Int. J. School. Health. 2023; 10(3)
Published online 2023 July.

Original Article

Quantitative Evaluation of Skeletal Muscle Fat Content by Measuring Echo Intensity Using Ultrasonography in Preschool Children

Chika Oya[1*], MSc; Erina Muramatsu[2], PhD; Keisuke Teramoto[2], PhD

[1]Humanitec Junior College, Japan
[2]Aichi University of Education, Japan

Corresponding author: Chika OYA, MSc; Postal address: Minami Hamada 4-21, Yokkaichi, Postal code: 510-0066, Mie, Japan. **Tel:** +81-59-3568170; **Fax:** +81-59-3560036; **Email:** o.chika@jc-humanitec.ac.jp

Received: December 15, 2022; **Revised:** January 20, 2023; **Accepted:** February 27, 2023

Abstract

Background: Quantitative analysis of echo intensity (EI) is a reliable method for discriminating between normal and neuromuscular disease-affected muscles in children. However, the effects of increased skeletal muscle fat mass and total body fat mass in healthy preschool children are yet to be determined. The purpose of this study was to compare the EI of skeletal muscles and local fat accumulation in healthy preschool children by gender and body shape using ultrasonography.
Methods: Three- to six-year-old children (145 boys and 122 girls) without any known neuromuscular disorder were included in this cross-sectional research, during the period of 2015 to 2018 in Miyazaki prefecture, Japan. Anthropometry, body composition, and EI of the biceps brachii muscle (BB) and quadriceps femoris (RF) muscle were measured by ultrasonography. Differences in anthropometric measurements and body composition divided by two types of criteria using age and sex-specific percentiles were analyzed using two-way ANOVA ($P<0.05$).
Results: EI at the BB of overweight children (above 90th percentile) was 9.82±3.76 for boys and 10.87±3.69 for girls. EI at the BB of normal children (10th-90th percentile) was 6.02±2.62 for boys and 6.53±2.92 for girls. EI at the BB of thin children (below 10th percentile) was 5.61±2.17 for boys and 6.52±3.49 for girls. EI at the BB of overweight children is higher than normal and thin children ($P<0.001$). EI at the RF of overweight children (above 90th percentile) was 16.97±5.12 for boys and 18.84±3.41 for girls. EI at the RF of normal children (10th-90th percentile) was 15.32±4.55 for boys and 16.20±3.98 for girls. EI at the RF of thin children (below 10th percentile) was 14.94±3.90 for boys and 14.58±4.94 for girls. EI at the RF of overweight children is higher than normal and thin children ($P=0.024$). These results were also similar for the groups created using the 25th and 75th percentiles, with the above 75th percentile group showing clearly higher values of EI.
Conclusions: Our results suggested that the accumulation of adipose tissue within skeletal muscle varies with the degree of obesity. It seems reasonable to conclude that an extreme increase in body fat might be linked to higher visceral and skeletal muscle fat (ectopic fat content).

Keywords: Quantitative, Skeletal muscle, Fat content, Echo intensity, Ultrasonography, Obesity

How to Cite: Oya C, Muramatsu E, Teramoto K. Quantitative Evaluation of Skeletal Muscle Fat Content by Measuring Echo Intensity Using Ultrasonography in Preschool Children. Int. J. School. Health. 2023;10(3):2-11. doi: 10.30476/INTJSH.2023.98323.1298.

1. Introduction

Childhood obesity has emerged as an important public health issue worldwide and is a crucial determinant of obesity risk (1, 2). In the last two decades, the prevalence of overweight has increased 2.6 times (from 5.0% to 12.9%) in Japanese boys and 2.5 times (from 5.0% to 12.5%) in Japanese girls, as assessed by using body mass index (BMI) (from 5 to 18 years old) (3). The factors responsible for the escalation in the rate of obesity include extreme and rapid changes in lifestyle, physical activity, and diet that accompany urbanization and rapid economic development. In other words, prolonged periods of inactivity coupled with reduced exercise time have led to a sedentary lifestyle. Furthermore, an increase in high-fat food intake has also contributed to the global increase in obesity (4). Previous studies exploring the correlation between BMI levels and obesity among children indicated that being overweight in childhood is related to subsequent obesity in adulthood, which, in turn, is associated with a higher risk of morbidity and mortality (2, 5-7). Additionally, we found that obese preschool children (>90th percentile of age- and sex-specific BMI) had increased visceral fat (preperitoneal fat thickness, Pmax) with higher BMI values, i.e., a greater tendency toward obesity (8).

In recent years, ectopic fat, as well as visceral fat, has been identified as fat that affects health, one of which is skeletal muscle fat. Muscle biopsy and magnetic resonance imaging (MRI) are the traditional methods used for quantifying muscles and understanding muscular pathologies. Both of these techniques are unsuitable for use in children, as biopsy is an invasive procedure, and MRI often requires sedation, in addition to being a time-taking procedure. Echo intensity (EI) using ultrasonography has recently been used as a method to measure skeletal muscle fat content. Stock and colleagues (9) showed that EI is a new and promising tool to examine skeletal muscle fat quality, even though some areas will show sufficient evidence in the future.

