

## Research Article

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# Ultrasonography to measure abdominal fat: A future marker of cardiovascular risk?

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**Keywords:** Body composition; Abdominal fat; Diagnostic imaging; Female; Ultrasound.

**Abbreviations:** Cardiovascular Disease; VF: Visceral Abdominal Fat; BC: Body Composition; DXA: Dual-Energy X-Ray Absorptiometry; Scf: Abdominal Wall Subcutaneous Fat Tissue; VF: Visceral Fat Tissue; PPF: Preperitoneal Fat In The Epigastric Regions; LLL: Caudate Liver Lobe; HEC: Hyperinsulinemic Euglycemic Clamp; PPF: Preperitoneal Fat; WC: Waist Circumference.

## Introduction

Social, demographic and behavioral changes have directly influenced health, in addition to the causes of morbidity and mortality in individuals [1]. Transmissible and cardiovascular (CV) diseases have been the major global causes of death [2,3]. One-third of the female mortality rates have been attributed to CVD and the number of deaths in the age groups younger than 55 years have not shown a decrease, despite all existing US protocols for the prevention and treatment of CV diseases [4-6].

## Abstract

**Objectives:** To evaluate ultrasound measurements of abdominal fat and correlation with body composition and cardiovascular (CV) disease markers.

**Methods:** A pilot study with 37 females aged 18-40 years, body mass index <30 (BMI; kg/m<sup>2</sup>) and no history of illness or use of medication. All patients were assessed for insulin resistance using the Hyperinsulinemic Euglycemic Clamp (HEC) at baseline and at 12 months. Ultrasound measurements of abdominal fat, anthropometry, body composition (BC) obtained by densitometry (DXA) and serum parameters related to CV health were made by the same observer during two time periods, at baseline and after 12 months. Measurements of liver volume, abdominal wall subcutaneous fat tissue (ScF), preperitoneal fat (PPF) and visceral fat tissue (VF) were taken. Lipid and liver profile, apolipoprotein levels and biomarkers of CV health were analyzed. Spearman coefficient was used for correlation analysis. The significance level was set at 5%.

**Results:** A slight elevation of the mean weight, BMI and ultrasound measurements was observed at 12 months. In both time periods, there was a significant correlation between ScF and total serum cholesterol levels ( $r=0.54$ ), LDL-cholesterol ( $r=0.53$ ) and Apo B-100 ( $r=0.44$ ), BMI ( $r=0.85$ ), waist circumference (WC;  $r=0.84$ ) and hip circumference ( $r=0.75$ ), total mass ( $r=0.78$ ), fat mass percentage and total fat mass ( $r=0.82$  and  $r=0.87$ , respectively). PPF measurements showed a significant correlation with CRP in both time periods ( $r=0.44$ ) and WC ( $r=0.51$ ), while VF correlated with the waist/hip ratio ( $r=0.60$ ).

**Conclusions:** Ultrasound measurement of abdominal fat showed a good correlation with anthropometric and BC measurements, and CV markers in this non-obese female sample. It is a promising technique that should be tested in the largest number of individuals in other populations to determine the cutoff parameter as a potential early marker of CV risk.

The majority of CV events occur during people's normal routine, often outside a hospital or healthcare unit. Therefore, it becomes clear that primary prevention should be top priority. Changes in feeding habits [7], physical activity [8], postmenopausal hormone therapy [9] and preventive surgeries for the obese [6,10] have been described as factors that may reduce morbidity and mortality from CV diseases. On the other hand, the proposal for more effective preventive measures focused on predisposed individuals, requires screening methods for CV disease risk factors.

The probable cause of the increasing prevalence of CVD is the number of people with excessive weight in fat, particularly those with visceral abdominal fat (VF) [11-13]. VF is a known risk factor for CVD, metabolic diseases and some types of tumors [14-19]. It has been described that dysfunctional and hypertrophic adipocytes located in VF precede the predisposing inflammatory processes of CVD and thromboembolism [20].

