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original article

Fat-free mass in overweight and obese older women: analysis of concurrent validity of bioelectrical impedance equations

Massa livre de gordura em idosas com sobrepeso e obesidade: análise da validade concorrente de equações de impedância bioelétrica

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Abstract – Older adults, especially women, show a higher level of functional dependence and disability because of muscle and bone mass loss and of a progressive increase in body fat mass. These changes in body composition components have been observed by different techniques. The aim of this study was to analyze the concurrent validity of bioelectrical impedance equations obtained with the Valhalla device[7] and those proposed by Gray *et al.*[8] for the estimation of fat-free mass (FFM) in Brazilian elderly women. The sample (n=34; 60-71 years old, height of 140-162 cm) was divided into two groups (n=17) according to relative body fat (%BF) obtained by DXA: %BF ≤41 and %BF >41. DXA was used as the gold standard. All correlation coefficients were satisfactory (r>0.79). $FFM_{Valhalla}$ (%BF ≤41: 36.1±3.4 kg; %BF >41: 39.3±3.2 kg) did not differ (p>0.01) from FFM_{DXA} (38.7±3.7 kg) in either group. However, the standard error of the estimate (SEE) was slightly higher (2.114 kg) than the recommended one in the %BF >41 group. FFM_{Gray} differed (p<0.01) from FFM_{DXA} in the two groups, although the SEE was satisfactory (<1.8 kg) in the %BF ≤41 group. The residual scores indicated the absence of agreement between FFM_{Gray} and FFM_{DXA}, reaching 7.08 kg. Only 9% of the subjects had FFM estimated within an acceptable error when the equation of Gray *et al.*[8] was used, while this percentage was 82% when the Valhalla equation was used. The latter equation showed concurrent validity for overweight and obese older women.

Key words: Body composition; Electrical impedance; Older adult; Test validity Validation studies.

Resumo – *Os idosos têm apresentado maior dependência funcional e incapacidade, principalmente as mulheres, em decorrência da redução da massa muscular e óssea e do aumento progressivo da gordura corporal. Estas alterações nos componentes da composição corporal têm sido observadas por meio de diferentes técnicas. Nesse contexto, o objetivo neste estudo foi analisar a validade concorrente das equações de impedância bioelétrica para estimar a massa livre de gordura (MLG), do fabricante Valhalla[7] e de Gray et al.[8], em idosas brasileiras. A amostra (n= 34), com idade de 60-71 anos e estatura de 140-162cm, foi dividida em dois grupos (n= 17) por gordura relativa (G%) via DEXA: G% ≤41%, G% >41%. A DEXA foi usada como padrão ouro. Todos os coeficientes de correlação foram (r >0,79) satisfatórios. Nos dois grupos, a* $MLG_{Valhalla}$ *(G% ≤41: 36,1±3,4kg; G% >41: 39,3±3,2kg) não diferiu (p >0,01) da* MLG_{DEXA} *(38,7±3,7kg), no entanto, o EPE (2,114 kg) foi um pouco acima do recomendado no grupo G% >41. A* MLG_{Gray} *diferiu (p <0,01) da* $MLG_{DEXA,}$ *nos dois grupos, ainda que o EPE tenha sido satisfatório (<1,8 kg) no grupo com G% ≤41. Os escores residuais mostraram a falta de concordância da* MLG_{Gray} *com a* MLG_{DEXA}*, chegando até 7,08kg. Pela equação de Gray et al.[8] ,somente 9% teve a MLG estimada dentro de um erro aceitável, enquanto que, pela equação Valhalla, 82%. Esta equação apresentou validade concorrente para as idosas com sobrepeso e obesidade.*

Palavras-chave: *Composição corporal; Estudos de validação; Idoso; Impedância elétrica; Validade dos testes.*

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INTRODUCTION

Following a worldwide trend, two processes have occurred in Brazil since the 1980s, which have produced important changes in the profile of diseases in the population. One process, called "demographic transition", is characterized by a significant reduction in fertility and birth rates and a progressive increase in life expectancy. As a result, there is a progressive increase in the proportion of older adults compared to the other age groups, a trend that is expected to grow in the coming decades. The other process, characterized by important changes in the morbidity and mortality profile, is called "epidemiological transition", in which the occurrence of infectious diseases is reduced and morbidity and mortality due to chronic noncommunicable diseases are increased[1]. This transition is the result of different factors such as urbanization, access to health services, and significant lifestyle changes.

