Ultrasound measures of abdominal fat layers correlate with metabolic syndrome features in patients with obesity

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Summary
Objective: Abdominal fat ultrasound (US) is a simple clinical tool that may allow measures of fat depots not visible using common dual-energy X-ray absorptiometry (DEXA) or computerized tomography (CT) imaging. The aim of this study was to validate the technique, give measures of superficial and profound subcutaneous, preperitoneal, omental and perirenal (retroperitoneal) fat and correlate them with MS markers.

Methods: Sequential US measures of these five abdominal fat layers were done at 397 adults. Blood pressure (BP), body mass index (BMI), waist, body fat %, HOMA-IR index (homeostatic model assessment of insulin resistance), lipid profile and leptin were recorded. Metabolic syndrome (MS) was defined according to Cholesterol education programme adult treatment panel III (ATPIII) criteria.

Results: Subcutaneous and omental fat were increased among people with obesity, whereas preperitoneal and perirenal fat did not show any difference according to BMI or waist. Women showed thicker subcutaneous fat (both superficial and profound), whereas men had bigger omental fat. Both postmenopausal and diabetic patients had changes in omental fat only, whereas patients with fatty liver showed thicker preperitoneal and perirenal fat, as well. MS patients showed both thicker perirenal and omental fat. A cut-off of 54 mm in male (M)/34 mm in female (F) of omental fat and 22.5 mm (M)/12.5 mm (F) of perirenal fat could be predictive of later MS onset.

Conclusions: US is a valid method to measure all different abdominal fat depots. Omental and perirenal fat measures may classify patients at risk for MS. Preperitoneal fat depot may also correlate with fatty liver disease.

KEYWORDS
metabolic syndrome, omental, subcutaneous adipose tissue, ultrasound
1 | INTRODUCTION

Adipose tissue is widespread in human body, and its distribution probably determines its function. Under the skin, it may act as a protective barrier against infections, whereas in the breast, adipose tissue acts as a supporting architectural structure and an energy deposit store. However, its capacity of secreting specific adipocyte-derived hormones makes the adipose tissue the most extended endocrine system of our body, capable of controlling almost all homeostatic processes ranging from hunger control, energy expenditure, glucose and lipid metabolism, blood pressure control or modulation of procoagulative states, becoming then a crucial actor in cardiovascular risk pathogenesis. Abdominal obesity, measured through waist circumference, has been clearly associated with increased cardiovascular mortality. However, there are marked differences comparing different abdominal fat depots. High adiponectin secretion in the subcutaneous abdominal layer is protective in terms of cardiovascular risk reduction, whereas visceral fat is known to secrete many insulin-resistance deleterious cytokines such as TNF, IL6, leptin, resistin or visfatin.

Structured ultrasound imaging of abdominal adipose tissue is a cheap, nonradiation, easy-to-learn methodology, able not only to differentiate between superficial and profound subcutaneous fat layers but also to look easily into deeper layers (preperitoneal) fat, omental (intraperitoneal) fat and perirenal (retroperitoneal) fat, all of them included under the concept of 'visceral adiposity' measured using classic body composition techniques.

Despite its easy reproducibility, the published data are scarce and focused on single specific layers. To our knowledge, there is no previous work using ultrasound to assess, in a systematic way, all five consecutive abdominal fat depots, in order to define their ranges both in normal-weight, overweight and obese population. US-measured omental fat thickness has been associated with increased risk of fatty liver, type 2 diabetes and increased coronary artery disease, but a specific cut-off value to predict metabolic syndrome as a marker of cardiovascular risk has not been established so far.

Hence, the aim of this study was to evaluate the different sono-graphic abdominal fat layers and stablish their relationship with metabolic risk factors in a Spanish cohort of participants with average body weight to those with obesity.

