A critical evaluation of bioimpedance spectroscopy analysis in estimating body composition during GH treatment: comparison with bromide dilution and dual X-ray absorptiometry

Short title: BIS validation

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Abstract

Objective: To compare estimates by Bioimpedance Spectroscopy Analysis (BIS) of extracellular water (ECW), fat mass (FM), and fat free mass (FFM) against standard techniques of bromide dilution and DXA during intervention that causes significant changes in water compartments and body composition.

Methods: Body composition analysis using BIS, bromide dilution, and DXA was performed in 71 healthy recreational athletes (43 men, 28 women; aged 18-40 years; BMI 24±0.4 kg/m²) who participated in a double-blinded, randomized, placebo-controlled study of growth hormone (GH) and testosterone treatment. The comparison of BIS with bromide dilution and DXA was analyzed using linear regression and the Bland-Altman method.

Results: At baseline, there was a significant correlation between BIS and bromide dilution derived estimates for ECW, and DXA for FM and FFM (p<0.001). ECW by BIS was 3.5±8.1 % lower compared to bromide dilution, while FM was 22.4±26.8 % lower and FFM 13.7±7.5 % higher compared to DXA (p<0.01). During treatment, the change in ECW was similar between BIS and bromide dilution, whereas BIS gave a significantly greater reduction in FM (19.4±44.8 %) and a greater increase in FFM (5.6±3.0 %) compared to DXA (p<0.01). Significant differences in body composition estimates between the BIS and DXA were observed only in men, particularly during the treatment that caused greatest change in water compartments and body composition.

Conclusion: In healthy adults, bioimpedance spectroscopy is an acceptable tool for measuring ECW, however BIS overestimates FFM and substantially underestimates FM compared to DXA.
INTRODUCTION
Measurement of body composition is central to many aspects of patient care and can be assessed by several methods, with dual energy X-ray absorptiometry (DXA) considered the common reference method. The classical three-compartment model of body composition consists of fat mass (FM), fat free mass (FFM) and bone minerals. The FFM can be then divided further into extracellular water (ECW) and a functional cellular compartment predominantly composed of muscle, the body cell mass (BCM). Thus, measuring ECW is of major importance in assessing treatment effect on muscle cell mass. DXA is a valid and reliable method for measuring FM and FFM, whereas for assessment of ECW tracer or dilution methods usually are used. Bromide dilution is a well-established and validated method for estimation of ECW in humans. However, use of DXA and bromide dilution are limited due to cost, invasiveness, lack of portability and the need of trained operators.

Bioelectrical Impedance Analysis (BIA) has become an increasingly popular alternative for the assessment of body composition due to the relatively inexpensive equipment, portability, ease of use and absence of health risks to volunteers. BIA provides an indirect estimate of ECW and total body water, from which FFM is determined by the use of hydration constant and FM is then calculated by subtracting FFM from the total body weight. The measurements are derived based on resistivity coefficients that are gender specific. Thus, the estimates of FFM and FM by BIA depend on many variables.

Different BIA techniques (single-, multi-frequency and Bioimpedance Spectroscopy) can be used for assessment of body composition. The single-frequency approach in BIA has poor precision of estimates, which is only partly corrected by introducing multiple-frequency BIA technique. It has been suggested that BIS is more accurate because it uses a spectrum of frequencies and the Cole-Cole model in its estimations. A few studies have compared BIS with DXA in assessing body composition, showing disagreement between the methods.
in measuring FM and FFM. There is a paucity of data that systematically validates BIS in assessing all aspects of body composition during interventions that causes changes in water compartments, FM and FFM.

