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RESEARCH ARTICLE

Changes in the fluid volume balance between intra- and extracellular water in a sample of Japanese adults aged 15–88 yr old: a cross-sectional study

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Ohashi Y, Joki N, Yamazaki K, Kawamura T, Tai R, Oguchi H, Yuasa R, Sakai K. Changes in the fluid volume balance between intra- and extracellular water in a sample of Japanese adults aged 15–88 yr old: a cross-sectional study. *Am J Physiol Renal Physiol* 314: F614–F622, 2018. First published December 6, 2017; doi: 10.1152/ajprenal.00477.2017.—The fluid volume balance between intracellular water (ICW) and extracellular water (ECW) gradually changes with age and various medical conditions. Comprehension of these physiological changes would aid in clinical decision-making related to body fluid assessments. A total of 1,992 individuals (753 men and 1,239 women) aged ≥15 yr included in this study had their body composition measurements performed at training gyms in 2014. We developed a regression formula to assess the association of age with the ratio of ECW to ICW in these subjects. The mean ages of male and female subjects were 51.2 ± 15.2 and 57.4 ± 15.2 yr, and their mean body mass indexes were 23.4 ± 3.3 and 21.1 ± 2.8 kg/m^2, respectively. The total fluid volumes of male and female subjects were 39.6 ± 4.9 and 27.7 ± 3.0 liters, whereas the percent body fat mass per kilogram of body weight were 19 and 26%, respectively. The ECW-to-ICW ratio increased with age because of the steeper decrease in the ICW content than in the ECW content, especially after the age of 70 yr. The regression formulas used for calculating the age-adjusted ECW/ICW ratio were as follows: $0.5857 + 7.4334 \times 10^{-6} \times (\text{age})^2$ in men and $0.6062 + 5.5775 \times 10^{-6} \times (\text{age})^2$ in women. In conclusion, the fluid imbalance between ICW and ECW contents is driven by decreased cell volume associated with aging and muscle attenuation. Therefore, our proposed formula may serve as a useful assessment tool for the calculation of body fluid composition.

body composition; cell volume; extracellular fluid; fluid balance; intracellular fluid

INTRODUCTION

Multifrequency bioelectrical impedance analysis (MF-BIA) can easily and effectively distinguish between intra- and extracellular components. The values for total body water (TBW), intracellular water (ICW), and extracellular water (ECW) content measured by MF-BIA show a high correlation with the values measured by isotopic dilution technique and dual-energy X-ray absorptiometry (3). In the body composition technique, the hydration component comprises of 73.3% of the lean body mass (LBM), and the fat content is calculated by subtracting LBM from the body weight (33). Standard hydration status is distributed between ICW and ECW at a ratio of 62:38 in healthy adults (34). In 1989, Schoeller reviewed the change in TBW with aging (29). At birth, TBW accounted for ≥80% of the LBM (12). During growth, the total amount of TBW increased with body size, whereas the percent of TBW in LBM gradually decreased to reach 73.3% at maturity. During subsequent aging, the hydration component continued to change (13, 29). Several studies have concluded that the change in TBW with age is mostly because of decreased cell volume (14, 17, 28, 30). However, some studies do not support the hypothesis that loss of cell volume is a part of the normal aging process (7, 16). In this regard, the decrease in cell volume may be partly countered by the retention of muscle mass over a lifespan because of the water content of muscles and viscera (6). In fact, the ratio of ECW to ICW, which is substantially similar to the ratio of ECW to TBW, has been associated with nutritional status (31) and adverse outcomes (5, 27, 32). In hydration assessment, the ECW-to-TBW ratio is often used as an indicator of volume overload (1, 11, 21). However, the ECW/TBW ratio is influenced by decreased cell volume and excess ECW (15, 18, 25). It is uncertain whether the measured ECW content is within the optimal range. Furthermore, excess hydration component may underestimate the fat content resulting from an increase in LBM containing a hydration component. In short, the reference values of body fluid composition considering the changes with aging remain unknown, which has hampered studies in this area. Understanding about the optimal fluid volume balance may help guide pharmacokinetics research regarding drug distribution in the body, the administration of intravenous fluids to patients, or heart failure management, since this disease is largely the result of the expansion of the ECW compartment.

We hypothesize that there is a universal physiological change in the fluid volume balance between ICW and ECW with aging in healthy subjects. In the present study, we aimed to *1*) assess the change in the fluid volume balance between ICW and ECW with aging and *2*) adjust the ratio of ECW to ICW by age.

MATERIALS AND METHODS

Study design. The present study was a cross-sectional study. We used sample data obtained from InBody Japan (Kameido, Tokyo, Japan) after entering into a nondisclosure agreement. The unlinkable anonymized dataset included 1,992 individuals (753 males, age:

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15–88 yr and 1,239 females, age: 16–85 yr) with MF-BIA body composition measurements that were obtained from four training gyms in Tokyo or Kanagawa prefectures in Japan. The gym membership agreement confirmed that the participants did not suffer from any health problems that would hinder exercise or prevent them from engaging in exercise. Most of the participants practiced their daily routine activities and exercise schedule. They were randomly recruited by advertisements between September and October 2014. Informed consent for handling personal information was obtained from all participants. The exclusion criterion was the presence of artificial implants, such as cardiac pacemakers and replacement joint that may interfere with accurate bioimpedance measurements. No interviews were conducted to identify the medical condition or any comorbidities, such as blood pressure and severe cardiac, pulmonary, or musculoskeletal disorders in the participants.

This study protocol was submitted to and approved by the Ethics Committee of the Faculty of Medicine, Toho University, Tokyo, Japan (approval no.: A16137), and it adhered to the principles of the Declaration of Helsinki.