Ultrasonography is a noninvasive, portable, and sedation-free option for visualizing muscles in children. It is well known that disrupted muscle architecture and fat infiltration lead to an increased EI (echogenicity index) of muscle on ultrasonography (10). Grayscale analysis, based on a histogram, calculates the average gray value of the muscle region of interest and can be used to quantify EI (11). Quantitative analysis of EI and muscle thickness (MT) is a reliable method for distinguishing between normal musculature and neuromuscular diseases in children (12). On ultrasound images, normal muscle tissue is expected to have a low EI, appearing black. However, EI increases due to fat infiltration into muscle tissue caused by decreased physical activity, as well as fibrosis caused by neuromuscular diseases, and such muscles appear white on ultrasound images. In a previous study, we showed that EI in healthy adult men without exercise habits was significantly higher than that in those with exercise habits (13). We also found that habitual exercising reduced EI (14). Previous study suggested that low levels of physical activity may lead to excess skeletal muscle fat content in the calves and thighs of children (15). However, it is still unclear whether total fat accumulation (whole or visceral) in early childhood affects skeletal muscle fat content and the relationship between the three.

The purpose of this study was to compare the EI of skeletal muscles and local fat accumulation in healthy preschool children, categorized by gender and body shape (thin, normal, and overweight), using ultrasonography.

2. Methods

2.1. Participants

We conducted a quantitative evaluation for measuring the participants. We recruited healthy children from the same kindergartens in Miyazaki prefecture, Japan for this study. An explanation of the experiment was given to the parents of approximately 350 children in the kindergarten. Participation in the measurement was voluntary, and informed consent was obtained from the parents of 301 children before the study commencement. The data used in this analysis were obtained from a cross-sectional sample of 267 participants, including 145 boys and 122 girls between the ages of three and six years during the period of 2015 to 2018. We excluded 34 children from the study who had missing data for the exposure and outcome variables. All participants were free of serious diseases, family history of neuromuscular and physical growth disorders, as well as any known pathologies or physical handicaps. This study was conducted in compliance with the Declaration of Helsinki, Ethical Guidelines for Clinical Research, and Act on the Protection of Personal Information. Ethical approval for this research was obtained from the Research Ethics Committee of Aichi University of Education, Japan. The children were divided into different age groups based on their age at the time of measurement. For instance, children aged between 3.0 and 3.9 years, on the date of measurement, were included in the 3-year group.

In this study, we classified the groups according to the percentile (%ile) value of each sex and age group classified by body fat percentage (%fat) values. In other words, the %fat values were arranged by group. We used %fat value instead of BMI to more strongly reflect the rate of body fat accumulation of the whole body. We used two types of criteria using percentiles to classify body types. The reason for this was that the classification criteria of body shape (thin and overweight) depended on the country and research articles (16), and we also wanted to compare EI according to the degree of body shape. The normal-fat content group consisted of children with %fat between 10th and 90th %ile, the low-fat content group (thin group) contained individuals having %fat below 10th %ile, and the high-fat content group (overweight group) consisted of participants with %fat above 90th %ile, for both boys and girls. Further dividing the children into three groups using quartile ranges, we classified the reference-fat content group as having %fat between 25th and 75th %ile, the low-fat tendency group as having %fat below 25th %ile, and the high-fat tendency content group as having %fat above 75th %ile.

2.2. Measurements

All measurements were carried out in a room with a temperature of approximately 23°C after at least one hour of eating, with the subjects wearing thin undergarments. Height was measured to the nearest 0.1 cm with a height meter and body weight was measured to the nearest 0.02 kg using a calibrated balance-beam scale. BMI (kg/m^2) was calculated using the formula: body weight/ height^2. The upper arm, thigh, and abdominal circumferences were measured with a metric plastic tape while the children stood upright. The upper arm circumference was measured at the biceps brachii as two-thirds of the distance from the acromion to the antecubital crease of the right arm. The thigh circumference was measured at the quadriceps femoris as the distance between the midpoint of the anterior superior iliac spine and the proximal end of the patella. The abdominal circumference was measured at the horizontal navel position. All anthropometric measurements were carried out by a trained examiner according to accepted standard techniques (17).

Body composition was estimated using a two-component model. Impedance was determined using a four-terminal impedance analyzer (AO-90, Applied Office, Japan). During the measurements, the participants wore no shoes or socks and were laid in a supine position on a bed with their limbs extended away from the trunk of their body. After cleaning all skin contact areas with alcohol, current electrodes (Red Dot-2330; 3M Health Care, USA) were placed on the dorsal surfaces of the distal metacarpals and metatarsals of the right hand and right foot, respectively. Detector electrodes were also applied at the right pisiform prominences of the wrist and between the medial and lateral malleoli of the ankle. The bioelectrical impedance analyzer generated an excitation current of 500 mA at a single frequency of 50 kHz. The fat-free mass (FFM, kg) was calculated using the equation of Masuda and Komiya (18), and the age- and gender-specific constants for the total body water (TBW) component (hydration) of FFM in children were calculated via the equation by Fommon and colleagues (19). Furthermore, fat mass (FM) was calculated as the difference between body weight and FFM.

