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## Original Article

# Validity of using multi-frequency bioelectrical impedance analysis to measure skeletal muscle mass in preschool children

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**Abstract.** [Purposes] Although it is recommended to develop a habit of physical activities, there is no easy way to measure skeletal muscle mass in preschool children, which cause the difficulty of evaluation. The purpose of this study was to examine the validity of body composition including the skeletal muscle mass assessment using multi-frequency bioelectrical impedance analysis by comparing body fat mass obtained by using multi-frequency bioelectrical impedance analysis method and body mass index formulas. [Subjects and Methods] Ninety-four children were surveyed for age, height, weight, grip strength, maximum occlusal force, thickness of muscle and fat mass (masseter and lower limb), body fat mass, skeletal muscle mass, and calf circumference. We assessed additional parameters, which were thought to be related to skeletal muscle mass, to ensure validity. [Results] A strong correlation was found in body fat mass values obtained using the multi-frequency bioelectrical impedance analysis method and those obtained using the body mass index formulas. Additionally, strong correlation coefficients were found between the skeletal muscle mass/height obtained using the multi-frequency bioelectrical impedance analysis method and grip strength and calf circumference. [Conclusion] Our results indicate that skeletal muscle mass can be reliably measured using the multi-frequency bioelectrical impedance analysis method in preschool children.
**Key words:** Bioelectrical impedance analysis, Skeletal muscle mass, Preschool children

## INTRODUCTION

In Japan, a decline in basic motor skills of children has been recognized. The Ministry of Education, Culture, Sports, Science, and Technology[1] recommended that preschool children engage in physical activity for at least 60 minutes daily. It is important to develop the habit of physical activity to improve one's life expectancy right from preschool age[2, 3]. Skeletal muscle mass is a principal factor of body movement, but there are few reports on skeletal muscle mass of preschool children. This is because there is no easy way to measure skeletal muscle mass in preschool children. Previously, skeletal muscle mass could be measured by using magnetic resonance imaging (MRI), computed tomography (CT), dual energy X-ray absorptiometry (DEXA), and multi-frequency bioelectrical impedance analysis (MF-BIA). Owing to their high costs, length of time required for examination, and radiation exposure, MRI, CT, and DEXA are unsuitable for use in children under the age of 6 years. However, MF-BIA is inexpensive, requires a short time, and is non-invasive; it is also a simple evaluation

method of body composition. However, the validity of MF-BIA in children under 6 years old has not been determined yet[4]. Currently, BIA is generally used to calculate the body composition using electrical resistance values obtained at a single frequency (SF-BIA), and it has been validated in children under 6 years old[5, 6]. As SF-BIA measures the impedance at 50 kHz, it cannot penetrate tissue completely; thus, SF-BIA cannot measure the entire muscle volume[7]. Conversely, MF-BIA uses several current frequencies to measure intracellular water and extracellular water, which are measured more accurately. Using the MF-BIA method, it is possible to measure body composition, including the skeletal muscle mass and body fat mass. Although there is no way to compare the skeletal muscle mass obtained using the MF-BIA method, the body fat mass calculated using the MF-BIA method can be compared with that calculated from body mass index (BMI) formulas[8]. The BMI formula[8] reported by Komiya used to calculate the body fat mass and body fat-free mass were derived from the following three methods: 1) the formula reported by Lohman[9], which involves weight and the log-transformed sum of skinfold thickness; 2) formula according to Houtkooper et al.[10], which is based on body density and body resistance derived by SF-BIA; and 3) formula based on total body water, as determined by using deuterium oxide[11] and SF-BIA[6]. Komiya estimated the body fat mass index (FMI, fat mass/height$^2$) as the average of body fat mass values from these three methods, and the BMI formulas[8] were derived from regression analysis of the FMI based on BMI. The BMI formulas[8] are as follows: body fat mass (kg) = (0.408 BMI − 3.937) height$^2$ (m), for boys; body fat mass (kg) = (0.462 BMI − 4.432) height$^2$ (m), for girls. This formula has been used by the Japanese national growth survey of preschool children and others[12, 13] to estimate the body fat mass and body fat-free mass. The primary purpose of the present study was to investigate the validity of body composition including the skeletal muscle mass assessment using MF-BIA by comparing body fat mass obtained by the MF-BIA method with the BMI formulas[8].

