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FULL PAPER

Liver in the analysis of body composition by dual-energy X-ray absorptiometry

^{1,2}A BAZZOCCHI, MD, PhD, ¹D DIANO, MD, ²U ALBISINNI, MD, ³G MARCHESINI, MD, ¹G BATTISTA, MD and ^{4,5}G GUGLIELMI, MD

¹Department of Specialized, Diagnostic, and Experimental Medicine, University of Bologna, Sant'Orsola Malpighi Hospital, Bologna, Italy

²Diagnostic and Interventional Radiology, IRCCS Rizzoli Orthopaedic Institute, Bologna, Italy

³Unit of Metabolic Diseases and Clinical Dietetics, University of Bologna, Sant'Orsola Malpighi Hospital, Bologna, Italy

⁴Department of Radiology, University of Foggia, Foggia, Italy

⁵Department of Radiology, Scientific Institute "Casa Sollievo della Sofferenza" Hospital, Foggia, Italy

Address correspondence to: Professor Giuseppe Guglielmi

E-mail: giuseppe.guglielmi@unifg.it

Objective: To investigate the predictive value for hepatic steatosis of a new software for the quantification of visceral fat by dual-energy X-ray absorptiometry (DXA) and to design new regions of interest (ROIs).

Methods: Adult volunteers were prospectively screened for hepatic steatosis by ultrasonography to obtain a well-balanced population according to the presence/absence of the disease. 90 adult patients without steatosis and 90 with steatosis (mild, 53.3%; moderate, 37.7%; and severe, 10.0%) were recruited. On the same day, all subjects were submitted to blood testing and to anthropometric and whole-body DXA for body composition evaluation. A new software for android visceral fat assessment was employed, and six new "liver-suited" ROIs as well as two modified android ROIs were designed. Their association with steatosis grade was tested by correlation analysis.

Results: Fat mass (FM) of the new ROIs showed the highest correlation coefficients with steatosis grade ($\rho = 0.610$ – 0.619 ; $p < 0.001$), which was also confirmed by multivariate analysis. On the whole population, the new ROIs maintained the highest predictive role for liver steatosis, with areas under the receiver operating characteristic curve up to 0.820 ± 0.032 . Inter- and intra-operator agreement for the new ROIs was excellent ($k = 0.915$ – 1.000 and $k = 0.927$ – 1.000).

Conclusion: New ROIs could be designed, standardized and implemented in DXA whole-body scan to provide more specific and predictive values of hepatic lipid content.

Advances in knowledge: This is the first study to investigate the predictive value for hepatic steatosis of visceral and regional FM assessed on the hepatic site by DXA in comparison with ultrasonography, anthropometry and surrogate markers derived by previously validated algorithms (fatty liver index).

The study of body composition has gained huge importance in the past few years. The increased availability of imaging techniques and the development of new methods for the assessment of body composition have been essential in the expansion of research fields as well as in enhancing the clinical impact and potentials of *in vivo* body composition analysis.^{1,2}

Adipose tissue and lipid content of different tissues (the muscle, liver etc.) have been increasingly investigated, and now the feeling in the scientific community is that most information about lipid storage, metabolism and associated risks for health has yet to be discovered. Generally, metabolism and the physiopathology associated with fat and adipose tissue seem to represent a special cornerstone in the comprehension and the development of a wide spectrum of

diseases and this issue is probably still underestimated. It has been demonstrated that adipose tissue as an active endocrine organ and its distribution are more considerable than the total amount of body fat in determining the risk of obesity and related metabolic and cardiovascular diseases. For instance, it is well known how visceral and subcutaneous fat depots have different metabolic meanings, in terms of functions and risks for health.^{3–5}

The result of improved tools and new concepts in the whole-body and regional analysis of body composition led scientists to focus on specific sites of fat depots.^{6–8}

The liver is central in the management of metabolism. Fatty liver disease includes non-alcoholic fatty liver disease (NAFLD) and alcoholic liver disease (ALD), and both of

these are increasing in prevalence. Each of which represents a histological spectrum that extends from isolated steatosis to steatohepatitis and cirrhosis. The pathogenesis of NAFLD and ALD involves cytokines, adipokines, oxidative stress and apoptosis. A subset of ALD and NAFLD will develop alcoholic steatohepatitis and non-alcoholic steatohepatitis (NASH), respectively, with serious consequences for health.

