A Comparison of Bioelectrical Impedance Analysis with Deuterium Dilution Technique for Body Fat Assessment in School-age Children

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ABSTRACT

The aimed of this study was to validate the accuracy of single (SF-BIA) and multi-frequency (MF-BIA) bioelectrical impedance analysis against the deuterium dilution technique (D_2O) in the assessment of body fat of children. Two hundred and fifty children, a purposive sampling approach were used to recruit children, aged 7-11 years-old, from 8 primary public schools in Nakhon Pathom province. Body weight and height were measured in all children and nutritional status were classified using BMI-Z- score. Total body fat of children were measured using SF-BIA, MF-BIA and D_2O. Bland and Altman analysis was applied to determine the limit of agreement and bias between methods. SF-BIA and MF-BIA provided the significantly lower percentage total body fat (TBF) compared to TBF from D_2O ($p < 0.05$) in both genders. Both BIA approach also provided the significantly lower values of TBF in normal weight and the overweight children. The SF-BIA explained the estimates of TBF as 53% for boys and 72% for girls against TBF by D_2O with the limit of agreement between -17.41% to 12.51% for boys and between -14.04% to 5.24% for girls. For MF-BIA, the estimates of TBF was 58% for boys and 74% for girls against TBF by D_2O with the limits of agreement were -17.06% to 12.78% for boys and -14.70% to 8.78% for girls. Bioelectrical impedance analysis provided the underestimated TBF compared to D_2O in school-aged children and that greater bias was detected in the overweight children. Thus, nutritional status should be considered when the technique is applied.

Keywords: body fat, deuterium dilution technique, bioelectrical impedance analysis, school-age children, nutritional status

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Introduction

Measurement of body composition in children is importance for as the principle technique could quantity both fat mass and fat free mass that have implication for health risk[1]. Many reference methods are used to estimate body composition at the individual level. These included underwater weighing (UWW) technique, air displacement plethysmography (ADP), dual-energy X-ray absorptiometry (DEXA) and deuterium dilution technique[2-3]. In addition, computed tomography and magnetic resonance imaging (MRI) technique also provide more detail information about body fat distribution[4-5]. However, these methods are limited by requirements for expensive equipment, time consuming and highly trained operators, thus, this may not be applicable for field settings. Bioelectrical impedance analysis (BIA) is considered one non-invasive technique that has been used to estimate body composition in both clinical and community settings[6]. The traditional BIA involves a whole-body measurement of impedance at a single frequency (50-kHz) for the purpose of estimating total body water (TBW) and fat free mass (FFM). Many equations have been published using single frequency to estimate body composition and the accuracy of values depend on various factors such as age, gender, ethnicity and level of body fatness[7-8]. Since the cross-sectional of each body part and both intracellular and extracellular water have the marked effect on impedance, therefore, the segmental BIA.[9] With is method, principally, the low level of 500-800 μA measure total body impedance. At frequency of 50 kHz, BIA really measure of extracellular water (ECW) rather than TBW, but ECW is highly correlated with TBW and fat free mass in healthy individual[10] and multi-frequency BIA devices[11] was subsequently developed to improve the measurement of resistance for body segment. For multi-frequency BIA, the divides are designed to scan a wide range of frequencies (1, 5, 50, 250, 500 to 100 kHz). Theoretically, this approach provides estimates of ECW, ICW and TBW[12]. The use of stable isotope deuterium oxide (D_2O) is another technique for measuring total body water (TBW). The method is based on D_2O concentration in biological fluid which is serve as marker for TBW from which FFM and body fat mass are derived and the results are being considered as "gold standard" for the study[13]. Previous studies have compared the results of body composition derived from BIA with that of isotopic dilution method. Study by Suprasongsin et al. in normal weight children and young adults showed the TBW values derived from heavy water tracer ($H_2{}^{18}O$) were highly correlated with impedance index ($r=0.94$, $p<0.001$)[14]. Bray et al. demonstrated that isotopic dilution and body density models provide estimates within 2% of the estimated provided by the 4-compartment model whereas BIA method did less well in American adolescents[15]. Resende et al. have shown that BIA overestimated FFM and TBW and underestimated fat mass in obese subjects and there was no agreement for FFM, fat mass or TBW provided by between BIA and dilution methods[16].