There are many studies[14–16] about the relationship between physical fitness and oral function such as the occlusal force in elderly people, and the relationship between size of jaw muscle and limb muscle, however, there are few reports in children. Therefore, we assessed maximum occlusal force and thickness of masseter to the additional variables. Other assessed additional variables were age, height, weight, grip strength, thickness of lower limb muscle, thickness of fat mass (masseter and lower limb), body fat mass, skeletal muscle mass, and calf circumference. We assessed these variables, which were thought to be related to skeletal muscle mass, to ensure the validity of the MF-BIA method.

## SUBJECTS AND METHODS

This study was approved in 2014 by the Ethics Committee of Showa University, School of Dentistry (issue no.: 2014-015). Written consent was obtained from the parents of the children enrolled in the study.

This study had a cross-sectional design. The survey was conducted between January 2015 and February 2015.

Participants were 94 children (41 boys, 53 girls; average age: 5 years ± 0.9 months, range: 3 to 6 years) from three preschools in Kashima City, Ibaraki prefecture, Japan.

The school staff distributed the consent forms and collected the ones that were completed. Parents were invited to speak in person with the researchers if they had any questions. Of 110 people who completed the consent form, we excluded 5 children who were absent on the test day, 5 who were unable to complete the tests because of crying and/or refusal, and 6 who had malocclusion of their molars. None of them had any illnesses or a known medical history of orthopedic dysfunctions that could affect the administered tests.

Data on the subjects' age, gender, height, weight, and medical history were collected on interview sheets from the parents.

The grip strength was measured using a hand-held Jamar dynamometer® (Patterson Medical Co., Nottinghamshire, UK). We followed the manufacturer's instructions to set the position of the dynamometer, and we told the children to squeeze their grip as hard as possible. Two successive trials were conducted to estimate the grip strength, and the maximum value was included in the analysis.

The maximal occlusal force was measured using a pressure-sensitive film (Dental Prescale® 50H type SS size, Fuji Photo Film, Tokyo, Japan). Children were seated on a height-adjustable chair with their heads upright, and their Frankfurt horizontal plane was parallel to the floor. Children were instructed to bite the film with maximum force. Two successive trials were conducted; subsequently, the used films were stored in a cool, dark place before analysis. The occlusal force was analyzed using the Occluzer® (Fujifilm, Tokyo, Japan). Of the two values, the maximum occlusal force was selected.

Muscle thickness and fat mass were measured using ultrasonography (Miru-Cube®, 6-MHz linear array transducer; Global Health, Kanagawa, Japan). Ultrasonograms of the right side of the masseter and lower limb muscles were obtained using a real-time scanner. Children were seated on a height-adjustable chair with their heads upright. Before the measurements, we instructed the children to clench their teeth so that the examiners could palpate the origin to estimate the thickest part of the masseter[17]. Examiners oriented the probe perpendicular to the thickest part of the masseter and the biggest part of the lower limb. Evaluation criteria were calibrated to account for inter-investigator differences. The relaxed muscles were also measured.

The body fat mass and skeletal muscle mass were measured using a body composition analyzer (InBody S10®; Biospace Co., Ltd., Seoul, Korea). This device uses multiple frequencies (1 kHz, 5 kHz, 50 kHz, 250 kHz, 500 kHz, and 1,000 kHz) of BIA technology and contains 8-point tactile electrodes that were attached to the left and right thumb, middle finger, and ankles. Children were seated in an upright position; their arms and legs did not come into contact with each other, and they
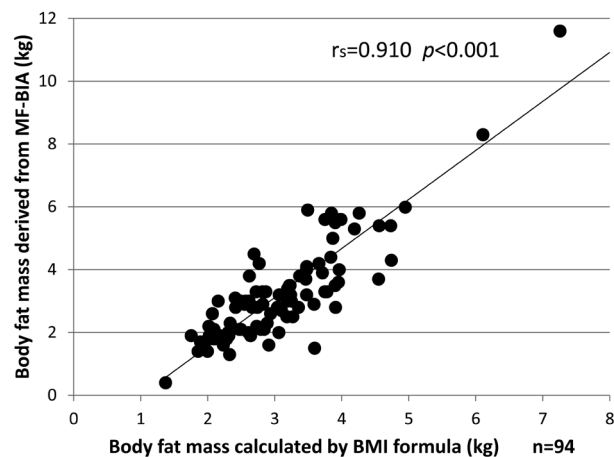
**Fig. 1.** Relationship of body fat mass calculated by the multi-frequency bioelectrical impedance analysis method and body mass index (BMI) formulas[8]

BMI formulas[8]:
Boys' Fat Mass (kg) = (0.408 BMI − 3.937) Height$^2$ (m)
Girls' Fat Mass (kg) = (0.462 BMI − 4.432) Height$^2$ (m)

did not talk or move during the measurement, which lasted 2 minutes. To control the research setting, all children were tested in the morning.

