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Evaluation and Validation of Fat Thickness Measurement Using Ultrasonography in Male Rats with Obesity Fat Thickness Measurement in Obese Male Rats

Sulistyani Kusumaningrum*, Ambar Mudigdo, Bambang Purwanto, Dono Indarto, Florensia Syntesa Haryta, Maryama Aisyah Pribadi
Universitas Sebelas Maret, Indonesia
Email : maryama@gmail.com*

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ABSTRACT

Obesity is a global health concern and a major risk factor for metabolic and degenerative diseases. Conventional anthropometric measurements cannot adequately characterize variations in adipose tissue distribution, highlighting the potential value of imaging techniques. This study aimed to evaluate and validate ultrasonography (US) for measuring subcutaneous fat thickness in an experimental rat model of obesity. A completely randomized experimental design was employed, involving 24 male Sprague-Dawley rats divided into control and obesity groups (n = 12 per group). The obesity group received a high-fat, high-fructose (HFHFr) dietary intervention for 30 days. Body weight (BW), body length (BL), Rohrer index (RI), body fat content (BFC), subcutaneous fat thickness (SFT), and wet fat weight (WFW) were assessed. The obesity group exhibited significant increases in BW, RI, BFC, SFT, and WFW compared with the control group. An SFT threshold of ≥1.57 mm corresponded to the RI-based obesity classification, yielding a sensitivity of 83.33% and specificity of 100%. The optimal SFT cutoff for identifying obesity was 1.54 mm. Ultrasonographically measured SFT demonstrated strong positive correlations with BW (r = 0.886), RI (r = 0.889), BFC (r = 0.888), and WFW (r = 0.852). These findings indicated that the 30-day HFHFr dietary intervention was associated with increased adiposity and that portable ultrasonography provided a promising noninvasive method for assessing abdominal subcutaneous fat thickness in male Sprague-Dawley rats. Further research is needed to establish the reproducibility of these measurements, validate their diagnostic performance in larger animal samples, and determine their potential relevance to assessing abdominal adiposity in humans.

INTRODUCTION

Obesity has become a major global public health challenge in the 21st century. According to the World Obesity Atlas 2023, the global prevalence of obesity was projected to increase from 14% in 2020 to 24% by 2035, with approximately 2 billion people expected to be living with obesity by 2035. In the Southeast Asian region, the prevalence of obesity among children and adolescents was projected to increase substantially between 2020 and 2035, from 3% to 11% among girls and from 5% to 16% among boys. Similarly, adult obesity prevalence was projected to increase from 4% to 10% among men and from 8% to 16% among women

during the same period. In Indonesia, obesity prevalence was projected to increase from 5.8% to 7.9% by 2035 (Lobstein et al., 2023).

Obesity is a major risk factor for metabolic and degenerative diseases, including cardiovascular disease, type 2 diabetes mellitus, dyslipidemia, obstructive sleep apnea, and several types of cancer (Masood & Moorthy, 2023). Cardiovascular diseases (CVDs) are the leading cause of death worldwide, accounting for approximately 17.9 million deaths annually (World Health Organization, 2021). In Indonesia, CVDs were responsible for an estimated 651,481 deaths in 2019, representing a substantial national disease burden (World Heart Federation, n.d.). Obesity is also strongly associated with type 2 diabetes mellitus, a metabolic disorder characterized by chronic hyperglycemia resulting from insulin resistance, impaired pancreatic β-cell function, or a combination of both (GBD 2021 Diabetes Collaborators, 2023).

Two major theoretical frameworks have been proposed to explain the pathogenesis of obesity: the energy balance model (EBM) and the carbohydrate-insulin model (CIM) (Hall et al., 2022; Ludwig & Ebbeling, 2018). The EBM emphasizes the role of sustained positive energy balance, in which energy intake exceeds energy expenditure, resulting in the accumulation of excess adipose tissue (Hall et al., 2022). In contrast, the CIM proposes that dietary carbohydrates, particularly those with a high glycemic load, influence hormonal responses that promote energy storage in adipose tissue and may subsequently increase hunger and food intake. Appetite regulation involves complex interactions between peripheral hormones, including ghrelin, and hypothalamic pathways involving neuropeptide Y (NPY) and agouti-related peptide (AgRP) neurons (Sangiorgi et al., 2021). Accordingly, weight management strategies based on the EBM generally emphasize reducing energy intake and increasing energy expenditure, whereas the CIM highlights the potential role of reducing dietary glycemic load in regulating fat accumulation (Ludwig et al., 2022).

