A novel method for measuring subcutaneous adipose tissue using ultrasound in children – interobserver consistency

Adela Chiriţă-Emandi1, Maria Camelia Papa2, Liliana Abrudan2, Mihaela Amelia Dobrescu3, Maria Puiu1,2, Iulian Puiu Velea4, Corina Paul4

1Department of Genetics, "Victor Babeș" University of Medicine and Pharmacy, Timișoara, Romania
2Department of Genetics, "Louis Titracanu" Emergency Hospital for Children, Timișoara, Romania
3Department of Genetics, University of Medicine and Pharmacy of Craiova, Romania
4Department of Pediatrics, "Victor Babeș" University of Medicine and Pharmacy, Timișoara, Romania

Abstract

Background: Currently, sufficiently accurate field methods for body composition assessment in children are missing. The ultrasound method for assessing adipose tissue thickness has been used extensively in sport medicine. However, there are no studies looking at the reliability of this method in non-athletic children. This paper aims to determine the inter-observer reliability in measuring the uncompressed subcutaneous adipose tissue thickness using ultrasound, in children. Subjects, Materials and Methods: Forty healthy children (20 males, 20 females), median age 11.85 years (5.3 to 18.1 years) were evaluated. Median body mass index standard deviation score (BMI SDS) was -0.13 (-3.9 to 4). Three observers used a Hosand BX 2000 Ultrasonic Adipometer to measure uncompressed subcutaneous adipose tissue thickness at three sites: triceps, subscapular, supraspinale. A single experienced observer used the three sites to also measure the compressed adipose thickness using a skinfold caliper. Results: Individual observer deviations from the mean value of the three observers in adipometer measurement had a standard deviation of 1.74 mm, 92.8% were less than 3 mm. Analysis separated by individual anatomical sites showed high reliability values for triceps: linear regression $R^2=0.84$, $p=0.000$; intraclass correlation coefficient (ICC)=0.92 and standard error of measurement (SEM)=0.63. The values at supraspinale site were $R^2=0.82$, $p=0.000$, ICC=0.89 and SEM=1.17, while for subscapular the values were lower: $R^2=0.79$, $p=0.000$, ICC=0.78 and SEM=1.02. The body fat percentage (BF%) calculated using skinfold measurements was highly correlated with the BF% calculated by the adipometer ($R=0.92$, $R^2=0.83$, $p=0.000$). The Pearson’s correlation between BMI SDS and BF% calculated from skinfold was $R=0.52$, $R^2=0.28$, $p=0.001$, while for the adipometer it was $R=0.53$, $R^2=0.27$, $p=0.000$. Conclusions: This novel ultrasound measurement technique can be used with good accuracy and reliability to measure uncompressed subcutaneous adipose tissue thickness in children, sustaining its application for research and clinical purposes, however larger studies are needed.

Keywords: ultrasound, uncompressed subcutaneous adipose tissue thickness, children, reliability.

Introduction

Childhood obesity is a major health concern worldwide, but especially in developing countries [1, 2]. Changes in body composition related to obesity have a dramatic impact on metabolism. Adipose tissue is nominated to be a key factor modulating lipid and glucose homeostasis [3]. Thus, the need to have accessible and reliable tools to evaluate body composition and adipose tissue thickness in particular, is extremely important.

Anthropometry is an important tool for nutritional assessment, which involves the external measurements of morphological traits of humans [4]. However, anthropometric data are not free from measurement errors. The magnitude of measurement errors is essential in interpreting results of anthropometric evaluations. There are mainly two types of possible errors: repeated measures providing different values (unreliability, imprecision); and measurements deviating from true values (inaccuracy, bias) [5]. Imprecision is due largely to observer error, and may be approximated by performing repeated anthropometric measures on the same subjects and calculating the standard error of measurement (SEM), coefficient of reliability ($R$), and intraclass correlation coefficient (ICC). A major factor influencing inaccuracy is poor training in taking measurements. Training in anthropometry is at present far from perfect, and great improvement is needed in developing appropriate protocols for nutritional anthropometry training.

