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## Article



# Validity of Bioimpedance Spectroscopy in the Assessment of Total Body Water and Body **Composition in Wrestlers and Untrained Subjects**

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Abstract: Bioimpedance spectroscopy (BIS) is an easy tool to assess hydration status and body composition. However, its validity in athletes remains controversial. We investigated the validity of BIS on total body water (TBW) and body composition estimation in Japanese wrestlers and untrained subjects. TBW of 49 young Japanese male subjects (31 untrained, 18 wrestlers) were assessed using the deuterium dilution method (DDM) and BIS. De Lorenzo's and Moissl's equations were employed in BIS for TBW estimation. To evaluate body composition, Siri's 3-compartment model and published TBW/fat-free mass (FFM) ratio were applied in DDM and BIS, respectively. In untrained subjects, DDM and BIS with de Lorenzo's equation showed consistent TBW estimates, whereas BIS with Moissl's equation overestimated TBW (p < 0.001 vs. DDM). DDM and BIS with de Lorenzo's equation estimated FFM and percent of fat mass consistently, whereas BIS with Moissl's equation over-estimated and under-estimated them (p < 0.001 vs. DDM). In wrestlers, BIS with de Lorenzo's and Moissl's equations assessed TBW similarly with DDM. However, the Bland-Altman analysis revealed a proportional bias for TBW in BIS with de Lorenzo's equation (r = 0.735, p < 0.001). Body composition assessed with BIS using both equations and DDM were not different. In conclusion, BIS with de Lorenzo's equation accurately estimates the TBW and body composition in untrained subjects, whereas BIS with Moissl's equation is more valid in wrestlers. Our results demonstrated the usefulness of BIS for assessing TBW and body composition in Japanese male wrestlers.

Keywords: anthropometry; deuterium; body mass index (BMI)

## 1. Introduction

Bioimpedance spectroscopy (BIS) is used to measure body water content based on Hanai's mixture theory by measuring the resistance of extracellular and intracellular components [1]. Previous studies have shown that BIS has good accuracy to estimate body water content in patients and healthy subjects with a reference to a "gold-standard" isotope dilution method and that BIS is a better than traditional single-frequency bioimpedance method because it allows for adjustments in the variations of intracellular and extracellular water [2–4]. In addition, BIS can be used to assess body composition, since hydration of fat-free mass (FFM) in both athletes and non-athletes is similar to the theoretical value (~73%) [5].

Regarding the estimation algorithm of BIS for body water assessment, de Lorenzo et al. proposed an estimation equation, which involved standard anthropometric coefficients (i.e., geometrical shape of the body ( $K_B$ ) and body density ( $D_b$ )) derived from enlisted people [6]. However, using standard anthropometric coefficients can potentially become a source of error, since actual anthropometric values may not be correct in certain types of subjects. This problem has been noted in lean and obese subjects, where total body water (TBW) assessed by BIS was underestimated in the former and overestimated in the latter [7]. To reduce the estimation error due to the incompatibility of anthropometric coefficients, Moissl et al. proposed the correction for coefficients using a body mass index (BMI) [7].

In trained subjects and athletes, it has been reported that BIS with standard anthropometric coefficients could accurately assess body water content [8–10]. However, using algorithm applying standard anthropometric coefficients for athletes is questionable because anthropometric characteristics vary between athletes in different sports. For example, wrestlers are presumed to have unique anthropometric characteristics compared with other athletes and untrained subjects, such as their arms being relatively more muscular than their legs [11,12].

In this study, the validity of BIS in measuring TBW and body composition was investigated in Japanese wrestlers and untrained subjects using two estimation equations: de Lorenzo's equation, which used the standard anthropometric coefficients, and Moissl's equation, which used BMI-corrected anthropometric coefficients. We hypothesized that de Lorenzo's equation would be valid in untrained subjects and that it should have an estimation error in wrestlers because of the differences in their anthropometric characteristics. In addition, the estimation error may be reduced by using Moissl's equation because it is compatible with anthropometric characteristics in wrestlers.

## 2. Materials and Methods

#### 2.1. Subjects

Forty-nine subjects participated in this study, which consisted of 31 male untrained subjects and 18 male college wrestlers. Each subject was fully informed of all the risks and discomforts associated with this study before a written informed consent to participate was given. The study procedure was approved by the ethical committee of Fukuoka University (17–10–06, approved on 8 March 2018) and Japan Institute of Sports Sciences (2014 No. 036, approved on 27 October 2014, and 2016 No. 015, approved on 27 May 2016). Most college non-athletes were sedentary or recreationally active but did not perform regular physical training. Athletes belonged to the East Japan Collegiate Wrestling League and participated in international-level, national-level, and regional-level matches.

