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ORIGINAL ARTICLE

Ultrasound, anthropometry and bioimpedance: a comparison in predicting fat deposition in non-alcoholic fatty liver disease

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Abstract
Purpose The aim of our study was the evaluation of anthropometric measurements [waist circumference and sagittal abdominal diameter (SAD)] and abdominal bioelectrical impedance analysis (BIA) (ViScan, TANITA) in comparison to several abdominal ultrasonographic (US) measurements to estimate visceral fat deposition and liver steatosis in a population of 105 subjects.
Methods All 105 patients underwent a complete anthropometric evaluation, blood sample for the determination of total cholesterol, HDL cholesterol, triglycerides, glucose, insulin, high-sensitivity C-reactive protein, BIA and US measurements (peritoneal, pre-peritoneal, peri-renal, para-renal and peri-hepatic fat thickness).
Results All the ultrasonographic markers considered in our study are related to the presence of non-alcoholic fatty liver disease (NAFLD), and so is true for SAD. Comparing ROC curves, peritoneal fat tissue thickness, SAD and ViScan visceral index are significantly better than waist circumference in predicting the presence of NAFLD (AUC 0.79 ± 0.04; 0.81 ± 0.05; 0.82 ± 0.04 vs 0.76 ± 0.05, respectively).
Conclusions According to our data, various methods may be useful in evaluating NAFLD, but only ViScan visceral index, US peritoneal fat thickness and SAD are better than waist circumference. Among them, SAD is the most promising, due to its small cost and time consumption.

Keywords Ultrasound · Bioimpedance · Abdominal fat · NAFLD

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is a chronic liver disease with histological features similar to alcohol-induced liver damage, but occurring in the absence of significant alcohol consumption (<20 gr/die) [1, 2]. Often regarded as a benign disease, NAFLD may lead to more severe outcomes. The true prevalence of NAFLD is unknown [3], but is reported between 3 and 30 % in the general population worldwide [4–6], with the prevalence increasing to about 60–70 % in obese patients [7]. Obesity, insulin resistance and metabolic syndrome (MS) are strongly associated with NAFLD and severity of the disease [8].

The distribution of body fat has a more important role in obesity-associated comorbidities because intra-abdominal visceral fat accumulation plays a central role in the development of metabolic syndrome and related diseases [8–11]; a precise and reliable estimation of visceral fat may be important for risk stratification and the identification of patients having high risk of NAFLD and its histological severity [12].

A precise measurement of visceral fat requires imaging techniques (CT or MRI) that are scarcely available and not applicable in routine clinical practice. Waist circumference has been proposed as a surrogate for the estimation of visceral fat in clinical practice [13]. Waist is inexpensive and widely usable, but its correlation with visceral fat deposition may be questionable at least in morbid obese

women [14]. Other anthropometric indexes have been suggested as more reliable [for example sagittal abdominal diameter (SAD)] [15]. Among instrumental techniques, bioelectrical impedance analysis (BIA) has been applied to quantify abdominal fat [16]. Abdominal ultrasonography is reliable, repeatable, less expensive and has been proposed to detect visceral fat deposition [17]. Among ultrasonographic indices of viscerality, peritoneal fat thickness is considered the gold standard [15], but many other different echographic approaches have been proposed to evaluate visceral fat, for example peri-renal and para-renal fat [18] and peri-hepatic adipose tissue thickness [19]. Therefore, the search for more reliable clinical indicators for visceral fat accumulation may be considered still open.

To our knowledge, in the literature there is no other study that has compared all these anthropometric, bioelectrical methods and different parameters evaluated with abdominal ultrasonography for evaluating visceral fat deposition and its relationship with NAFLD. Therefore, the aim of our study was the evaluation of anthropometric measurements (waist circumference and SAD) and abdominal BIA (Vi-Scan, TANITA) in comparison to several abdominal US measurements of visceral fat deposition and liver steatosis in a sample of normal weight and obese subjects.