Different nutritional anthropometric measures have different precisions. Weight and height have the highest precision with correct training of observers. The body mass index (BMI) calculated from weight and height is by far the most used method for assessing obesity in children, by comparison to a reference growth chart. However, the use of BMI alone is cautioned in athletes or persons with certain medical conditions (e.g., sarcopenia) where body weight may be significantly modified by changing proportions of muscle and fat masses [3, 6, 7]. BMI does not provide direct data on adipose tissue. Additionally, if assessing results in follow-up after a lifestyle intervention, the change in BMI may not accurately reflect body composition changes.

Waist and hip circumference show strong between-observer differences, and should, if possible, be carried out by a single observer [8]. Skinfolds can be associated with fairly large measurement error, some authors consider that interpretation is problematic [4, 9]. Currently, sufficiently accurate field methods for body composition assessment in children are missing. The ultrasound method...
for assessing adipose tissue thickness has been used extensively in sport medicine [10–14]. However, there are no studies looking at the reliability of this method in non-athletic children.

This paper aims to determine the inter-observer reliability in measuring the uncompressed subcutaneous adipose tissue thickness using ultrasound in children and to compare this method to skinfold.

Subjects, Materials and Methods

Participants

A group of 40 healthy children (20 males, 20 females), median age 11.85 years (range 5.3 to 18.1 years) were evaluated. Median body mass index standard deviation score (BMI SDS) was -0.13 (range -3.9 to 4). Measurements were done in the morning of the last day of hospital stay; all patients had a good state of health. Patients with known chronic conditions affecting growth and development or with gastrointestinal disorders or other disorders that affect the hydration state were not included in the study.

Observers

A single observer trained in taking accurate measurements, according to international measurement techniques [15] had performed the anthropometric measurements (height, weight, waist circumference, skinfold) in all the children. The three observers taking the uncompressed subcutaneous adipose tissues thickness with the adipometer were trained in the ultrasound technique and were highly experienced, having done more than 500 previous measurements.

Anthropometric measurements method and evaluation procedures

All measurements were made during the morning hours. Height was determined using a SECA telescopic stadiometer, and body mass was determined using a calibrated SECA mechanical column scale with round dial. We calculated BMI for each child and the BMI SDS following the World Health Organization (WHO) reference 5–19 years [16].

We used the WHO cut-offs for interpretation, as follows:
- overweight: +1 to +2 (equivalent to BMI 25 kg/m² at 19 years);
- obesity: +2 (equivalent to BMI 30 kg/m² at 19 years);
- thinness: -2 to -3;
- severe thinness: <-3.

For waist circumference we used a plastic measuring tape and the reference from Fryar et al. [17].

Adipose tissue morphology

Adipose tissue is one of the best represented connective tissues in the body. An example of adipose tissue morphology is provided in Figure 1. Most of the adipose tissue is composed of unilocular round, oval or polyhedral adipocytes with dimensions up to 150 μm, organized in lobules (Figure 1A). Although it was considered to be a tissue with low metabolic activity, this tissue has many vessels and nerves (Figure 1B).

Excessive fat tissue develops in people with obesity. In our study, we measured the compressed adipose tissue thickness by using skinfold calliper and uncompressed adipose tissue with an ultrasonic measurement technique.

Figure 1 – (A) Adipose tissue [Hematoxylin–Eosin (HE) staining, ×200]; (B) Adipose tissue with blood vessels and nerves (HE staining, ×100).