In clinical and community settings, obesity is commonly assessed using anthropometric measurements, including body weight (kg), height (cm), body mass index (BMI; kg/m^2), waist circumference (cm), and waist-to-height ratio, with a value above 0.5 commonly used as a screening threshold for increased cardiometabolic risk. In experimental animals, obesity-related assessments include the Rohrer index (g/cm^3), Lee index ($g^{1/3}/cm$), TM index ($g/cm^{2.823}$), body fat content ($g/cm^{2.823}$), and visceral fat mass (g) (Lee et al., 2011). However, anthropometric indices alone cannot adequately characterize differences in adipose tissue distribution. Therefore, imaging techniques, including dual-energy X-ray absorptiometry (DXA) and computed tomography (CT), are used to assess body composition and regional fat accumulation. DXA provides measurements of fat mass, lean soft tissue mass, and bone mineral content, with high precision under standardized conditions (Baek et al., 2020). CT enables detailed assessment of regional adipose tissue distribution, including pericardial, intrathoracic, and epicardial fat, which may contribute to cardiometabolic risk assessment (Eastwood et al., 2013). Nevertheless, both DXA and CT involve exposure to ionizing radiation, making radiation protection an important consideration, particularly during repeated examinations (Cornacchia et al., 2020). Ultrasonography (US) offers an alternative imaging modality that uses nonionizing sound waves to visualize anatomical structures and assess soft tissues (Wang et al., 2021). Its advantages include relatively low cost, portability, rapid

examination, and the absence of ionizing radiation. However, standardized ultrasonographic thresholds for classifying obesity, particularly in experimental animal models, remain insufficiently established (Wang et al., 2014). Therefore, this study aimed to evaluate and validate portable ultrasonography for measuring abdominal subcutaneous fat thickness in male rats with experimentally induced obesity.

METHOD

Materials

Fructose powder and commercial broiler feed (Comfeed) were purchased from local suppliers in Surakarta, Indonesia. Distilled water was obtained from the Technical Implementation Unit (UPT) Laboratory at Universitas Sebelas Maret (UNS), Surakarta. Alcohol swabs were purchased from PT Kimia Farma, Jakarta, while ultrasound gel was supplied by the Department of Radiology at Dr. Moewardi General Hospital, Surakarta. Subcutaneous fat thickness was measured using a Philips CX50 portable ultrasound system equipped with a 12-MHz linear transducer. Male Sprague-Dawley rats were obtained from CV Dunia Kaca, Ngargoyoso, Karanganyar Regency, Indonesia.

Experimental Design

This was a pilot study to generate an obesity rat model, which used a completely randomized design. Sample size was calculated using the formula: $(np-1) – (p-1) \geq p2$, n = rat number, and p = rat groups (Fraenkel & Wallen, 1993). We established two groups (control and obesity) to use at least three male rats per group. Because we evaluated whether a USG device could be used to examine fat thickness, we decided to use 12 male rats per group, which fulfilled inclusion criteria: two months old and weighted ± 300 g BW. Ethical clearance was approved by the Commission Ethics of Health Research, General Hospital (RSUD) Dr. Moewardi, Surakarta, with number 2.094/IX/HREC/2025.

Development of Obesity Rat Model

Before the research study began, all rats were adapted to four different cages for 7 days with 12 dark and light cycles, ambient temperature, and controlled humidity. High-fat high fructose (HFHFr) diet used in this study was adopted from a previous study (Sundari et al., 2022). The rat diet consisted of broiler-2 feed (BR-2), beef fat, duck egg yolk, chicken liver, and butter. Ten % (w/v) fructose was added into the drinking water, which was given to rats ad libitum. The control group was given the BR-2 feed (10% from BW) for 30 days, while the obesity group was given HFHFr feed with the same amount as the control group.

Measurements of Rohrer Index and Body Fat Content

Monitoring BW and body length were routinely checked every morning using a BW scale (Sonic, Jakarta) and a stadiometer (Measure King, Jakarta). The Rohrer Index was calculated using Rohrer Index = {BW (g)/Naso-anal length (cm)3}x 103, in which the Rohrer Index was ≥ 30 g/cm3. Meanwhile, body fat percentage was calculated using a formula = 0,581 x TM Index-22,03, in which the TM Index was = BW (g)/Naso-anal length (cm)2,823 x 103 (Lee et al., 2011). After all rats were sacrificed, adipose tissues were removed from the abdominal region and directly measured using the weight scale. The collected adipose tissues were stored at -20oC before further analysis.