Although abdominal fat is estimated by anthropometric and body composition (BC) measurements using total body densitometry (DXA), computed tomography (CT) scan has been the gold standard of VF assessment. CT is an expensive technique that emits some level of radiation [21]. Regional fat compartments were measured with the aid of new software (iDXA) for BC assessment. A study of females and males aged 18-70 years compared abdominal fat compartments by iDXA and ultrasound measurements. It was concluded that ultrasound is reliable for visceral fat estimation [22].

Ultrasonography (US) is a widely available and safe exam, with a high reproducibility. The aim of this study was to assess fat measurements by ultrasound and its correlation with anthropometry, BC data and laboratory parameters related to CV health in young non-obese females, without a known history of illness.

## Methods

A pilot study that used secondary data from a study conducted from February 2011 to February 2013 in the Ultrasonography Unit of the Department of Obstetrics and Gynecology, State University of Campinas (UNICAMP) School of Medicine, Campinas, Brazil. The project was approved by the Ethics Committee. All female participants signed a consent term prior to the beginning of the study.

37 female participants received follow-up during 12 months for the assessment of insulin resistance. The study project was registered in ClinicalTrials.gov, under number NCT01527526. All had undergone the Hyperinsulinemic Euglycemic Clamp at baseline and at 12 months. The M-value was calculated which corresponded to glucose consumption at steady-state. M values <4mg/kg/min were defined as diagnostic for insulin resistance.

Inclusion criteria were age, ranging from 18-40 years; body mass index <30 (BMI, kg/m<sup>2</sup>); fasting blood glucose <100 mg/dL and OGTT (75 g oral glucose) at 120 minutes <140 mg/dL. Exclusion criteria were breastfeeding; first-degree family history of diabetes mellitus (DM); history of DM 1 or 2; systemic arterial hypertension (SAH); hyperthyroidism or hypothyroidism; chronic kidney failure; and any organ transplantation; use of corticosteroids, antipsychotics, thiazidics or statins; females with hirsutism and/or hyperandrogenism, polycystic ovarian syndrome (PCOS) and a previous history of bariatric surgery or omentectomy.

The variables evaluated were anthropometry (weight, body mass index (BMI; kg/m<sup>2</sup>), waist/hip circumference and waist/hip ratio), body composition (BC) assessed by the dual-energy X-ray absorptiometry (DXA) technique using the Lunar DPX bone densitometer device (GE Healthcare Lunar Corporation, WI, USA). All measurements were taken at baseline and at 12 months. Anthropometric measurements were always made by

the same observer; for BC the interobserver coefficient of variation for fat mass measurement was 0.7%

Blood samples following a 12-hour fast were collected at baseline and at 12 months.

Total cholesterol, HDL-chol and triglycerides were measured by the colorimetric method (CHOD-PAP and GPO-PAP; Roche Diagnostics, Mannheim, Germany). LDL-chol concentration was calculated by the Friedewald equation [LDL cholesterol mg/dL = total cholesterol – HDL cholesterol – (triglycerides/5)]. Leptin and adiponectin measurements were performed by commercial immunoassay kits (Human Leptin “Dual Range and Human Adiponectin ELISA; Merck Millipore, Darmstadt, Germany), apolipoprotein measurements were performed by turbidimetry using PowerWave XS (BioTek, Winooski, USA) and Tina-quant APO A-I and Tinaquant APO B reagents (Roche, Indianapolis, USA). Free fatty acids were measured using the WAKO enzymatic colorimetric kit (Dusseldorf, Germany); interleukin-6 and TNF-alpha (Human IL-6 Quantikine E and TNF-alpha Quantikine HS; R&D Systems, Minneapolis, USA), and RCP was evaluated by the Nephelometry method, using the BN ProSpec System (Dade Behring, Liederbach, Germany) and Siemens CardioPhase hs CRP kit (Erlangen, Germany). Liver enzymes Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Gamma-glutamyltransferase (GGT) were measured by automated assays (COBAS- Roche, USA).