Nowadays, NAFLD is the most common cause of chronic liver disease in North America; it is estimated that 30% of the population of the USA has NAFLD and that this percentage will rise with increasing obesity in the USA and other Western countries.⁹ Patients affected by NAFLD seem to have a higher mortality from non-liver-related, as well as liver-related, causes of death, than does the general population. Patients with NAFLD present different clinical course. The majority of them have a stable disease, with isolated steatosis and an indolent course with no progression to advanced liver disease or clinical sequelae; about 30% with isolated steatosis will progress to NASH. Of those with NASH, approximately 20% develop cirrhosis, and of those with cirrhosis, 30–40% become worse and succumb to liver-related death over a 10-year period.¹⁰ NAFLD is associated with obesity, diabetes and insulin resistance and is considered the liver manifestation of the metabolic syndrome. Recent studies have also pointed out an increased risk for cardiovascular disease;^{11,12} it is not clear if this is owing to shared risk factors, or if NAFLD independently contributes to the development of cardiovascular disease. Many of the risk factors for NAFLD are known, but the underlying pathogenesis is still incompletely understood.

Liver biopsy currently represents the diagnostic gold standard for NAFLD.¹⁰ However, several non-invasive diagnostic strategies have been proposed as a potential diagnostic tool (ultrasound, CT, MRI and ultrasound elastography). Each technique presents different accuracies (lower for ultrasound) and limitations (radiation exposure and high costs for CT and MRI, respectively). Furthermore, the role of ultrasound elastography for the assessment of NAFLD is controversial.¹⁰ Hepatologists and other clinicians tend to look at the liver as at the “centre” of the human body, thus considering the fatty liver primarily as a first potential step to the worst diffuse and focal liver diseases. Therefore, the crosstalk between the liver, immunomediated processes (e.g. inflammation) and the whole “metabolic system” is sometimes forgotten or put on a second level. In other words, the sign and the features of the fatty liver should be also primarily considered from a wider point of view as something is going wrong with the “boiler” of the metabolic system.

On the other hand, in the field of liver imaging, radiologists took a step backwards focusing on diffuse liver disease and “adiposity”. Nevertheless they are actually gaining an increasing awareness of that, and beginning to consider and perform a regional body composition evaluation.^{13,14} To further support this trend, as well, in the field of cardiac imaging, more and more studies have been published concerning epicardial fat.^{15,16} Someone also studied body composition analysis as it is not only organ-based.¹⁷

Today, imaging as well as other clinical tools available for the assessment of body composition are not only aiming at fat mass (FM)/lean mass (LM) differential evaluation but also at visceral fat/subcutaneous amount quantification. Efforts in studying “quantity” and “quality” of different fat depots in different anatomical regions have been made. This emerged hot topic has become increasingly popular, but it often needs expansive techniques to be investigated, with consequent bounds fixed to research field. Although CT and MRI have been shown to be useful in distinguishing and quantifying fat tissue depots, dual-energy X-ray absorptiometry (DXA) is less expensive and invasive, and technological improvements of DXA are focusing on body composition.^{18–20}

DXA is a valid, fast, reproducible and accurate technique for the evaluation of both regional and total body composition at molecular level. Among the advantages of DXA, whole-body and regional body composition analysis capabilities are always mentioned. Moreover, a new software has been recently proposed to separately assess the visceral compartment of android fat.¹⁹ The “android” region has been defined as the portion of the abdomen included between the line joining the two superior iliac crests and extended cranially up to 20% of the distance between this line and the chin (Figure 1). DXA regions of interest (ROIs) have been proposed on the main basis of anatomical landmarks. With the exception of android region (and gynoid region—a portion of legs leaving from the femoral great trochanter, directed caudally up to a distance double of the android region), no one considered the opportunity to create new ROIs of hot metabolic regions such as the liver, to provide an estimate with a higher predictive value on lipid amount at this site.

The aim of this study was to analyse the amount of visceral adipose tissue (VAT), to develop new liver-tailored DXA ROIs and to determine their predictive value for hepatic steatosis. This opportunity might considerably increase the potential of DXA in the clinical setting.

METHODS AND MATERIALS

Ethics statement

This prospectively designed, cross-sectional, case–control, single-centre study was approved by our institutional review board (Sant’Orsola Malpighi Hospital, Bologna, Italy) and was carried out according to the principles of the Declaration of Helsinki. All patients signed an informed consent before participation.

Patients

90 patients with ultrasonographically detected steatosis and 90 adult healthy volunteers (control group) were recruited. The two groups were matched by sex, age and body mass index (BMI) ($\pm 2 \text{ kg m}^{-2}$). Pregnant females and subjects carrying implantable devices, foreign bodies or those who had been recently submitted to diagnostic tests using nuclides or barium or radio-opaque substance were not considered. Excluded were also patients presenting with cirrhosis, ascites or pleural effusions, any disease involving the upper abdominal regions or the lower chest fields (e.g. focal liver lesions,

Figure 1. Dual-energy X-ray absorptiometry whole-body analysis. According to the regional assessment of body composition the figure shows upper limbs (U), trunk (T) and lower limbs (L). A, android; G, gynoid.

pneumonitis etc.) or any other condition potentially modifying body composition (e.g. cancer).