Within this context, in older adults, especially women, show a higher level of functional dependence and disability[2] because of the loss of muscle and bone mass and of the progressive increase in body fat[5]. These conditions increase the risk of falls, fractures, recurrent hospitalization, and mortality.

The alterations in body composition components have been observed by different techniques. Dual-energy X-ray absorptiometry (DXA) has been used to estimate different body components. Evidence indicates its validity to estimate fat-free mass (FFM) in older adults[6]. In view of its high cost, time spent, radiation, and need for a well-trained technician, DXA is not an option for large-scale application. In contrast, the practicality and low cost of bioelectrical impedance analysis (BIA) facilitate its application to large samples and its use in field studies. Furthermore, equations containing predictors obtained by anthropometric measurements and BIA have been developed to estimate FFM more accurately as a function of certain physical, age, sexual and ethnic characteristics.

Among the existing equations developed for older adults, two recommended in classical studies did not have their concurrent validity tested in Brazilian elderly women, i.e., the equations of Valhalla[7] and Gray *et al.*[8]. Therefore, the objective of the present study was to analyze the concurrent validity of these equations to estimate FFM in Brazilian elderly women.

METHODOLOGICAL PROCEDURES

Sample

The sample consisted of 34 women aged 60 to 71 years who lived in different satellite towns of Brasília, DF, Brazil. All women of the study had migrated from different regions in the northeast and mid-west of Brazil to Brasília and volunteered to participate in the study. The participants were informed about the objective and procedures adopted during data collection. All women read and signed the free informed consent form after all doubts about the procedure involved had been resolved. The study was approved by the Research Ethics Committee of Universidade Católica de Brasília, DF (04/2005).

According to Pedhauzer[9], in cross-validation studies the necessary number of subjects should correspond to 20% or more of the sample size that gave origin to the equation. Hence, the minimum number of subjects in the present study was 13 for the equation of Gray *et al.*[8] and 15 for the Valhalla equation[7].

Data collection

The volunteers were asked to adhere to the following protocol prior to the measurements: fasting for 4 h; no ingestion of coffee of alcoholic beverages 24 h before measurement; no strenuous physical activity; no use of diuretics, and to empty the bladder and bowels before measurement. During data collection, the participant was asked if she had followed the guidelines and if one or more had been ignored, data were collected on another day.

A single person made all measurements on the same day. First, body weight and height were measured, followed by DXA and BIA (Biodynamics 310). The room temperature during collection ranged from 20 to 26°C. Al participants were normally hydrated. Height was measured with a Filizola to the nearest 0.5 cm and body weight was measured with a scale to the nearest 100 g according to the procedures described by Gordon *et al.*[10].

The Hologic QDR-1500, software v.5.67 was used for the measurement of fat-free mass and relative fat by DXA. The algorithms of the software, which are used to obtain the values of body composition components, are unknown. The procedures described in the manual of the device were followed. Whole-body scans were obtained with the volunteer lying still with the elbows and knees extended. To guarantee the quality of the measurements, the device was calibrated weekly and daily. A phantom scan was performed for weekly calibration and quality control testing for daily calibration. The calibrations were carried out according to the procedures described in the manual of the equipment.

Resistance was estimated by BIA (Biodynamics 310) according to the instructions of the manual. This device is equipped with a software that provides the values of the body composition components, but the algorithms used to estimate these components are unknown. Resistance (R), body weight (BW) and height (H) were used in the equation of Gray *et al.*[8] and in another equation reported by Lohman[7]. However, this equation is given in the body of a table entitled “Bioelectrical impedance equations used in Valhalla bioimpedance analyzers”. Certainly, this is an equation of the manufacturer Valhalla and not of Lohman and will from now on be called “Valhalla equation”.

