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Research Article

A cross-sectional analysis of dietary intake and abdominal visceral fat content measured by ultrasound sonography

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ABSTRACT

Background: The assessment of abdominal visceral fat (VF) warrants more applicable screening methods to detect individuals at risk of developing comorbidities. The purpose of this study was to identify a practical ultrasound sonography (US) protocol that can accurately estimate abdominal VF area (VFA, cm^2) and to examine how much of the abdominal VFA can be explained by dietary intake.

Methods: A 3-day dietary food recall followed by a dual-energy X-ray absorptiometry (DEXA) and US scan were performed in 30 young adults.

Results: The developed regression equation ($F[4, 25] = 46.869$, $P = 0.001$) was: (37.677 + [1.456 × Age] − [26.963 × Sex] − [11.336 × Region 4] + [13.554 × Region 6]), where regions 4 and 6 = transverse transducer placement 2 cm to the left and right of the superior border of the umbilicus, respectively (thickness defined as the distance in cm from the internal surface of the abdominal muscle to the anterior aortic wall). The regression equation had high accuracy (adjusted $R^2 = 0.864$) and test reliability ($r = 0.927$, $P < 0.001$) at estimating abdominal VFA (31.4 ± 21.4 cm^2) when compared to the abdominal VFA (31.1 ± 21.1 cm^2) measured by DEXA. The intake of added sugars, only in males, was positively associated ($r = 0.671$, $P = 0.048$) with abdominal VFA.

Conclusions: Sex, age, and two abdominal regions are sufficient to estimate abdominal VFA in healthy adults. Examining added sugar consumption

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over time could aid in identifying individuals with a greater risk of developing a greater abdominal VF content.

1. Introduction

A high abdominal visceral fat (VF) content is a concern because of its proximity to vital organs in the abdomen, making abdominal VF the most harmful type of fat within the body. The excess accumulation of abdominal VF results in a myriad of health problems that range from maladaptive metabolic disturbances to organ malfunction.[1] Although excess subcutaneous fat is detrimental and should not be ignored,[2] this manuscript focuses on the need for accurate, reliable, and accessible methods for assessing abdominal VF because, to date, there is a lack of applicable methods to examine it. The premise is to develop accessible methods for early screening of excess abdominal VF accumulation. Because abdominal VF is developed within the abdomen, it can take significant time to manifest as changes in abdominal volume. For example, an individual can have what is considered a healthy body mass index (BMI) (around 22 kg/m^2) and be asymptomatic of major comorbidities like type II diabetes and hypertension. However, if this individual maintains a sedentary lifestyle and is malnourished, their abdominal VF area (VFA) can increase above 100 cm^2 regardless of their otherwise healthy BMI. As previously documented, an abdominal VFA above 100 cm^2 predisposes individuals to type II diabetes and hypertension.[3,4] This demonstrates that BMI would not be an adequate measure to identify risk factors for an individual's health who faces a greater risk than those with higher BMI but an abdominal VFA below 100 cm.[2,5,6] The reason such situations can occur is because the total body fat percentage can increase linearly as abdominal VFA increases,[7] but abdominal VFA can also increase disproportionally to total body fat percentage.[8,9]

Understandably, the assessment of abdominal VF in healthy BMI individuals is not routinely performed but should receive more attention. A challenge with this recommendation is that validated assessments for abdominal VF are expensive, involve radiation, and are relatively inaccessible for regular use. Among research studies lending support to increasing the use of abdominal VF assessment in everyday healthcare, comparisons of computed tomography (CT), the gold standard for assessing body composition, and dual-energy X-ray absorptiometry (DEXA) have shown strong correlations in the accuracy of abdominal VF content estimations.[10,11] Other studies have shown that ultrasound sonography (US) can estimate abdominal VF content as accurately as a CT or DEXA scan.[12–15] However, the use of US is limited by its difficulty. Advanced technical expertise is necessary for the assessment of abdominal VF content when employing US. A few US protocols have been developed to assess abdominal VF content,[12,14,16,17] but no standardized method has been well-defined or validated. This is one major hurdle that has prevented US from becoming widely used to assess abdominal VF content and gaining popularity among practitioners. If validated, however, US can serve as a significant tool to assess people's body composition due to its portability, increased accessibility, and lack of radiation exposure that allows for increased frequency of use.