## Procedures for ultrasound fat assessment

Participants were in the supine position and all measurements were taken in triplicate from frozen screen images. Cards were used for image occlusion. After the end of the exam, measurements were retrieved from the file and the arithmetic mean was calculated. All measurements were performed by the same observer and the average coefficient of intraobserver variation was always lower than 5%.

A Toshiba Xario machine and multifrequency probes were used. Convex probes ranged from 3.0 to 6.0 MHz and linear probes ranged from 6.6 to 9.0 MHz. A convex probe was used to measure the liver, and visceral fat when the use of a linear probe was not feasible and to compare echogenicity of the liver with kidney/spleen echogenicity. A linear probe was used to measure abdominal wall fat above the umbilicus (ScF), visceral fat in the mesogastric (VF) and preperitoneal fat in the epigastric regions (PPF).

ScF was measured in centimeters (cm) in the region immediately above the umbilical scar, in the xiphoid umbilical line. Its measurement was considered from the skin to the linea alba, in the region between the rectus abdominus muscles, during expiration [15,23] (Figure 1). PPF and VF were measured in two regions of the xiphoid umbilical line. VF was measured in the region immediately above the umbilical scar and was considered the extension in cm from the linea alba to the anterior wall of the aorta, during diastole and at the end of expiration. PPF was measured in the epigastric region, from the linea alba to the surface of the left liver lobe, at the end of expiration. Measurements were determined from frozen amplified images that occupied 2/3 of the screen (Figures 2 & 3).

Measurement of the right liver lobe (RLL) was calculated

by the mean of three longitudinal distances, from the lower border of the right liver lobe to the upper border proximal to the diaphragm, in the right hemiclavicular line. Measurement of the caudate liver lobe (LLL) was obtained by the anteroposterior distance placing the transducer in the right paramedian epigastric region [24]. To evaluate liver echotexture, the cortical regions of the kidneys or spleen were used for comparison [25, 26].

### Statistical analysis

The mean, standard deviation and median of all measurements and Spearman's correlation coefficient were used to correlate ultrasound measurements with anthropometric/body composition measurements and laboratory test results. Measurements taken at baseline and at 12 months in the same female sample were analyzed. The significance level of 5% was adopted.

### Results

The mean age of the female patients was 28.8 (±5.7) years, a little more than half of these females were self-reported as non-white (59.4%) and had >8 years of school education (54.0%) (data not shown). Mean weight, BMI and abdominal measurements increased slightly at 12 months and there was no variation in the mean M-value measured by HEC (Table 1).

Table 2 shows the statistically significant correlations between variables measured by ultrasonography and body composition/anthropometric measurements. ScF measurement was significantly correlated with body weight (r= 0.78), BMI (r= 0.85), total mass (r= 0.78), fat mass percentage (r= 0.82), total fat tissue (r= 0.87), waist circumference (r= 0.84) and hip circumference (r= 0.75) at the beginning of the study. After 12 months, the same correlations remained significant. BMI and waist measurements had the highest values, emerging a correlation with a new variable--the waist/hip ratio (r= 0.66) (Table 2).

VF measurement had a significant correlation with waist/hip ratio in both time periods (r= 0.60 and r= 0.50 at baseline and at 12 months, respectively) (Table 2). PPF measurements had a significant correlation with anthropometric and body composition variables at baseline. In both time periods, correlation was only maintained for waist circumference (r= 0.51 and r= 0.34 at baseline and at 12 months, respectively) (Table 2).

Concerning serum markers, the ScF measurement had a significant correlation with total cholesterol concentrations at baseline and at 12 months (r= 0.51 and r= 0.54, respectively), LDL cholesterol (r= 0.42 and r= 0.53, respectively) and Apo B-100 (r=0.44) (Table 3). PPF had a positive correlation with CRP in both time periods (r=0.44 and r=0.41), while VF had a positive correlation with LDL-chol at baseline (r=0.34). Measurements of LLL showed a negative correlation with HDL-chol (r= -0.37) and free fatty acids (r= -0.39) only at baseline. Measurements of LHD and echotexture had no correlation with the variables studied (data not shown).

