

Original Article

Assessment of Abdominal Fat Distribution in Non-Alcoholic Fatty Liver Disease by Magnetic Resonance Imaging: a Population-based Study

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Abstract

Background: To investigate the association between non-alcoholic fatty liver disease (NAFLD) and quantitative measures of central adiposity in the general population using a semi-automated method on magnetic resonance imaging (MRI) data.

Methods: Subjects were recruited from Golestan Cohort Study. Two groups of 120 individuals with and without fatty liver were randomly selected based on findings of ultrasound. Non-invasive diagnosis of NAFLD was made by combination of ultrasound and MRI. Various anthropometric indices including body mass index (BMI), waist-to-hip ratio (WHR) and waist-to-height ratio (WHtR) were measured. Segmentation and calculation of visceral (VFA) and subcutaneous fat area (SFA) were performed on three levels of MRI slices using semi-automated software.

Results: A total of 109 individuals fulfilled the NAFLD criteria, while 92 subjects were selected as the control group. All obesity measures, except for SFA, were significantly higher in subjects with NAFLD compared to controls. Significant associations were found between NAFLD and adiposity indices, except for SFA, with the highest odds ratio observed in WHR (OR: 3.37, CI: 1.40–3.70, $P < 0.001$). VFA also had the greatest correlation with ultrasound ($r = 0.523$, $P < 0.001$) and MRI ($r = 0.546$, $P < 0.001$) indicators of NAFLD.

Conclusions: Quantitative measures of visceral adiposity are associated with NAFLD, while subcutaneous fat measures are poor indicators for identifying NAFLD. Compared to conventional anthropometric indices, VFA best correlates with ultrasound and MRI criteria of fatty liver.

Keywords: Abdominal fat segmentation, general population, non-alcoholic fatty liver disease, subcutaneous fat, visceral fat

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Introduction

Non-alcoholic fatty liver disease (NAFLD) encompasses a spectrum of disorders from simple steatosis to nonalcoholic steatohepatitis (NASH) and liver cirrhosis.¹ NAFLD has been considered as a manifestation of the metabolic syndrome² with an incidence varying from 1%–3% in children³ to 40% in individuals older than 70 years.⁴ Several diagnostic methods are available for diagnosis of NAFLD. Although biopsy is the gold standard for diagnosis, biochemical and imaging methods are widely used to non-invasively detect fatty liver.^{5,6} Currently, ultrasonography (US) is widely used for imaging diagnosis of fatty liver; however, magnetic resonance imaging (MRI) has been shown to detect lower levels of fat deposition within liver compared to US.⁷

Visceral obesity is identified as one of the main factors contributing to development of NAFLD.^{8,9} Several studies have assessed the relationship between visceral fat and NAFLD yielding conflicting results. Some have found an association between visceral fat quantity and fatty liver,^{10,11} while others have not.^{12,13} US has been used for estimation of visceral fat; however, it is limited by its inability to measure fat volume and technical difficulties in very obese patients. Likewise, computed tomography (CT) is limited by causing unnecessary radiation, although visceral fat area measured by CT scan has been reported to have strong association with the metabolic syndrome.¹⁴ MRI seems to be more appropriate for quantitative measurement of

abdominal fat tissue and segmentation of fat to subcutaneous and visceral compartments.¹⁵ Previous studies have been performed to develop accurate imaging methods for quantitative measurement and segmentation of abdominal fat tissue.¹⁶⁻²⁰ In comparison with manual segmentation, semi- or full-automated segmentation is less time consuming and is less subject to operator-dependent bias.

The first objective of this population-based study was to develop a semi-automated algorithm for segmentation of visceral (VFA) and subcutaneous fat area (SFA) in subjects with and without NAFLD to determine the accuracy of quantitative measures of central obesity on MRI for prediction of hepatic steatosis. The second objective was to compare the quantitative measures of VFA and SFA with other conventional anthropometric indices including body mass index (BMI), waist-to-hip ratio (WHR) and waist-to-height ratio (WHtR).

Materials and Methods

Study design & subjects

In this cross-sectional study, subjects from the general population were randomly selected from a community-based, randomized clinical trial, PolyIran-L (ClinicalTrials.gov identifier: NCT01245608) which is assessing the effects of a combination pill (Polypill) on the natural history of NAFLD among more than 1500 individuals aged over 50 residing in Gonbad City, northeastern Iran.^{21,22} The clinical trial of PolyIran-L is nested in the Golestan Cohort Study (GCS).

