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Intrahepatic Fat Analysis Using Dual-Energy X-ray Absorptiometry and Its Relation to Liver Ultrasound Findings: A Case Series of 100 Patients at a Single Facility

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Abstract- New methods to study body composition, such as dual-energy X-ray absorptiometry (DXA), are of great support for the diagnosis of diseases such as obesity. Previously, liver-tailored DXA radiological regions of interest (ROI) have been designed to evaluate liver fat content; however, whether those observations correlate with liver ultrasound imaging findings has not been described. The goal of this study was to correlate liver ultrasound findings with intrahepatic fat content quantified by liver-tailored DXA ROIs, and to determine their predictive value for hepatic steatosis. Medical records of 100 patients were included. The majority was female (77%); mean age 40.3±10.5 years. Body mass index (BMI) was: normal: 23%, overweight: 48%, and obese: 29%. Bivariate analysis suggests a relationship between intrahepatic fat content > 31% (by DXA) and BMI, exhibiting relevant differences between BMI categories, which were also associated with body fat percentage (BFP) and visceral adipose tissue (VAT), independent of sex, and consistent with ultrasound findings and biochemical liver function tests. Thus, our results strongly suggest that there is a likely consistent association between an intrahepatic fat content percentage >30% and BFP, VAT, and BMI; which is consistent with ultrasound imaging findings, providing quantitative data that may lead to reduced observer bias.

Keywords- *Obesity, Body Composition, Dual-Energy Absorptiometry, DXA, Fatty Live*

I. INTRODUCTION

Overweight and obesity negatively impact human health (1). Therefore, over the past few years, the study of human body composition has gained importance, and the greater availability of new tools and methods to measure and assess body composition have been of great support for the diagnosis and approach of diseases such as obesity, among others (2, 3). The ability of adipose tissue to "invade" other tissues, such as muscle and liver, has drawn more interest as a field of study in order to understand the impact of states of excess adiposity and associated risks on diseases such as fatty liver FL. In general, adipose tissue has been proposed to be the cornerstone for the understanding, as well as for the development, of a wide range of chronic diseases (4).

Previous studies have shown that the distribution and endocrine activity of adipose tissue play a more relevant role than total body fat in as risk factors of obesity and the associated metabolic and cardiovascular diseases (5). For instance, it is widely known that visceral and subcutaneous fat deposits have different metabolic effects that are associated with cardiovascular risk (6, 7, 8).

The prevalence of FL is on the rise (9). Pathogenesis of FL involves cytokines, adipokines, hepatokines, oxidative stress, and apoptosis (10, 11), which have been associated with obesity and insulin resistance, and thus with an increased risk of cardiovascular disease (12, 13). However, it is unclear whether this is owed to common risk factors, or because fatty liver acts as an independent risk factor of cardiovascular disease (14).

Currently, liver biopsy evaluation is the gold standard for the diagnosis of liver disease (15). Nonetheless, several non-invasive approaches such as ultrasound (US), computed tomography (CT), magnetic resonance imaging (MRI), and ultrasound elastography (USE) have been proposed as potential alternative non-invasive diagnostic tools. Each of these techniques exhibits different levels of precision –lower for US- and limitations such as the exposure to radiation and high costs of CT and MRI, respectively.

An emerging metabolic liver disease should be considered as a trigger for multiple systemic diseases, therefore, interference between the liver, immune-mediated responses (e.g. inflammation), and the entire "metabolic system" is occasionally forgotten or given a secondary role (16).

The field of liver imaging has contributed to reduce the number of biopsies, however, by focusing mostly on diffuse liver disease and adiposity, the contributions of this field have stagnated. Despite this, radiologists have acquired knowledge about liver imaging and interpretation of liver fat content, and have begun to include regional evaluation of body composition into these exams (17, 18). Similarly, in the field of cardiac imaging regional body composition is also being included as part of regular assessments, and several studies on quantification of epicardial adipose tissue have been recently published (19,20).