ECW measurements are of central importance in dissecting hormone effects on body composition, in particular on muscle mass, as approximate estimation of the functional compartment of muscle mass can be obtained by subtracting ECW from the FFM. Growth hormone (GH) and testosterone are known for their anabolic effects. However GH, particularly when combined with testosterone administration, results in fluid retention. Therefore, during these interventions, measuring FFM by DXA will not accurately reflect changes in functional muscle mass but rather may reflect an increase in ECW content. As BIS may be a convenient, fast and cost effective tool to measure ECW, providing also assessment of FFM and FM, we aimed to compare estimates by BIS of ECW, FM and FFM against bromide dilution and DXA by examining the agreement between the methods while assessing GH and testosterone effects in healthy adults in a previously published study.

METHODS

Study design

We performed a double-blinded, randomized, placebo-controlled study of 96 healthy recreationally-trained athletes aged 18—40 years who were in regular training (≥ 2 sessions per week) for the last 12 months. None of the women received any hormonal birth control medication. The data on body composition assessed by DXA and bromide dilution have been previously published. Women (n = 28) were randomized into 2 groups: (i) GH (2 mg/d sc; n = 15) or (ii) placebo (n = 13). Men (n = 43) were randomized into 4 treatment groups: (i) GH (2 mg/d sc) plus testosterone (T 250 mg/week im; n = 11), (ii) GH plus placebo T (n = 9), (iii) T plus placebo GH (n = 11), and (iv) double placebo (n = 12). Body composition
assessment with DXA, bromide dilution assay, and BIS were done at baseline (week 0), and during treatment (week 8), and these measurements were done on the same day. Due to equipment availability, BIS measurements were undertaken only on 53 subjects (female = 22, male = 31) at baseline and 71 subjects (female = 28, male = 43) during treatment. The St Vincent’s Hospital Human Research Ethics Committee approved the study and all subjects provided written informed consent before participation. The study was registered with the Australian New Zealand Clinical Trials Registry (ACTRN012605000508673).

**Height and Weight**

Body weight was measured to the nearest 0.1 kg using an electronic scale (Tanita BWB-600) without shoes. Height was measured to the nearest 0.5 cm standing without shoes using a stadiometer (Holtain Ltd, Crymmych, Pembs, UK).

**DXA**

FM and FFM were measured by DXA (Model DPX, software version 3.1, Lunar Radiation Corp, Madison, WI). The coefficients of variation (CV) for FFM and FM are 1.4% and 2.9%, respectively.

**Bromide Dilution Assay**

Serum bromide was measured by HPLC. The serum samples were de-proteinated by centrifugation through a filtration unit with a cut-off size of 10 kDa (Amicon YM10, Millipore Corp., Bedford, MA). The protein-free ultra-filtrate was passed through an anion exchange column (IC-Pak A, Waters Corp., Milford, MA) at a flow rate of 0.35 ml/min and a detection wavelength of 195 nm. ECW was calculated from the change in the serum bromide concentration 140 minutes after injection of a known amount of bromide using the formula reported by Miller et al. 16: ECW (liters) = 0.9 x 0.95 x Br dose (mmol)/Δ Br serum (mmol/liter), where Δ Br serum is the change in serum bromide concentration, 0.9 is the correction factor for non-extracellular distribution of bromide, and 0.95 is the correction
factor for Donnan equilibrium. For ECW inter-assay and intra-assay CVs were 1.6% and 0.3%, respectively.

**BIS**

Body composition was assessed by BIS using the ImpediMed Ltd model SFB7 analyser (ImpediMed Ltd, Queensland, Australia). SFB7 is a bioimpedance spectroscopy device that scans 256 frequencies from 4 kHz to 1000 kHz. The measurement is based on assumption that an electric current at low frequencies cannot permeate cells and travels through extracellular space only, so is used to measure extracellular water (ECW). An electric current at high frequencies permeates cell membranes and is used to measure total body water (TBW). BIS estimates body composition using the mathematical model based on a Cole-Cole analysis. It uses the Hanai mixture theory which models the body as a series of cylinders each having specific resistivity and gender specific resistivity coefficients, which along with height and weight are used to calculate body fluid compartments. The following resistivity coefficients were used: pECW 310.0 and 316.2, and pICW 1018.0 and 1023.5 for males and females, respectively. FFM is then derived by dividing TBW with the hydration constant 0.732 (FFM = TBW/0.732). FM is estimated by subtracting FFM from the total body weight. Measurements are taken with subjects in supine position after a 5 min rest with their arms by their sides, but separated from their body with their palms down. Two electrodes placed on the dorsal surface of the right hand/wrist, and another two electrodes on the right foot/ankle according to the manufacturer’s instructions. Measurements were repeated twice and the average was taken as the measured value. Inter-assay and intra-assay CVs for ECW were 2.2% and 0.2%, for FFM 1.8% and 0.3%, and for FM 4.3% and 1.2%, respectively.