An initial US examination was performed on subjects enrolled in PolyIran-L study. Based on ultrasound results, two groups of 120 subjects were randomly selected from participants with and without fatty liver. Subjects were excluded if they had positive markers for viral hepatitis B and C or history of alcohol abuse or habitual consumption. Laboratory markers of metabolic syndrome were measured as followed: fasting blood sugar (FBS), hemoglobin A1c (HbA1c), alanine transaminase (ALT), aspartate transaminase (AST) and lipid profile (cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL) and low-density lipoprotein cholesterol (LDL)).

BMI was calculated as weight (in kg) divided by height squared (in meters). WHR was defined as waist circumference divided by hip circumference (both in centimeters). Likewise, WHtR was defined as waist circumference divided by height (both in centimeters). Waist circumference was measured at the midpoint between the lower costal margin and the iliac crest. Hip circumference was measured at the level of maximal protrusion of the gluteal muscles.

The study was carried out between September 2011 and March 2014. Written informed consent was obtained from all participants. This study was approved by the ethics committee of the Digestive Disease Research Institute, Tehran University of Medical Sciences, based on the ethical principles of human research and experimentation.

Ultrasound

Liver ultrasound was carried out using an Accuvix XQ ultrasound unit (Medison, Seoul, Korea) equipped with a 3-7 MHz curved-array transducer. Individuals were examined in supine position following at least 6 hours of fasting. Fatty liver was suggested using ultrasonographic scoring method originally

described by Hamaguchi and colleagues,²³ which provides high sensitivity (91.7%) and specificity (100%) for diagnosis of fatty liver. Scores of this ultrasound protocol included hepatorenal echo contrast and/or liver brightness (0 to 3), deep attenuation (0 to 2), and vascular blurring (0 to 1). Fatty liver diagnosis required a minimum total score of 2, including minimum hepatorenal echo contrast and/or bright liver score of 1. An experienced radiologist (A.R.), who was blinded to the participants' characteristics, performed ultrasound examinations.

Magnetic resonance imaging

All subjects underwent MRI exam to confirm the presence or absence of NAFLD. MRI was performed using a 1.5T unit (Symphony, Siemens, Erlangen, Germany) with a 8-channel phased array body coil. Non enhanced transverse T1-weighted in-phase (IP) and opposed-phase (OP) breath-hold spoiled gradient-echo (GRE) MRI sequences were acquired for evaluation of steatosis by using repetition time (TR) range of 100–120 milliseconds/echo time (TE) of 2.4 milliseconds (OP) and 4.8 milliseconds (IP), flip angle of 70 degrees, slice thickness of 8 mm, interslice gap of 0 mm, matrix of 256 × 160, field of view of 400 cm², and acquisition time of 23 seconds. MRI covered the whole abdomen from lower lung to pelvic inlet at the level of L5-S1.

Image analysis for estimation of hepatic fat

IP/OP images were transferred to a computer workstation for post processing. Image analysis was performed by a consensus between two experienced radiologists (A.H., A.R.) with more than 10 and 4 years of experience in this field, respectively. They were blinded to the patients' clinical and ultrasound findings. Signal intensity values were measured on eighteen circular ROIs in liver (four in the right and two in the left lobe at each of three sections of above, below and at the level of main portal vein). Likewise, nine circular ROIs were drawn on spleen including three ROIs at each of three sections to adjust for the effect of T2* decay between IP and OP images (Figure 1). A home-made software was designed using MATLAB (Math works, Natick, MA) for anatomic placement of ROIs by precise localization of the selected ROIs in IP images on the corresponding OP images. Each circular ROI had an area of 1.5–2 cm², placed in an area of liver/spleen appearing homogeneous and devoid of vessels. The mean signal intensity values of 18 ROIs in liver and 9 ROIs in spleen obtained from IP and OP images were calculated. Relative signal intensity loss was calculated in the described software according to the following previously published formula²⁴: $(SI_{in} - SI_{out})/SI_{in} \times 100$, where SI is the mean liver signal intensity divided by the mean spleen signal intensity, SI_{in} is in-phase signal intensity, and SI_{out} is OP signal intensity.