In addition to providing differential assessment of body fat mass (FM) and lean mass (LM), the currently available imaging and clinical tools for the evaluation of body composition also provide quantification of visceral and subcutaneous fat. While CT and MRI have shown to be useful in differentiating and quantifying adipose tissue deposits, dual-energy X-ray absorptiometry (DXA) has the benefits of being less invasive and of lower costs, and recent technical improvements have focused on achieving greater precision and accuracy to assess body composition (21,22,23,24).

Previous studies have reported DXA as a valid, fast, and reproducible technique to accurately measure body composition both regional and total body composition at the molecular level (25). In addition to regions of interest (ROI) based on anatomical landmarks - including android and gynoid regions, Guglielmi and colleagues designed, standardized and implemented new DXA ROIs for the evaluation of hepatic fat content (25).

The aim of this study was to correlate liver US findings with those provided by the quantification of intrahepatic fat with using liver-tailored DXA ROIs, and to determine their predictive value for hepatic steatosis. This approach may provide evidence to further support the use of DXA in the clinical setting.

II. MATERIALS AND METHODS

A. Study design

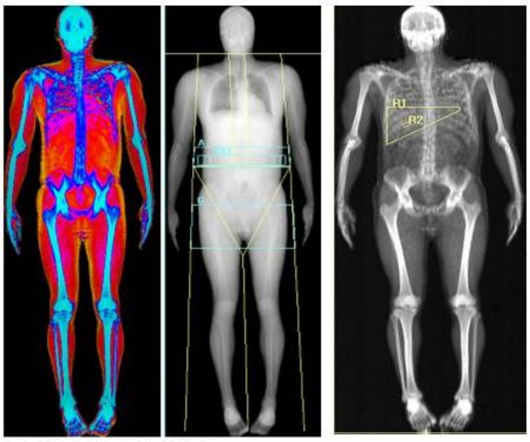
This was a retrospective observational, case series study of 100 patients attending our Obesity, Dysmetabolism and Sports Center (COD2) in Medellin, Colombia, during the first trimester of 2019. All patients were members of the contributory health scheme of the General System of Social Security and Health. Patients meeting the following criteria were included in this study: age > 18 years, and being evaluated as part of the dysmetabolism and obesity program at Las Americas Clinic. Patients presenting chronic diseases (high blood pressure, type 2 diabetes mellitus, dyslipidemia, COPD, alcoholism or alcohol intake > 40 gr/week), patients taking any type of antidiabetic drugs –including metformin-, corticoids, or psychiatric medications were excluded from this study.

B. Study variables

Epidemiological variables collected included sex (male and female), and age (>18 years); anthropometric and diagnostic variables were body mass index (BMI) (range: 19 - 40 Kg/m2), lean mass (LM), fat mass (kg, and percentage), and visceral adipose tissue (VAT) content. These variables were measured in a body composition exam using a Hologic Discovery W Densitometer system (Hologic APEX software, version 4.0.2), DXA, and ROIs based on the study by Guglielmi et al (24), and were analyzed by a single expert technologist certified in body analysis. In ROI-1 (R1), horizontal plane of the hepatic profile was defined from the eighth right costal arch to the left midclavicular line, and the vertical plane up to the last costal arch, except in patients with breast implants where the area involving implants was excluded. In ROI-2 (R2), the area within the hepatic profile between the tenth intercostal space and right midclavicular line (Figure 1).

In addition, abdominal US was performed by an expert radiologist with over 12 years' experience using a Toshiba Aplio™-400 Ultrasound machine (transducer cover 3.5), who classified imaging findings of hepatic alterations as: without fatty infiltration or normal, mild, moderate, or severe fatty liver infiltration. Additionally, biochemical analysis of hepatic enzymes (ALT, AST) was also performed at the central high quality laboratory at the Las Americas Clinic.