Measurement of Subcutaneous Fat

A portable USG device (Philips C5x) was used to measure abdominal fats, which referred to previous studies (Wagner, 2013; Marzola et al., 2016). In brief, the abdominal skin of rats was shaved with approximately 5 cm diameter and evenly applied for a USG gel. A Radiologist measured abdominal fat before and after administration of the HFHFr diet.

We compared the measurement of subcutaneous fat using the Rohrer Index and USG, which were evaluated with sensitivity and specificity values. The formula for sensitivity value was a/(a+c), while the specificity value was d/(d+b). Using both methods, we also used the positive predictive value: a/(a+b) and the negative predictive value: d/(d+c) to identify false positive and negative fat thickness measurements. The a,b,c, and d words were rat numbers with obesity, without obesity, false negative, and true negative, respectively. We also used the ROC curve to analyze the SFT limit value in determining whether rats could be considered obese. The formula for the cut-off value was Youden's J Statistic = Sensitivity + Specificity – 1.

Statistical Analysis

Collected data were analyzed using IBM SPSS (Statistical Package for the Social Sciences) Statistic 25 and presented as mean ± standard deviation. Mean comparison within groups was analyzed using a dependent T-test while between groups was analyzed using the independent T-test with p-value < 0.05 considered significantly different.

USG correlation test was conducted using Pearson Correlation Test with the condition that the data is normally distributed. The r result is used to determine the level of correlation between USG and BB, RI, BFC and WFW.

RESULTS AND DISCUSSION

Administration of HFHFr increased BW, RI, and BFC in male rats

We used HFHFr to induce obesity in male Sprague Dawley rats. Figure 1a-d indicated that HFHFr administration for 30 days significantly increased mean BW, RI, and BFC compared to the obesity group before treatment (p=0.001). Before HFHFr treatment, the mean BW of control group (315.91 ± 26.39 g) was higher than the mean BW of obesity group (299 ± 52.79 g) and it wasn't significant with p=0.332. After 30 days treatment, the mean BW of the obesity group (392 ± 40.44 g) was increased significantly compared to the mean BW of obesity group at day 1 treatment (p=0.001) and control group at day 3 (311.66 ± 58.64 g; p=0.001). In contrast to BW, the mean BL in control group (22.42 ± 1.16 cm) was lower than the mean BL of obesity group (21.87 ± 0.71 cm) and it wasn't significant (p= 0.183). The mean BL of the control group after 30 days of treatment (23.67 ± 0.98 cm) was higher than the mean BL of the obesity group (23.25 ± 0,89 cm), and it wasn't significant with p=0.289. Before HFHFr treatment, the mean RI of the control group (27.83 ± 1.09 g/cm3) was lower than the mean RI of the obesity group (28.51 ± 0.61 g/cm3), but it wasn't significant with p=0.074. After given HFHFr, the mean RI of the obesity group (31.11 ± 0.89 g/cm3) was higher than the mean RI of the control group (23.33 ± 2.42 g/cm3). The mean RI of the obesity group on day 30 was significantly compared with the mean RI of the obesity group on day 1 (p=0.001) and the control group on day 30 (p=0.001). The mean BFC of the control group on day 1 (6.00

± 1.13 g/cm2.823) was lower than the mean BFC of the obesity group on day 1 (6.56 ± 0.56 g/cm2.823), but it wasn't significant with p=0.143. After 30 days, the mean BFC of the obesity group was increased (9.50 ± 0.84 g/cm2.823), but the mean BFC of the control group was decreased (1.70 ± 2.50 g/cm2.823), and it was significant with p=0.001.

