EVALUATION OF THE TESTER'S INFLUENCE ON THE RESULTS OF AIR DISPLACEMENT PLETHYSMOGRAPHY

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Abstract. The tester was reported as a potential source of variability in air displacement plethysmography (ADP). This work evaluates the impact of the tester on body fat percentage (%*BF*) assessments *via* ADP using the BOD POD system. We analyzed sets of consecutive ADP trials conducted on the same subject. A pair of randomly assigned testers performed 5 sets of 12 trials. Sets differed in subject preparation (eating, drinking, and bathroom visits). The results were analyzed using the Bland-Altman method, curve fitting, and the two-sample *t*-test. In a second protocol, a team of 6 testers performed 10 measurements each, in random order, and the mean values of their readings were compared *via* one-way analysis of variance (ANOVA). The two-sample *t*-test indicated no significant difference between the mean values of %*BF* recorded by the two testers who conducted the first protocol (P = 0.51). Moreover, intra- and intertester Bland-Altman plots were similar. The mean values of the readings of 6 testers differed by less than 0.46 %*BF*. According to one-way ANOVA, these differences were not significant (P = 0.33). The influence of the tester on ADP results is statistically insignificant and smaller than the technical error of measurement of the BOD POD.

Key words: Body composition, body fat percentage, body volume, resting metabolic rate, total energy expenditure.

INTRODUCTION

The BOD POD® Body Composition Tracking System is a commercially available air displacement plethysmography (ADP) instrument for assessing body fat and fat-free mass by measuring body mass (*BM*) and body volume (*BV*) [6]. The BOD POD is user- and subject-friendly. For about a minute, the subject sits still and breaths normally in a hermetically closed chamber, while the air in the chamber is compressed periodically by an oscillating diaphragm. A sensor measures the pressure changes, and the BOD POD software calculates the volume of air in

Received: July 2019;
in final form August 2019.

ROMANIAN J. BIOPHYS., Vol. 29, No. 4, P. 101–112, BUCHAREST, 2019

the chamber. Lacking time for heat exchange, this air undergoes adiabatic transformations, except for the air in the lungs and near the skin, which suffers isothermal transformations [6]. Thus, ADP measurements involve a complex interplay of thermodynamic phenomena influenced by the environment, as well as by the subject [8].

Body composition assessments by the BOD POD were found in very good agreement with hydrostatic weighing [7, 14]. They displayed moderate deviations from dual-energy X-ray absorptiometry [8, 20], which were more pronounced for underweight and overweight subjects [13]. The BOD POD also demonstrated an excellent repeatability, with a technical error of measurement ranging from 0.8 to 1.1 %*BF* [1, 3, 4, 8, 10, 17, 22].

The tester has also been reported as a possible source of error in body composition assessments using the BOD POD [8, 15]. Indeed, the consistency of the procedures performed by the tester might be important for precise results. How fast the door is closed, or how far and for how long the door is opened between the measurements that compose one trial, might influence the results. Controlling such aspects, however, would make the instrument less user-friendly. Although the measurement protocol devised by the manufacturer seeks to minimize the errors related to the tester's performance [5], there is insufficient evidence in the literature regarding their magnitude. To assess the impact of the tester on ADP results, in this work we analyze series of consecutive trials conducted on the same subject.

MATERIALS AND METHODS

We performed several series of body composition tests on one subject (male, 48 years, body mass index (*BMI*) = (body mass)/(height^2) = 21.9 kg/m^2). Focusing on one subject we sought to minimize biological variability. Before being tested, the subject provided a written informed consent. Our study was approved by the Committee for Scientific Research Ethics of the ”Victor Babeș” University of Medicine and Pharmacy Timișoara, România.

BOD POD MEASUREMENTS

Body composition was evaluated using a BOD POD® Gold Standard Body Composition Tracking System (COSMED USA, Inc., CA, USA), with BOD POD software version 5.3.2. Scale calibration and system quality check were carried out daily.

