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ORIGINAL ARTICLE

Measurement of longitudinal changes in body composition during weight loss and maintenance in overweight and obese subjects using air-displacement plethysmography in comparison with the deuterium dilution technique

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Background: Air-displacement plethysmography (ADP) may be a valid and practical technique to assess body composition in a clinical setting.
Objective. This study aimed to assess longitudinal changes in body composition using ADP and to compare it with the deuterium dilution technique.
Design: The study was a 6-months dietary intervention, consisting of four phases. The first month, subjects were fed in energy balance (phase I). This was followed by 1 month with an energy intake of 33% of energy requirements (phase II), followed by 2 months at 67% of energy requirements (phase III) and 2 months of ad libitum intake (phase IV). Body composition was assessed using ADP (Bod Pod) and deuterium dilution at baseline and at the end of each phase. The baseline analysis included 111 subjects (88 female). Sixty-one subjects (50 female) completed all measurements and were included in the longitudinal analysis.
Results: At baseline, the fat mass (FM) as assessed with the Bod Pod was on average 2.3 ± 4.2 kg (mean ± 2 s.d.) higher than that assessed with deuterium dilution. The difference in FM between techniques increased significantly with increasing FM ($R^2 = 0.23$; $P < 0.001$). Both techniques showed significant changes in FM over time $P < 0.001$). On average, FM as assessed with the Bod Pod was 2.0 kg higher than with deuterium dilution ($P < 0.001$). During phase II, there was a significant interaction between time and method, meaning that the Bod Pod showed a larger decrease in FM than deuterium dilution.
Conclusions: The Bod Pod was able to detect all changes in the body composition, but consistently measured a higher FM than deuterium dilution.
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Keywords: Bod Pod; weight loss; body composition; deuterium dilution; validity

Introduction

Measurement of body composition is important for the assessment of nutritional status and disease risk. Air-displacement plethysmography (ADP) has gained increasing popularity as an alternative for underwater weighing (UWW) to assess total body volume, from which total body density and the percentage of fat mass (%FM) can be calculated. The advantage of ADP is its ease of use and short assessment duration with a lower burden for the subjects as compared with UWW. In addition, UWW has extensively been used for research purposes but its use in a clinical setting is restricted.

In the clinical setting, accurate assessment of body composition remains a challenge. It is often limited to the use of double indirect methodologies such as bio-electrical impedance analysis or skinfold measurements. The major drawback of these techniques is the limited accuracy, especially at the individual level.[1] Dual energy X-ray absorptiometry (DEXA), originally developed to assess bone mineral density, has proven useful to assess body fatness in the clinic. A DEXA scan is fast and the radiation dose is minimal, but estimates of %FM may display marked

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individual differences from those obtained by the criterion four-compartment (4C) model.[2–4] ADP can be used for a wide variety of patients, given the low burden, the rapid acquisition time, good reproducibility[5,6] and non-invasiveness of the assessment. Therefore, ADP may be valuable as a clinical tool to assess body composition.

For a technique to be applicable in a clinical setting, it needs to be able to accurately detect changes over time, for example, to monitor the effect of an intervention. It has previously been shown by our laboratories that there was no difference in %FM as assessed by UWW compared with the deuterium dilution method, both in normal weight and obese subjects.[7] In addition, it was shown that when UWW was compared with deuterium dilution before and after weight loss in obese subjects, both techniques showed the same change in fat-free mass (FFM) over time.[8] Therefore, the aim of this study was to assess the validity of the Bod Pod (Life measurement, Inc., Concord, CA, USA) to detect changes in body composition during a period of weight loss and weight maintenance in overweight and obese subjects, and to compare it with the deuterium dilution technique as the reference.

Methods

Design

The study was a 6-months dietary intervention, consisting of four phases. The first month (phase I), subjects were fed in energy balance. The second month (phase II), subjects received 33% of normal energy requirements and the next 2 months (phase III) 67% of energy requirements. The last 2 months (phase IV), energy intake was ad libitum. Body composition was determined with ADP (Bod Pod) and deuterium dilution at baseline and at the end of each phase.