Assessment of body fluid composition. We assessed the body composition measurements and simultaneously evaluated the anthropometric measurements before gym exercise. The body surface area (BSA) was estimated using the equation of Du Bois and Du Bois (9). The measurements of the body composition components, such as TBW, ICW, ECW, protein, minerals, and body fat mass (BFM), were performed using a Multifrequency Bioelectrical Impedance Analyzer InBody 770 scanner (InBody, Seoul, Korea). The microprocessor-controlled switches and impedance analyzer were activated, and the segmental resistances of the arms, trunk, and legs were measured at six frequencies (1, 5, 50, 250, 500, and 1,000 kHz). Thereby, the resistance of 20 segments was measured for each individual. The sum of the measurements for each body segment was then used to calculate TBW, ICW, ECW, and other components using built-in MF-BIA software. Muscle mass was converted in the skeletal muscle mass index by dividing the weight by the height squared. The participants were asked to remove additional clothing items such as shoes, coats, and sweaters and to maintain a standing position, according to the manufacturer's instructions; the scanner with a high-resolution touch-screen was used to analyze their body composition with a measurement time of 60 s. Furthermore, we developed a regression formula to assess the association between the age and ratio of ECW to ICW for calculating an age-adjusted ECW-to-ICW ratio. In addition, standardized ECW was calculated by $0.613 \times$ measured ICW value as the optimal ratio of 62:38 in ICW and ECW.

Validation of our proposal formula for calculating age-adjusted ECW/ICW ratio. Eligible individuals from the entire database were simply divided by the first half of the group and the last half of the group in chronological order to create a development and validation dataset, respectively, referring to a previous study (23). Next, we developed a regression formula for calculating an age-adjusted ECW/ICW ratio by using the first half of the population and assessed validation of our proposal formula for calculating the age-adjusted ECW/ICW ratio by using the last half of the population.

Statistical analyses. The data were analyzed with JMP 12.2.0 Statistical Software (SAS Institute, Cary, NC). The measured values are expressed as means ± SD and percentages. The frequency distribution of TBW, ICW, and ECW is plotted in the histograms. Statistical significance for the two groups was assessed using the Wilcoxon rank-sum test for continuous variables. Statistical significance for the mean values of associated factors among the quartile groups was assessed using a linear regression model. The quadratic least-squares regression was used to identify the correlation between age and the ratio of ECW to ICW, as well as to obtain the predicted estimates and 95% prediction intervals of the age-adjusted ECW/ICW ratio at different levels of age and gender. $P < 0.05$ was considered statistically significant.

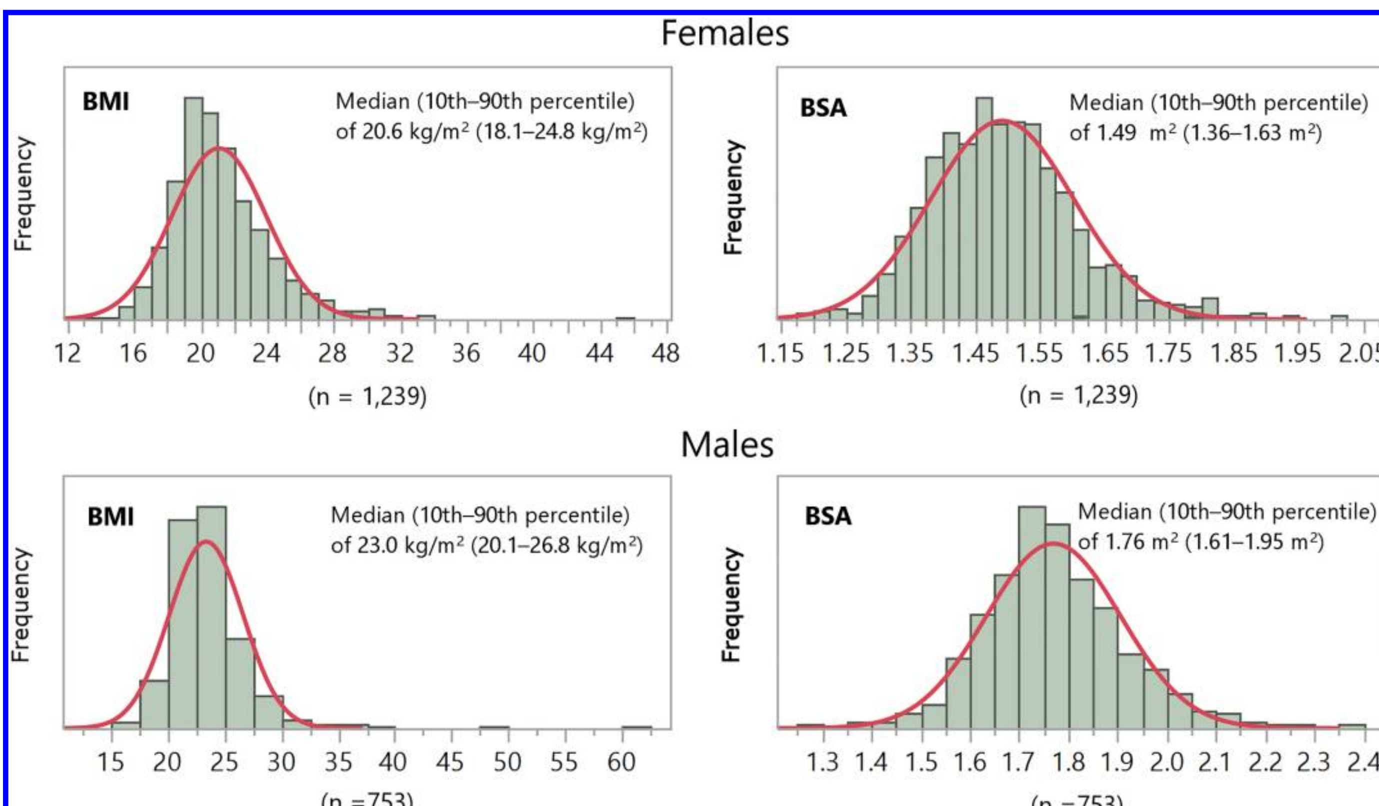

Fig. 1. Frequency distribution of body mass index (BMI) and body surface area (BSA) by gender.

