A Novel Ultrasound Analytical Approach: A Tool for Fat Patterning and Prediction of Body Fat

Un Nuevo Enfoque Analítico por Ultrasonido: Una Herramienta para el Patrón de Grasa y la Predicción de la Grasa Corporal

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NJOKU, C. O.; OBASI, A. F.; OKPOSHI, F. T.; ANWARA, C. E. & STEWART, A. D. A novel ultrasound analytical approach: A tool for fat patterning and prediction of body fat. *Int. J. Morphol.*, 43(1):175-181, 2025.

SUMMARY: Simple, inexpensive, safe, reliable and accurate methods are suitable for routine check of body composition. This study aimed to establish that a novel ultrasound can accurately quantify subcutaneous fat at different locations of the body, and using air displacement plethysmorgraphy (Bod pod) as a reference can predict body fat. A sample of 91 adults (58 males and 33 females), age 18 – 44 y was recruited for the research. All participants took part in the Bod pod measurements. Each participant's body mass was obtained using Bod pod system electronic scale. Each participant was landmarked with a demographic pencil on the 9 selected sites on the right side of the body. Each site was scanned using an ultrasound scanner and flat 10 MHz probe. The air displacement plethysmorgraphy quantified the fat mass in kg and % fat of the participants. The ultrasound quantified fat depth at each site both with connective tissue interspersed and without connective tissue. Lateral Thigh had greatest fat depth while Brachioradialis had the least fat depth. Females had greater mean SAT depth than males at all the sites for both included and excluded connective tissue. Ultrasound fat depth for each region both including and excluding connective tissue, with a gender term to account for male/female differences was used to predict % fat from the Bod pod determination using Stepwise linear regression. Body fat can be predicted in a mixed sample with high accuracy. The exclusion of imbedded connective tissue and removal of compression may make ultrasound approach more valid.

KEY WORDS: Body fat; Ultrasound; Subcutaneous fat; Air displacement plethysmorgraphy.

INTRODUCTION

Routine surveillance of body composition relies on inexpensive, portable and convenient methods to detect changes in body composition or estimate those derived from other reference methods (Dempster & Aitkens, 1995). This ranges from simple anthropometry (field methods), Bioelectric Impedance Analysis (BIA) (laboratory methods) to highly complex methods such as computed tomography (CT) and magnetic resonance imaging (MRI) (Reference Methods) as discussed by Ackland *et al.* (2012).

MRI and CT, and at two compartment model, air displacement plethysmorgraphy (ADP) are considered more accurate and precise than others; hence they form the basis for comparing and evaluating other methods (Ackland *et al.*, 2012) but they are not available in every laboratory due to their high cost, and in addition, CT exposes the subject or

participant to high doses of ionization as reported by Ackland *et al.* (2012).

In a scientific review paper conducted by Fields *et al.* (2015), ADP was reported as a body composition tool that can be used to track body composition changes from childhood to adulthood, primarily because of its validity, reliability across volumes between 25 and 150 L, and its ease of application. Its comparability working modalities with Under Water weighing (UWW) was reported by Going (2005) though they are not fully interchangeable. In measuring human adults, Wells *et al.* (2000), found that the Bod pod reliability (0.11 L) was better than that of UWW (0.16 L) while Dewit *et al.* (2000), reported body volume of 0.07 L with Bod pod and 0.15 L with UWW. Reporting reliability of percentage body fat (%BF), Nuñez *et al.* (1999),

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reported high reliability of ADP for prediction of %BF. Utter & Hager, (2008) and Johnson *et al.* (2012), separately found good validity of ultrasound when compared to: UWW and ADP, respectively.

To many people, ultrasound is only known as biomedical diagnostic equipment. However, ultrasound has been used as a tool for body composition assessment since the mid-1960s (Bullen et al., 1965). It operates at a frequency greater than 20 KHz but, for ultrasonic imaging it generally uses frequencies greater than 2MHz. Its imaging principle is based on reflection of ultrasound waves from tissue in the pathway of the beam. The degree of sound waves reflection is based on changes in acoustic impedance between the two tissue interfaces (in body composition analysis this is muscle - fat), and a high acoustic impedance produces high sound reflection rather than transmission. The transducer detects the reflected waves and converts them into electrical signals which are transformed into images. Since the sound waves are reflected, the processor calculates the tissue depth as shown below

Distance = speed \times time/2 (mm)

This is an example of 'A'-mode imaging, amplitude modulation, which involves one-dimensional information between the single line from the transducer and reflector. Other modalities involve a two-dimensional by the way of B-mode or brightness modulation which an array of transducers generates multiple echoes which are usually graded in grey scale image reported by Starck *et al.* (2001).