Subjects

One hundred and forty-eight subjects (115 female) aged 19–65 years, with an average BMI of 31.9 (range 23.7–54.3) were recruited for this study. One hundred eleven subjects (88 female) completed both the Bod Pod assessment and the deuterium dilution at baseline and were included in the cross-sectional analysis. Sixty-one subjects (50 female) completed the body composition assessment with both the Bod Pod and deuterium dilution at all five time-points, and were included in the longitudinal analysis. Subject characteristics at baseline are shown in Table 1.

The study was approved by the medical ethics committee of Maastricht University Medical Centre and registered in the public trial registry CCMO (http://www.ccmo-online.nl).

Anthropometry

Height was measured to the nearest 0.1 cm (Seca-stadiometer, model 220, Hamburg, Germany). Body weight was measured to the nearest 0.1 kg with subjects in bathing suit after an overnight fast using a calibrated scale included in the Bod Pod technology.

Table 1 Subjects' characteristics, data of the sample used for the cross-sectional analysis ($n = 111$)

	Mean ± s.d. (baseline)		
	Male	*Female*	*All*
N	23	88	111
Age (y)	45 ± 11	42 ± 10	43 ± 10
Height (m)	180 ± 8	166 ± 8	169 ± 9
Body mass (kg)	97.6 ± 13.3	88.6 ± 16.6	90.4 ± 16.3
BMI (kg m^{-2})	30.2 ± 3.6	32.1 ± 5.7	31.7 ± 5.4
TBW	50.9 ± 5.8	37.3 ± 5.2	40.1 ± 7.7
Db	1.033 ± 0.014	1.001 ± 0.012	1.008 ± 0.018
%FM$_{deuterium}$	28.2 ± 5.0	41.7 ± 5.1	38.9 ± 7.5
%FM$_{Bod\ Pod}$	29.5 ± 6.4	44.5 ± 5.9	41.4 ± 8.5
Difference %FM$_{Bod\ Pod-deuterium}$	1.3 ± 2.3	2.8 ± 2.2	2.5 ± 2.3

Abbreviations: BMI, body mass index; D_b, body density; TBW, total body water; %FM, percentage fat mass.

Body composition

Total body water was measured using deuterium dilution according to the Maastricht protocol.[9] A background urine sample was collected in the evening before the consumption of ~75 ml of deuterium-enriched (4.25%) water resulting in an enrichment of 50–150 ppm. In the morning, after an overnight fast, the second voiding was collected. To calculate the FFM, a hydration fraction of FFM of 0.73 was used.[10,11]

The Bod Pod was used to measure body volume according to the manufacturer's instructions and described by Dempster *et al*.[12] All subjects wore tightly fitting bathing suits and a swim cap. Subjects had not engaged in exercise at least 1 h before the test. Before subjects entered the Bod Pod, a standard two-point calibration was performed using an empty chamber and a known volume of 50 l. Subsequently, the subject entered the Bod Pod and was asked to sit still. The average of two repeated measures of body volume was used. If the two measurements differed by more than 150 ml, a third measurement was performed. If any two of the three measurements agreed within 150 ml, the mean value of those two was used for calculations, according to the recommended test procedure.[12] Thoracic gas volume was predicted using the equations incorporated in the Bod Pod software. The %FM was calculated using the 2C equation of Siri.[13]

To test the reproducibility of the Bod Pod, three repeated measures (entire test procedure including calibration) were performed for 95 out of 148 subjects at baseline.

Statistics

To test the precision of the Bod Pod, the coefficient of variation was calculated for the three repeated measurements in 95 subjects.

At baseline, ADP was compared with deuterium dilution using linear regression analysis and a Bland–Altman plot. In addition, Lin's concordance correlation was computed. This concordance correlation coefficient evaluates the agreement between two readings (from the same sample) by measuring the variation from the 45° line through the origin (the concordance line).[14] The possible effect of gender on differences between techniques was tested using an independent samples *t*-test and ANCOVA, with the difference between techniques as the dependent variable, the mean of both techniques as the co-variable and gender as a fixed factor.

Longitudinally, the change in fat mass (FM) and %FM over time and the difference between techniques were assessed using factorial repeated measures ANOVA with 'time' and 'method' as the independent variables. The assumption of sphericity was tested using Mauchly's test and, if violated, the degrees of freedom were corrected using Greenhouse–Geisser estimates of sphericity. Statistical significance was set at $\alpha < 0.05$.