Ultrasound images of the anterior upper arm and anterior thigh were obtained using a B-mode ultrasonography device (SDD-Prosound2, Aloka, Japan), based on the study by Pillen and colleagues (12). The measurement was set at a 53 dB gain and was done using a linear transducer of 7.5 MHz. All ultrasound measurements were performed by one skilled measurer who was proficient and had several accepted research articles on skeletal muscle fat content. The participants were asked to lie on the bed in a supine position and relax. The transducer was placed perpendicular to the muscle at the predefined circumference measurement points. The pressure of the transducer on the skin was minimized by using a large amount of contact gel. Muscle and subcutaneous fat thicknesses were measured using an electronic caliper included in the ultrasonography device. For the anterior upper arm, the distance from the skin to the boundary between the subcutaneous fat and the biceps brachii muscle was measured as the upper arm subcutaneous fat thickness (mm). Similarly, for the anterior thigh, the distance from the skin to the boundary between the subcutaneous fat and rectus femoris muscle was measured as the thigh subcutaneous fat thickness (mm). The target muscle for measuring the EI of the anterior upper arm was the biceps brachii muscle, and that of the anterior thigh was the rectus femoris muscle. Measurements of EI for a single site were taken at least twice, and the average value was used as the measurement result. The mean EI was defined using a calculated 8-bit grayscale analyzer with standard histogram functions using Adobe Photoshop Elements (Adobe, USA). For each image, the software was used to select the largest possible region of the target muscle, not including the bone or fascia, and the mean EI (pixels) in each region was calculated (black=0, white=255). Each image was analyzed multiple times, and the intraclass correlation coefficient for these analyses was 0.99.

The thickness of the preperitoneal and subcutaneous fat layers was measured using the abdominal ultrasonography method described by Suzuki and co-workers (20). All images were scanned longitudinally from the xiphoid process to the umbilicus using an ultrasonography device. Scanning was performed in the optimal position according to previous literature (20), and images were taken with the liver surface almost parallel to the skin during breath-holding. The probe was touched as lightly as possible to prevent compression of the fat layer, as well as for the upper arm and thigh images. The maximum thickness of the preperitoneal fat layer (Pmax) and the minimum thickness of the subcutaneous fat layer (Smin) were measured directly on the screen with the help of an electronic caliper.

2.3. Statistical Analysis

No statistical sample size calculations were conducted. SPSS version 27.0 (IBM, Japan) was used to conduct all statistical analyses in this study. The measurement results were presented as mean values and standard deviations. Differences in sex, age, and percentages of physical characteristic variables were examined using t-tests between the two groups and ANOVA for the rest. Differences were considered significant at P values less than 0.05. The interrelationships between each measurement were investigated using Pearson's correlation coefficients.

Furthermore, we structured our paper in accordance with the STROBE Checklist for cross-sectional studies (21).

3. Results

The study included 3-6 years healthy children, and we excluded those with incomplete data for the exposure and outcome variables (28 out of 301 children were excluded). These excluded subjects were unable to complete all the measurements, and thus were shown as excluded. Table 1 presents the physical characteristics and body composition of the study participants categorized by gender. There were no gender-related differences in anthropometric characteristics, such as age (boys: 65.5±10.1, girls: 65.2±10.1, P=0.844), height (boys: 109.7±6.9, girls: 108.7±6.3, P=0.225), weight (boys: 19.04±3.66, girls: 18.63±3.19, P=0.333), BMI (boys: 15.7±1.6, girls: 15.7±1.6, P=0.905), and the upper arm (boys: 16.0±1.4, girls: 16.3±1.5, P=0.097) and thigh circumferences (boys: 30.9±3.5, girls: 32.1±3.3, P=0.005). However, girls exhibited significantly higher fat content than boys, including higher FM (boys: 3.73±1.78, girls: 4.60±1.65, P<0.001), as well as thickness of abdominal (Pmax, boys: 3.66±1.31, girls: 4.16±1.54, P=0.005) and subcutaneous fat layer (Smin, boys: 2.77±1.54, girls: 3.53±1.67, P<0.001). In contrast, EI values were not significantly different between the two genders (BB, boys: 6.35±2.93, girls: 6.96±3.30, P=0.110; RF, boys: 15.44±4.55, girls: 16.30±4.11, P=0.110).

Table 2 shows the results of fat content indices divided by quartile ranges of fat percentage for boys and girls, while Table 3 shows the results divided by the 10th percentile and 90th percentile for boys and girls, respectively.

Table 1: Comparison of physical characteristics with statistical data for gender-related differences

	Boys		Girls		Sex, P
Subject number	145		122		
Age, month	65.5	±10.1	65.2	±10.1	0.844
Height, cm	109.7	±6.9	108.7	±6.3	0.225
Weight, kg	19.04	±3.66	18.63	±3.19	0.333
Body mass index, kg/m^2	15.7	±1.6	15.7	±1.6	0.905
Circumference					
Abdminal, cm	50.7	±4.4	50.3	±4.0	0.443
Upper Arm, cm	16.0	±1.4	16.3	±1.5	0.097
Thigh, cm	30.9	±3.5	32.1	±3.3	0.005
Fat-free mass, kg	15.31	±2.09	14.02	±1.75	<0.001
%	81.2	±4.9	75.9	±4.5	<0.001
Fat mass, kg	3.73	±1.78	4.60	±1.65	<0.001
%	18.8	±4.9	24.1	±4.5	<0.001
Abdminal fat layer					
Subcutaneous fat layer, mm	2.77	±1.54	3.53	±1.67	<0.001
Preperitoneal fat layer, mm	3.66	±1.31	4.16	±1.54	0.005
Subcutaneous fat layer					
Upper arm, mm	2.06	±0.84	2.39	±0.99	0.004
Thigh, mm	5.26	±1.66	6.30	±1.79	<0.001
Muscle echo intensity					
Biceps brachii muscle, pixel	6.35	±2.93	6.96	±3.30	0.110
Rectus femoris muscle, pixel	15.44	±4.55	16.30	±4.11	0.110

