CHAPTER 5

RESULTS

*Opposite associations of abdominal and thigh adiposity with liver fat in overweight and obese women

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Running Head: Body fat distribution and liver fat

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ABSTRACT

Our purpose was to analyze the independent associations of abdominal and thigh AT compartments with liver fat. We further investigate the relations of proinflammatory and atherothrombotic metabolic syndrome features with liver fat. Abdominal and thigh adipose and muscle tissue distribution, and liver and spleen attenuation were assessed by computed tomography in 140 overweight and obese women. Blood lipids and insulin resistance markers, as well as atherothrombotic and proinflammatory risk factors were also measured. Thigh subfascial AT (TSFAT) was inversely associated with liver fat ($p<0.05$), independently of age and BMI. Contrarily, a higher sagittal diameter and a larger visceral adipose tissue (VAT) area were related with liver fat ($p<0.05$). Additionally, increased fasting insulin, triglycerides, plasminogen activator inhibitor-1, liver transaminases, and uric acid concentrations, as well as higher total-cholesterol/HDL-cholesterol and LDL-cholesterol/HDL-cholesterol ratios were independently associated with liver fat ($p<0.05$). These associations remained significant after adjustment for VAT ($p<0.05$). We conclude that thigh subfascial AT was inversely associated with liver fat, suggesting that this thigh AT depot may play a protective role against hepatic ectopic fat storage in overweight and obese women. Furthermore, these results reinforce the contribution of an abdominal obesity phenotype associated with a diabetogenic, inflammatory and an atherothrombotic metabolic profile to liver lipotoxicity.

Keywords: Body composition, liver fat, visceral and thigh adiposity, metabolic syndrome

INTRODUCTION

Obesity-related comorbidities seem to be more closely related to body fat distribution rather than the total amount per se (19). Abdominal obesity is a predictor of a higher metabolic risk, assuming insulin resistance (IR) the common link between visceral adiposity and dyslipidemia (10, 22, 35), type 2 diabetes mellitus (DM) (7, 18), liver fat (4), hypertension (33), and other cardiovascular diseases (CVD) (23, 30, 33). Two major pathophysiological hypotheses have been advanced to explain metabolic disturbances in abdominal obese individuals. It has been proposed that neuroendocrine perturbations, mediated by hypothalamic-pituitary-adrenal (HPA) axis stimulation, also known as stress, are responsible for IR and abdominal obesity (8, 9). Moreover, alterations in cortisol secretion, inhibition of steroid and growth hormones production, and stimulation of sympathetic nervous centers are some of the dysfunctions which may precipitate metabolic disturbances (8). Conversely, according to the "portal" hypothesis, the increased lipolytic activity in visceral adipocytes, lead to an augmented release of free fatty acids (FFA) into portal circulation, promoting liver fat storage, which is accompanied by hepatic metabolism disturbances and IR (6, 18, 48).

In this context, abdominal obesity has been associated with ectopic fat storage, defined as fat accumulation outside "classical" AT depots, such as heart, skeletal muscle, pancreas, and liver (47). Moreover, liver fat seems to be associated with obesity, increased concentrations of plasma FFA, as well as with the IR degree, both in obese and type 2 DM patients (21, 40, 48). Furthermore, liver-to-spleen ratio (LSR), a reliable index of liver fat (31), has been independently associated not only with visceral adiposity (4, 37, 38), but also with hepatic IR (18, 21, 27, 38), dyslipidemia, and several other metabolic syndrome features (4, 18, 29, 38). Emerging from a combination of several disturbances, including increased liver FFA influx and synthesis, decreased FFA oxidation and very-low-density

lipoprotein (VLDL) secretion, and a low chronic inflammatory state, hepatic steatosis has also been related with IR and major CVD risk factors (3, 27).

However, although evidence has been highlighting the independent contributions of both visceral adiposity and liver fat to an increased metabolic risk in obese or type 2 DM patients, it is not totally clear if liver fat is additionally associated with other specific inflammatory and atherothrombotic metabolic syndrome features. On the other hand, despite the recognized relevance of abdominal obesity to ectopic liver fat storage, little is known about the relationships of both specific abdominal and thigh AT compartments and liver fat. Moreover, one study developed in type 2 DM patients has reported that thigh subfascial AT was correlated with both liver fat and IR (18). Therefore, based on previously defined criteria (31), the current study examined the separate contributions of abdominal and thigh AT compartments to liver fat in overweight and obese women. Additionally, it was further investigated the independent associations of liver fat with metabolic syndrome proinflammatory and atherothrombotic clinical features.