The subject was asked to refrain from alcohol consumption and intense exercise for 12 hours before the tests. Using a wall-mounted stadiometer, we measured the subject's height to the nearest 0.5 cm, while he was standing barefoot with his Frankfort plane in horizontal position. Body mass was measured using the

scale connected to the BOD POD, at the full precision of the scale ($\pm 10^{-3}$ kg). During ADP tests, the subject wore a form-fitting swimsuit and tight acrylic swim cap. To minimize posture-related variability [18], the subject adopted a well-defined position in the BOD POD chamber, with hands resting on his knees, and straightened back not touching the backrest of the seat. We ran the BOD POD application to predict thoracic gas volume, to measure body mass and body volume, to calculate %*BF* using the Siri formula [21], and to estimate the resting metabolic rate (*RMR*) [16] and total energy expenditure (*TEE*) [5].

Two testers with 2 years of ADP experience conducted 5 sets of 12 trials. An equal number of measurements were assigned randomly to both testers: identical cards, numbered 1 to 12, were placed face-down on a table; the testers extracted, alternatively, 6 cards, representing the labels of measurements they had to do. For the first set of measurements, the subject came after overnight fasting and urinated a few minutes before being tested. The second set was done right after consuming 500 mL unsweetened tea. The third set started two hours later, after having the bladder voided. The fourth set started 30 minutes after lunch. The fifth set started two hours after lunch, after urinating. Thus, the sets 1, 3, and 5 were in accord with the operator's manual [5].

Then, a team of 6 testers performed 60 consecutive trials on the same subject. To avoid test order effects, the testers were assigned in a random order: cards numbered from 1 to 60 were placed on a table and each tester picked 10 cards specifying the numbers of the allotted trials.

STATISTICAL ANALYSIS

Results were analyzed using the Statistics Toolbox of MATLAB 7.13 (The MathWorks, Natick, MA, USA). The significance level of hypothesis tests was set to 0.05.

We performed a two-sample *t*-test to decide whether the results recorded by the first two testers came from independent random samples of normal distribution with equal means and equal variances.

We also did a Bland-Altman analysis (i) by plotting the difference versus the mean of $n = 30$ pairs of %*BF* values recorded by the two testers, (ii) by calculating the bias, defined as the mean value of the differences, $\overline{D}$, and (iii) by calculating the 95% limits of agreement, $\overline{D} \pm 1.96 SDD$, where SDD denotes the standard deviation of the differences [2]. We also computed 95% confidence intervals (*CI*) for the bias and for the limits of agreement [11]. The *CI* of the bias was expressed in terms of these quantities as $\overline{D} \pm t \cdot SDD/\sqrt{n}$, where $SDD/\sqrt{n}$ is the standard error of the bias, and t denotes the argument of Student's probability density function with $n - 1$ degrees of freedom for which this function takes the value 0.05.

We used the formula of the standard error of the limits of agreement, $SDD\sqrt{3/n}$, to express the *CI* of the lower limit of agreement (LLA) as $LLA \pm t \cdot SDD\sqrt{3/n}$; a similar expression holds also for the upper limit of agreement (ULA) [2, 9].

One-way analysis of variance (ANOVA) was employed to point out significant differences between the results recorded by the 6 testers, as well as to evaluate the impact of drinking and eating prior to an ADP trial. To assure that ANOVA is applicable, we used the Jarque-Bera test [12] to check that the data are normally distributed.

We employed the Bonferroni algorithm to identify data sets whose mean %*BF* values differed significantly from the mean of set 1; all other data sets were combined to form a sample of correctly recorded data. For this sample, we calculated the mean, standard deviation, standard error, skewness, and kurtosis of %*BF*, *BM*, *BV*, body density (*BD*), fat mass (*FM*), fat free mass (*FFM*), *RMR* and *TEE*. We also calculated the coefficient of variation, $CV = (SD/MEAN)\cdot 100\%$, where $MEAN$ and SD denote the sample mean and standard deviation, respectively.

RESULTS

To assess errors in body composition estimates related to the tester's performance, we sought to minimize biological variability by monitoring one subject *via* consecutive ADP trials. Measurements were performed in 5 sets of 12 trials; within each set, two randomly assigned testers performed 6 trials each.

In Figs. 1 and 2, empty markers represent the results recorded by Tester 1 (T1), whereas the solid markers refer to Tester 2 (T2).

