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THE DISCREPANCY OF FAT-FREE MASS MEASURED BY DUAL ENERGY X-RAY ABSORPTIOMETRY AND AIR-DISPLACEMENT PLETHYSMOGRAPHY VARIES WITH AGE AND ADIPOSITY – IS IT RELATED TO FAT INFILTRATION?

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Abstract: *Background:* The discrepancy of fat-free mass (FFM) measured by dual energy x-ray absorptiometry (DXA) and air-displacement plethysmography (ADP) could be attributed to the erroneous inclusion of inter-muscular adipose tissue (IMAT) which increases with age and adiposity, as part of FFM measured by DXA. We, therefore, attempted to examine whether this disagreement was related to age and adiposity. *Methods:* One hundred and seventy two participants aged 20 to 76 years, were examined by ADP and DXA to measure their body density (BD) and FFM respectively. FFM was also derived from BD using the 2-compartment model where: (FM+FFM)/BD = FM/FM density + FFM/FFM density. The FFM and FM density was assumed to be 1.1000 and 0.9007 respectively. The difference between DXA-measured and ADP-derived FFM was calculated and the association between this difference with age, gender, body mass index (BMI), Waist-hip ratio (WHR) was examined by multiple linear regression. *Results:* The DXA-measured FFM was 2.2 kg (2.3 %FFM) higher than the ADP-derived FFM. In multivariate analysis, higher BMI, higher WHR and older age was significantly associated with greater difference between DXA-measured and ADP-derived FFM. *Conclusion:* DXA-measured FFM was higher than that derived by the 2-compartment model using ADP BD measurement. This variation was significantly associated with older age, general and central adiposity. Comparison of DXA-measured muscle mass across a wide range of age and adiposity should take this into consideration. Fat infiltration into skeletal muscles in older and more obese adults may contribute to the unexplained discrepancy between the two methods.

Key words: Dual energy x-ray absorptiometry, air-displacement plethysmography, Bod Pod, body density, inter-muscular adipose tissue, elderly.

Introduction

Many studies have attempted to validate ADP against one of the reference methods, DXA, with respect to their agreement in the quantification of fat mass (FM) (1-9). These two methods, however do not agree often (3-9). Most investigators concluded that ADP was either valid in comparison to DXA (1, 2) or it under-estimated fat mass (3-6); while two other studies found the disagreement in the opposite direction (8, 9). Koda et al examined a sample of 721 participants and revealed a mixed result: ADP overestimated FM in men but underestimated that in women (7). In two validation studies against 4-comparment model, ADP, on the contrary, overestimated FM (9, 10). Therefore whether ADP is a valid measurement of FM, and if not, the direction of disagreement of ADP against the reference method are still uncertain.

Many sources of bias have been proposed to explain the disagreement between the two methods, which include invalidity of the assumption that fat-free mass (FFM) density is a constant and technical inconsistency in ADP (11). The variation in total body water (9) and bone mineral content of FFM (7) have been proposed to explain the overestimation of FM by ADP. However, this proposal could not account for the opposite findings of underestimation in other studies (3-6). In all, the major portion of this disagreement remains unexplained (11).

To our knowledge, most if not all studies compared ADP to DXA in terms of FM quantification. Age-associated muscle loss, or FFM loss, however, is increasingly being recognized as a prevalent phenomenon in old age (12-15) and is associated with

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adverse health outcome and physical limitation (16-21). Therefore it is timely to conduct comparison between various methods with special attention to FFM measurement. Furthermore, adipose tissue deposition in muscles can occur in both inter- and intramuscular space, termed as inter-muscular adipose tissue (IMAT), which has been observed to be related to poorer strength and limited physical function (22-26). Though regarded as one of the reference methods, DXA is subject to bias too (27-29). Viewing from another perspective, it may be equally plausible that DXA bias can partially contribute to the discrepancy. The erroneous inclusion of IMAT as FFM by DXA may alternatively explain the discrepancy between the two methods, which has not been addressed in the past.

We hypothesized that the discrepancy in FFM measurement by DXA and ADP could be attributed to this erroneous inclusion of IMAT by DXA. If this hypothesis is true, then the disagreement will vary with age and adiposity because IMAT is associated with old age and obesity (22-26). We therefore attempted to examine the discrepancy between DXA-measured and ADP-derived FFM and test whether it varied with age, general adiposity as representated by body mass index and central adiposity as represented by waist hip ratio.

Methods

One hundred and seventy two participants aged 20 to 76 years were recruited by displaying posters at the Faculty of Medicine notice boards located at the School of Public Health, the Clinical Science Building in Prince of Wales Hospital, and the Department of Sports Science and Physical Education, The Chinese University of Hong Kong. They were university students, hospital or university staff. The older persons were recruited through the younger subjects, university students, hospital or university staff. The study was approved by The Chinese University of Hong Kong Clinical Research Ethics Committee, and all participants gave informed consent.

