Determination of Percent Body Fat by the Newly Developed Sulfur Hexafluoride Dilution Method and Air Displacement Plethysmography

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Summary The reliability and validity of two newly developed densitometric methods for determining the human body volume and percent body fat (%FAT), the sulfur hexafluoride dilution method (SHF) and air displacement plethysmography (ADP), were evaluated in comparison with the underwater weighing method (UWW). Seven healthy male volunteers (age 31 to 44, mean height 166.0cm, weight 61.4kg) participated in this study. The same-day test-retest coefficients of variation (CVs) for body volume and %FAT measurements were not significantly different among the three methods. SHF and UWW showed a strong correlation in terms of body volume and %FAT, with the correlation coefficients (r) being 0.9997 and 0.986, respectively. The correlation between ADP and UWW was slightly weaker (r=0.9997 for body volume and 0.907 for %FAT). However, body volumes measured by SHF and ADP were significantly different from that by UWW when compared by mean values. Such differences were also found for %FAT measurements. The regression lines of body volume measured by SHF and ADP on that by UWW were almost equivalent to the line of identity. However, those of %FAT measured by SHF and ADP on that by UWW were significantly different from the line of identity. Because the reliability of SHF and ADP appeared to be high, further validation and improvement are required and worth doing.

Key Words gas dilution, plethysmography, underwater weighing, body volume, percent body fat

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Densitometry is currently considered the most valid method in estimating human body composition without invasion. The underwater weighing method (UWW) has been popular as the standard procedure, against which newer methods should be assessed (1). However, the requirements of skill and patience by subjects throughout this procedure limit those who can undergo it. Among several densitometric techniques developed as surrogates of UWW (2–8), the recent two volumetric techniques equipped with new devices have some advantages: the gas dilution method (9) using sulfur hexafluoride, SF₆, which is pharmacologically inert (SHF) (10), and air displacement plethysmography (ADP) (11, 12). We tested their validity and reliability for evaluating body composition regarding UWW as the gold standard.

**Materials and Methods**

To detect \( r = 0.9 \) at the 5% significance level with 80% power in a two-sided test (13), 7 healthy male volunteers (aged 31–44) were examined after obtaining their informed consent. The Ethical Review Committee at the Medical Research Institute, Tokyo Medical and Dental University approved the study design. All measurements were made on the same day. The room temperature was kept from 20 to 22°C during the experiment. First, the body weight of each subject was determined in air on a balance scale to the nearest 20 g.

In the body volume measurement system using SF₆ dilution (BSF-200, SHF, Shimadzu, Kyoto, Japan) (9), each subject (wearing swimwear) was examined twice (after 12-h overnight fasting and urination). A control valve released a small amount of gas after attaining equilibrium when the subject’s body heat increased the pressure in the chamber. This mechanism enabled the SF₆ analyzer to detect gas concentration free from the effect of pressure change. The body volume was derived as \( V = V_0 + V_1 - (V_1 \times 10^6)/X \), \( V_0 \), the volume of the chamber (195 L); \( V \), subjects’ body volume (wearing swimwear); \( V_1 \), the amount of diffused SF₆ after attaining equilibrium. Therefore, the SF₆ gas concentration (\( X, \text{ppm} \)) is \( X = \{V_1/(V_0 - V + V_1)\} \times 10^6 \). If the difference between the first and second volumetric values exceeded 0.5%, the average of the two closest values was recorded after the third measurement was conducted.

One new air displacement plethysmograph (BOD POD, ADP, Life Measurement Instruments, Concord, CA, USA) (11, 12) comprises a single structure containing two chambers. A seat forms a common wall separating the front (test) and rear (reference) chambers (hereinafter C_T and C_R). A volume-perturbing element in the form of a moving diaphragm is mounted between the two chambers. During operation, the diaphragm oscillates back and forth to create sinusoidal volume perturbations that lead to small and complementary pressure fluctuations in the two chambers. Fourier coefficients are used to calculate pressure amplitude at the frequency of oscillation. Since the pressure variations are quite small compared with ambient pressure, the ratio of the volume of C_T to that of C_R is equal to the ratio of C_R amplitude to C_T amplitude according to Poisson’s Law. Thus the volume...
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of an object in $C_T$, $y$, is obtained from the equation $y = ax + b$, where $x$ is $C_R : C_T$ ratio (pressure amplitude). The parameters, $a$ and $b$, are determined by the two-point calibration process at 0 and 50 L.

During the 12-s measurement, the subject was requested to breathe normally. Then the subject held a breathing tube in his mouth to measure lung air volume (thoracic gas volume). The examiner observed the display of the subject’s normal breathing on the computer screen for a moment and signaled verbally to him just before airway occlusion. At this time, the subject alternately contracted and relaxed the diaphragm muscle while airway and chamber pressures were simultaneously recorded.