Comparison of Subcutaneous Fat Thickness in Control and Obesity Rat Groups and Comparison of Wet Fat Weight and Subcutaneous Fat Thickness Results using Ultrasonography

Figure 2a-b indicated that HFHFr is able to increase SFT. This can be seen in Figure 2b where the SFT of the obesity group (2.18 mm) is significantly thicker compared to the SFT of the control group (1.23 mm). This shows that USG can present the difference in SFT between the control group and the obese group. Figure 3a-b indicated that male rats given HFHFr increased in SFT and WFW. Before HFHFr treatment, the mean SFT of the control group (1.17 ± 0.09 mm) was lower than the obesity group (1.23 ± 0.1 mm), and it wasn't significant with p=0.130. After 30 days of treatment, the mean SFT of the obesity group (1.72 ± 0.19 mm) increased significantly compared to the mean BW of the obesity group at day 1 treatment ($p<0.001$) and the control group at day 30 (1.29 ± 0.07 mm) with $p<0.001$. After 30 days of male rats given HFHFr treatment, the mean WFW of the obesity group (4.98 ± 1.01 g) was higher than the mean WFW of the control group (2.31 ± 0.33 g), and it was significant with $p<0.001$.

Diagnostic Test of Ultrasonography Compare to Rohrer Index

Table 2 compares subcutaneous fat measurements using the RI calculation and the USG measurement. It was found that 12 male rats indicated obesity using the RI calculation, but two male rats in the obesity group had less than 1.57 mm SFT, while all male rats in the control groups had less than 1.57 mm SFT. This data is used to measure sensitivity, specificity, and positive and negative predictive values. After calculating subcutaneous fat (in the subcutaneous fat section), the sensitivity and specificity values were 83.33% and 100%, respectively. The positive and negative predictive values were 100% and 85.71% respectively. Further ROC analysis was used to determine a cut-off value for obesity (figure 4). We got ≥ 1.54 mm as a cut-off value for obesity.

Correlation between SFT with BW, RI, BFC and WFW

Figure 5a-d illustrates the correlation between subcutaneous fat thickness (SFT) measured using ultrasonography and other variables, including body weight (BW), Rohrer Index (RI), body fat content (BFC), and wet fat weight (WFW). The correlation between ultrasonography and BW (Figure 5a) was significantly different ($p < 0.001$) with a positive correlation of 0.886, indicating a strong relationship between ultrasonography and BW, where an increase in SFT corresponds to a higher BW in rats. Similarly, the correlation between ultrasonography and RI (Figure 5b) was also significantly different ($p < 0.001$) with a positive correlation of 0.889, suggesting a very strong relationship between ultrasonography and RI, where a thicker SFT is associated with a higher RI value. The correlation between ultrasonography and BFC (Figure 5c) was significantly different ($p < 0.001$) with a positive correlation of 0.888, indicating a strong relationship between ultrasonography and BFC, where a thicker SFT corresponds to a higher BFC in rats. Additionally, the correlation between

ultrasonography and WFW (Figure 5d) was significantly different ($p < 0.001$) with a positive correlation of 0.852, demonstrating a strong correlation between ultrasonography and WFW, where an increase in SFT is associated with a greater WFW in rats. The correlation level table is presented in table 1.

Table 1. Correlation Coefficient Interpretation Guidelines

Coefficient Interval	Level of Correlation
0,00 – 0,199	Very low
0,20 – 0,399	Low
0,40 – 0,599	Moderate
0,60 – 0,799	Strong
0,80 – 1,000	Very strong

Diagnostic Test of Ultrasonography Compare to Obesity Index

Table 2. Determinations of specificity, sensitivity, positive predictive value and negative predictive value between obesity index and ultrasound value.

		Obesity Index		Total
		Obese	**Not Obese**	
Ultrasound	**Obese**	10^a	0^b	10
	Not Obese	2^c	12^d	14
Total		12	12	24

Nd. A male rat in the obesity group with 30.05 OI calculation had 1.57 mm SFT. The a, b, c and d indicated rat number with obesity, without obesity, false negative and true negative respectively