On the day of the experiment, the subjects arrived at the laboratory in the morning. They were asked not to consume any food or drink for at least 10 h before the experiment. However, they were allowed to drink water. Vigorous physical activities and alcoholic beverages were restricted one day before the experiment. TBW was estimated using the deuterium ( $D_2O$ ) dilution method and BIS at the same time. Subsequently,  $D_b$  was measured using air displacement plethysmograph or underwater weighting, as described previously [5].

### 2.2. $D_2O$ Dilution Method

The  $D_2O$  dilution method, as described by previous studies [13,14], was used for the estimation of TBW (TBW<sub>D2O</sub>). The first urine samples were obtained, and subjects subsequently ingested 0.06 g/kg body mass of  $D_2O$  diluted with 20 times water. The bottle used to administer the solution was then rinsed twice with about 30 mL of water that was also ingested. Within 2 h after  $D_2O$  ingestion,

an additional 300 mL of water was ingested. The second and third urine samples were obtained 3 h and 4 h after D<sub>2</sub>O ingestion, respectively. The urine samples were stored at -30 °C for later analysis. D<sub>2</sub>O abundance in the samples was measured using an isotope-ratio mass spectrometer (Hydra 20–20 Stable Isotope Mass Spectrometer, Sercon, Crewe, UK). The average standard deviations of the Hydra 20–20 in the analyses were reported to be 0.7‰ for <sup>2</sup>H [15]. A hydrogen gas-water equilibration method using platinum catalysts was used to measure the ratio of <sup>2</sup>H and <sup>1</sup>H isotopes [16]. The accuracy of the analyses was calibrated and checked by measuring three different working water standards (low, -57.59%, middle, 490.64‰, and high, 872.40‰ delta V-SMOW unit) within each batch of samples. The <sup>2</sup>H dilution space was determined by dividing the dose of administered <sup>2</sup>H. TBW was calculated as <sup>2</sup>H dilution space divided by 1.041 for the dilution space measured by <sup>2</sup>H [14]. Test-retest variability (coefficient of variation) for TBW assessment using the isotope dilution method is 0.6% in our laboratory [17].

## 2.3. Bioimpedance Spectroscopy (BIS)

A BIS system (SFB7, ImpediMed, Pinkenba, QLD, Australia) was used to estimate TBW. Two injection electrodes (Red Dot, 3M Health Care, St. Paul, MN, USA) were placed on the right side of the body, on the dorsal surface of the right hand and right foot, proximal to the metacarpophalangeal and metatarsophalangeal joints, respectively. Sensing electrodes were then placed on the right side of the body at the middle of an imaginary line on the dorsum of the wrist, joining the bony prominences of the radius and ulna, and at the middle of the anterior surface of the ankle, on an imaginary line joining the medial and lateral malleoli. The measurements were done in a temperature-controlled room (21–22  $^{\circ}$ C) with the subjects lying in a supine position for 15 min.

The frequency range of BIS was 3 kHz to 1 MHz. Resistance at infinity high frequency ( $R_{\infty}$ ) and at zero frequency ( $R_0$ ) were obtained from the Cole plot [18] using analytical software (Bioimp Software, Impedimed, Pinkenba, QLD, Australia), with the analytical setting as described in previous studies [19,20]. The resistance of the extracellular component ( $R_e$ ) was equivalent to  $R_0$ , and resistance of the intracellular components ( $R_i$ ) was calculated as 1/(( $1/R_{\infty}$ ) – ( $1/R_0$ )). TBW was estimated from  $R_e$  and  $R_i$  by using two estimation equations proposed by de Lorenzo et al. [6] and Moissl et al. [7].

de Lorenzo's equation was embedded into the SFB7 system. TBW calculation with de Lorenzo's equation (TBW<sub>SFB7</sub>) was conducted using the following formula [6].