Equation of Gray *et al.*[8]:

$$FFM_{kg} = 0.00151\,(H^2) - 0.0344\,(R_{\Omega}) + 0.14\,(BW_{kg}) - 0.158\,(Age_{years}) + 20.387.$$

Valhalla[7] equation:

$$FFM_{kg} = 0.474\,(H^2_{cm} / R_{\Omega}) + 0.18\,(BW_{kg}) + 7.3.$$

Statistical analysis

First, the Kolmogorov-Smirnov test was applied to test the normality of the data, which showed a normal distribution. Descriptive statistics was used to characterize the sample and the independent *t*-test (p≤0.05) to compare the two groups stratified according to the relative amount of body fat (%BF). The criteria suggested by Lohman[7] were used to determine agreement between the Valhalla[7] and Gray *et al.*[8] equations and DXA for estimating FFM in older women: Pearson's linear correlation coefficient (r) >0.79; paired *t*-test (*t*), where $t_{calculated} < t_{tabulated}$ (p>0.01), and standard error of the estimate (SEE = $s\sqrt{1 - R^2}$) > 1.8 kg for the prediction of FFM. Finally, analysis of residual scores as proposed by Bland and Altman[12] was performed. The data were analyzed using the licensed Statistical Package for the Social Sciences (SPSS)-IBM program, version 22.0.

RESULTS

We decided to divide the sample into two groups, one with %BF ≤41 and one with %BF >41 using the median of the sample itself. Gray *et al.*[8] showed that FFM estimated by BIA is overestimated compared to that obtained by hydrostatic weighing in more obese subjects and that this differences is smaller in subjects with %BF <41.1%. The characteristics of these two groups are shown in Table 1. The groups were similar in age and height (p>0.05) and differed in terms of the other variables (p≤0.05).

Table 1. Descriptive characteristics of older women stratified according to relative body fat.

Variable	%BF ≤41 (n=17)			%BF >41 (n=17)		
	$\bar{x}$	Minimum	Maximum	$\bar{x}$	Minimum	Maximum
Age (years)	64.3 ± 3.3[a]	60	71	64.6 ± 3.3[a]	60	69
Height (cm)	151.1 ± 5.1[a]	141	158	152.8 ± 6.1[a]	140	162
Body weight (kg)	57.7 ± 6.1[a]	48.2	71.5	70.8 ± 8.9[b]	54.3	98.8
BMI (kg/m²)	25.2 ± 2.2[a]	22.8	29.8	30.4 ± 3.7[b]	22.9	38.5
%BF (DXA)	35.6 ± 3.4[a]	30.2	41.0	45.1 ± 3.4[b]	41.1	50.9

%BF: relative body fat; BMI: body mass index; DXA: dual-energy X-ray absorptiometry. Means followed by the same superscript letter do not differ (p>0.05, independent *t*-test)

Table 2 shows the statistical results of the comparison of the BIA equations and DXA. All correlation coefficients were satisfactory (r>0.79)[7]. In the two groups, FFM obtained with the Valhalla equation (%BF ≤41: 36.1 ± 3.4 kg; %BF >41: 39.3 ± 3.2 kg) did not differ (p>0.01) from that obtained by DXA (38.7 ± 3.7 kg). However, the SEE (2.114 kg) was slightly higher than the recommended one[7] in the group with higher %BF. Obviously, the criteria were met[7] when the whole sample was considered (n=34) (SEE = 1.828 kg). FFM obtained with the equation of Gray *et al.*[8] differed significantly (p<0.01) from the FFM obtained by DXA in the two groups, although the SEE was only satisfactory in the group with %BF ≤41.

Table 2. Validation of equations to estimate fat-free mass in older women stratified according to relative body fat.

Variable	%BF ≤41 (n=17)					
	$\bar{x}$	r[a]	R	t	p	SEE
FFM DXA (kg)	37.0 ± 3.3					
FFM Valhalla[7] (kg)	36.1 ± 3.4	0.916	0.84	2.733	0.015	1.360
FFM Gray *et al.*[8] (kg)	32.3 ± 4.1	0.927	0.86	11.881	0.000	1.274
	%BF >41 (n=17)					
FFM DXA (kg)	38.7 ± 3.7					
FFM Valhalla[7] (kg)	39.3 ± 3.2	0.838	0.70	-1.171	0.259	2.114
FFM Gray *et al.*[8] (kg)	35.5 ± 4.0	0.787	0.62	5.238	0.000	2.390

FFM: fat-free mass; %BF: relative body fat; SEE: standard error of the estimate; DXA: dual-energy X-ra y absorptiometry. [a]: p<0.0005; r: Pearson's correlation coefficient; *t*: *t*-test; p: p value (probability).

Residual analysis (Figure 1) supports the results shown in Table 2, demonstrating that only 9% of the subjects had FFM estimated within an acceptable error when the equation of Gray *et al.*[8] was used, while 82% of the sample had their FFM estimated with excellent accuracy when the Valhalla equation was used.