Concerning UWW, the standard procedure was adopted (1) with a UWW meter (AD6204, A & D, Tokyo, Japan). Each subject was measured five times and the maximum value was adopted. The water temperature was maintained at about 36°C. Residual lung volume was measured by the oxygen-dilution technique (14).

One author (H.I.) had completed measurement by SHF and ADP as the sole examiner. Another author (T.F.) had been responsible for the entire process of UWW.

The means and standard deviations (SDs) of body volume, body density (body weight/body volume), percent body fat (%FAT) (by Brozek equation (15)), and lean body mass (LBM) were compared among the three methods. The reliability of measuring body volume and %FAT was evaluated by calculating the coefficient of variation (CV) for repeated measurements. To test whether the CV differed among the methods, the Wilcoxon sign-rank test was used. The first and second measurements of each method (e.g., body volume$_{SHF1}$ vs. body volume$_{SHF2}$) were compared by the paired $t$-test.

Linear regression analyses were performed with body volume or %FAT by UWW as a dependent variable and by SHF or ADP as an independent variable to determine whether the regression line differed significantly from the line of identity (e.g., body volume$_{UWW} = $body volume$_{SHF}$) by using a simultaneous test of slope and intercept. The agreement between methods for individual subjects was assessed by using the method of Bland and Altman (16).

Results

Table 1 shows the subjects’ basic characteristics. The means of weight, height, and body mass index were similar to those of the average Japanese of the same age (17) and smaller than those of Caucasians (18).

There was no significant difference in the measurements of body volume and %FAT between the first and second trials in any of the three methods, i.e., SHF, ADP, and UWW. The between-trial CVs: for body volume, 0.15±0.09 for SHF, 0.09±0.05 for ADP, and 0.16±0.04 for UWW; for %FAT, 3.76±3.68 for SHF, 3.73±4.26 for ADP, and 4.27±2.48 for UWW, respectively (mean±SD, %). These CVs did not differ significantly among the three methods.

The means of body volume and body fat determined by SHF were higher and
Table 1. Characteristics of subjects (n=7).

| Variables             | Mean ± SD  |
|-----------------------|------------|
| Age (year)            | 36.4 ± 4.2 |
| Weight (kg)           | 61.4 ± 10.5|
| Height (cm)           | 166.0 ± 5.1|
| Body mass index (kg/m²)| 22.3 ± 3.5 |

Table 2. Means and standard deviations of body volume, body density, body fat, and lean body mass determined by three densitometric methods.

| Variables                  | UWW Mean ± SD | SHF Mean ± SD | p-value | ADP Mean ± SD | p-value |
|----------------------------|---------------|---------------|---------|---------------|---------|
| Body volume (L)            | 58.3 ± 10.6   | 58.8 ± 10.8   | 0.011   | 57.8 ± 10.1   | 0.017   |
| Body density (g/mL)        | 1.055 ± 0.015 | 1.047 ± 0.019 | 0.004   | 1.065 ± 0.018 | 0.014   |
| Body fat (%)               | 18.9 ± 6.3    | 22.4 ± 8.2    | 0.005   | 14.9 ± 7.3    | 0.014   |
| Body fat (kg)              | 12.0 ± 5.6    | 14.3 ± 7.0    | 0.011   | 9.7 ± 5.6     | 0.017   |
| Lean body mass (%)         | 81.1 ± 6.3    | 77.6 ± 8.2    | 0.005   | 85.2 ± 7.3    | 0.014   |
| Lean body mass (kg)        | 49.4 ± 6.3    | 47.1 ± 5.9    | 0.011   | 51.8 ± 6.0    | 0.017   |

SHF, SF₆ dilution method (BSF-200); ADP, air displacement plethysmography (BOD POD).

*Difference between SHF or ADP and UWW in the paired t-test.