Semi-automated measurement of visceral and subcutaneous fat

A semi-automated method was developed for segmentation of abdominal adipose tissue from MR images. Prior to the segmentation, the images were preprocessed to remove signal inhomogeneities of the voxel caused by bias field effect. A nonparametric coarse to fine approach was used for automatic correction of this artifact, which allowed bias fields to be modeled with different frequency ranges without user supervision. Then, the adipose tissue was labeled across the abdomen by unsupervised classification using fuzzy c-means (FCM) clustering. FCM

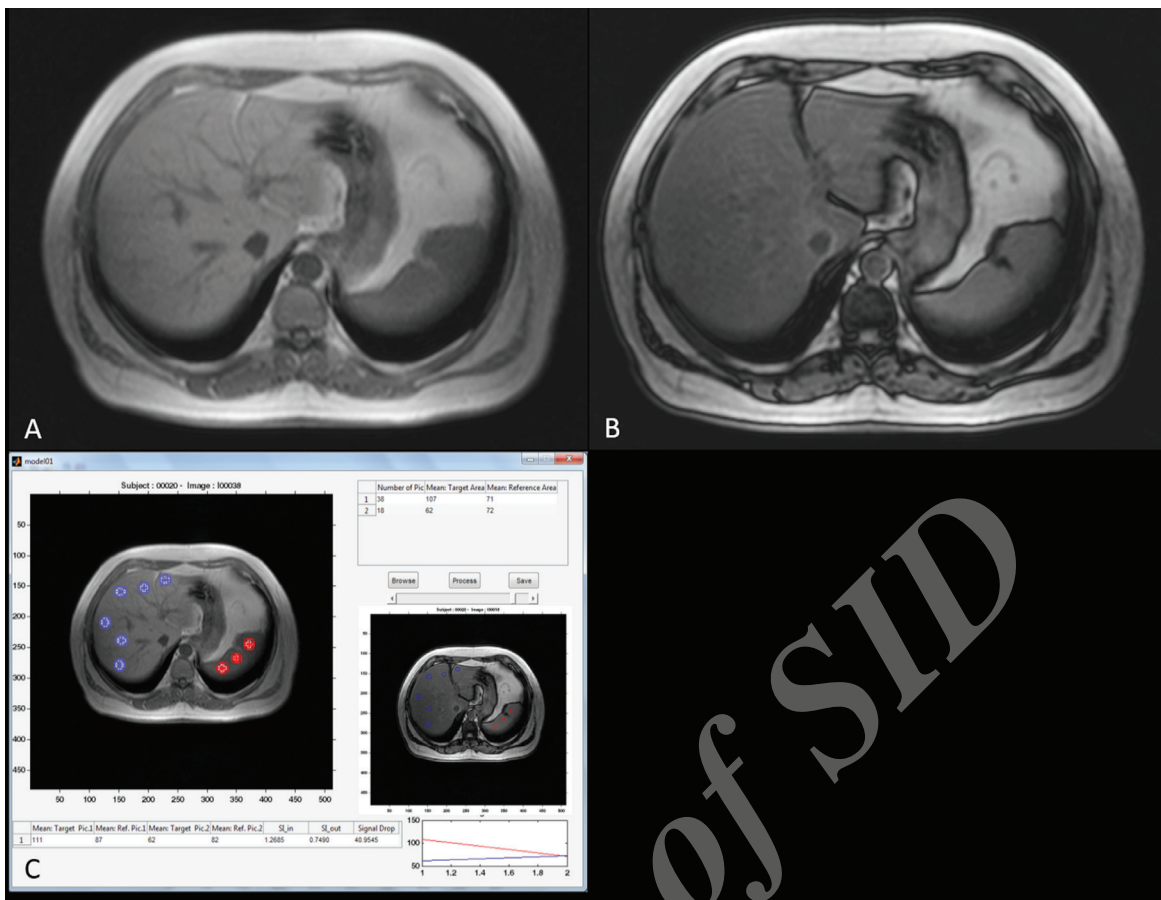


Figure 1. Transverse T1-weighted in-phase (A) and opposed-phase (B) images of the liver in a 54 year old woman with NAFLD. (C) Calculation of relative signal intensity loss using a home-made software by drawing 6 ROIs in liver (4 in right and 2 in left lobe) and 3 ROIs in spleen at each slice on in-phase image to automatically localize the same pixels on corresponding opposed phase image. This was repeated for two other slices. According to the average signal intensity of 18 ROIs in liver and 9 ROIs in spleen (reference organ), relative signal intensity drop was calculated as about 41% suggestive of steatosis.

clustering computes three masks as background, muscle and fat tissue. By incorporating signal intensity information, the abdomen boundary was segmented and the visceral adipose tissue was separated from the subcutaneous adipose tissue by means of active contours and level set algorithm.