All cases were first submitted to ultrasonography (Technos MPX; Esaote, Genoa, Italy) by the same skilled radiologist for the quantification of hepatic steatosis as detailed below. On the same day, they were submitted to whole-body examination by last-generation DXA equipment (Lunar iDXA™; GE Healthcare, Madison, WI; enCORE™ 2011 software v. 13.6) for the total and regional assessment of FM, non-bone LM and bone mineral content (and density), according to the “classical” three-compartment molecular model of body composition.²¹ DXA examinations were performed by an expert technician in a standardized manner, according to manufacturer recommendations and guidelines.

Waist circumference (WC) was measured in centimetres at the midpoint between the lowest rib bone on the sides and the iliac crest using a flexible tape to the nearest 0.1 cm, in standing subjects with underwear, at the end of a normal expiration.

Gamma-glutamyl transferase (GGT), high-density lipoprotein (HDL) cholesterol and triglycerides (TGs) were also measured by standard laboratory methods after 12 h fasting. Values were combined with anthropometric measurements to calculate the fatty liver index (FLI) and visceral adipose index (VAI).^{22,23} FLI predicts the likelihood of steatosis, based on an algorithm including TGs, GGT, BMI and WC. VAI calculates visceral adiposity on the basis of WC, BMI,

TGs and HDL. VAI values are strongly associated with cardiometabolic risk.²³

Dual-energy X-ray absorptiometry analysis

The scanner was calibrated daily using a standard calibration block supplied by the manufacturer. All metal items were removed before densitometry. The subjects (in underwear and a cloth gown) were placed in a supine position with arms at sides slightly separated from the trunk and correctly centred on the scanning field. ROIs, as defined by the analytical program, included six different body districts: total body, trunk, upper limbs, lower limbs, android region and gynoid region (Figure 1). Visceral fat analysis was performed by CoreScan® (GE Healthcare), a new software option for the assessment of visceral FM and volume in the android region. The iDXA values have coefficients of determination (r^2) vs CT of 0.957 (0.959 for females and 0.949 for males).¹⁹

A consensus of radiologists and hepatologists joining the study set the limit and rules to draw new liver-suited ROIs, as shown in Figure 2. Eight new ROIs were independently reproduced on acquired DXA images by two radiologists with expertise in body composition analysis. One of them blindly repeated the analysis after 7 days.

ROI-1 was designed by drawing a right triangle in the right hypocondrium with the major leg along a line extending over the diaphragmatic dome to the left up to two-thirds of the ribcage, and the minor leg tangential to the external margin of the 11th rib. ROI-2 was defined as a three-cornered area on the

Figure 2. The figure shows the new regions of interests [android liver (A liver)-1 and A liver-2, and region of interest (ROI)-1 to ROI-6] designed as detailed in the main text.

right hypocondrium, similar to ROI-1, with a different upper leg getting through the right costophrenic sinus. ROI-3 was drawn tracing the hepatic profile. ROI-4 was identical in dimensions to ROI-2, with the major leg getting over the diaphragmatic dome. ROI-5 was drawn following the right profile of the liver and represents the largest part of the right lobe. ROI-6 was a circular area positioned at the hepatic dome.

Android liver-1 ROI was obtained by shifting the traditional android region up to the maximum limit allowed by the software. In order to move the rectangular ROI (and the related visceral fat assessment) further up, an android liver-2 ROI was created, shifting the line getting through the chin up to over the head. Thus android liver-2 was a rectangular ROI similar to the traditional android one, with the same proportion but with a different upper bounding line getting through the armpits.

DXA parameters of FM and FM%, as well as LM, were investigated on the traditional and new DXA ROIs and were correlated with the ultrasound score of steatosis.

Ultrasonographic assessment

Abdominal ultrasound assessment was performed using a convex probe (3.5–5 MHz). Steatosis was evaluated on the basis of the liver tissue brightness in comparison with cortical parenchyma of the right kidney and graded as absent (grade 0), mild (grade 1), moderate (grade 2) and severe (grade 3) (Table 1).²⁴

Analysis of data

All data were analysed in order to (a) describe body composition; (b) study the correlation between the grade of steatosis with both the conventional and the new DXA ROIs; (c) determine the predictive value of ROIs for steatosis in a multivariate system; (d) define the cut-offs for each of the DXA parameters with the highest accuracy for steatosis; (e) evaluate the correlation between the new designed DXA ROIs and FLI; (f) compare the DXA-derived parameters of central adiposity (trunk FM, android FM, VAT, android liver-1 FM and VAT, android liver-2 FM and VAT) with BMI, WC and VAI values. All analyses were adjusted by age, sex and BMI. Conventionally, subjects with BMI <25 kg m⁻² were classified as normal weight