Table 2: Comparison of fat content and muscle thickness by ultrasonography divided by 25th and 75th percentiles of body fat percentage

	Boys			Girls			Sex		%ile		Sex*%ile	
	%Fat <25%ile	25-75%ile	75%ile<	%Fat <25%ile	25-75%ile	75%ile<	F	P	F	P	F	P
Subject number	36	73	36	30	62	30						
Body mass index, kg/m²	14.53±0.79	15.44±0.90	17.38±1.93	14.52±0.91	15.38±0.75	17.41±1.90	0.004	0.949	101.3	<0.001	0.027	0.973
Fat mass, kg	2.20±0.47	3.38±0.58	5.95±2.10	3.05±0.47	4.34±0.68	6.69±1.72	36.3	<0.001	200.7	<0.001	0.239	0.788
%	13.2±2.0	18.5±1.7	25.1±4.0	18.6±1.8	24.0±1.5	29.9±3.1	295.0	<0.001	403.8	<0.001	0.505	0.604
Abdminal fat layer												
Subcutaneous fat layer, mm	2.11±0.57	2.43±0.76	4.11±2.39	2.61±0.74	3.10±0.85	5.32±2.24	21.2	<0.001	63.1	<0.001	1.29	0.278
Preperitoneal fat layer, mm	3.31±0.99	3.47±1.28	4.41±1.41	3.95±1.72	3.79±1.10	5.15±1.75	10.6	<0.001	18.2	<0.001	0.634	0.531
Subcutaneous fat layer												
Upper arm, mm	1.59±0.56	1.97±0.65	2.74±1.01	1.77±0.62	2.25±0.70	3.30±1.17	11.7	<0.001	52.0	<0.001	1.13	0.326
Thigh, mm	4.23±1.10	5.05±1.24	6.71±1.91	4.98±1.13	6.16±1.38	7.92±1.87	30.3	<0.001	60.5	<0.001	0.488	0.615
Muscle echo intensity												
Biceps brachii muscle, pixel	5.99±2.42	5.70±2.73	8.00±3.19	5.44±2.79	6.76±2.97	8.88±3.57	1.47	0.226	17.0	<0.001	1.75	0.176
Rectus femoris muscle, pixel	14.93±4.52	15.32±4.51	16.18±4.69	15.18±4.36	16.37±3.96	17.26±4.06	1.96	0.162	2.399	0.093	0.211	0.810

Table 3: Comparison of fat content and muscle thickness by ultrasonography divided by 10th and 90th percentiles of body fat percentage

	Boys			Girls			Sex		%ile		Sex*%ile	
	%Fat <10%ile	10-90%ile	90%ile<	%Fat <10%ile	10-90%ile	90%ile<	F	P	F	P	F	P
Subject number	14	117	14	12	98	12						
Body mass index, kg/m²	13.99±0.65	15.51±1.01	18.94±1.97	14.07±1.06	15.45±0.84	19.01±2.09	0.028	0.866	155.5	<0.001	0.077	0.926
Fat mass, kg	1.76±0.29	3.49±0.95	7.70±2.31	2.68±0.44	4.42±0.98	8.04±1.95	12.1	<0.001	192.8	<0.001	0.853	0.428
%	11.0±1.3	18.5±2.9	29.1±3.6	16.8±1.5	24.0±2.8	32.7±3.4	83.9	<0.001	234.5	<0.001	1.32	0.270
Abdminal fat layer												
Subcutaneous fat layer, mm	2.07±0.53	2.51±0.85	5.59±3.15	2.35±0.73	3.25±1.08	6.95±2.25	11.6	<0.001	96.8	<0.001	1.30	0.275
Preperitoneal fat layer, mm	3.46±1.20	3.53±1.27	4.94±1.18	3.31±1.07	4.10±1.46	5.53±1.78	1.71	0.192	14.8	<0.001	0.823	0.440
Subcutaneous fat layer												
Upper arm, mm	1.40±0.37	2.02±0.76	3.09±1.00	1.51±0.72	2.34±0.77	3.72±1.49	5.27	0.022	40.0	<0.001	0.708	0.494
Thigh, mm	3.75±1.03	5.13±1.29	7.88±2.14	4.64±0.97	6.23±1.54	8.57±2.17	10.3	0.002	51.6	<0.001	0.273	0.761
Muscle echo intensity												
Biceps brachii muscle, pixel	5.61±2.17	6.02±2.62	9.82±3.76	6.52±3.49	6.53±2.92	10.87±3.69	2.27	0.133	23.7	<0.001	0.144	0.866
Rectus femoris muscle, pixel	14.94±3.90	15.32±4.55	16.97±5.12	14.58±4.94	16.20±3.98	18.84±3.41	0.937	0.334	3.81	0.024	0.434	0.648

The 25th and 75th percentile calculations, as well as the 10th and 90th percentile calculations, revealed gender differences in most indices of fat content, except EI. The results depended on body size, as there were no gender differences in EI and no interaction effects. Additionally, significant differences in classification by each percentile were observed for all parameters except EI of the rectus femoris muscle (boys, %Fat<25%ile: 14.93±4.52, 25-75%ile: 15.32±4.51, 75%ile<: 16.18±4.69; girls, %Fat<25%ile: 15.18±4.36, 25-75%ile: 16.37±3.96, 75%ile<: 17.26±4.06, F=2.399, P=0.093) by the 25th and 75th percentile calculations. The upper muscle groups exhibited significantly higher percentile values.

