

# Reproducibility of ultrasonography as an assessment tool for abdominal adipose tissue

## *Reprodutibilidade da ultrassonografia como ferramenta de avaliação do tecido adiposo abdominal*

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**Abstract** - The growing demand for practical, accessible, and clinically meaningful diagnostic strategies to assess visceral adiposity has spurred the exploration of alternative methods for evaluating body composition. This study aimed to examine the intra- and inter-rater reproducibility of ultrasonography (US) in precisely estimating abdominal adipose tissue within both its visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) compartments. Conducted between 2020 and 2021, this methodological study encompassed a diverse group of adult individuals, aged 20 to 59, representing both sexes. The assessment of VAT and SAT involved a US scan across three distinct anatomical sites within the abdominal region. The analysis of ultrasound measurements exhibited robust intra- and inter-rater concordance, quantified by the Intra-Class Correlation Coefficient (ICC) exceeding the threshold for excellent agreement ( $\geq 0.971$ ). This strong agreement was reaffirmed by the Bland-Altman plots, while the linear regression line underscored the consistent symmetry among the measurements. The present study confirms the high reproducibility of ultrasound in estimating visceral adiposity, an important predictor of cardiovascular and metabolic risk.

**Key words:** Intra-abdominal fat; Subcutaneous fat, abdominal; Ultrasonography.

**Resumo** - O aumento na demanda por estratégias diagnósticas práticas, acessíveis e clinicamente relevantes na avaliação da adiposidade visceral impulsionou a utilização de métodos alternativos de avaliação da composição corporal. Este estudo objetivou avaliar a reproducibilidade intra-avaliadores e interavaliadores da ultrassonografia (USG) na estimativa do tecido adiposo abdominal, nos seus compartimentos do tecido adiposo visceral (TAV) e tecido adiposo subcutâneo (TAS). Trata-se de um estudo metodológico, realizado entre 2020 e 2021, com indivíduos adultos, com idade entre 20 e 59 anos, de ambos os sexos. O TAV e o TAS foram avaliados por USG através de uma varredura da região abdominal em três sítios anatômicos abdominais. A avaliação da reproducibilidade das medidas de USG mostrou alta concordância intra-avaliadores e interavaliadores, com Coeficiente de Correlação Intraclass (CCI) na faixa de concordância excelente ( $\geq 0,971$ ), confirmada pelos gráficos de Bland-Altman, e adequada simetria entre as medidas evidenciadas pela reta de regressão linear. O presente estudo confirma a alta reproducibilidade da USG na estimativa da adiposidade visceral, importante preditor do risco cardiovascular e metabólico.

**Palavras-chave:** Gordura Intra-abdominal; Gordura subcutânea abdominal; Ultrassonografia.

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## INTRODUCTION

Abdominal obesity is recognized as one of the primary risk factors for the appearance of cardiovascular and metabolic complications. Abdominal adipose tissue, in its visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) compartments, has been identified as an organ with significant endocrine and metabolic activity. Consequently, there is a growing interest within the scientific community in quantifying intra-abdominal adiposity<sup>1,2</sup>.

A variety of methods are available for evaluating body fat and its distribution. Imaging examinations are particularly recommended due to their capacity for in-depth analysis of body composition<sup>3-5</sup>. Computed tomography (CT) and magnetic resonance imaging (MRI) are considered reference procedures for assessing VAT, as they offer the capability to accurately quantify subcutaneous and visceral fat separately<sup>3,4</sup>.

Nevertheless, these diagnostic tests possess disadvantages that reduce their application in clinical practice and large-scale epidemiological studies. These disadvantages include high expenses, restricted accessibility, potential exposure to ionizing radiation (in the context of CT), as well as the intricate and complex methodology associated with these techniques<sup>6,7</sup>.

The growing demand for practical, accessible, and clinically relevant diagnostic strategies in assessing visceral adiposity has driven the adoption of alternative methods for evaluating body composition. In this regard, ultrasound (US) has increasingly been employed as a non-invasive imaging method for assessing intra-abdominal adipose tissue<sup>5</sup>.