Results

Cross-sectional data

The coefficient of variation of three repeated measurements ranged between 0.01 and 0.2% for body volume, and between 0.07 and 2.8% for absolute FM.

At baseline, there was a high correlation between the %FM as assessed with the Bod Pod and the deuterium dilution reference method ($R^2 = 0.93$, $P < 0.001$; Figure 1). Lin's concordance correlation was 0.91. A Bland–Altman plot showed that on average the %FM as assessed with the Bod Pod was 2.5 ± 4.6% (mean ± 2 s.d.) higher than with deuterium dilution. Linear regression showed that the difference in %FM between techniques significantly increased with increasing %FM ($R^2 = 0.20$, $P < 0.001$; Figure 2a). Figure 2b shows the Bland–Altman plot for absolute FM (kg). The mean difference was 2.3 ± 4.2 (mean ± 2 s.d.) and the difference in FM between techniques significantly increased with increasing FM ($R^2 = 0.23$; $P < 0.001$).

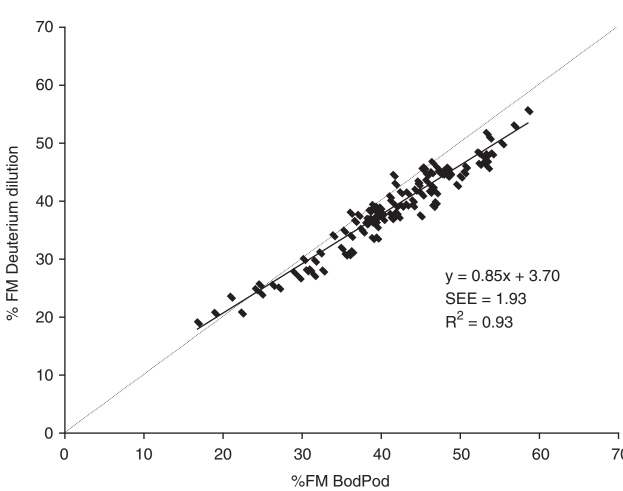

Figure 1 Regression plot showing the relation between the %FM as assessed using the Bod Pod and deuterium dilution method.

A *t*-test showed that the difference between techniques was higher in women than in men (2.8 ± 2.2 versus 1.3 ± 2.3; $P < 0.01$). Further analysis using ANCOVA revealed that after correcting for the mean %FM, gender was no longer significant. Therefore , the gender difference was explained by the higher %FM in women and not related to gender *per se*.

Longitudinal data

Results for all subjects included in the longitudinal analysis ($n = 61$) are shown in Table 2.

There was a significant change in FM over time ($P < 0.001$). Contrasts revealed a significant decrease in FM during phase I, II and III ($P < 0.001$) and a regain of FM during phase IV ($P < 0.05$; Figure 3a). The Bod Pod consistently measured a