In 2016, Muller et al., reported a novel ultrasound technique for accurate and reliable measurement of Subcutaneous Adipose Tissue (SAT) which uses image texture algorithm to quantify fat and was standardized by an ad hoc working group on body composition, health and performance under the auspices of the International Olympic Committee (IOC) Medical Commission. Eight new sites on the right side of the body (covering the arm – distal triceps, forearm - brachioradialis, abdomen - upper and lower abdomen, thigh - front and lateral thigh, back - erector spinae, and leg - medial calf were developed in a new protocol to maximise the image quality. Obtainable accuracy was 0.2 mm, reliability $R^2 = 0.998$, SEE = 0.55 mm, ICC (95 % CI) =0.998. This level of accuracy is unprecedented, and has enabled the group to produce highly detailed images of small ribbons of fat which are not visible with comparable techniques. It has also elucidated and quantified the extent of connective tissue development within the fat of skinfolds.

Taken together, the discipline of body composition has several methods and techniques with varying complexity

and ease of use available to researchers. Each of these techniques also makes assumptions that affect its suitability for different conditions; hence a single technique is unlikely to be ideal in all circumstances. Several techniques are prohibitively expensive and inaccessible to many laboratories. As reviewed by Ackland *et al.* (2012), these assumptions in both the two component models and multicomponent models create scope for affordable laboratory and field methods of body composition that are convenient, practicable, reliable and accurate. Hence, this study involved the combination of ADP and ultrasound in fat patterning and prediction of body fat in healthy adults.

MATERIAL AND METHOD

Recruitment of participants. A sample of 91 adults (58 males and 33 females), age 18 - 44 years was recruited for the research based on advertisement via a body composition flier and via e-mails seeking healthy adults for a single session body composition measurements. To ensure that the participants were fully hydrated, they were made to drink at least 60 cL water 20 min before the commencement of the measurements.

Every measurement was carried out during the day in a single session visit to the in purpose-designed research laboratories N516e and N516f of the Sir Ian Wood Building, RGU, Garthdee campus. In addition to the information sheet given to each participant in advance, a brief explanation of the measurements and the opportunity to ask questions related to the study was given to all participants on their arrival. All that was involved in the research study was explained to the participants, and all those who wished to continue gave their consent to participate by signing the consent form.

Ethical approval. All these research investigations were approved by the Robert Gordon University, (RGU) Research Ethics Committee.

Inclusion criteria. Only apparently healthy adults within the age range of 18-60 years were allowed to participate in the research. However, the accessible age range was 18-44 y, which it was decided to implement, as there was an abundance of available individuals within this range. Such individuals are more likely to be healthy than older individuals (who might otherwise have skewed the data).

Exclusion criteria. Owing to hormonal changes during pregnancy and the consequence on altered tissue masses, distribution and densities, pregnant women were excluded. The pregnant women were identified based on oral reporting of those affected.

Procedures for Bod pod measurements (ADP). All participants [91 adults (58 males and 33 females)] involved in this research took part in the air displacement plethysmography measurements. Each participant's body mass was obtained using Bod pod system electronic scale (Tanita Corp., Tokyo, Japan), which was calibrated with a 20 kg weight to measure the body mass to the nearest 0.01 kg. A double chamber calibration was performed when the chamber was empty using a standard 50 l cylinder.

The participant, in the required clothing, was asked to sit in the chamber for volume measurement after which the door of the Bod pod was closed (Fig. 1). Following the manufacturer's instructions, the 20-second volume measurement commenced with the participant relaxed and breathing normally. Two trials were performed on each participant and the average of the two measured volumes was taken as the participant's volume as per the manufacturer's instructions. Rarely, if the two trials did not meet the reproducibility criteria, then a third trial was performed and the two closest volumes averaged. However, if none of the measurements agreed with each other (within 150 mL, that is equivalent to 0.2 %), then the whole system was recalibrated and the measurements repeated.



Fig. 1. Cross section illustrating components of the Bod pod system

The thoracic gas volume (TGV) of each of the participants was predicted using the age, sex and height factors based on healthy adults' reference values as recorded by Crapo *et al.* (1982). In contrast to UWW, which requires maximal exhalation to residual volume, Bod pod lung volume is typically the average lung volume at normal tidal breathing.