Since data of body fatness among Thai school-age children are less available. Therefore, the aims of this study was to 1) to compare body fatness of school-aged children using three methods; ie single-frequency bioelectrical impedance analyzer (SF-BIA) multi-frequency bioelectrical impedance analyzer (MF-BIA) and deuterium dilution technique (D_2O) and (2) to determine the agreement between the BIA method and deuterium dilution technique in body fat values among children.

Materials and Methods

Subjects

Two hundred and fifty school-age children, a purposive sampling approach were used to recruit children, aged 7-11 years-old, from 8 primary public schools of 3 districts (Phutthamonthon, Nakorn Chaisri and Sampran) in Nakorn pathom province. This study was a part of the control and prevention of childhood malnutrition in Thailand, TC Regional project supported by IAEA[17]. All subjects were healthy at the time of study and who were pre-puberty and free from any diagnosed medical condition. The study protocol was approved by The Committee on Human Rights Related to Human Experimentation, Mahidol University (COA.No. MU-IRB 2008/067.2608). Written consent forms were obtained from parents and children prior to the beginning of the study.

Methods

Anthropometry

On the day of the study, body weight of each child was measured by trained staff using digital weighing scale (Seca 882, weighing capacity 0-180 kg, Hamburg, Germany) to the nearest 100 g. in shorts and T-shirts without shoes. Height was measured with stadiometer to the nearest 0.1 cm (Holtain Ltd, Crymmych, Dyfed, Britain) without shoes. Body mass index (BMI) was calculated as weight (kg) divided by squared height (m^2). Nutritional status the subjects were classified into three group; normal weight was defined by BMI-Z-scores of > -2SD to 1SD, overweight by BMI-Z-scores of >1SD to 2SD and obese as BMI-Z-scores of >2SD' 2007 WHO growth reference [18].

Body composition assessment

Bioelectrical impedance analysis

The BIA measurement was performed using a single frequency leg-to-leg bioelectrical impedance analyzer (Tanita^R Model BC-532, Japan). The subjects were asked to void their bladder prior to measurement. The information of age, gender and height were entered into the software program. Then the subjects stand over the platform for approximate 1 minute until the results of total body fat appeared on the scale. Then, body fat of subjects were also determined using multi-frequency BIA on that day. For measurement, after the age, height and gender were entered in the software program, subject was asked to stand on the platform of bioelectrical impedance analyzer (In Body 720, Biospace Co, Ltd, Seoul, Korea) for approximate 1- 2 minutes. Measurement was performed using 4 pairs of electrodes with passing current into the analyzer's handles (thumb and palm electrodes) and floor scale (ball of foot and heel electrodes).

Deuterium dilution technique (D_2O)

Total body water (TBW) was assessed by using deuterium dilution technique (D_2O), this technique as described by Colley[19]. Before consuming a dose of isotope, a 5 mL of sample urine was collected to determine basal ^2H concentration in the body. An oral dose of 0.5 g per kg body weight (10% ^2H-labelled water) was given orally to the subject. A second urine sample was collected 5 hours later to allow the complete equilibrium within the body water compartments. All urine samples were kept at -20 °C until analysis. The enrichments of pre-and post dose urine samples were sent to Queensland University of Technology, Australia for analysis using isotope ratio mass spectrometry (IRMS, Hydra Model; PDZ Europa, Crew, Cheshire, UK) and determination of TBW was calculated using a correction factor as ~4 % because of deuterium exchange with the non-aqueous hydration in the body[20]. The TBW was determined using the following equation:

$$\text{TBW (kg)} = \frac{TA}{a} \times \frac{(Ea\text{-}Et)}{Es\text{-}Ep} \times \frac{1}{1.04}$$

Where "T" was the amount of plain water in which the dose was diluted in grams, "A" was the amount of dose taken by the subject, "a" was the amount of dose in grams retained for MS analysis and Ea, Et, Ep and Es were the isotopic enrichment in delta units relative to standard mean ocean water of the diluted dose, the water used, the pre-dose urine and the post-dose urine samples. The constant 1.04 was used to adjust for the non-aqueous exchange of H-atoms in the body. FFM was derived from TBW using Lohman's age-and gender-specific constants for hydration of the FFM for children[21]. The FM and %BF can be calculated based on the two compartment model of body composition from the FFM as follows:

