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Fat-free mass prediction equation for Moroccan women in the 1st trimester of pregnancy using bioelectrical impedance analysis and deuterium oxide dilution

A. Hamrani[1], H. Belghiti[2], K. El Kari[1], A. Barkat[1,3], H. Aguenaou[1] and N. Mokhtar[1]

[1]Unité Mixte de Recherche en Nutrition et Alimentation - URAC39 (Université Ibn Tofaïl, BP 133 Kenitra 14000 - Centre National de l'Energie, des Sciences et des Techniques Nucléaires, BP 1382 RP Rabat 10001), Maroc; [2]Unité de nutrition-Service d'hygiène et médecine de collectivité, Hôpital Militaire d'Instruction Mohammed V, Rabat, Maroc; [3]Equipe de Recherche en santé et nutrition du couple mère- nouveau-né, Faculty of Medicine and Pharmacy, Rabat, Maroc.

Objectives: Determination of body composition by deuterium oxide dilution, development and validation of bioelectrical impedance prediction equation for Moroccan women in the 1st trimester of pregnancy.
Subjects and methods: 26 pregnant women in the 1st trimester aged 19-40 years from urban area were included. Anthropometric parameters were measured, total body water, fat-free mass and fat mass were determined by deuterium oxide dilution, whereas resistance and reactance were measured at 50 kHz by bioelectrical impedance.
Results: The prediction equation is: fat-free mass by ^2H_2O in kg = 0.788 × Height^2 in cm^2/Resistance in Ω + 0.279 × Body weight in kg - 6.824; with R^2 = 0.92 and standard error of the estimate = 2.06 kg. The bias = -1.26 kg, confidence interval was -2.80, +0.26 kg and the pure error was 2.53.
Conclusions: The BIA equation is accurate, precise and valid to be used to assess body composition in Moroccan pregnant women during the 1st trimester.

Keywords: body composition; Moroccan pregnant women; deuterium oxide dilution; bioelectrical impedance equation

Introduction

Maternal body composition during pregnancy may be related to pregnancy outcomes. Lean body mass and total body water (TBW) during pregnancy were related to intrauterine growth in several studies [1, 2], and short maternal stature and low pre-pregnancy weight were reported as to increase the risk of low birth weight, intra-uterine growth retardation [3], and preterm delivery [4]. During a normal pregnancy, it is well-known that there is progressive fluid retention with a subsequent increase in TBW [5] and in plasma volume. The plasma volume correlates with birth weight in both humans and animals [6]. Most studies of pregnancy rely on simple anthropometric assessment of body composition on the basis of one or two skinfold-thickness measurements, usually in combination with body weight [7-8]. Multifrequency bioelectrical impedance analysis (BIA) is a safe and noninvasive method to estimate body composition, it measures the impedance to the flow of an electrical current through the body and uses empirical linear regression models to predict TBW and fat-free mass (FFM) [9]. Many studies validated the use of this method for estimating TBW in humans [10-11]. The validity of BIA equations is uncertain among different ethnicities, especially African populations [12-13] as well as during pregnancy and HIV-infection, which are conditions of altered body composition and water distribution [14]. It is widely accepted that maternal weight gain during pregnancy is necessary for proper gestational development of the fetus [15]. The changes in maternal body composition during pregnancy in healthy women have been reviewed [16].

Assessment of TBW in human pregnancy represents a challenge for any noninvasive conductivity technique. In addition to changes in water volume and distribution, body geometry and hematocrit val-

Address for correspondence: Abdeslam Hamrani, Université Ibn Tofaïl, Faculté des Sciences, Département de Biologie, Laboratoire de Nutrition et Alimentation. BP 133 Kenitra 14000 Maroc.
Tel: 00212668995453 Fax: 00212537711846
Email: salamhamrani@hotmail.com

ues also change [15]. The use of the deuterium oxide dilution is a reference method to assess the validity of BIA measurements. The dose of deuterium given and its subsequent enrichment in body fluids can be used to calculate TBW and subsequently FFM. The difference between the body weight and the FFM yields the fat mass (FM). The method is safe and has been validated widely among different populations including pregnant women [17]. During pregnancy the water content of FFM increases [19]. Therefore the deuterium oxide dilution is not recommended for assessment of body composition in women in the second and third trimesters of pregnancy. The conventional hydration coefficient, 0.732, is generally used in lactating women and women in the 1st trimester of pregnancy [20]. The aims of the present study were to determine body composition by deuterium oxide dilution, to develop and validate a BIA equation assessing FFM in Moroccan women in the 1st trimester of pregnancy.