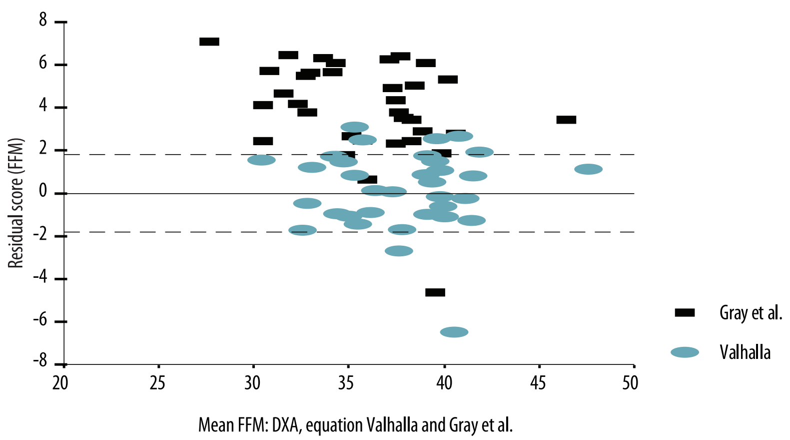

Figure 1. Residual analysis of fat-free mass (FFM) obtained by dual-energy X-ray absorptiometry (DXA) and by the equations of Gray et al.[8] and Valhalla[7]. The upper and lower dotted lines indicate the limit of validation (SEE <1.8 kg).

DISCUSSION

The development of an accurate method that can be applied on a large scale is essential to monitor FFM in older women. In this respect, using the criteria proposed by Lohman[7] as parameters, the present results indicate that the Valhalla equation shows concurrent validity to estimate FFM in both overweight (%BF ≤41) and obese (%BF >41) older women.

The equation of Gray *et al.*[8] has been recommended in classical textbooks[7,13]. However, its concurrent validity could not be demonstrated for the present sample, since a difference (p≤0.01) was observed between mean FFM estimated with this equation and that obtained by DXA. Residual analysis showed wide individual differences between the two procedures.

These findings agree with those reported by Stolarczyk *et al.*[11] who used hydrostatic weighing as the gold standard.

The SEEs obtained with the Valhalla equation are lower than that of the original equation (2.8 kg). The correlations are of high magnitude and the coefficient of determination indicates that 70 and 84% of the FFM estimated in overweight and obese women, respectively, is explained by the BIA equation. This demonstrates the concurrent validity of this equation for women aged 50 to 71 years, with $\%BF_{DXA}$ of 30 to 51%. The standard deviations indicate that the sample is represented as a whole in terms of DXA. Furthermore, residual analysis (Figure 1) indicates that 82% of the women had their FFM estimated with excellent accuracy, with an SEE less than 1.8 kg, demonstrating the excellent concurrent validity of the Valhalla equation. The same does not apply to the equation of Gray *et al.*[8] whose residuals indicate that most (91%) women had their FFM estimated with an SEE of 2 to 7.5 kg, an unacceptable fact.

The stability of the regression coefficient for each predictor variable in the model can contribute substantially to the determination of concurrent validity. The stability of this coefficient from one population to another mainly depends on the number of subjects used for the development of the original prediction equation. Very stable coefficients are obtained when the ratio of subjects for each predictor variable is at least 20:1[9]. A proportion of 25:1 was used for the Valhalla equation in a sample of women aged 50 to 70 years, while Gray *et al.*[8] used a proportion of 15:1. Therefore, the age homogeneity of the original sample and the high proportion of women for each predictor variable greatly favored the concurrent validity of the Valhalla equation for the sample of this study. In contrast, the age heterogeneity (22 to 74 years) of the original sample, wide variation in %BF (19.5 to 59%) and low proportion of subjects for each predictor variable (15:1) contributed to the fact that the equation of Gray *et al.*[8] did not exhibit concurrent validity.

Another factor that could explain the concurrent validity of the Valhalla equation is the use of H^2/R, which is a better predictor than H^2 or R alone. H^2/R has shown a high predictive power for both FFM[14-15] and skeletal muscle mass[16]. BIA is based on the principle that the resistance offered to the passage of an electrical current is related to the square length of the conductor. In this respect, Hoffer *et al.*[14] and Lukaski *et al.*[15] showed that total body water volume is correlated with H^2/R in humans. This was confirmed in the present study in which the Valhalla equation, which uses H^2/R as the regression coefficient, exhibited validity, while the equation of Gray *et al.*[8], which uses H^2 and R as separate predictors, did not. Furthermore, the level of hydration may have also played a role since it tends to decrease with increasing age[17], thus affecting the regression coefficient of resistance, given the characteristics of the original sample and the sample of the present study.