2 | DESIGN AND METHODS

2.1 | Participants

During 2017–2018, patients sent from Endocrinology to the Radiology Department for any ultrasound imaging (mostly thyroid, parathyroid and ovaries) were offered a sonographic assessment of superficial and intraabdominal fat. From 516 patients that gave their informed consent, the 397 that had anthropometric data, analytical measures and clinical diagnoses correctly recorded, were enrolled.

2.2 | Anthropometric data

Abdominal perimeter was measured in centimetre, from top of both iliac crest and measures >88 cm in female and >102 cm in male were considered as major criteria for MS definition.

Total body fat was measured by multipolar bioelectrical impedanciometry (Inbody 530©), using 15 different measures (30-s assessment) at three different frequencies (5–50–500 kHz) in left arm, right arm, trunk, left leg and right leg.

2.3 | Clinical data

Type 2 diabetes was diagnosed according to ADA guidelines. Abnormal blood pressure was defined as >130 mmHg for systolic and >85 mmHg for diastolic blood pressure, and dyslipidemia was defined as serum triglycerides >150 mg/dl or HDL-cholesterol <40 mg/dl in male and <50 mg/dl in female. Fatty liver (NASH) was defined as elevated liver enzymes in the blood tests records and loss of differential ultrasound echogenicity between liver and right kidney parenchyma. Metabolic syndrome was defined according to ATPIII definition criteria.

2.4 | Laboratory measurements

In the overweight and obese patients, measurements of basal serum insulin and leptin (enzyme immunoanalysis BEST2000 from DBC Diagnosis Biochem Canada, Inc. 0.50 ng/ml sensibility) were recorded at the time the echography was performed. HOMA index was also calculated in these subgroups and HOMA-IR > 4.05 p90 was considered as marker of insulin-resistance.

2.5 | Ultrasound measurements

A General Electric Logic E© with a high frequency 12 MHz linear probe (superficial fat measures) and a 5 MHz convex probe (intraperitoneal and retroperitoneal fat measures) was used for the abdominal fat ultrasound assessment. Following a strict standardized protocol, thickness (mm) of (a) total subcutaneous tissue, divided into superficial and profound subcutaneous layers by the fascia superficialis, (b) preperitoneal fat, measured from the linea alba to the parietal layer of the peritoneum (Figure 1), (c) visceral fat, including both omental and mesenteric fat and ranging from the peritoneal line to the anterior wall of the abdominal aorta (Figure 2), and (d) perirenal fat, starting from the renal cortex to the triangle formed by liver pole and abdominal wall musculature (for the right perirenal depot) (Figure 3), were sequentially measured. All these measures were made where the abdominal aorta ultrasound imaging bifurcates into the iliac arteries, position corresponding to the fourth lumbar vertebra level (L4), using the usual reference point when measuring visceral fat with CT and MRI. Good correlation between CT assessed intraabdominal fat...
**FIGURE 1** Echographic superficial, profound subcutaneous fat and preperitoneal fat. Subcutaneous fat is divided by the fascia superficialis (strong white line) and preperitoneal fat (between the linea alba and the peritoneum). (1) superficial subcutaneous fat, (2) profound subcutaneous fat and (3) preperitoneal fat (signalled with a red square in the atlas scheme). From Atlas de anatomía humana Sobotta. Volumen I. Ferner, H. y Staubesand, J. Ed. Médica Panamericana (1982). ISBN 84-85320-25-5

**FIGURE 2** Echographic visceral/omental fat (dotted line and signalled with a red square in the atlas scheme). From Atlas de anatomía humana Sobotta. Volumen I. Ferner, H. y Staubesand, J. Ed. Médica Panamericana (1982). ISBN 84-85320-25-5

**FIGURE 3** Echographic right perirenal fat (both right and left signalled with a grey square and pointed, in the atlas scheme). From Atlas de anatomía humana Sobotta. Volumen I. Ferner, H. y Staubesand, J. Ed. Médica Panamericana (1982). ISBN 84-85320-25-5
compared to sonographic measurements had been described at this cut-off level ($r = 0.74; p < .001$).7