Beyond a sedentary lifestyle, an individual's diet has a strong relationship with the development of abdominal VF. Excess caloric intake is not the only factor that will increase someone's likelihood of excess abdominal VF accumulation. Rather, the quality of the diet and its composition plays a significant role. Most Western-style diets place a greater hepatic postprandial metabolic reliance due to greater consumption of added sugars, fats, and salt, compared to a Mediterranean or a reduced ultra-processed diet.[18,19] The increased intake of these three nutrients promotes upregulation of processes such as *de novo* lipogenesis [18,20] that exacerbate the development of abdominal VF. That is in part why the pervasiveness of modernized food production has been seen with a consistent rise in obesity and abdominal VF adiposity. With the need to increase awareness about the importance of abdominal VF content and its assessment, the primary goal of this study was to further develop previously postulated US protocols and exemplify the potential that US has in determining abdominal VF content. The secondary goal of this study was to investigate the relationship between participants' dietary intake, specifically saturated fat and added sugars, with abdominal VFA.

2. Methods

2.1. Participants and design

Prospective recruitment for this research study was open to all age, sex, and ethnic groups. The only exclusion criterion was pregnancy. An a priori power analysis conducted with G*Power 3.1.9.7 (Universität Kiel, Germany) [21] using the squared correlation coefficients of similar studies [12,16,17] revealed that 13 participants were needed to achieve a power of 84% with four predictors for a multiple regression analysis using an alpha level of 0.05. A total of 30 healthy (no reported comorbidities), white adults were randomly recruited to participate in this study that consisted of a single visit with consecutive measurements. The sex breakdown for participants was nine males (27.1 ± 11.8 years) and 21 females (21.5 ± 3.6 years). Prior to any data collection, participants gave their written informed consent to participate in the study which was approved (#1741657) by the Institutional Review Board at Baylor University. The study procedures and all data collection were conducted at Baylor University's Body Composition and Vascular Ultrasound Imaging Laboratory following the principles embodied in the *Declaration of Helsinki*. Participants fasted for a minimum of 8 h, while normal water intake was allowed prior to the US and DEXA scans.

2.2. Baseline values and body composition

Height (cm) and weight (kg) were assessed on a standard dual-beam balance scale with a height measuring rod (#703, Seca, Chino, CA, USA). Heart rate and blood pressure were measured on the non-dominant arm with an automated device (E-Sphyg 2, American Diagnostic Corporation, Hauppauge, NY, USA). Fat-free mass (FFM, kg), fat mass (FM, kg), and abdominal VFA were determined via DEXA (Horizon, Hologic, Marlborough, MA, USA). During the DEXA scan, participants rested in a supine position with both legs and arms against their bodies. From the total body scan, abdominal VFA was calculated with the built-in software provided by the manufacturer. Briefly, a rectangular area between the superior border of the iliac crests, superior border of L3, and lateral walls of the subcutaneous fat layer, was created for each participant. With this positioning, L4 was centered within the rectangular area, where abdominal VFA was assessed. Utilizing those landmarks is critical to have a standardized protocol, where L4 corresponds to the area with the greatest abundance of abdominal VFA development.[22]

In addition, the waist-to-hip ratio (WHR) was calculated by dividing the circumference in cm at the midpoint between the lowest point of the ribcage and the upper border of the iliac crest by the circumference in cm of the hips at the widest point. The girth measurements were performed in triplicate with a standard Gulick tape and recorded after normal exhalation while participants stood with their feet together and arms relaxed by their sides. If there was more than a 0.5 mm difference between the measurements, a fourth measurement was obtained. Measurements were averaged and assessed with at least 1 min in between measurements.

2.3. Ultrasound sonography

The thickness of nine different abdominal regions was measured via US (Logiq S7 Pro, GE Healthcare, Chicago, IL, USA) while participants rested in a supine position. All US measurements were collected using a 3.5 MHz phased-array transducer (3Sp-D, GE Healthcare, Chicago, IL, USA) in triplicate and averaged. The abdominal regions were measured with the built-in electronic caliper feature at the end of a normal exhalation with minimal/no pressure placed against the skin with the transducer. Because every ultrasound assessment was completed by the same trained researcher with over 12 months of experience working with a certified (ARDMS) ultrasound technician, no intraclass correlation determination between assessments was performed.