FM (kg) = Body weight – FFM

%BF = (Body weight – FFM)/Body weight × 100

Statistical analysis

All data were analyzed using Statistical Package for Social Science (SPSS; version 20.0 SPSS Inc., Chicago, IL, USA). The data expressed as mean ±SD. Kolmogorov Smirnov test was used to verify the normality of the data and Wilcoxon matched-pair signed-ranks test was applied to determine significant mean difference of body fat between different methods. Pearson correlation coefficients were applied to determine the relationship of BIA results to that of deuterium dilution technique. Linear regression analysis was used to assess agreement and bias between TBF derived from different techniques. Bland and Altman[22] plots was applied to indicate the agreement between results obtained from BIA and isotopic dilution technique. Limit of agreement expressed as 2 SD above and below the bias were determined.

Results

The characteristics of subjects are shown in Table 1. A total 250 children (119 boys and 131 girls) participated in the study. Subjects' age ranged between 7-11 years. The BMI of children ranged between 13.5-28.9 kg/m^2 for boys and 13.5-33.6 kg/m^2 for girls. No significant mean difference in child's age, body weight, height and BMI values between genders. The percentage of total body fat among children by three methods. For both genders, total body fat (TBF) values derived from SF-BIA and MF-BIA were significantly lower than that from D_2O. For girls, SF-BIA also provided the significantly lower mean TBF value ($p<0.05$) when compared to TBF from MF-BIA.

Table 1 Characteristics of Children and Percentage of Total Body Fat among Children Using Deuterium Dilution Technique (D_2O) and Bioelectrical Impedance Analysis (BIA) Techniques.

	Boys	Girls	p
No of subjects (n)	119	131	
Age (yrs.)	9.2±0.88 (7.6-11.0)	9.2±0.89 (7.6-10.8)	0.883
Weight (kg)	33.6±10.4 (17.0-61.0)	34.2±10.2 (19.0-76.0)	0.455
Height (cm)	133.9±7.4 (110.0-150.6)	134.3±7.8 (116.6-151.1)	0.675
BMI (kg/m^2)	18.4±4.3 (13.5-28.9)	18.7±4.0 (13.5-33.6)	0.304
D_2O	22.7±10.4	28.1±8.8	25.5±10.0
SF-BIA	20.2±9.8*	23.7±8.6*	22.0±9.4*
MF-BIA	20.5±11.1*	25.2±10.1*¥	23.0±10.8*

Values were mean ±SD

Values in the parentheses were min-max.

* Significantly different from deuterium dilution technique, at $p<0.05$

¥ Significantly different from SF-BIA, at $p<0.05$

D_2O; deuterium dilution technique

SF-BIA; single frequency bioelectrical impedance analysis

MF-BIA; multi-frequency bioelectrical impedance analysis

When the nutritional status of children was categorized using the international growth reference, Table 2 has shown that both SF-BIA and MF-BIA provided significantly lower TBF ($p<0.05$) compared to that of D_2O in normal weight and overweight in both genders. For obese girls, significantly lower TBF was found in SF-BIA compared to D_2O but the values were not difference between MF-BIA and D_2O.

Table 2 Percentage of Total Body Fat among Children Having Different Nutritional Status Using D_2O, SF-BIA and MF-BIA.