Our proposed sequential protocol would start from the deeper layers to the most superficial ones. Using a convex probe, perirenal fat had been measured with the patient laying on the left side. Then, with the patient upside-down, abdominal aorta should be localized, moving down the probe until the iliac bifurcation appears. A measure of the omental fat can then be done at that specific point, trying to minimize the pressure over the abdomen. At the same external abdominal area (can be drawn on the skin), the explorer must switch to the linear probe and measure consecutively preperitoneal, profound and superficial subcutaneous fat depots. The correlation coefficient of the mean ultrasound distance assessed by two different sonographers at baseline is 0.94 ($p < .001$), with a mean difference 0.40 cm (SD 0.90), and a coefficient of variation of 5.40%, indicating good reproducibility of the ultrasound measurements.7

### 2.6 | Statistical analysis

The statistical study was carried out using the statistical software package SPSS version 19.23 Quantitative variables were revised to follow or not normal distribution in order to compare their means and their confidence index (95%) through the Student's $t$ test or the non-parametric Mann-Whitney's U test, in different groups according to age, gender, menopause status, presence of fatty liver, type-2 diabetes and dyslipidemia. ANOVA test to analyse the different fat layers within different BMI categories (normal, overweight and obesity) and Jonkaert's test to check 'two by two' relationship (normal to overweight, normal to obesity and overweight to obesity) was used. To study the association between the quantitative variables, Pearson's $r$ test for normally distributed variables and Spearman's $r$ test for those with no normal distribution were calculated. Simple regression analysis was used to predict quantitative changes between related variables. ROC (receiver operating characteristic) curve analysis, split by gender, was done to establish a cut-off point either for omental or perirenal fat that could predict metabolic syndrome, in advance. Specificity and sensibility were calculated using the Youden index. Area under the curve (AUC) > 0.50.

## 3 | RESULTS

### 3.1 | Clinical and demographic data

Age ranged from 16 to 77 years old (mean 53 ± 13). A total of 28% were male and 72% female, 64% of which postmenopausal. And 49% were obese people, 32% were overweight and 19% had normal weight. Abdominal perimeter ranged from 70 to 133 cm. Mean abdominal perimeter was 110 ± 11 in male (range 84–133 cm) and 101 ± 11 in female (range 70–130). A total of 19% were type 2 diabetic patients, 41% had fatty liver, 30% had dyslipidemia, 27% patients had treated hypertension and 74% patients met metabolic syndrome criteria.

### 3.2 | Fat layers measures and correlations

Systematic measures of all five abdominal fat layers, split by gender, are shown in Table 1. Women showed thicker subcutaneous fat depots (both superficial and profound), whereas men had significantly bigger omental and perirenal fat (both left and right) ($p < .0001$). Preperitoneal fat layer was similar in both genders.

Superficial subcutaneous fat correlates with profound subcutaneous layer ($r = 0.58; p < .0001$) and total subcutaneous showed a negative correlation with omental fat depot ($r = −0.14; p = .017$). No other significant correlations between layers were found.

Subcutaneous layers (both superficial profound and total subcutaneous), left perirenal and omental fat, are all thicker as BMI category increases. This relation is also valid in a two-by-two analysis (normal to overweight, normal to obese and overweight to obese patients’ relationship) (Table 2). No significant differences in preperitoneal fat and right perirenal fat according to BMI categories were found.

Both omental and perirenal fat showed a significant correlation both with BMI ($r = 0.53; p < .001$ for omental fat and $r = 0.35 p < .0001$ for right and $r = 0.29 p < .0001$ for left perirenal fat) and abdominal perimeter ($r = 0.55; p < .001$ for omental fat and $r = 0.39; p < .0001$ for right and $r = 0.35; p < .0001$ for left perirenal fat). Every 10-mm increase in US-measured omental fat corresponds to a 2-cm increase in the abdominal perimeter measurement in men or 2.4 cm in women.

