Validation of bioelectrical impedance spectroscopy to measure total body water in resistance trained males

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Abstract

The three-compartment (3-C) model of physique assessment (fat mass, fat-free mass, water) incorporates total body water (TBW) whereas the two-compartment model (2-C) assumes a TBW of 73.72%. Deuterium dilution (D₂O) is the reference method for measuring TBW but is expensive and time consuming. Multi-frequency bioelectrical impedance spectroscopy (BIS SFB7™) estimates TBW instantaneously and claims high precision. Our aim was to compare SFB7 with D₂O for estimating TBW in resistance trained males (BMI >25kg/m²). We included TBW_{BIS} estimates in a 3-C model and contrasted this and the 2-C model against the reference 3-C model using TBW_{D₂O}. TBW of 29 males (32.4 ± 8.5 years; 183.4 ± 7.2 cm; 92.5 ± 9.9 kg; 27.5 ± 2.6 kg/m²) was measured using SFB7 and D₂O. Body density was measured by BODPOD, with body composition calculated using the Siri equation. TBW_{BIS} values were consistent with TBW_{D₂O} (SEE = 2.65L; TE = 2.6L) as were %BF values from the 3-C model (BODPOD + TBW_{BIS}) with the 3-C reference model (SEE = 2.20%; TE = 2.20%). For subjects with TBW more than 1% from the assumed 73.72% (n=16), %BF from the 2-C model differed significantly from the reference 3-C model (Slope 0.6888; Intercept 5.093). The BIS SFB7™ measured TBW accurately compared to D₂O. The 2C model with an assumed TBW of 73.72% introduces error in the estimation of body composition. We recommend TBW should be measured, either via the traditional D₂O method or when resources are limited, with BIS, so that body composition estimates are enhanced. The BIS can be accurately used in 3C equations to better predict TBW and BF% in resistance trained males compared to a 2C model.

Key words: deuterium dilution, total body water, bioelectrical impedance spectroscopy, body fat percentage

Introduction

Accepted methods used to derive a two-compartment (2-C) model of body composition separate the body into two chemically distinct compartments: fat mass (FM) and fat free mass (FFM) (Withers, Laforgia, & Heymsfield, 1999a; Withers et al., 1998). The application of 2-C model approaches carries several assumptions including that the total body water (TBW) content of the fat free mass (FFM) is 73.72% and that FM and FFM have densities of 0.9007 g/cm³ and 1.1000 g/cm³, respectively (Brožek, Grande, Anderson, & Keys, 1963). As a result, these methods do contain error due to the biological variability in these assumed constants (Siri, 1961). However, a three-compartment (3-C) model, which combines measures of body density and TBW rather than assuming a constant TBW, affords greater validity and is shown to be closer to the reference method in body composition assessment (J. Wang & Pierson, 1976; Withers et al., 1999a). Deuterium oxide dilution (D₂O) is the reference standard for laboratory based TBW measurements but it is expensive and time consuming due to the need to allow at least four to six hours for the deuterium to equilibrate throughout the body (Buchholz, Bartok, & Schoeller, 2004; Colley, Byrne, & Hills, 2007; Lichtenbelt, Westerterp, & Wouters, 1994). It also requires a high level of technical expertise for laboratory analysis. In comparison, multi frequency bio-electrical impedance spectroscopy (BIS) is a tool that has been applied for measurement of TBW in non-athletic populations as it is safe, non-invasive, portable, user friendly, cost effective and provides instantaneous results (J. Moon et al., 2008). Additionally, BIS with its estimates of extracellular water plus total body water may provide more accurate estimates of total body water than single frequency bioelectrical impedance analysis (BIA).

The SFB7 BIS (Impedimed Limited, Brisbane, Australia) is a relatively new device for measuring total body water and is reported to be more sensitive than previous methods (J. Moon et al., 2008; Patel, Matthie, Withers, Peterson, & Zarowitz, 1994). Although there is information available on TBW measurement of healthy males and females, (Armstrong et al., 1997; J. Moon et al., 2008; Patel et al., 1994; Van Loan & Mayclin, 1992) as well as overweight and obese individuals (J. R. Moon et al., 2009; Pateyjohns, Brinkworth, Buckley, Noakes, & Clifton, 2006; Thomson, Brinkworth, Buckley, Noakes, & Clifton, 2007) and athletes using BIS technology, (Svantesson, Zander, Klingberg, & Slinde, 2008; Utter & Lambeth, 2010) the validity of this machine to measure TBW on

larger athletes, such as resistance trained males with proportionally higher FFM and thus TBW, has not been explored (Modlesky et al., 1996; Van Loan & Mayclin, 1992).