Methods

Subjects

The study was performed in a group of 26 women in the 1st trimester of pregnancy aged 19 to 40 years who were recruited from different health centers in two Moroccan cities (Temara and the capital Rabat). In the total sample (N=26), 13 women were randomly selected to develop the equation, while the remaining 13 were taken for the cross-validation sample. The gestational age was determined by ultrasound done by the gynecologist. Pregnant women were randomly selected and this study included only those who were free from any pathology or any other condition that could alter body composition before the 14th week of amenorrhea. This inclusion has been confirmed by one or more of the following techniques: questioning, blood glucose levels determination, serology and blood pressure measurements. All selected subjects provided their signed consents. The ethics committee of the ministry of health had approved the study.

Anthropometric measurements

Anthropometric measurements were performed by a trained nurse according to Lohman's recommendations [21]. Body weight was taken using a mechanical flat scale (150 ± 0.1 kg) with round dial (Seca 750, Germany), subjects were barefoot and dressed in minimal clothes. Standing height was measured with a wall mounted stadiometer (Seca,Germany) to the nearest millimeter (200 ± 0.1cm). Body mass index (BMI, kg/m^2) was calculated [22] as body weight (in kg) divided by the squared height (in m^2).

Bioelectrical impedance analysis

After an overnight fast, participants were asked to wear clothing free of metal and to remove their shoes, jewelry, and hair clips. They were also asked to empty their bladder and instructed to lie in the supine position with their hands at their sides and their legs separated. Four self-adhesive electrodes were placed on the dorsum surface of the right hand and foot. All measurements were performed according to Lukaski [28]. Resistance and reactance were measured at 50 kHz with multifrequency BIA (4000 Bodystat Quadscan) according to the manufacturer's instructions. BIA equipment calibration was periodically performed with an electrical resistor.

Deuterium oxide dilution

Body composition (TBW, FFM and FM) of subjects were measured by deuterium oxide dilution. Body water was sampled in the form of saliva and the enrichment of deuterium was measured by FTIR [23]. After an overnight fast, the saliva pre-dose (T0: background) was collected with absorbent cotton put in the mouth. The saturated cotton was removed from mouth and the saliva was extracted using a 20 ml disposable syringe. Participants received an oral dose of 99.8% ^2H_2O (15g for each subject) using a straw. Dose bottles were rinsed two times and the rinsing water was also taken by the participant to ensure that all ^2H_2O was consumed. The dose administration time was recorded. Three post-doses (T_3, T_4 and T_5) were taken at 3, 4 and 5 hours after the administration of ^2H_2O. The final enrichment was determined as an average of the three enrichments corresponding to the three post-doses (T_3, T_4 and T_5). The saliva samples (T_0, T_3, T_4 and T_5) were stored separately in screw-capped tubes and in a cool bag to avoid cross-contaminations and then transported to the laboratory. All samples were frozen (less than -20°C) until analysis. For the FTIR deuterium enrichment measurement, the saliva samples were centrifuged and the supernatant was taken and introduced through the FTIR-cell by a 1 ml disposable syringe.

The body composition was calculated as following [24]:

$$\text{Deuterium space (l)} = \text{Dose amount (g)} \times 10^{-3} / (\text{Enrichment (ppm)} \times 10^{-6})$$

$$\text{TBW (l)} = \text{Deuterium space (l)} / 1.041$$

TBW was used to estimate FFM according to hydration factor provided by Pace and Rathbun [25]. In vivo studies in adults indicate that there is no effect of aging on the constant up to age 70 years [26, 27].