### 3.3 | Fat layers in different clinical situations

Patients with recorded diagnosis of Fatty liver showed bigger amounts of all preperitoneal ($p < .01; CI, 1.05–4.33$), omental ($p < .001; CI, 13.60–30.60$) and perirenal fat (both left $[p < .01; CI, 1.80–13.60]$ and right $[p < .001; CI 2.30–11.28]$), compared to nondiagnosed fatty liver patients. No changes were seen when analysing subcutaneous fat depots. In fatty liver diagnosed patients, a significant correlation between preperitoneal fat and omental fat depots ($r = 0.43; p = .001$) as well as between preperitoneal and perirenal fat ($r = 0.22; p = .001$ for left perirenal and $r = 0.25 p = .001$ for right perirenal fat) was found.

Menopausal women showed significant increases only in omental fat ($p = .002; CI 6.25–25$ mm) as also occurs in Type-2 diabetic patients ($p = .04; CI, 6–23$ mm). Serum glucose level (mean 106 ± 37 mg/dl) also showed a significant correlation only with the omental fat depot ($r = 0.22; p < .05$). A significant correlation between omental fat measure and HOMA-index was also found ($r = 0.43; p < .001$). Perirenal fat did not show any correlation with HOMA index.

No statistical differences were found comparing fat depots in the group of patients suffering hypertension.
Patients with recorded dyslipidemia did not show any difference in the fat layers distribution compared to those with normal lipid profile. Only HDL-cholesterol (mean 63 ± 20 mg/dl in women and 49 ± 13 mg/dl in men) showed a significant negative correlation with omental fat thickness ($r = -0.22; p < .05$) and left perirenal fat ($r = -0.23; p < .0001$). No correlations were found comparing total cholesterol (205 ± 46 mg/dl), LDL (124 ± 37 mg/dl) or triglycerides (126 ± 64 mg/dl) with any abdominal layer.

Multivariate analysis ($R^2 = 0.53$) including age, BMI, abdominal perimeter, serum glucose, HDL-cholesterol, HOMA-index, type 2 diabetes or fatty liver diagnoses as predicting values with omental fat thickness as dependent value only shows a significant association with the abdominal perimeter measure ($\beta = 0.49$ $p < .01$).

Patients fulfilling ATPIII criteria of metabolic syndrome showed both thicker perirenal ($p = .024; CI, 0.80–8.70$ mm in left perirenal; and $p = .02; CI, 1.60–7.04$ mm in right perirenal) and omental ($p = .001; CI, 14–26$ mm) fat depots.

3.4 Predictive analysis

ROC curves analysis showed a cut-off point for omental fat measurement of 54 mm (S: 85% E: 65% AUC 0.78; $p < .001$) in male and 37 mm (S: 73% E: 55% AUC 0.71; $p < .001$) in female to predict later development of metabolic syndrome features (Figure 4).

For left perirenal fat measurement, the cut-off point is 22.50 mm (S: 73% E: 70% AUC 0.70; $p = .02$) in male and 12.50 mm (S: 72% E: 57% AUC 0.63; $p = .03$) in female and 14.50 mm (S: 74% E: 54% AUC 0.65; $p < .001$) in female (AUC 0.64; $p = n.s$ in male) for right perirenal to predict later development of metabolic syndrome features (Figure 5).

Correlations between fat layers and anthropometric or analytical parameters were maintained when thickness was adjusted by total body fat %.

4 DISCUSSION

This is the first study, to our knowledge, which used ultrasound imaging in a standardized manner, to assess all the five different abdominal fat layers, in a large population.