To evaluate changes in whole-body fat accumulation and energy intake (EI), since EI was significantly higher for individuals with different body shapes, Figure 1 displays the correlation between the EI of the biceps brachii muscle and the body fat percentage. The results showed a significantly positive correlation for both boys and girls, indicating that EI, which refers to fat infiltration into muscle tissue, increased as fat accumulated in the body. In Figure 2, the correlation between the EI of the biceps brachii muscle and the EI of the rectus femoris muscle is presented separately for boys and girls. The results revealed a significant positive correlation between the two indices, indicating that an increase in EI of

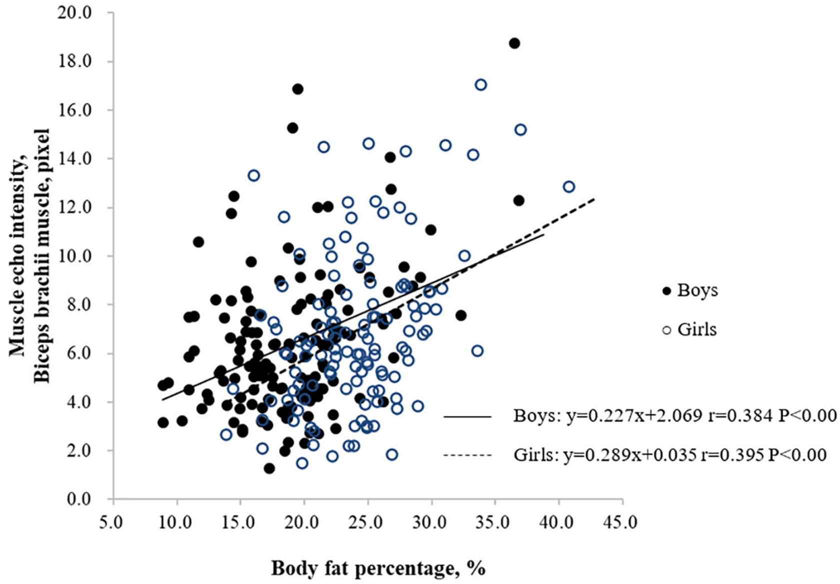

Figure 1: The figure shows the correction between body fat percentage and muscle echo intensity of biceps brachii muscle.

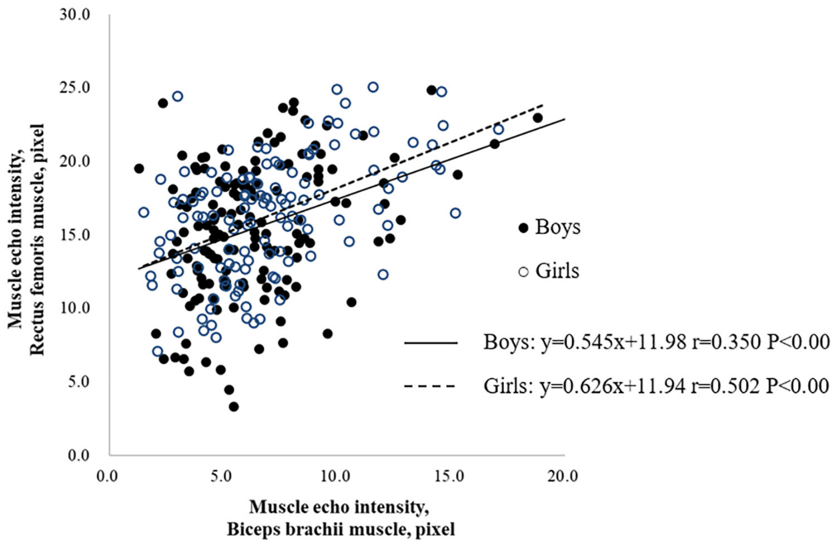

Figure 2: The figure shows the correlation between echo intensity of biceps brachii muscle and rectus femoris muscle.

the upper limbs was accompanied by an increase in EI of the lower limbs.

4. Discussion

In the present study, when the EI was divided by the 10th percentile and 90th percentile of body fat percentage, the result was significantly higher in the high-fat group (above 90th percentile) than the normal-fat group (10-90th percentile) and the low-fat group (below 10th percentile). Similarly, the high-fat group (above 75th percentile) had significantly higher EI than the standard fat group (25-75th percentile) and the low-fat group (below 25th percentile).

Furthermore, our data indicated that EI of the rectus femoris muscle was proportionally related to the %fat of the whole body. This finding was similar to that of our previous study, which showed that inactivity increased EI values in healthy young adults (13). Therefore, it can be concluded that preschoolers may have increased skeletal muscle fat mass due to increased total body fat mass. Furthermore, there were significantly higher values of the abdominal fat layer, especially Pmax, which is strongly related to visceral fat accumulation, as total body fat accumulation increased. This result was consistent with the results of our previous study (8).