Methods

A total of 105 randomly selected subjects (56 males and 49 females) with a mean age of 52.8 ± 14.7 years (range 23–82 years) participated in the study. Twenty-six patients hospitalized for acute medical problems in the Clinica Medica 1 unit of the Padova University Hospital, Italy, 42 patients attending for the first time the outpatients' service for dyslipidemic disorders of the same institution and 37 healthy subjects undergoing a routine screening were enrolled from April 2009 to July 2010. Exclusion criteria were known liver disease, severe kidney disease, recent acute myocardial infarction, uncontrolled diabetes mellitus, weight change of more than 3 kg during the last 3 months, current treatment with insulin-sensitizing drugs like metformin and glitazones, weight-loss drugs like rimonabant, sibutramina ed orlistat, beta-blockers and oral lipid-lowering drugs, and alcohol assumption of more than 20 gr/die. Given the observational nature of the study, not involving active treatments or diagnostic procedures that are not routinely used in clinical practice (blood samples and ultrasound are obtained for clinical purposes), no formal institutional review board approval was requested. All subjects gave their informed consent to the use of their clinical data. Patients were evaluated in the same morning and after a 12-h overnight fast with anthropometry and abdominal ultrasonography.

Anthropometry

All anthropometric measurements were performed with the subjects wearing light clothes without shoes. Height was measured to the nearest 0.01 m using a calibrated wall-mounted stadiometer. Body weight was determined to the nearest 0.05 kg using a calibrated balance beam scale. BMI was calculated as weight (kg) divided by the height-squared (m^2). Body circumferences were measured with a flexible tape, with the subject in the upright position at the end of a gentle expiration, at the following levels: waist (midway between the lower rib margin and the superior anterior iliac spine) and hip (widest circumference over the great trochanters). Sagittal abdominal diameter (SAD) was determined at the highest point of the abdominal surface with the subject in the supine position and during normal breathing by means of a specifically made instrument [15].

Metabolic variables

Venous blood samples were obtained after a 12-h overnight fast for the determination of the following metabolic parameters: total cholesterol, HDL cholesterol, triglycerides, glucose, insulin, high-sensitivity C-reactive protein (hs-CRP). All analytical determinations have been performed at the Department of Laboratory Medicine of University Hospital of Padova, Italy. Fasting plasma glucose, total cholesterol, HDL cholesterol and triglycerides were measured in plasma samples (lithium-heparin) using an enzymatic assay automatized on the Modular DP (Roche Diagnostics, Mannheim, Germany). Plasma insulin concentration was determined with a chemiluminescent assay automatized (IMMULITE® 2000 (Medical System S.p.A., Genova, Italia). Insulin resistance was calculated according to the homeostasis model assessment (HOMA-IR). Hs-CRP concentrations were measured with a high-sensitivity immunonephelometric assay (Cardiophase® hsCRP, Siemens Healthcare Diagnostics, Malvern, PA, USA) automatized on the BN II analyzer (Siemens Healthcare Diagnostics, Malvern, PA, USA). LDL cholesterol was calculated according to the Friedewald's formula except when triglycerides were >400 mg/dl.

Abdominal ultrasonography

All the patients underwent liver ultrasonography performed by the same operator with a Toshiba Aplio XV scanner (Toshiba Corporation, Tokyo, Japan) using a 3.5 MHz convex probe. Evaluation of the liver includes many scans taken and recorded: transverse epigastric; oblique right subcostal; oblique right subcostal; longitudinal involving the right kidney in the mediaxillary line, and two

intercostal to visualize the right lobe and right kidney if not visualized in longitudinal scan. The gain and time-gain compensations had to be considered optimal if the parenchymal echoes were as bright as possible while vascular structures were kept anechoic, according to Needleman [20]. Patients were classified as NAFLD positive or negative according to the presence/absence of bright liver. The diagnosis of "bright liver" was based on abnormally intense, high-level echoes arising from the hepatic parenchyma, and was graded on a three-grade scale, defined as follows: grade 1 = increased echogenicity or bright liver with normal visualization of diaphragm and intrahepatic vessel borders; 2 = increased echogenicity with posterior beam attenuation, but with slightly impaired visualization of the intrahepatic vessels and diaphragm; and 3 = marked increase in echogenicity and marked posterior beam attenuation resulting in failure to demonstrate the intrahepatic vessels, diaphragm, and posterior right lobe of the liver [21]. The measurements included pre-peritoneal, peritoneal, peri-renal, para-renal and peri-hepatic fat. Pre-peritoneal fat thickness was measured with the modified criteria of Suzuki et al. [22]. The pre-peritoneal fat thickness was measured from the external face of the recto-abdominal muscle to the skin surface, between xiphoid process and umbilicus, with the probe placed perpendicular to the skin surface and scanning longitudinally along the midline of the abdomen. Peritonal fat thickness was measured from the internal face of the recto-abdominal muscle to the anterior wall of aorta, with the convex probe transversely placed perpendicular to the skin in the midline of abdomen [17]. Peri-renal fat was determined as the distance between the kidney and the Gerota's fascia, and the para-renal fat was calculated as the distance between the renal fascia and the abdominal muscle [18]. We finally measured peri-hepatic fat as the thickness of the adipose tissue comprised between the abdominal muscular layer and the surface of the liver [19]. The probe was placed between the ribs along the mid-axillary line and the ultrasound beam was aimed toward the right branch of the portal vein. We measured the echogenic tissue between the hyper-echogenic line of the deeper surface of the abdominal muscle and the liver surface. This assessment represents a modification of the measurement of the subcutaneous tissue thickness by Riley et al. [23] who measured the distance between the skin surface and the hepatic surface, thus including the muscular layer. All ultrasonographic measurements were taken twice, and the mean value was calculated for analysis. A standard approach with the patient lying in the supine position with the arms placed above the head was used. All ultrasound measurements of fat thickness were made in the expiratory phase of a quiet respiration. The application of the transducer on the body surface was done without pressure.