US enables the assessment of body compartments across several sections, simplifying abdominal region scans and a comprehensive understanding of the distribution of subcutaneous and visceral adipose tissues<sup>4,8</sup>. Several studies have shown a strong correlation between US with MRI and CT, positioning it as an alternative technique that is valuable, accessible, and safe for evaluating abdominal adipose tissue<sup>9,10</sup>.

However, the accuracy of the effective US screening depends on the technical skill of the evaluator<sup>6,11</sup>. As a result, researchers continually seek to verify the reliability of this diagnostic method in both quantifying and characterizing abdominal adiposity. Thus, the central aim of this study was to evaluate the consistency among different evaluators and within the same evaluator in estimating abdominal adiposity (both VAT and SAT) through the use of US imaging.

## METHODS

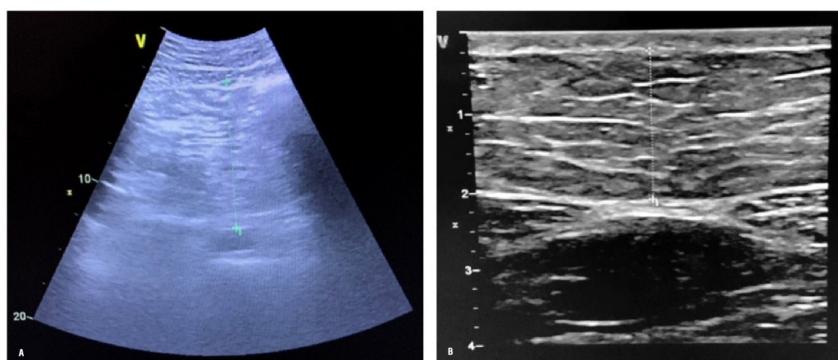
This is a methodological study conducted between 2020 and 2021. The participants were adults aged between 20 and 59 years, encompassing both sexes, and they were recruited from a public hospital in Northeast Brazil. Individuals with physical or clinical conditions that could hinder anthropometric measurements and ultrasound assessments were excluded from the study. Such conditions included edema, ascites, anasarca, hepatomegaly, splenomegaly, pregnancy, women who had given birth within the last 6 months before the study, and those who had undergone abdominal surgery within the past 6 months.

The body mass index (BMI) was calculated using the equation: Weight (kg)/Height (m)<sup>2</sup>. Height was measured using a portable stadiometer (Tonelli Ltda.), with a precision of 1mm. Participants were measured in an upright

position, with feet together and barefoot, arms hanging loosely at their sides, heels, back, and head touching the vertical column of the equipment, and the head oriented in the Frankfurt plane. Weight was measured using a digital scale (Model FILIZOLA), with a capacity of 150 kg and a precision of 100 grams. Participants were barefoot, in an upright position, wearing minimal clothing, and facing the measurement scale.

Waist Circumference (WC) was measured using an inelastic, flexible tape measure with an accuracy of 0.1 cm. The measurement was taken directly on the skin in a horizontal plane around the abdomen, at the narrowest point between the iliac crest and the last rib<sup>12</sup>. Anthropometric measurements were collected in duplicates and repeated when the measurement error between them exceeded 1 cm or 100 g. The final measurement used was the average of the two closest values.

The VAT and SAT were assessed using ultrasound, with the Vivid T8 Pro Color Doppler Ultrasound System (GE, P.O., Asia). The visceral fat thickness was estimated by measuring, in centimeters, the greatest distance between the inner (deep) face of the rectus abdominis muscle and the anterior wall of the aorta. For subcutaneous fat thickness, the distance in centimeters between the skin and the upper surface of the linea alba was measured<sup>9,13</sup> (Figure 1).



**Figure 1.** **A:** Greatest distance between the inner face of the rectus abdominis muscle and the anterior wall of the aorta. **B:** Distance between the skin and the upper surface of the linea alba.

All participants were evaluated in a supine position, with their right arm raised, and having fasted for a minimum of 4 hours<sup>9,14</sup>. The convex electronic transducer at a frequency of 3.5 MHz and the linear transducer at a frequency of 6.0 MHz were positioned transversely, aiming for a longitudinal scan from the xiphoid process to the umbilical level along the linea alba<sup>8,15</sup>.