Due to the convenience of BIS to measure TBW, if this tool could be validated for larger athletes, the routine monitoring of body composition using a 3-C model would be possible, facilitating an opportunity to reliably monitor small, but potentially important, changes in body composition. More data are required on large muscular individuals to determine if this device is a valid tool for measurement of TBW, which is a highly variable component of the total body mass in larger athletes. We propose that this modified 3-C TBW_{BIS} model may be a more accurate measure of physique than the traditional 2-C model when both are compared against the reference standard of the 3-C model where TBW is estimated using D₂O (TBW_{D2O}).

The primary aim of this study was to assess the validity of the SFB7 BIS against the D₂O method for estimating TBW in resistance trained males. A second aim was to determine the accuracy of body composition (BF%) estimates obtained using the 3-C model using TBW_{BIS}. A comparison of this accuracy with that obtained from a 2-C model using BOD POD, in which TBW is held constant at a value of 73.27% of the FFM, was assessed by comparison with the BF% values obtained using the reference method 3-C model using TBW_{D2O}.

Methods

After providing initial baseline measurements of stretch stature, body mass and urine specific gravity (USG) to determine hydration status, all subjects ingested a D₂O tracer and, after an equilibrium period, had TBW measurements determined by the reference method (Schoeller, Dietz, van Santen, & Klein, 1982) and the BIS device, SFB7. The BF% of all subjects was determined with the Siri formula (Siri, 1961) using body density values estimated by BOD POD.

Subjects

Thirty Caucasian large muscular males, with at least two years resistance training experience, volunteered to participate in this study. One of the post sample laboratory readings for D₂O was found to be unreliable (greater than three standard deviations from the mean) and this individual was excluded from further analysis. The characteristics of the remaining 29 individuals are given in Table 1.

Subjects were informed of the nature and possible risks of the investigation before giving their written informed consent. The investigation was approved by the human research ethics committee of the University of the Sunshine Coast. Stretch stature was measured with a stadiometer (Harpender, Holtain Limited, Crymych, United Kingdom) to the nearest 0.1cm, using techniques previously described (Marfell-Jones, Stewart, & Carter, 2006). Body mass was measured with a calibrated scale to the nearest 0.01kg (SECA GMBH, Germany). Hydration status was measured by analysis of waking urine samples for specific gravity using a refractometer (UG-Alpha, Atago Corporation, Japan).

Deuterium oxide

A D₂O tracer was used as the reference method to measure TBW. After voiding the bladder and providing a baseline urine sample for subsequent analysis, the overnight (eight hours) fasted subjects ingested a predetermined volume of D₂O diluted to 10% (v/v) concentration with deionised water, calculated as 0.5g·kg⁻¹ body mass. An equilibrium period of four hours without food, fluid or exercise followed and, at the completion of this stage, subjects voided their bladders. This was followed immediately by the consumption of a 375ml liquid protein supplement (Musashi P30) and 80g snack (Musashi Bulk P30 protein bar). After another two hour equilibrium period subjects provided a urine sample in a sterile specimen container resulting in a six hour period between administration of the tracer and sampling. All samples were labelled and frozen at -4°C before analysis with an isotope-ratio mass spectrometer (Hydra 20/20, Sercon, Cheshire, United Kingdom) using the Equilibration IRMS method to determine TBW values as previously described (Colley et al., 2007).

Air displacement plethysmography

Within four hours of the D₂O ingestion, assessment of body density was undertaken using BOD POD (BOD POD, Life Measurement Instruments, Concord, CA, USA) following the recommended procedures of the manufacturer (Dempster & Aitkens, 1995). After initial calibration, subjects were weighed in minimal clothing on an electronic scale. The resulting body mass value as well as stretch stature, gender and age were incorporated into an equation by the software to estimate a predicted thoracic lung volume (V_{TG}). The subject cohort consisted of healthy male adults deemed acceptable for use of predicted V_{TG} estimations (Crapo, Morris, Clayton, & Nixon, 1982).