With respect to the effect of the amount of body fat on the estimation errors of FFM using the equations compared to DXA, the errors tended to be higher in women with %BF >41 (SEE = 2.114 kg) compared to those with

%BF ≤41 (SEE = 1.360 kg). These results agree with the study of Glaner[18], in which the concurrent validity of BIA equations were tested compared to DXA using a tetrapolar model, as well as with the results of comparison of bipolar BIA compared to DXA[19].

In general, few of the equations developed in other countries exhibit concurrent validity for Brazilian subjects. In a similar study[18], the only equation showing concurrent validity (compared to DXA) for adult men was a specific equation for individuals with elevated body fat (%BF ≥20%). On the one hand, this demonstrates that models for BIA should include the level of %BF of the subjects for more accurate prediction. This appears to be confirmed in the present study since the sample also had high %BF (30 to 51%). On the other hand, studies indicate that DXA tends to overestimate %BF compared to computed tomography[20], plethysmography[21], four-component model[22,23], hydrostatic weighing[22,24], anthropometry[25], and BIA[26]. These two critical points leave gaps and indicate the need for more conclusive evidence, i.e., whether BIA models for the estimation of FFM should indeed be structured according to groups with relatively homogenous %BF, and whether DXA is only accurate to quantify bone mineral density.

One important limitation of this study and of any other study designed to elucidate the concurrent validity of these equations is the lack of an unquestionable gold standard. DXA has been indicated in the scientific literature as the gold standard for the quantification of %BF and FFM. However, the manufacturers of these devices themselves only recommend DXA for the quantification of bone density[27], a fact increasing the probability of error and reducing the validity of the results. Nonetheless, *in vitro* experiments found no differences (p≥0.05) in the estimates of different body components[28,29], a fact justifying the use of this method in the present study.

CONCLUSION

The Valhalla[7] equation exhibits concurrent validity to estimate FFM in overweight and obese Brazilian women with the same demographic characteristics as those of the present sample. Using this equation, FFM was estimated with an acceptable error (SEE <1.8 kg) in 82% of the sample. The results suggest that the Valhalla is a suitable alternative of relatively low cost to accurately estimate FFM and consequently %BF in Brazilian women. The equation of Gray *et al.*[8] did not exhibit concurrent validity.

REFERENCES

1. Brasil. Ministério da Saúde. A vigilância, o controle e a prevenção das doenças crônicas não-transmissíveis/DCNT no contexto do Sistema Único de Saúde brasileiro. 2005. Available from: <http://bvsms.saude.gov.br/bvs/publicacoes/DCNT.pdf> [2012 Jul 12].
2. Souza CC, Valmorbida LA, Oliveira, JP; Borsatto AC, Lorenzini M, Knorst MR, et al. Mobilidade funcional em idosos institucionalizados e não institucionalizados. Rev Bras Geriatr Gerontol 2013; 16(2):285-93.