Table 1. Steatosis grading assessed by abdominal ultrasound²⁴

Score	Description
0: no steatosis	Normal echogenicity of liver parenchyma; normal visualization of diaphragm and intrahepatic blood vessels
1: mild steatosis	Slightly increased echogenicity of liver parenchyma; normal visualization of diaphragm and intrahepatic blood vessels
2: moderate steatosis	Markedly increased echogenicity of liver parenchyma; slightly impaired visualization of diaphragm and intrahepatic vessels
3: severe steatosis	Severely increased echogenicity of liver parenchyma, with poor or no visualization of diaphragm and intrahepatic vessels and posterior part of the right liver lobe

(if $<18.5 \text{ kg m}^{-2}$ as underweight), those with BMI between 25 and 30 kg m^{-2} as overweight and with BMI $\geq 30 \text{ kg m}^{-2}$ as obese.²⁵

Statistical notes

The normal distribution of continuous variables was analysed for skewness; normal ranges were considered for values between -2 and $+2$. Data were reported as frequencies (%) or mean $\pm$ standard deviation. Spearman rank analysis was used to evaluate correlations between body composition parameters measured by different techniques. The area under the receiver operating characteristic curve (AUROC) of FM measurements that presented the best p value at Spearman rank correlation test were used to evaluate the accuracy for the prediction of steatosis. The standard error of the mean of AUROC and optimal cut-off values were also estimated; the AUROC was compared by means of the z distribution. The analysis was performed in both males and females of different BMI classes (normal weight, overweight and obese) and in the whole population sample.

All new DXA ROIs were also tested one by one in the stepwise multiple regression analysis, having the ultrasound grading of steatosis as a continuous dependent variable. Since four sets of variables were tested, two-tailed p -values were adjusted according to Duncan's multiple as to $p' = 1 - \sqrt[n-1]{1-p}$, where $p = 0.050$ and $n = 4$.²⁶ The final significant limit was therefore set at $p < 0.017$.

Intra- and interoperator agreements were evaluated by means of the Cohen κ statistic. An excellent agreement was defined for κ values >0.750 according to Fleiss.²⁷

MedCalc® v. 11.4.2 statistical package (MedCalc Software, Mariakerke, Belgium) was used for the analysis.

RESULTS

The selected population comprised 98 males and 82 females, aged 20–75 years (mean, 49.7 ± 12.2 years), with the BMI ranging from 18 to 53 kg m^{-2} (mean, $33.7 \pm 3.6 \text{ kg m}^{-2}$). 74 cases (41.1%) were normal weight, 68 (37.8%) overweight and 38 (21.1%) obese patients. Liver fat was classified as mild in $>50\%$ of cases with ultrasound-detected steatosis, with only 10% classified as severe.

The distribution of all DXA parameters was normal in the total population. Their general features and body composition parameters are described in Table 2a,b and in Figure 3. All DXA parameters showed a statistically significant correlation with the grading of steatosis (Table 3). The correlation was much lower for lower limbs and gynoid FM and FM% (but nonetheless significant, $p < 0.001$). The most significant correlations were demonstrated between ultrasound and FM parameters of ROI-1, ROI-2 and ROI-3 and by android liver-2 and android liver-1, VAT and android (all, $p < 0.0001$). All these correlations were independent of sex, age, BMI and subcutaneous adipose tissue (SAT), with the exception of VAT, whose algorithms depend on SAT. Trunk FM (and FM%) was dependent on sex.

In a multivariate analysis of the whole population, the following variables were retained in the final model of both forward and backward stepwise methods: ROI-2 FM, android liver-2 FM%, gynoid FM% and ROI-5 FM% (standardized coefficient, 0.722, 0.434, -0.248 and -0.483 , respectively; $p < 0.001$). When the multivariate analysis was repeated adding the new ROIs one by one, only the added ROI remained in the final model.

In the AUROC analysis, ROI-1 showed the highest accuracy in predicting steatosis (0.820 ± 0.032). Generally, the accuracy of the ROIs decreased slightly when the analysis was limited to patients with high BMI. In particular, the ROIs focusing on the liver area (*i.e.* ROI-1 and ROI-2) were more accurate in predicting steatosis in subjects with low BMI, whereas larger ones (*i.e.* android, android liver) were more accurate for individuals with high BMI (Table 4). The AUROC values ranged from $0.929 (\pm 0.072)$ (ROI-2 in obese males) to $0.551 (\pm 0.115)$ (android liver-1 in overweight females). However, the very high value of AUROC in obese males might be driven by the limited number of subjects free from steatosis in that group (Table 4, Figure 3).