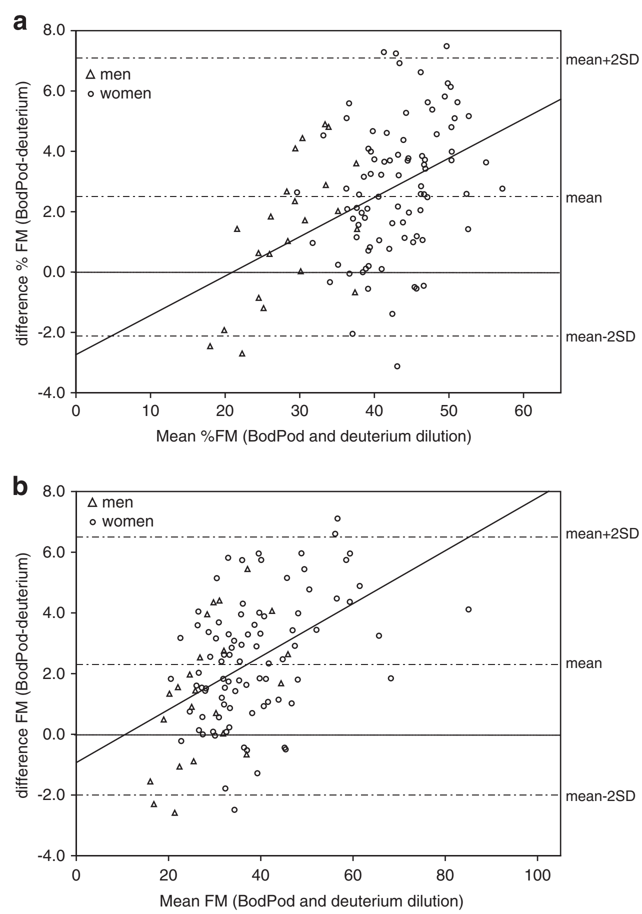

Figure 2 (**a**) Bland–Altman plot showing the difference in %FM between the Bod Pod and deuterium dilution method as assessed at baseline. Mean difference (2.5%) and limits of agreement (±4.6%) are indicated. Although separate symbols are used for men and women, the regression line (full line) is based on all subjects combined ($R^2 = 0.20$). (**b**) Bland–Altman plot for absolute FM (kg). Mean difference (2.3) and limits of agreement (±4.2) and the regression line ($R^2 = 0.23$) are indicated.

Table 2 Subjects' characteristics, data of the sample used for the longitudinal analysis ($n=61$ (11 m per 50f); age = 45 ± 10; height = 1.68 ± 0.07)

	Mean ± s.d. (longitudinal)				
	Baseline	*1 month*	*2 months*	*4 months*	*6 months*
Body mass (kg)	87.3 ± 12.6	85.9 ± 12.5	81.7 ± 12.2	80.2 ± 12.1	80.9 ± 11.9
BMI ($kg\,m^{-2}$)	31.0 ± 4.4	30.5 ± 4.3	29.0 ± 4.2	28.5 ± 4.1	28.7 ± 4.0
TBW	38.6 ± 5.8	38.7 ± ± 5.6	37.6 ± 5.4	37.9 ± 5.6	38.0 ± 5.6
Db	1.007 ± 0.017	1.009 ± 0.017	1.014 ± 0.019	1.016 ± 0.020	1.016 ± 0.020
$FM_{deuterium}$	34.4 ± 9.8	32.9 ± 9.9	30.3 ± 9.6	28.3 ± 9.7	28.8 ± 9.6
$FM_{Bod\ Pod}$	36.7 ± 10.7	35.5 ± 10.6	31.9 ± 10.6	30.2 ± 10.5	30.5 ± 10.4
$\%FM_{deuterium}$	39.1 ± 7.3	37.9 ± 7.6	36.6 ± 7.7	34.8 ± 8.2	35.1 ± 8.2
$\%FM_{Bod\ Pod}$	41.7 ± 8.3	40.9 ± 8.4	38.6 ± 9.0	37.2 ± 9.4	37.3 ± 9.2
Difference $\%FM_{Bod\ Pod-deuterium}$	2.6 ± 2.4	3.0 ± 2.4	2.0 ± 3.1	2.4 ± 3.2	2.1 ± 2.7

Abbreviations: BMI, body mass index; D_b, body density; TBW, total body water; %FM, percentage fat mass.

higher FM than the deuterium dilution method did (mean difference 2.0 kg; $P<0.001$; Figure 3a). There was a significant interaction between time and method during phase II ($P<0.001$; Figure 3a), in which the Bod Pod showed a larger decrease in FM than the deuterium dilution method did. In Figure 3c, the change in FM from baseline is shown.

The %FM significantly changed over time ($P<0.001$). Contrasts revealed a significant decrease in %FM during phase II and III ($P<0.001$; Figure 3b). The %FM as assessed with the Bod Pod was consistently higher than with deuterium dilution (mean difference 2.4%; $P<0.001$; Figure 3b). There was a significant interaction between time and method ($P<0.001$; Figure 3b) during phase II. The Bod Pod showed a larger decrease in %FM than the deuterium dilution method did.

To provide an estimate of the ability of the Bod Pod to detect individual changes in %FM, Table 3 shows the correlations and mean bias and limits of agreement (2 s.d.) for the change in %FM during each of the four phases of the study.