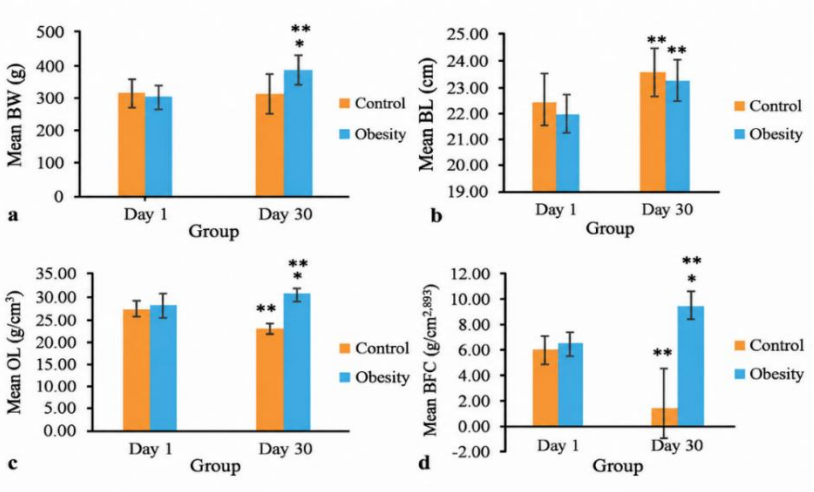


Figure 1. Body Weight, Body Length, Rohrer Index, and Body Fat Content Before and After the 30-Day Dietary Intervention

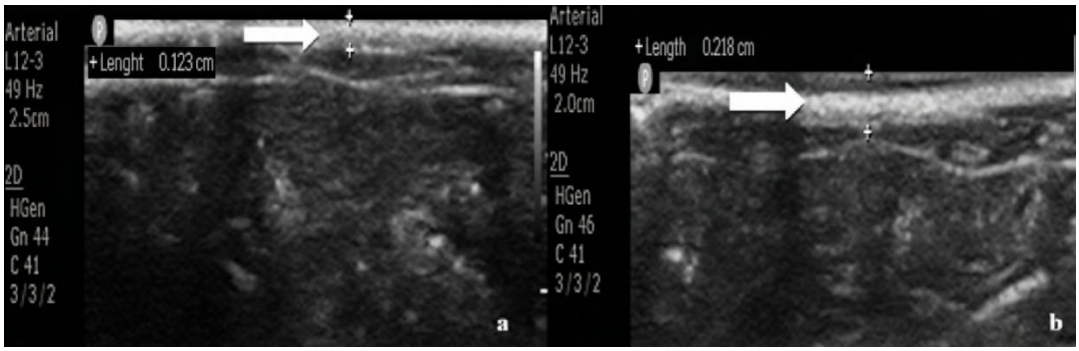


Figure 2. Representative Ultrasound Images of Subcutaneous Fat Thickness in Control and Obese Rats

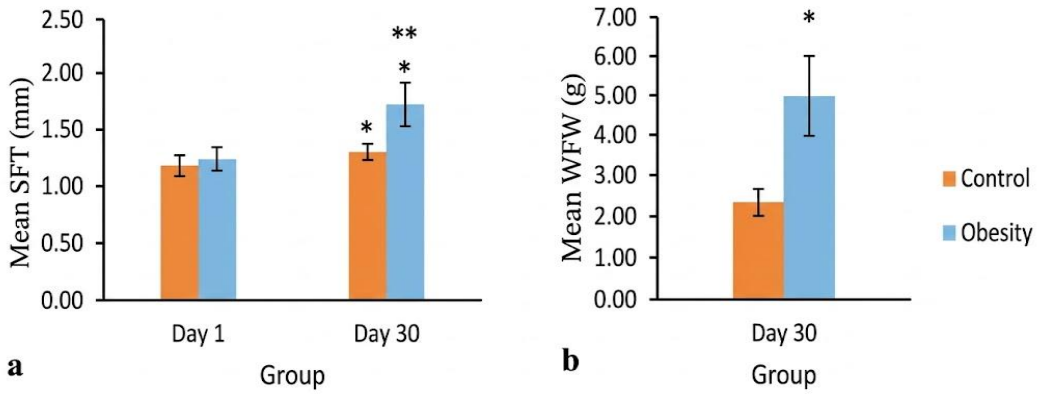


Figure 3. Subcutaneous Fat Thickness Before and After the Dietary Intervention and Wet Fat Weight at Day 30 in Control and Obese Rats