$$ECW_{SFB7} = k_{ECW, SFB7} \left(\frac{L^2 \sqrt{Wt}}{R_e}\right)^{\frac{2}{3}}$$
(1)

$$k_{ECW, SFB7} = \frac{1}{100} \left( \frac{K_B^2 \rho_{ECW}^2}{D_b} \right)^{\frac{1}{3}}$$
(2)

where ECW<sub>SFB7</sub> is the extracellular water volume (liters), Wt is the body weight (kg), L is the height (cm), and  $\rho_{ECW}$  is the  $\rho$  of extracellular water (ohm·cm).  $\rho_{ECW}$  was determined as 273.9 ohm·cm from the default setting of the analytical software. K<sub>B</sub> was identified as 4.03 from the National Institute of Advanced Industrial Science and Technology (AIST) anthropometric database of young Japanese adult men [21]. D<sub>b</sub> was taken at 1.050 g/mL in conformity with a previous study [6] and the default setting of analytical software.

$$\left(1 + \frac{\text{ICW}_{\text{SFB7}}}{\text{ECW}_{\text{SFB7}}}\right)^{\frac{3}{2}} = \left(\frac{\text{R}_{\text{e}} + \text{R}_{\text{i}}}{\text{R}_{\text{e}}}\right) \left(1 + \frac{\text{k}_{\rho}\text{ICW}_{\text{SFB7}}}{\text{ECW}_{\text{SFB7}}}\right)$$
(3)

$$k_{\rho} = \frac{\rho_{ICW}}{\rho_{ECW}} \tag{4}$$

where ICW<sub>SFB7</sub> is the intracellular water volume (liters) and  $\rho_{ICW}$  is the  $\rho$  of intracellular water (ohm·cm).  $\rho_{ICW}$  was determined as 937.2 ohm·cm from the default setting of the analytical software.

$$TBW_{SFB7} = ECW_{SFB7} + ICW_{SFB7}$$
(5)

Moissl's equation applied variable coefficients according to the individual's BMI. TBW calculation with Moissl's equation (TBW<sub>SFB7</sub>) was conducted using the following formula [7].

$$ECW_{BMI} = k_{ECW, BMI} \left(\frac{L^2 \sqrt{Wt}}{R_e}\right)^{\frac{2}{3}}$$
(6)

where

$$k_{ECW, BMI} = \frac{a}{BMI} + b \tag{7}$$

$$ICW_{BMI} = k_{ICW, BMI} \left(\frac{L^2 \sqrt{Wt}}{R_i}\right)^{\frac{5}{3}}$$
(8)

where

$$k_{ICW,BMI} = \frac{c}{BMI} + d$$
(9)

$$TBW_{BMI} = ECW_{BMI} + ICW_{BMI}$$
(10)

The equation parameter was determined as a = 0.188, b = 0.2883, c = 5.8758, and d = 0.4194 in accordance with previous studies [7].

## 2.4. Body Composition

From TBW<sub>D2O</sub>, FFM, and fat mass percentage were assessed using Siri's three-compartment (3C) model with Db (FFM<sub>3C</sub> and %Fat<sub>3C</sub>) [22]. In the 3C model, FFM was determined by subtracting the fat mass from the body weight, which was the sum of TBW<sub>D2O</sub> and fat-free dry solid. From TBW<sub>SFB7</sub> and TBW<sub>BMI</sub>, FFM (FFM<sub>SFB7</sub> and FFM<sub>BMI</sub>) and fat mass percentage (%Fat<sub>SFB7</sub> and %Fat<sub>BMI</sub>) were estimated using the previously reported TBW/FFM ratio (0.721 for untrained subjects and 0.723 for wrestlers) [5].

## 2.5. Statistical Analysis

All data were expressed as a mean  $\pm$  standard deviation (SD). The Shapiro–Wilk test was used to confirm normality. *T*-tests were applied to evaluate the differences between non-athletes and athletes. To investigate the relationship between TBW estimated with BIS and a D<sub>2</sub>O dilution method, linear regression analysis was performed. To assess the accuracy of TBW estimated using BIS, Lin's concordance correlation coefficient (CCC) was calculated [23]. The CCC ( $\rho$ c) includes the factor of precision ( $\rho$ ) and accuracy (Cb), i.e.,  $\rho c = \rho \times Cb$ .  $\rho$  is Pearson's correlation coefficient, and Cb is a bias correction factor that measures how far the best-fit line deviates from a line at 45 degrees. Agreement between methods were assessed using Bland-Altman analysis. Differences were considered statistically significant at p < 0.05. Statistical calculations were performed using Microsoft Excel 2016 (Microsoft Corp., Redmond, WA, USA) and SPSS version 21.0 software (IBM, Armonk, NY, USA).