The first set of US measurements (regions 1–3): The first set of measurements replicated Hirooka et al.'s protocol [14] to estimate abdominal VFA. The measured abdominal regions in this protocol

included: (1) the distance between the internal surface of the abdominal muscle (ISAM) to the splenic vein, (2) the distance between the ISAM to the posterior aortic wall on the umbilicus, and (3) the thickness of the fat layer of the posterior right renal wall in the right posterior perinephric space. For region 1, Doppler was used if there were difficulties differentiating between the splenic vein and the superior mesenteric vein. For region 3, there were no difficulties collecting the measurements while participants were lying supine; however, if needed, participants can be placed on their side to increase the visibility of the kidney. The collected measurements (regions 1–3) were then inputted in Hirooka's abdominal VFA regression equation (VFA_{e1}) [14] to compare VFA_{e1} against our calculated abdominal VFA measured by DEXA (VFA_d).

The second set of US measurements (regions 4–9): The second set of measurements aimed to estimate abdominal VFA via the regression equation developed by the current study (VFA_{e2}). In the current study, the protocol utilized by Stolk et al., [12] Pimanov et al., [16] and Ribeiro-Filho et al. [23] were combined to make the transducer placements more specific to each abdominal region and increase the replicability for future studies and within participants. For instance, instead of using any location within 1–5 cm superior to the umbilicus, the current study defined 6 specific abdominal regions, which were 2 cm and 5 cm to the left, up, and right from the superior border of the umbilicus (Fig. 1A) while using a transverse view. At each abdominal region, the thickness was defined as the ISAM to the anterior aortic wall (Fig. 1B). The selected thickness included peritoneal fat, and therefore, mesenteric fat of the small intestine and omental fat were included within each measurement. The transducer placements covered the same area used to outline abdominal VFA within the DEXA scan. For accuracy, each abdominal region was measured and marked with a skin-friendly pen prior to the US assessments after participants rested in a supine position for 5 min.

2.4. Imaging analysis and data recording

After the completion of each participant's visit, all images (DEXA and US) were saved. After all 30 participants completed their visit, every image was analyzed in a blinded manner by a single researcher to minimize random errors and maintain internal consistency of measurements.

2.5. Dietary analysis

During each participant's visit, they were instructed on how to correctly and precisely record quantities and portion sizes of their meals, snacks, and fluids consumed on a paper log. Participants recorded their dietary intake for 3 days (2 weekdays and 1 weekend day). Each dietary log was analyzed using the

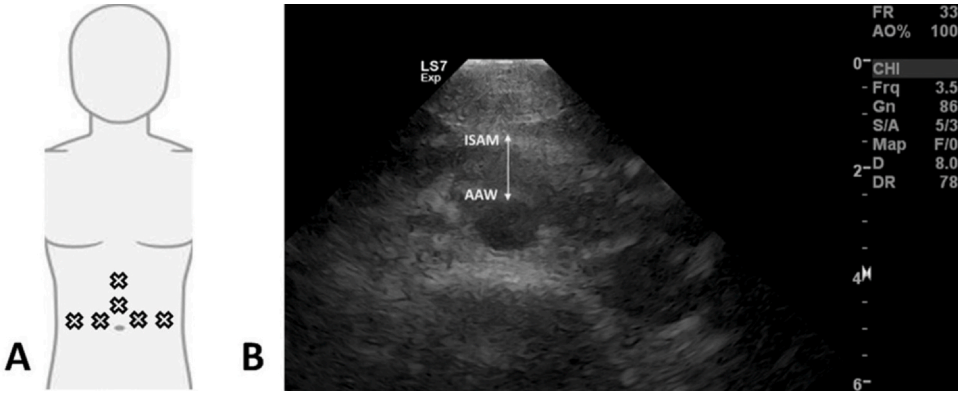

Fig. 1. (A) Denotes the probe placement for the second set of ultrasound measurements. (B) Denotes the distance used in the second set of ultrasound measurements to define thickness. From superior to inferior, the yellow arrow depicts the distance from the internal surface of the abdominal muscle (ISAM) to the anterior aortic wall (AAW).