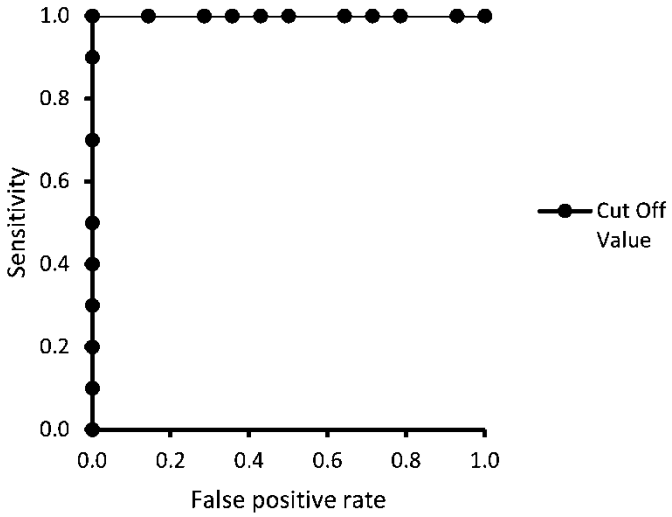


Figure 4. Receiver Operating Characteristic Curve for Ultrasound-Measured Subcutaneous Fat Thickness in Detecting Obesity

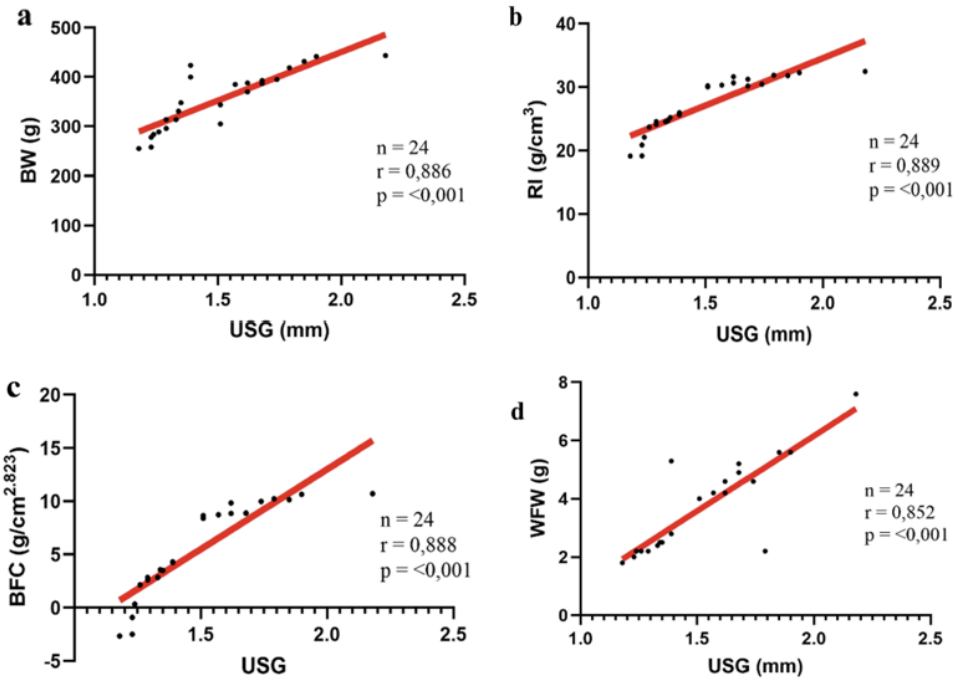


Figure 5. Correlations of Ultrasound-Measured Subcutaneous Fat Thickness with Body Weight, Rohrer Index, Body Fat Content, and Wet Fat Weight

We have evaluated BW, BL, RI, BFC, WFW, and SFT in male rats treated with HFHFr for 30 days. The mean BW of the obesity group significantly increased by 93 ± 14.05 g compared to the mean BW of the control group, which decreased by 4.25 ± 5.85 g. The increased BW corresponds to the increased RI values (≥30 g/cm3). Furthermore, the BFC and WFW measurements in the obesity group (9.50 ± 0.84 g/cm2.823 and 4.98 ± 1.01 g, respectively) follow the increased BW and RI values. The RI values in the obesity group also match the SFT measurements (≥ 1.57 mm), which have 83.33% and 100% sensitivity and specificity values, respectively. The cut-off of obesity using the SFT measurements was 1.54 mm. In addition, there is a positive correlation indicating a strong relationship between USG and BW, RI, BFC and WFW (0.886, 0.889, 0.888 and 0.852, respectively). Overall, our findings suggest that the USG device can be used to measure fat thickness in obese rats.