	Boys			Girls		
	Normal	**Overweight**	**Obese**	**Normal**	**Overweight**	**Obese**
No of subjects	73	16	30	77	22	32
D_2O	17.01±6.29	27.95±10.05	33.56±8.49	22.99±6.21	31.58±4.70	38.14±6.02
SF-BIA	13.70±3.76*	22.88±3.35*	34.61±5.16	17.54±3.58*	28.05±3.31*	35.66±4.47*
MF-BIA	12.96±.12*	24.91±4.39	36.55±5.09	18.78±6.80*	29.53±5.72*	37.55±4.61

*Significantly different from D_2O, at $p<0.05$

Normal weight; BMI Z-score -2SD to 1SD

Overweight; BMI Z-score >1SD to 2SD

Obese; BMI Z-score >2SD

D_2O; deuterium dilution technique

SF-BIA; single frequency bioelectrical impedance analysis

MF-BIA; multi-frequency bioelectrical impedance analysis

The regression for TBF values derived from SF-BIA and MF-BIA against TBF by D_2O technique was shown in Table 3. In this study, the SF-BIA explained the estimates of TBF as 53% for boys and 72% for girls against TBF by D_2O and MF-BIA explained the estimates of TBF as 58% for boys and 74% for girls against TBF by D_2O.

Table 3 Regression of Percentage Body Fat by SF-BIA and MF-BIA Against Percentage Body Fat by D_2O.

BIA	R	R^2	Intercept (%)	SEE (%)
SF-BIA				
Boys	0.73	0.53	7.05±1.51*	7.2
Girls	0.85	0.72	7.72±1.20*	4.7
MF-BIA				
Boys	0.76	0.58	8.05±1.46*	6.8
Girls	0.86	0.74	7.54±1.16*	4.5

*Intercept significantly different from zero, or slope significantly different from 1

R^2; coefficient of determination

SEE; standard error of estimation

D_2O; deuterium dilution technique

SF-BIA; single frequency bioelectrical impedance analysis

MF-BIA; multi-frequency bioelectrical impedance analysis

Bland-Altman plot was applied to assess the agreement between BIA and the D_2O methods. For both genders, the difference between SF-BIA and D_2O plotted against the mean of the SF-BIA and D_2O and the difference between MF-BIA and D_2O plotted against the mean of the MF-BIA and D_2O are shown in Figure 1. The limit of agreement for SF-BIA were -17.41% to 12.51% to for boys and -14.04% to 5.24% for girls. The limit of agreement for MF-BIA were -17.06% to 12.78% for boys and -14.70% to 8.78% for girls.