Subjects were given a brief description of the procedure before entering the chamber for the first of two sequential body volume measurements, wearing only lycra clothing and a swim cap, with all metal objects removed prior to measurement. If the difference between these two measurements was >150mL a third measurement was taken. Body density was calculated by the BOD POD's software system (COSMED V5.3.2) and an estimate of BF% was obtained using the simple 2-C model defined by the Siri equation (Siri, 1961) as described by Withers et al (Withers et al., 1998).

Bioelectrical impedance spectroscopy

Immediately prior to the six hour equilibrium period, TBW was measured using the SFB7 (ImpediMed, Brisbane, Australia) device. All metal jewellery or accessories and electronic devices were removed prior to establishing the sites for measurement. Each subject was required to remain in a supine position on a yoga mat for a minimum of fifteen minutes prior to measurement, with arms and legs abducted to 30 and 45 degrees, respectively. Sites of attachment for the electrodes (ImpediMed, Brisbane, Australia) were first shaved and cleaned with alcohol wipes before the dual-tab electrodes were attached as follows: one electrode was attached centrally on the top side of the wrist in alignment with the ulnar head and 5cm lower on the dorsal surface of the hand. The second electrode was attached centrally on the dorsal surface of the ankle between the lateral and medial malleoli and 5cm lower on the dorsal surface of the foot which is in accordance with previous guidelines (J. R. Moon et al., 2010). The SFB7 was calibrated as per the manufacturer's instructions with each participant's stature, body mass, age and gender programmed into the unit. The SFB7 measures impedance using 256 frequencies between 4 and 1024kHz to estimate TBW based on a Cole-Cole plot (Cornish, Ward, Thomas, Jebb, & Elia, 1996). Three measurements were taken consecutively and the mean of these used in subsequent analysis.

Statistical Analysis

Two approaches were used to compare, and thus validate, the TBW estimates from the SFB7 with the reference TBW readings obtained using the standard D₂O method. Firstly, statistical analysis was performed using validity by linear regression, meaning if the two methods are the same then the slope = 1 and the intercept = 0, using SPSS version 21.0 (SPSS, Inc., Chicago, IL, USA). Secondly, Bland and Altman methods (Martin Bland & Altman, 1986) were used to identify the 95% limits of agreement between the reference and predicted TBW.

For the regression analysis, the TBW values predicted from the BIS device, SFB7 were compared with the reference TBW values (D₂O). The following statistical methods described by Moon et al (2008) were used: the constant error (CE = actual TBW (D₂O) – predicted TBW (BIS)); Pearson's correlation coefficient, r , the standard error of estimate (SEE = $SD \sqrt{(1-r^2)}$); and the total error (TE = $\sqrt{(\sum[\text{predicted} - \text{actual}]^2 / n)}$ (J. Moon et al., 2008).

The three methods of obtaining BF% estimates (2-C model based on body density, 3-C model with TBW_{BIS} and the 3-C model with TBW_{D2O}) were compared using a repeated measures analysis of variance. Differences between estimates using each of the experimental methods (3-C with TBW_{BIS} and 2-C based on body density) and the reference BF% estimates (using the 3-C model with TBW from D₂O) were explored using paired t -tests and graphical analysis. Graphical methods were used to identify the impact of inter-individual variability in the TBW on the accuracy of estimate. A separate analysis was done on individuals who were greater than 1% from the assumed constant of 73.72% TBW ($n = 16$).

Results

All subjects produced urinary specific gravity values less than 1.020 (mean $\pm$ SD, 1.020 ± 0.005) and were deemed to be euhydrated.

The reference D₂O values are presented in Table 2 with the results of the validation analysis. In all subjects, TBW_{BIS} estimates were not significantly different from TBW_{D2O} ($p > 0.05$) with regard to slope, tested against one and intercept, tested against zero (Figure 1). TBW_{BIS} estimates compared with TBW_{D2O} revealed an r value of 0.9, SEE of 2.65L and 95% confidence limits (CL) of -5.57 to 5.09L (Table 2). The overall difference between the TBW content of the FFM from the D₂O method and the BIS method for mean and standard deviation was minimal being $73.65\% \pm 1.62\%$ and $73.38\% \pm 1.28\%$ respectively.