Procedures for ultrasound measurements. The landmarking strategy of the Medical Commission's working group in body composition for the International Olympic Committee was followed (Muller *et al.*, 2016). This strategy relates measurement sites to bony landmarks via distances which are exact proportions of stature. Each participant was landmarked with a demographic pencil on the 9 selected sites on the right side of the body indicated in Muller *et al.* (2016), (Fig. 2). Each site was scanned using an ultrasound scanner and flat 10 MHz probe (Telemed, Vilnius, Lithuania) after introduction of a 5 mm layer of gel between the head of transducer and the skin to eliminate any air artefact. The reflected sound waves generated an image of the superficial tissues.



Fig. 2. Illustration of landmark locations for ultrasound assessment, where Br = Brachioradialis; DT= Distal triceps; UA= upper abdomen; LA= lower abdomen; EO =External Oblique; ES =Erector spinae; LT=Lateral thigh; AT= Anterior thigh; MC= Medial calf

All measurements with ultrasound were carried out in supine or prone position depending on the landmark's position and the images analyzed using FAT software v3.2 (Graz, Austria). This involved calibrating images, identifying a region of interest which extended from the epidermis to the muscle fascia, and introducing a 'seed' to identifiable areas of fat. The seed's grayscale and texture values are varied to allow it to 'invade' neighboring tissues in the region of interest. **Statistical Analysis.** All data was expressed in mean \pm standard deviation. Independent sample t-test was used to compare fat depth at every site between males and females. Stepwise linear regression analysis was used to determine explained variance between different measures and to derive predictive equation between ADP and ultrasound from measurements. Statistical analysis was performed using SPSS version 21 (SPSS Inc. Chicago, IL).

RESULTS

Subcutaneous adipose tissue depth assessed by B-mode ultrasound. 58 males and 33 females were measured for SAT depth at 9 different sites using ultrasound images and a novel analytical approach for quantifying fat by Müller et al. (2016). The measurement sites were defined as follows: AT - anterior thigh, EO - external oblique, DT - distal triceps, ES - erector spinae, LA - lower abdomen, MC - medial calf, BR - brachioradialis, UA - upper abdomen, and LT lateral thigh. The results quantified fat depth at each site both with connective tissue interspersed (included) and without connective tissue (excluded) (Fig. 3). LT had greatest fat depth and descended through LA, UA, AT, EO, DT, ES, MC, and BR had the least fat depth (Fig. 3). Fat depth at each site was compared in males and females as shown in Figures 4 and 5 with females maintaining greater mean SAT depth than males at all the sites for both included and excluded connective tissue.

Table I.	Physical	characteristics	of	participants
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	Males (n =58)		Females(n =33)	
	Mean	SD	Mean	SD
Age (year)	27.8	7.5	23.6	4.2
Stature (cm)	177.5	6.8	165.5	6.7
Weight (cm)	77.9	13.0	60.4	9.4
$BMI (kg/m^2)$	24.8	3.6	22.1	3.0
Waist girth (cm)	83.4	9.6	72.3	7.2
Hip girth (cm)	97.7	8.4	94.9	6.7
% Fat	21.9	8.0	23.0	8.7

Prediction of percentage fat using SAT depots. Ultrasound fat depth for each region both including and excluding connective tissue structures within the adipose tissue, with a sex term to account for male/female differences in fat patterning was used to predict % fat derived from the Bod pod determination. Stepwise linear regression optimised the prediction of % BF as follows.

$$\%$$
 fat = (1.046 × EO) + (1.295¥DT) + (6.556 × sex) +7.40

Where ($R^2 = 0.849$; SEE = 2.91; P < 0.001), where EO and DT are the external oblique and distal triceps sites, both excluding the thickness of connective tissue structures, and 0 and 1 term for females and males, respectively.



Fig. 3. Bar chart showing mean SAT in healthy adults with (included) and without (excluded) connective tissues. Blue bars represent SAT including subcutaneous tissues and red bars represent SAT excluding subcutaneous tissue. The sites used includes AT (anterior thigh), BR (brachioradialis), DT (distal deltoid), EO (external oblique), ES (erector spinae), LA (lower abdomen), LT (lateral thigh), MC (medial calf) and UA (upper abdomen), error bar = 1SD.



Fig. 4. Subcutaneous Adipose tissue map depicting fat depth (mm) (excluded) of male and female at different sites (n=58 males and 33 females).