Table 3 (on the right side) shows correlation between FLI and FM at the new DXA ROI level. All parameters under investigation significantly correlated with FLI ($\rho = 0.827$ – 0.923 ; $p < 0.0001$); the best correlation was achieved by FM android liver-1. VAT android liver-1 and VAT showed the highest correlation with VAI, whereas BMI poorly correlated with VAI ($\rho = 0.326$ and 0.331 , respectively).

Inter- and intra-operator agreements were excellent to perfect for all the new ROIs ($k = 0.915$ – 1.000 and $k = 0.927$ – 1.000 , respectively), with no statistical differences between ROIs.

DISCUSSION

The study shows that DXA may be confidently used for the evaluation of liver steatosis, with the new software and new ROIs identified in this study. They correlate closely with standard techniques, including anthropometry, ultrasonography and surrogate markers derived by previously validated algorithms.

The interest in fatty liver diseases, particularly NAFLD, has exponentially grown in the past few years, in both the clinical and the imaging setting. The disease is at the centre of the clinical arena for two main reasons: (a) the high prevalence in the

Table 2a. Population and values of body composition (mean $\pm$ standard deviation): anthropometric parameters

Population	Sex, number (%)	Males		98 (54.4%)
		Females		82 (45.5%)
	Age (years)	49.7 $\pm$ 12.2 (20–75)		
	Body mass index (kg m ⁻²)	33.7 $\pm$ 3.6 (18–53)		
Fatty liver index		40.2 $\pm$ 33.0 (2–100)		
Visceral adipose index		1.9 $\pm$ 1.3 (0–5)		
Ultrasound	Steatosis, number (%)	Absence		90 (50.0%)
		Presence		90 (50.0%)
		Grade	Mild	48 (53.3%)
			Moderate	33 (37.7%)
			Severe	9 (10.0%)

general population and the potential progression to more severe diffuse liver disease;^{28,29} (b) the key role of the liver and its function in the development of metabolic diseases and in their management.³⁰

The assessment of visceral fat has always been considered a critical point for all techniques measuring body composition, including DXA. Although DXA, as a projective method, cannot directly determine the effective volume of scanned tissue, it is considered a very accurate and reproducible tool for the analysis of body masses and composition. Significant correlations were demonstrated between DXA and the gold standard techniques at the organ-tissue level for fat and lean volume and mass (*i.e.* CT and MRI).^{19,31} DXA is largely available, owing to large use in the field of osteoporosis and metabolic bone diseases. The equipment has been enlarged to house obese subjects up to >200 kg, with wider field of view, and the new technological developments may now address organ composition in specific metabolic disorders. Hence, DXA may now be proposed as a routine clinical method in this field.^{18–20}

The majority of ROIs in DXA analysis are anatomically designed (*e.g.* trunk, upper limbs and lower limbs). In the past, DXA measurements of android and gynoid regions have been proposed to correlate better with visceral abdominal tissue and to improve risk assessment for metabolic and cardiovascular diseases.³² Therefore, android and gynoid regions have been fully integrated in almost all new-generation DXA instruments. Although these

Table 2b. Population and values of body composition (mean $\pm$ standard deviation): densitometric parameters

	Site of assessment	FM (g)	FM%	LM (g)
Dual-energy X-ray absorptiometry	Whole body	28,923 $\pm$ 13,171	36.1 $\pm$ 10.4	49,184 $\pm$ 10,066
	Upper limbs	3138 $\pm$ 1387	34.1 $\pm$ 12.4	6107 $\pm$ 1844
	Lower limbs	9058 $\pm$ 4223	34.1 $\pm$ 10.8	16,974 $\pm$ 3770
	Trunk	15,387 $\pm$ 7773	38.4 $\pm$ 11.4	22,966 $\pm$ 4419
	Gynoid	5302 $\pm$ 2268	42.0 $\pm$ 12.3	7105 $\pm$ 1753
	Android	2688 $\pm$ 1632	40.2 $\pm$ 13.8	3543 $\pm$ 762
	Android liver-1	1561 $\pm$ 1163	28.2 $\pm$ 13.5	3491 $\pm$ 752
	Android liver-2	2000 $\pm$ 1421	27.7 $\pm$ 13.1	4736 $\pm$ 1103
	VAT	1333 $\pm$ 987	–	–
	VAT liver-1	601 $\pm$ 452	–	–
	VAT liver-2	746 $\pm$ 584	–	–
	Subcutaneous adipose tissue	1386 $\pm$ 1008	–	–
	ROI-1	1349 $\pm$ 636	38.9 $\pm$ 12.2	2003 $\pm$ 491
	ROI-2	944 $\pm$ 492	40.2 $\pm$ 12.2	1318 $\pm$ 391
	ROI-3	1029 $\pm$ 523	39.7 $\pm$ 12.1	1457 $\pm$ 385
	ROI-4	607 $\pm$ 439	25.1 $\pm$ 12.7	1661 $\pm$ 512
	ROI-5	305 $\pm$ 215	23.4 $\pm$ 13.5	933 $\pm$ 215
	ROI-6	151 $\pm$ 105	23.0 $\pm$ 13.9	474 $\pm$ 62