## 3. Results

## 3.1. Subject Characteristics

The subjects' characteristics are presented in Table 1. In untrained subjects, age and height were significantly high (p = 0.006 and p = 0.002, respectively), and weight, BMI, and Db were significantly small compared to wrestlers (p < 0.001, p < 0.001, p = 0.003, respectively).

Variable	Unit	Untrained ( <i>n</i> = 31)					Wrestlers ( $n = 18$ )			
Age	year	23	±	4	(20–37)	21	±	1 †	(19–22)	
Height	cm	173.4	±	6.5	(161.3–192.1)	167.8	±	3.7 <sup>+</sup>	(160.4–173.4)	
Weight	kg	63.9	±	6.2	(50.3-82.4)	72.2	±	8.3 ++	(62.9–91.6)	
BMI	kg/m <sup>2</sup>	21.2	±	1.7	(18.0–25.1)	25.6	±	2.1 **	(22.6-31.4)	
D <sub>b</sub>	g/mL	1.065	±	0.009	(1.049–1.083)	1.074	±	0.011 +	(1.037–1.086)	

Table 1. Anthropometric characteristics.

Values are mean  $\pm$  SD (range). BMI, body mass index. D<sub>b</sub>, body density. <sup>+</sup> p < 0.05 and <sup>++</sup> p < 0.001 vs. untrained subjects.

## 3.2. Body Resistance, Total Water Content

Body resistance and body water content measured using BIS and the  $D_2O$  dilution technique are presented in Table 2.

Variable	Unit	Untra	ined (1	ı = 31)	Wrestlers ( $n = 18$ )			
Re	Ohm	642	±	71	506	±	45 <sup>++</sup>	
R <sub>i</sub>	Ohm	1226	±	213	852	±	111 **	
TBW <sub>D2O</sub>	Liter	38.1	±	3.8	46.0	±	4.5 **	
TBW <sub>SFB7</sub>	Liter	38.1	±	4.3	46.3	±	5.4 **	
ECW <sub>SFB7</sub>	Liter	16.0	±	1.9	18.6	±	2.1 ++	
ICW SFB7	Liter	22.1	±	2.7	27.6	±	3.5 **	
TBW <sub>BMI</sub>	Liter	39.4	±	4.0 **	46.2	±	4.9 **	
ECW <sub>BMI</sub>	Liter	15.6	±	1.8	18.1	±	2.0 ++	
ICW <sub>BMI</sub>	Liter	23.8	±	2.5	28.1	±	3.1 **	

Table 2. Body resistance and body water content.

Values are mean  $\pm$  standard deviation. \*\* p < 0.001 vs. TBW<sub>D2O</sub> within group. <sup>++</sup> p < 0.001 vs. untrained subjects. R<sub>e</sub>, resistance of extracellular components. R<sub>i</sub>, resistance of intracellular components. TBW, total body water. ECW, extracellular water. ICW, intracellular water.

TBW, ECW, and ICW assessed using the D<sub>2</sub>O dilution method and BIS in wrestlers were significantly larger than those in untrained subjects (all p < 0.001). In untrained subjects, TBW<sub>BMI</sub> was significantly larger than TBW<sub>D<sub>2</sub>O</sub> (p < 0.001). However, there were no differences between TBW<sub>D<sub>2</sub>O</sub> and TBW<sub>SFB7</sub> or TBW<sub>BMI</sub> in wrestlers.

## 3.3. Body Composition

Body composition estimated from TBW measured using Siri's 3C model and BIS are presented in Table 3.

**Table 3.** Body composition estimated from total body water (TBW) measured using the D<sub>2</sub>O dilution technique and bioimpedance spectroscopy (BIS).

Variable	Unit	Untra	Untrained ( $n = 31$ )			Wrestlers $(n = 18)$			
FFM <sub>3C</sub>	kg	53.0	±	5.1	63.3	±	5.8 ++		
%Fat <sub>3C</sub>	%	16.9	±	3.5	12.1	±	4.3 **		
FFM <sub>SFB7</sub>	kg	52.8	±	6.0	64.0	±	7.5 ++		
%Fat <sub>SFB7</sub>	%	17.3	±	4.7	11.3	±	4.6 ++		
FFM <sub>BMI</sub>	kg	54.6	±	5.6 **	63.9	±	6.7 <sup>++</sup>		
%Fat <sub>BMI</sub>	%	14.4	±	4.9 **	11.3	±	5.1 <sup>+</sup>		