**Statistical Analysis**

Regression analysis was used to determine the level of relative agreement between the different techniques. Bland-Altman analysis with paired t-tests was used to determine the
absolute limits of agreement between the body composition parameters assessed by DXA, bromide dilution and BIS. Data are presented as means with standard deviations, unless otherwise stated. A p value of less than 0.05 was considered significant. Statistical Analysis was performed using SPSS Statistics 20 (IBM Corp., NY, US).

RESULTS
The baseline characteristics are summarised in Table 1.

ECW
At baseline, there was a significant correlation between the bromide dilution assay and BIS for measuring ECW ($r^2=0.84$, $p<0.001$). When compared to bromide dilution, ECW measured by BIS was significantly lower by $0.7 \pm 1.6$ L ($p<0.01$; Fig 1A).

During treatment, there was a significant correlation between the bromide dilution assay and BIS for assessing the amount of ECW ($r^2=0.84$, $p<0.001$). ECW measured by BIS was significantly ($p<0.05$) lower by $0.6 \pm 2.0$ L compared to ECW measured by bromide dilution.

BIS assessment of changes in ECW during treatment showed significant correlation with bromide dilution ($r^2=0.35$; $p<0.001$). There was no significant difference in assessing changes in ECW between BIS and bromide dilution during treatment with GH, testosterone or combined hormone administration (Fig 2A and 3A). There was no significant gender effect on changes in ECW content during hormone administration when comparing BIS with bromide dilution technique (Fig 3A).

Thus, when compared to the reference method, BIS under-estimated absolute levels of ECW by $3.5 \pm 8.1\%$ at baseline, whereas the change in ECW during treatment was similar when measured by either BIS or bromide dilution.
At baseline, there was a significant correlation between DXA and BIS for estimating FM ($r^2=0.79$; $p<0.001$). When compared to DXA, FM measured by BIS was significantly lower by $3.9 \pm 3.7$ kg ($p<0.001$; Fig 1B).

During GH and testosterone administration, FM estimated by BIS significantly correlated with DXA ($r^2=0.69$; $p<0.001$). BIS derived FM was significantly lower by $5.0 \pm 4.5$ kg compared to that derived by DXA ($p<0.001$).

Changes in BIS derived FM during treatment significantly correlated with those derived by DXA ($r^2=0.24$; $p<0.001$). However, the change in BIS derived FM was significantly lower by $1.2 \pm 3.0$ kg ($p<0.01$; Fig 2B). When the change in FM was analysed separately for each of the treatment groups, the difference between the methods were confined to participants receiving GH, particularly when GH was combined with testosterone, which was highly significant ($p=0.001$; Fig 3B). Moreover, this difference was noted in men but not in women (Fig 3B).

Overall, when compared to DXA, BIS estimate of FM was consistently lower by $22.4 \pm 26.8$ % at baseline, and by $40.6 \pm 47.9$ % during treatment. Moreover, the measured reduction in FM during treatment was by $19.4 \pm 44.8$ % greater with BIS. In a subgroup of male participants receiving GH and testosterone combined administration, the reduction in FM was $71.4 \pm 46.1$ % greater as estimated by BIS than by DXA.