**Table 1:** Mean and standard deviation of some sample variables at baseline and at 12 months.

Variables	Baseline	12 months
Weight Kg, mean (SD)	61.8 (8.4)	63.0 (8.4)
BMI kg/m <sup>2</sup> , mean (SD)	24.2 (3.2)	24.7 (3.1)
ScFI cm, mean (SD)	24.3 (10.3)*	26.3 (9.7)
PPF cm, mean (SD)	12.6 (4.1)*	14.5 (8.6)
VF cm, mean (SD)	32.2 (10.8)*	34.0 (11.5)
M-value mg/kg/m <sup>2</sup> , mean (SD)	5.6 (1.5)	5.5 (1.7)

SD: Standard deviation. \*Missing: 1 (abdominal fat measurements calculated at baseline with 36 females). BMI: Body Mass Index; Scf: Subcutaneous Abdominal Fat; PPF: Preperitoneal Epigastric Fat; VF: Visceral Mesogastric Fat. M-Value: Measured By Hyperinsulinemic Euglycemic Clamp (M<4= insulin resistance).

**Table 2:** Significant correlations between ultrasound measurements and DXA anthropometric and body composition measurements, at baseline and after 12 months.

Variables	ScF*	Baseline	VF*	LLL*	12 Months		
		PPF*			ScF	PPF	VF LLL
Weight	0.7807	0.4672			0.6499		
p value	<.0001	0.004			<.0001		
BMI	0.8517	0.4599			0.7470		0.3937
p value	<.0001	0.004			<.0001		0.0159
Total mass	0.7899	0.4271			0.6284		
p value	<.0001	0.009			<.0001		
% Fat mass	0.8244	0.4263			0.6334		
p value	<.0001	0.009			<.0001		
Total fat tissue	0.8723	0.4977			0.6794		
p value	<.0001	0.002			<.0001		
Waist circumference	0.8455	0.5184			0.7931	0.3473	0.4440
p value	<.0001	0.001			<.0001	0.035	0.005
Hip circumference	0.7520	0.4274			0.5306		
p value	<.0001	0.009			0.0007		
Waist/hip ratio			0.6066	0.3465	0.6654		0.5083
p value			<.0001	0.0384	<.0001		0.0013

Spearman's correlation coefficient. BMI: Body Mass Index. ScF: Subcutaneous abdominal fat. PPF: Preperitoneal epigastric fat. VF: visceral mesogastric fat. LLL: Left liver lobe. \*Missing: 1 (abdominal fat measurements of 36 females calculated at baseline).

**Table 3:** Significant correlations between ultrasound measurements and serum markers at baseline and at 12 months.

Variables	ScF*	Baseline	VF*	LLL*	ScF	12 Months		
		PPF*				PPF	VF	LLL
Total Cholesterol	0.5130	-	-	-	0.5423	-	-	-
<i>p value</i>	0.001				0.000			
HDL-chol	-	-	-	-0.3780	-	-	-	-
<i>p value</i>				0.002				
LDL-chol	0.4222	-	0.3449		0.5320	-	-	-
<i>p value</i>	0.001		0.003		0.000			
Triglycerides	0.3595	-	-	-	-	-	0.3632	-
<i>p value</i>	0.003						0.027	
ALT	-	-	-	-	-	-	0.3824	-
<i>p value</i>							0.019	
Gama GT	-	-	-	0.3753	-	0.4016	0.4408	-
<i>p value</i>				0.024		0.013	0.006	
CRP	0.4951	0.4427	-	-	-	0.4122	-	-
<i>p value</i>	0.002	0.006				0.011		
APO-A	-	-	-	-	-	0.3681	-	-
<i>p value</i>						0.025		
APO-B	0.4479	-	-	-	0.4446	-	-	-
<i>p value</i>	0.006				0.005			
Interleukin-6	-	-	-	-	-	-	-	-
<i>p value</i> Free fatty acids	-	-	-	-0.3947	-	-	-	-
<i>p value</i>				0.018				

Spearman's coefficient correlation. BMI: Body Mass Index. ScF: Subcutaneous Abdominal Fat. PPF: Visceral Epigastric Fat. VF: Visceral Mesogastric Fat. LLL: Left Liver Lobe. \*Measurements of 36 females taken at baseline.