Children were seated on a height-adjustable chair, and the calf circumference was measured using standard procedures.

To validate the InBody S10® measurements of children aged 3 to 6 years, Spearman rank correlation coefficients between body fat mass obtained from the InBody S10® and by using the BMI formulas[8] were calculated.

Differences between participants' values and average values of Japanese children for height and weight were evaluated using the one sample t-test. Nonparametric tests were performed, because assumptions of the normal distribution of all parameters were rejected in the Shapiro-Wilk test. Spearman rank correlation coefficients were used to establish the relationship between the body fat mass calculated using the BMI formulas[8] and BIA method. Values are shown as a mean ± standard deviation. The Kruskal-Wallis test was used to evaluate differences between children of different ages. In addition, Spearman rank correlation coefficients were used to establish the relationship among the test results. A p value less than 0.05 was considered statistically significant.

## RESULTS

A comparison of participants' height and weight to that from the Japanese National growth data[18] showed no significant differences (p>0.05).

Figure 1 shows the correlation between body fat mass derived from the BMI formulas[8] and that calculated from the BIA method. A strong significant correlation was observed between the two methods for the body fat mass (p<0.001, $r_s$=0.910). Table 1 shows the mean values of age-related differences. As shown in Table 1, the height, weight, grip strength, maximum occlusal force, thickness of the lower limb muscle, thickness of fat mass at the masseter, skeletal muscle mass, and calf circumference showed the main effect of age.

Correlation coefficients between skeletal muscle mass/height and age, height, weight, grip strength, thickness of the lower limb muscle, and calf circumference were 0.624, 0.729, 0.796, 0.724, 0.414, and 0.733, respectively (p<0.001; Table 2).

## DISCUSSION

In this study of children between 3 to 6 years old, a strong significant correlation was found between the body fat mass values obtained by the MF-BIA method and those obtained using the BMI formulas[8]. MF-BIA can measure body composition, including the skeletal muscle mass and body fat mass. To accurately investigate the validity of body composition obtained from the skeletal muscle mass assessment method using MF-BIA, DEXA and/or MRI should be used. However, in this study, the MF-BIA method was not compared to DEXA and/or MRI. This was because DEXA and/or MRI carry a risk of radiation exposure and/or require a long measurement time; hence, DEXA and/or MRI in this study was considered unethical for use in preschool children. In addition, our study did not evaluate SF-BIA methods or the skinfold thickness method because of the long examination periods required and the burden of stress on preschool children. BMI formulas[8] can calculate body fat

**Table 1.** Measurements grouped by age

| | 3 years old (n=23) | 4 years old (n=24) | 5 years old (n=24) | 6 years old (n=23) | Age difference[a] p-value (n=94) |
|---|---|---|---|---|---|
| Height (cm) | 95.7 ± 2.97 | 101 ± 4.36 | 109 ± 2.49 | 114 ± 5.44 | ** |
| Weight (kg) | 14.6 ± 1.78 | 16.4 ± 2.64 | 18.9 ± 1.71 | 21.1 ± 5.13 | ** |
| Grip strength (kg) | 3.87 ± 1.20 | 5.03 ± 1.30 | 6.92 ± 1.64 | 8.08 ± 1.57 | ** |
| Maximum occlusal force (N) | 171 ± 80.9 | 218 ± 129 | 224 ± 92.9 | 271 ± 129 | * |
| Thickness of the masseter muscle (mm) | 11.0 ± 1.37 | 9.83 ± 1.52 | 10.3 ± 2.05 | 10.7 ± 2.06 | - |
| Thickness of the lower limb muscle (mm) | 29.2 ± 5.31 | 31.9 ± 5.78 | 34.4 ± 6.51 | 35.9 ± 5.12 | * |
| Thickness of fat mass at the masseter (mm) | 6.26 ± 0.85 | 5.58 ± 1.11 | 5.54 ± 1.00 | 5.39 ± 1.17 | * |
| Thickness of Fat mass at the lower limb (mm) | 5.48 ± 1.02 | 5.00 ± 1.15 | 5.29 ± 1.46 | 5.52 ± 1.41 | - |
| Skeletal muscle mass (kg) | 5.22 ± 0.70 | 6.08 ± 0.93 | 7.29 ± 1.11 | 8.03 ± 1.51 | ** |
| Body fat mass (kg) | 2.72 ± 1.36 | 3.13 ± 1.41 | 3.40 ± 1.15 | 4.30 ± 3.01 | - |
| Calf circumference (cm) | 21.9 ± 1.80 | 22.5 ± 1.89 | 24.0 ± 1.38 | 24.7 ± 2.90 | ** |

[a] Kruskal-Wallis test, *$p<0.05$, **$p<0.01$
Data are presented as a mean ± standard deviation.