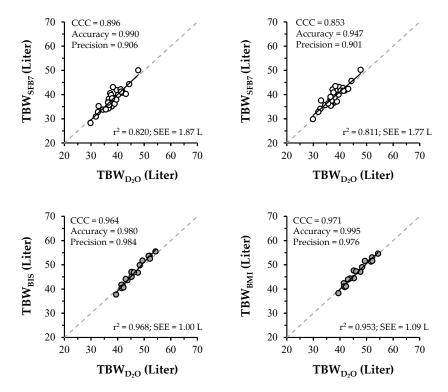
Values are mean  $\pm$  SD. \*\* p < 0.001 vs. 3C model within group. <sup>†</sup> p < 0.05 and <sup>++</sup> p < 0.001 vs. untrained subjects. FFM, fat-free mass. %Fat, % fat mass.

 $FFM_{3C}$ ,  $FFM_{SFB7}$ , and  $FFM_{BMI}$  in wrestlers were significantly higher than those in untrained subjects (all p < 0.001).  $\%Fat_{3C}$ ,  $\%Fat_{SFB7}$ , and  $\%Fat_{BMI}$  in wrestlers were significantly lower than

those in untrained subjects (p < 0.001, p < 0.001, p = 0.046, respectively). In untrained subjects, FFM<sub>BMI</sub> was significantly higher than FFM<sub>3C</sub> (p < 0.001), and %Fat<sub>BMI</sub> was significantly lower than %Fat<sub>3C</sub> (p < 0.001). In wrestlers, FFM<sub>SFB7</sub> and FFM<sub>BMI</sub> were not different compared to FFM<sub>3C</sub>, and %Fat<sub>SFB7</sub> and %Fat<sub>BMI</sub> were not different when compared to %Fat<sub>3C</sub>.

## 3.4. Regression and CCC Analysis

Results of regression and CCC analysis are shown in Figure 1. Coefficient of determination ( $R^2$ ) for TBW<sub>SFB7</sub> and TBW<sub>BMI</sub> were 0.820 and 0.811, respectively, in untrained subjects and were 0.968 and 0.953, respectively, in wrestlers. The standard error of estimate for TBW<sub>SFB7</sub> and TBW<sub>BMI</sub> were 1.87 L and 1.77 L, respectively, in untrained subjects and 1.00 L and 1.09 L, respectively, in wrestlers. The CCC values were 0.896 and 0.853 for TBW<sub>SFB7</sub> and TBW<sub>BMI</sub>, respectively, in untrained subjects and 0.964 and 0.971 for TBW<sub>SFB7</sub> and TBW<sub>BMI</sub>, respectively, in wrestlers.

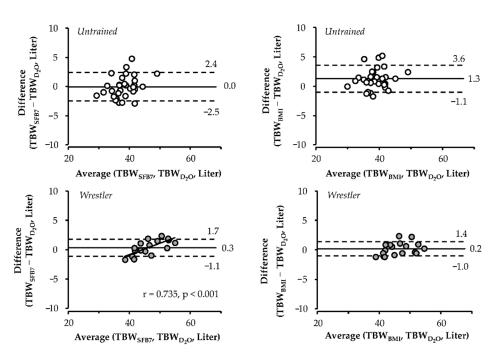


**Figure 1.** Relationship between  $\text{TBW}_{D_2O}$  and  $\text{TBW}_{SFB7}$  or  $\text{TBW}_{BMI}$ .  $\bigcirc$ , non-athletes. •, athletes.  $r^2$  and SEE represent coefficient of determination and standard error of estimates, respectively.

## 3.5. Bland-Altman Analysis

Bland–Altman plot for TBW<sub>SFB7</sub> and TBW<sub>BMI</sub> in relation to TBW<sub>D2O</sub> is shown in Figure 2. In untrained subjects, the mean bias was significantly smaller for TBW<sub>SFB7</sub> than for TBW<sub>BMI</sub> (p < 0.001). The mean bias for TBW<sub>SFB7</sub> was 0.0 L (95% CI, -0.7 to 0.6 L) and that for TBW<sub>BMI</sub> was 1.3 L (95% CI, 0.5 to 2.1 L). The 95% limit of agreement (±1.96 SD) for TBW<sub>SFB7</sub> was -2.5 to 2.4 L and that for TBW<sub>BMI</sub> was -1.1 to 3.6 L.