According to Fig. 1 (top panel) *BM* drops steadily, with an average slope of 35.9 g/h, and 95% confidence interval (*CI*) of (–37.7, –34.0) g/h, obtained by fitting the time dependence of *BM* with a piecewise linear function. More precisely, the fit function was $BM = a \cdot t + b_1$ for the first set, $BM = a \cdot t + b_2$ for the second set, and so on. Here t is time, a is the slope, whereas $b_1, b_2, ..., b_5$ are the intercepts of the lines that fit data sets 1, 2, ..., 5, respectively. The intercepts are $b_1 = 71.86$ kg, *CI* (71.85, 71.88) kg; $b_2 = 72.37$ kg, *CI* (72.35, 72.39) kg; $b_3 = 71.66$ kg, *CI* (71.64, 71.68) kg; $b_4 = 72.92$ kg, *CI* (72.88, 72.95) kg; $b_5 = 72.79$ kg, *CI* (72.76, 72.83) kg.

Differences of intercepts are equal to the abrupt changes in *BM*; increments resulted from drinking (at 10 a.m.) and having lunch (between 2:30 and 3:30 p.m.), whereas decrements resulted from urinating. These events affected the subject's *BV*, too (Fig. 1, bottom panel): 66.960 ± 0.049 L in the first set and 67.521 ± 0.073 L in the second set, recorded after the subject drank 0.5 L tea.

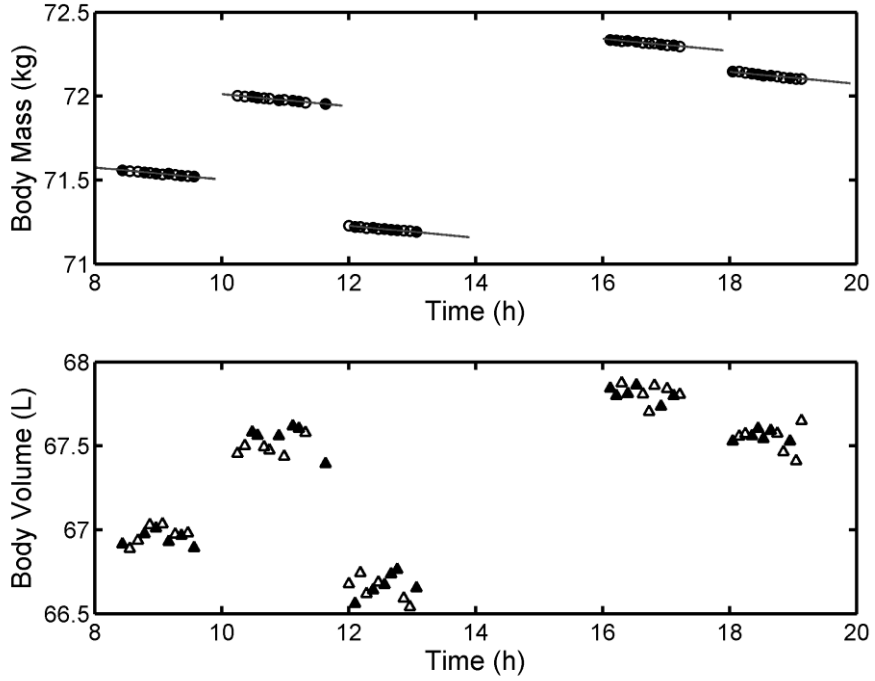

Fig. 1. Time dependence of the subject's body mass (top) and body volume (bottom). Empty (solid) markers depict the results of measurements performed by the first (second) tester. In the top panel, solid lines represent the fit of data by a piecewise linear function. Abrupt changes in body mass and body volume were caused by drinking (at 10:00), eating (at 14:30) and urinating (at 11:55 and 17:40).

Figure 2 plots body composition data from 5 sets of ADP trials. The resting metabolic rate (*RMR*) and total energy expenditure (*TEE*) were estimated by the BOD POD software, taking into account the subject's self-assessed, low level of physical activity [5].

To check whether the tester has a statistically significant impact on %*BF* estimates, we applied a two-sample *t*-test, which did not question the validity of the null hypothesis that the data recorded by the two testers had equal means and equal variances ($P = 0.51$).