MATERIALS AND METHODS

Subjects

Participants in this investigation were 140 pre-menopausal Caucasian women, recruited from community by public advertisement for a 2-year weight management program, as described earlier in detail (44). Study inclusion criteria required that the subjects were no currently pregnant, older than 24 years, had a body mass index (BMI)>24.9 kg/m^2, were not under any medication that could affected weight, body composition or liver metabolism, had no clinical or laboratory evidence of liver or spleen disease, and had no history of cancer in the last five years. Ongoing hormonal medication, history of CVD, stroke, hypertension, type 2 DM, Cushing syndrome, hormonal dysfunction, as well as

resting and exercise abnormal electrocardiograms were defined as exclusion criteria. Subjects that were undertaking oral medication to treat hypertriglyceridemia, hyperglycemia or hypercholesterolemia were also excluded. All subjects were informed about the purpose, nature and study design before giving their fully written consent to participate. The study protocol was performed according to the principles of the Helsinki Declaration and was approved by The Human Subjects Institutional Review Board of the Faculty of Human Movement, Technical University of Lisbon.

Body Composition Assessments

Anthropometric variables.

Height was measured to the nearest 0.1 cm with a stadiometer (Seca, Hamburg, Germany). Body mass was measured to the nearest 0.01 kg on a previous calibrated scale after removing shoes and clothes. Abdominal sagittal diameter (SD), waist circumference (WC) and hip circumference (HC) measurements were obtained by standard procedures (24). BMI was calculated as weight divided by height squared (kg/m^2) and waist-to-hip ratio (WHR) was defined as the WC divided by HC.

Dual energy X-ray absorptiometry (DXA).

Trunk fat mass (TFM), TBFM, and total body lean mass (TBLM) were measured by DXA (QDR-1500 Hologic, Inc. Waltham, MA). The intra-observer coefficient of variation (CV) for TBFM and TBLM was 2.0% and 1.7%, respectively. A 0.5% technical error for %TBFM was obtained as calculated in 2 repeated measures performed on 10 subjects.

Measurement of abdominal adipose tissue distribution.

With the subjects supine and arms extended above their head, a single cross-sectional CT (Siemens, Somaton Plus) image at L4-L5 inter-vertebral space was acquired to measure abdominal AT compartments, as described elsewhere (19). All images were obtained using

120kVp, 480 mA, 512×512 matrix with a 48-cm field of view. Total abdominal adipose tissue (TAAT), abdominal subcutaneous adipose tissue (Ab SAT), superficial and deep Ab SAT, and VAT areas were measured. The boundary between VAT and Ab SAT was defined using the abdominal and oblique muscles in continuity with the deep fascia of the paraspinal muscles and the anterior aspect of the vertebral body (12). The subcutaneous fascia was used to differentiate Ab SAT into its superficial and deep compartments (19).

Measurement of thigh adipose tissue and muscle distribution.

Using same scan parameters, contiguous 7-mm-thick cross-sectional images of both legs were obtained between the inferior ischial tuberosity and the superior border of the patella. Total thigh adipose tissue (TTAT), total thigh subcutaneous AT (TTSAT), thigh subfascial AT (TTSFAT,) and muscle tissue areas and attenuations were measured. The tissues volumes (cm^3) identified in each image were calculated by multiplying the image thickness (7 mm) by the tissue area (cm^2). Thigh AT volume (litters) were than converted to mass units (kilograms) multiplying the volume by the assumed constant fat density (0.92 kg/L) (41). Total thigh muscle mass was also calculated multiplying volume by the constant density assumed for adipose tissue-free skeletal muscle (1.04 kg/L) (41). From the thigh scans performed, it was selected a single slice located at the mid-point distance between both anthropometric markers described to image mid-thigh AT and muscle tissue distribution.

Measurement of liver fat.