FM, fat mass; FM%, fat mass percentage; LM, non-bone lean mass; ROI, region of interest; VAT, visceral adipose tissue.

Figure 3. Distribution of steatosis according to body mass index classes (normal weight, overweight and obese).

two regions are designed on the basis of anatomical landmarks, they are not essentially defined by metabolic concepts. For these reasons, a new software has been recently developed allowing an

estimation of visceral fat in the android region. The software automatically quantifies FM over the muscular wall in the android region (subcutaneous FM); then visceral fat is calculated

Table 3. Spearman rank correlation test between grading of steatosis and weight, body mass index (BMI), fat mass (FM) and fat mass percentage (FM%) at different sites; on the right side, the correlations between fatty liver index (FLI) and FM, as assessed by the new designed regions of interest (ROIs)

	Steatosis (grading)		Steatosis (grading)		FLI	
	FM		FM%			
	ρ	p -value	ρ	p -value	ρ	p -value
Weight	0.479	0.000				
BMI	0.526	0.000				
Whole body	0.518	0.000	0.396	0.000		
Upper limbs	0.482	0.000	0.298	0.000		
Lower limbs	0.327	0.000	0.206	0.006		
Trunk	0.556	0.000	0.489	0.000		
Gynoid	0.342	0.000	0.212	0.004		
Android	0.560	0.000	0.524	0.000		
Android liver-1	0.577	0.000	0.581	0.000	0.923	0.000
Android liver-2	0.592	0.000	0.564	0.000	0.904	0.000
VAT	0.573	0.000				
VAT liver-1	0.513	0.000			0.835	0.000
VAT liver-2	0.523	0.000			0.827	0.000
Subcutaneous adipose tissue	0.393	0.000				
ROI-1	0.619	0.000	0.622	0.000	0.886	0.000
ROI-2	0.616	0.000	0.634	0.000	0.886	0.000
ROI-3	0.610	0.000	0.633	0.000	0.874	0.000
ROI-4	0.522	0.000	0.530	0.000	0.877	0.000
ROI-5	0.500	0.000	0.478	0.000	0.851	0.000
ROI-6	0.538	0.000	0.521	0.000	0.839	0.000

VAT, visceral adipose tissue.

by the subtraction of this amount from the total android FM.¹⁹ After validation of the technical caveats of CoreScan, our study is one of the first investigations to carry out the assessment of visceral fat by this software. The study went even further, designing new, metabolically sound ROIs, focusing on the liver for its pivotal action on whole-body metabolism. The study also tested the complete set of conventional and new DXA ROIs, in order to evaluate the relationships between FM at different body sites and liver steatosis.

The features of the liver and its anatomical position made us test the possibility to get reliable measurements with DXA. The sole limitation is represented by the posteroanterior projective scan of DXA. DXA technical and physical language is based on a molecular assessment model, subsequently translated into a three-compartment mass model (Figure 4); thus, DXA is directly dependent on lipid assessment.

The anatomical shape of the liver and its visceral compartment are particularly suitable for DXA measurement. The abdominal region where the liver is housed is the one with the highest

density of visceral organs, with a minimum filling by connective tissues, such as adipose (although the amount of fat may be considerable in obese subjects), especially at the right lobe and cranial levels. Moreover, as is known, the subcutaneous tissues around this abdominal–thoracic transition zone are also lower than those found in the more caudal abdominal sections.³³

The new liver ROIs were projected following several criteria: (1) to limit the scanned area to the liver, (2) to avoid as much as possible the inclusion of other-than-liver tissues in the field of analysis, (3) to set ROI landmarks to maximize repeatability and reproducibility, and (4) to create ROIs looking at potential integration in the next software generation. ROI-1 to ROI-6, and particularly ROI-3, complied with criteria 1; ROI-5 and ROI-6 were even more focused on the exclusion of other splanchnic components (*i.e.* vessels, organs and fat) and enhanced criterion 2 but suffered from a reduced proportion between their mass and SAT. Although some ROIs partly extended outside the liver region, they were much easier to standardize and more reproducible. Therefore, they remain the best candidates for the evaluation of liver fat by the DXA software. Android liver-1

