., 2005),* although more recently Stevenson and van Tets (*2008*) found excellent agreement in North American voles. The magnitude of the inaccuracy is not large, about 6%, but, perhaps not unexpectedly, is much larger when only small (< 5 g) amounts of fat are being quantified. Inaccuracy of DXA is not particular to its use in small animals and it appears to have similar degrees of inaccuracy when being used for soft-tissue measurement in humans (*Lohman, 1996*) and, consequently, is not yet regarded as a reference method for body composition analysis. Although accuracy may be questionable, particularly for FM, measurement precision of both BIS and DXA is very high and the techniques are widely used.

A number of deficiencies in the present study should be acknowledged. The DXA instrument used, although having software specific for small animal analysis, is primarily designed for use in humans. Instruments specifically optimised for small animal use have been manufactured (e.g. Lunar PIXImus3, GE Lunar, Madison, WI, USA), but are no longer commercially available. It is likely, therefore, that in the future DXA analysis of laboratory animals will be undertaken using instruments designed primarily for bone mineral determination in humans. BIS and DXA instruments were used from single manufacturers. To the author's knowledge there is, at present, only one manufacturer of full frequency scanning BIS instruments and three major manufacturers of DXA. It is widely recognised that body composition predictions can vary with from instrument to instrument or from software version to software version, both within manufacturers and between manufacturers, for both DXA and impedance analysers *(Oldham, 1996; Johnston et al., 2005)*. The correction equation generated here, to facilitate comparison of prediction between DXA and BIS, should only be considered valid for these particular instruments and software versions.

Prediction of body composition from BIS measurements was accomplished using resistivity coefficients determined in rats of the same strain and fed upon the identical diets. The resistivity coefficients were calculated using tracer dilution as the reference methods for both TBW and ECW (see Introduction). Agreement of prediction with DXA measurements is likely to be improved, where resistivity constants have been generated, by using DXA to predict TBW. This has proved to be the case in studies in humans *(Ward et al., 2008)* and similar studies need to be undertaken in laboratory animals.

Comparison of body composition data between methods was based upon whole body measurements with both DXA and BIS. BIS, when calibrated using tracer dilution, provides a prediction of whole body composition, since the tracers are assumed to distribute throughout their respective total body fluid compartments, irrespective of their anatomical locations. In contrast, some DXA manufacturers,

e.g. GE Healthcare for the PIXIMus2 instrument, recommend that the head and tail be excluded from the analysis. Since no such recommendation is made by Norland for the instrument used in the present study, and to facilitate direct comparison with BIS, the whole animal was included in the analysis. This may not be appropriate for other DXA instruments. Both BIS and DXA provide rapid, minimally-invasive methods for the analysis of body composition of laboratory animals consistent with accepted standards for the use of animals for scientific purposes. Both methods require the animal to be immobilised for a short period of time, 1-3 minutes for BIS and up to 15 minutes for DXA on the instrument used here, although more recent fan-beam DXA equipment provides shorter scan times. Periods of anaesthesia of this duration are readily achievable with inhalation anaesthetics, such as isoflurane, or injectables, as used here, and may be repeated at intervals during longitudinal experimentation. The necessity to insert needles in the BIS technique may be seen by some as a disadvantage, but it should be noted that extremely fine gauge needles may be used and are inserted only 2-3 mm sub-dermally. BIS and DXA have a valuable role in the assessment of body composition in animals, despite reservation over their absolute accuracy. The lack of commercial availability of small animal DXA instruments may lead to a greater use BIS. The study described here is particularly useful in facilitating the comparison of data obtained by DXA with that, which can be obtained from the emerging technique of BIS.

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