Table 1. *Demographic characteristics of the study population*

Characteristics	Overall (*n* = 1,992)	Males (*n* = 753)	Females (*n* = 1,239)
Age, yr	55.1 ± 16.8	51.2 ± 18.6	57.4 ± 15.2
Height, cm	161.3 ± 8.8	169.6 ± 6.8	156.3 ± 5.5
Body wt, kg	57.4 ± 11.2	67.0 ± 9.6	51.6 ± 7.5
BMI, kg/m^2	22.0 ± 3.2	23.4 ± 3.3	21.1 ± 2.8
BMI <18.5 kg/m^2, *n* (%)	186 (9.3)	15 (2.0)	171 (13.8)
BMI 18.5–24.9 kg/m^2, *n* (%)	1,471 (73.8)	539 (71.6)	932 (75.2)
BMI 25.0–29.9 kg/m^2, *n* (%)	300 (15.1)	180 (23.9)	120 (9.7)
BMI ≥30.0 kg/m^2, *n* (%)	35 (1.8)	19 (2.5)	16 (1.3)
Body surface area, m^2	1.60 ± 0.18	1.77 ± 0.14	1.49 ± 0.11
TBW			
Liters	32.2 ± 6.9	39.6 ± 4.9	27.7 ± 3.0
% in body wt	56.1 ± 5.5	59.4 ± 4.9	54.1 ± 4.9
ICW			
Liters	19.9 ± 4.4	24.6 ± 3.1	17.0 ± 1.9
% in body wt	34.7 ± 3.6	36.9 ± 3.2	33.3 ± 3.1
ECW			
Liters	12.3 ± 2.5	15.0 ± 1.8	10.7 ± 1.1
% in body wt	21.4 ± 2.0	22.4 ± 1.8	20.8 ± 1.9
Protein			
kg	8.6 ± 1.9	10.6 ± 1.3	7.4 ± 0.8
% in body wt	15.0 ± 1.6	16.0 ± 1.4	14.4 ± 1.4
Skeletal muscle mass index, kg/m^2	9.1 ± 1.3	10.5 ± 1.0	8.3 ± 0.7
Minerals			
kg	3.1 ± 0.6	3.6 ± 0.5	2.7 ± 0.3
% in body wt	5.3 ± 0.5	5.4 ± 0.5	5.3 ± 0.5
Body fat mass			
kg	13.6 ± 5.5	13.2 ± 5.9	13.8 ± 5.3
% in body wt	23.6 ± 7.5	19.2 ± 6.7	26.2 ± 6.8
Ratio of ECW to TBW	0.382 ± 0.008	0.378 ± 0.008	0.385 ± 0.006

n, No. of subjects; BMI, body mass index; TBW, total body water; ICW, intracellular water; ECW, extracellular water

RESULTS

Sample characteristics. The frequency distributions of body mass index (BMI) and BSA are shown in Fig. 1, which exhibited a nonnormal distribution. The medians (10th–90th percentile) of BMI and BSA were 23.0 kg/m^2 (20.1–26.8 kg/m^2) in males and 20.6 kg/m^2 (18.1–24.8 kg/m^2) in females and 1.76 m^2 (1.61–1.95 m^2) in males and 1.49 m^2 (1.36–1.63 m^2) in females, respectively. The population characteristics of the subjects are presented in Table 1. The mean age of the subjects was 55.1 ± 16.8 yr (51.2 ± 18.6 yr in males vs. 57.4 ± 15.2 yr in females, $P < 0.001$), and the mean BMI was 22.0 ± 3.2 kg/m^2 (23.4 ± 3.3 kg/m^2 in males vs. 21.1 ± 2.8

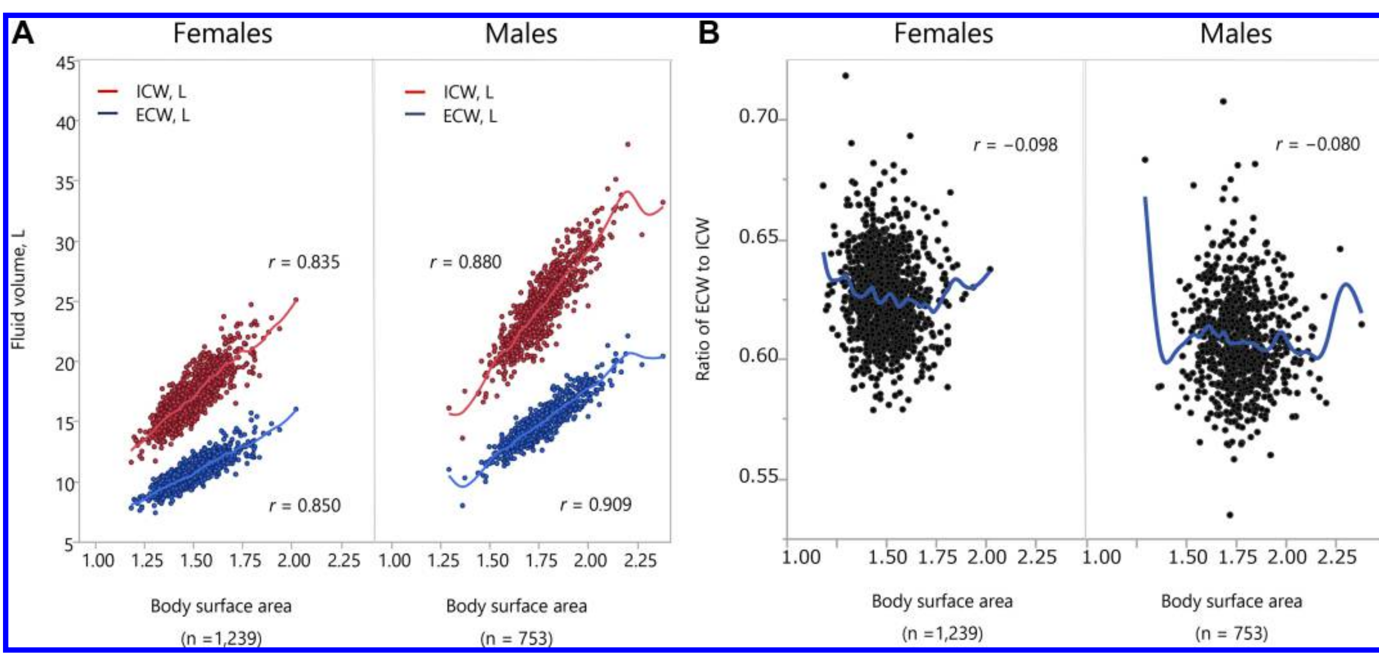

Fig. 2. *A*: fluid volume of intra- and extracellular water by body size. *B*: fluid volume balance of intra- and extracellular water by body size. Plot depicting intracellular and extracellular water by body surface area. ICW, intracellular water; ECW, extracellular water.