A 7-mm-thick cross-sectional image at T11-T12 vertebral space was acquired to measure both liver and spleen CT attenuations, which were determined by calculating the mean Hounsfield units (HU) of three regions of interest (ROI) (liver ROI had ~120 mm^2, located 2 in right lobe and 1 in left lobe; spleen ROI had ~75 mm^2). All ROI were consistently selected in peripheral parenchyma areas, as previously described (18), away from artefacts, major blood vessels and other areas of inhomogeneity. The ratio of mean

liver to spleen attenuation values, defined as liver-to-spleen ratio (LSR) is a reliable index of liver fat (31). Fatty liver is defined when LSR<1 (18).

Measurements reliability.

The reliability for abdominal and thigh adipose and muscle tissue compartments was calculated in 30 women chosen randomly from all subjects. Same technician performed the repeated analyses on same images, separated by 3 months. The intra-observer coefficient of variation (CV) for VAT and TAAT were, respectively, 0.9% and 0.7%. For Ab SAT, deep and superficial Ab SAT, the intra-observer CV were, respectively, 0.8%, 2.8% and 3.1%. For mid-thigh muscle tissue, total mid-thigh AT and subfascial AT (SFAT) the intra-observer CV were 0.1%, 0.4% and 2.5%, respectively. Reliability for liver and spleen measurements was also examined, being the CV for LSR 2.2%.

Image analysis.

Based on image morphology, CT data was analyzed by specific analysis software (Slice-O-matic, Version 4.2, Tomovision, Montreal, Canada). It was used a combination of watershed techniques and edge detection filters (28). Standard HU ranges for adipose tissue (-190 to -30 HU) and skeletal muscle (-29 to 150 HU) were used to compute the tissue segmentation (36). It was also measured, in both legs, the high-density (31 to 150 HU) and low-density muscle areas (-29 to 30 HU) (15). Thigh fascia was used as boundary to demarcate the subcutaneous AT from SFAT (18).

Blood Analysis

After a 12-hour overnight fast, venous blood samples were obtained from antecubital vein. Measurement of triglycerides (TG), uric acid, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were made by enzymatic colorimetric methods. Fasting blood insulin was determined by

electrochemiluminescence immunoassay (ECLIA), glycemia was assessed by hexoquinase method and interleukin-6 (IL-6) was measured by chemiluminescence immunoassay. Tumor necrosis factor-alpha (TNF-α) was measured using a high-sensitivity enzyme-linked immunosorbent assay (ELISA) principle. Plasminogen activator inhibitor-1 (PAI-1) was measured in iced citrated plasma using the Coatest PAI method (enzyme immunoassay - EIA).

Hemoglobin A1c (Hb A1c) was determined by high-pressure liquid chromatography (HPLC). Adiponectin, leptin and urinary cortisol were measured by radioimmunoassay (RIA). Microalbuminuria and C-reactive protein (CRP) plasma concentrations were measured by a high-sensitivity particle-enhanced turbidimetric assay. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined by a kinetic method.

Blood Pressure

After a 5-minute rest, systolic and diastolic blood pressures (BP) were measured in seated position with a semiautomatic oscillometric recorder (Dinamap, Critikon, Tampa, FL). A suitable cuff size was applied to participant's upper arm and the mean of three measurements in each arm was calculated.

Statistical Analysis

Unless otherwise is indicated, data are presented as means±SD. It was studied the variables normality and homocedasticity. When necessary, log transformations were used to normalize distributions. Multiple linear regressions, adjusted for age and BMI, were performed to study the independent associations of liver-to-spleen ratio with major metabolic syndrome features, and proinflammatory and atherothrombotic disturbances.

Adjusting for the same variables, independent associations of LSR with anthropometric markers, abdominal and thigh adipose and muscle tissue compartments were also studied.

In order to facilitate the comparisons of the results obtained in the multiple linear regression models, standardized beta values were presented. To determine how much the independent variables were linearly related to one another, it was studied the multicolinearity by statistic tolerance ($1-R^2$), being the stability of the regression model disturbed by multicolinearity if tolerance is inferior to 0.1. Statistical significance was set at $p<0.05$. Statistical analyses were performed using the SPSS version 13.0 for Windows (SPSS, Chicago, IL, USA).