## Discussion

This study of non-obese females showed that ultrasound measurements of ScF was strongly correlated with body composition and anthropometric measurements. Study participants had a negative history of known diseases and laboratory parameters assessed at the beginning of the study were within the normal range. This result allows us to affirm that ultrasonography may be used to assess ScF and offered advantages over anthropometric measurements. Previous studies that described anthropometric measurements had the highest interobserver error, did not reflect the location of fat deposition in a reliable manner and did not correlate with variations in body weight [15,27,28,29] while BMI in non-obese females may not reflect VF deposition, decreasing the perception of CV risk [30] DXA assessment of BC is expensive and the method is not available in the majority of health services. In general, it is most frequently indicated for females older than 60 years to measure bone mass. Furthermore, even when available the device may not always distinguish between different abdominal fat deposits [31].

Previous studies have associated VF deposition with increased body weight [15,28] and increased risk for CVD [32]. In this study, we did not find any correlation between these variables, which may be explained by the characteristics of the non-obese female sample. Regarding the weak correlation encountered between ultrasound VF measurements and anthropometric measurements and the lack of correlation with fat measured in BC, we could speculate that visceral fat at baseline induced a "safe" deposition, located in the subcutaneous tissue. We can also speculate that inflammatory biomarkers could already have been affected at this stage, which was shown in our study. It has been described that VF has a particular metabolism, limited by the intra-abdominal space. It accumulates by hypertrophy of adipocytes, through mechanisms that are not fully understood, and is capable of shifting excess fat to muscles

and subcutaneous tissue deposits. In contrast, subcutaneous fat deposition occurs through adipogenesis and precedes an increase in VF, playing a protective role in the beginning of body weight gain [33].

The study has some strengths and limitations. The strengths were the evaluation of a nonobese female cohort with measurements obtained with ultrasound by only one experienced observer and two measurements taken 12 months apart. These characteristics demonstrated that some results were repeated with strong correlation in both time periods of assessment. It is possible that the results related to CVD markers such as LDL-chol, Apo B-100 and C-RP had weaker correlations with ultrasound measurements due to the sample characteristics (non-obese, apparently healthy females). On the other hand, limitations are those of a pilot study, and results should be tested by other studies using a larger number of individuals in different populations.

There are no studies on the amount of abdominal fat that can be regarded as physiological or normal for an individual. Studies on the values above which it would be considered a higher risk for metabolic diseases or CVD are also lacking. Studies to confirm or refute whether ultrasound measurement of abdominal fat deposition may occupy a role in the prediction of CVD risks must be conducted. In case of affirmative results, professionals dedicated to imaging diagnostics will become involved in screening for individual indicators of CVD risk.

Since it shows early variation in fat gain, ultrasound measurement of abdominal ScF may be conducted during abdominal ultrasound ordered for other indications, permitting longitudinal comparisons to detect any changes. Ultrasound is a widely available, low-cost method, with a safe application. Therefore, assessment of ScF could be encouraged and included in the standard report, irrespective of test indication.



Future studies need to be conducted with a larger number of females to assess ultrasound use for the measurement of abdominal fat thickness in different populations and age groups, with and without comorbidities and determine measurements of maximum thickness, velocity of increased fat deposition or cut-off value indicating CVD risk. Studies should propose to standardize the best locations for visceral fat measurement. It is most important to specifically study the deposition of subcutaneous fat located above and below the Scarpa's fascial layer, since its relation is modulated by body weight gain [34,35].

## Conclusion

On ultrasound assessment of abdominal fat, there was a strong correlation between body composition and anthropometric measurements. Due to the high prevalence of CVD in females and the pronounced effect of well-known CV risk factors on this population, it may be important to carry out studies with practical procedures that are new, easy, inexpensive and available for the detection of alarm signals to prevent these diseases. Ultrasound as a screening method for assessment of fat deposition is quite promising.

**Conflict of interest:** The authors declare that they have no conflict of interest.

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