those by ADP lower than those by UWW, and the means of body density and LBM were just the opposite (Table 2). All these differences were statistically significant. The differences of body volumes between UWW and the other methods, i.e., [body volume_BODY_UWW] and [body volume_BODY_ADW], were 0.5 ± 0.4 (95% confidence interval [CI], 0.2 to 0.8) and −0.5 ± 0.4 (95% CI, −0.9 to −0.1), respectively (mean ± SD, L). The differences of %FAT between UWW and the other methods, i.e., [%FAT_BODY_UWW] and [%FAT_BODY_ADW], were 3.57 ± 2.18 (95% CI, 1.5 to 5.6) and −4.03 ± 3.10 (95% CI, −6.9 to −1.2), respectively (mean ± SD, %). Figure 1 shows the regression line and the line of identity. The slope and intercept of body volume measured by SHF (A) and ADP (B) were not significantly different from 1 and 0, respectively. However, regarding %FAT, the slope of SHF (C) and the intercept of ADP (D) were significantly different from 1 and 0, respectively. Figure 2 demonstrates the agreements between body volume_BODY_UWW and body volume_BODY_ADW, body volume_BODY_SHF and body volume_BODY_UWW, %FAT_SHF and %FAT_UWW, and %FAT_ADW and %FAT_UWW for individual subjects. The differences of body volume measurements between UWW and the other methods (e.g., body volume_BODY_SHF − body volume_BODY_UWW) and those of %FAT measurements between UWW and the other methods (e.g., %FAT_SHF − %FAT_UWW)
Fig. 1. Associations between measurements by the underwater weighing method (UWW) and those by the SF₆ dilution method (SHF) or the air displacement method (ADP): (A) body volumes measured by UWW and those by SHF; (B) percent body fats obtained by UWW and those by SHF; (C) body volumes measured by UWW and those by ADP; (D) percent body fats obtained by UWW and those by ADP. The solid line is the line of identity; the dashed line is the regression equation. The slope and intercept of body volume measured by SHF (A) and ADP (B) were not significantly different from 1 and 0, respectively. In regard to %FAT, however, the slope of SHF (C) and the intercept of ADP (D) were significantly different from 1 and 0, respectively. The corresponding r- and p-values are given in the figure.

%FAT_UWW were plotted against the mean body volume and mean %FAT of the two methods. The mean difference ± 2SD of body volume and %FAT establish limits of agreement that are -0.2 to 1.2 L for body volume_SHF, -0.3 to 1.4 L for body volume_ADPI, -0.79 to 7.9 for %FAT_SHF, and -10.1 to 2.17 for %FAT_ADPI. There appears to be an increasing trend in %FAT_SHF − %FAT_UWW as %FAT increases (p = 0.018).

Discussion

The average CVs of body volume and %FAT were almost the same or slightly
The agreement of SHF and ADP with UWW for individual subjects assessed by the method of Bland and Altman (16): (A) mean (x axis) and difference (y axis) of two body volumes measured by SHF and UWW; (B) mean (x axis) and difference (y axis) of two body volumes measured by ADP and UWW; (C) mean (x axis) and difference (y axis) of two %FATs measured by SHF and UWW; (D) mean (x axis) and difference (y axis) of two %FATs measured by ADP and UWW. Dashed lines are the mean difference ± 2SD.

smaller than those by UWW, indicating that the same-day reliability of SHF and ADP were as good as that of UWW.

This study showed the validity of SHF and ADP for a determination of body volume. The %FAT by SHF was highly correlated with that by UWW, being consistent with a previous report (9). However, the present study implied that SHF tends to underestimate %FAT, especially in fatter subjects. The correlation coefficient of %FAT between ADP and UWW in this study ($r = 0.907$) was smaller than that reported by McCrory et al ($r = 0.96$) (12). The results of the simultaneous test of slope and intercept indicated the tendency of ADP to overestimate %FAT in the present subjects. The $C_T$ volume of ADP developed in the United States was too large for the average Japanese. The body size of our male subjects (height

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160–174 cm, weight 45.4–72.0 kg) was smaller than that of McCrory's subjects (12) (height 166–191 cm, weight 64.6–129.0 kg). According to Matsumura (19), a correlation coefficient of %FAT was observed as 0.958 between ADP and UWW among Japanese sumo wrestlers (height 166.8–185.9 cm, weight 78.0–150.6 kg), and it was 0.563 among Japanese athletes (height 165.8–176.0 cm, weight 50.0–60.8 kg). For introducing ADP to Asian populations, including Japanese, CT volume should be adjusted to their body sizes. The chamber volume of SHF was small relative to ADP, since the apparatus was originally made for the average Japanese.

The use of SHF or ADP would eliminate the necessity to totally immerse the subject in water, a disadvantage with UWW. The shorter time of performance was another advantage of SHF and ADP (about 10 min and 15 min, respectively) compared with UWW (no less than 40 min). Consequently, these methods realized a densitometric measurement even on the handicapped population, such as people with mental retardation (20).

Being superior to ADP, SHF requires no special effort by subjects. Measurement of the lung volume required in ADP is not always easy for subjects and examiners, and subjects need to be well trained to obtain reproducible results. In this regard, employing the value of lung volume predicted from age and height may be helpful in ADP.

To estimate body composition, bioelectrical impedance analysis (BIA) has been also validated in adult populations (21, 22). However, it is indicated that BIA overestimated %FAT in the thin subjects and underestimated it in fatter ones (23). Moreover, the daily fluctuation of water content because of exercise, dehydration, eating, and drinking may affect the results (23). This is because BIA's principle is not densitometry, but resistance to a mild electric current related to total body water (24).

SHF and ADP are far easier than UWW to perform, which can extend the range of subjects to those who cannot stand a stressful procedure. Because reliability of both SHF and ADP appeared to be high, further validation and improvement are required and worth doing.

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