As a reference for conducting the abdominal region scan, the measurements of VAT and SAT were taken at the following external landmarks: a) The narrowest point between the iliac crest and the last rib (VAT<sub>1</sub>, SAT<sub>1</sub>)<sup>12,16</sup>; b) The midpoint between the last rib and the iliac crest (VAT<sub>2</sub>, SAT<sub>2</sub>)<sup>17,18</sup>; and c) 1 cm above the umbilical scar (VAT<sub>3</sub>, SAT<sub>3</sub>)<sup>13,19</sup>. The measurements were taken while the individual was in an exhalation phase and without applying pressure on the abdomen, to avoid underestimating the results. Each measurement was conducted in triplicate and repeated if the measurement error exceeded 0.1 cm<sup>9,17</sup>.

The measurements were carried out by two trained evaluators in strict adherence to the technical protocols for body composition assessment using ultrasound. Evaluator 1, a certified fitness expert, had undergone prior training

conducted by a radiologist physician. Evaluator 2, a nutritionist, received a comprehensive four-day training under the guidance of Evaluator 1.

The data were entered into an Excel spreadsheet and subsequently imported into the Statistical Package for the Social Sciences (SPSS) version 22.0 (SPSS Inc., Chicago, IL, USA) for statistical analysis. To describe the sample, the data were presented in terms of median and interquartile range (25th and 75th percentiles).

Intra-rater reproducibility was determined by considering the triplicate measurements obtained by the primary evaluator at two different time points. On the other hand, inter-rater reproducibility (between the two evaluators) was estimated by using the mean as the central measurement for the three readings obtained by each evaluator. Similarly, the intra-rater reproducibility was determined by a single evaluator at two separate time points, under the three anatomical sites assessed.

The assessment of intra- and inter-rater reproducibility of the US was carried out by calculating the Intra-Class Correlation Coefficient (ICC) and generating a Bland-Altman scatter plot<sup>20</sup>, which involved checking the symmetry of measurements within the reference range on the plot using a linear regression line. The statistical significance level adopted for the analyses was 0.05.

## RESULTS

US imaging for assessing abdominal adiposity was conducted on a non-probabilistic sample of 15 individuals, whose demographic and body composition characteristics are presented in Table 1.

**Table 1.** Characteristics of the sample of 15 adult individuals recruited from a public hospital in Northeast Brazil during the period from 2020 to 2021, categorized by sex.

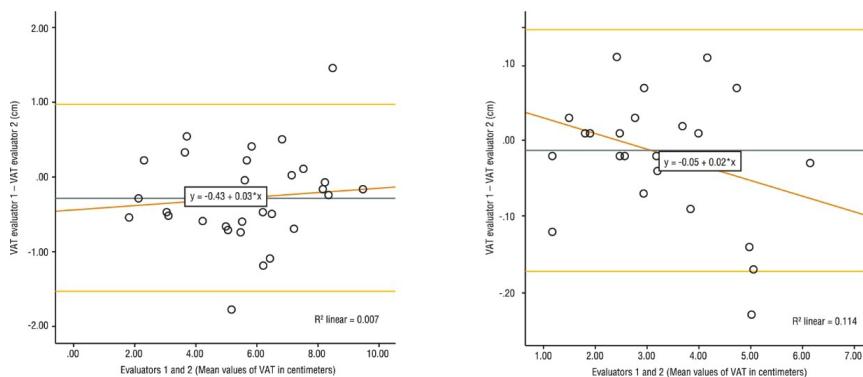
	Men (4)	Women (11)
	Median (1st quartile – 3rd quartile)	Median (1st quartile – 3rd quartile)
Age (Years old)	40 (29 - 48)	43 (39 - 51)
Weight (Kg)	94.7 (70.2 - 110.4)	69.2 (55.6 - 91.7)
BMI (Kg/m <sup>2</sup> )	29.6 (21.8 - 33.1)	26.0 (24.6 - 36.3)
WC (cm)	95.3 (71.5 - 105.6)	83.8 (81.0 - 102.4)
VAT (cm)	6.5 (3.6 - 9.8)	3.5 (2.0 - 7.5)
SAT (cm)	2.7 (0.8 - 2.6)	3.3 (1.8 - 5.3)

Note. BMI: Body mass index; WC: Waist circumference; VAT: Visceral adipose tissue; SAT: Subcutaneous adipose tissue.