Table 2. *Characteristics of individuals separated by quartiles of the ratio of extracellular to intracellular water*

Characteristics	*Quartile 1* (*n* = 368 males and 130 females)	*Quartile 2* (*n* = 192 males and 304 females)	*Quartile 3* (*n* = 108 males and 385 females)	*Quartile 4* (*n* = 85 males and 420 females)	*P* for Trend
Females					
Age, yr	44.0 ± 14.9	52.1 ± 14.3	56.8 ± 13.9	66.0 ± 11.8	<0.001
Height, cm	157.1 ± 5.7	157.0 ± 5.1	156.4 ± 5.6	155.5 ± 5.6	<0.001
Body wt, kg	53.1 ± 7.5	52.0 ± 6.7	51.2 ± 7.4	51.2 ± 8.0	0.06
BMI, kg/m^2	21.5 ± 2.7	21.1 ± 2.6	20.9 ± 2.6	21.2 ± 3.1	0.47
Body surface area, m^2	1.52 ± 0.11	1.50 ± 0.10	1.49 ± 0.11	1.48 ± 0.11	<0.001
TBW					
Liters	29.1 ± 3.1	28.1 ± 2.7	27.6 ± 2.9	26.9 ± 3.0	<0.001
%TBW in BW	55.3 ± 4.8	54.5 ± 4.7	54.4 ± 4.7	53.2 ± 5.3	<0.001
ICW					
Liters	18.2 ± 2.0	17.4 ± 1.7	17.0 ± 1.8	16.4 ± 1.9	<0.001
%ICW in BW	34.6 ± 3.0	33.8 ± 2.9	33.4 ± 2.9	32.3 ± 3.2	<0.001
ECW					
Liters	10.9 ± 1.2	10.7 ± 1.0	10.6 ± 1.1	10.6 ± 1.2	0.023
%ECW in BW	20.7 ± 1.8	20.7 ± 1.8	20.9 ± 1.8	20.8 ± 2.1	0.42
Protein					
kg	7.9 ± 0.9	7.5 ± 0.7	7.3 ± 0.8	7.1 ± 0.8	<0.001
%Protein in BW	15.0 ± 1.3	14.6 ± 1.3	14.4 ± 1.3	14.0 ± 1.4	<0.001
Skeletal muscle mass index, kg/m^2	8.8 ± 0.8	8.4 ± 0.7	8.2 ± 0.7	8.0 ± 0.7	<0.001
Minerals					
kg	2.8 ± 0.3	2.7 ± 0.3	2.7 ± 0.3	2.6 ± 0.3	<0.001
%Minerals	5.4 ± 0.5	5.3 ± 0.5	5.3 ± 0.5	5.2 ± 0.6	0.003
BFM					
kg	13.2 ± 5.1	13.5 ± 4.8	13.6 ± 5.0	14.5 ± 5.8	<0.001
%BFM in BW	24.4 ± 6.5	25.6 ± 6.4	25.9 ± 6.4	27.6 ± 7.2	<0.001
Ratio of ECW to TBW	0.375 ± 0.003	0.380 ± 0.002	0.385 ± 0.002	0.391 ± 0.004	<0.001
Males					
Age, yr	39.6 ± 15.6	56.3 ± 14.6	66.0 ± 10.4	71.3 ± 9.2	<0.001
Height, cm	170.3 ± 7.3	169.1 ± 5.9	168.7 ± 6.4	168.0 ± 6.5	0.002
Body wt, kg	67.5 ± 9.3	66.6 ± 10.3	67.4 ± 8.8	65.6 ± 9.8	0.20
BMI, kg/m^2	23.3 ± 3.6	23.2 ± 3.1	23.7 ± 2.5	23.2 ± 2.9	0.92
Body surface area, m^2	1.78 ± 0.14	1.76 ± 0.14	1.77 ± 0.13	1.74 ± 0.14	0.027
TBW					
Liters	40.7 ± 4.8	38.9 ± 4.7	38.7 ± 4.6	37.5 ± 4.7	<0.001
%TBW in BW	60.6 ± 4.9	58.9 ± 4.3	57.6 ± 4.4	57.5 ± 5.0	<0.001
ICW					
Liters	25.5 ± 3.0	24.2 ± 2.9	23.8 ± 2.8	22.8 ± 2.9	<0.001
%ICW in BW	38.0 ± 3.1	36.5 ± 2.7	35.5 ± 2.7	35.0 ± 3.1	<0.001
ECW					
Liters	15.1 ± 1.8	14.8 ± 1.8	14.9 ± 1.8	14.7 ± 1.8	0.026
%ECW in BW	22.5 ± 1.8	22.4 ± 1.6	22.1 ± 1.7	22.5 ± 2.0	0.38
Protein					
kg	11.0 ± 1.3	10.4 ± 1.3	10.3 ± 1.2	9.9 ± 1.3	<0.001
%Protein in BW	16.4 ± 1.4	15.8 ± 1.2	15.3 ± 1.2	15.1 ± 1.3	<0.001
Skeletal muscle mass index, kg/m^2	10.8 ± 0.9	10.3 ± 0.9	10.2 ± 0.8	9.8 ± 0.9	<0.001
Minerals					
kg	3.7 ± 0.5	3.6 ± 0.5	3.5 ± 0.5	3.4 ± 0.4	<0.001
%Minerals	5.6 ± 0.5	5.4 ± 0.5	5.3 ± 0.4	5.2 ± 0.5	<0.001
BFM					
kg	12.1 ± 5.9	13.6 ± 5.9	15.0 ± 5.3	14.9 ± 6.2	<0.001
%BFM in BW	17.4 ± 6.7	20.0 ± 5.9	21.8 ± 6.0	22.1 ± 6.7	<0.001
Ratio of ECW to TBW	0.375 ± 0.003	0.380 ± 0.002	0.385 ± 0.002	0.391 ± 0.004	<0.001

n, No. of subjects; BW, body weight; BFM, body fat mass.

kg/m^2 in females, $P < 0.001$). The fluid volumes in male and female subjects were 39.6 ± 4.9 vs. 27.7 ± 3.0 liters, respectively, and the percentages of BFM in the body weight in males and females were 19 and 26%, respectively. A significant difference was observed in the ratio of ECW to TBW between male and female subjects (0.378 ± 0.008 vs. 0.385 ± 0.006, $P < 0.001$).