RESULTS

Subject's anthropometric and body composition characteristics are presented in **Table 1**. Most of the subjects were obese, revealing BMI, as well as abdominal and thigh AT compartments wide variation ranges. An increased WC was observed in 43.9% of the subjects (11). VAT was the minor constituent of abdominal AT area (23.6%). Superficial and deep Ab SAT areas were similar, comprising each one, approximately half of total Ab SAT depot. While TTAT mass represented 57.9% of total thigh mass, LDM was the smallest compartment (19.8%) of mid-thigh muscle area. The fatty liver prevalence observed was 2.9%.

In **Table 2** are presented the metabolic syndrome clinical outcomes. Accordingly to the ATP III criteria (11), hypertriglyceridemia was found in 22.3% of the subjects, while hypertension assumed 21.4% prevalence. Despite lower HDL-C concentrations were found in 44.5% of the subjects, only 0.7% revealed hyperglycemia.

Table 1. Subject characteristics (n=140)

	Mean±SD	Range
Anthropometric data		
Age, y	38.3±0.5	25.0-49.0
Weight, kg	78.1±1.0	59.1-107.8
BMI, kg/m^2	30.4±0.3	25.1-45.2
WC, cm	87.2±0.8	71.1-123.4
Waist-to-hip ratio	0.78±0.01	0.64-0.99
Sagittal diameter, cm	20.5±0.2	16.3-31.0
Fat mass		
TFM, kg	18.0±0.4	9.4-32.3
TBFM, kg	36.1±0.7	23.5-60.3
TBLM, kg	41.2±4.62	29.7-55.6
Abdominal adipose tissue		
TAAT, cm^2	470.9±12.1	211.9-910.8
VAT, cm^2	111.3±4.3	24.9-266.8
Ab SAT, cm^2	353.6±9.1	145.0-633.4
Superficial, cm^2	192.2±5.0	90.6-384.2
Deep, cm^2	161.8±5.4	54.7-344.9
Thigh compartments		
Thigh AT, cm^2	270.7±6.9	132.9-509.1
Thigh SAT, cm^2	261.6±6.8	129.4-501.6
Thigh SFAT, cm^2	3.5±0.2	1.0-11.9
Muscle, cm^2	234.3±2.6	176.3-324.7
HDM, cm^2	189.3±2.3	145.7-264.4
LDM, cm^2	32.8±0.9	15.7-80.0
TTAT, kg	8.4±2.1	4.0-14.8
TTSAT, kg	7.9±2.1	3.8-14.0
TTSFAT, kg	0.6±0.2	0.3-1.5
TTMT, kg	6.1±0.9	4.4-10.3
Liver and spleen variables		
Liver attenuation, HU	59.8±0.8	-5.6-71.0
Spleen attenuation, HU	46.4±0.4	34.0-57.5
LSR	1.30±0.02	-0.11-1.82

Values are presented as means ± SD. BMI, body mass index; WC, waist circumference; TFM, trunk fat mass; TBFM, total body fat mass; TBLM, total body lean mass; TAAT, total abdominal adipose tissue; VAT, visceral adipose tissue; Ab, abdominal; SAT, subcutaneous adipose tissue; Thigh SAT, mid-thigh subcutaneous adipose tissue; SFAT, subfascial mid-thigh adipose tissue; HDM, mid-thigh high-density muscle; LDM, mid-thigh low-density muscle; TTAT, total thigh adipose tissue; TTSAT, total thigh subcutaneous adipose tissue; TTSFAT, total thigh subfascial adipose tissue; TTMT, total thigh muscular tissue; HU, Hounsfield units; LSR, liver-to-spleen ratio.