At baseline, there was a significant correlation between DXA and BIS for estimating FFM ($r^2=0.91$; $p<0.001$). However, BIS derived FFM was by $7.2 \pm 3.8$ kg higher than when measured with DXA ($p<0.001$; Fig 1C).
During treatment, BIS also significantly correlated with DXA in assessing the amount of FFM ($r^2=0.91$, $p<0.001$). When compared to DXA, BIS derived FFM was significantly higher by $7.9 \pm 4.8$ kg ($p<0.001$).

Changes in BIS derived FFM during the treatment showed significant correlation with those derived by DXA ($r^2=0.6$; $p<0.001$). When compared to DXA, there was a significantly greater increase by $1.0 \pm 3.1$ kg in BIS derived FFM ($p<0.05$; Fig 2C). Assessing each treatment subgroup, the over-estimation of changes in FFM were significant only in participants receiving combined GH and testosterone administration ($p<0.01$; Fig 3C). As with FM, the difference between the methods in assessing FFM was noted only in men but not in women (Fig 3C). The difference between the methods correlated significantly with the change in FFM during the treatment ($r^2=0.11$; $p=0.015$), reflecting greater overestimation of FFM by BIS in subjects with the highest increase in FFM.

We next calculated body cell mass (BCM), a functional cellular compartment within FFM, which is derived by subtracting ECW from the FFM. As with FFM, there was a significantly greater increase in BCM measured by BIS compared to the reference methods (Fig 3D). The difference between the methods in assessing BCM was noted only in men, in whom during combined GH and testosterone administration BIS over-estimated change in BCM by $2.8 \pm 1.2$ kg ($p<0.001$; Fig 3D).

Overall, when compared to DXA, BIS estimate of FFM at baseline was $13.7 \pm 7.5$ % higher, during the treatment $13.8 \pm 7.6$ % higher. The change in FFM was significantly different between the methods only in men, over-estimating the change in FFM by $5.6 \pm 3.0$ % and in BCM by $5.0 \pm 2.6$ % during combined GH and testosterone administration.
DISCUSSION

In this study of body composition, BISP-derived estimates of ECW, FM and FFM correlated significantly with those obtained by bromide dilution and DXA in healthy young adults. However, BISP-derived measurements of ECW and FM were significantly lower and FFM significantly higher compared to the reference methods. Treatment with GH, testosterone or both increased ECW, FFM and reduced FM. BIS-derived measurements of increase in ECW were similar to that estimated by bromide dilution. However, BIS recorded a significantly greater reduction in FM and a greater increase in FFM compared to DXA. These treatment differences in FM and FFM between methods were evident in men but not in women.

BIS utilizes a spectrum of frequencies between 4 kHz and 1000 kHz, and employs Hanai mixture conductivity theory and the Cole-Cole model in estimating body fluid compartments, which is regarded more accurate than SFBIA or MFBIA. The estimates are based on assumptions on body shape, tissue density, hydration, and are derived from resistivity coefficients that are gender-specific. FFM is then estimated by the use of a hydration constant and with FM derived by subtracting FFM from the total body weight. Thus, the quantification of body composition by BIS depends on many factors and coefficients that can potentially result in measurement bias. A study in patients with GH deficiency reported that the bias can be diminished by applying unisex resistivity coefficients derived specifically from this patient population. As resistivity coefficients influence measurements by BIS, there is a need to develop coefficients derived from large populations, and incorporating factors, such as age, gender, hydration status and BMI. Thus, the assumptions and coefficients inherent are likely factors that underlie errors in estimating fluid compartments and body composition by BIS.
This is the first placebo-controlled study comparing BIS to bromide dilution, a classical method for quantifying ECW, in a healthy population of lean men and women who received GH and/or testosterone administration. Our results show good agreement between the two methods. At baseline, there was only a 0.7 L difference in ECW which represents a 3% lower estimate by BIS. Importantly, the change in ECW during 8 weeks of GH, testosterone and combined hormone administration showed no significant difference between the methods. Two previous studies evaluated acute shifts in extracellular water, one involving a hydration/dehydration regimen in healthy men over 4 days and the other in men undergoing surgery. Both these studies reported good agreement between BIS and bromide dilution \(^{22, 23}\). Thus, BIS is a reliable and accurate method for assessing changes in ECW.