The FFM measurement by DXA and the BD measurement by ADP for each participant were conducted on the same day. They were advised to continue with their usual daily diet but alcohol, coffee, tea and moderate to heavy exercise were not allowed 24 hours prior to the examination to maintain normal hydration status.

Air displacement plethysmography

The Bod Pod system (Life Measurement Instruments, Concord, CA, USA) measures air pressure fluctuation inside the chamber before and after the participant sits inside (30, 31). By applying Poisson's Law, which describes pressure-volume relationship under various temperature conditions, the chamber air volume with the participant inside can be calculated with the observed chamber pressure fluctuations. Body volume was calculated by subtracting the volume of air inside the chamber with the subject inside from the volume of air in the chamber when it was unoccupied.

A two-step calibration was undertaken according to the manufacturer manual before each measurement. The volume of the empty chamber and that of a 50-L-calibration cylinder was measured in the first and second step respectively. The participants were dressed in swimming suit and hat to minimize the isothermal air volume, and they were instructed to sit inside the Bod Pod chamber motionlessly for one minute. Two repeated volume measurements were performed and the mean was taken for analysis. The participant's lung volume was estimated from age and height as part of the Bod Pod procedure, using predictive equations of the manufacturer (32). Body weight was measured by the Bod Pod electronic scale. Their body density was calculated, dividing the body weight by the body volume.

FFM was then derived from the 2-compartment model where:

(FM + FFM) / BD = FM / FM density + FFM / FFM density

The FFM and FM density was assumed to be 1.1000 and 0.9007 respectively. [33]

Dual Energy X-ray Absorptiometry

FFM was measured by DXA using a Hologic QDR4500A densitometer (Hologic, Bedford, MA., USA) according to the protocol, described by Heymsfield et al. (34). The maximum coefficient of variation for FFM is 0.84%. During the course of the study, the DXA system was regularly matched to quality assurance scans to ensure there was no drift.

Body Mass Index (BMI)

Stature and body weight were measured to calculate BMI (body weight in kilograms divided by the square of stature in meters). The participants were asked to stand upright without shoes and look straight ahead, and their standing heights were measured by the Holtain Harpenden stadiometer (Holtain Ltd, Crosswell, UK). Body weight was measured, with the participants wearing a light gown, by the Physician Balance Beam Scale (Health-O-Meter, Inc., Alsip, IL, USA).

Statistical Method

The DXA-measured and BD-derived FFM were compared by paired t-test. The association of this discrepancy with age, gender, and adiposity measurements, namely body mass index (BMI), and waist-hip ratio (WHR) was tested by univariate analysis.

The effect of BMI on this difference was further examined by multiple linear regressions with adjustment for age, sex and predicted lung volume. The effect WHR was tested similarly in a separate multivariate model. All tests were two-sided, and p values less than .05 were considered statistically significant. Statistical analysis was conducted using SPSS version 15.0.

Results

The mean age (SD) of all participants was 43.6 (12.9) years and seventy six (44.1%) of them were male. (Table1). The DXA-measured FFM was 2.2 kg (2.3 %FFM; %FFM = FFM / body weight x 100%) higher than the ADP-derived FFM. (Table 2, paired t-test, p<0.001) This disagreement varied significantly with age, gender, BMI and WHR on univariate analysis. (Table 3) With one standard deviation increase in age, the disagreement widened by 0.97 kg. (Table 3) In men, the disagreement was 1.2 kg (1.2 %FFM) more than that in women. (Table 3 and Table 2; 2.9 kg in men vs. 1.7 kg in women; 3.0 %FFM in men vs. 1.8 %FFM in women) The adiposity measurements, namely BMI and WHR, were associated positively with the difference between DXA-measured and ADP-derived FFM before and after adjustment for age, gender and lung volume (Tables 3 and 4). With increasing age, the discrepancy widened in both univariate (Table 3) and multivariate analysis after adjustment for gender, lung volume and BMI. ($p < 0.001$)

Table 1
Baseline characteristics of the participants

	Men Mean (SD) N = 76	Women Mean (SD) N = 96	Overall Mean (SD) N = 172
Age (year)	44.8 (13.8)	42.7 (12.1)	43.6 (12.9)
Body Weight (kg)	68.8 (12.4)	54.3 (9.2)	60.7 (12.9)
Body Mass Index	24.3 (3.7)	22.4 (3.4)	23.2 (3.7)
Waist Hip Ratio	0.89 (0.06)	0.80 (0.06)	0.84 (0.07)
Predicted Lung Volume (litre)	3.6 (0.27)	2.9 (0.27)	3.2 (0.44)
Body Density	1.04 (0.018)	1.02 (0.015)	1.03 (0.18)