$$\text{FFM (kg)} = \text{TBW (l)} / 0.732$$

$$\text{FM (kg)} = \text{Body weight (kg)} - \text{FFM (kg)}$$

$$\text{FM (\%)} = \text{FM (kg)} \times 100 / \text{Body weight (kg)}$$

Statistical analysis

All analyses were done with the statistical package for the social sciences (SPSS, version 17.0). Data were presented as mean ± standard deviation and ranges. The normality was tested by Kolmogorov-smirnov test. Independent and paired sample t-test were used to test significance. *P*-values < 0.05 were considered significant. The stepwise linear regression method was used to predict FFM in kg as dependent variable and body weight in kg, squared height in cm²/Resistance in Ω as independent variables. Tolerance (T) and the variance inflation factor (VIF) measures of collinearity were used. Bland and Altman analysis was used to test agreement between FFM measured by deuterium oxide dilution and that calculated by BIA equation.

Results

Twenty-six Moroccan women in the 1st trimester of pregnancy were included in this study. In the total sample, descriptive statistics of age, anthropometric and body composition parameters, resistance and reactance measured at 50 kHz by BIA are presented in Table 1. The means comparison of all parameters between prediction and validation sample among Moroccan woman in the 1st trimester of pregnancy were performed by independent sample t-test which showed that there were no differences between these two subsamples (*P*-values ≥0.05) (Table 2).

Regression analysis of the model developed from prediction sample (n=13) in Moroccan women in the 1st trimester of pregnancy are presented in Table 3. The final model is FFM in kg determined by deuterium oxide dilution = 0.788 × Height² in cm²/Resistance measured at 50 kHz + 0.279 × Body weight in kg - 6.824; with R^2 = 0.92 and standard error of the estimate = 2.06 kg.

In addition, the use of paired sample t-test in the validation sample, showed that there was no significant difference between FFM measured by deuterium oxide dilution using FTIR (42.0±5.7 kg) and that estimated with BIA equation (43.2±6.5 kg) with a *P*-value = 0.097. Furthermore, in the total sample, there was no difference between the FFM and FM obtained with the validated BIA equation and those measured by deuterium oxide dilution (Table 4).

According to linear regression curve (Figure 1a), in the validation sample, the intercept (+ 7.065) was not different to zero and the slope (0.807) was not differ-

Table 1. Descriptive statistics of different parameters of Moroccan women in the 1st trimester of pregnancy (N=26).

Parameters	Mean ± standard deviation	Range
Age, years	26.8±5.7	19.0 to 40.0
Body weight, kg	65.2±10.9	48.0 to 100.0
Height, m	1.59±0.05	1.5 to 1.7
Height²/R_{50}, cm²/Ω	39.0±5.3	31.8 to 49.6
BMI, kg/m²	25.7±4.1	17.6 to 36.7
TBW, l	30.3±4.5	22.84 to 38.7
TBW, %	46.8±4.7	38.7 to 58.3
FFM, kg	41.5±6.1	31.3 to 53.0
FFM, %	64.0±6.5	53.0 to 79.9
FM, kg	23.7±7.3	12.8 to 47.0
FM, %	35.9±6.4	20.13 to 47.0
R_{50}, Ω	662.3±87.9	533.9 to 857.0
Xc_{50}, Ω	70.2±21.5	33.0 to 160.6

Results are presented as mean ± standard deviation and range; BMI, body mass index; TBW, total body water; FFM, fat-free mass; FM, fat mass; R_{50}, resistance at 50 kHz; Xc_{50}, reactance at 50 kHz.

Table 2. Means comparison of all parameters between prediction and validation sample of Moroccan woman in the 1st trimester of pregnancy.

Parameters	Prediction sample (n=13)	Validation sample (n=13)	p-values
Age, years	26.8±7.2	26.8±3.9	0.97
Body weight, kg	62.0±9.9	68.4±11.3	0.14
Height, m	1.60±0.05	1.58±0.05	0.28
Height²/R_{50}, cm²/Ω	38.7±5.7	39.3±5.0	0.76
BMI, kg/m²	24.1±3.8	27.2±3.8	0.05
TBW, l	29.9±4.9	30.6±4.2	0.67
TBW, %	48.4±4.9	45.1±4.0	0.07
FFM, kg	40.9±6.7	42.0±5.7	0.67
FFM, %	66.3±6.7	61.8±5.5	0.07
FM, kg	21.1±6.3	26.4±7.4	0.06
FM, %	33.6±6.7	38.2±5.5	0.07
R_{50}, Ω	677.4±92.0	647.1±84.5	0.39
Xc_{50}, Ω	73.8±27.8	66.5±12.8	0.40

Results are presented as mean ± standard deviation; BMI, body mass index; TBW, total body water; FFM, fat-free mass; FM, fat mass; R_{50}, resistance at 50 kHz; Xc_{50}, reactance at 50 kHz; *P*-values < 0.05 were considered significant.