We also performed a Bland-Altman analysis by plotting the differences between randomly assigned pairs of readings vs. the means of the same pairs (Fig. 3). In these plots, horizontal lines depict the bias (solid line) and the limits of agreement (dotted lines).

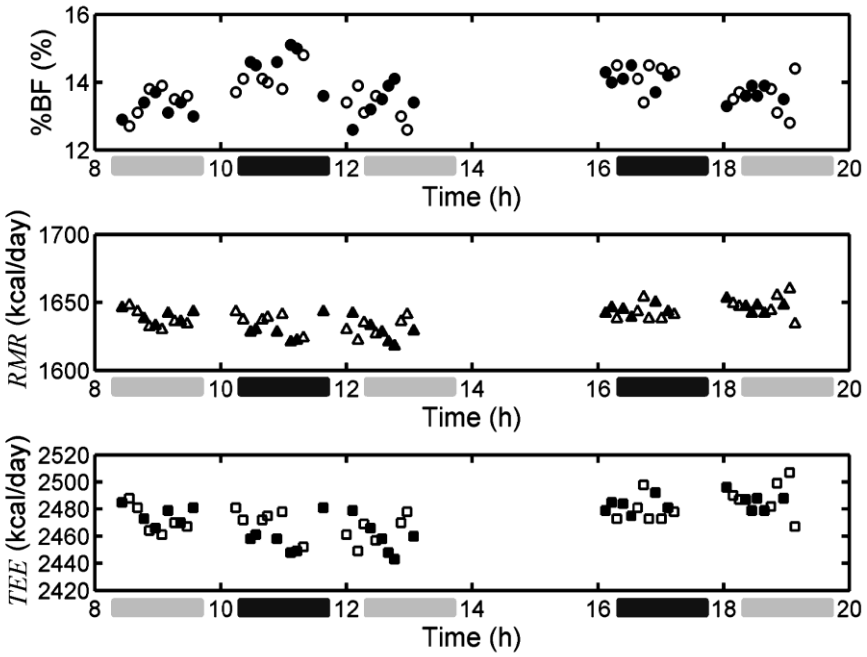

Fig. 2. Time dependence of percentage body fat (top), resting metabolic rate (middle) and total energy expenditure (bottom). Empty and solid markers plot data recorded by Tester 1 and Tester 2, respectively. Grey (black) bars below the time axis indicate sets of measurements performed in accord (at odds) with the manufacturer's instructions.

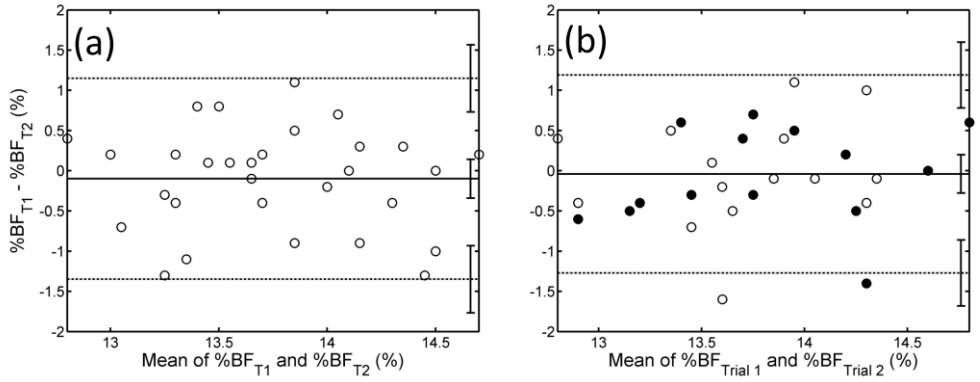

Fig. 3. Intertester (a) and intratester (b) Bland-Altman plot of differences vs. means of %*BF* recorded by two testers for the same subject (5 sets of 6 measurements by each tester). Pairs of measurements were randomly assigned within each set. In panel (b), differences between pairs of readings by Tester 1 (Tester 2) are depicted as empty (solid) markers. The solid line represents the bias, whereas dotted lines represent the limits of agreement. Vertical error bars on the right depict 95% confidence intervals (*CI*) of the quantities represented by the horizontal lines they cross.