software “Food Processor” (Esha Research, Salem, OR, USA) in a blind and randomized order. The average daily dietary intake for macronutrients, types of fat, and types of sugar were extracted and compared to the FM and abdominal VFA of participants. Importantly, this study is a cross-sectional analysis, and utilizing a 3-day dietary recall approach might not best represent the effect of longitudinal dietary intake on adiposity. However, a 3-day dietary recall approach increases both the validity and reliability of dietary recalls by avoiding the participant's subjectiveness/under-reporting that arises when recalling longer periods without the intention of remembering the information for later reporting.[24] If a study utilizes dietary recalls to track VF content changes over time, we recommend employing a repeated measures design with multiple time points of 3-day recall periods.[25]

2.6. Statistical analysis

All data were analyzed using IBM SPSS (Version 27.0, IBM Corp., Armonk, NY, USA) and are reported as mean ± standard deviation (SD) with a significance set at $P < 0.05$. A paired sample *t*-test was utilized to compare the estimation of VFA_{e1} against VFA_d. In addition, a Pearson correlation analysis was utilized to assess the relationship between anthropometric variables and the second set of US measurements with VFA_d.

Stepwise linear regression was utilized to develop a regression equation with VFA_d as the dependent variable. To construct the model, the second set of US measurements, along with age, sex, anthropometric variables, and vital signs were initially added to determine their impact against VFA_d. Thereafter, the model was re-created by only including meaningful variables, until only the significant variables were maintained to avoid inflating the model results. The developed regression equation was utilized to estimate the VFA of participants, referred to as VFA_{e2}. A Pearson correlation analysis was utilized to assess the relationship between VFA_{e2} and VFA_d because although it would be ideal to compare the level of agreement between VFA_{e2} and VFA_d via a Bland-Altman plot, this was not possible due to the difference in measurement units. A paired sample *t*-test was utilized to compare the estimation of VFA_{e2} against the VFA_d. Lastly, Pearson correlation analysis was utilized to examine the relationship between dietary intake and overall body composition. All data was normally distributed, had the independence of residuals, and met the assumption of normality assessed via Q-Q plots. The dataset was complete and no missing data points had to be corrected.

3. Results

3.1. Characteristics of the study population

The mean age (23.2 ± 7.4 years), BMI (22.3 ± 3.2 kg/m^2), systolic (119.2 ± 12.5 mmHg [1 mmHg = 0.133 kPa]) and diastolic (70.3 ± 9.1 mmHg) blood pressure, and resting heart rate (66.5 ± 13.3 bpm) suggested that all participants (Table 1) were healthy young adults. For body composition, the mean FFM (51.3 ± 13.0 kg) and FM (14.3 ± 3.7 kg) yielded an average body fat percentage of 22.3% ± 6.0% for all participants combined. Lastly, the mean WHR was 0.75 ± 0.50.

3.2. Replicated US protocol: Region 1–3 (first set of US measurements)

The first set of US measurements is displayed in Table 2. VFA_{e1} was significantly different than VFA_d ($t[29] = 14.957$, $P < 0.001$). Thereby, the regression equation replicated from another study (VFA_{e1}) was not accurate at estimating abdominal VFA in the population of this study (Table 2).

Table 1
Breakdown of participant's characteristics.

Variables	Males (n = 9)	Females (n = 21)
Age (years)	27.1 ± 11.8	21.5 ± 3.6
Body mass index (kg/m^2)	25.1 ± 4.0	21.2 ± 1.8
Systolic blood pressure (mmHg)	128.0 ± 13.4	115.5 ± 10.3
Diastolic blood pressure (mmHg)	70.2 ± 12.2	70.3 ± 7.8
Resting heart rate (bpm)	60.0 ± 11.9	69.3 ± 13.1
Fat-free mass (kg)	67.7 ± 9.8	44.3 ± 5.9
Fat mass (kg)	13.0 ± 5.0	14.9 ± 2.9
Body fat percentage (%)	15.6 ± 3.5	25.2 ± 4.2
Abdominal visceral fat area (cm^2)[a]	55.2 ± 24.5	20.8 ± 5.6
Waist-to-hip ratio	0.81 ± 0.05	0.73 ± 0.04

Data are presented as mean ± standard deviation. bpm: beats per minute.
[a] Abdominal visceral fat area was measured via Dual-energy X-ray absorptiometry scan and referred to as VFA_d throughout the manuscript.