Discussion

In this study population of overweight and obese subjects, the %FM as assessed with the Bod Pod was highly correlated with, but higher than, the %FM as measured with the deuterium dilution technique. The difference between techniques increased with increasing %FM. Longitudinally, the Bod Pod was able to detect changes in %FM over time, but showed a larger change in %FM after energy restriction (phase II) than the deuterium dilution method did.

Many techniques are available to assess body composition, but few of these are applicable for routine clinical practice. For obvious reasons 3 or 4C models, based on UWW, isotope dilution and/or DEXA have limited applicability outside research settings. As a 2C model, UWW was the gold standard for the assessment of body composition, but complete submersion of many patient populations is not feasible for daily practice. Deuterium dilution is accurate and places a very low burden on the subjects, but time, expertise and expensive equipment is needed for analysis of the urine (or saliva or blood) samples. DEXA has become popular as a clinical tool given its fast acquisition time, the low burden for the subjects and limited exposure to radiation. It also has the advantage of providing an estimate of body fat distribution. However, when DEXA is compared with the 4C model, the mean bias in %FM can range from −1.7 to 3.9%[15–18] with limits of agreement as large as 6.7%.[18] The Bod Pod may therefore be a good alternative for DEXA in the clinical setting.

Cross-sectional analysis

This study showed a mean difference in %FM between the Bod Pod and deuterium dilution of 2.5% and limits of agreement of 4.6%. Other studies comparing ADP with deuterium dilution in overweight and obese subjects are scarce. In agreement with this study, Jebb *et al.*[19] also found an overestimation of the %FM by ADP compared with deuterium dilution in a sample of overweight and obese women, although the difference was even higher (4.4%). Given that both UWW and ADP are measures of body volume, many studies have compared ADP with UWW. Demerath *et al.*[6] summarised the results of these studies, showing mean differences varying between −4.0 and +2.0% and individual-level differences in the order of ±6–8%. A point of concern is the increase in bias with increasing %FM. A review by Fields *et al.*[20] also included whether or not trends were observed in the Bland–Altman analyses when the Bod Pod was compared with UWW. Of the 12 studies in adults included in the review, three studies found an upward trend,[21–23] that is, overestimation of %FM by the Bod Pod at higher levels of %FM. Some of these studies also included some overweight or obese subjects, but none of them had a large study population with an average %FM as high as this study.[5,24–26] It is interesting to notice that the regression line, indicated in the Bland–Altman plot, crosses the line of zero difference at a %FM of 21%, which is a normal %FM for a healthy population of normal weight men and women. This suggests that for normal weight subjects there may have been no difference in %FM between techniques.

Fields *et al.*[20] suggested to test for a systematic effect of gender, independent of %FM, on differences between ADP

