Characteristics of body water distribution in healthy adults measured by multi-frequency bioelectrical impedance analysis

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Abstract
Objective: To investigate the characteristics of the ratio of extracellular water (ECW) to total body water (TBW) volume (ECW/TBW) in a large group of healthy adults, measured by multi-frequency bioelectrical impedance (bioimpedance) analysis (MF-BIA). Subjects and methods: The correlation between ECW and TBW was studied in 957 healthy adults who underwent general medical examinations. Differences between measured and predicted ECW from ECW–TBW correlation equations (ΔECW) were calculated, and possible factors for non-zero ΔECW were explored. To investigate the influence of percent fat mass (%FM) on ECW/TBW, the ECW/TBW values of “lean” and “obese” groups, classified by %FM, were compared. ECW/TBW was also compared between “non-obese” and “obese class I-II” groups, classified by the body mass index for both genders. Results: ECW and TBW showed strong positive correlations in both genders. ΔECW was within ±0.2 L and increased with advancing age; ECW/TBW also increased. There were no significant differences in ΔECW/TBW between the “lean” and “obese” groups in either gender, or between the “non-obese” and “obese class I-II” groups in the female group. Conclusions: ECW/TBW measured by MF-BIA was considered to be an index of body water distribution in healthy adults ranging from “lean” to “obese class I-II,” which is not significantly affected by body fat.

Keywords healthy adult, MF-BIA, ECW/TBW, ECW–TBW correlation, ΔECW, aging

Introduction
Bioelectrical impedance (bioimpedance) analysis (BIA) is a method that enables the estimation of body water volume from physical electric resistance to weak alternating current; it is used widely to measure the body composition in healthy subjects [1]. In particular, multi-frequency BIA (MF-BIA) has been applied as a method to evaluate body water volume and nutritional status in obese subjects, athletes, and patients undergoing hemodialysis (HD), who are all presumed to have fluid distributions different from general healthy subjects [1–5].

MF-BIA allows an accurate estimation of the extracellular water (ECW) volume and total body water (TBW) volume, based on electrical currents measured at either low frequency (ECW) or high frequency (TBW) [1, 6]. In addition, as the ratio of ECW to TBW (ECW/TBW) was found to be proportional to the ratio of physical electric resistance to low- and high-frequency alternating current [7], ECW/TBW can be used as an indicator of the electrical characteristics of the human body.

Previously, ECW/TBW was used as a classification index of excessive fluid volume regarding mortality in patients with chronic kidney disease (CKD), heart failure (HF), or critical illness [8]. In addition, ECW/TBW of healthy subjects, measured by MF-BIA, was used as an index of body water distribution to estimate appropriate body weight, that is, dry weight in patients undergoing HD who cannot self-regulate body water content [9, 10]. Other interesting findings include increasing ECW/TBW values in healthy subjects with increasing age [9] and higher ECW/TBW values in female subjects than in males [11]. Thus, ECW/TBW is
considered an index of body water distribution that is affected by age, gender, and individual body composition. However, these previous studies had some critical limitations; their subject numbers were relatively small (i.e., less than 150), gender differences of ECW/TBW were not evaluated [9], and subjects were limited only to the elderly [11]. To the best of our knowledge, no previous studies have examined the relationship between ECW and TBW or body composition measured by MF-BIA for a large number of healthy subjects in detail, and thus the characteristics of ECW/TBW for healthy subjects have not yet been fully investigated.

In this study, we aimed to clarify the characteristics of ECW/TBW by examining in detail the relationship between ECW and TBW in a large number of healthy adults, measured using MF-BIA.

**Methods**

**Subjects**

In total, 957 healthy adults who underwent general medical examinations at the General Health Care Center of Kurashiki Central Hospital were included in this study (487 males and 470 females). All subjects had normal renal function, that is, an estimated glomerular filtration rate [12] (eGFR) ≥ 60 mL/min/1.73 m$^2$. The body composition, specifically water distribution, of all subjects was investigated using MF-BIA.

The protocol for this retrospective study was approved by the ethics committee of the Kurashiki Central Hospital (approval number: 2229).