Several reports showed a relationship between physical activity and skeletal fat content in healthy adults (22, 23). Similarly, we previously reported the relationship between low physical activity and high EI or high body fat accumulation in adults (13). Fat contained in the skeletal muscles has been reported to be strongly associated with obesity, type 2 diabetes, and metabolic syndrome (24). Physical inactivity has also been shown to be a risk factor for greater fat infiltration into growing skeletal muscles (25).

Johnson and colleagues (26), in their MRI-based study, found that children with quadriplegic cerebral palsy had greater skeletal muscle fat content in the midthigh region than healthy children. This was significantly associated with their lower level of physical activity. This finding was consistent with the observation made in another study conducted on children with low muscle mass and disabilities that limited their participation in physical activities (27).

A worldwide epidemic of childhood obesity has unfolded in the last few decades due to an increase in sedentary lifestyle among children, which is associated with a higher risk of lifestyle-related diseases (28). It has been reported that a sedentary lifestyle deregulates fat oxidizing enzymes, resulting in reduced ability to oxidize fat (29). In recent years, physical fitness and motor ability in children have declined significantly compared to several decades ago in Japan (30). A major reason for this decline may be the lack of opportunities and suitable places for children to play and develop their physical fitness. Limiting sedentary behaviors, such as prolonged television watching, coupled with increased physical activity in children, is an important public health strategy for preventing childhood obesity (28).

Increased body fat in children can cause visceral fat accumulation and fat infiltration into muscles, leading to pathological changes in the muscular system. Rech and co-workers (31) reported a negative relationship between bone and local fat accumulation, such as visceral fat and fat within skeletal muscle in children, similar to observations made in adult individuals. Ectopic fat accumulation can lead to serious pathologies even in childhood, which may persist in adulthood (2, 7). In adults, EI has been noted to be negatively correlated with muscle strength. The association between muscle quality assessed via EI and muscle strength was found to be unaffected by age or MT (32). Muscle mass (muscle size) and muscle quality (composition of fibrous and adipose tissue within the muscle) have been shown to contribute independently to muscle strength in middle-aged and older adults (31). Although it was not possible to measure muscle strength or physical activity in preschool children in the previous study, the intramuscular fat content may have a small compounding effect on the decline of motor skills in children.

Assessment for visceral fat and ectopic fat accumulation in childhood is typically performed using MRI and other techniques. However, they are rarely evaluated unless symptoms of pathological changes due to excessive accumulation occur. The measurement of skeletal muscle mass using ultrasonography can be easily performed in children.

It has been reported that EI on ultrasonography may be an important tool for assessing the structural aspects of skeletal muscle tissue, as it is significantly correlated with fat infiltration (10, 33). Fat infiltration into muscles has been documented to increase the risk of neuromuscular diseases such as Duchenne muscular dystrophy, spinal muscle atrophy, and congenital myopathies. This is attributable to the disruption of muscle structure due to the replacement of muscle cells by fat and connective tissue (34).

Only a few studies have examined intramuscular fat content in healthy children using muscle echo intensity. Knowledge regarding the reference values of EI is necessary to describe the normal muscle architecture in children and to understand the corresponding decline in skeletal muscle quality and reduction in EI. Furthermore, it is essential to examine whether physical activity (total energy expenditure and physical activity level) affects EI so that the information can be applied to school health and preventive medicine programs during childhood. Therefore, continuous measurement using a simple assessment method such as ultrasonography is important from the perspective of preventive medicine in childhood. We also believe that it is necessary to identify the growth changes and critical points of the assessment index.

4.1. Limitations

The number of participants and measured parameters in this study might be insufficient to clarify this relationship because we did not conduct statistical sample size calculations, and it is difficult to collect a large number of pediatric subjects. Moreover, the system settings differ between separate ultrasound machines, which can affect the resulting echo intensity. Therefore, further studies are needed to compare the values obtained with different instruments, as noted in previous study (9), as well as to validate the findings of the present study. We believe that this study will be positioned as one of the studies that will help prevent the incidence of metabolic syndrome and locomotive syndrome from childhood to adulthood. Furthermore, we will continue our research with improved measurement parameters, and we expect that this study will serve as a basis for future studies in this area.

5. Conclusions

This study aimed to compare echo intensity (EI) and local fat accumulation by ultrasonography in thin, normal, and overweight healthy preschool children. The results of this study showed that the accumulation of adipose tissue within skeletal muscle varied with the degree of obesity. Extreme increases in body fat were associated with increases in visceral and ectopic fat, including skeletal muscle fat mass. From the viewpoint of preventive medicine, it is clear that attention should be paid to local fat accumulation due to increased body fat mass, even during childhood, as this can lead to health issues during adulthood.

Funding

This work was supported by the Japan Society for the Promotion of Science (Keisuke TERAMOTO, Grant Numbers: JP16K0182).

Ethical Approval

This study was conducted in compliance with the Declaration of Helsinki, Ethical Guidelines for Clinical Research, and Act on the Protection of Personal Information. This study received ethical approval from the Research Ethics Committee of the Aichi University of Education, Japan. Before the measurements, informed consent was obtained from the parents of all participants.

Acknowledgement

We appreciate the time and effort spent by our volunteers.

Conflict of Interests: None declared.