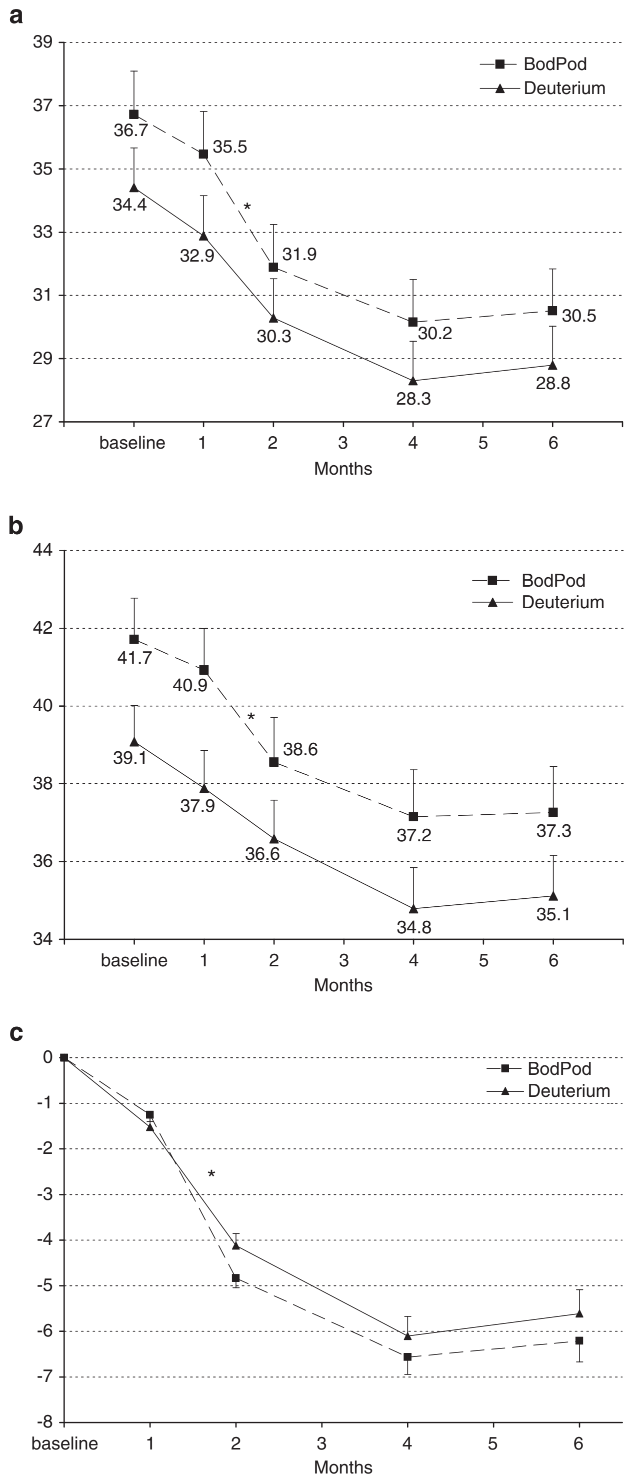

Figure 3 Change in FM (**a**) and %FM (**b**) over time as measured with the Bod Pod and deuterium dilution method. There was a significant difference between the Bod Pod and deuterium dilution method at all time points. Error bars represent standard error (s.e.). (**c**) Shows the change in FM from baseline; the error bars indicate the standard error (s.e.). *During phase II, the change in FM (**a**) and %FM (**b**) was different between the Bod Pod and deuterium dilution (time*method interaction, $P<0.001$).

Table 3 Correlations and mean bias ± 2 s.d. for changes in %FM during the four phases between the Bod Pod and deuterium dilution (mean bias is Δ Bod Pod—Δ deuterium)

	R	*Bias ± 2 s.d.*
Change %FM phase I	0.11	0.4 ± 4.0
Change %FM phase II	0.26*	−1.1 ± 4.1
Change %FM phase III	0.46**	0.4 ± 5.2
Change %FM phase IV	0.20	−0.2 ± 4.5

*$P<0.05$; **$P<0.01$.

and other techniques. In the current population of overweight and obese subjects, the range in %FM was sufficiently large in men as well as in women. The analysis showed that the gender difference disappeared when %FM was included as a covariable, suggesting that the gender difference was explained by the higher %FM in women and not related to gender *per se*.

Longitudinal analysis

For a technique to be applicable in a clinical setting, it needs to accurately detect changes over time. Our results show that the Bod Pod systematically showed a higher %FM but on average detected the same changes in the %FM as deuterium dilution, except during the very low energy diet (Phase II). When subjects received 33% of their energy requirements, %FM dropped with 1.3% according to the deuterium dilution method and 2.3% according to the Bod Pod (Figure 3). This observation may be partly due to the higher overestimation in %FM by the Bod Pod at higher fat percentages (Figure 2). Although statistically significant, this difference is relatively small and on average, the changes in %FM over time were quite similar for both techniques. However, as shown in Table 3, limits of agreement (Δ Bod Pod—Δ deuterium) range between 4 and 5%, and the correlations between the deltas of both techniques are low. Jebb *et al.*[19] assessed changes in %FM during weight loss and regain in overweight women using ADP and deuterium dilution. When compared with the 3C model (based on body density from ADP and total body water from deuterium dilution), the bias in the delta %FM between ADP and the 3C model during weight loss was -0.87 ± 3.03 (mean ± 2 s.d.). These limits of agreement are slightly smaller than in the current study, which is not surprising as the comparison was made against the 3C model, which included density from ADP.