Ultrasound measurement is an affordable methodology, noninvasive and easy to use. In fact, most endocrinologists are already

| TABLE 1 | Abdominal fat layers thickness according to gender distribution |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Fat Layer Thickness, mm | General Population n = 397 | Women n = 285 | Men n = 112 | $p$ |
| Total subcutaneous | 20 | 21 (18–27) | 19 (12–25) | n.s |
| Superficial subcutaneous | 9 | 10 (7–11) | 7 (6–10) | <.05 |
| Profound subcutaneous | 11 | 12 (10–15) | 9 (6–15) | <.0001 |
| Preperitoneal | 11 | 11 (7–14) | 11 (8–14) | n.s |
| Right perirenal | 22 | 18 (12–23) | 29 (22–36) | <.001 |
| Left perirenal | 18 | 12 (7–16) | 28 (16–37) | <.0001 |
| Omental (visceral) | 51 | 44 (34–57) | 70 (57–86) | <.0001 |

Note. Results expressed as median (mm) and IQA (interquartile amplitude) (between arrows). $p$ ANOVA (comparing sex distribution).

| TABLE 2 | Abdominal fat layers thickness according to BMI categories |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| BMI | Median Range, mm |
| Total subcutaneous | Normal | 42 (36–48) |
| Overweight | 43 (35–49) |
| Obesity | 63 (51–76) | .001 |
| Superficial subcutaneous | Normal | 44 (37–51) |
| Overweight | 43 (35–49) |
| Obesity | 63 (51–76) | .002 |
| Profound subcutaneous | Normal | 44 (37–51) |
| Overweight | 46 (39–53) |
| Obesity | 63 (52–77) | .008 |
| Pre-peritoneal | Normal | 40 (34–46) |
| Overweight | 55 (48–62) |
| Obesity | 58 (50–65) | .37 |
| Right Perirenal | Normal | 40 (34–46) |
| Overweight | 54 (48–60) |
| Obesity | 56 (47–65) | .83 |
| Left Perirenal | Normal | 39 (34–45) |
| Overweight | 51 (46–55) |
| Obesity | 56 (47–64) | .05 |
| Omental (visceral) | Normal | 36 (32–39) |
| Overweight | 47 (39–53) |
| Obesity | 64 (47–82) | .001 |

Note. Results expressed as median (mm) and IQA (interquartile amplitude) (between arrows). $p$ Jonkaert’s test (BMI categories ‘two-by-two’ comparison).
assessing thyroid gland morphology at the patient’s bed side and are keen on linear (high frequency) probe use for superficial imaging.

Linear probe ultrasound of abdominal fat is the only available technique that allows us to differentiate the superficial from the profound subcutaneous adipose tissue depots. Thicker superficial subcutaneous layer may act as a protective barrier for infections, just below the skin, and may also be an important energy storage depot, especially in women. This sexual dymorphism, also found in our population, lean, overweight or obese, is present in almost all mammals.1

On the other side of the fascia superficialis, a rudimentary structure in humans, yields the profound subcutaneous tissue. Highly vascularized and innervated, it is rich in preadipocytes and stem cells.24 Brown adipocytes (in periscapular or supratendinous location) are also located in this profound subcutaneous layer.25

Adiponectin, a protective cytokine in terms of cardiovascular risk and metabolic syndrome pathogenesis, is secreted in higher amounts in this particular fat depot. According to our results, subcutaneous depots seem to be thicker in nonfatty liver patients and shows a negative correlation with omental fat, enhancing the idea of a protective layer, compared to deeper depots.4

Ultrasound imaging is the only way to measure preperitoneal fat. This layer cannot be described through DEXA, CT or MRI imaging of abdominal fat, being included in the wider concept of ‘visceral adiposity’. Little is known about its function.

Preperitoneal fat has been related to increased risk of malignancies in women.26 As previously published,27 an association between fatty liver disease and preperitoneal fat thickness was found. Therefore, for those with less ultrasound use experience, assessing preperitoneal fat by using a simple linear probe might be an easy way to assess deeper visceral adiposity (fatty liver and perirenal fat), at the bed side of our obese patients. It is important to highlight that preperitoneal fat measures should be made at L4 level in a standardized manner. Fat is much thicker close to the xiphoid appendix and thinner inside the pelvic area, where most of the gynaecological measures are made.26

In this study, another significant correlation between fatty liver and perirenal fat depot thickness has also been described.