Skinfold assessment

Skinfold measurements were done using a Harpenden calliper, three times by a single observer and the average value was noted for each child. For subscapular and triceps skinfold reference we used the paper from Addo & Himes [18]. Tanner stage was also assessed. For calculating body fat percentage (BF%) from skinfold measurements, we used Slaughter et al.’s skinfold-thickness equations [19] as shown as follows:

• for Tanner stage 1–2 male: BF% = 1.21 (triceps + subscapular) – 0.008(triceps + subscapular)² – 1.7;
• for Tanner stage 3 male: BF% = 1.21 (triceps + subscapular) – 0.008(triceps + subscapular)² – 3.4;
• for Tanner stage 4–5 male: BF% = 1.21 (triceps + subscapular) – 0.008(triceps + subscapular)² – 5.5;
• for all females: BF% = 1.33 (triceps + subscapular) – 0.013(triceps + subscapular)² – 2.5.
Ultrasonic measurement technique and evaluation procedures

Measurements were made in standing position. We used a Hosand BX 2000 Ultrasonic Adipometer in ‘default’ setting that uses a 2.5-MHz transmitter (manufactured by HOSAND TECHNOLOGIES s.r.l., Italy, kindly provided for the study period, by MST Solutions SRL Timișoara). An example of the software algorithm that we used to evaluate thickness of adipose tissue and the actual ultrasound image that the software interprets during the same assessment is presented (Figure 2, A and B). The first spike corresponds to the delineation of adipose tissue and muscle; while the second for delineation of muscle and bone.

Measurements using the BX 2000 adipometer were done by applying the device to the skin; while previously, a thin coating of water-soluble gel was applied to the contact surface of the device. The transducer was positioned carefully to avoid compression of the subcutaneous fat. The transducer was held parallel to the direction of the skinfold in order to assure precise depth analyses, as an angulation of interfaces different than 90 degrees – may result in a transmission parallax error [20]. During the measurement, the BX 2000 adipometer was glided slightly back and forth along the skin surface (approximately 5 mm from the measurement site) to provide local averaging of the measurements, over a period of 3 to 5 seconds. Subcutaneous adipose thickness was then calculated using the prediction equations supplied by the manufacturer’s computer algorithm. The measurements were made at the three anatomic ISAK sites (protocol of the International Society for the Advancement of Kinanthropometry [21]) on the left side of the body: triceps, subscapular, supraspinale (above iliac crest). The skin sites were not set with mark on the skin. Each observer set the site before each measurement.

Ethics

Permission to undertake the study was provided by the Ethics Commission of the “Victor Babeș” University of Medicine and Pharmacy, Timișoara.

On parent of each child had discussed the aims and methods of the study with the investigators and signed the informed consent form.

The Hospital Management also approved the study protocol.

Statistical methods

Statistical analysis was performed with SPSS (IBM SPSS Statistics ver. 22). Descriptive statistics provide mean, standard deviation, minimum and maximum for all variables. Descriptive characteristics are also show for male and female separately, significant differences were sown in bold, \(p\)-values <0.05 were considered significant. Reliability assessment was done using intraclass correlation coefficient (ICC, alpha two way mixed, absolute agreement), Cronbach’s alpha and standard error of measurement [\(\text{SEM}=\text{SD} \times \sqrt{1-\text{Cronbach’s } \alpha}\)] were calculated for all and for each uncompressed subcutaneous adipose tissue thickness measurement, at three ISAK sites. ICC and Cronbach’s \(\alpha\) values between 0.7–0.9 were considered representative of a good reliability, while a value above 0.9 was considered representative for a very good reliability. The closer the SEM is to 0, the higher is the reliability [22]. Linear correlation fit line and 95% CI (confidence interval) lines from regression analysis as well as Pearson’s regression coefficient squared \(R^2\) are given.

Results

Descriptive statistics are presented in Table 1 and further detailed into males and females separately in Table 2.
Mean age was 11.85 years (SD=3.73), while mean BMI SDS was -0.13, (SD=1.54). The mean triceps, subscapular and supraspinale adipometer measurements represent the mean value of the three observers for each child. Significant differences were observed between male and female in calculated body fat percentage from skinfold and adipometer, females presenting higher mean values compared to males. Females also showed significantly greater mean values in adipometer measurements at triceps and subscapular sites. Our cohort of children included 28 (70%) normal weight children, five (12.5%) underweight, five (12.5%) overweight, while two (5%) were obese according to WHO cut-offs. BMI SDS in respect to age and gender of children is presented (Figure 3).