**Measurement of body composition by MF-BIA**

A body composition analyzer (InBody$^\text{®}$ 730; InBody Japan Inc., Tokyo, Japan) was used to measure the body composition of all subjects. The device measures the physical electrical resistance across five body segments (arms, trunk, and legs) by measuring electrodes, which were placed at 8 points (segmental method), using 6 different frequencies (1, 5, 50, 250, 500, and 1,000 kHz) to estimate body water volume. Basic data for each subject, including height, age, and sex, were entered into the analyzer, following which body weight, body mass index (BMI) [kg/m$^2$], intracellular water (ICW) [L], extracellular water (ECW) [L], TBW [L], ECW/TBW [–], fat-free mass (FFM) [kg], fat mass (FM) [kg], and percent fat mass (%FM) [%] were measured by the analyzer, with the subject in a standing position.

**Data analyses**

First, age, height, body composition measured by MF-BIA, and eGFR were compared between the male and female groups. Next, correlations between ECW, TBW, and each set of measurement data were studied to seek an ECW–TBW correlation equation; correlations between ECW/TBW and other measurement data were also examined. Differences between measured ECW and estimated ECW (based on the ECW–TBW correlation equation and measured TBW), were calculated, with ΔECW being equivalent to measured ECW–estimated ECW; possible factors causing non-zero ΔECW were explored using multiple regression analysis. Finally, to evaluate the effect of body fat mass and degree of obesity on ECW/TBW, ECW/TBW results were compared between the lean group (%FM ≤ 15% for males, %FM ≤ 20% for females) and the obese group (%FM ≥ 30% for males, %FM ≥ 35% for females) for each age group. ECW/TBW was also compared between the non-obese group (18.5 ≤ BMI < 25 kg/m$^2$) and the obese class I–II group (30 ≤ BMI < 40 kg/m$^2$) for each gender [13].

**Statistical analyses**

All statistical analyses were performed using PASW Statistics 17.0 (SPSS Inc., Chicago, IL, USA). Linear regression analysis was applied to correlations between ECW, ECW/TBW, and other measurement data, and Student’s t-test was used to compare between groups. In addition, multiple regression analysis using a stepwise method was applied to determine the major factors contributing to non-zero ΔECW with the largest coefficient of determination; an explanatory variable common to both gender groups in the model was regarded as a major factor for ECW fluctuation. All data were presented as means ± standard deviation, and p < 0.05 was considered statistically significant.

**Results**

**Relationship between ECW, TBW, and other measurement data**

Table 1 shows age, height, body composition measured by MF-BIA, eGFR, and correlation coefficients between ECW and measurement data. Height, body weight, BMI, ICW, ECW, TBW, and FFM were significantly higher in the male group than in the female group, while ECW/TBW, %FM, and eGFR were significantly higher in the female group than in the male group. Both groups showed a significant correlation between ECW and age, height, body weight, BMI, ICW, ECW, TBW, and FM. Among these factors, the strongest positive correlation (r = 0.990, p < 0.001 for males and r = 0.992, p < 0.001 for females) was observed between ECW and age, height, body weight, BMI, ICW, ECW, FFM, and FM. Further, correlation equations for ECW/TBW were obtained by rearranging the ECW–TBW correlation equations (Equations 1-B, 2-B).
Healthy male group (m):

\[ ECW_m = 0.365 \times TBW_m + 0.581 \quad (r = 0.990, p < 0.001) \]  
\[ (Eq. 1-A) \]

\[ ECW_m/TBW_m = 0.365 + 0.581/TBW_m \]  
\[ (Eq. 1-B) \]

Healthy female group (f):

\[ ECW_f = 0.384 \times TBW_f + 0.088 \quad (r = 0.992, p < 0.001) \]  
\[ (Eq. 2-A) \]

\[ ECW_f/TBW_f = 0.384 + 0.088/TBW_f \]  
\[ (Eq. 2-B) \]

There were significant correlations between ECW and ECW/TBW in the male group and between ECW and %FM and eGFR in the female group; however, these correlation coefficients were relatively small.