In the intertester analysis (Fig. 3a), the readings of Tester 1 (T1) are compared with those of T2. Within each set of measurements, pairs were built randomly, by extracting numbered cards. For example, we extracted one of numbers 1 to 6 to decide which reading of T2 to compare with the first reading of T1; then we extracted another card to decide which reading of T2 to compare with the second reading of T1, and so on. The intertester bias was −0.1%, *CI* (−0.34%, 0.14%), depicted on the right by the error bar that cuts the solid horizontal line; *LLA* was −1.35%, *CI* (−1.77%, −0.93%), whereas *ULA* was 1.15%, *CI* (0.73%, 1.57%).

The intratester analysis compares pairs of %*BF* readings by T1 (Fig. 3b, empty markers) and T2 (Fig. 3b, solid markers). For each tester, we built three random pairs from the 6 values recorded during each set of measurements. We extracted three cards from the pack of cards numbered 1 to 6, three cards from the pack numbered from 7 to 12, and so on. From the first set, for instance, trials 1, 3, and 6 (the extracted numbers) were compared with the remaining trials, 2, 4, and 5, respectively. The intratester bias was −0.04%, *CI* (−0.28%, 0.20%); *LLA* was 1.27%, *CI* (−1.68%, −0.86%), whereas *ULA* was 1.19%, *CI* (0.78%, 1.60%).

The results presented so far were recorded by two testers who had comparable experience in ADP (2 years). Although they worked individually, the question arises whether their readings agree because of their similar expertise, or because the instrument is insensitive to the operator's performance. To address this question, we extended our team of testers with 4 inexperienced members. After about 10 hours of training, the novices joined T1 and T2 in a set of 60 trials. The corresponding results are shown in Fig. 4.

Again, body mass decreased linearly, with a slope of −32.8 g/h, *CI* (−33.2, −32.4) g/h and an intercept of 72.51 kg, *CI* (72.51, 72.52) kg. The coefficient of determination was $R^2 = 0.998$, indicating that the linear fit function accounts for 99.8% of the relationship between body mass and time.

Body volume also displayed a linear decreasing trend, with a slope of −23 mL/h, *CI* (−32.4, −13.5) mL/h and intercept 67.9 L, *CI* (67.8, 68.1) L. Nevertheless, $R^2 = 0.289$ shows that the linear relationship describes only 28.9% of the variation of body volume with time; the remaining variability of the data stems from volume measurement errors.

Body fat percentage did follow a linear trend, too, with a slope of 0.05 %*BF*/h, *CI* (−0.013, 0.12) %*BF*/h and intercept of 13.8 %*BF*, *CI* (13.1, 14.6) %*BF*. Nevertheless, $R^2 = 0.042$ indicates that the linear relationship describes merely 4.2% of the time dependence of %*BF* recorded in successive ADP trials.

Different markers in Fig. 4 are randomly intermixed, suggesting that different testers obtain similar results while using the BOD POD. The mean values of %*BF* readings by testers T1, T2, ..., T6 were 14.44%, 14.39%, 14.38%, 14.45% 14.18%,

and 14.64%, respectively. To assess whether the differences between these are statistically significant, we performed a one-way ANOVA test; it did not reveal significant differences between the mean %*BF* readings of the 6 operators ($P = 0.33$).