3.3. Developed US protocol

The correlation coefficients between the second set of US measurements with BMI, WHR, and body composition assessed via DEXA are presented in Table 3.

In addition, stepwise linear regression was utilized to identify the best predictive equation for VFA_d. Age, sex, abdominal region 4, and abdominal region 6 were identified as the statistically significant predictors for VFA_{e2}, $F(4, 25) = 46.869$, $P < 0.001$. The developed equation (VFA_{e2}) had high accuracy (adjusted $R^2 = 0.864$), and the generated regression equation was: (37.677 + [1.456 × Age] − [26.963 × Sex] − [11.336 × Region 4] + [13.554 × Region 6]), where Age = years, Sex: 1 = male or 2 = female, and region 4/6 = transducer placement 2 cm to the left and right of the superior border of the umbilicus, respectively. The regression equation was utilized to estimate VFA_{e2} (31.4 ± 21.4 cm^2), which was significantly correlated ($r = 0.927$, $P < 0.001$) with VFA_d (31.1 ± 21.1 cm^2). There were no significant differences between VFA_{e2} and VFA_d ($t[29] = 0.712$, $P = 0.865$) whose distribution is illustrated in Fig. 2.

Table 2
First set of ultrasound sonography measurements.

Value	Replicated ultrasound protocol			DEXA
	Region 1 (cm)	Region 2 (cm)	Region 3 (mm)	Abdominal visceral fat area (cm^2)
Mean	2.49 ± 0.77	3.66 ± 0.85	6.05 ± 1.59	–
Estimated abdominal visceral fat area (cm^2)		78.72 ± 14.59		31.11 ± 21.06[a]

Data are presented as mean ± standard deviation. The presented estimated abdominal visceral fat area is referred to throughout the manuscript as VFA_{e1}, which is an equation that uses regions 1–3 that was replicated from a different manuscript and was not able to predict VFA_d measured via dual-energy X-ray absorptiometry in the participants of this study. Region 1: the thickness between the internal surface of the abdominal muscle to the splenic vein. Region 2: the thickness between the internal surface of the abdominal muscle to the posterior aortic wall at the umbilicus. Region 3: the thickness of the fat layer of the posterior right renal wall in the right posterior perinephric space. VFA_{e1} vs. VFA_d –: not applicable. DEXA: dual-energy X-ray absorptiometry.
[a] $P < 0.01$.

Table 3
Correlations matrix between body composition variables and the second set of ultrasound sonography measurements.

Abdominal region and mean value (cm)	Variables				
	BMI (kg/m^2)	Waist to hip ratio	Fat-free mass (kg)	Fat mass (kg)	DEXA abdominal visceral fat area (cm^2)
Region 4 2.54 ± 0.69	0.626[a]	0.597[a]	−0.167	−0.206	0.584[a]
Region 5 2.76 ± 0.78	0.419[b]	0.561[a]	−0.221	−0.322	0.556[a]
Region 6 2.55 ± 0.78	0.606[a]	0.559[a]	−0.182	−0.234	0.706[a]
Region 7 2.63 ± 0.67	0.537[a]	0.480[a]	−0.131	−0.097	0.613[a]
Region 8 2.80 ± 0.71	0.448[b]	0.470[a]	−0.012	−0.069	0.425[b]
Region 9 2.82 ± 0.81	0.652[a]	0.596[a]	−0.141	−0.140	0.670[a]

Data are presented as mean ± standard deviation and Pearson correlation coefficients. Region 4/5/6: transducer placement 2 cm to the left, up, and right from the superior border of the umbilicus. Region 7/8/9: transducer placement 5 cm to the left, up, and right from the superior border of the umbilicus. Thickness for abdominal regions is defined as the internal surface of the abdominal muscle to the anterior aortic wall. BMI: body mass index; DEXA: dual-energy X-ray absorptiometry.
[a] $P < 0.001$.
[b] $P < 0.05$.