Segmentation accuracy was assessed by comparing the segmented images with those manually segmented by an expert operator (M.G) as reference. Correlation between automated segmented images and reference segmented images was 0.90 ± 0.048 . Dice coefficient and Jacard metric were also examined with correlation of 0.92 ± 0.049 and 0.85 ± 0.079 , respectively.

Finally, the expert operator corrected some automatic segmentation errors and then, VFA and SFA were calculated in cm^2 at L3-L4, L4-L5 and L5-S1 levels. Mean correction and calculation time was about 4 minutes (Figure 2).

In the automated technique, all measurements will be done automatically by the designed program, but in the semi-automated method that was applied in this study, some parts of the process will be facilitated manually by an operator. As mentioned before, after automatic segmentations of visceral and subcutaneous fat using designed software, the fat containing particles of fecal materials within the lumen of colon, which had signal intensities similar to visceral fat, were removed manually by an expert operator (semi-automated technique).

Definitions

Non-invasive diagnosis of NAFLD was made if subjects with ultrasound diagnosis of fatty liver showed at least 6% relative signal intensity loss in OP images.^{24,25} Control (non-NAFLD) group consisted of subjects with normal ultrasound (total score of 0 or 1) and less than 6% relative signal intensity loss.

Statistical Analysis

Numerical data are expressed as means \pm standard deviation (SD). The significance of differences in variables between NAFLD and control groups was examined using Student's T-test or Pearson's Chi-square test. Multivariable logistic regression analysis was performed to compare the association of VFA, SFA and other obesity indices with NAFLD. Since anthropometric indices are highly correlated with each other, they were tested separately in multivariable logistic regression models. Variables with a *P* value less than 0.2 in univariate analysis were included in the multivariable model. Pearson coefficient correlation was used to assess the correlation between obesity indices with ultrasound and MRI findings of fatty liver. A *P* value less than 0.05 was considered as significant in this study. Statistical analysis was performed using SPSS program, version 17.0 (SPSS Inc., Chicago, Ill., USA).

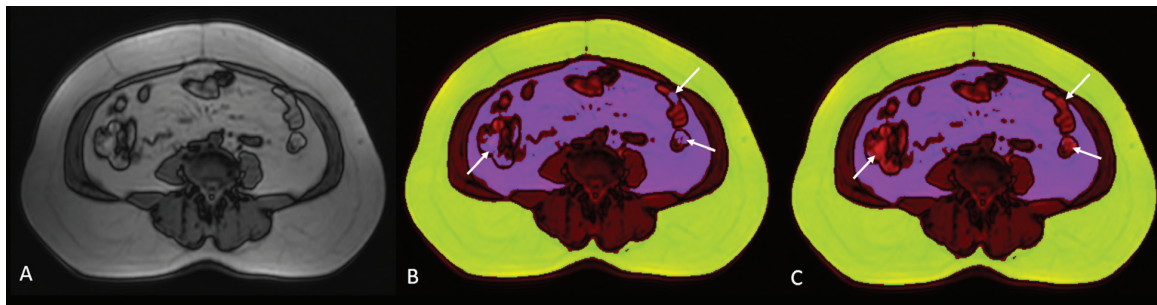


Figure 2. Quantification of VFA and SFA. **(A)** Transverse T1-weighted opposed-phase image of a 59 year old man at the L4-L5 level. **(B)** Automatic segmentation of VFA and SFA with color coding to illustrate them in green (SFA) and purple (VFA). **(C)** Manual correction of errors due to fat containing material within the bowel loops (arrows) to finalize the segmentation of abdominal fat.