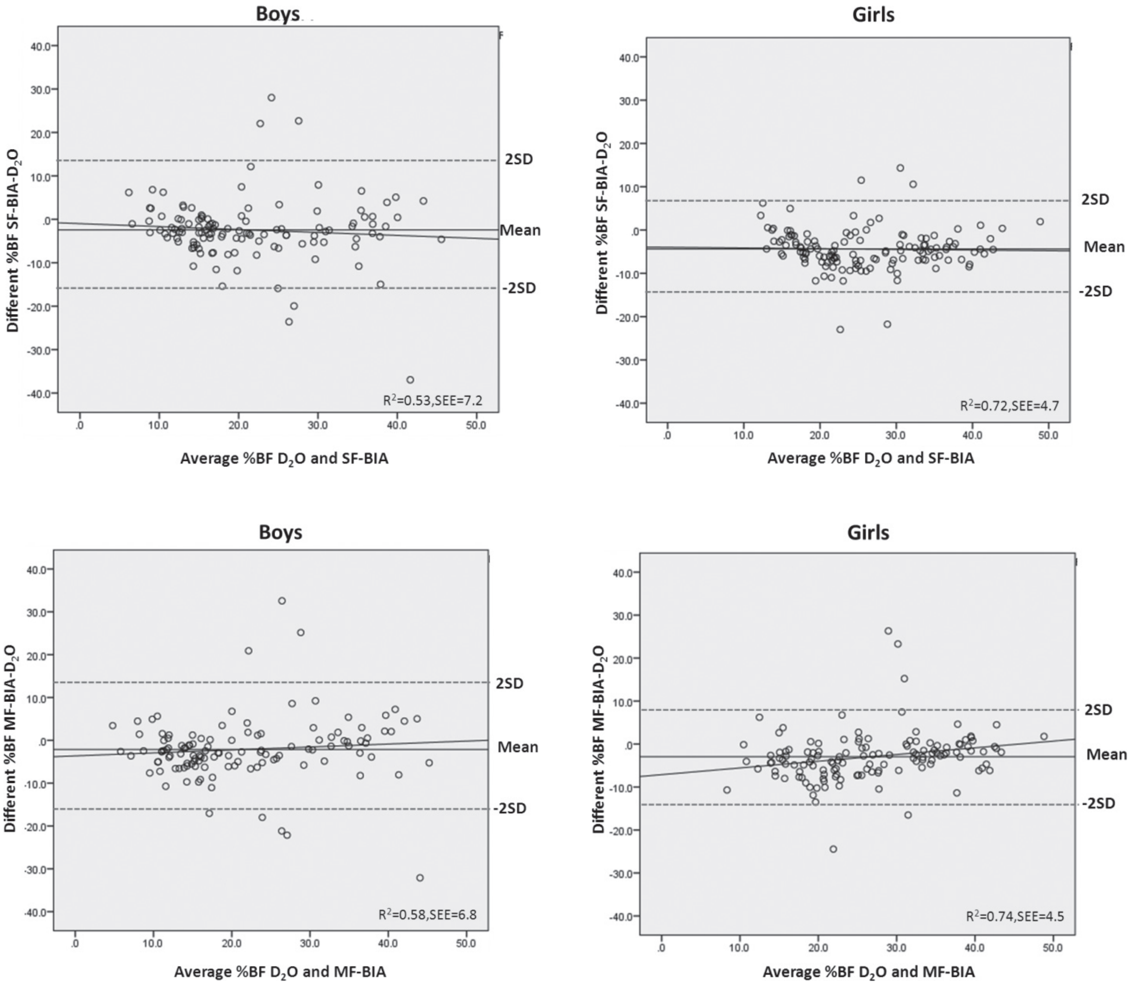

Figure 1 Bland-Altman Plot for the Difference in Percentage Body Fat of Children Measured by SF-BIA, MF-BIA and D_2O.

Discussion

Many reference methods are used to assess body composition. Multi-component model, underwater weighing, isotopic dilution technique and dual-energy X-ray absorptiometry (DXA) are most reliable methods to accurately measure total body fat[2,23]. The deuterium dilution is a reference technique for measuring TBW and that can be used to determine body fat. This technique is considered by several investigators as the “gold standard” for body composition measurement. However, this technique are expensive and time-consuming and require extensive training of technicians. However, since bioelectrical impedance analyzer is considered as an inexpensive, non-invasive device and commonly used in epidemiological survey for estimating TBW, FFM and percentage body fat, therefore, the values derived from this device was validated against the reference method. Our results found that percentage body fat from SF-BIA and MF-BIA were underestimated compared with that from deuterium dilution technique in both gender. This was according to the study by Khan et al. which found that leg-to-leg BIA provided the significantly underestimated TBW in boys and percentage body fat in grils[24]. Regarding the effect of nutritional status, our results also revealed that BIA provided the underestimated percentage body fat in the normal weight and the more pronounced effect was seen among the overweight children. Study by Resende et al. which measured body composition in obese adolescents found that MF-BIA overestimated FFM and TBW and underestimated fat mass although there were the highly positive correlation between BIA and D_2O methods in body fat mass. In addition, results from Bland-Altman plot in Figure 1 shown the deviation obtained by two methods. The relationship between TBF by both BIA and TBF by D_2O were significantly deviated from the line of identity in both genders. Precision of the individual was determined from the R^2 and standard error of estimate (SEE) as shown in Table 3. The TBF by SF-BIA explained 53% and 72% of variance in TBF by D_2O and had SEE of 7.2 and 4.7 for boys and girls, respectively. Whereas, TBF by MF-BIA explained 58% and 74%, SEE of 6.8 and 4.5 for boys and girls, respectively. It was noted that wide variation of TBF obtained from both BIA approach was observed in boys than in girls. These results was contrary to Heitmann's[25] studies found that gender differences occur when estimating body composition. The differences in hormonal secretion have impacts in body composition as well. Besides, changes in hormonal state in females during the menstrual cycle should be taken in the consideration[26].