(a) (b) (c)



Whole Body Analysis

TBAR353 - NHANES BCA
F.S. 68.00% -10.00%
MCS 73.2% Water

Región	Fat mass (g)	Lean mass (g)	Total (g)	%
GLOBAL	22109.7	70651.2	92760.8	23.8
R1	1521.3	4446.6	5967.9	25.5
R2	27.9	85.8	113.7	24.5
NETAVG	1521.3	4446.6	5967.9	25.5

Region	Area(cm²)	BMC (g)	BMD (g/cm²)
GLOBAL	2515.93	3308.32	1.315
R1	107.81	81.09	0.752
R2	0.00	0.00	0.000
NETAVG	107.81	81.09	0.752

Figure 1. (A) Total body image with three basic compartments, (B) Location of ROI-1 and ROI-2, and (C) fat and lean content measurement report in ROI-1 and ROI-2 excluding bone content in ROI-2

C. Statistical analysis

Data were collected, tabulated, analyzed using Microsoft Excel software (Mac version16.16.5 (181209)). Descriptive statistics were used, and results are shown as frequencies and measures of central tendency and dispersion.

D. Ethics statement

This study complied with resolution N° 8430 of 1993 of the Colombian Ministry of Health and Social Protection, and is classified as "no risk" research since it "employs techniques and methods of retrospective chart review and no intervention or intended modification of biological, physiological, psychological, or social variables of the individuals participating in the study is performed, nor does it identify or treat sensitive aspects of their behavior". During the course of this study, investigators guaranteed that the principle of respect, dignity, and protection of rights and wellbeing of study participants prevailed. All participating individuals or legal representatives provided signed informed consent to participate in this study. Data extracted from medical records was kept confidential and de-identified prior to performing analysis. All members of the research team carefully maintained patient confidentiality and did not disclose any information that may potentially identify any of the participating individuals.

III. RESULTS

Data from 100 patients were extracted from medical records. In this series, the majority of participants were female (77%), with a mean age 40.3 (±10.5) years. Anthropometric analysis was performed and BMI values were normal for 23% of patients, overweight for 48%, and 29% were classified as obese.

Body composition analysis showed that body fat percentage (BFP) ⩽ 33% was observed in 5.2% of female participants, while 4.3% of male participants exhibited a BFP ⩽ 25%. Regardless of BMI, 94% of the study population presented BFP above normal ranges in both genders. Visceral adipose tissue ⩽ 100 cm2 was observed in 41% of the population, while VAT was ⩾ 100 cm2 in the remaining 59% of participating individuals. Finally, lean mass in both genders was found to be within normal ranges for both males (46.4 ± 15.2 kg) and females (38.7 ± 5.5 kg).

While biochemical analysis of hepatic enzymes alanine transaminase (ALT) and aspartate transaminase (AST) showed mean normal levels in both male and female participants (AST in males 34.8 ± 19.6 UI/l, and females 24.1 ± 14.8 UI/l), individual analysis of these enzymes showed a dramatic increase in specific cases, which was also associated with alterations in ultrasound findings.

In male participants, ultrasound imaging findings were normal/without fatty infiltration in 47.8% o participants, while mild steatosis was found in 30.4%, moderate steatosis in 17.3%, and one case of severe steatosis (4.3%). On the other hand, females exhibited ultrasound imaging findings consistent with normal/without fatty infiltration in 37.6% of cases, mild steatosis was found in 55.8%, moderate steatosis in 5.19%, and also one case of severe steatosis (1.2%) was identified. The relationship between liver lobe size and elevation of hepatic transaminases was consistent with ultrasound findings, since both parameters were found to be increased (liver lobe > 150mm, and a 2.5-fold increase in levels of transaminases).

Liver fat content measured by DXA, and adopting the previously mentioned ROIs, showed that mean ROI-1 was 31.6% and 34.4% in males and females, respectively. Similarly, no significant changes were found for ROI-2, which was 28.6% and 28.0% in males and females, respectively (Table 1).