Results

All 120 subjects with initial ultrasound diagnosis of fatty liver accepted to participate in the study and underwent MRI. Consequently, 109 cases (mean age 56.3 ± 5.4 years) fulfilled MRI criteria of fatty liver and were considered as the NAFLD group. Of 120 individuals with initial normal ultrasound, two were excluded due to contraindications for MRI and six refused to undergo MRI. Therefore, of 112 participants with normal ultrasound, 92 subjects (mean age 57.9 ± 6.5 years) were not demonstrated to have fatty liver on MRI (control group).

Univariate analysis

Table 1 shows the association between demographics, risk factors of metabolic syndrome and all obesity measures with the presence of fatty liver in subjects with and without NAFLD. On univariate analysis, BMI was significantly higher in the NAFLD

compared to the control group ($P < 0.001$). Participants with NAFLD were more likely to have higher WHR than controls ($P < 0.001$). Likewise, subjects with NAFLD were more prone to have higher WHtR than control subjects ($P < 0.001$). VFA showed significantly higher values in the NAFLD group in comparison with controls ($P < 0.001$). There was no significant difference in SFA values between NAFLD and non-NAFLD groups ($P = 0.45$).

Mean values of FBS and HbA1c were statistically higher in the NAFLD group ($P = 0.03$ and $P = 0.001$, respectively). Serum levels of triglyceride were significantly higher in participants with NAFLD ($P < 0.001$). Likewise, lower levels of serum HDL were more frequent in NAFLD subjects ($P = 0.002$). Statistically higher serum levels of ALT and AST were found in the NAFLD than the control group ($P < 0.001$ and $P = 0.01$, respectively). Meanwhile, participants with and without NAFLD did not exhibit any significant difference in terms of serum cholesterol and LDL levels.

Table 1. Univariate analysis for association of demographics, obesity indices and risk factors of metabolic syndrome in participants with and without NAFLD.

Risk factors	With NAFLD (n = 109)	Without NAFLD (n = 92)	P-value
Gender (F/M)	57/52	49/43	0.891
Age (yrs.), mean (SD)	56.35 (5.4)	57.98 (6.5)	0.055
BMI (kg/m ²), mean (SD)	29.83 (3.3)	27.63 (4.2)	<0.001*
WHR, mean (SD)	1.01 (0.06)	0.95 (0.07)	<0.001*
WHtR, mean (SD)	0.64 (0.06)	0.59 (0.07)	<0.001*
VFA (cm ²), mean (SD)	429.80 (157.3)	305.08 (135.7)	<0.001*
SFA (cm ²), mean (SD)	631.13 (195.7)	605.08 (291.5)	0.452
Total cholesterol (mg/dL), mean (SD)	216.82 (41.0)	215.02 (40.4)	0.765
LDL (mg/dL), mean (SD)	119.87 (43.42)	126.15 (34.4)	0.283
HDL (mg/dL), mean (SD)	54.66 (13.7)	61.88 (16.9)	0.002*
Triglyceride (mg/dL), mean (SD)	186.18 (114.14)	130.13 (62.9)	<0.001*
FBS (mg/dL), mean (SD)	114.67 (36.3)	103.28 (38.2)	0.039*
HbA1C (%), mean (SD)	2.22 (0.9)	0.81 (0.4)	0.001*
ALT (IU/mL), mean (SD)	33.56 (26.7)	20.89 (9.9)	<0.001*
AST (IU/mL), mean (SD)	24.53 (13.3)	20.35 (8.8)	0.011*

BMI = body mass index; WHR = Waist to hip ratio; WHtR = waist to height ratio; VFA = visceral fat area; SFA = subcutaneous fat area; LDL = low density lipoprotein; HDL = High density lipoprotein; FBS = fasting blood sugar; HbA1C = glycated hemoglobin; ALT = alanine aminotransferase; AST = aspartate aminotransferase.

Table 2. Association of each obesity index with NAFLD in separate multivariable logistic regression models.

Obesity index	Odds Ratio (95% CI)	P-value**
BMI (per 1 kg/m ²)	1.13 (1.04–1.23)	0.005*
WHR (per 0.1)	3.37 (1.93–5.88)	<0.001*
WHtR (per 0.1)	2.27 (1.40–3.70)	0.001*
VFA (per 1 dm ²)	1.05 (1.02–1.07)	<0.001*
SFA (per 1 dm ²)	N/A	N/A

* Statistical significance ($P < 0.05$). **Adjusted P -value for age, HDL = triglyceride, FBS and HbA1C. BMI = body mass index; WHR = Waist to hip ratio; WHtR = waist to height ratio; VFA = visceral fat area; SFA = subcutaneous fat area.