Across all subjects there was no significant difference ($p > 0.05$) between BF% values estimated using the 3-C model (TBW_{BIS} + BOD POD) and those estimated from the 3-C model (TBW_{D2O} + BOD POD) (Fig 2). The correlation coefficient between the two sets of BF% estimates from the 3-C model (TBW_{BIS} + BOD POD) and the 3-C model (TBW_{D2O} + BOD POD) was 0.90 with an SEE of 2.20% and 95% CL of -3.99 to 4.31% (Table 3). In Figure 3, for all subjects BF% measurements estimated using the 2-C model (BOD POD) with an assumed TBW of 73.72% were significantly different from the 3-C model

($TBW_{D2O} + BOD_{POD}$), (Intercept = 3.302; $p = 0.0573$ and slope = 0.816; $p = 0.0256$). The BF% values from the 2-C model (BOD_{POD}) compared with the 3-C model ($TBW_{D2O} + BOD_{POD}$) revealed an r value of 0.87, SEE of 2.34% and 95% CL of -4.87 to 4.85% (Table 3).

Figure 4 shows that for 16 subjects, whose TBW values were greater than 1% from the assumed 73.72%, there was no significant difference between BF% estimated using the 3-C model ($TBW_{BIS} + BOD_{POD}$) and BF% values from the 3-C model ($TBW_{D2O} + BOD_{POD}$). However, for those same 16 subjects (Figure 5), BF% estimates using the 2-C model (BOD_{POD}) were significantly different from BF% values obtained from the 3-C model ($TBW_{D2O} + BOD_{POD}$) (intercept = 5.093; $p = 0.0811$ and slope = 0.689; $p = 0.0300$). In Table 4, the correlation coefficient between the two sets of BF% values from the 3-C model ($TBW_{BIS} + BOD_{POD}$) and the 3-C model ($TBW_{D2O} + BOD_{POD}$) was 0.87 with an SEE of 2.24% and 95% CL of -3.781 to 4.596%. In comparison, the 2-C model (BOD_{POD}) revealed an r value of 0.76, SEE of 3.01% and 95% CL of -6.428 to 6.442% compared to the 3-C model ($TBW_{D2O} + BOD_{POD}$).

Discussion

The primary outcome of this study was that the BIS device, SFB7, provided a valid estimate of TBW in a small sample of resistance trained males when compared to the reference D_2O method, as reflected by the strong correlation and the low prediction error which compare favourably to similar studies (Armstrong et al., 1997; J. Moon et al., 2008; J. R. Moon et al., 2010). The impact on BF% using the TBW_{BIS} values in a 3-C model was minimal as shown by the minimal bias between the methods (0.33%). Ultimately, the difference introduced with the use of a 3-C model using TBW_{BIS} values was smaller than that in the 2-C model which is in agreement with previous studies (Collins et al., 1999; Withers et al., 1998). This strongly suggests that the use of a 3-C model in body composition assessment is more accurate than a 2-C model because it accounts for variability of TBW - the largest percentage of FFM (Withers et al., 1998).

Our findings, using all 29 subjects, are consistent with those of Moon et al (J. Moon et al., 2008) who reported a strong correlation ($r = 0.90$) between the deuterium dilution and BIS techniques for the measurement of TBW in healthy (J. Moon et al., 2008) and over-fat (Armstrong et al., 1997) individuals. The prediction error (SEE) for TBW values in these studies was similar to that observed in the current investigation ($SEE = 2.65L$) (Withers, Laforgia, & Heymsfield, 1999b). Other studies, using

a similar BIS device to measure TBW, the XiTron 4000B, found a strong association and comparable results to the present study in young healthy adults ($r = 0.98$) (Collins et al., 1999) and in healthy male college students, ($r = 0.96$, $SEE = 2.23L$) (Thomson et al., 2007). Although all indirect methods of body composition assessment contain errors of prediction, the reported error of 2.65L for TBW in this study suggests that the BIS device is a valid and reliable predictor of TBW in resistance trained males (J. Moon et al., 2008). It is noted that BIS may be a better predictor of extracellular water than TBW but is deemed a valid method for estimating TBW in groups of healthy individuals such as resistance trained males (Buchholz et al., 2004). Additionally, when determining BF% in a 3-C model of body composition assessment ($SEE = 2.20\%$), the error is lower than that Siri proposed was likely from a 2-C method using body density only where TBW is assumed, rather than measured (3.8%) in the general population (Siri, 1961).