Excessive consumption of high carbohydrates and fats leads to weight gain and accumulation of abdominal fat. In vivo experiments, the RI and BFC are important parameters for evaluating obesity. Our results indicated that BW and RI increased by 93 ± 14.05 g and 31.11 ± 0.89 g/cm3 in male rats that received HFHFr for 30 days. The increased BW in our study is higher than the increased BW in a previous study (79.8 ± 7.7 g), but the RI in our study is lower than that of a previous study (34.22 ± 89 g/cm3). In addition, the BFC in our study (9.50 ± 0.84 g/cm2.823) was lower than in the previous study (11.33 ± 0.90 g/cm2.823) (Sundari et al., 2022). The different rat strains and initial BW perhaps cause discrepancies in both studies. The initial mean BW in our study was 299 ± 52.79 g at 8 weeks old, whereas the

mean BW in the previous study was 140.80 ± 3.32 g at 4-5 weeks old. Therefore, male rats in our study are older than those in the previous study, which results in lower growth and development. We used the Sprague-Dawley rat strain, while the previous study used the Wistar rat strain (Sundari et al., 2022). Energy consumption, weight gain, and fat gain are more pronounced in Wistar rat strains compared to the Sprague Dawley rat strains after receiving HFHFr (Marques et al., 2016). Another study reported that Wistar rat strains tend to accumulate more fats in adipose tissues receiving HFHFr compared to the Sprague Dawley rat strain (Miranda et al., 2018).

The RI indicator is commonly used for the evaluation of the obesity rat model, but it has a weakness in that it cannot determine the heterogeneity of fat deposition in the body. Therefore, another laboratory test, such as a radiological examination, is required. We found that obese male rats had >30 g/cm3 RI and ≥ 1.51 mm SFT with 83.33% and 100% sensitivity and specificity values. The cut-off for obesity was ≥ 1.54 mm SFT, which matched with 4.98 ± 1.01 g WFW. The SFT measurement using a portable USG has not been established yet, but another study showed that wet abdominal adipose tissues in the HFD rat group (9.53 ± 0.90 g) were significantly higher than that of the normal rat group with a given standard diet (6.06 ± 0.80 g) (Rusdiana et al., 2022). In addition, the average body fat weight in obese rats was 54.019 ± 14.32 g using the DXA, which was higher than that of wet tissues (39.29 ± 16.445 g) collected from subcutaneous, epididymal, kidney, and intestine tissues (54.019 ± 14.32 g) (Yeu et al., 2019). Therefore, the body fat measurement using USG can be an alternative method to evaluate obesity and body fat composition in obese rats.

Although the portable USG can be implemented for obesity evaluation, our study has several limitations. At first, portable USG for experimental animals was not available in our laboratory facilities, so we used a portable USG for human babies and the measurement of superficial body organs. Secondly, we must take care of rats properly when using the portable USG because it can cause stress and harm the researchers. Finally, the SFT measurement has to be conducted by researchers with expertise in radiological examination.

CONCLUSION

Administration of a high-fat, high-fructose (HFHFr) diet for 30 days increased body weight (BW), Rohrer index (RI), body fat content (BFC), and wet fat weight (WFW) in male Sprague-Dawley rats. Portable ultrasonography (US) demonstrated high sensitivity and specificity for assessing abdominal subcutaneous fat thickness in obese male rats. Further studies are needed to validate the reproducibility and diagnostic accuracy of this technique and to determine its potential applicability to the assessment of abdominal adiposity in humans. Appropriate anesthesia or sedation may be considered during ultrasonographic examinations to minimize animal movement, reduce stress, improve measurement consistency, and ensure researcher safety, subject to established animal welfare protocols.

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CONFLICT OF INTEREST

The authors declare that this study was conducted without any commercial or financial relationships that could be interpreted as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

Sulistyani Kusumaningrum[1] and Ambar Mudigdo[2]: research conceptualization, methodology design, experiment execution, data collection, data analysis, manuscript drafting, and manuscript revision. Bambang Purwanto[3], Dono Indarto[4], Florensia Syntesa Haryta[5] and Maryama Aisyah Pribadi[6]: research supervision, validation of research results, provision of scientific input, and manuscript review and revision. All authors have read and approved the final version of the manuscript.

GENERATIVE AI STATEMENT

The authors declare that generative artificial intelligence (AI) was used in the preparation of this manuscript to help improve linguistic clarity, correct grammar, and refine the writing structure. Generative AI was not used to generate, manipulate, or analyze research data. The entire content of the manuscript has been reviewed and verified by the author(s). The author(s) are fully responsible for the accuracy, integrity, and final content of the manuscript.

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