SD = standard deviation

Table 2
FFM and FFM difference between DXA and ADP method

	Men Mean (SD) N = 76	Women Mean (SD) N = 96	Overall Mean (SD) N = 172
DXA-measured FFM (kg)	54.2 (7.5)	37.9 (4.9)	45.1 (10.2)
%FFM (%)	79.1 (5.4)	69.8 (5.3)	73.9 (7.1)
ADP-derived FFM (kg)	51.2 (7.3)	36.2 (4.2)	42.8 (9.4)
%FFM (%)	76.0 (8.2)	67.9 (7.0)	71.5 (8.5)
Difference between DXA-measured and ADP-derived FFM (kg)	2.9 (2.6) *	1.7 (1.9) *	2.2 (2.3) *
%FFM (%)	3.0 (3.6) *	1.8 (3.1) *	2.3 (3.4) *

* $p < 0.001$; SD = standard deviation; %FFM = FFM / body weight x 100%

Table 3
Effect of age, gender and adiposity on the difference between DXA-measured and ADP-derived FFM (Univariate analysis)

	Unit (SD)	Difference (kg) (95% CI)	p-value
Age	12.9 years	0.97 (0.51, 1.19)	< 0.001
Female	-	-1.22 (-1.91, -0.53)	0.001
Body Mass Index	3.7 kg/m^2	1.41 (1.13, 1.69)	< 0.001
Waist Hip Ratio	0.07	1.35 (1.07, 1.64)	<0.001

Values are the change in the difference (kg) between DXA-measured and ADP-derived FFM per one SD increase of the independent variable; SD = standard deviation; CI = confidence interval

Table 4
Effect of adiposity on the difference between DXA-measured and ADP-derived FFM (Multivariate analysis)

	Unit (SD)	Difference (kg) (95% CI)	p-value
Body Mass Index	3.7 kg/m^2	1.27 (1.01, 1.54)	< 0.001
Waist Hip Ratio	0.07	1.38 (0.99, 1.77)	<0.001

Values are the change in the difference (kg) between DXA-measured and ADP-derived FFM per one SD increase of the independent variable; SD = standard deviation; CI = confidence interval. Adjusted for age, sex and predicted lung volume

Discussion

The disagreement between the DXA-measured and ADP-derived FFM widened with both general (BMI) and central (WHR) adiposity. IMAT similarly has been demonstrated to vary linearly with the total body fat mass, (35) and therefore it may be plausible that IMAT may partly account for the discrepancy between the two methods. IMAT increases with age and we have observed that older age was similarly associated independently and positively with the overestimation of FFM by DXA. This, additionally, may strengthen our postulation that the erroneous inclusion of IMAT by DXA would overestimate FFM. Interpretation or comparison of DXA-measured FFM in subjects across a wide range of age and BMI should take into consideration the possibility of overestimation in those who are older and overweight. We have employed multiple linear regressions to generate a formula to quantify how the overestimation varies with age and BMI. The regression equation is:

Overestimation of FFM by DXA measurement = 0.056 x Age (years) + 0.36 x BMI – 8.52; and therefore, for a 70-year-old adult with a BMI of 30, the overestimation amounts to 6.20 kg. If a considerable portion of it is IMAT, the associated metabolic and functional adverse effects could be considerable.

The FFM density has been demonstrated to vary with age, ethnicity and muscularity (36), which however, is assumed to be constant by the Brozek's equation (33). We are, therefore, uncertain whether the observed disagreement may be alternatively accounted for by the invalidity of this assumption. Furthermore, it is plausible that IMAT might have partially contributed to the variation of the FFM density observed by Prior et al. (36). The calculation of BD requires the measurement of lung volume which was derived from age and height by the prediction equations of the Bod Pod manufacturer. This estimation might have introduced another source of disagreement between the two methods if its accuracy varies with age and adiposity. To minimise this bias, the predicted lung volume had been adjusted for in the multivariate analysis but the disagreement persisted. This may suggest that the predicted lung volume is not a major source of error.

The association of age and adiposity with the variation in the disagreement between DXA-measured and ADP-derived FFM is only a surrogate evidence to support the notion that the erroneous inclusion of IMAT contributes to the discrepancy between the two methods. Our hypothesis to explain the discrepancy between the two methods could preferably be examined by direct imaging quantification of IMAT. Future work with imaging methods is warranted to relate the amount of IMAT and the disagreement between these two methods.

Conclusion

DXA-measured FFM was higher than that derived by the 2-compartment model using ADP measurement. This variation was significantly associated with older age, general and central adiposity. Comparison of FFM across a wide range of age and adiposity should take this into consideration. Fat infiltration into skeletal muscles in older and more obese adults may contribute to the unexplained discrepancy between the two methods.

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