In wrestlers, there was no significant difference in the mean bias between TBW<sub>SFB7</sub> and TBW<sub>BMI</sub> (p = 0.629). The mean bias for TBW<sub>SFB7</sub> was 0.3 L (95% CI, -0.3 to 1.0 L) and that for TBW<sub>BMI</sub> was 0.2 L (95% CI, -0.3 to 0.8 L). The significant correlation between average and difference of TBW<sub>D2O</sub> and TBW<sub>SFB7</sub> (r = 0.735, p < 0.001) showed proportional bias for TBW<sub>SFB7</sub>, but not for TBW<sub>BMI</sub>. The 95% limit of agreement for TBW<sub>SFB7</sub> was -1.1 to 1.7 L and that for TBW<sub>BMI</sub> was -1.0 to 1.4 L.



**Figure 2.** Bland-Altman plot for TBW<sub>SFB7</sub> and TBW<sub>BMI</sub> in relation to TBW<sub>D20</sub>.  $\bigcirc$ , non-athletes. •, athletes. The trend line represents mean bias and the regression line between parameters as illustrated by a coefficient of correlation (r). The upper and lower dashed line represents a 95% limit of agreement.

## 4. Discussion

We evaluated the validity of BIS with de Lorenzo's and Moissl's equations against the  $D_2O$  dilution method in Japanese college wrestlers and untrained subjects. BIS with de Lorenzo's equation accurately estimated TBW and body composition in untrained subjects. However, systematic bias was seen in wrestlers. BIS with Moissl's equation provided an accurate estimation of the TBW and body composition without systematic bias in wrestlers.

In untrained subjects, no significant difference was found between TBW<sub>SFB7</sub> and TBW<sub>D20</sub>. Bland–Altman analysis showed a small mean bias (=0.0 L) and no systematic bias for TBW<sub>SFB7</sub>. The mean bias and limit of agreement of TBW<sub>SFB7</sub> in untrained subjects was better than those in a previous study [24]. Our results primarily indicate that BIS with de Lorenzo's equation can correctly assess TBW in untrained Japanese subjects. In calculating for TBW<sub>SFB7</sub>, we used  $K_B = 4.03$  and D<sub>b</sub> = 1.050. K<sub>B</sub> is determined using only the length and circumference of each body segment (arm, trunk, and leg), and numerous studies have used  $K_B = 4.3$ , which is determined from anthropometric values in the enlisted Caucasian [6,25]. However,  $K_B$  of 4.3 is not applicable to Japanese subjects because the length of their anthropometric features and arm/trunk/leg circumference ratio are different from those of Caucasians. Japanese subjects have relatively shorter limbs and smaller trunk circumferences [26]. If 4.3 was used instead of 4.03, the  $TBW_{SFB7}$  would have been overestimated by about 1.5 L by our trial calculation. Our data showed that the actual D<sub>b</sub> was 1.065 g/mL (range, 1.049–1.083 g/mL, Table 1) in untrained subjects, which is 1.5% higher than the proposed value. However, we calculated that this difference was small as it only had a negligible effect on body water estimation (less than 100 mL). Incidentally, we confirm that the use of measured  $D_b$  instead of the proposed  $D_b$  did not improve the bias between TBW<sub>SFB7</sub> and TBW<sub>D2O</sub>. Therefore, the values of K<sub>B</sub> and D<sub>b</sub> used in this study were appropriate for Japanese untrained male subjects and helped in improving the validity of TBW<sub>SFB7</sub>.

In contrast to TBW<sub>SFB7</sub>, TBW<sub>BMI</sub> overestimated TBW<sub>D2O</sub> by more than 1.0 L in untrained subjects. This error was directly implicated in the estimation error in body composition. Overestimation of TBW<sub>BMI</sub> was likely related to differences in anthropometric characteristics between races. Originally, correction formula of the TBW<sub>BMI</sub> was determined to fit the body water content of Caucasians [7]. However, Caucasians have a larger muscle mass, which results in more intracellular water, than Asians,

even though their BMI were similar [26,27]. Therefore, relatively small muscle mass in Japanese untrained subjects more than initially assumed should cause overestimation of  $TBW_{BMI}$  in this study.