Convex (low frequency) probe, commonly used in any visceral ultrasound imaging (liver, gallbladder, and kidney) allows us to measure visceral fat and perirenal fat.

Visceral fat is a sum of both omental (surrounding bowels) and mesenteric fat (fixing bowels to the lumbar area and giving a structural support). Despite some images show the mesenteric fat as hyper-echogenic with linear tracts (more fibrotic) and the omental as
hypoechogenic dotted areas, it remains not possible at present, to give separate approximative measures of them. Again, for accuracy measurement, L4 (abdominal aorta bifurcation sonography) should be the selected cut-off level and the explorer should not pressure too much on the abdomen wall. Published papers show a good correlation between intraabdominal fat assessed with CT compared to sono-

graphic measurements (r = 0.74; p < .001).7

CT or MRI measures of visceral fat have been widely associated with cardiovascular risk factors in number of papers.3 Ultrasound measures of omental fat have been clinically validated and show strong correlations with fatty liver disease,12 type-2 diabetes13 and coronary artery disease.14,15 Our findings (significant correlations with HDL-cholesterol, serum glucose levels, menopause, fatty liver or type-2 diabetes or metabolic syndrome diagnoses) support these previous published works.

DEXA/CT or magnetic resonance imaging (MRI) measures probably overestimate visceral fat compartment because neither preperitoneal nor perirenal (retroperitoneal) fat cannot be segregate.

The same complian could be done to abdominal perimeter, embracing all together: subcutaneous, preperitoneal, intra peritoneal and retroperitoneal fat, in a single measure. However, in our study, only ultrasound omental fat (perirenal fat shows a very weak correlation) shows a significant correlation with the abdominal perimeter in a multivariate analysis, highlighting the importance of waist circumference measure in the clinical assessment of obesity-associated cardiovascular risk. For every centimetre increased of omental fat corresponds an increase of 2.00–2.40 cm in the abdominal perimeter.

With the discovery of glucose-sodium transporters inhibitors beneficial effects on cardiovascular issues, kidney is becoming a major player in insulin-resistance pathophysiology.28 Because adipokines secreted in the retroperitoneal area, may probably interact with the kidney in a paracrine manner,29 assessment of the perirenal fat using ultrasound imaging may help us to understand its role.

Some preliminary work published in children show a marked correlation between ultrasound measured perirenal adiposity and increased intima media thickness as a surrogate marker of cardiovascular risk.30 In our population, only left perirenal fat showed correlation with metabolic syndrome ATPIII criteria (especially abdominal perimeter and HDL-cholesterol levels) and was thicker in fatty liver diagnosed patients but failed to show correlations with HOMA-index. The relationship of perirenal fat relationship with MS is mainly seen in left perirenal depot. Underestimation of right perirenal fat due to fatty liver pressure on it might be the reason.

Is there a maximum clinically acceptable size for some of these depots? Based on our results, a proposed cut-off level of 55 mm of omental fat in male and 34 mm in female seems to be predictive of metabolic syndrome associated cardiovascular risk markers (type 2 diabetes, low-HDL and hypertension).

For left perirenal layer, the predictive cut-off would be 22.50 mm in male and 12.50 mm in female. Again, these cut-off measures should be confirmed in populations at-risk and its value as a prognostic marker should be evaluated in larger studies.

This work has some important limitations. Despite published data,7 concerns about intra and interobserver variation and reproducibility are raised. This study is focused on a northern Spanish population, with a nonbalanced number of men/women and predominance of obese subjects and specific subset of metabolic patients has not been formerly defined before recruitment.

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CONFLICT OF INTEREST
All the authors have declared no having any conflict of interest and send the form to the corresponding author.

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