References

1. Simmonds M, Llewellyn A, Owen CG, Woolacott N. Predicting adult obesity from childhood obesity: a systematic review and meta-analysis. Obes Rev. 2016;17(2):95-107. doi: 10.1111/obr.12334. PubMed PMID: 26696565.
2. Guo S, Chumlea WC Roche AF, Gardner JD, Siervogel RM. The predictive value of childhood body mass index values for overweight at 35y. Am J Clin Nurt. 1994;59(4):810-9. doi: 10.1093/ajcn/59.4.810. PubMed PMID: 8147324.
3. Inokuchi M, Matsuo N, Takayama JI, Hasegawa T. Official Japanese reports significantly underestimate prevalence of overweight in school children: Inappropriate definition of standard weight

and calculation of excess weight. Ann Hum Biol. 2009;36(2):139-45. doi: 10.1080/03014460802635213. PubMed PMID: 19194805.
4. Rosiek A, Frąckowiak Maciejewska N, Leksowski K, Rosiek-Kryszewska A, Leksowski Ł. Effect of Television on Obesity and Excess of Weight and Consequences of Health. Int J Environ Res Public Health. 2015;12(8):9408-26. doi: 10.3390/ijerph120809408. PubMed PMID: 26274965; PubMed Central PMCID: PMC4555288.
5. Singh AS, Mulder C, Twisk JWR, van Mechelen W, Chinapaw MJM. Tracking of childhood overweight into adulthood: a systematic review of the literature. Obes Rev. 2008;9(5):474-88. doi: 10.1111/j.1467-789X.2008.00475.x. PubMed PMID: 18331423.
6. National Task Force on Childhood Obesity, National Center for Women's and Children's Health, Ding ZY. National epidemiological survey on childhood obesity, 2006. Zhonghua Er Ke Za Zhi. 2008;46(3):179–84. PubMed PMID: 19099704. Chinese.
7. Must A, Jacques P, Dallal G, Bajema C, Dietz W. Long-term morbidity and mortality of overweight adolescents-a follow-up the Harvard Growth Study of 1922 to 1935. New Engl J Med. 1992;327(19):1350-5. doi: 10.1056/NEJM199211053271904. PubMed PMID: 1406836.
8. Teramoto K, Ishikawa T, Yamashita R, Oya C, Muramatsu E, Iezaki K, et al. Assessment of Abdominal Fat Accumulation in Preschooler Using Ultrasonography. J Human and Living Environment. 2015;22(2):33-42. doi: 10.24538/jhesj.22.2_103. Japanese.
9. Stock MS, Thompson BJ. Echo intensity as an indicator of skeletal muscle quality: applications, methodology, and future directions. Eur J Appl Physiol. 2021;121(2):369-380. doi: 10.1007/s00421-020-04556-6. PubMed PMID: 33221942.
10. Reimers K, Reimers CD, Wagner S, Paetzke I, Pongratz DE. Skeltal muscle sonography: a correlative study of echogenicity and morphology. J Ultrasound Med. 1993;12(2):73-7. doi: 10.7863/jum.1993.12.2.73. PubMed PMID: 8468739.
11. Scholten RR, Pillen S, Verrips A, Zwarts MJ. Quantitative ultrasonography of skeletal muscles in children: normal values. Muscle Nurve. 2003;27(6):693-8. doi: 10.1002/mus.10384. PubMed PMID: 12766980.
12. Pillen S, Scholten RR, Zwarts MJ, Verrips A. Quantitative skeletal Muscle ultrasonography in children with suspected neuromuscular disease. Muscle Nurve. 2003;27(6):699-705. doi: 10.1002/mus.10385. PubMed PMID: 12766981.
13. Teramoto K, Iezaki K, Suda K, Oya C, Muramatsu E, Sugiyama S. Effects of habitual exercise to skeletal muscle fat content in young adult. Bulletin of Aichi University of Education, Art, Health,and Physical Education, Home Economics, Technology and Creative Arts. 2016;65:39-44. Japanese.
14. Teramoto K, Suda K, Kataoka Y, Oya C, Muramatsu E, Iezaki K. Effects of Short-term Moderate Training on Visceral Fat Accumulation and Skeletal Muscle Fat Content. J Human and Living Enviirronmeent. 2021;28:21-28. Japanese.
15. Farr JN, Loan MDV, Lohman TG, Going SB. Lower physical activity is associated with skeletal muscle fat content in girls. Med Sci Sports Exerc. 2012;44(7):1375-81. doi: 10.1249/MSS.0b013e31824749b2. PubMed PMID: 22217562; PubMed Central PMCID: PMC3819115.
16. Junior CASA, Martins PC, Aznar LAM, Silva DAS. Reference growth curves to identify weight status (underweight, overweight or obesity) in children and adolescents: systematic review. Br J Nutr. 2023;25:1-13. doi: 10.1017/S0007114522003786. PubMed PMID: 36695353.
17. Komiya S, Eto C, Otoki K, Teramoto K, Shimizu F, Shimamoto H. Gender differences in body fat of low- and high-body-mass children: relationship with body mass index. Eur J Appl Physiol. 2000;82(1-2):16-23. doi: 10.1007/s004210050646. PubMed PMID: 10879438.
18. Masuda T, Komiya S. A prediction equation for total body water from bioelectrical impedance in Japanese children. J Physiol Anthropol Appl Human Sci. 2004;23(2):35–9. doi: 10.2114/jpa.23.35. PubMed PMID: 15067189.
19. Fommon SJ, Haschke F, Ziegler EE, Nelson SE. Body composition of reference children from birth to age 10 years. Am J Clin Nutr. 1982;35:1169-75. doi: 10.1093/ajcn/35.5.1169. PubMed PMID: 7081099.
20. Suzuki R, Watanabe S, Hirai Y, Akiyama K, Nishide T, Matsushima Y, et al. Abdominal wall fat index, estimated by ultrasonography, for assessment of the ratio of visceral fat to subcutaneous fat in the abdomen. Am J Med. 1993;95(3):309-14. doi: 10.1016/0002-9343(93)90284-v. PubMed PMID: 8368228.
21. STROBE Statement-Checklist of items that should be included in reports of cross-sectional studies. STROBE Checklist. Available from: https://www.strobe-statement.org/checklists.