To the best of our knowledge, this is first study demonstrating the validity of BIS with de Lorenzo's and Moissl's equations in assessing TBW in wrestlers. TBW<sub>SFB7</sub> and TBW<sub>BMI</sub> were equivalent, and both were not different from  $TBW_{D_2O}$ . However, the Bland–Altman analysis revealed a proportional bias for TBW<sub>SFB7</sub>. This result partly contradicts previous results showing no systematic bias in TBW<sub>SFB7</sub> with reference to the isotope dilution method in athletes [8–10] and supports our hypothesis that the BIS algorithm using standard anthropometric coefficients causes an estimation error in some athletes. Arakawa et al. reported that the arm segment was significantly more developed than any other body segment in female wrestlers compared to elite athletes engaged in other sports [11]. Theoretically, hypertrophy of the arm relative to the trunk and the leg leads to alteration of  $K_B$ . Thus, unique muscle development in wrestlers may have led to the difference between the actual and the postulated  $K_B$ , which then resulted in the proportional bias in TBW<sub>SFB7</sub>. In wrestlers, overestimation in TBW<sub>BMI</sub> was not found. Since the ratio of the muscle volume was greater in wrestlers than untrained subjects [12], it can be presumed that wrestlers were better aligned to Moissl's equations made for Caucasian. Moreover, TBW<sub>BMI</sub> did not show any systematic bias. TBW<sub>BMI</sub> was originally made to adjust various anthropometric characteristics of healthy subjects and patients. However, our results support the notion that BMI correction contributes to bridging the gap between standard and actual anthropometric characteristics in wrestlers.

Our results show a small mean bias and no systematic bias in TBW<sub>BMI</sub> and TBW<sub>SFB7</sub> with reference to TBW<sub>D2O</sub> for wrestlers and untrained subjects, respectively. This finding shows that BIS can accurately estimate TBW of Japanese, at least at a population-mean level. However, the Bland–Altman analysis also showed few liters of the limit of agreement in TBW<sub>SFB7</sub> and TBW<sub>BMI</sub> with reference to TBW<sub>D2O</sub> (Figure 2). The validity of BIS at the individual level was confirmed using CCC with factors of precision and accuracy in this study. CCC for TBW<sub>SFB7</sub> and TBW<sub>BMI</sub> in non-athletes were 0.896 and 0.853, which indicates poor strength of agreement, whereas, in athletes, these were 0.984 and 0.976, which indicates substantial strength of agreement [28]. Therefore, similar to previous studies [10,29,30], we presumed that BIS with de Lorenzo's and Moissl's equations are relatively less valid at the individual level.

In this study, we estimated body composition using BIS by using hydration in FFM, which has been reported in Japanese athletes and untrained subjects [5]. Our data showed that estimated body composition using BIS were similar to those calculated using Siri's 3C model, if TBW was accurately assessed. This result means that FFM hydration reported in a previous study [5] was true for our subjects. In addition, BIS can be used in body composition assessment as a replacement for the isotope dilution method. Notably, the demonstration of a good accuracy in the assessment of TBW and body composition by BIS with Moissl's equation in wrestlers is crucial because they try to maintain the weight and muscle volume. Moreover, several wrestlers undergo dehydration weight loss before competitions. Easy and accurate assessment of TBW and body composition by BIS may help athletes and coaches manage body conditions.

This study had some limitations. Although our data indicated that Moissl's equation, which included correction in accordance with anthropometric characteristics, improved the TBW estimation only in wrestlers, the anthropometric characteristics of the athletes was more varied. Moreover, this study was conducted only in male subjects to ignore effects of menstrual edema on BIS accuracy. Therefore, additional studies are necessary to confirm the variability of BIS with Moissl's equation in female athletes, athletes who have different anthropometric characteristics, and athletes of other races. Additionally, we determined the K<sub>B</sub> value from a Japanese anthropometric database. However, we did not measure the actual K<sub>B</sub> for each subject. We believe that this is one of the potential reasons why BIS has relatively less validity at the individual level. Future studies should be conducted to show that using the actual anthropometric coefficients improves the estimation error of BIS at the individual level in Japanese subjects.

### 5. Conclusions

BIS with de Lorenzo's equation accurately estimates the TBW and body composition in male Japanese untrained subjects. However, a systematic bias is seen in male Japanese wrestlers. BIS with Moissl's equation improves the systematic bias and provides accurate estimation of the TBW and body composition in wrestlers likely because BMI correction bridges the gap between the standard and actual anthropometric characteristics. The accurate assessment of TBW and body composition using BIS can facilitate the management of body conditions in wrestlers.

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