Fluid volume balance between ICW and ECW. The fluid volume of ICW and ECW was strongly correlated with body size (Fig. 2*A*), and the ICW slope showed a steeper increase than the ECW slope. However, the correlation between BSA and the ECW/ICW ratio was extremely small in this study population ($r = -0.080$ in males and $r = -0.098$ in females) (Fig. 2*B*).

The factors of age, anthropometric measurements, and body composition were separated by quartiles of the ratio of ECW to ICW (Table 2). *Quartiles 1*, *2*, *3*, and *4* were categorized with ECW/ICW ratios of <0.605, 0.606–0.619, 0.620–0.631, and ≥0.632, respectively. The proportion of female subjects tended to increase from *quartile 1* to *4*, whereas the proportion of male subjects was observed to be inversely proportional to the ECW/ICW ratio. A higher ECW/ICW ratio was more probable

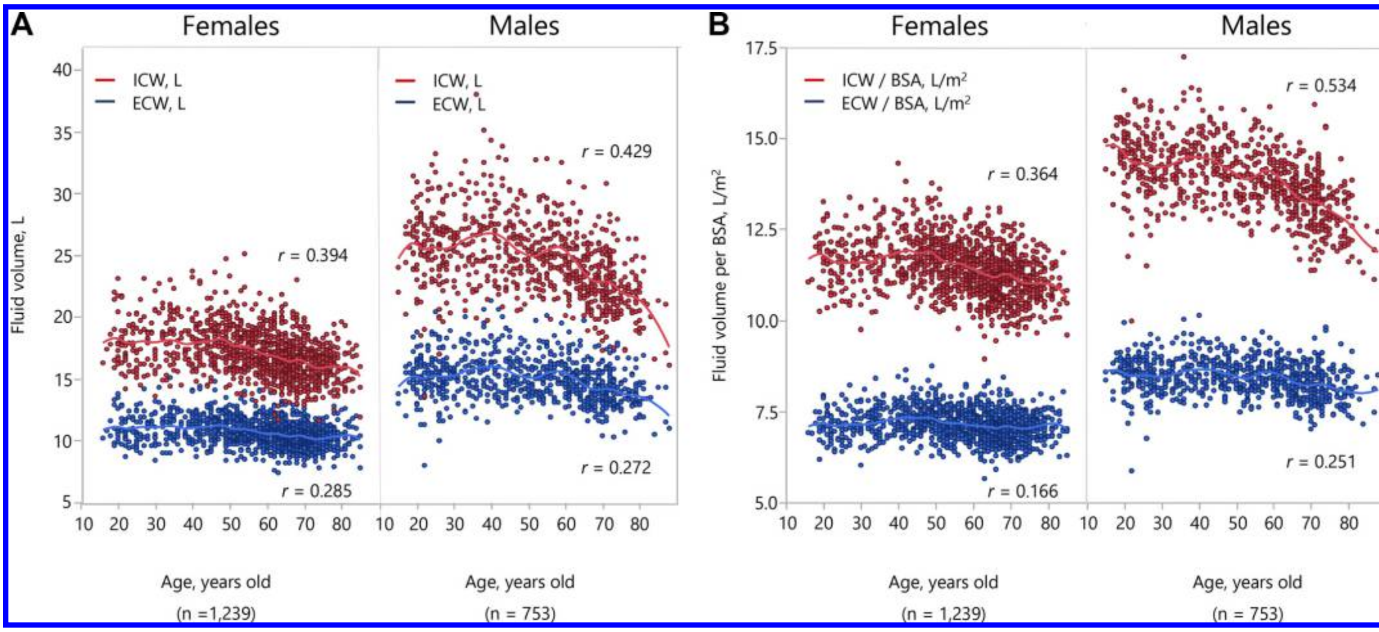

Fig. 3. *A*: fluid volume of intra- and extracellular water by age. *B*: fluid volume adjusted for body surface area by age. Plot depicting intracellular and extracellular water by age.

in older individuals and in those with a smaller body size and lower skeletal muscle mass index; however, this ratio was not associated with BMI. As shown in Fig. 3*A*, the total fluid volume was more likely to be higher in male than that in female subjects and also tended to gradually decrease with age in both genders. Interestingly, the ICW slope ($r = -0.429$ in males and $r = -0.394$ in females) was steeper than the ECW slope ($r = -0.272$ in males and $r = -0.285$ in females). These age trends in the ICW and ECW contents were observed even in the fluid volume per BSA (Fig. 3*B*). As shown in Fig. 4, *A* and *B*, the fluid volume was maximized between the ages of 30 and 40 yr in both genders (41.8 and 29.4 liters in males and females, respectively). Furthermore, the advanced age groups tended to have lower fluid volume compared with those belonging to the age groups of 40–49 yr ($P < 0.001$). The upward trend in the ratio of ECW to ICW occurred in female subjects aged ≥50 yr and in male subjects aged ≥40 yr, with the trend steepening after 70 yr in both genders. Consequently, the ratio of ECW to ICW increased with age because of the decreasing ICW contents (Fig. 5, *A* and *B*). The regression formulas for the association between the ECW/ICW ratio and age are shown below:

$$\text{Age-adjusted ECW/ICW ratio in males} = 0.5857 + 7.4334 \times 10^{-6} \times (\text{age})^2 \quad (1)$$

$$\text{Age-adjusted ECW/ICW ratio in females} = 0.6062 + 5.5775 \times 10^{-6} \times (\text{age})^2 \quad (2)$$

Table 3 shows the predicted estimates and the prediction intervals of the age-adjusted ECW/ICW ratio in the 10-yr age groups. The SEs of the quadratic least-square regression coefficient in male and female subjects were 0.2825×10^{-6} and 0.2516×10^{-6}, respectively. The SEs of the *y*-intercept in the