TABLE I. ANTHROPOMETRIC, DENSIOMETRIC, ECOGRAPHIC, AND BIOCHEMICAL CHARACTERISTICS OF THE STUDY POPULATION

VARIABLE	n	M (n=23)	F (n=77)
Age (years)		40.45 (±10.4)	40.29 (±10.6)
BMI [a]			
Normal weight	23	2 (8.69%)	21 (27.27%)
Overweight	48	12 (52.17%)	36 (46.75%)
Obesity I	23	7 (30.43%)	16 (20.77%)
Obesity II	6	2 (8.69%)	4 (5.19%)
Obesity III	0	0 (0.00%)	0 (0.00%)
BFP [b]			
NORMAL [c]	5	1 (4.34%)	4 (5.19%)
ABNORMAL [d]	95	22 (95.65%)	73 (94.80%)
VAT [e]			
<100 cm2	41	5 (21.73%)	36 (46.75%)
>100 cm2	59	18 (78.26%)	41 (53.24%)
Hepatic examination by DXA (%)			
R1 [f]		31.6 (±6.0)	34.4 (±9.9)
R2 [g]		28.6 (±10.5)	30.3 (±11.8)
LM (Kg) [h]		46.4 (±15.2)	38.7 (±5.5)
Hepatic enzymes			
AST (IU/l)		34.8 (±19.6)	24.1 (±14.8)
ALT (IU/l)		35.2 (±20.1)	28.0 (±23.8)
Ultrasound findings			
No alterations	40	11 (47.8%)	29 (37.6%)
Mild	50	7 (30.4%)	43 (55.8%)
Moderate	8	4 (17.39%)	4 (5.19%)
Severe	2	1 (4.34%)	1 (1.29%)
Size: Right LL (cm) [i]		137.6 (±65.8)	136.8 (±70.2)

[a]Body mass index (kg/m2), [b]total body fat percentage, [c] M: males = <25%, F: females = <33%, d H = >25%, M = >33%, e Visceral adipose tissue (cm2), [f] total liver fat percentage ROI-1, [g] non-bone liver fat percentage ROI-2, [h] Lean mass (Kg), [i] Right liver lobe

Next, bivariate analysis –including all patients- was performed for the variables of body composition and ultrasound findings. In males, our results suggest a close relationship between having a BFP > 25% and different hepatic alterations, since 54.5% of male participants with BFP > 25% exhibited hepatic alteration in ultrasound imaging. Similarly, in 67.5% of females with BFP > 33% presented mild fatty infiltration of the liver. It is worth to point out that one of the female patients with a BFP < 33% exhibited ultrasound findings of mild fatty infiltration despite presenting liver lobe measurements and levels of hepatic enzymes within normal ranges.

Regarding BMI, it is noteworthy that 22.7% of the normal weight population exhibited ultrasound findings consistent with mild and moderate fatty infiltration, while VAT < 100cm2 was associated with fatty infiltration in 36.5% of cases (Table 2). This was further confirmed by performing bivariate analysis of the hepatic fat content estimated by DXA at the ROIs of interest (ROI-1 and ROI-2) and body composition findings. For this analysis, BMI was categorized as normal weight, overweight, class I obesity, and class II obesity. According to these categories, fat content in ROI-1 was 25.9%, 34.47%, 38.84%, and 39.83%, respectively. Similarly, these differences among categories were also observed for fat content in ROI-2, being 20.48%, 31.81%, 34,37%, and 34.11%, respectively. This association was independent of sex. Thus, this analysis suggested an association of BMI with hepatic fat percentage >31%.