Table 1 shows the correlation coefficients between ECW/TBW and body composition. A significant positive correlation was observed between ECW/TBW and age in both genders; this correlation coefficient was the largest among all measurement data (Table 1; Figures 2a, b; Equations 3, 4).

Healthy male group:

\[ ECW_{m}/TBW_m = 0.00035 \times Age + 0.36081 \quad (r = 0.618, p < 0.001) \]  
\[ (Eq. 3) \]

Healthy female group:

\[ ECW_{f}/TBW_f = 0.00024 \times Age + 0.37307 \quad (r = 0.450, p < 0.001) \]  
\[ (Eq. 4) \]
ECW–TBW correlation equation and factors contributing to ECW fluctuation

ΔECW was calculated as $0.01 \pm 0.24 \text{ L}$ for males and $-0.01 \pm 0.15 \text{ L}$ for females. The deviation between estimated ECW based on the ECW–TBW correlation equation and measured ECW was within a range of approx. $\pm 0.2 \text{ L}$. Tables 2a and b show the results of multiple regression analysis using the stepwise method, with ΔECW as the objective variable and 7 parameters of interest (age, height, body weight, BMI, FM, %FM, and eGFR) as explanatory variables. To account for collinearity between explanatory variables in the obtained models, age, height, and eGFR in the male group, and age and body weight in the female group were extracted as independent relative factors related to ΔECW. Therefore, ΔECW and age were strongly correlated in both gender groups. In addition, when the linear regression between ΔECW and age (Figures 3a, b; Equations

Table 2 Multiple regression analysis for factors related to ΔECW (using the stepwise method)

| Explanatory variables | Partial regression coefficient (B) | Standard error (SE B) | Standardized partial regression coefficient (β) |
|-----------------------|------------------------------------|-----------------------|-----------------------------------------------|
| a                     |                                    |                       |                                               |
| Age [years]           | 0.014                              | 0.001                 | 0.677**                                       |
| Height [cm]           | 0.012                              | 0.001                 | 0.289**                                       |
| Body weight [kg]      | —                                  | —                     | —                                             |
| BMI [kg/m²]           | —                                  | —                     | —                                             |
| FM [kg]               | —                                  | —                     | —                                             |
| %FM [%]               | —                                  | —                     | —                                             |
| eGFR [mL/min/1.73m²]  | 0.002                              | 0.001                 | 0.108*                                        |
| Multiple coefficient of determination ($R^2$) | | | 0.357** |

| b                     |                                    |                       |                                               |
| Age [years]           | 0.005                              | 0.001                 | 0.337**                                       |
| Height [cm]           | —                                  | —                     | —                                             |
| Body weight [kg]      | 0.007                              | 0.001                 | 0.373**                                       |
| BMI [kg/m²]           | —                                  | —                     | —                                             |
| FM [kg]               | —                                  | —                     | —                                             |
| %FM [%]               | —                                  | —                     | —                                             |
| eGFR [mL/min/1.73 m²] | —                                  | —                     | —                                             |
| Multiple coefficient of determination ($R^2$) | | | 0.216** |

a: male subjects, b: female subjects. *: p < 0.05; **: p < 0.001.
5, 6) was determined, its correlation coefficient was the largest among correlations with measurement data, with the exception of ECW/TBW (Table 1).

Healthy male group:
\[ \Delta \text{ECW}_{\text{m-age}} = 0.011 \times \text{Age} - 0.609 \ (r = 0.522, \ p < 0.001) \]
(Eq. 5)

Healthy female group:
\[ \Delta \text{ECW}_{\text{f-age}} = 0.006 \times \text{Age} - 0.361 \ (r = 0.411, \ p < 0.001) \]
(Eq. 6)

Figure 3: Relationship between ΔECW and age.