Table 3. Regression analysis of the model developed from prediction sample (n=13) in Moroccan women in the 1st trimester of pregnancy.

Variables included in the model	Regression coefficient (SE)	R^2	SEE	p-value	Collinearity Statistics Tolerance	VIF
Intercept	- 6.824 (4.467)	0.92	2.06	<0.0001		
Height²/R_{50}, cm²/Ω	0.788 (0.125)				0.70	1.43
Body weight, kg	0.279 (0.072)				0.70	1.43

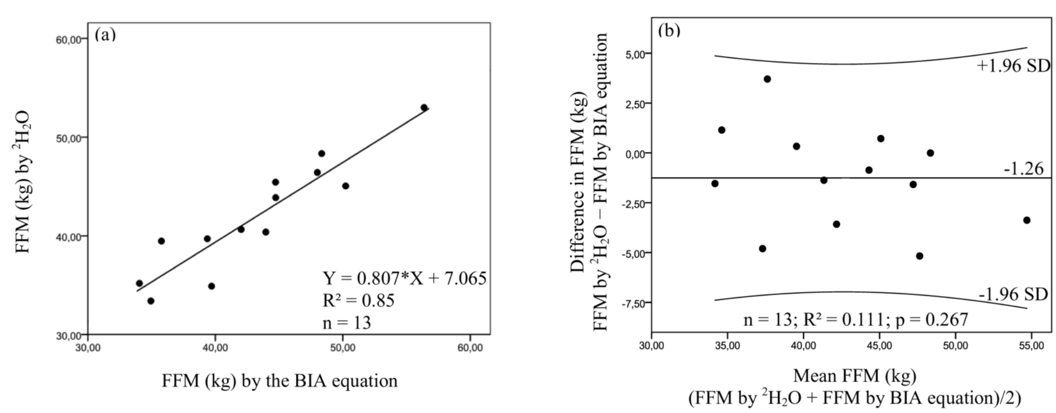
R_{50}, resistance at 50 kHz; SE, standard error; SEE, standard error of the estimate; VIF, variance inflation factor.

Table 4. Body composition by deuterium oxide dilution and by BIA equation.

Parameters	Deuterium oxide dilution (n=13)	BIA equation (n=13)	p-values
FFM, kg	42.0±5.7	43.2±6.5	0.10
FFM, %	61.8±5.5	63.4±4.1	0.15
FM, kg	26.4±7.4	25.1±6.1	0.10
FM, %	38.2±5.5	36.6±4.1	0.15

Results are presented as mean ± standard deviation; FFM, fat-free mass; FM, fat mass; BIA, bioelectrical impedance analysis; *P*-values < 0.05 were considered significant.

Figure 1. A – FFM by deuterium oxide dilution (^2H_2O) plotted against FFM by BIA equation applied in the validation sample (n=13). **B** – Bland and Altman analysis of the differences between FFM by deuterium oxide dilution and FFM predicted by BIA equation against the averages between these two methods.



ent to 1.0. The R^2 was 0.85. Bland and Altman analysis revealed that the bias expressed as the mean of the difference in FFM measured by deuterium oxide dilution using FTIR and that determined by BIA equation was -1.26±2.53 kg. The limits of agreement, defined as mean ± 1.96 SDs, were -6.218, +3.698 kg of FFM (Figure 1b). Moreover, there was no significant association (P = 0.267) between the means difference and the average of the two measurements of FFM obtained by deuterium oxide dilution using FTIR and that determined by the validated BIA equation (Figure 1b).