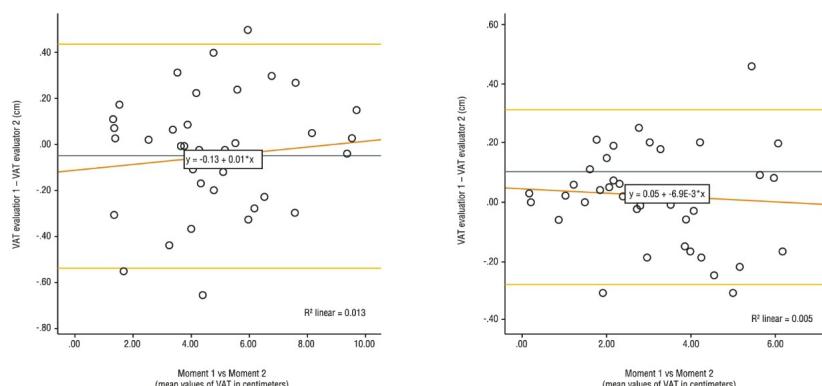
The assessment of US measurements' reproducibility demonstrated strong intra- and inter-rater agreement, with an ICC falling within the range of excellent concordance ( $\geq 0.971$ ). This was further substantiated by the Bland-Altman plots, which exhibited suitable symmetry among measurements, as evidenced by the linear regression line. This can be observed in Figures 2 to 3.

## DISCUSSION

The assessment of US measurements' reproducibility demonstrated strong intra- and inter-rater agreement, with an ICC falling within the range of excellent concordance ( $\geq 0.971$ ). This was further substantiated by the Bland-Altman plots, which exhibited suitable symmetry among measurements, as evidenced by the linear regression line. This can be observed in Figures 2 to 3.



**Figure 2.** Inter-rater reproducibility of visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) measurements obtained by US in adult individuals of both sexes from 2020 to 2021.



**Figure 3.** Intra-rater reproducibility of visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) measurements obtained by US at two different time points in adult individuals of both sexes from 2020 to 2021.

With the growing employment of US for assessing the distribution and quantification of abdominal fat, particularly intra-abdominal adiposity, the importance of methodological evaluation and standardization has escalated. This is crucial for reducing and managing sources of error, thereby guaranteeing dependable and high-quality outcomes<sup>4,21</sup>. As an evaluator-dependent method, researchers aim to not only achieve methodological standardization but also assess the accuracy and reliability of USG. These factors are considered sources of variability that need to be minimized to enhance the precision of this method<sup>21</sup>.

Our investigation assessed technical aspects regarding the use of US in characterizing abdominal adiposity. Through abdominal region scanning, the study demonstrated high intra- and inter-rater agreement in evaluating the reproducibility of VAT and SAT measurements taken at various anatomical sites within the abdomen. This confirms the excellent reproducibility of this imaging assessment method, regardless of the specific external anatomical site considered.

Other researchers have reported reliability results similar to those found in our study<sup>9,17,22</sup>. In a study that examined the consistency of using US to evaluate abdominal fat compartments in healthy individuals, utilizing the same VAT and SAT measurement method as employed in our research, Mauad et al.<sup>9</sup> discovered strong reproducibility. They identified exceptional agreement coefficients between examiners, both within the same examiner and across different examiners. The absolute values estimated were 0.94 for SAT measurement and 0.99 for VAT measurement, respectively.

Schlecht et al.<sup>22</sup> assessed the reproducibility of US in quantifying abdominal adipose compartments and emphasized the high intra- and inter-examiner reproducibility. They reported intra-examiner ICCs exceeding 0.99 for both VAT and SAT measurements, and inter-examiner ICCs of 0.989 for SAT and 0.998 for VAT measurements. Studies that assessed intra- and inter-examiner variability in quantitative measures of abdominal fat content within a population at high risk of type 2 diabetes, using US, reported coefficients of variation between observers ranging from 3.4% to 4.0% for VAT and from 4.2% to 9.5% for SAT<sup>17</sup>.