Table 3: Comparison of ECW/TBW between lean and obese groups across six age groups

| Age groups [years] | Lean (%FM ≤ 15%) (n = 52) | Obese (%FM ≥ 30%) (n = 62) | p-value |
|--------------------|-----------------------------|-----------------------------|---------|
|                    | ECW/TBW                     | ICW:ECW                     | ECW/TBW | ICW:ECW |         |
| ≤ 39 (n =39)       | 0.35700 ± 0.00518 (n = 13)  | 1.67:1                      | 0.37433 ± 0.00416 (n = 3) | 1.67:1 | 0.824 |
| 40–49 (n = 97)     | 0.37733 ± 0.00498 (n = 15)  | 1.65:1                      | 0.37567 ± 0.00531 (n = 12) | 1.66:1 | 0.414 |
| 50–59 (n = 127)    | 0.38429 ± 0.00582 (n = 7)   | 1.60:1                      | 0.37944 ± 0.00642 (n = 16) | 1.64:1 | 0.098 |
| 60–69 (n = 167)    | 0.38730 ± 0.00380 (n = 10)  | 1.58:1                      | 0.38253 ± 0.00534 (n = 19) | 1.61:1 | <0.05 |
| 70–79 (n = 54)     | 0.38686 ± 0.00367 (n = 7)   | 1.59:1                      | 0.38658 ± 0.00438 (n = 12) | 1.59:1 | 0.886 |
| 80 ≤ (n = 3)       | — (n = 0)                   | —                           | —       | —       | —      |

b

| Age groups [years] | Lean (%FM ≤ 20%) (n = 34) | Obese (%FM ≥ 35%) (n = 103) | p-value |
|--------------------|-----------------------------|-----------------------------|---------|
|                    | ECW/TBW                     | ICW:ECW                     | ECW/TBW | ICW:ECW |         |
| ≤ 39 (n =18)       | — (n = 0)                   | —                           | 0.38100 ± 0.00141 (n = 3) | 1.62:1 | —      |
| 40–49 (n = 107)    | 0.38417 ± 0.00415 (n = 12)  | 1.60:1                      | 0.38694 ± 0.00380 (n = 16) | 1.58:1 | 0.083 |
| 50–59 (n = 163)    | 0.38400 ± 0.00469 (n = 13)  | 1.60:1                      | 0.38597 ± 0.00571 (n = 39) | 1.59:1 | 0.226 |
| 60–69 (n = 128)    | 0.38967 ± 0.00455 (n = 6)   | 1.57:1                      | 0.38741 ± 0.00542 (n = 34) | 1.58:1 | 0.309 |
| 70–79 (n = 49)     | 0.38350 ± 0.01061 (n = 2)   | 1.61:1                      | 0.39280 ± 0.00365 (n = 10) | 1.55:1 | 0.436 |
| 80 ≤ (n = 5)       | — (n = 1)                   | —                           | —       | —       | —      |

a: male subjects, b: female subjects

Effect of body fat (%FM or BMI) on ECW/TBW

Tables 3a and b show the ECW/TBW of lean (52 male and 34 female subjects) and obese (62 male and 103 female subjects) groups, classified by %FM, for six age groups. Although the ECW/TBW of the lean male group aged in their 60s was significantly higher than that of the obese group, in other age groups of either gender, there were no significant differences in ECW/TBW between lean and obese groups.

Table 4 shows measurement data for the non-obese group.
(304 male and 337 female subjects) and obese class I–II group (18 male and 11 female subjects) classified by BMI. In males, the ECW/TBW values and average age of the non-obese group were significantly higher than those of the obese class I–II group; however, there were no significant differences in females.

Discussion

This study revealed that both ECW and TBW values in healthy adults, measured by MF-BIA, were strongly correlated, and that deviation between measured ECW and estimated ECW is influenced by aging. In addition, ECW/TBW has been demonstrated to be an index that is not greatly affected by body fat content.