Discussion

The present study findings are important information on anthropometric parameters and body composition characteristics (TBW, FFM, FM) assessed by deuterium oxide dilution as a reference standard method for Moroccan women in the 1st trimester of pregnancy. This method is one of the most reliable methods to obtain accurate measures of total body fat, including underwater weighing, air displacement plethysmography and dual-energy X-ray absorptiometry (DXA) [30, 31]. Computed tomography and magnetic resonance imaging have been also shown to provide information about body fat distribution [32]. The BIA is rapid, simple, noninvasive, portable, inexpensive, does not require extensive training and has been shown to be fairly accurate in comparison to the most precise methods [33].

Herbert V et al. [34] reported that BIA is a good monitoring method of longitudinal changes in body fluid compartments in pregnant women and a good predictor of normal and abnormal adaptations throughout pregnancy, even in the early stages. However, several researchers have reported that fat distribution discrepancies between ethnic groups could diminish the accuracy of body composition values obtained with BIA [35, 36]. For Moroccan pregnant women in the 1st trimester, there was a need to develop an equation using BIA, deuterium oxide dilution and some anthropometric parameters, to accurately estimate body compostion. In 1996, BIA has also been reported as a useful technique for body composition analysis in healthy individuals [37]. Baumgartner et al. [38] recommended that impedance measurements must be made with the same type of analyzer used in developing the prediction equation to be applied. Regular calibration of impedance instruments is critical for obtaining accurate and reliable measurements. In this study, we used the same calibrated BIA unit for the total sample to avoid inaccurate measurements of body composition. Changes in body composition assessed by BIA differ significantly from the reference method used [39-40]. FFM and FM values obtained with equations developed for specific populations are more accurate and precise [41]. To develop a specific model for our population, the data were split into two subsamples and the means comparison by independent sample t-test showed that there were no significant differences. This non significance proves that the data splitting was adequate. The model developed in the present study is the first specific and validated BIA equation for Moroccan pregnant women in the 1st tirmester. This BIA equation (R^2 = 0.92; SEE = 2.06 kg) showed better statistics parameters compared to those obtained with other equations used for the same population, such as the two anthropometric equations of Anne P et al. [42], one for determining the change in body fat during pregnancy (R^2 = 0.73) and another for determining fat mass at term (R^2=0.89). Kupka R et al. [43] predicted four equations estimating TBW obtained by using deuterium oxide dilution for pregnant women during the 1st trimester, the first model included only $Height^2/R_{50}$ with a R^2=0.653, the second model included only anthropometry (R^2=0,634), the third model includes anthropometry and $Height^2/R_{50}$ with a R^2=0.750 and the full model had a (R^2=0.769). On the other hand, the regression analysis between FFM values estimated with the BIA equation validated in the present study and that measured by deuterium oxide dilution (Figure 1a) showed that the BIA equation developed for Moroccan women in the 1st trimester was accurate and with a good precision. In addition, there was no difference between the mean of body fat percentage calculated by BIA equation and that measured by deuterium oxide dilution (Table 4). The Bland and Altman approach [44] was based on comparing the results of two methods by plotting their difference, obtained in a group of subjects, against their average. The methods that most closely estimated the real value would be likely to have a difference that was close to or equal to zero and to have a small variation. In our study there was no significant bias (-1.26±2.53 kg, p>0.05). This result confirms that our BIA equation is valid to be used to estimate fat-free mass in Moroccan pregnant women in the 1st trimester of pregnancy.

In this study, we had a small sample size mainly due to the recruitment difficulties, for example many women did not accept to drink deuterated water and many women refused to wait for more than three hours for post-doses sampling. However we remain confident of the quality of our findings because deuterium dilution method is accurate and can be performed on a small sample size [20]. In addition, previous studies in the literature have been performed on a similar or smaller sample size [45-48] using deuterium dilution technique for body composition assessment and the results were comparable.

In summary, we developed a specific BIA equation for use in Moroccan women in 1st trimester of pregnancy using deuterium oxide dilution as reference standard method. We then applied the equation among our population to test its validity and reliability.

Conclusion

The BIA equation is accurate, precise and valid to be used to assess body composition and nutritional status in Moroccan women in the 1st trimester of pregnancy.

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Conflict of interest: All authors declare having no conflict of interest.

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