Table 2. Subject metabolic syndrome characteristics (n=140)

	Mean±SD	Range
Fasting insulin, μIU/mL	8.22±0.32	2.40-17.9
Fasting glycemia, mg/dL	89.48±0.65	73.0-113.0
Triglycerides, mg/dL	101.48±4.86	32.0-329.0
TC, mg/dL	194.74±3.86	101.0-307.00
HDL-C, mg/dL	54.09±1.05	29.0-91.0
LDL-C, mg/dL	123.50±3.54	45.0-255.0
TC/HDL-C ratio	3.74±1.11	2.04-9.55
LDL-C/HDL-C ratio	2.38±0.08	0.94-6.13
Systolic BP, mm Hg	120.65±1.43	90.0-175.0
Diastolic BP, mm Hg	75.83±0.93	50.0-101.0
ALT, IU/L	18.16±0.46	9.0-43.0
AST, IU/L	16.18±0.60	5.0-44.0
CRP, mg/dL	0.45±0.03	0.03-1.14
IL-6, pg/mL	10.32±0.56	0.80-31.50
TNF-α, pg/mL	3.87±0.23	0.90-14.10
PAI-1, ng/mL	21.18±2.01	1.0-100.0
Fibrinogen, mg/dL	369.38±6.48	201.0-552.0
Hb A1c, %	4.87±0.04	4.0-7.0
Uric acid, mg/dL	4.39±0.97	2.40-8.50
Microalbuminuria, μg/min	2.73±0.70	0.50-89.80
Cortisol, ug/day	41.04±1.69	6.0-105.0
Leptin, ng/mL	32.92±43.33	0.90-167.40
Adiponectin, ng/mL	9.18±6.44	2.93-41.00
Apo A1/Apo B100 ratio	1.74±0.05	0.78-3.31

Values are presented as means ± SD. TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, Low-density lipoprotein cholesterol; BP, blood pressure; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha; PAI-1, plasminogen activator inhibitor-1; Hb A1c, hemoglobin A(1c); Apo A1, apolipoprotein A1; Apo B, apolipoprotein B100.

Age was not associated with LSR (β=0.118, $p>0.05$). However, both weight and BMI were inversely associated with LSR, even when adjusting for age (β=-0.235, $p<0.01$; β=-0.225, $p<0.01$, respectively). The results of simultaneously entering each anthropometric and body composition marker to predict LSR, adjusting for age and BMI, are shown in **Table 3**. Higher sagittal diameter values were independently related with a lower LSR, representing

an increased liver fat storage. Similar associations were observed when using liver attenuation as dependent variable. Despite not significant, WHR, as well as total body and trunk fat mass revealed an inverse association tendency with liver fat. In further analysis, after adjusting for HC, a larger WC was related with a lower LSR (β=-0.203, p<0.05).

Table 3. Independent contributions (standardized betas) of anthropometric and body composition markers to liver-to-spleen ratio, adjusted for age and BMI.

	Liver-to-spleen ratio	Percentage of variance explained** (%)
WC, cm	-0.229	7.7#
HC, cm	0.125	7.0#
WHR	-0.145	7.9#
SD, cm	-0.383*	10.1#
TFM, kg	-0.221	7.4#
TBFM, kg	-0.111	6.5#
TBLM, kg	-0.077	6.7#

BMI, body mass index; WC, waist circumference; HC, hip circumference; WHR, waist-to-hip ratio; SD, sagittal diameter; TFM, trunk fat mass; TBFM, total body fat mass; TBLM, total body lean mass. Age did not have any independent significant contribution to the anthropometric studied variables.
** Variance explained by age, BMI and the studied variable.
# Independent significant contribution of BMI, $P < 0.01$.
* $P < 0.05$.
† $P < 0.01$.
‡ $P < 0.001$.

In **Table 4** are presented the independent contributions of abdominal AT depots and thigh body composition compartments to LSR, after adjustment for age and BMI. Higher VAT areas were associated with a lower LSR. On the contrary, a higher thigh SFAT area was related with a higher LSR. These associations remained significant when using liver attenuation as dependent variable and adjusting for the same confounders. Furthermore, thigh SFAT remained positively associated with LSR, independently of WC (β=0.166, p<0.05).

Table 4. Independent contributions (standardized betas) of abdominal adipose tissue depots and thigh body composition compartments to liver-to-spleen ratio adjusted for age and BMI.