Abdominal bioelectrical impedance analysis

Abdominal bioelectrical impedance analysis (BIA) was performed with the use of a bioelectrical abdominal fat analyzer (AB-140 ViScan) (Tanita Corporation, Tokyo, Japan). The ViScan consists of a rigid electrode belt that is placed on the bare midriff of the subject. The belt has two pairs of injecting and sensing electrodes placed directly on the skin at the umbilicus in the sagittal plane and uses dual-frequency BIA technology (6.25 and 50 kHz) to take bioelectrical measurements. The following abdominal body composition values are derived from extrapolation of impedance measures using inbuilt software: trunk fat percentage on a scale of 5.0–75.0 % (0.1 % g graduation); visceral fat level on a scale of 1–59 arbitrary units (0.5 graduation); estimated waist circumference (1 cm graduation). The method has a high reproducibility and required <1 min for data acquisition. Abdominal body composition values obtained with ViScan have been compared to total abdominal adipose tissue and intra-abdominal adipose tissue measured by magnetic resonance imaging (MRI) in a cross-sectional validation study including 74 participants (40 females and 34 males with BMI between 18.5 and 39.6 kg/m^2) [16]. In this study, the ViScan-derived percentage trunk fat was found to be strongly associated with MRI-derived total abdominal fat ($r = 0.938$; $p < 0.001$), explaining 88 % of the variance in total abdominal fat. The ViScan-derived visceral fat level also correlated strongly with MRI-derived intra-abdominal adipose tissue ($r = 0.731$; $p < 0.001$).

Statistical analysis

Results are expressed as mean ± standard deviation. All variables were tested for normal distribution and skewed variables were logarithmically transformed. Unpaired Student's t test was used to compare numerical variables in positive and negative NALFD subjects. Chi square was used for non-continuous variables. The relationships between anthropometric, abdominal BIA, ultrasonographic measurements and NALFD were tested in a logistic regression analysis, adjusted for age, sex and waist circumference. In all statistical analysis, a p value <0.05 was considered to indicate statistical significance.

ROC curves are compared each other according with formula of Hanley and McNeil [24, 25], actually considered the most rigorous statistical analysis for comparing ROC curves. Statistical analysis was performed with the SPSS statistical package, version 16.0 (SPSS, Chicago, IL).

Results

Patients' anthropometric and biochemical parameters are summarized in Table 1, considering both total population

Table 1 Anthropometric and biochemical parameters in total population and according to the presence of NAFLD (data are mean ± standard deviation; frequency for discrete variables)

Parameter	Total population *n*:105	NAFLD+ *n*:51	NAFLD− *n*:54
Age (years)	53.1 ± 14.6	**56.2 ± 12.4**[1]	**50.2 ± 15.9**
Sex M/F (%)	56/49 (53.3/46.7)	35/16 (68.6/31.4)	21/33 (38.9/61.1)
BMI (kg/m^2)	29.2 ± 5.3	**31.3 ± 4.5**[2]	**27.3 ± 5.4**
Waist (cm)	102 ± 15	**108 ± 10**[2]	**96 ± 16**
SAD (cm)	23.0 ± 4.6	**25.2 ± 3.0**[2]	**20.7 ± 4.7**
Col-Total (mg/dl)	215 ± 47	219 ± 54	212 ± 40
Col-HDL (mg/dl)	46 ± 14	**41 ± 9**[2]	**51 ± 17**
Col-LDL (mg/dl)	136 ± 40	139 ± 45	134 ± 35
TG (mg/dl)	132 ± 84	**162 ± 103**[2]	**105 ± 48**
TG/HDL ratio	3.4 ± 3.0	**4.4 ± 3.8**[2]	**2.5 ± 1.7**
Glycemia (mg/dl)	99 ± 21	**104 ± 25**[1]	**94 ± 14**
HOMA-IR	1.9 ± 1.4	**2.4 ± 1.5**[2]	**1.2 ± 0.8**
log hs-CRP (mg/dl)	0.87 ± 1.32	**0.94 ± 1.43**[1]	**0.26 ± 1.34**
Hypertension (Y/N)	83	**49**[3]	**34**