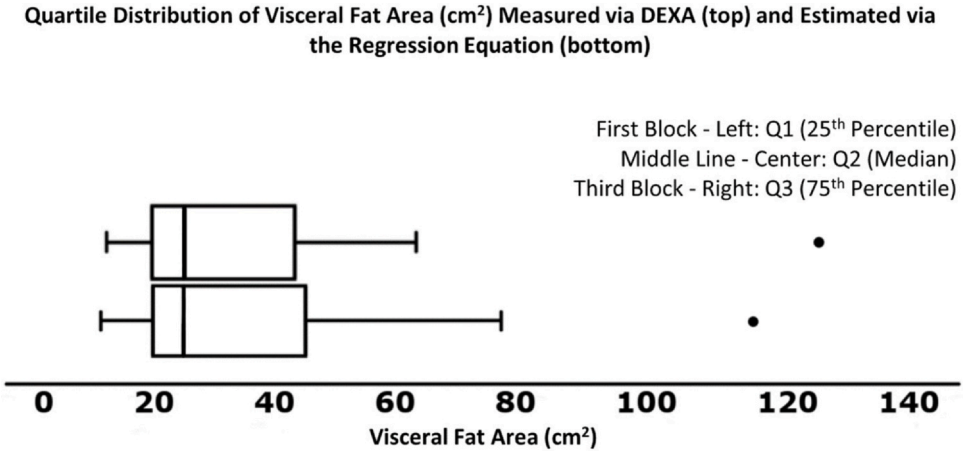

Fig. 2. Represents the visceral fat area (cm^2) distribution estimated via dual-energy X-ray absorptiometry (top) and estimated via the regression model that uses age, sex, and two ultrasound sites (bottom). There were no significant differences between distributions. With or without the two outliers, the data distribution remained the same, therefore, outliers were kept denoting the true distribution.

3.4. Dietary intake

The breakdown of the dietary intake for all participants based on sex is presented in Table 4. Moreover, the correlation analysis provided minor inferences regarding dietary intake and body composition, such as: in males, their added sugar intake was positively correlated with their VFA_d ($r = 0.671$, $P = 0.048$) and although not significant, a similar trend was seen with their VFA_{e2} ($r = 0.622$, $P = 0.074$). In females, their carbohydrate ($r = 0.552$, $P = 0.012$) and protein ($r = 0.553$, $P = 0.011$)

Table 4
Breakdown of participant's dietary intake.

Variable	Males (n = 9)	Female (n = 21)
Energy intake (kcal)	2414.5 ± 369.9	1743.7 ± 520.8
Protein (g)	134.1 ± 20.3	79.8 ± 24.9
Fat (g)	97.3 ± 18.9	65.8 ± 25.0
Carbohydrates (g)	249.6 ± 63.3	212.2 ± 86.3
Sugar (g)	73.8 ± 27.9	89.51 ± 57.4
Added sugar (g)	17.4 ± 5.9	32.6 ± 8.9
Monosaccharides (g)	8.7 ± 5.2	5.6 ± 1.8
Disaccharides (g)	8.5 ± 5.7	3.7 ± 1.1
Saturated fat (g)	31.5 ± 9.2	18.9 ± 7.0
Monosaturated fat (g)	19.1 ± 9.2	10.3 ± 2.3
Polyunsaturated fat (g)	10.0 ± 4.2	6.2 ± 4.5
Cholesterol (mg)	534.9 ± 181.2	243.3 ± 164.7

Data are presented as mean ± standard deviation.

intake was positively associated with their FFM, while their protein ($r = -0.451$, $P = 0.046$) intake was negatively associated with their body fat percentage.

4. Discussion and conclusion

In this study, we replicated the US protocol utilized by Hirooka to estimate abdominal VFA.[14] The approach by these researchers is unique since it specified reference points for the US assessment. We tested Hirooka et al.'s proposed regression equation which was developed through a stepwise approach with our recorded measurements following their methodology. However, we were unable to accurately estimate the abdominal VFA of our participants. There was a significant difference between VFA_{e1} and VFA_d, where the VFA_{e1} overestimated the actual VFA_d of our participants. It is possible that the inaccurate estimate of abdominal VFA from Hirooka's regression equation was due to inherent differences in the studied cohorts. The participants in this study were healthy young adults whereas most participants in Hirooka et al.'s study had ongoing chronic diseases like chronic hepatic disease and gastrointestinal disease. These two diseases are associated with increased abdominal VF deposition,[26] so Hirooka et al.'s regression equation might have accounted for pathologic abdominal VF accumulation/differences in peritoneal fat that was not present in our participants. It is likely that different US protocols should be developed to estimate abdominal VF content for different populations, including healthy and diseased individuals. Alternatively, an ideal regression equation could be made to account for normal and atypical abdominal VF accumulation. If so, the abdominal regions that are used must be scrutinized when developing a widely applicable US protocol.