	Liver-to-spleen ratio	Percentage of variance explained** (%)
TAAT, cm^2	-0.234	8.4
Ab SAT, cm^2	-0.136	6.8
Superficial, cm^2	0.014	6.3
Deep, cm^2	-0.133	7.3
VAT, cm^2	-0.241*	9.2
Mid-thigh AT, cm^2	0.148	7.1
Mid-thigh SAT, cm^2	0.129	7.1
Mid-thigh SFAT, cm^2	0.295†	12.5
Muscle, cm^2	0.005	6.2
HDM, cm^2	-0.036	6.4
LDM, cm^2	0.145	7.3
TTAT, kg	0.136	8.2
TTSAT, kg	0.040	6.8
TTSFAT, kg	0.089	7.2
TTMT, kg	-0.051	6.9

TAAT, total abdominal adipose tissue; Ab, abdominal; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; AT, adipose tissue; SAT, subcutaneous adipose tissue; SFAT, mid-thigh subfascial adipose tissue; HDM, high-density muscle; LDM, low-density muscle; TTAT, total thigh adipose tissue; TTSAT, total thigh subcutaneous adipose tissue; TTSFAT, total thigh subfascial adipose tissue; TTMT, total thigh muscular tissue. While BMI revealed an independent contribution to all body composition variables ($P < 0.01$), age did not have any independent contribution to the studied variables.

** Variance explained by age, BMI and the studied variable.

* $P < 0.05$.

† $P < 0.01$.

‡ $P < 0.001$.

Independent associations of metabolic syndrome components, and inflammatory and atherothrombotic risk factors to liver fat, adjusting for age and BMI, are presented in **Table 5**. Higher fasting insulin, TG, liver transaminases, PAI-1 and uric acid concentrations, as well as higher TC/HDL-C and LDL-C/HDL-C ratios were associated with lower LSR values.

Table 5. Independent contributions (standardized betas) of metabolic syndrome features, proinflammatory and atherothrombotic risk factors to liver-to-spleen ratio, adjusted for age and BMI.

	Liver-to-spleen ratio	Percentage of variance explained** (%)
Fasting insulin, μIU/mL	-0.218*	10.1
Fasting glycemia, mg/dL	-0.069	6.6
Triglycerides, mg/dL	-0.257†	11.9
TC, mg/dL	-0.067	6.5
HDL-C, mg/dL	0.107	7.2
LDL-C, mg/dL	-0.021	6.2
TC/HDL-C ratio	-0.284‡	13.1
LDL-C/HDL-C ratio	-0.181*	9.0
Apo A1/Apo B100 ratio	0.067	6.5
Systolic BP, mm Hg	0.047	6.6
Diastolic BP, mm Hg	0.177*	9.4
ALT, IU/L	-0.437‡	24.8
AST, IU/L	-0.346‡	17.8
CRP, mg/dL	-0.006	3.6
IL-6, pg/mL	-0.054	6.1
TNF-α, pg/mL	0.013	6.1
PAI-1, ng/mL	-0.208*	9.7
Fibrinogen, mg/dL	-0.011	6.3
Hb A1c, %	0.001	6.2
Uric acid, mg/dL	-0.178*	8.9
Microalbuminuria, μg/min	0.071	6.7
Cortisol, ug/day	-0.096	7.1
Leptin, ng/mL	-0.085	6.8
Adiponectin, ng/mL	0.041	6.4

All variables were entered in the regression models as continuous variables. TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CRP, C-reactive protein; IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha; PAI-1, plasminogen activator inhibitor-1; Hb A1c, hemoglobin A(1c). While age did not have any independent contribution to the studied variables, BMI revealed an independent contribution to all metabolic syndrome features ($P < 0.01$).

** Variance explained by age, BMI and the studied variable.

* $P < 0.05$.

† $P < 0.01$.

‡ $P < 0.001$.

These metabolic risk factors remained significantly associated with LSR, independently of VAT ($p<0.05$). The explained variance for each metabolic risk factor studied to LSR varied between 3.6% and 24.8%, showing higher values for liver transaminases, TC/HDL-C ratio and fasting insulin. When adjusting for age and BMI and using liver attenuation as dependent variable, similar associations were found, excepting fasting insulin and uric acid ($p>0.05$).