Relationship between ECW and TBW and other measurement data

It has been reported previously that body water distribution in healthy adults is maintained at a nearly constant level, and that relative increases in ECW can reflect edema [14]. Therefore, it may be possible to understand changes in body water distribution by observing a relative increase/decrease of ECW against TBW. In this study, ECW showed the strongest correlation with TBW. The average ECW/TBW can also be estimated from the measured TBW based on Equations 1-B and 2-B, obtained by rearranging ECW–TBW correlation equations (Equations 1-A and 2-A). It was confirmed that ECW/TBW varied within a range of 0.365 + 0.581/TBW for males and 0.384 + 0.088/TBW for females. The mean TBW was 38.2 ± 4.5 L (33.7–42.7 L) for males and 27.3 ± 3.2 L (24.1–30.5 L) for females, while the fluctuation range of ECW/TBW was estimated to be 0.379–0.382 for males and 0.387–0.388 for females. Thus, comparing the average ECW/TBW values between the two groups, ECW/TBW showed lower values and a relatively wide fluctuation range in males, and higher values and a relatively narrow fluctuation range in females. Considering that females generally have less muscle mass and lower ICW values than males [15], a relative increase in ECW against ICW may lead to the rise of ECW/TBW in females, where TBW is the sum of ICW and ECW.

When examining the relationship between ECW/TBW and age, it was found that ECW/TBW for both gender groups increased with age, similar to previous findings by Lopot et al. [9]. Furthermore, fluctuations in ECW/TBW with increasing age were found to be larger in males than in females (Figures 2a, b). The ratio of ICW to ECW (ICW:ECW) for both gender groups tended to decrease with age, this trend being more pronounced in males (Tables 3a, b). It was thus inferred that decreases in muscle mass with increasing age lead to relative increases in ECW, resulting in increased ECW/TBW. Furthermore, the fluctuation range of ECW/TBW may be wider in males due to a larger decrease in muscle mass with age [15].

Factors contributing to differences between measured ECW and estimated ECW

The fluctuation range of ΔECW in the ECW–TBW correlation equation was confirmed to be approx. ±0.2 L, which is relatively small. From the results of multiple regression analysis, it was suggested that age was the major factor behind ΔECW fluctuation. ΔECW was correlated with age, increasing by 0.011 L/year and 0.006 L/year in the male

| Measurement data | Male subjects | p-value | Female subjects | p-value |
|-------------------|---------------|---------|-----------------|---------|
| Age [kg]          | 56.9 ± 11.4   | <0.05   | 56.5 ± 9.8      | 0.916   |
| Height [cm]       | 168.8 ± 5.7   | 0.698   | 155.6 ± 5.9     | 0.972   |
| Body weight [Kg]  | 63.4 ± 6.0    | <0.001  | 52.2 ± 5.2      | <0.001  |
| BMI [kg/m²]       | 22.2 ± 1.6    | <0.001  | 21.5 ± 1.7      | <0.001  |
| ICW [L]           | 23.0 ± 2.4    | <0.001  | 16.7 ± 1.7      | <0.01   |
| ECW [L]           | 14.2 ± 1.4    | <0.001  | 10.5 ± 1.0      | <0.001  |
| TBW [L]           | 37.2 ± 3.7    | <0.001  | 27.2 ± 2.7      | <0.001  |
| ECW/TBW [–]       | 0.38110 ± 0.00608 | <0.05 | 0.38648 ± 0.00532 | 0.086 |
| FFM [kg]          | 50.2 ± 5.0    | <0.001  | 36.8 ± 3.7      | <0.001  |
| FM [kg]           | 13.3 ± 3.8    | <0.001  | 15.4 ± 3.5      | <0.001  |
| %FM [%]           | 20.7 ± 5.1    | <0.001  | 29.3 ± 5.1      | <0.001  |
| eGFR [mL/min/1.73 m²] | 79.1 ± 12.5  | 0.387   | 81.2 ± 13.3     | 0.342   |

Non-obese group: male vs. female subjects, †: p < 0.05; ‡: p < 0.01; ††: p < 0.001.
Obese class I–II group: male vs. female subjects, *: p < 0.05; **: p < 0.01; ***: p < 0.001.
and female groups, respectively. ECW/TBW also increased by 0.0003/year and 0.0002/year in the male and female groups, respectively, as a result of the rise in ΔECW (Equations 3, 4).