The TBW CE or bias in our study (-0.48L) was smaller than those from results from similar literature of healthy males with bias values of -0.80 L (J. Moon et al., 2008) and -3.33L (Armstrong et al., 1997). This suggests that there are few systematic errors or inaccuracies in the estimations of TBW_{BIS} using the SFB7 in resistance trained males and that this device can be considered a suitable substitute for the D_2O method. Additionally, although the SFB7 over and under predicted TBW by as much as 5L, the results are again comparable to similar studies validating this method (Armstrong et al., 1997; J. Moon et al., 2008). Individuals with high muscle mass have atypical density and composition due to the high water content of the FFM so that, on a relative scale, the limits of agreement are acceptable (van Marken Lichtenbelt, Westerterp, Wouters, & Luijendijk, 1994; Z. M. Wang et al., 1998). Nonetheless, a limitation of this study is that some inaccuracy may be introduced by the TBW equation used by the SFB7 software because the variables used to calculate TBW are unknown. Further limitations of this study include the use of a small sample size of one athletic group with no comparisons made with single frequency BIA.

In agreement with previous investigations for estimation of %BF, (J. Moon et al., 2009) the 2-C model using BOD POD with an assumed TBW value was found to be significantly different from the 3-C model $TBW_{D_2O} + BOD_{POD}$. Although the bias for the 3-C model (CE = 0.33%) was higher than for the 2-C model (CE = 0.03%) which differs from similar validation research (3-C, CE = 0.07; 2-C, CE = 0.81), (J. Moon et al., 2009) the precision of the 2-C model was lower. This is demonstrated by the limits of agreement in all subjects for the 3-C model (-3.99% and 4.31%) whereas the limits for the 2-

C model were -4.87% and 4.85%. Further, in 16 subjects whose TBW was found to be more than 1% outside the assumed constant of 72.73%, the limits of agreement were wider for the 2-C model (-6.428 to 6.442%) than the 3-C model (-3.781 to 4.596%). These indicate smaller under- and over-predictions for BF% are achievable by using a 3-C model over a 2-C model as seen in Figures 5 and 6. Due to the wider limits of agreement in the 2-C model, it may lack precision to identify individual BF% in groups of resistance trained males due to a high variance in TBW and FFM hydration in this group of individuals (Z. M. Wang et al., 1998; Withers et al., 1998).

Although the SFB7 and other BIS devices have been validated against D₂O dilution techniques (Armstrong et al., 1997; J. Moon et al., 2008; J. R. Moon et al., 2009; Patel et al., 1994; Van Loan & Mayclin, 1992; van Marken Lichtenbelt et al., 1994) to the best of the researchers' knowledge no other study has validated the SFB7 device against the reference D₂O method for TBW estimation in resistance trained males. The results of our study suggest that the SFB7 is an important tool for TBW measurement in individuals with high FFM and the values can be used to create an accurate 3-C model of body composition for BF%. The use of TBW values from the SFB7 in a 3-C model for BF% is shown to be valid and more accurate than using a 2-C model of %BF with an assumed value of 73.72% for TBW. On an individual basis, the 3-C model is more accurate with narrow limits of agreement and can therefore, negate the need for inclusion of an assumed value for TBW which contains the most variability in FFM. Over half of the subjects' TBW values were more than 1% from the assumed value of 73.72%, therefore, when BIS values were included in the estimate of body composition (%BF), an enhancement of physique traits was identified compared to the 2-C model. In practice, the BIS can be accurately used in 3C equations to better predict TBW and BF% in resistance trained males compared to a 2C model. Future research directions could include monitoring change in body composition using the same methods in resistance trained males as well as other populations.

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Figure 1 Regression Analysis of TBW; Residual versus predicted TBW D₂O vs MF-BIS

Figure 2 Regression Analysis of BF% in a 3-C model (TBW_{MF-BIS} + BOD POD) vs 3-C model (TBW_{D2O} + BOD POD) (n = 29); Residual vs Predicted BF% in a 3-C model (TBW_{MF-BIS} + BOD POD) vs 3-C model (TBW_{D2O} + BOD POD) (n = 29)

Figure 3 Regression Analysis of BF% in a 2-C model (BOD POD) vs 3-C model (TBW_{D₂O} + BOD POD) (n = 29); Residual vs Predicted BF% in a 2-C model (BOD POD) vs 3-C model (TBW_{D₂O} + BOD POD) (n = 29)