Individual observer deviations from the mean value in adipometer measurements (all three sites, 40 children, 360 measurements) of the three observers are shown (Figure 4). SD of individual deviations from the mean is 1.74 mm with extremes ranging from -7.70 to 9.33 mm. Only 26 (7.2%) measurements had a deviation from the mean of more than 3 mm.

Three hundred sixty individual adipometer measurements taken by three observers on 40 children, at three ISAK sites were plotted against the mean values of the observers, \( R^2=0.77, p=0.000 \) (Figure 5A). The linear regression line and 95% CI’s lines are also shown (Figure 5, A–F). Data from Figure 4A is separated according to individual ISAK sites, subscapular, triceps and supraspinale respectively; 120 measurements in each figure (Figure 5, B–D). At subscapular site (Figure 5B), \( R^2=0.79, p=0.000, \text{ICC}=0.78 \) and SEM=1.02. At triceps site (Figure 5C), the values were \( R^2=0.84, p=0.000, \text{ICC}=0.92 \) and SEM=0.63. The values at supraspinale site (Figure 5D) were as follows: \( R^2=0.82, p=0.000, \text{ICC}=0.89 \) and SEM=1.17.
The skinfold measurements are plotted against the mean adipometer measurements at all sites all observers (120 measurements; \( R^2=0.51, p=0.000 \)) (Figure 5E). Body fat percentage obtained from the formula using skinfold measurements were plotted against the body fat percentage calculated by the adipometer (40 measurements; Pearson \( R=0.913, R^2=0.83, p=0.000 \)) (Figure 5F).

Bivariate correlations were performed for all variables, however only those that were relevant and statistically significant are presented. The Pearson correlation between BMI SDS and body fat percentage calculated from skinfold was \( R=0.52, R^2=0.28, p=0.001 \). Between BMI SDS and body fat percentage from the adipometer Pearson \( R=0.53, R^2=0.27, p=0.000 \). Measurements from adipometer correlated very well with measurements from skinfold as following: at triceps, \( R=0.71, p<0.001 \); at supraspinale, \( R=0.79, p<0.001 \); but not at subscapular, \( R=0.49, p<0.001 \).

The \( R^2 \), linear regression line and 95% CI’s lines are shown in Figure 5, A–F.

### Discussion

Measuring the thickness of subcutaneous fat tissue is important for several medical specialists such as pediatricians, nutritionists, endocrinologists, or others when assessing nutritional status, including obesity and monitoring dietary manipulation. Subcutaneous fat has been considered to play a role in energy provision, thermo-regulation and therмоinsulation. Recently, main function of subcutaneous fat is changing from energy store to cytokine and growth factor production related with innate immunity and cell growth [23]. Measuring the thickness of subcutaneous adipose tissue layer in a precise, quick and simple way for daily use in the clinic, or outside of the medical office and in research is highly needed.

### Current assessment methods for subcutaneous adipose tissue

BMI is the most commonly used parameter for epidemiological studies on obesity; however, in the individual pediatric patient, especially from 10 years onwards, it gives only a limited insight to the degree of obesity [24]. Nonetheless, in certain circumstances, such as after an lifestyle intervention the BMI may not accurately depict specific shifts between lean and adipose tissue compartments, and individuals may therefore present with weight stability while gaining lean mass and losing fat, or vice versa [25].