Bold values are statistically significant values of p

t test for unpaired data: NAFLD+ vs − [1]$p < 0.05$, [2]$p < 0.005$, χ^2 NAFLD+ vs − [3]$p < 0.005$

Table 2 Ultrasound measurements, BIA parameters and SAD in total population and according to the presence of NAFLD (data are mean ± standard deviation)

US fat thickness	Total population	NAFLD+	NAFLD−
Pre-peritoneal (mm)	18.3 ± 7.2	18.4 ± 7.5	18.2 ± 7.0
Peritoneal (mm)	67.8 ± 25.2	**80.0 ± 22.7**[2]	**56.0 ± 21.8**
Peri-renal (mm)	9.5 ± 6.8	**12.4 ± 7.2**[2]	**6.7 ± 4.9**
Para-renal (mm)	11.6 ± 8.5	**15.6 ± 8.5**[2]	**7.9 ± 6.7**
Peri-hepatic (mm)	5.6 ± 2.5	**6.7 ± 2.5**[2]	**4.5 ± 1.9**
US Right liver lobe (cm)	15.4 ± 2.3	**16.3 ± 2.1**[2]	**14.5 ± 2.1**
ViScan visceral index (a.u.)	15.7 ± 7.5	**19.4 ± 7.0**[2]	**12.0 ± 6.0**
ViScan trunk (%)	37.6 ± 9.7	38.6 ± 8.4	36.5 ± 10.7
ViScan waist (cm)	104 ± 16	**108 ± 10**[1]	**101 ± 10**
SAD (cm)	23.0 ± 4.6	**25.2 ± 3.0**[2]	**20.7 ± 4.7**

Bold values are statistically significant values of p

t test for unpaired data: NAFLD+ vs − [1]$p < 0.05$, [2]$p < 0.005$

Table 3 Logistic regression, adjusted for age, sex and waist circumference

US fat thickness	Beta	p
Pre-peritoneal (mm)	−0.02	0.64
Peritoneal (mm)	**0.03**	**0.05**
Peri-renal (mm)	**0.12**	**<0.05**
Para-renal (mm)	**0.10**	**<0.05**
Peri-hepatic (mm)	**0.30**	**<0.05**
Right liver lobe (cm)	**0.25**	**<0.05**
ViScan visceral index (a.u.)	0.12	0.09
ViScan trunk(%)	−0.19	0.62
ViScan waist (cm)	−0.01	0.77
SAD (cm)	**0.40**	**<0.05**

Bold values are statistically significant values of p

and two subgroups (according to the ultrasonographic diagnosis of NAFLD).

Differences were found between the two groups in BMI, waist circumference, SAD and prevalence of hypertension; in NAFLD group there was a significant higher level of triglycerides, Col-HDL, glycemia and HOMA index. This is the typical metabolic pattern of insulin resistant patients; no significant difference was found for Col-LDL level. Also, CRP was higher in NAFLD patients.

Table 2 summarizes the ultrasound measurements of intra-abdominal fat, the parameters obtained by abdominal bioelectrical impedance analysis (BIA) and the sagittal abdominal diameter (SAD).

According to the presence of NAFLD, we found significant differences in peritoneal fat ($p < 0.005$) peri- and para-renal fat ($p < 0.005$), in peri-hepatic fat ($p < 0.005$), in right liver lobe ($p < 0.005$), ViScan visceral index (a.u.) ($p < 0.005$), in ViScan Waist ($p < 0.05$) and SAD ($p < 0.005$). We did not find significant differences for pre-peritoneal ($p = 0.42$) and ViScan Trunk ($p = 0.33$).