Table 3. Correlation between obesity indices and ultrasound and MRI indicators of steatosis.

Obesity index	Ultrasound score of fatty liver		Relative signal intensity loss on in-phase and opposed-phase images	
	Pearson coefficient (r)	P-value	Pearson coefficient (r)	P-value
BMI	0.370	<0.001*	0.165	0.019*
WHR	0.421	<0.001*	0.313	<0.001*
WHtR	0.391	<0.001*	0.227	0.002*
VFA	0.523	<0.001*	0.546	<0.001*
SFA	0.097	0.17	0.026	0.712

* Statistical significance ($P < 0.05$). BMI = body mass index; WHR = waist to hip ratio; WHtR = waist to height ratio; VFA = visceral fat area; SFA = subcutaneous fat area.

Multivariate analysis

To determine the independent effect of BMI, WHR, WHtR, VFA and SFA, multivariate logistic regression analyses were performed, following demonstration of associations in univariate analysis by including each obesity index in separate models (Table 2).

In the final models, following adjustment for other covariates, significant associations were found between WHR, WHtR, BMI and VFA and the presence of NAFLD in order of decreasing odds ratio, while SFA did not show any statistically significant association. There were no significant interactions between any of the obesity measures with presence of NAFLD when they were entered either one-by-one or together into each model.

Pearson coefficient correlation

We evaluated the correlation between various obesity indices of this study with ultrasound and MRI criteria of fatty liver (Table 3). Significant correlations of a moderate nature were demonstrated between VFA and ultrasound scores of fatty liver ($r = 0.523$), as well as relative signal intensity loss in MRI ($r = 0.546$) (both $P < 0.001$) with the highest coefficients among all obesity measures in this study. We also found significant weak to moderate correlations between BMI, WHR and WHtR with ultrasound and MRI manifestations of fatty liver, while SFA did not exhibit any statistically significant correlation.

Discussion

In this population-based study, a useful method was developed based on MRI data for quantification of distribution of visceral and subcutaneous adiposity. Meanwhile, the associations between NAFLD and quantitative measures of central obesity and other anthropometric indices were also investigated. While SFA did not have any significant association with the presence of NAFLD, increment in VFA related well to NAFLD. These findings are consistent with the results of similar studies implicating that

individuals with visceral adiposity are more likely to develop NAFLD.^{10,26} Conventional anthropometric indices including BMI, WHR and WHtR were also strong predictors of the presence of NAFLD, with WHR showing the highest odds ratio. Of all obesity indices, increase in VFA was the best correlate of ultrasound and MRI criteria of fatty liver.

In this population-based study, liver biopsy could not be done due to ethical and practical concerns, so we applied a non-invasive technique to choose NAFLD and control subjects by combination of ultrasound and MRI findings. The inherent risk of biopsy also makes it unattractive for subjects from the general population. Furthermore, the results of biopsy are based on a small sample representing approximately 1/50,000th of the liver and on the other hand fat deposits in a non-uniform and heterogeneous pattern in liver. Therefore, biopsy results cannot be attributed to the whole liver, while MRI can provide more information about the heterogeneous distribution of steatosis. Accordingly, by applying a non-invasive protocol, the NAFLD group consisted of those with positive findings of fatty liver in both ultrasound and MRI examinations. In contrast, control subjects included those without findings of fatty liver in both ultrasound and MRI assessments.

Visceral fat deposition is termed the most important risk factor for development of NAFLD.²⁷ Bahl *et al.* showed that VFA is a stronger correlate of fatty liver compared to BMI.²⁸ It has been reported that the ratio of CT-measured visceral fat thickness (VAT) to subcutaneous fat thickness (SAT) is an independent predictor of cardiometabolic risk.²⁹ The higher association of VAT/SAT ratio with prevalence of metabolic syndrome has also been demonstrated.³⁰ According to our data, VFA was demonstrated to be accurate for detection of hepatic steatosis. Although BMI has been often used for estimation of obesity, it has poor correlation with visceral adiposity and it does not provide any information about the anatomic distribution of accumulated fat. Several alternative adiposity indices such as WHR and WHtR have been more strongly correlated with adiposity-related morbidity.