Therefore, the relative increase of ECW due to aging may be a contributing factor to the differences between the measured and estimated ECW, based on the ECW–TBW correlation equation; however, the measured difference was small.

**Effect of %FM and BMI on ECW/TBW**

Using a 3-compartment body composition model in which the water content of adipose tissue was included in FFM, Chamney et al. demonstrated that ECW/TBW of healthy subjects increased with increasing %FM [16], also revealing that the evaluation of body water distribution may be biased if ECW/TBW is set to a constant value as an index of body water distribution. This may be due to the fact that ECW/TBW increased with increasing ECW in FFM because of the large water content in obese subjects with a large volume of adipose tissue. In the current study, a significant positive correlation was found between ECW/TBW and %FM in females; however, the correlation coefficient was small (0.110), and there was no significant difference in ECW/TBW due to differences in %FM in males, except in those aged in their 60s, or in females of any age. Therefore, the influence of %FM on the ECW–TBW correlation was small for both genders in this study.

No significant difference was observed for ECW/TBW between non-obese and obese class I–II groups (classified by BMI) in females; however, ECW/TBW values of the obese class I–II group in males were significantly lower than those in the non-obese group, in contrast with previous findings by Chamney et al. [16]. There are some possible reasons for this discrepancy. There was no significant difference in age between non-obese and obese class I–II female groups, whereas the mean age of the non-obese male group was significantly higher than that of the obese class I–II group in this study. Therefore, ECW/TBW may be less strongly affected by BMI than by age.

As BMI also increases in subjects with large muscle mass, it may not always accurately reflect the degree of obesity [17]. When comparing between the obese class I–II groups for both genders, there were no significant differences between the BMI values of both the groups; however, the %FM was significantly lower for males than for females (Table 4). This indicates that in males, the %FM is low even when BMI is high, while FFM is high. In groups containing many subjects with high muscle mass and high BMI, ECW/TBW may be reduced due to the large ICW. This may explain the low ECW/TBW values observed in males with high BMI. Furthermore, since this study included only one subject with extreme obesity (class III) with BMI ≥ 40 kg/m², the effect of BMI on ECW/TBW in obese class III subjects could not be evaluated. Therefore, further investigation is needed to clarify the influence of body fat on ECW/TBW in obese class III subjects.

To summarize, although the effect of body fat on ECW/TBW in obese class III subjects could not be evaluated in depth, ECW/TBW in healthy subjects from lean to obese class I–II is suggested as an index of body water distribution, which is less affected by the %FM and BMI.

**Clinical applicability of the ECW–TBW correlation equation**

As it was shown that the average ratio of ECW to TBW (ECW/TBW) in healthy adults obtained from the ECW–TBW correlation equation is maintained within a narrow range of about ±0.2 L (as a result of fluctuations in ECW), a quantitative evaluation of body water distribution may be possible based on deviation from this “normal range.” For example, an estimation of “ideal ECW” can be derived from the ECW–TBW correlation equation, based on the measured TBW, not only in healthy subjects but also in patients with excessive ECW. Next, deviations can be obtained for both healthy subjects and patients with excessive ECW. Finally, the results obtained from healthy subjects and patients with excessive ECW can be compared. This approach would be extremely useful for the quantitative evaluation of excessive ECW. Furthermore, excessive ECW may be estimated more accurately by factoring in increases in ECW with age. This method may allow us to quantitatively evaluate excessive ECW not only in patients with HD but also in patients with abnormal body water distributions (e.g., those with CKD, HF, and critical illness [8, 14, 18]).

**Conclusions**

ECW and TBW in healthy adults, measured by MF-BIA, showed a strong correlation; this correlation varied within a narrow range of ±0.2 L between measured and estimated ECW. A major factor contributing to this fluctuation is thought to be a relative increase in ECW with increasing age. Although ECW/TBW also fluctuated depending on TBW and age, the fluctuation range was narrow. It was thus suggested that, in healthy adults ranging between lean and obese class I–II, ECW/TBW can be an indicator of body water distribution, which is not greatly affected by body fat.

**Conflict of interests** The authors declare that they have no conflicts of interests related to this study.

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