Stolk et al. [12] suggested that reference points at the lower end of the rib cage should be considered when estimating abdominal VFA. This is a good approach since it defines a specific location for transducer placement. However, only the most medial (centered) placements were recommended since the lateral placements were less applicable and more difficult to locate due to intestinal gas within the ascending and descending colon. Pimanov et al.[16] replicated the reference points suggested by both Hirooka et al., [14] Stolk et al., [12] and Ribeiro-Filho et al. [23] where the transducer was placed 2 cm above the superior border of the umbilicus with a distance defined as the ISAM to the anterior aortic wall. In the current study, we also utilized the reference point 2 cm above the superior border of the umbilicus. We added other reference points including 2 cm to the left and right of the superior border of the umbilicus and 5 cm to the left, above, and right of the superior border of the umbilicus to determine if these locations for transducer placement could provide good estimates of abdominal VFA. All these reference points were labeled as the second set of US measurements. Among these points, two had the greatest

ability to estimate abdominal VFA and were included in our regression equation (VFA_{e2}): 2 cm to the left and right of the superior border of the umbilicus while using a transverse view.

It was surprising that WHR had no significant statistical impact on the estimation of VFA_{e2} in the developed regression equation. WHR is often used as an indicator of abdominal VF content, but it did not apply to our participants. To be considered, VFA_{e2} was created with VFA_d as the dependent variable. WHR was correlated with VFA_d ($r = 0.753$, $P < 0.001$), however, in the final regression model, the partial correlation ($r = 0.041$, $P = 0.835$) between them was insignificant. This could suggest that the usefulness of WHR as one common indicator of abdominal VF content is negated once direct measures of intra-abdominal fat content are introduced, such as those collected via ultrasound. Therefore, in our sample, WHR was not a direct marker of abdominal VFA, and suggests that the applicability of WHR depends on the population that it is utilized for. Even without WHR, our regression equation shows benefits since it only requires the measurement of two specific points to successfully estimate abdominal VFA. Our choice and identification of the two transducer placements make our protocol easy to reproduce. Moreover, the depth of the reference points can be easily measured since the walls of the abdominal muscles and the aorta are well-defined and can be easily recognized due to their hyperechoic outline compared to the regions within the vicinity of the US scan. This was true even with participants who reported constipation and had excess noise in the lateral abdominal sites. The success of our US protocol for estimating abdominal VFA is limited by our sample size, low male representation, and all-white participants. Even though an a priori power analysis indicated that only 13 participants were needed to achieve statistically high power, a much larger group is required to develop a regression equation that can be broadly used. Our developed regression equation accounts for both sex and age and can help to control for the uneven sex distribution. Although sex was accounted for, race was not considered as a factor that could influence the results. It is known that race may influence abdominal VF deposition. With that into consideration, the main goal was to demonstrate that US is a promising approach to estimating abdominal VFA in individuals, and we recommend that future studies utilize our US protocol as a guide and replicate it in larger and more diverse cohorts to determine its applicability and consistency among other researchers and populations, including populations with comorbidities.

On the other hand, the secondary goal of this study was to examine the relationship between the dietary intake of participants and their abdominal VF content. Diets that are abundant in processed foods increase the likelihood of excessive fat and sugar consumption.[27] Of these two macronutrients, saturated fats are thought to be major contributors to abdominal VF development due to their metabolization promoting de novo lipogenesis in the liver.[28] Saturated fat increases abdominal VF content to a greater extent than polyunsaturated fats,[28] and its consumption is also linked to the development of insulin resistance.[29] In addition, since the source of saturated fat is often found with added sugars, the consumption of both jointly facilitates the upregulation of cortisol in adipocytes which promotes abdominal VFA accumulation.[30] Within added sugar consumption, fructose is the main promoter in the development of abdominal VF content.[31] Under that premise, our data had no relation between abdominal VFA and the intake of saturated fat in either sex, and only demonstrated a positive relationship with the intake of added sugar in males. However, the sample size of males was only nine and utilizing a correlation analysis should be done with caution. For this finding, the data had no outliers and was linear. We recommend that future studies consider the intake of added sugar in males as a potential determining variable of VFA in males that warrants a bigger sample size if used as a potential longitudinal risk factor marker of abdominal VF content accumulation.