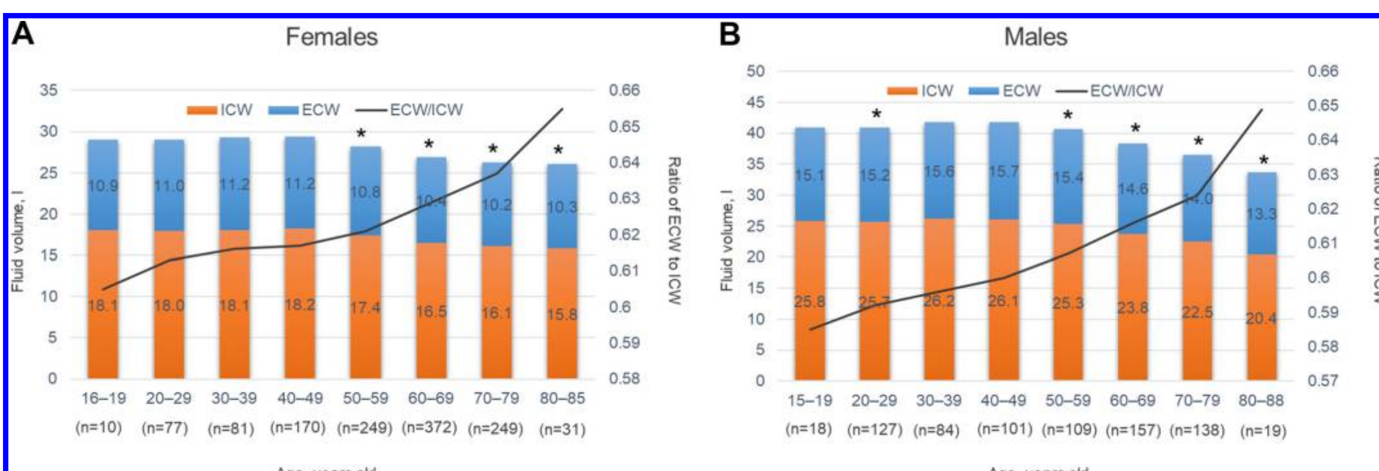

Fig. 4. Fluid volume balance of intra- and extracellular water in 10-yr age groups in females (*A*) and males (*B*). *$P < 0.05$ compared with total body water, including intracellular water and extracellular water in the 40- to 49-yr age group.

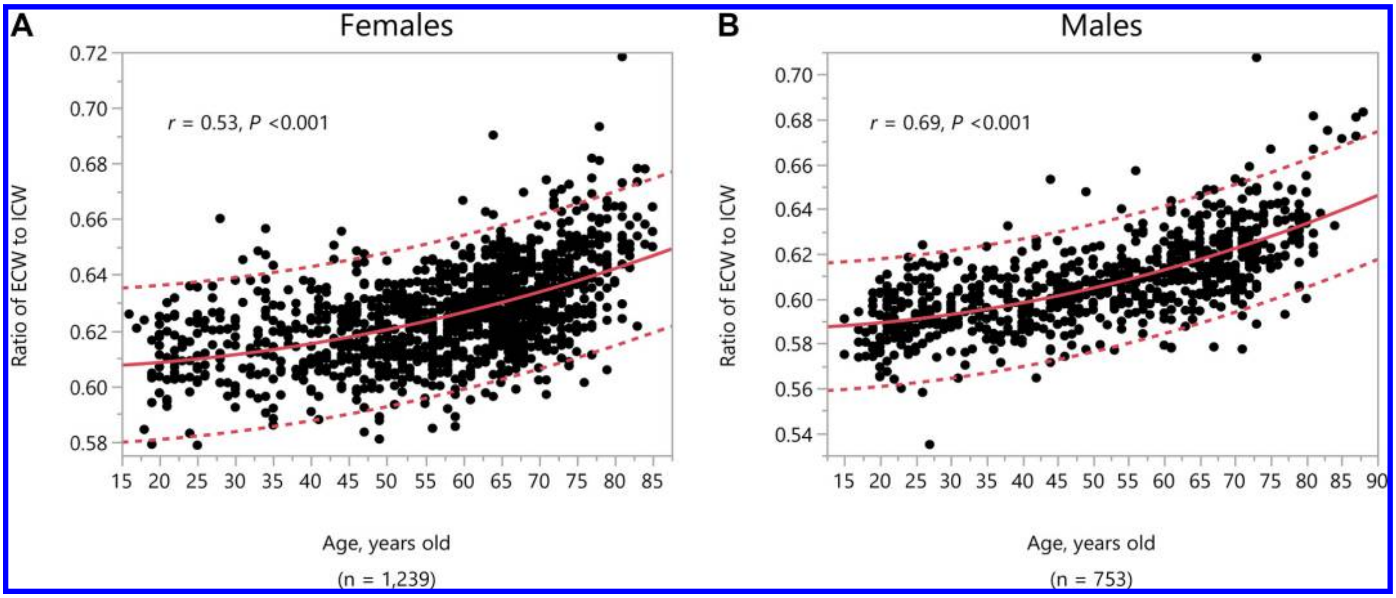

Fig. 5. Effects of age on fluid volume balance of intra- and extracellular water in females (*A*) and males (*B*). Mean (solid line), 5th, and 95th percentiles (broken line) for the ECW/ICW ratio by age.

formulas for male and female subjects were 0.000989 and 0.000974, respectively.

Validation of our proposal formula for calculating age-adjusted ECW/ICW ratio. The measured ECW/ICW ratio and the formulas for calculating the age-adjusted ECW/ICW ratio in the first half group and in the last half group are shown in Table 4. The age-adjusted ECW/ICW ratio calculated by the proposal formula in the validation set was close to that in the development set. The developed age-adjusted ECW/ICW ratio model demonstrated a good discriminative power in the validation population.

Standard ECW content and ECW content corrected by age-adjusted ECW/ICW ratio. The gap between the measured ECW content and the standard ECW content, calculated using the formula 0.613 × measured ICW as an indicator of volume overload, increasingly widened with age in our study population because the ECW content did not adapt to decreased ICW content. Understandably, the ECW content corrected by the age-adjusted ECW/ICW ratio calculated using our proposed formula minimized the influence of age and gender (Fig. 6).

Table 3. *Predicted values and 95% prediction intervals of age-adjusted ECW/ICW ratio in 10-yr age groups*

		Age-Adjusted ECW/ICW ratio		
Age Group, yr	*n*	5th percentile	Predicted value	95th percentile
Females	1,239			
16–19	10	0.580	0.608	0.636
20–29	77	0.582	0.609	0.637
30–39	81	0.585	0.613	0.640
40–49	170	0.590	0.618	0.645
50–59	249	0.595	0.623	0.651
60–69	372	0.602	0.630	0.657
70–79	249	0.609	0.637	0.664
80–85	31	0.616	0.644	0.672
Males	753			
15–19	18	0.560	0.589	0.617
20–29	127	0.562	0.591	0.619
30–39	84	0.567	0.595	0.624
40–49	101	0.572	0.601	0.629
50–59	109	0.580	0.608	0.637
60–69	157	0.589	0.617	0.646
70–79	138	0.597	0.626	0.654
80–88	19	0.608	0.636	0.665

n, No. of subjects.