All the ultrasonographic markers of visceral obesity considered in our study seem to be related to the presence of NAFLD, and so is true for ViScan visceral index and SAD. These correlations remain true adjusting for age, sex and waist circumference. In fact we found a significant correlation between the presence of NAFLD and peritoneal

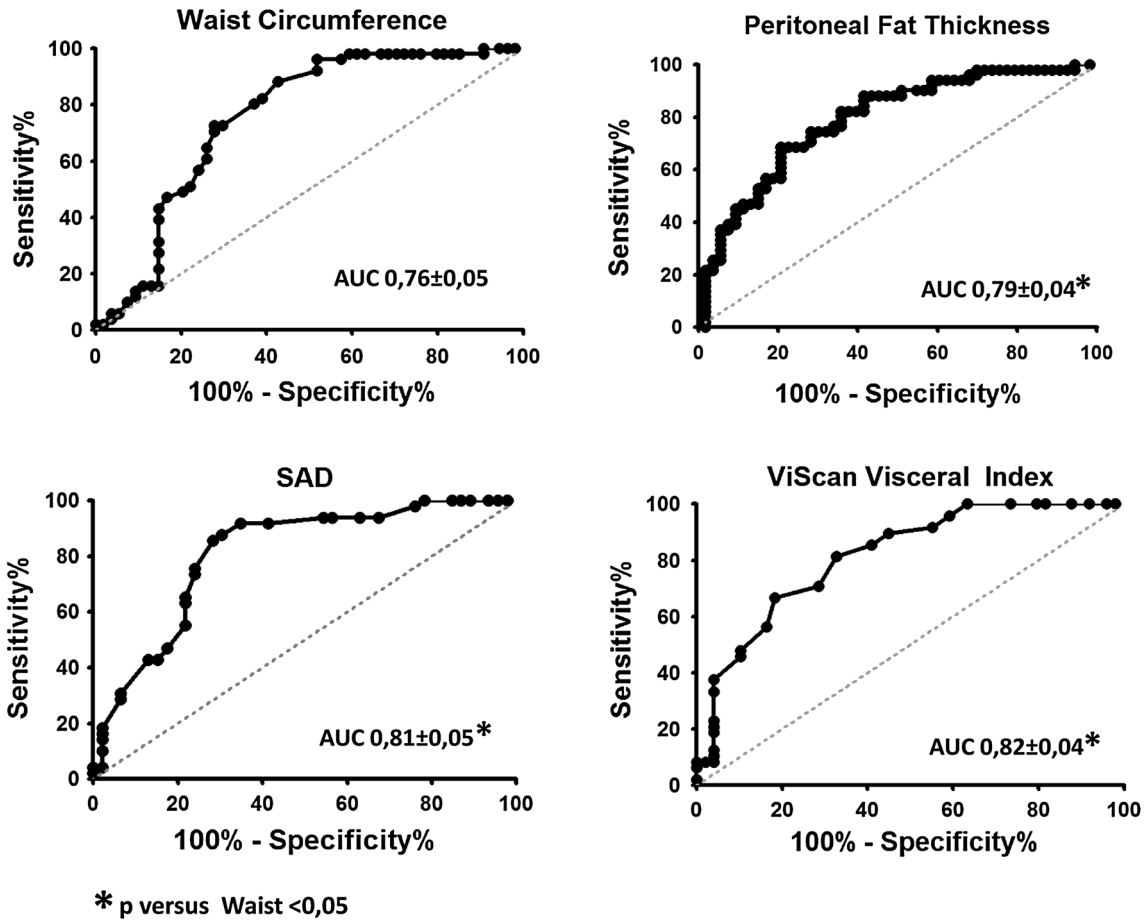

Fig. 1 AUC of ROC curves (data expressed as AUC value ± SD) and differences with waist circumference (*p* evaluated according to Hanley and McNeil)

fat (beta = 0.03 $p = 0.05$), peri-renal fat (beta = 0.12 $p < 0.05$), para-renal fat (beta = 0.10 $p < 0.05$), peri-hepatic fat (beta = 0.30 $p < 0.05$) and SAD (beta = 0.40 $p < 0.05$), but not ViScan parameters (Table 3).

To assess the ability of each parameter to predict the presence of NAFLD, we calculated ROC curves for SAD, ultrasound and BIA parameters (Fig. 1); then we compared them to waist circumference and to peritoneal fat thickness.

Peritoneal fat tissue thickness, SAD and ViScan visceral index AUC are significantly different from waist circumference AUC. Compared to peritoneal fat thickness AUC, the ultrasonographic index for visceral fat considered our reference method, and all the parameters share the same diagnostic accuracy in predicting the presence of NAFLD, with no significant differences in AUC.