TABLE II. BIVARIATE ANALYSIS OF BODY COMPOSITION AND ULTRASOUND FINDINGS

Ultrasound findings	No alterations	Mild	Moderate	Severe	total
	N (%)	N (%)	N (%)	N (%)	N (%)
BMI					
Normal weight	14 (35%)	7 (14%)	2 (8.7%)	0	23 (100%)
Overweight	22 (55%)	22 (44%)	4 (8.33%)	0	48 (100%)
Obesity I	3 (7.5%)	18 (36%)	1 (4.35%)	1 (4.35%)	23 (100%)
Obesity II	1 (2.5%)	3 (6%)	1 (16.67%)	1 (16.67%)	6 (100%)
Subtotal BMI	40 (40%)	38 (38%)	8 (8%)	2 (2%)	100 (100%)
BFP Females					
< 33%	3 (75%)	1 (25%)	0	0	4 (100%)
> 33%	26 (35.61%)	42 (57.53%)	4 (5.48%)	1 (1.37%)	73 (100%)
Subtotal BFP	29 (37.66%)	43 (55.84%)	4 (5.19%)	1 (1.3%)	77 (100%)
BFP Males					
< 25%	1 (100%)	0	0	0	1 (100%)
> 25%	10 (45.45%)	7 (31.81%)	4 (18.18%)	1 (4.55%)	22 (100%)
Subtotal BFP	11 (47.82%)	7 (30.43%)	4 (17.39%)	1 (4.35%)	23 (100%)
VAT					
< 100 cm2	26 (63.41%)	14 (34.14%)	1 (2.43%)	0	41 (100%)
> 100 cm2	14 (23.72%)	36 (61.01%)	7 (11.86%)	2 (3.38%)	59 (100%)
Subtotal VAT	40 (40%)	50 (50%)	8 (8%)	2 (2%)	100 (100%)

Furthermore, taking into account VAT, the relationship between intrahepatic fat was maintained in the previously mentioned ranges. In patients with VAT < 100cm2, mean ROI-1 was 27.61%, and mean ROI-2 was 24.21%. On the other hand, patients with VAT > 100cm2, an increase in mean fat content in ROI-1 and ROI-2 was observed, being 38.14% and 33.91%, respectively. Similarly, an association between fat content in ROIs and BFP was observed when differentiating by sex; males with BFP > 25% exhibited mean fat content in ROI-1 and ROI-2 of 31.68% and 29.1%, respectively, while women with BFP > 33% mean fat content in ROI-1 and ROI-2 was 35.71% and 31.46%, respectively (Table 3).

TABLE III. BIVARIATE ANALYSIS OF LIVER FAT CONTENT IN ROIS AND BODY COMPOSITION

Liver fat content	n	ROI-1 (%)	ROI-2 (%)
IMC			
Normal weight	23	25.9 (±9.65)	20.48 (±10.93)
Overweight	48	34.47 (±7.94)	31.81 (±10.6)
Obesity I	23	38.84(±7.01)	34.37 (±9.66)
Obesity II	6	39.83 (±4.39)	34.11 (±9.11)
BFP Females			
< 33%	4	12.05 (±1.67)	9.6 (±1.6)
> 33%	73	35.71 (±8.64)	31.46 (±11.08)
BFP Males			
< 25%	1	30.4 (±0)	17.4 (±0)
>25%	22	31.68 (±6.15)	29.1 (±10.49)
VAT			
< 100 cm2	41	27.61 (±8.8)	24.21 (±11.58)
> 100 cm2	59	38.14 (±6.78)	33.91 (±9.75)

ROI: Radiologic regions of interest; BMI: body mass index; BFP: total body fat percentage; VAT: visceral adipose tissue.

IV. DISCUSSION

Currently, the prevalence of fatty liver in patients lacking clearly established risk factors and otherwise healthy, has significantly increased. Therefore, it is pertinent in patients with high levels of adiposity to consider this possibility when approaching these cases. Ultrasound imaging study of the liver is a valuable diagnostic tool that offers well established techniques for the evaluation of alterations of the liver such as comparative echogenicity of hepatic parenchyma and adjacent structures such as diaphragm, spleen, and kidneys; measurement of the right liver lobe, and measurement of the caudate lobe, among others. In addition to being operator-dependent, ultrasound imaging studies also depend on patients characteristics, since they may make difficult to perform the test, or to visualize certain structures, hence contributing to high variability in results. That said, and taking into account that the gold standard for diagnosis of inflammatory or infiltrating diseases of the liver is the liver biopsy, and that other radiological alternatives are of high cost, low access, and high radiation, the development of novel tools that are more accessible, of low cost, low radiation, and provide quantitative data that allow clinicians to set starting point and follow up for their patients would be of great help in a pathology that does not currently have quantitative fat tracking options and we do not rely on liver enzymes or US for this fatty evaluation. For this reason, this observational, retrospective, case series study was carried out as a single-center study in which data from a heterogeneous sample of patients –given their different anthropometric and body composition characteristics, without previously identified risk factors were analyzed. Diagnostic DXA and ultrasound imaging of the liver, and biochemical analysis of liver enzymes were performed according to our institutional protocols.