DISCUSSION

This study revealed that the fluid volume balance between ICW and ECW is significantly associated with age. Thus, the ECW-to-ICW ratio is primarily driven by decreased cell volume, particularly its steepening trend after the age of 70 yr. The change in the ECW/ICW ratio with age can be calculated using a quadratic regression formula.

The total fluid volume depends on age, gender, and body size. In particular, ICW content may be associated with the muscle component in healthy subjects. In men, the TBW content was observed to be relatively constant during early adulthood, followed by a gradual decline at the rate of 0.3 kg/yr until reaching a nadir between the ages of 70 and 80 yr. In women, the TBW content was observed to remain during young adulthood and middle age; however, there was a dramatic decrease of 0.7 kg/yr after the age of 70 yr (29). Cell volume is regulated by apoptosis, a morphological hallmark of programed cell death that may also be associated with cell shrinkage (2). Apoptotic cell shrinkage is mediated through an active efflux of Na^+ and K^+ through the Na^+-K^+-ATPase pump and Ca^+-dependent K^+ channels, respectively (22). MF-BIA directly measures the resistance and reactance in the body, which is associated with cell membrane integrity and fluid distribution between the intra- and extracellular spaces. Approximately 75% of the muscles and viscera are composed of water (6); organ aging (14) and sarcopenia (24, 36) may be associated with the loss of ICW content, whereas the downward ECW slope with aging is mild in healthy subjects. This universal loss of ICW content may play a role in the change in the balance between ICW and ECW content, which disturbs the standard ICW/ECW ratio of 62:38. In the present study,

Table 4. *Measured ECW/ICW ratio and age-adjusted ECW/ICW ratio in the first half group and in the last half group*

	First Half Sep. 13–Oct. 8, 2014 (*n* = 996)	Last Half Oct. 8–Oct. 21, 2014 (*n* = 996)
Females, *n*	610	629
Measured ECW/ICW ratio	0.579 ± 0.017	0.583 ± 0.017
Regression formula for age-adjusted ECW/ICW ratio	$0.6059 + 5.6651 \times 10^{-6} \times (\text{age})^2$	$0.6065 + 5.4770 \times 10^{-6} \times (\text{age})^2$
SE of regression coefficient	0.3393×10^{-6}	0.3743×10^{-6}
SE of *y*-intercept	0.00132	0.00144
Males, *n*	386	367
Measured ECW/ICW ratio	0.608 ± 0.021	0.608 ± 0.019
Regression formula for age-adjusted ECW/ICW ratio	$0.5861 + 7.6250 \times 10^{-6} \times (\text{age})^2$	$0.5850 + 7.3196 \times 10^{-6} \times (\text{age})^2$
SE of regression coefficient	0.4006×10^{-6}	0.4000×10^{-6}
SE of *y*-intercept	0.00135	0.00145

n, No. of subjects.

fluid volume peaked during the 40 s and gradually decreased in advanced age for both male and female subjects. This trend was observed even in the ICW content adjusted for BSA, suggesting that ICW content is influenced by not only total body size but also each cell volume. As a reference, we determined the predicted values and the 95% prediction intervals of the age-adjusted ECW/ICW ratio in a 10-yr age group. The reference values of body fluid composition in consideration of its changes with aging can serve as a useful indicator to assess fluid volume imbalance.

Disorders of fluid volume balance are often experienced in diseases such as cardiac impairment and kidney failure. However, the optimal fluid volume remains controversial (4, 8). The balance between the ICW and ECW changes with age as the percentage of ECW content in the body fluid composition increases, implicating decreased ICW content as the primary driver of this percentage change. We speculated that a basal ICW content is associated with the reserve capacity for volume overload. In fact, brain natriuretic peptide is associated with lower BMI and a higher ECW/ICW ratio (26). In healthy adults, ECW content constitutes ~33–40% of the TBW content (10) and is determined by the absolute amounts of sodium and water. The ECW content is regulated by the activity of the renin-angiotensin-aldosterone and sympathetic nervous systems, as well as the secretion of natriuretic peptides. In clinical practice, we multidirectionally judge the fluid volume status based on vital signs, findings of physical examinations, serum sodium balance, and alterations of these hormones. Even if body fluid composition is measured using the MF-BIA device, it is uncertain whether measured ECW content can be considered as being of euvolemic status. Our proposal formula may be applicable in the calculation of optimal ECW content. The optimal ECW content can be corrected by the age-adjusted ECW/ICW ratio, which may resolve the serious problem of