DISCUSSION

Using the image texture analytical approach, Bmode ultrasound is an established, highly accurate and reliable tool for subcutaneous adipose tissue patterning in healthy adults (Muller *et al.*, 2016). The data from the present study on fat patterning in healthy adults indicate that the lateral thigh site had the greatest subcutaneous fat



Fig. 5. Subcutaneous Adipose tissue map depicting fat depth (mm) (included) of male and female at different sites. (n=58 males and 33 females).

deposition in both males and females while brachioradialis had the least subcutaneous fat deposition both with connective tissue included and excluded as shown in Figure 3. A comparison of fat patterning between males and females indicated that females had higher subcutaneous fat deposition (both including and excluding connective tissue) at all the nine sites scanned: brachioradialis, distal triceps, upper abdomen, erector spinae, external oblique, lower abdomen, lateral thigh, anterior thigh, and medial calf (Figs. 4 and 5). Males had the highest SAT deposition at the lower abdomen including connective tissue (14.7 mm) and females had the highest SAT deposition at the lateral thigh (19.4 mm). The male and female participants demonstrated the similar fat distribution at the lower abdomen (with 1.0 mm difference) and the greatest difference at the lateral thigh. The male-female difference was greatest at the lateral thigh when the connective tissue was included (8.8 mm difference). The observed total connective tissue within the regions of interest was 9.1 mm in males and 10.4 mm in females. While it is not possible to attribute causality to this difference, it can be speculated that more connective tissue will be present when the adipose tissue both in relation to its depth and when it is subjected to greater elastic strain. If this were the case, the male participants might be more active, but have less adipose tissue which requires stabilization, so these factors may cancel out resulting to no or little difference in connective tissue between males and females. The ultrasound method optimizes the acquisition of good images with distinct boundaries and accuracy. Using the fat patterning, a predictive equation was developed for quantifying % body fat in healthy adult using air displacement plesthymography (Bod pod) % fat as a criterion with comparable predictive capability to many published equations.

Precision and reliability in measurements of SAT are very important in assessment of body composition, especially in fat patterning, determination of the health status, health surveillance and evaluating treatment of obese and anorexic patients. It can be used to compare SAT distribution between groups and to, assess the result of weight intervention therapy. For over five decades, assessment of SAT through ultrasound has been used (Bullen, et al. 1965). Bullen et al. (1965) reported an excellent reliability of r=0.98. Although Haymes et al. (1976), (who worked with 20 women and 17 men with r=0.88) and Borkan et al. (1982) (who worked on 39 men at 15 body sites) had reported skinfold thickness to have better interobserver reliability than ultrasound, many improvements had been made in ultrasound over the years. On the contrary, Selkow et al. (2011), in their study (13 men and 7 women, age = 26.9 ± 5.4 years, height = $173.9 \pm$ 7.3 cm, mass = 77.4 ± 16.1 kg), had compared ultrasound and skinfold thickness and found the skinfold thickness to overestimate subcutaneous fat thickness as compared with ultrasound, especially as subcutaneous fat thickness levels increased. However, these studies did not report inter-tester TEMs. Diniz et al. (2009), also found ultrasound to present a good interobserver reproducibility in 50 patients in the evaluation of abdominal and visceral fat by means of measurements of the thickness of subcutaneous, visceral and perirenal fat. Although Diniz et al. (2009), measured at the end of normal expiration; they did not employ any intertester TEMs. It is important to recognize that all such studies have adopted on-screen caliper approaches to measure tissue depth as individual measurements.

The use of the novel technique of the present study has several advantages. It utilizes regions of interest and generates several hundred depth measures within it, and automatically generates a statistical characteristic of the region (mean, median, SD, minimum and maximum values). The approach uses a thick layer of gel, which has not been specifically identified in previous work (although it is recognized that this may have been applied in practice). Crucially, however, the major advance of the Müller et al. (2009), approach is its ability to quantify the imbedded connective tissue which cannot be achieved with the use of a skinfold caliper. This in combination with the appropriately selected sites chosen to enable the highest quality images make the new novel technique ideal for formation of predictive equations against any criterion approach. In the present study, air displacement plethysmography was selected, and is the gold standard for volume and % fat determination via the 2 compartment model. Some limitations include the fact that tissues' densities vary according to age; ethnicity and the activity of an individual (Stewart, 2003). This alters bone density and ultimately the average density of the fat free mass.