Discussion

Non-alcoholic liver disease is the most common cause of chronic liver disease, with a prevalence up to 30 % in the general population. Even if considered as a benign disease, it may lead to more severe outcomes, such as cirrhosis and hepato-cellular carcinoma.

The relationship between NAFLD and metabolic abnormalities is well known, so that often it is referred to as an additional element of the metabolic syndrome. Insulin resistance and visceral obesity play a fundamental role in the development of NAFLD, as for many other obesity-related disorders.

A correct evaluation of visceral obesity is essential for risk stratification for the development of NAFLD. Liver fat content is related to the presence of visceral fat and not to the presence of obesity itself.

While CT and MRI scan provide very precise quantification of intra-abdominal fat, they are expensive and not routinely useful (CT also exposes patients to ionizing radiation). In our study, we utilized some non-invasive parameters, both anthropometric and instrumental (impedentiometric and ultrasonographic). Data show that all our indices of visceral fat are significantly different in NAFLD patients compared to the control group; no differences were

found, as we expected, in subcutaneous fat and in trunk fat (measured with ViScan). This confirms the relationship between intra-abdominal fat and steatosis: more fat, more severe is the steatosis. When compared to each other, the diagnostic performances in predicting fatty liver are significantly better (compared to waist circumference) for peritoneal fat thickness, SAD e ViScan visceral index; even though very different from each other (SAD is an anthropometric measurement; US peritoneal fat thickness and ViScan are instrumental parameters), they have similar diagnostic performance (considered in our paper as AUC in the ROC curve); none of them obtains significantly better result. The simplest and the most used method for the estimation of visceral fat accumulation is the waist circumference; however, it is not able to distinguish between visceral and subcutaneous fat (that has not a role in insulin resistance) and may misclassify individuals in terms of visceral adipose tissue [26]. Even with these limitations, we decided to compare the diagnostic performance in the detection of NAFLD of our anthropometric and instrumental parameters to waist circumference, due to its lower cost and simplicity. Our data show that only peritoneal fat thickness (among the ultrasound parameters) and ViScan visceral index are better (even if more expensive and time consuming) than waist circumference. Another promising parameter is SAD, which measures the antero-posterior diameter of the abdomen. Its relationship with visceral adipose tissue is based on the observation that subcutaneous fat is displaced inferiorly by gravity [27]. Few studies confirmed their association with metabolic abnormalities [28] or visceral fat [29]. Our data suggest that SAD is related to the presence of NAFLD than waist. Compared to ultrasound and ViScan, SAD is a valuable tool in the diagnosis of NAFLD, while being certainly quicker and cheaper.

In our study we did not measure visceral fat deposition with CT or MRI, so we cannot compare the diagnostic performance of anthropometry, ultrasound and BIA to these two imaging techniques that at the moment are considered the gold standard for evaluating intra-abdominal fat. Even if more accurate than ultrasound, CT and MRI are expensive and time consuming. In the last years, the need for a more rapid, simple and widely usable technique has led to an improved use of ultrasound. In the last few years, some papers have tried to identify the more accurate ultrasound parameter for evaluating intra-abdominal fat deposition and for predicting liver steatosis [12]. In our knowledge, no other study has directly compared ultrasound parameters with anthropometry and bioelectrical impedance analysis. Our data suggest that ultrasound parameters are as good as other techniques in predicting NAFLD. Among the various ultrasound parameters, all of which are related—according to the literature—to metabolic alterations typical of insulin resistance, the peritoneal fat thickness would seem to be better than other ultrasound parameters as diagnostic ability [30, 31].

One of the limitations of our study is due to the single operator performing ultrasound examination; this led to a high accuracy due to huge experience but losing the evaluation of inter-operator variability. Another limitation is the absence of histological data of degree of steatosis. Even if we applied a well-validated ultrasound protocol described by Saverymuttu [21], our data on liver fat deposition are only indirect; moreover, we have no biochemical index of NAFLD (i.e. fatty liver index or liver fat score). Interesting field of further investigation could be the role of ultrasound, BIA and anthropometry in predicting the evolution from simple NAFLD to NASH.

Even with the caveat pointed out above, the comparison between ultrasound, ViScan and SAD in diagnosis and grading NAFLD shows that many techniques may be useful, but only ViScan visceral index, US peritoneal fat thickness and SAD are better than waist circumference. Among them, SAD is the most promising, due to its small cost and time consumption.

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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