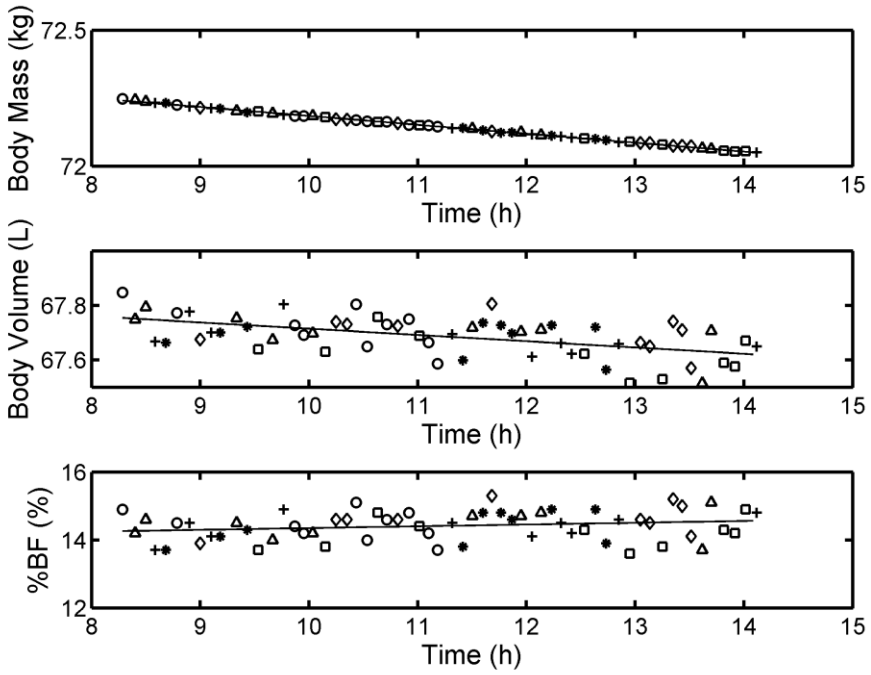

Fig. 4. Body mass (top), body volume (middle) and body fat percentage (bottom) vs. time in a contiguous set of 60 BOD POD trials conducted by 6 randomly assigned testers (T1 to T6). Different markers depict the results recorded by different testers: T1-circles, T2-plus signs, T3-stars, T4-triangles, T5-squares, T6-diamonds. Solid lines show the linear regression of the corresponding data.

Our study design also illustrates the sensitivity of ADP to subject preparation. The P values of the Jarque-Bera tests were larger than 0.05, giving no reasons to reject the null hypothesis that body mass, body volume, %*BF*, *RMR* and *TEE* are normally distributed. The mean %*BF* obtained in the 5 sets of measurements were 13.3% (set 1), 14.3% (set 2), 13.4% (set 3), 14.2% (set 4), and 13.6% (set 5), and one-way ANOVA indicated significant differences between them ($P = 7.63\times10^{-8}$). According to the Bonferroni algorithm, the mean %*BF* of sets 2 and 4 differed significantly from the mean %*BF* of set 1.

We combined the results of sets 1, 3, and 5 into one sample of correct measurements (done with the subject prepared according to the manufacturer's instructions [5]). Table 1 presents the descriptive statistics of this sample.

Table 1

Descriptive statistics of the sample of correctly recorded data, composed of the results of measurement sets 1, 3, and 5. Variables of interest are per cent body fat (%*BF*), body mass (*BM*), body volume (*BV*), fat free mass (*FFM*), resting metabolic rate (*RMR*), and total energy expenditure (*TEE*).

Statistical quantities	**%*BF* (%)**	***BM* (kg)**	***BV* (L)**	***FFM* (kg)**	***RMR* (kcal/day)**	***TEE* (kcal/day)**
Mean value	13.4	71.62	67.06	62.01	1639	2474
Standard deviation	0.431	0.384	0.380	0.395	9.71	14.7
Standard error	0.072	0.064	0.063	0.066	1.62	2.44
Skewness‡	–0.132	0.327	0.348	–0.124	–0.075	–0.047
Kurtosis‡	2.519	1.504	1.590	2.683	2.586	2.622
P value Jarque-Bera‡	0.768	0.062	0.070	0.878	0.852	0.886
CV (%)†,‡	3.213	0.536	0.567	0.637	0.593	0.592

†Abbreviations: CV, coefficient of variation;
‡These quantities are dimensionless.

For a normal distribution, skewness is zero [19], whereas kurtosis is 3. These quantities, as well as the *P* values of the Jarque-Bera test, are consistent with the normal distribution of %*BF*, *BD*, *FM*, *FFM*, *RMR*, and *TEE*. For *BM* and *BV*, however, the assumption of normality is questionable even though the *P* values marginally exceed 0.05. In the light of Figs. 1 and 2, this result is not surprising: unlike %*BF* (Fig. 2, top panel), the mean *BM* and *BV* differ from one set of measurements to another (Fig. 1). The one-way ANOVA test also revealed significant differences between the mean values of *BM* and *BV* in different sets ($P < 0.001$).