There are several techniques to measure the thickness of subcutaneous adipose tissue. The most widely used is the skinfold caliper technique [26]. The technique is simple to use but has some limitations, such as increased skinfold compressibility with age, imprecision, unsatisfactory reproducibility between observers and inability to measure skinfold thickness at some sites in obese people [27, 28]. Computed tomography (CT) has been used to assess the changes in muscle and adipose tissue [29]. CT has some limitations such as exposure to ionizing radiation, high cost, time-consuming and difficult availability in some settings. Magnetic resonance imaging (MRI) is also used for assessing subcutaneous and visceral fat [30]. Although not irradiating, it is an expensive, time-consuming technique that is not available in many settings. Dual-energy X-ray absorptiometry (DXA) is a widely used method for the measurement of the fat, fat-free soft tissue and bone mineral compartments of the body. Body composition measurements using newer generation dual-energy X-ray absorptiometry machines compared between different manufacturers and compared with earlier instruments continue to show differences that may be unacceptable [31]. Bioelectrical impedance analysis (BIA) and quantitative magnetic resonance (QMR) have been developed to assess of body composition in the obese population [32]. BIA measures the body impedance (the effective resistance of a circuit), and is based on the varying resistance of different tissues types within the body. The impedance measure predicts total body water content and fat-free mass, from which the fat mass can be calculated. It can be conducted through varying frequencies, each with varying degrees of accuracy in predicting %BF. However, body fat will be generally underestimated in severely obese individuals [33]. QMR is another emerging technique; it measures differences in hydrogen atom emission signals originating from fat, fat-free tissue, and
free water. This tool is sensitive to small changes in fat mass compared with other measures, and has shown significant promise in the assessment of body composition in the obese population [34]. Presently, this is a high cost method has low availability. The ultrasound technique for assessing adipose tissues thickness has been developed for many years [35] and has been used largely in sports medicine. It is non-invasive, non-radiating, cheap, rapid and can also be applied in a medical office or in a school setting. We performed this study to test the interobserver reliability in order to extend its usage of this method to the pediatric clinical practice.

Figure 5 – Regression lines and confidence intervals for measurements: (A) 360 adipometer measurements plotted against the mean values of each observer for each child; Adipometer measurements at ISAK site subscapular (B), triceps (C) and supraspinale (D) are plotted against mean values of each observer for each child (120 measurements); (E) Skinfold measurements are plotted against mean adipometer measurements at all sites, all observers (120 measurements); (F) Body fat percent (BF%) from skinfold measurements plotted against the BF% calculated by the adipometer (40 measurements).
Participants

The majority of children included in the study were normal weight (70%). The distribution of normal weight, overweight, obese and underweight mimics to some extent the one in the general Romanian pediatric population [36, 37]. There was a difference between genders in regards to body composition, girls showing significantly more adipose tissue thickness and increased body fat percentage, compared to boys. Adiposity distribution is highly related to pubertal stage, in our study, 65% of boys and girls had Tanner stage 3, 4 or 5. The findings are not surprising, many other studies found similar results [38, 39]. Aside from pubertal stage and gender, there are many factors influencing growth and development like genetic factors, environmental factors, nutritional factors, psychosocial status of the children that were not accounted for in this study. Characterization of fat distribution in relation to gender, age, pubertal stage or other factors influencing children’s development goes beyond the scope of this paper.

Choice of measurement sites

In the present study, the three ISAK skinfold sites were chosen also for ultrasound measurements because the triceps and subscapular sites are known as an international standard for skinfold, and because comprehensive skinfold data in children already exist for these sites [9, 18, 40, 41]. Thus, allowing comparison between uncompressed subcutaneous adipose tissue measurements using ultrasound and skinfold measurements. However, we must take into account that there is high heterogeneity in skinfold compression at various sites and among individuals, and additionally skin thickness varies between sites [27, 42]. In addition, we chose the supraspinale site because it is also used in many studies in children and adults, except there are no reference data for this site in children [43–47].

We chose to use the computer algorithm of the adipometer Hosand BX 2000 Ultrasonic Adipometer to control the output of the subcutaneous adipose tissue segmentation algorithm in order to eliminate human variability in interpreting the images of embedded fibrous tissues. However, analysis and interpretation of complex biological structures may not be replaced in all cases by computer algorithms. Conversely, the computer algorithm could provide accurate and easy use for any observer in the field, with just a brief training.

Interobserver reliability of uncompressed subcutaneous adipose tissue measurements using ultrasound

The adipometer measurements performed in our study had small individual observer deviations from the mean value, the majority of them were no more than 3 mm.