In contrast to the good agreement between BIS and bromide dilution in measuring ECW, there was a marked disagreement in FM and FFM estimates between BIS and DXA. At baseline, BIS derived estimate of FM was 22% lower and FFM 14% higher than those obtained by DXA in normal subjects. Previous studies comparing BIA and DXA have also reported similar bias \(^{9-12, 24}\). Importantly, our study observed that the reduction in FM and increase in FFM during GH and testosterone treatments quantified by BIS substantially differed from that of DXA. The largest discrepancy was for a change in FM, with the reduction in FM almost 20% greater than by DXA. Collectively, BIS significantly overestimates FFM and underestimates FM compared to DXA.

The observed difference between body composition estimates by BIS and DXA was particularly evident in men receiving GH alone or in combination with testosterone. These treatments resulted in the greatest increase in FFM, most of which is fluid retention \(^{14}\). The impact of body composition change in determining BIS accuracy is supported by a weight loss study, which showed greater disagreement between BIS and the reference methods for TBW and ECW measures in patients with the greatest weight loss \(^{25}\). As the amount of body...
water determines FFM and FM estimates by BIS, changes in tissue water may be associated 
with larger discrepancies between BIS and DXA derived measures of body composition.
Moreover, as TBW is comprised of ECW and ICW, the substantial difference in FFM and 
FM between the methods in the face of no significant discrepancy between BIS and bromide 
dilution in ECW estimate, may reflect bias in ICW measurements by BIS. Thus, bias in water 
compartment assessment by BIS may be of the utmost importance in determining body 
composition measurements.

BIS estimates are based on the assumption that TBW comprises 73.2% of FFM and that 
this proportion does not change. There is strong evidence that the hydration constant of FFM 
is not fixed and varies with many factors including the degree of adiposity. For muscle, a 
major component of the FFM, the average water content is 76% in normal-weight individuals 
but only 66% in obese subjects. Thus, the hydration of the FFM is influenced by the degree 
of adiposity. The increase in hydration derived from increase in TBW in our cohort was 
approximately 3 % during GH or combined GH and testosterone administration in men. 
Since BIS-derived FFM is estimated by dividing TBW by the hydration constant, an increase 
in hydration reduces the derived measure of FFM. Assuming that the average TBW in men is 
45 L in our cohort, an increase in the hydration constant by 3% from 0.73 to 0.75 reduces 
FFM by 2.5 kg. Thus, the use of a fixed hydration coefficient introduces a systematic error 
that is likely to explain in part the overestimation of the FFM by BIS.

Our analysis uncovered interesting gender difference for change in soft tissue composition 
estimates. We speculate that the gender difference may be related to differences in gender-
specific response to GH intervention, resulting in a smaller water retention in women 
compared to that in men. As hydration in women would have increased less than in men, it 
can be predicted that FFM show better agreement with DXA in women than in men. Gender-
related differences in responsiveness to GH may also introduce additional systematic bias. In
our study, the absolute increase in DXA-derived FFM was lower in women than in men (2.7 kg vs 3.5 kg), in whom combined treatment with testosterone further increased FFM (6.4 kg). Thus, the increase in FFM was far greater in men than in women. As a component of the increase in FFM is ECW, the parallel increase in hydration will lead to a greater estimate of FFM by BIS, as was observed. More studies are required to develop hydration and gender-specific correction factors to improve application the accuracy of estimating body composition by BIS in health and disease.