Studies that assessed the methodological validity of US also reported satisfactory outcomes. Pimanov et al.<sup>23</sup> evaluated the correlation between VAT measurements obtained using ultrasound at different anatomical points and computed tomography (CT) in individuals with metabolic syndrome (MS). They identified a statistically significant correlation between the ultrasound and CT techniques in assessing VAT. In line with these findings, Gadalla et al.<sup>24</sup> reported a high correlation between VAT and SAT measurements obtained through ultrasound and CT, with correlation coefficients of 0.921 for VAT and 0.988 for SAT, confirming the role of ultrasound in providing valid estimates of various abdominal adipose tissues.

These findings strengthen the role of US as a reliable tool for monitoring abdominal adiposity. In addition to its proven high reproducibility and accuracy, ultrasound offers further benefits such as accessibility, minimal invasiveness, ease of use, and the absence of ionizing radiation exposure. These advantages enable periodic reevaluation, making US a precious method for tracking abdominal fat changes over time<sup>25,26</sup>. Furthermore, the use of US has the potential to detect even subtle changes in different abdominal adipose compartments. It allows for scanning the abdominal region, leading to a better understanding of the distribution of SAT and VAT<sup>4,8</sup>.

Therefore, we emphasize the potential of using US as a precise and safe tool for the non-invasive monitoring of subcutaneous and visceral adipose tissues. When used at the appropriate time, it can contribute to the early implementation of preventive actions against complications associated with ectopic accumulation of body fat.

Our study has some limitations that need to be acknowledged. The sampling method employed was non-probabilistic, and the participants were selected through voluntary enrollment. Since this study constitutes a subset of a cross-sectional investigation, the relatively small participant pool and the recruitment process itself could be viewed as limitations. The constrained sample size precludes meaningful stratification, leading to diminished statistical power and potential implications for the external generalizability of the outcomes.

Nonetheless, it is noteworthy that our findings align with numerous previously published results. To address these potential limitations, we opted to employ two distinct reliability assessment methods in our data analysis framework. This approach aimed to offer both confirmatory insights and a more comprehensive understanding of the outcomes, ultimately enhancing the comparability of our results with those derived from other studies in the literature that employed at least one of the two reproducibility analysis methods used in our investigation.

We underscore this investigation's positive aspects, including the evaluators' prior training and the comprehensive abdominal scanning approach. The enabled measurements of VAT and SAT at various abdominal anatomical points and, consequently, facilitated an assessment of reproducibility across different

sites within the abdominal region. We recognize that conducting additional intra- and inter-rater agreement analyses stratified by VAT and SAT subgroups would contribute to the discussion on this topic. Such analyses could assess the reproducibility variability of measurements based on the proportion of subcutaneous and visceral adipose tissues. However, the limited number of study participants could somewhat constrain the statistical power of certain analyses. Consequently, reliability studies of this nature should ideally be undertaken with a broader spectrum of participants, encompassing diverse age groups, genders, ethnicities, and states of normal weight, undernutrition, and overweight.

## CONCLUSION

Our study demonstrated strong intra- and inter-rater reliability of the ultrasound procedure for analyzing different abdominal adipose tissues, confirming the high reproducibility of this method in estimating visceral adiposity. Visceral adiposity is a significant predictor of cardiovascular and metabolic risk.

## Compliance with ethical standards

### Funding

This study received financial support from the *Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco – FACEPE* (Financial code: IBPG-0303-4.05/19).

### Ethical approval

Ethical approval was obtained from the Ethics and Research Committee on Human Beings of the University of Pernambuco (UPE), being approved under protocol number 2.630.006/2018, and the protocol was written in accordance with the standards established by the Declaration of Helsinki.

### Conflict of interest statement

The authors have no conflict of interests to declare.

### Author Contributions

Conception and design of the experiment: CPSP and ASD. Realization of the experiments: NFS and FLA. Data analysis: NFS and CPSP. Contribution with reagents/research materials/analysis tools: FLA. Article Writing: NFS, ASD and CPSP. All authors read and approved the final version of the manuscript.

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