An important aspect in assessing changes over time is the precision of a technique. The Bod Pod did show excellent precision as the coefficient of variation in body volume of three repeated measures in a sub-sample of 95 subjects ranged between 0.01 and 0.2%. Good precision of the Bod Pod is in agreement with previous data.[5]

We chose to compare the Bod Pod with deuterium dilution and not against the 3C model calculated from body density and total body water. In that case, body density would be included in both the 2C (ADP) and the 3C model, resulting in smaller biases, narrower limits of agreement and stronger correlations. Obviously, the deuterium dilution technique is also not free of potential error, either technical or related to the hydration fraction of FFM. Previous results from our lab have shown that there was no difference in %FM as assessed by deuterium dilution and UWW, both in normal weight and obese subjects.[7] Both techniques also showed the same changes in FFM over time.[8] With regard to the sampling protocol, an overnight equilibration time has been shown to be preferable over a 4–6 h equilibration as the latter protocol was insufficient to reach complete isotope equilibration.[27,28] Although some of the deuterium may already have washed out during equilibration, this does not outweigh the advantage of having subjects at rest and fasted. The overnight protocol has been proven to produce the most consistent results.[27,28] It is well-known that the hydration of FFM may change acutely during weight loss, for example due to changes in glycogen stores. It is not expected that these changes would persist over a longer period of time (weeks) when on a balanced diet. Evans *et al*.[29] showed that there was no change in the density of the FFM and water, mineral and protein fractions of the FFM after a diet or diet plus exercise intervention.[29] The hydration fraction of FFM is higher in children and may change with disease, but is otherwise remarkably stable.[30] Nevertheless, it remains a potential source of error, as a change in the hydration fraction would cause error in the calculation of %FM from ADP as well as deuterium dilution.

Regarding precision of deuterium dilution, the coefficient of variation to assess total body water with deuterium dilution using isotope ratio mass spectrometry is 1%.[31,32]

Errors could be related to the underlying assumptions, as already discussed, or technical errors. Regarding the latter, it has previously been published that when a range of known volumes is measured in the Bod Pod (plastic containers ranging from 10 to 150 l), both accuracy and precision are significantly lower with volumes below 40 l and higher for higher volumes.[33] The same study also showed that precision was independent of the subject's fat percentage (range in %FM was 5–50%). Therefore, a higher BMI (higher volume) should increase accuracy and precision.

In this study, lung volume was predicted. It has previously been shown that there was no difference between predicted and measured lung volume.[33,34] However on an individual basis, %FM can deviate by 3% between the predicted and the measured lung volume, but systematic under or overprediction of lung volume, or even gender differences do not explain this discrepancy.[33] As the prediction of lung volume is based on height, age and gender and not on body mass, it is unlikely that this would cause the systematic difference observed in this study. Nevertheless, no difference between measured and predicted lung volume in the Bod Pod does not necessarily mean that the predicted value represents the true value. An overprediction in the lung volume would result in an overprediction of the %FM.

Body volume measured by the Bod Pod also needs to be corrected for the surface area artefact, calculated from the body surface area. However, it has already been shown that any error in the predicted body surface area has very little impact on the %FM (0.1%).[33,35]

In clinical practice, the currently accepted standard to assess body composition is DEXA. This study shows that the individual error (2 s.d.) in assessing changes in %FM with the Bod Pod is 4–5% when compared with the deuterium dilution method as the reference. To assess changes in %FM with weight loss, these limits of agreement are about the same (mean difference ± 2 s.d., 1.3 ± 4.2%;[10]) or lower (mean difference ± 2 s.d., 0.6 ± 8.9%;[36]) than those reported for DEXA. Mahon *et al*.[36] also assessed changes in %FM during weight loss with ADP and showed a smaller mean difference (0.4%), but larger limits of agreement (−10.1 to 10.9%) than the current study. In those studies, the 4C-model was used as the reference technique.

As a 3C or 4C model is not feasible in many clinical or research settings, the second best option is to use a 2C-model. DEXA has been proven useful in clinical studies, but intra- and inter-instrument variability, even from the same manufacturer, remains a problem.[37] This study shows that ADP may be a good alternative for DEXA to assess body composition in a clinical setting. However, care should be taken when assessing obese subjects with the Bod Pod, given the observed overestimation in %FM at higher levels of obesity. This warrants future research.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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