A reason for discrepancies between BIS and DXA may arise from assumptions used in DXA measurement of different tissue compartments. DXA estimates FFM on the basis of greater attenuation of X-rays going through lean tissue compared to fat tissue, not taking tissue water content into account. An increase in weight in parallel with an increase in FFM measured by DXA has been reported after normal saline infusion, reflecting errors in assessing FFM by DXA \(^{27}\). Moreover, assessment of DXA compared to the four-compartment model has reported bias that varies according to the sex, size, fat amount, and disease state of the subjects, showing that DXA is unreliable for patients who undergo significant changes in body composition \(^{28, 29}\). Thus, DXA is only assumed to be the reference standard method for measuring body composition. Measurement bias by DXA compared to the four-compartment model should be taken into account when comparing accuracy of other methods for body composition measurements, such as BIS, particularly when assessing significant changes in body composition.

In summary, BIS accurately estimates ECW, markedly under-estimates FM and over-estimates FFM in healthy young adults. Importantly, when compared to DXA, the reduction in FM and increase in FFM during intervention was significantly greater by BIS. There was a gender difference for changes in FM and FFM, with the highest discrepancy between the methods in men who received combined GH and testosterone administration. This
discrepancy may reflect use of incorrect resistivity coefficients and/or hydration constant for deriving body composition estimates by BIS. The results reflect differences between BIS and the reference methods undertaken in healthy recreational athletes. The findings may be different in hypopituitary patients.

This is the first study systematically comparing BIS to bromide dilution and DXA in a healthy population of lean men and women undergoing intervention that results in significant changes in tissue water and body composition. We conclude that BIS is an accurate, time and cost efficient method for estimating ECW, however it over-estimates FFM and substantially under-estimates FM compared to DXA.

ACKNOWLEDGEMENT

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References


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**Figure legends**

**Figure 1.** Bland-Altman plots comparing baseline ECW (A), FM (B) and FFM (C) by BIS with bromide dilution (ECW) and DXA (FM and FFM). The dotted line represents mean difference between the methods. The shaded area represents the upper and lower limit of agreement (± 1.96 SD).

**Figure 2.** Bland-Altman plots comparing mean changes in ECW (A), FM (B) and FFM (C) during the treatment measured by BIS, bromide dilution (ECW) and DXA (FM and FFM). The dotted line represents mean difference between the methods in assessing change in ECW, FM and FFM during GH, testosterone (T), combined hormone or placebo administration. The shaded area represents the upper and lower limit of agreement (± 1.96 SD).

**Figure 3.** Mean changes in ECW (A), FM (B), FFM (C) and BCM (D) during the treatment with GH, testosterone, combined hormone or placebo administration. Grey bars represent measurements by bromide dilution or DXA, black bars represent measurements by BIS. Data are presented as means with SEM. BCM, body cell mass; Pl, placebo; GH, growth hormone; T, testosterone.
Table 1. Baseline characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Women (n = 28)</th>
<th>Men (n = 43)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>29.4 ± 1.2</td>
<td>27.1 ± 0.8</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>167.4 ± 1.2</td>
<td>181.9 ± 1.0</td>
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<tr>
<td>Weight (kg)</td>
<td>64.0 ± 2.0</td>
<td>82.8 ± 2.5</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>22.8 ± 0.6</td>
<td>24.9 ± 0.6</td>
</tr>
<tr>
<td>IGF-I (µg/l)</td>
<td>124.8 ± 6.9</td>
<td>114.9 ± 6.4</td>
</tr>
<tr>
<td>Testosterone (nmol/l)</td>
<td>1.3 ± 0.1</td>
<td>23.4 ± 1.1</td>
</tr>
<tr>
<td>ECW (l) by Bromide Dilution</td>
<td>15.6 ± 0.3</td>
<td>21.8 ± 0.6</td>
</tr>
<tr>
<td>Fat-Free Mass (kg) by DXA</td>
<td>42.3 ± 0.9</td>
<td>64.0 ± 1.3</td>
</tr>
<tr>
<td>Fat Mass (kg) by DXA</td>
<td>18.5 ± 1.3</td>
<td>15.1 ± 1.3</td>
</tr>
</tbody>
</table>

Data are presented as means ± standard error of the mean.