**Table 2.** Results of the correlations

| | Age | Height | Weight | Grip strength | Maximum occlusal force | Thickness of the masseter muscle | Thickness of the lower limb muscle | Thickness of fat mass at the masseter | Thickness of fat mass at the lower limb | Skeletal muscle mass/height | Body fat mass/height | Calf circumference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Age | — | 0.892** | 0.725** | 0.792** | 0.301** | -0.057 | 0.408** | -0.296** | -0.025 | 0.624** | 0.155 | 0.490** |
| Height | | — | 0.876** | 0.779** | 0.286** | 0.059 | 0.429** | -0.249* | 0.102 | 0.729** | 0.252* | 0.669** |
| Weight | | | — | 0.725** | 0.191 | 0.141 | 0.407** | -0.111 | 0.214* | 0.796** | 0.551** | 0.869** |
| Grip strength | | | | — | 0.271** | -0.081 | 0.392** | -0.297** | -0.012 | 0.724** | 0.155 | 0.601** |
| Maximum occlusal force | | | | | — | 0.149 | 0.051 | -0.058 | -0.113 | 0.190 | -0.002 | 0.071 |
| Thickness of the masseter muscle | | | | | | — | 0.169 | 0.202 | 0.219* | 0.123 | 0.146 | 0.141 |
| Thickness of the lower limb muscle | | | | | | | — | -0.135 | -0.093 | 0.414** | 0.127 | 0.330** |
| Thickness of fat mass at the masseter | | | | | | | | — | 0.236* | -0.162 | 0.149 | -0.038 |
| Thickness of fat mass at the lower limb | | | | | | | | | — | 0.002 | 0.408** | 0.325** |
| Skeletal muscle mass/height | | | | | | | | | | — | 0.159 | 0.733** |
| Body fat mass/height | | | | | | | | | | | — | 0.547** |
| Calf circumference | | | | | | | | | | | | — |

Spearman rank correlation coefficient; *$p<0.05$, **$p<0.001$ (n=94)

mass and body fat-free mass. Thus, we compared the body fat mass obtained from MF-BIA with the BMI formulas[8], and we thought this was an appropriate approach to this epidemiological study. Our study's findings suggest that the values obtained using the MF-BIA method are reliable in children aged 3 to 6 years old.

In addition, to ensure the validity of the MF-BIA method, we compared other variables to the skeletal muscle mass. In this study, correlation coefficients were significantly greater for grip strength and calf circumference. According to previous studies[19, 20], the grip strength and calf circumference are predictors of total muscle strength in children and adolescents. These results also confirm the validity of the MF-BIA method for measuring skeletal muscle mass. Besides the BIA technique, simple measurements such as the grip strength and circumference are highly recommended for determining the skeletal muscle mass in preschool children. Thickness of the lower limb was less strongly correlated with skeletal muscle mass than calf circumference, which indicated the difficulty of measuring thickness of the lower limb in preschool children. Fat width[21] in preschool children affects the measurement of thickness of the muscle, which can minimize the relationship between skeletal muscle mass and thickness. The maximum occlusal force and grip strength were measured in isometric contraction in this study. However, the maximum occlusal force was not correlated with skeletal muscle mass. According to previous studies, early childhood is a period when the ability of balance develops[22], and bite force in preschool children is reported to be associated with body balance[23]. The chewing muscles contribute to the stability of the head as antigravity muscles. Therefore, in childhood, the development of antigravity function in mastication muscles may be related to factors that increase the bite force.

MF-BIA in preschool children enabled us to evaluate measurements of the skeletal muscle mass. Besides the MF-BIA, however, the grip strength and calf circumference are convenient indicators of skeletal muscle mass in the clinical setting. In the future, a longitudinal study using the MF-BIA method introduced in this study may be necessary. The MF-BIA method may also be useful for population screening. In addition, the MF-BIA method can measure more parameters than the SF-BIA method; therefore, we recommend it for use in future research in preschool children, as it is widely used in adults[24].

A limitation of this study was its cross-sectional design. Longitudinal studies are needed in the future to generate detailed information about muscle development. Additionally, only 94 children were included in this study; thus, more knowledge could be obtained by increasing the number of subjects in similar future studies.

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