Table 4. Accuracy of several regional fat mass (best ρ value) in the prediction of presence/absence of steatosis: area under the receiver operating characteristic curve (AUROC) $\pm$ standard error of the mean of AUROC (and cut-off values)

	All subjects			Normal weight			Overweight			Obese		
	Total (n = 180)	Males (n = 98)	Females (n = 82)	Total (n = 74)	Males (n = 40)	Females (n = 34)	Total (n = 68)	Males (n = 41)	Females (n = 27)	Total (n = 38)	Males (n = 17)	Females (n = 21)
ROI-1	0.820 $\pm$ 0.032^a (939 g)	0.844 $\pm$ 0.042 ^a (939 g)	0.794 $\pm$ 0.048 (935 g)	0.802 $\pm$ 0.062^a (574 g)	0.803 $\pm$ 0.090 ^b (495 g)	0.798 $\pm$ 0.088 (574 g)	0.670 $\pm$ 0.066 (1126 g)	0.665 $\pm$ 0.088 (1096 g)	0.643 $\pm$ 0.109 (1126 g)	0.753 $\pm$ 0.093 (2196 g)	0.857 $\pm$ 0.101 (2196 g)	0.741 $\pm$ 0.109 (1907 g)
ROI-2	0.818 $\pm$ 0.032 ^a (633 g)	0.848 $\pm$ 0.041^a (626 g)	0.786 $\pm$ 0.049 (633 g)	0.788 $\pm$ 0.064 ^a (373 g)	0.807 $\pm$ 0.090^b (405 g)	0.791 $\pm$ 0.089 (370 g)	0.686 $\pm$ 0.065 (686 g)	0.681 $\pm$ 0.086 (626 g)	0.655 $\pm$ 0.108 (686 g)	0.758 $\pm$ 0.092 (1295 g)	0.929 $\pm$ 0.072^a (1647 g)	0.667 $\pm$ 0.122 (1284 g)
ROI-3	0.813 $\pm$ 0.032 (690 g)	0.836 $\pm$ 0.043 ^a (673 g)	0.795 $\pm$ 0.048 (690 g)	0.773 $\pm$ 0.065 (451 g)	0.768 $\pm$ 0.095 ^a (535 g)	0.783 $\pm$ 0.090 (390 g)	0.666 $\pm$ 0.066 (690 g)	0.649 $\pm$ 0.090 (673 g)	0.673 $\pm$ 0.106 (690 g)	0.720 $\pm$ 0.100 (1806 g)	0.732 $\pm$ 0.133 ^a (1806 g)	0.704 $\pm$ 0.116 (1806 g)
Android liver _{1,2}	0.804 $\pm$ 0.033 (1595 g)	0.817 $\pm$ 0.045 (1595 g)	0.799 $\pm$ 0.048 (1326 g)	0.731 $\pm$ 0.069 (872 g)	0.682 $\pm$ 0.104 (1450 g)	0.791 $\pm$ 0.083 (545 g)	0.674 $\pm$ 0.066 (1880 g)	0.665 $\pm$ 0.088 (1996 g)	0.649 $\pm$ 0.109 (1325 g)	0.791 $\pm$ 0.085 (3774 g)	0.911 $\pm$ 0.080 (3774 g)	0.759 $\pm$ 0.105 (3428 g)
Android liver ₁	0.790 $\pm$ 0.034 (1212 g)	0.814 $\pm$ 0.045 (1212 g)	0.780 $\pm$ 0.050 (1085 g)	0.692 $\pm$ 0.071 ^a (569 g)	0.652 $\pm$ 0.106 ^a (1212 g)	0.692 $\pm$ 0.089 (569 g)	0.655 $\pm$ 0.067 (1473 g)	0.690 $\pm$ 0.085 (1706 g)	0.551 $\pm$ 0.115 (1450 g)	0.808 $\pm$ 0.081 (3054 g)	0.875 $\pm$ 0.095 (3054 g)	0.778 $\pm$ 0.100 (3054 g)
Visceral adipose tissue	0.786 $\pm$ 0.034 (669 g)	0.834 $\pm$ 0.043 ^a (1097 g)	0.749 $\pm$ 0.052 (731 g)	0.723 $\pm$ 0.069 (329 g)	0.687 $\pm$ 0.093 ^b (519 g)	0.707 $\pm$ 0.099 (319 g)	0.656 $\pm$ 0.067 (798 g)	0.659 $\pm$ 0.088 (947 g)	0.583 $\pm$ 0.113 (640 g)	0.684 $\pm$ 0.106 (1945 g)	0.893 $\pm$ 0.088 (2284 g)	0.616 $\pm$ 0.129 (2181 g)
Android	0.781 $\pm$ 0.035 ^a (1827 g)	0.779 $\pm$ 0.049 ^a (1827 g)	0.779 $\pm$ 0.049 (1827 g)	0.673 $\pm$ 0.072 ^a (1037 g)	0.597 $\pm$ 0.108 ^b (1667 g)	0.758 $\pm$ 0.094 (1037 g)	0.645 $\pm$ 0.068 (2728 g)	0.596 $\pm$ 0.074 (2685 g)	0.679 $\pm$ 0.106 (2066 g)	0.764 $\pm$ 0.091 (4828 g)	0.788 $\pm$ 0.112 (4828 g)	0.704 $\pm$ 0.116 (4471 g)