DISCUSSION

Our primary findings were that a higher thigh SFAT area was associated with either a higher LSR or a lower liver attenuation, representing a lower liver fat storage, independently of age and BMI. Furthermore, we found that for a given WC, increased thigh SFAT areas were also significantly related with a higher LSR. To our knowledge, these associations between thigh SFAT and both LSR and liver attenuation are novel observations that may suggest an indirect preventive role of this thigh AT depot against ectopic liver fat storage in overweight or obese women. Moreover, it has been suggested that femoral-gluteal AT may function as a "sink" for circulating FFA (13). When compared with visceral adipocytes, these thigh adipocytes are less sensitive to stimulated lipolysis and reveal a relatively high lipoprotein lipase activity, important in FFA uptake from the circulation (32). Hence, these metabolic characteristics may prevent liver lipotoxicity and counteract the inevitable physiologic cascade observed in abdominal obese subjects, responsible for IR and other secondary metabolic disturbances, such as multiple proinflammatory cytokine response. Interestingly, several studies have been reporting that peripheral fat mass (PFM) is an independent predictor of a lower health risk (42, 43). This protective PFM role in metabolic disturbances and atherogenesity may be, in part, explained by adiponectin insulin sensitizing effects (49). In fact, it has been suggested that thigh SAT, a major contributor for circulating

adiponectin, may mediate these counteracting effects (20). However, rather than thigh SAT, our observations support the notion that that TTSFAT may confer a metabolic protection against detrimental ectopic fat storage in the liver.

However, previous studies have been associating mid-thigh SFAT not only with liver fat (18), but also with IR (15, 18). In fact, in a recent study developed with 83 type 2 DM patients, it was observed that fatty liver was not only inversely related with subfascial AT of skeletal muscle but also with visceral adiposity (18), independently of the effects of VAT and BMI. In this context, more than interpreting these results as an evidence suggesting a causative role of thigh SFAT in fatty liver pathogenesis, the authors have proposed that, SFAT together with fatty liver are special adiposity depots related with IR pathogenicity in type 2 DM. Therefore, these results obtained in type 2 DM patients contrast with our results verified in overweight and obese women, suggesting that this body composition area warrants more research.

The role of abdominal obesity on ectopic liver fat storage and consequent metabolic abnormalities has been a purpose of several studies. Indeed, in a study with 144 patients with hepatic steatosis, clinically characterized by hepatocyte fat infiltration and often described as fatty liver, BMI was the unique independent predictor of the steatosis degree (2). Another two studies have also reported that, both in obese patients (25) and in living liver donors (34), BMI was associated with the steatosis severity. Conversely, we found that, independently of age, a higher weight and BMI were associated, in this sample of overweight and obese women, with a lower LSR. On the other hand, in a study with 221 chronic hepatitis C patients (1), VAT rather than BMI, was a significant predictor of hepatic steatosis. Indeed, abdominal obesity markers, such as WC (21, 37), WHR (16, 37), VAT (4), VAT/TAAT ratio (4), and Ab SAT (37) seem to be highly correlated with liver fat. In our study, after adjusting for HC, a larger WC was related with liver fat. Furthermore, when

adjusting for age and BMI, higher VAT areas, as well as an increased SD were significantly associated with liver fat, emphasizing the abdominal obesity relevance to liver lipotoxicity (47). This relevance already suggested in previous observations (18), was clinically reinforced in a recent study which reported that surgical VAT removal could reverse hepatic IR (5). The link between abdominal adiposity and liver fat storage may be explained by the fact that FFA are more easily mobilized from visceral AT rather than Ab SAT depots, draining directly into the liver via portal circulation (17). The increased FFA liver influx may induce hepatic steatosis, which might be responsible for other metabolic disturbances, such as increased liver FFA and TG-rich lipoproteins synthesis, adipocyte proliferation failure, insufficient hepatocyte FFA oxidation (26, 38, 47). In addition, liver lipotoxicity may be accompanied by a low chronic inflammatory state, which can promote the future progression to non-alcoholic steatohepatitis (NASH) (26). Despite evidence has been demonstrating the VAT-derived FFA contribution to these pathophysiologic cascade, a recent overview have also highlighted the role of FFA released from abdominal subcutaneous adipocytes into systemic circulation to these hepatic disturbances (14). In this context, the results of our study are consistent with some emerging observations (4), suggesting that liver fat is associated not only with abdominal obesity, but can also reflect an unfavourable metabolic syndrome profile.