For estimation of fat content by DXA, we specifically used two ROIs described by Guglielmi and colleagues (25) for the estimation of fat content, with the possibility of analyzing their association with biochemical liver profile, body composition, and ultrasound imaging of the liver. Our results suggest a direct relationship between BMI and BFP with the intrahepatic fat percentage (Table 3). Moreover, all the variables subjected to bivariate analysis support cut-off points that allowed a quantitative approximation of fatty infiltration. In class II obese patients, fatty infiltration was associated with a discrete elevation of hepatic transaminases (AST 42.8 IU/l, and ALT 59 IU/l), and with an increased average size of right liver lobe of 146 mm (min. 140 mm, max. 155 mm).

Analysis of discrepant cases indicated two important aspects that may affect outcome; 1) previous history of aesthetic plastic surgery –which may alter segmental BFP and thus total BFP; and 2) observer variability, since a strict protocol for ultrasound evaluation of the liver is not currently enforced at our institution, as evidenced by the 30% of cases lacking hepatic measurements (at least right lobe) and the comparison with 3 associated organs. In many cases, this was due to the inability to visualize such structures, constituting one of the limitations of our study. This was also evident when comparing imaging data with levels of liver enzymes. Nonetheless, cases of mild fatty infiltration usually exhibited normal or a discrete elevation of levels of hepatic enzymes.

On the other hand, data not included in this study showed a consistent alteration in insulin curves of patients with normal or slightly elevated BMI with low muscle mass. This could explain our results of high content of intrahepatic fat in the absence of other findings, given the direct association of increased insulin resistance and decreased insulin sensitivity with fatty infiltration, understanding the pathophysiology of the adipose tissue-liver axis (26).

Altogether, this calls for a serious and interesting discussion to achieve a more complete and comprehensive approach of these patients, which is not limited to hemodynamic and mechanical techniques that are easily reduced to vital signs, weight, and height. On the contrary, accumulating body of evidence strongly suggests the need of constant evaluation and understanding of body composition as a clinical parameter as relevant as blood pressure, even more so when it could provide complementary quantitative data that may guide patient goal setting and to individualize intervention in different entities.

While the goal of our study was not to establish normal values of intrahepatic fatty content, nor to exclude or replace validated diagnostic imaging, our results do provide complementary data suggesting that parameters such as total and segmental body fat content, total and segmental body muscle content, and total and segmental visceral fat content should be taken into account, in addition to the analysis of structures such as the liver. It is important to highlight that liver fat content, as measured by DXA (in males: ROI-1 > 31% and ROI-2 > 29%; in females: ROI-1 > 34% and ROI-2 > 31%), was associated with ultrasound imaging findings, which may guide the clinician to determine and prioritize the type of intervention according to the main types of body composition identified. For instance, in this case series adipocytic and sarcopenic biotypes were identified, and while both can be associated to similar comorbidities, therapeutic intervention varies among biotypes.

In conclusion, our results strongly suggest that there is a likely consistent association between an intrahepatic fat content percentage –measured by DXA (ROI-1 and ROI-2)- greater than 30% and BFP, VAT, and BMI; which in addition was associated with ultrasound imaging findings and provided quantitative data that may lead to reduced observer bias.

Furthermore, we propose that body composition evaluated by densitometry should be reassessed in order for it not to be limited to fat, bone, and muscle compartments, but rather to broaden its analysis, understand the retrieved data, and to apply appropriate strategies tailored for individual patients.

Our study contributes to the field of diagnostic imaging applied to body composition analysis and its relationship with other clinical entities. This requires continuity and the comparison of novel techniques or approaches with the established diagnostic gold standards in order to perform correlations and to unravel novel diagnostic parameters that are less invasive, low risk, accessible and affordable. Larger population studies are required to determine the normal range of intrahepatic fat content in different populations, and its impact on human health.

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Conflict of Interest

The authors declare no conflict of interest.

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