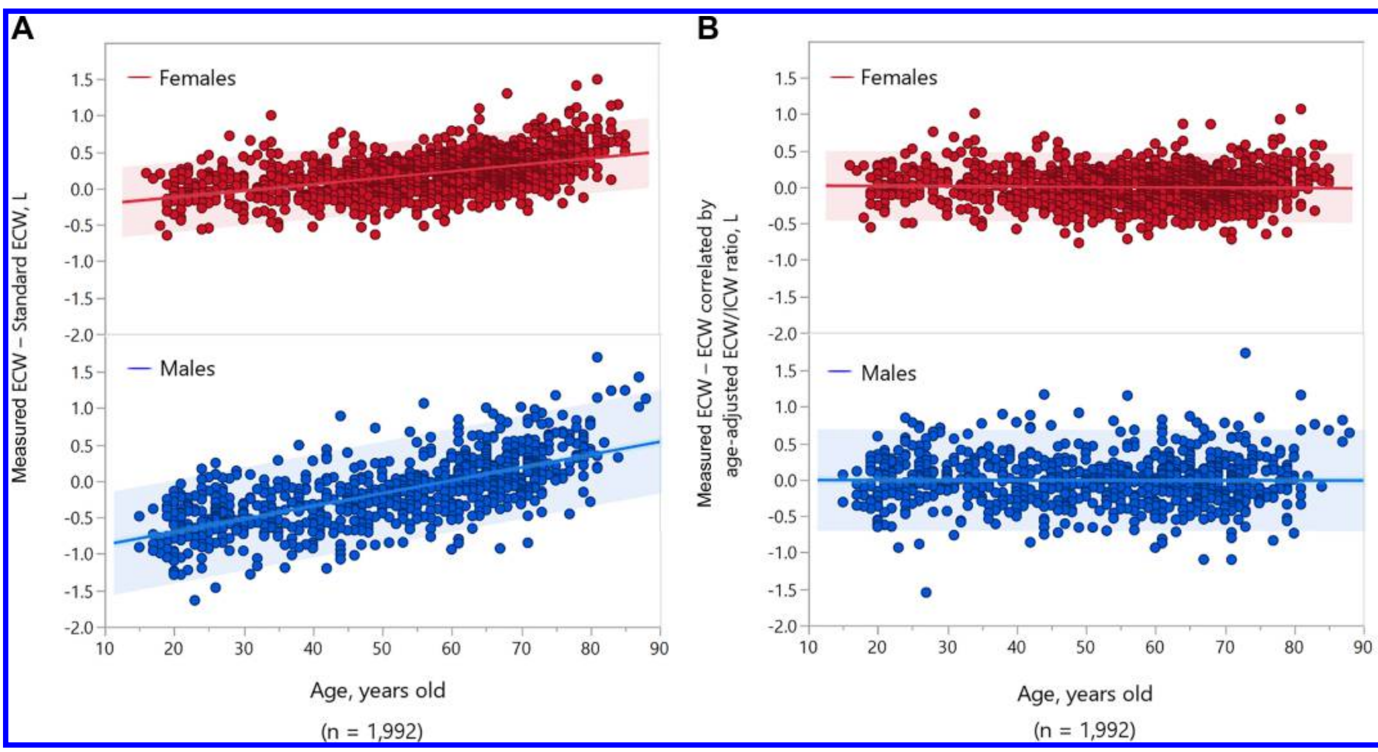

Fig. 6. *A*: difference between measured ECW content – standard ECW content. *B*: difference between measured ECW content – ECW content corrected by age-adjusted ECW/ICW ratio. BIA, bioelectrical impedance analysis.

having to consider a standard range of the ECW/TBW ratio of 0.360–0.390 as the optimal hydration status.

Some studies reported that the ECW/ICW ratio may increase in obese individuals (19, 20), which conflicted with our findings. The ICW slope tended to be a steeper increase than the ECW slope along with BSA (Fig. 2*A*). As a result, BSA had inversely a small correlation with the ECW/ICW ratio. Theologically, the ECW/ICW ratio should increase in severe obese conditions without an increase in the muscle component as the ECW content increases in the large body size subjects. This association may be notably observed in sarcopenic obesity. In this regard, we could not fully validate this assumption. In the present study, BMI and BSA were plotted in a nonnormal distribution. Especially, individuals with BMI $\geq$30 kg/m^2 were less prevalent than those in the Japanese general population (2.5 vs. 4.3% in males and 1.3 vs. 3.0% in females, respectively). Our proposed formula may yield accurate data in male subjects with BMI of 20.0–27.0 kg/m^2 and female subjects with BMI of 18.0–25.0 kg/m^2 as the basis of the 10th–90th percentile of BMI in the study population. According to the Organisation for Economic Co-operation and Development's Obesity Update 2017, the obesity rate in Japan is the lowest in the 35-member countries. The BMI distribution in this study population was slightly better than that in the National Health and Nutrition Examination Survey conducted in 2015. Future study is required to evaluate the unique fluid volume balance between ICW and ECW in obese populations, including those in any other developed countries.

The present study has several limitations. First, this study population was not confirmed to be healthy. For instance, the skeletal muscle mass index in this study population was higher than that in a sample of Japanese healthy adults aged 40–79 yr old, including 16,379 men and 21,660 women (10.8 $\pm$ 0.9 vs. 8.0 $\pm$ 0.7 kg/m^2 in men and 9.1 $\pm$ 1.3 vs. 6.3 $\pm$ 0.6 kg/m^2 in women) (35). Second, this present study was performed using existing sample data obtained from the InBody Japan Inc. Medical condition, and the presence of comorbidities was not fully evaluated in the preliminary survey. However, we believe that most training gym users are free of any serious disease; therefore, our study population is almost representative of healthy adults or health-conscious individuals. In contrast, few obese populations were included in this study, which may have hampered our results related to changes in the fluid volume balance resulting from obesity. Especially, if a patient showed an extreme decrease in the ICW content because of severe sarcopenia or sarcopenic obesity, the ECW content corrected by the age-adjusted ECW/ICW ratio may be underestimated. Third, we did not analyze the racial and ethnic differences among the subjects, since only Japanese individuals were included in this study. Fourth, if the ICW content is influenced by any parameters besides age and muscle mass, our proposed formula may derive an incorrect result for the fluid volume imbalance between ICW and ECW. For example, if a patient is overhydrated and develops hyponatremia because of an excess intake of sodium-free water, the excessive fluid volume is most likely redistributed on the basis of the ratio of ICW to ECW. In such a case, a patient may be exposed to increased ICW content. We recommend that body composition be measured after correcting the fluid volume imbalance as much as practicably possible. Still, we believe that our proposed formulas will help clinical decision-making in terms of body fluid assessments.

Future studies are required for validating the proposal formulas using other populations to create sophisticated formulas for adjusting the racial and ethnic differences within a large study population and for testing the utility of the proposal formulas in patients with volume overload.

In conclusion, the fluid imbalance between ICW and ECW is driven by decreased cell volume that occurs with age and muscle attenuation, as well as with volume overload. This trend is particularly steeper after the age of 70 yr. Our proposed formula for calculating the age-adjusted ECW/ICW ratio may be a useful assessment tool for the calculation of body fluid composition, and it can be applicable to the quantitative analysis of volume overload in patients with fluid volume imbalance.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors. Sample data were provided by InBody Japan Inc. after entering into a nondisclosure agreement.

AUTHOR CONTRIBUTIONS

Y.O. conceived and designed research; Y.O., K.Y., T.K., R.T., and H.O. performed experiments; Y.O. and N.J. analyzed data; Y.O. and N.J. interpreted results of experiments; Y.O. prepared figures; Y.O. drafted manuscript; Y.O., N.J., K.Y., T.K., R.T., H.O., R.Y., and K.S. edited and revised manuscript; Y.O., N.J., K.Y., T.K., R.T., H.O., R.Y., and K.S. approved final version of manuscript.

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