22. Gilsanz V, Kremer A, Mo AO, Wren TA, Kremer R. Vitamin D status and its relation to muscle mass and muscle fat in young women. J Clin Endocrinol Metab. 2010;95(4):1595-601. doi: 10.1210/jc.2009-2309. PubMed PMID: 20164290; PubMed Central PMCID: PMC2853984.
23. Manini TM, Clark BC, Nalls MA, Goodpaster BH, Ploutz-Snyder LL, Harris TB. Reduced physical activity increases intermuscular adipose tissue in healthy young adults. Am J Clin Nutr. 2007;85(2):377-84. doi: 10.1093/ajcn/85.2.377. PubMed PMID: 17284732.
24. Blaak EE, van Aggle-Leijssen DP, Wagenmakers AJ, Saris WH, van Baak MA. Impaired oxidation of plasma-derived fatty acids in type 2 diabetic subjects during moderate-intensity exercise. Diabetes. 2000;49(12):2102-7. doi: 10.2337/diabetes.49.12.2102. PubMed PMID: 11118013.
25. Farr JN, van Loan MD, Lohman TG, Going SB. Lower physical activity is associated with skeletal muscle fat content in girls. Med Sci Sports Exerc. 2012;44(7):1375-81. doi: 10.1249/MSS.0b013e31824749b2. PubMed PMID: 22217562; PubMed Central PMCID: PMC3819115.
26. Johnson DL, Miller F, Subramanian P, Modlesky CM. Adipose tissue infiltration of skeletal muscle in children with cerebral palsy. J Pediatr. 2009;154(5):715-20. doi: 10.1016/j.jpeds.2008.10.046. PubMed PMID: 19111321; PubMed Central PMCID: PMC2963648.
27. Leroy-Willig A, Willig TN, Henry-Feugeas MC, Frouin V, Marinier E, Boulier A, et al. Body composition determined with MR in patients with Duchenne muscular dystrophy, spinal muscular atrophy, and normal subjects. Magn Reson Imaging. 1997;15(7):737-44. doi: 10.1016/s0730-725x(97)00046-5. PubMed PMID: 9309604.
28. Zhang G, Wu L, Zhou L, Lu W, Mao C. Television watching and risk of childhood obesity: a meta-analysis. Eur J Public Health. 2015;26(1):13-8. doi: 10.1093/eurpub/ckv213. PubMed PMID: 26604324.
29. Prior SJ, Joseph LJ, Brandauer J, Katzel LI, Hagberg JM, Ryan AS. Reduction in midthigh low density muscle with aerobic exercise training and weight loss impacts glucose tolerance in order men. J Clin Endocrinol Metab. 2007;92(3):880-6. doi: 10.1210/jc.2006-2113. PubMed PMID: 17200170.
30. Sugihara T, Kondo, M, Mori, S, Yoshida I. Chronological change in preschool children's motor ability development in Japan from the 1960s to the 2000s. International Journal of Sports and Health Science, 2006;4:49-56. doi: 10.5432/ijshs.4.49.
31. Rech A, Radaelli R, Goltz FR, Rosa LHT, Schneider CD, Rinto RS. Echo intensity in negatively associated with functional capacity in older women. Age. 2014;36(5):9708. doi: 10.1007/s11357-014-9708-2. PubMed PMID: 25167965; PubMed Central PMCID: PMC4453939.
32. Fukumoto Y, Ikezoe T, Yamada Y, Tsukagoshi R, Nakamura M, Mori N, et al. Skeletal muscle quality assessed from echo intensity is associated with muscle strength of middle-age and elderly persons. Eur J Appl Physiol. 2012;112(4):1519-25. doi: 10.1007/s00421-011-2099-5. PubMed PMID: 21847576.
33. Pillen S, Tak RO, Zwarts MJ, Lammens MMY, Verrijp KN, Arts IMP, et al. Skeletal muscle ultrasound:correlation between fibrous tissue echo intensity. Ultra Med Biol. 2009;35(3):443-6. doi: 10.1016/j.ultrasmedbio.2008.09.016. PubMed PMID: 19081667.
34. Heckmatt JZ, Dubowitz V. Diagnostic advantage of needle muscle biopsy and ultrasound imaging in the detection of focal pathology in a girl with limb girdle dystrophy. Muscle Nerve. 1985;8(8):705-9. doi: 10.1002/mus.880080813. PubMed PMID: 3903492.