The study showed over all sites a good interobserver reliability of measurements ($R^2=0.77$, ICC=0.815; Figure 5, A–D; Table 3).

For individual sites, the triceps had excellent reliability ($R^2=0.837$, ICC=0.907), while at supraspinale the reliability was also very good ($R^2=0.818$, ICC=0.892), at subscapular there was more imprecision ($R^2=0.688$, ICC=0.779). The deviations of observer values from each other depend on the exact positioning of the ultrasound probe (position, orientation and angle with respect to the skin) at a given site on the body surface. Visual interpretation of image by the observer and improved training should reduce the number erroneous evaluations.

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<th>Table 3 – Inter-observer reliability statistics for adipometer measurements in 40 children using ultrasound</th>
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ICC: Intraclass correlation coefficient; CI: Confidence interval; SEM: Standard error of measurement; BF%: Body fat percentage.

Importantly, the three ISAK anatomical sites have been selected for skinfold measurement and not for ultrasound. Müller et al. proposes that ideal sites for ultrasound measurement of subcutaneous adipose thickness should have simple structures; the thickness of the layer should not change appreciably in the vicinity, and their predictive value for total body fat should be high [10]. A systematic screening for optimal ultrasound sites on body surface does not yet exist. In Müller’s et al. studies on athletes, the ISAK sites on the limbs showed simple ultrasound structures [10, 11]. However, at the trunk sites, there were anatomical identification problems; at the abdomen, an intermediate fascia (Camper’s fascia) caused large measurement deviations between observers [10]. At the subscapular site, the presence of two visible bands in the ultrasound image instead of just one muscle-fascia boundary also led to image interpretation confusions [10]. Studies defining ultrasound sites not only by anatomical landmarks on the surface, but also by means of information contained in the ultrasound image should further enhance reliability [10].

Ultrasound versus skinfold measurement

Overall skinfold measurements (Figure 5E) showed relatively low correlation to the mean adipometer measurements $R^2=0.51$, $p=0.000$. However, looking at individual sites the correlation is strong: triceps, $R=0.71$, $p<0.001$; supraspinale, $R=0.79$, $p<0.001$; but not for subscapular, $R=0.49$, $p<0.001$. Nevertheless, the skinfold body fat percentage (Figure 5F) correlated very well with the body fat percentage calculated by the adipometer ($R=0.913$, $R^2=0.83$, $p=0.000$). There was a similar correlation between skinfold and adipometer when plotted against BMI SDS suggesting the clinical utility of the method.

The advantages of the ultrasound technique for assessing adipose tissues thickness are:

• it is a non-invasive, non-radiating technique;
• tissue thickness limits can range from 1 to 300 mm;
• quick evaluation process; subject involvement is minimal;
• it can be applied in a medical office or in a school setting;
• costs are low when compared with MRI or CT [11].
Further studies with extended numbers of children and observers should clarify the role of ultrasound in measuring adipose tissue thickness and its use in practice. This study has some limitations. The relatively small number of children assessed over a wide age range 6 to 19 years. The measurements were not compared with the CT, MRI, or DXA, which are considered the most accurate measurements for assessing adipose tissue percentage. We did not assess all factors that influence the children’s development; however, we excluded children with acute or chronic conditions that might affect growth or hydration state.

Conclusions

The study showed for the triceps and supraspinale sites have good reliability parameters, while at subscapular there was more imprecision. Skinfold measurements were correlated strongly at triceps and supraspinale, but not as good for subscapular. This novel ultrasound measurement technique can be used with good accuracy and reliability to measure uncompressed subcutaneous adipose tissue thickness in children, sustaining its application for research and clinical purposes, however larger studies are needed.

Conflict of interests

All authors have nothing to declare.

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Corresponding author
Mihaela Amelia Dobrescu, Lecturer, MD, PhD, Department of Genetics, University of Medicine and Pharmacy of Craiova, 2 Petru Rareș Street, 200349 Craiova, Romania; Phone +40351–443 561, Fax +40251–593 077, e-mail: ameliadobrescu@gmail.com

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