Indeed, we observed that higher insulin, TG, liver transaminases, uric acid and PAI-1 concentrations were independently associated with a lower LSR. Furthermore, higher TC/HDL-C and LDL-C/HDL-C ratios were also related with a lower LSR. These metabolic markers remained significantly associated with liver fat, independently of VAT (data not shown). Despite some evidence has been proposing that liver fat storage is normally preceded by VAT accumulation, our results are consistent with other observations reporting that liver fat remains associated with metabolic syndrome features independently of total and

visceral adiposity (29, 45). In this sense, our results suggest that hyperinsulinemia, hypertriglyceridemia and hypercholesterolemia are relevant to the metabolic cascade that mediates liver disturbances in overweight and obese women. Other studies developed with both insulin sensitive and insulin resistant subjects have also reported that liver fat was associated with IR (4) and TG concentrations (4, 7). Another study with type 2 DM patients reported that the presence of fatty liver was associated with higher degree of IR and dyslipidemia (18). Hepatic steatosis has also been associated with dyslipidemia, hyperinsulinemia, and IR, not only in obese subjects but also in lean subjects without glucose intolerance (27). Although the role of diabetes in hepatic steatosis and in its progression to NASH still remains unclear (26), the National Health and Nutrition Examination Survey (NHANES-III) has reported that simple IR features, such as fasting insulin, Hb A1c and C-peptide concentrations, as well as abdominal obesity markers were independently associated with ALT concentrations, the most sensitive indicator of liver cell integrity. In fact, increased liver transaminases concentrations are not only associated with obesity severity, but can also predict the liver injury degree (25, 39). On the other hand, it is noteworthy that hyperinsulinemia seems to play a key role in FFA metabolism and may inhibit hepatocyte mitochondrial beta-oxidation, which can additionally contribute to liver lipotoxicity. Furthermore, the inverse associations of both PAI-1 and uric acid with LSR observed in our study emphasize the ectopic liver fat storage relevance to inflammatory and atherothrombotic metabolic syndrome disturbances in overweight and obese women.

The role of some adipocytokines, such as leptin and TNF-α in hepatic steatosis has been also increasingly study. Recent studies have reported that leptin can mediate lean body tissues protection against lipotoxic damage (46), being also relevant in lipogenesis blocking, and in muscle insulin-sensitization and fatty acid oxidation enhancement (46). However, hyperleptinemia, commonly present in visceral obese patients, may aggravate IR and

promote liver fat storage. On the other hand, inflammatory endotoxins, such as TNF-α and IL-6, often overexpressed in obese patients or overweight subjects with type 2 DM, have also been associated with liver fat and NASH pathogenesis (26). Contrarily to the observed in a previous study with type 2 DM patients (18), in our study, nor LSR nor liver attenuation were independently associated with leptin, IL-6, TNF-α, and any other inflammatory and thrombotic risk factors studied.

The CT abdominal and thigh adipose and muscle tissue assessments, as well as the broad list of metabolic features measured are some of the strengths of this study. Additionally, participants were counseled to refrain from exercise at least 48 hours prior to blood sampling, avoiding metabolic acute exercise interferences. However, there are some limitations in our study that warrant reference. First, it is noteworthy that liver attenuation obtained by CT cannot quantify absolute liver fat because attenuation of each voxel is a function of its lipid, lean tissue and water composition. Therefore, variations in each one of the components may change the resultant attenuation, adding difficulties in data interpretation. Second, despite the rigorous protocol to obtain fasting blood samples, being controlled the stage of menstrual cycle to avoid lipid profile variations induced by phase changes, we did not control diet composition prior blood sampling.

In summary, contrarily to previous observations in type 2 DM patients, thigh subfascial AT was independent and inversely associated with liver fat in overweight and obese women, suggesting that this thigh AT compartment may play a preventive role against detrimental ectopic liver fat storage. Conversely, our results emphasize the contribution of a higher BMI and visceral AT, especially if associated with hyperinsulinemia, dyslipidemia, and an inflammatory and atherothrombotic profile to the metabolic cascade that mediates liver lipotoxicity in overweight and obese women.

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DISCLOSURES

The authors have no conflicts of interest to report in this research.

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