There was much variations to the traditional and serial BIA model when they were used. SF-BIA almost operate at a current frequency of 50kHZ. At this frequency, the impedance index (L^2/R) is directly related to the volume of TBW[27], therefore, the use of the impedance index to estimate FFM and body fat is based on hydration fraction of 73% in FFM. However, this fraction is not constant among individuals[21]. The SF-BIA devices are limited in their ability to distinguish body water distribution into intra-and extra-cellular water compartments. This is important to describe fluid shift and balance and the level of hydration for some individuals such as the elderly and patient with chronic renal failure [28-29]. With this approach, multi-frequency BIA devices have been developed through the use of impedance to improve the measurement of cellular body water. One meta-analysis from overall 16 studies demonstrated that based on weighted difference, the use of MF-BIA did not overestimate the TBW compared with reference value suggesting MF-BIA could provide the accurate estimation of TBW compartment for healthy and obese adults [30]. BIA devices can have a wide range of error[31] by underestimated percentage of body fat in lean subjects and overestimated in obese subjects[32]. However, when the BIA technique is applied for the determination of body composition, other biological factors should be considered. These included body build among ethnic groups[33], menstruation cycle[34], and type of exercise such as moderate-intensity exercise[35-36], etc. All these factors should be considered when the prediction equation.

Conclusion

Bioelectrical impedance analysis provided the underestimated body fat values in school-age children and that greater bias was detected in the overweight children. Also, various biological factors should be considered when the technique is applied for the determination of body composition in children.

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การเปรียบเทียบวิธีประเมินไขมันในร่างกายโดยใช้ Bioelectrical Impedance Analysis กับวิธี Deuterium Dilution Technique ในเด็กวัยเรียน

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บทคัดย่อ

วัตถุประสงค์ของการศึกษาเพื่อทดสอบความเที่ยงตรงของการประเมินไขมันในร่างกายเด็กด้วย SF-BIA และ MF-BIA กับวิธี Deuterium Dilution Technique (D_2O) เด็กวัยเรียน 250 คน อายุ 7-11 ปี สุ่มแบบเจาะจงจาก 8 โรงเรียนประถมศึกษา จังหวัดนครปฐม ชั่งน้ำหนักและวัดส่วนสูงและจำแนกภาวะโภชนาการโดยใช้ค่าดัชนีมวลกายต่ออายุ ประเมินไขมันด้วยวิธี SF-BIA, MF-BIA และ D_2O วิเคราะห์ผลโดยใช้ Bland and Altman plot เพื่อดูขอบเขตที่ยอมรับได้ ระหว่างวิธี BIA และ D_2O พบว่า ค่าไขมันในร่างกายที่ได้จาก SF-BIA และ MF-BIA มีค่าต่ำกว่าค่าวิธี D_2O อย่างมีนัยสำคัญทางสถิติ ($p<0.05$) ในทั้งสองเพศ ค่าไขมันในร่างกายที่จาก BIA ทั้งสองแบบ ต่ำกว่าวิธี D_2O อย่างมีนัยสำคัญทางสถิติในเด็กน้ำหนักปกติ และน้ำหนักเกิน วิธี SF-BIA อธิบายค่าประมาณการของไขมัน 53% ในเด็กชาย และ 72% ในเด็กหญิง เมื่อเทียบกับวิธี D_2O แสดงค่าขอบเขตที่ยอมรับได้ระหว่าง -17.41% ถึง 12.51% ในเด็กชาย และ -14.04% ถึง 5.24% ในเด็กหญิง สำหรับวิธี MF-BIA ค่าประมาณการของไขมัน 58% ในเด็กชาย และ 74% ในเด็กหญิง ค่าขอบเขตที่ยอมรับได้ระหว่าง -17.06% ถึง 12.78% ในเด็กชาย และ -14.70% ถึง 8.78% ในเด็กหญิง การประเมินไขมันในร่างกายเด็กวัยเรียนด้วยวิธี BIA ได้ค่าไขมันในร่างกายน้อยกว่าวิธี D_2O และมีค่าอคติมากกว่าในเด็กที่น้ำหนักเกิน ดังนั้นการนำวิธี BIA ไปใช้ควรคำนึงถึงภาวะโภชนาการด้วย

คำสำคัญ: ไขมันในร่างกาย, deuterium dilution technique (D_2O), bioelectrical impedance analysis (BIA), เด็กวัยเรียน, ภาวะโภชนาการ

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