3. Yu S, Umapathysivam K, Visvanathan, R. Sarcopenia in older people. Int J Evid Based Health care 2014;12(4):227-43.
4. Sapir-Koren R, Livshits G. Is interaction between age-dependent decline in mechanical stimulation and osteocyte–estrogen receptor levels the culprit for postmenopausal-impaired bone formation? Osteoporos Int 2013;24(6):1771-89.
5. Kuchibhatla MN, Fillenbaum GG, Kraus WE, Cohen HJ, Blazer DG. Trajectory classes of body mass index in a representative elderly community sample. J Gerontol A Biol Sci Med Sci 2013;68(6):699-704.
6. Kim J, Wang Z, Heymsfield SB, Baumgartner RN, Gallagher D. Total body skeletal muscle mass: estimation by a new dual-energy x-ray absorptiometry method. Am J Clin Nutr 2002;76(2):378-83.
7. Lohman TG. Advances in body composition assessment. Current issues in exercise sciences series. Champaign: Human Kinetics; 1992.
8. Gray DS, Bray GA, Gemayel N, Kaplan K. Effect of obesity on bioelectrical impedance. Am J Clin Nutr 1989;50(2):255-60.
9. Pedhauzer EJ. Multiple regression in behavioral research. New York: CBS College Publishing, 1982.
10. Gordon CC, Chumlea WC, Roche AF. Stature, recumbent length, weight. In: Lohman TG, Roche AF, Martorell R, editors. Anthropometric standardizing reference manual. Champaign: Human Kinetics Books; 1988. p. 3-8.
11. Stolarczyk LM, Heyward VH, Van Loan MD, Reano LM. The fatness-specific bioelectrical impedance analysis equations of Segal et al: are they generalizable and practical? Am J Clin Nutr 1997;66(1):8-17.
12. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurements. Lancet 1986;1(8476):307-10.
13. Heyward VH, Stolarczyk LM. Avaliação da composição corporal aplicada. São Paulo: Manole; 2000.
14. Hoffer EC, Meador CK, Simpson DC. Correlation of whole-body impedance with total body water volume. J AppI Physiol 1969;27(4):531-4.
15. Lukaski HC, Johnson PE, Bolonchuk WW, Lykken JGI. Assessment of fat-free mass using bioelectrical impedance measurements of the human body. Am J Clin Nutr 1985;41(4):810-7.
16. Rech CR, Salomons E, Lima LRA, Petroski EL, Glaner MF. Estimativa da massa muscular esquelética em mulheres idosas: validade da impedância bioelétrica. Rev Bras Med Esporte 2010;16(2):95-8.
17. Wang Z, Deurenberg P, Wang W, Pietrobelli A, Baumgartner RN, Heymsfield SB. Hydration of fat-free body mass: new physiological modeling approach. Am J Physiol 1999;276(6):995-1003.
18. Glaner MF. Validação cruzada de equações de impedância bioelétrica em homens. Rev Bras Cineantropom Desempenho Hum 2005;7(1):05-11.
19. Rech CR, Glaner MF. Acuracidade da estimativa de gordura corporal por meio da impedância bioelétrica bipolar em homens. Rev Bras Cineantropom Desempenho Hum 2011;13(2):1-7.
20. Salamone LM, Fuerst T, Visser M, Kern M, Lang T, Dockrell M, et al. Measurement of fat mass using DEXA: a validation study in elderly adults. J Appl Physiol 2000;89(1)345-52.
21. Sardinha LB, Lohman TG, Teixeira PJ, Guedes DP, Scott B. Comparison of air displacement plethysmography with dual-energy X-ray absorptiometry and 3 field methods for estimating body composition in middle-aged men. Am J Clin Nutr 1998;68(4):786-93.
22. Clasey JL, Kanaley JA, Wideman L, Heymsfield SB, Teates CD, Gutgesell ME, et al. Validity of methods of body composition assessment in young and older men and women. J Appl Physiol 1999;86(5):1728–38.
23. Wong WW, Hergenroeder AC, Stuff JE, Butte NF, Smith EO, Ellis KJ. Evaluating body fat in girls and female adolescents: advantages and disadvantages of dual-energy X-ray absorptiometry. Am J Clin Nutr 2002:76(2):384–9.

24. Clark RR, Kuta JM, Sullivan JC. Prediction of percent body fat in adult males using dual energy x-ray absorptiometry, skinfolds, and hydrostatic weighing. Med Sci Sports Exer 1993;25(4)528-35.
25. Glaner MF, Rosário WC. Validação cruzada de técnicas antropométricas para a estimativa da gordura corporal em homens. Lect Educ Fís Deportes (B. Aires) 2005;10(82).
26. Newton Junior RL, Alfonso A, York-Crowe E, Walden H, White AM, Ryan D et al. Comparison of body composition methods in obese African-American women. Obesity 2006;14(3):415-22.
27. Glaner MF. Absortometria de raio X de dupla energia In: Petroski EL, Pires Neto CS, Glaner MF, organizadores. Biométrica. Jundiaí: Fontoura. 2010. p. 229-47.
28. Svendsen OL, Haarbo J, Hassager C, Christiansen C. Accuracy of measurements of body composition by dual energy X-ray absorptiometry in vivo. Am J Clin Nutr 1993;57(5):605-8.
29. Makan S, Bayley HS, Webber CE. Precision and accuracy of total body bone mass and body composition measurements in the rat using X-ray-based dual photon absorptiometry. Can J Physiol Pharmacol 1997;75(1011):1257-61.

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