ROI, region of interest.

AUROC were compared by means of the z distribution.

The best values of accuracy are in bold.

^a $p < 0.05$.^b $p < 0.01$.

and android liver-2 were perfect for criterion 3; a potential solution to provide the full scan of the liver field by the two ROIs could be the acquisition of DXA images with upper limbs adducted, to separate the profile of limbs from the trunk. In spite of these drawbacks, the new ROIs were characterized by very high levels of inter- and intra-observer agreement. Finally, android liver-1 and android liver-2, as well as ROI-1 and ROI-2 (and ROI-3) fulfil the criterion for software integration (number 4).

Although SAT around the inferior part of the thorax is minimum (the so-called “minimum subcutaneous fat thickness” at the level of the xiphoid process), it may represent a significant mass in obese individuals, since DXA anteroposterior projection measures the whole amount of fat in the selected area (subcutaneous plus all sites of visceral fat). Also, the female breast may worsen the measurement and the correlation at this site, especially for large breast sizes. This element, and the VAT around the liver, may partially explain the relatively lower accuracy of DXA in overweight and obese patients, as well as in females. However, the correlation between DXA and ultrasonography in people with high BMI were maintained without statistical differences compared with normal-weight subjects.

Two important advantages of the new ROIs and visceral fat assessment by DXA CoreScan analysis also deserve mention: (1) the lack of radiation exposure for the patient; and (2) no extra time for machine use and operator analysis. All DXA data are acquired in a single whole-body scan, and unlike other techniques, and particularly imaging techniques, no further data acquisition and therefore no further costs in terms of biological exposure and time spent are necessary.

The DXA equipment used in this study provides a remarkable improvement in spatial and contrast resolution. The use of “point typing” analysis allows the operators to determine and to distinguish the type of tissue scanned. Thanks to the high and improved resolution of images provided by new DXA technologies (pixel size, $1.05 \times 0.6 \text{ mm}^{18}$), it is possible to extrapolate soft-tissue data even in small regions of whole-body scan. Although liver ROIs were manually drawn, a very high level of reproducibility and repeatability was achieved; in the future, software implementation could save time to integrate these new regional evaluations in the final report of DXA analysis.

Limits of this study are mainly represented by the manual track of new ROIs and by the comparison limited so far to ultrasound measures or surrogate markers of liver fat (FLI). Ultrasound is an operator-dependent technique, and the estimation of steatosis grading by ultrasound is a qualitative and subjective assessment; therefore, some variability in results depending on the operator may occur. A recent article has also raised some concerns in the evaluation of liver steatosis by the classical ultrasound four-grade system in an obese adolescent population.²⁴ A comparison with a stronger gold standard technique (e.g., MR spectroscopy, other MRI techniques, CT or biopsy) is urgently needed. As for FLI, the technique has

Figure 4. Body composition organization at molecular level. DXA, dual-energy X-ray absorptiometry.

been extensively validated for qualitative prediction of steatosis also in very large clinical settings,^{34–36} but its quantitative value is still pending. So far, the use of different standard techniques would have implied more limitations in the number and characteristics (e.g. obesity) of the population to enrol in this pilot study.

Another critical point of discussion is represented by the applicability of new liver ROIs for DXA equipment belonging to previous technological generations (owing to larger pixel size, point typing analysis is not always possible on whole-body images and several other technical issues).

Finally, this report will help stimulate research on DXA-assessed body composition information and stimulate the exploitation of the technique. Future studies comparing the new liver ROIs using MRI or biopsy are needed as well further research conducted in overweight and obese subjects. Moreover, the assessment of liver adiposity by the new ROIs must be proved valid in longitudinal studies, where ROIs are tested under conditions of weight gain or weight loss, as well as during incidence/remission of steatosis. The association between the new ROIs and cardiovascular risk factors will also become a fruitful area of research.

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