The purpose of examining the relationship between fat and sugar intake with abdominal VFA in this study was to denote that dietary intake needs to be incorporated in assessments related to body composition.[32,33] In the context of abdominal VFA, the goal would be to follow up with the males in this study over time to determine if the relationship between their intake of added sugar with abdominal VFA predisposed them to develop a higher abdominal VFA than the female group. Worth denoting, females in this study had a greater intake of added sugar than males, but females also had a lower total caloric intake. Since added sugars were not associated with female's abdominal VFA, it remains to be elucidated if their reduced caloric intake served as a "buffer" to prevent the increment of abdominal VFA. Following females over time would help to elucidate if their total caloric intake,[34] or if chronic high intake of added sugar,[35] is more important in the development of abdominal VFA within this cohort. Moreover, this cross-sectional analysis did not examine

the impact of physical activity and hormonal status, which could be another factor helping females to maintain a healthy abdominal VFA despite their higher intake of added sugars.[36–38] That is because despite dietary intake, being physically active and compounds like estrogen and growth hormone can independently promote abdominal VF content reductions. Denoting that the results found in this study could become more informative if these variables had been included at the time of data collection.

One last aspect to be considered is the cross-sectional nature of this study. Previous reports suggest that when it comes to anthropometric measures, these types of study designs resemble changes over time when compared to prospective designs.[39] While our study did not include a validation sample, it is promising that ultrasound sonography holds the potential to develop a generalizable equation to rapidly and accurately estimate one's abdominal VF content. We are not proposing that our VFA_{e2} is final and applicable across all populations. Rather, we present evidence that ultrasound sonography is a viable method to determine VF content. Considering that current common approaches, such as DEXA or skinfolds, have both benefits and limitations, it must be accepted that no method is infallible to being applicable across limited populations.[40] Therefore, it is important to continue advancing body composition assessments [41] with an upfront goal of ensuring the proposed methods are as applicable and meaningful as possible for large-scale studies and diverse populations. To achieve this, equal representation of males and females, along with diverse ethnic backgrounds must be accounted for, so that future results provide greater applicability to these larger cohorts.

In conclusion, this study identified two specific reference points for the US assessment of abdominal VFA that can be easily identified without extensive imaging expertise. Considering sex and age, a regression equation was developed that accurately estimated abdominal VFA in healthy young adults in comparison to abdominal VFA estimated by DEXA scans. From an applicability point of view, the proposed US protocol is promising and could be used to estimate abdominal VFA frequently and inexpensively without the risk of radiation exposure and the need for advanced imaging expertise. Over time, frequent screening for abdominal VFA can aid in the early detection of excess abdominal VF deposition and promote necessary interventions. Moreover, a diet with increased added sugar consumption was linked to greater abdominal VFA only in males. Although there is a direct link between the development of abdominal VFA with the intake of saturated fat and added sugars, cross-sectional analyses might not be sensitive enough to detect the impact of diet on abdominal VF content. Therefore, longer-term, strict analyses are warranted to identify dietary breakdowns that predispose individuals to a greater risk of developing comorbidities due to excess abdominal VF content that is not illustrated by BMI.

CRediT authorship contribution statement

Jose M. Moris: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Conceptualization. **Vineet K. Paidisetty:** Writing – review & editing, Writing – original draft, Formal analysis. **Haley A. Turner:** Writing – review & editing, Writing – original draft, Formal analysis. **Kylie Allen:** Writing – review & editing, Writing – original draft, Formal analysis. **Brandon W. Arnold:** Writing – review & editing, Writing – original draft, Formal analysis. **Yunsuk Koh:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Conceptualization

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

De-identified data presented in this manuscript is available upon reasonable request to the first author (J.M. Moris).

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