DISCUSSION

Data reported in the literature indicate that the performance of the tester might influence the results of BOD POD tests [2, 13]. We tackled this problem by analyzing time series of consecutive ADP trials performed by different testers on the same subject. In the first protocol, different sets of trials were conducted in different physiological states of the subject; therefore, in the Bland-Altman analysis, we selected random pairs of results within each set. In the second protocol, all trials were conducted after overnight fasting, at a steady pace, without breaks, so that physiological evolution of the subject was gradual. Both protocols indicated similar rates of *BM* loss, attributable to pulmonary and cutaneous evaporation [23].

During our measurements, *BM* decreased linearly, explaining the statistically significant differences in *BM* observed between successive trials in a vast study of the BOD POD's reliability [10]. In our work, the drop in *BV* was apparent on the time scale of hours (Fig. 4, middle panel), proving that the interpretation of Noreen and Lemon was correct: consecutive %*BF* estimates did not differ significantly in their study because both *BM* and *BV* decreased from one trial to the other [10].

Figures 1 and 2, as well as the large *P* value of the two-sample *t*-test, prove that the tester does not sway the ADP results. This conclusion is further supported by the similarity of the inter- and intratester Bland-Altman plots (Fig. 3).

We also ruled out the hypothesis that intertester agreement came from the similarity of the working habits of the two testers implied in the first protocol. Upon 10 hours of training distributed along one week, 4 inexperienced testers independently recorded results in good agreement with the experienced testers. Their readings differed by less than 0.46 %*BF*, whereas the technical error of measurement of the BOD POD was reported between 0.8 %*BF* [9] and 1.07 %*BF* [10].

Our findings are at odds with the results of Miyatake *et al*. regarding the reliability and validity of the BOD POD [13]. They found an average CV of 4.53 %*BF* when 3 testers did single trials on 5 subjects. By contrast, when one tester did duplicate trials on 5 subjects the average CV was 2.48 %*BF*, suggesting that employing different testers takes a toll on the reliability of ADP. However, as noticed by Fields *et al.*, the large intertester CV stems from one, presumably anomalous result. Without the one distrustful value, the intertester CV would have been 2.69 %*BF* [2].

Our analysis also evaluates measurement errors stemming from the pre-test preparation of the subject. Consistent results were obtained only when the subject was prepared for the test according to the BOD POD operator's manual [14]. Surprisingly, 0.5 L tea consumed by the subject induced a significant, 1% shift, in the mean %*BF*, similar to the shift caused by having lunch.

Nevertheless, in what concerns subject preparation the present work is merely a case study. For a lean person, whose $BD > 1$ g/mL, water ingestion reduces *BD*, inducing a positive shift in the measured %*BF*; for an obese individual, whose $BD < 1$ g/mL, water consumption is expected to cause an opposite shift in %*BF* assessed by ADP.

Further research is needed to challenge the measurement protocol over a wide range of body compositions and to quantify the effects of deviations from the manufacturer's guidelines [24]. Such studies would be important because subjects are often reluctant when asked to refrain from water intake for 2 hours. Indeed, even well-established laboratories of body composition analysis accept moderate water consumption prior to BOD POD testing [11].

CONCLUSION

This study demonstrates that the tester has no significant impact on the results of body composition assessments using the BOD POD. Besides being statistically insignificant, the differences between the mean values of body fat percentage recorded by different testers were at least twice smaller than the technical error of measurement of the BOD POD. Hence, even longitudinal studies,

that seek to detect slight changes in body composition, can be conducted by employing several testers.

Our work also illustrates the importance of subject preparation for a BOD POD test. Focused on the impact of drinking, eating, and bathroom visits, our analysis pleads for strictly respecting the manufacturer's guidelines.

Acknowledgements. This research has received funding from the "Victor Babeș" University of Medicine and Pharmacy, Timișoara, grant PII-C4-TC-2016, awarded to HGH.

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