Figure 4 Regression Analysis of BF% in a 3-C model (TBW_{MF-BIS} + BOD POD) vs 3-C model (TBW_{D2O} + BOD POD) for those subjects greater or less 1% from assumed constant TBW of 73.72% (n = 16); Residual vs Predicted BF% in a 3-C model (TBW_{MF-BIS} + BOD POD) vs 3-C model (TBW_{D2O} + BOD POD) (n = 16)

Figure 5 Regression Analysis of BF% in a 2-C model (BOD POD) vs 3-C model ($TBW_{D2O + BOD\ POD}$) for those subjects greater or less 1% from assumed constant TBW of 73.72% ($n = 16$); Residual vs Predicted BF% in a 2-C model (BOD POD) vs 3-C model ($TBW_{D2O + BOD\ POD}$) ($n = 16$)

Table 1. Descriptive characteristics of 29 male subjects

Age	Height	Mass	BMI	BD
(yr)	(cm)	(kg)	(kg/m ²)	(g/cm ³)
32.4 ± 8.5	183.4 ± 7.2	92.5 ± 9.9	27.5 ± 2.6	1.06 ± 0.0

BMI = Body Mass Index, BD = Body Density. Values are mean ± standard deviation.

Table 2. TBW measured by the D₂O method, with comparison to MF-BIS method (n = 29) $TBW_{D_2O} = \text{intercept} + \text{slope} \times TBW_{MF-BIS}$

Parameter	D ₂ O	MF-BIS
Mean (L)	55.9	55.4
SD (L)	6.0	5.4
Intercept (L)		0.052
Slope (L)		1.008
<i>r</i>		0.90
SEE (L)		2.65
CE(Bias) (L)		-0.48
Lower 95% Limit for CE (L)		-5.57
Upper 95% Limit for CE (L)		5.09

D₂O = deuterium oxide method, MF-BIS = ImpTM SFB7, SD = standard deviation, *r* = Pearson product-moment correlation coefficient, SEE = standard error of estimate, TE = total error, CE (Bias) = constant (mean) error, Limits = 95% limits of agreement (CE ± 1.96 SD of residual scores (predicted – actual)).

Table 3. Validation of a 3-C model (TBW_{MF-BIS} + BOD POD) and a 2-C model (BOD POD) for predicting BF% compared with a 3-C model (TBW_{D2O} + BOD POD) for all subjects (n = 29)

Parameter	3-C (D2O)	3-C (MF-BIS)	2-C (BOD POD)
Mean (%)	17.85	18.18	17.83
SD (%)	4.63	4.52	4.92
Intercept (%)		1.393	3.305*
Slope (%)		0.905	0.816*
r		0.90	0.87
SEE (%)		2.20	2.34
TE (%)		2.20	2.47
CE/Bias (%)		0.33	-0.03
Lower 95% Limit for CE (%)		-3.99	-4.87
Upper 95% Limit for CE (%)		4.31	4.85

*Significance at ($p < 0.05$), D₂O = deuterium oxide method, MF-BIS = ImpTM SFB7, BOD POD = air displacement plethysmography, SD = standard deviation, r = Pearson product-moment correlation coefficient, SEE = standard error of estimate, TE = total error, CE (Bias) = constant (mean) error, Limits = 95% limits of agreement (CE $\pm$ 1.96 SD of residual scores (predicted – actual)).

Table 4. Validation of a 3-C model (TBW_{MF-BIS} + BOD POD) and a 2-C model (BOD POD) for predicting BF% compared with a 3-C model (TBW_{D2O} + BOD POD) in subjects whose TBW was greater or less 1% from the assumed 73.72% (n = 16)

%BF (3C model)			
Parameter	3-C (D2O)	3-C (MF-BIS)	2-C (BOD POD)
Mean (%)	16.40	17.21	16.41
SD (%)	4.459	4.800	4.905
Intercept (%)		2.4182	5.0930 *
Slope (%)		0.8122	0.6888 *
r		0.87	0.76
SEE (%)		2.24	3.01
TE (%)		2.34	3.29
CE/Bias (%)		0.82	0.01
Lower 95% Limit for CE (%)		-3.781	-6.428
Upper 95% Limit for CE (%)		4.596	6.442

*Significance at ($p < 0.05$), D₂O = deuterium oxide method, BOD POD = air displacement plethysmography, SD = standard deviation, r = Pearson product-moment correlation coefficient, *SEE* = standard error of estimate, TE = total error, CE (Bias) = constant (mean) error, Limits = 95% limits of agreement ($CE \pm 1.96$ SD of residual scores (predicted – actual)).