Within the limitations of the 2-compartment approach, it is an ideal tool for prediction of % fat, as can be found in the low standard error of estimate (SEE = 2.91) and high explained variance (R2 = 0.845) in the prediction equation of this study. Although Durnin & Womersley (1974) and Jackson & Pollock (1978), provided earlier equations using skinfold thickness and UWW, they did not indicate explained variance (R2) in their equations. Eston *et al.* (2005), formed a predictive equation using skinfold thickness and DEXA and a 4C model using BIA and UWW in a sample of 31 females and 21 males and found explained variance to range from 0.67 to 0.96. This exposed the criticality of the thigh and calf sites for measurement, in addition to the more frequently used skinfold sites. The sites selected in the present study for the equation were above the external oblique muscle and distal triceps muscle both with imbedded connective tissue excluded. This suggests that discounting connective tissue within the site of measurement increased the accuracy of the determination of % fat. Although there have been equations derived from ultrasound using other criteria (Abe et al. 1994; Saito et al. 2003), the novel technique of the current study has several advantages especially in precision, accuracy, and validity (due to sampling of multiple measurements and convenience) (Muller et al. 2016).

CONCLUSION

Finally, apparently healthy adult females had greater % fat than their male counterparts, as well as still higher fat depth at every site measured. The use of ultrasound in combination with the software developed by Müller *et al.* (2009), was novel in the prediction of % fat air displacement plethysmography. As a predictor variable, ultrasound has equivalent accuracy and precision to much more expensive methods such as MRI and CT which are confined to limited medical facilities whose primary purpose is diagnostics. By contrast, the portability and affordability of ultrasound makes it suitable for research for field and clinical settings.

ACKNOWLEDGEMENTS

We acknowledge some peoples' contributions that made this research article a success such as Hau Yan Ng (Nikki) who cross-checked the data to avoid errors. We also appreciate our volunteers who readily gave their consent and complied with the measurement protocols. We also thank the Tertiary Education Trust Fund (TETFUND) Nigeria, for funding this project in Aberdeen through Ebonyi State University, Abakaliki, Nigeria. NJOKU, C. O.; OBASI, A. F.; OKPOSHI, F. T.; ANWARA, C. E. Y STEWART, A. D. Un nuevo enfoque analítico por ultrasonido: Una herramienta para el patrón de grasa y la predicción de la grasa corporal. *Int. J. Morphol.*, *43(1)*:175-181, 2025.

RESUMEN: Los métodos simples, económicos, seguros, confiables y precisos son adecuados para el control rutinario de la composición corporal. Este estudio tuvo como objetivo establecer que un nuevo ultrasonido puede cuantificar con precisión la grasa subcutánea en diferentes ubicaciones del cuerpo, y el uso de la pletismografía por desplazamiento de aire (Bod pod) como referencia puede predecir la grasa corporal. Se reclutó una muestra de 91 adultos (58 hombres y 33 mujeres), entre 18 y 44 años de edad para la investigación. Todos los participantes tomaron parte en las mediciones del Bod pod. La masa corporal de cada participante se obtuvo utilizando una báscula electrónica del sistema Bod pod. Cada participante fue marcado con un lápiz demográfico en los 9 sitios seleccionados en el lado derecho del cuerpo. Cada sitio fue escaneado utilizando un escáner de ultrasonido y una sonda plana de 10 MHz. La pletismografía por desplazamiento de aire cuantificó la masa grasa en kg y el % de grasa de los participantes. La ecografía cuantificó la profundidad de la grasa en cada sitio, tanto con tejido conectivo intercalado como sin tejido conectivo. La parte lateral del muslo tuvo la mayor profundidad de grasa, mientras que el braquiorradial tuvo la menor profundidad de grasa. Las mujeres tuvieron una mayor profundidad media de SAT que los hombres en todos los sitios, tanto para el tejido conectivo incluido como excluido. Se utilizó la profundidad de la grasa por ecografía para cada región, tanto incluyendo como excluyendo el tejido conectivo, con un término de sexo para tener en cuenta las diferencias entre hombres y mujeres, para predecir el % de grasa a partir de la determinación de la cápsula corporal mediante regresión lineal escalonada. La grasa corporal se puede predecir en una muestra mixta con gran precisión. La exclusión del tejido conectivo incrustado y la eliminación de la compresión pueden hacer que el enfoque ecográfico sea más válido.

PALABRAS CLAVE: Grasa corporal; Ecografía; Grasa subcutánea; Pletismografía por desplazamiento de aire.

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