Quantification of various parts of body fat depot by imaging

methods has gained considerable interest. Ultrasonography is a non-expensive method for estimation of visceral fat; however, it is limited by technical difficulties in obese individuals. MRI has been identified as the most promising tool for this purpose. Semi-automated and fully-automated methods have been described in several studies yielding accurate and reproducible results.¹⁶⁻²⁰ Kullberg *et al.* described a fully-automated approach for abdominal fat segmentation; however, their method was limited by measuring abdominal fat at a single level.²⁰ A reliable method for measuring total and visceral adiposity was introduced by Ross *et al.*³¹; they showed that visceral fat volume has a strong correlation with WHR. Ducluzeau *et al.*³² suggested single level VFA surface and VFA/SFA+VFA to be a better correlate of liver steatosis than BMI. Moreover, they found that WHR is the most accurate anthropometric measure to predict fatty liver. Their study was limited by small number of subjects without a control group in addition to the single level VFA and SFA measurements. Thomas *et al.*³³ showed that only multi-slice imaging provides accurate and consistent comparison of inter-subjects central adiposity; use of single-slice technique for estimation of abdominal fat can lead to significant misinterpretations. Bahl *et al.*²⁸ used water-suppressed T1-weighted sequence for manual measurement of VFA at three levels and suggested visceral fat to be a probable biomarker for hepatic steatosis. According to their data, VFA had 77% correlation with liver steatosis grade. They enrolled only 52 subjects which included 23 patients with HIV and HCV coinfection. Since HIV and its treatment may affect body fat distribution or independently induce hepatic steatosis, this might lead to confounding results. While the findings of present study are consistent with previously published data, it used a non-invasive highly-reproducible method for measuring fat area on MRI by developing a semi-automated software for multilevel measurement of visceral and subcutaneous fat. Our purpose was to use an unsupervised method with minimal user interaction. We demonstrated strong correlation between automated and reference manual segmentation.

The relationship between visceral fat and hepatic steatosis is not well understood. Visceral fat has been termed a paracrine organ as it releases several metabolically active substances into the bloodstream.³⁴ In contrast to subcutaneous fat, visceral fat has portal venous drainage and delivers free fatty acids (FFA) directly to liver, contributing to fat deposition in hepatocytes.³⁵ Visceral adipocytes are insulin resistant with subsequent increased lipolysis leading to FFA formation. Furthermore, visceral adipocytes initiate an inflammatory process through macrophage activation and subsequent cytokine production which induce insulin resistance as the cardinal feature of metabolic syndrome. All this together leads to excess FFA storage in liver and development of NAFLD.³⁶ Van der Poorten *et al.*⁸ found a direct association between visceral adiposity and liver fibrosis. They suggested that visceral fat accumulation has a direct toxic effect on liver through producing inflammatory cytokines. It has been shown that the severity of fatty liver is directly associated with visceral fat area regardless of BMI.¹⁰ Visceral fat thickness can also be used as an indicator for assessing response to treatment in subjects with NAFLD.²⁷

The strengths of this study include use of a community-based sample from a large cohort study with adjustments for potential confounders. By applying MRI data, a highly reproducible algorithm was provided for estimation of subcutaneous and

visceral fat area. Moreover, the relationship between hepatic steatosis and various anthropometric measures was assessed. The results of this study should be interpreted with a few limitations in mind; first, since individuals were selected from the general population, no biopsy was performed for identifying patients with liver fibrosis and NASH; however, a strict protocol was utilized using ultrasound and MRI to identify individuals with NAFLD. Second, only individuals older than 50 years of age were enrolled in this study; therefore, the results might not be generalizable to younger individuals.

In conclusion, the current study found significant associations between quantitative measures of visceral adiposity and NAFLD obtained from a reproducible semi-automated method, while subcutaneous fat measures were poor indicators of NAFLD. In addition, VFA was demonstrated to have the highest correlation with ultrasound and MRI indicators of hepatic steatosis above and beyond other conventional anthropometric indices in this study. It will be of interest to see how VFA correlates with the natural history of NAFLD.

Conflict of interest: None

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