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Visceral adiposity index is associated with significant fibrosis in patients with non-alcoholic fatty liver disease

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SUMMARY

Background
Metabolic factors have been associated with liver damage in patients with non-alcoholic fatty liver disease (NAFLD).

Aims
To test a new marker of adipose dysfunction, the visceral adiposity index (VAI), in NAFLD patients to assess whether or not it is associated with host factors, and to investigate a potential correlation with histological findings.

Methods
One hundred and forty-two consecutive NAFLD patients were evaluated by liver biopsy, and clinical and metabolic measurements, including insulin resistance with the homeostasis model assessment (HOMA), and VAI by using waist circumference, body mass index, triglycerides and HDL. Serum levels of TNFα, IL-6, adiponectin and leptin were also assessed. All biopsies were scored for NAFLD activity score (NAS) and its components, and for staging (Kleiner).

Results
By multiple linear regression analysis, VAI was independently associated with higher HOMA ($P = 0.04$), and fibrosis ($P = 0.04$). In addition, an independent association was found between higher VAI and lower adiponectin levels ($P = 0.002$). Higher HOMA (OR 1.149, 95% CI 1.003–1.316, $P = 0.04$), higher VAI (OR 1.446, 95% CI 1.023–2.043, $P = 0.03$), lobular inflammation (OR 3.777, 95% CI 1.771–8.051, $P = 0.001$), and ballooning (OR 2.884, 95% CI 1.231–6.757, $P = 0.01$) were correlated with significant fibrosis (F2–F4) on multiple logistic regression analysis. In particular, the prevalence of significant fibrosis progressively increased from patients with a VAI ≤ 2.1 and HOMA ≤ 3.4 (26%) to those with a VAI > 2.1 and HOMA > 3.4 (83%).

Conclusions
In NAFLD patients, visceral adiposity index is an expression of both qualitative and quantitative adipose tissue dysfunction and, together with insulin resistance, is independently correlated with significant fibrosis.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD), the hepatic manifestation of insulin resistance (IR), is a leading cause of chronic liver disease worldwide.[1, 2] A relevant proportion of NAFLD patients, particularly those with non-alcoholic steatohepatitis (NASH), may progress to cirrhosis and its complications,[1, 3] with liver necroinflammation[4, 5] and IR[5, 6] the strongest predictors of both severity of fibrosis, and progression of liver disease.

There has been increasing interest in the last few years in the role of visceral adipose tissue in both cardiometabolic disorders and NAFLD. In fact, studies have shown that visceral adipose tissue, originally considered a passive depot for energy storage, is able to secrete a variety of substances that regulate metabolism, inflammation and immunity, participating in the pathogenesis of the above-cited disorders.[7, 8] Visceral adiposity, when evaluated by magnetic resonance, the best estimation of visceral obesity, correlates with liver-fat accumulation in healthy subjects,[9, 10] and with severity of both inflammation and fibrosis in NASH.[11] The association between visceral obesity, evaluated using waist circumference (WC) measurement – a surrogate marker of visceral adiposity, and liver damage in terms of both the presence of steatosis and disease progression, has also been found in other studies on NAFLD and chronic hepatitis C (CHC).[12–15] However, in most of these studies, the effect of visceral obesity on the histological features of the liver disease was not corrected for IR. In addition, the use of WC to indicate visceral obesity is not entirely accurate, because WC alone does not help in distinguishing between subcutaneous and visceral fat mass,[16] the latter being the key factor in the development of metabolic alteration.

To overcome these problems, a recent study[17] elaborated an index, using both anthropometric [body mass index (BMI) and WC] and metabolic (TG and HDL) parameters. This index, called the visceral adiposity index (VAI), is thought to be capable of indicating both fat distribution and function, and has been proposed as a surrogate marker of adipose tissue dysfunction. VAI has been independently correlated with cardiometabolic risk in general population,[17] and with severity of both steatosis and necroinflammatory activity in patients with genotype 1 (G1) CHC.[18] In light of these facts, we aimed to assess the host factors associated with VAI, and its association with histological features in patients with a histological diagnosis of NAFLD.

MATERIALS AND METHODS

Patients

One hundred and forty-two consecutive patients with NAFLD, recruited at the Gastrointestinal & Liver Unit at the University Hospital in Palermo and fulfilling all inclusion and exclusion criteria detailed below were assessed. Patients were included if they had a histological diagnosis of NAFLD by liver biopsy done less than 6 months before enrolment. The diagnosis of NAFLD was based on chronically elevated ALT for at least 6 months, alcohol consumption of <20 g/day in the year before liver biopsy and evaluated by a questionnaire, and steatosis (>5% of hepatocytes) at histology with/without necroinflammation and/or fibrosis. Exclusion criteria were: (i) advanced cirrhosis; (ii) hepatocellular carcinoma; (iii) other causes of liver disease or mixed aetiologies (excessive alcohol consumption, hepatitis C, hepatitis B, autoimmune liver disease, Wilson's disease, hemochromatosis, α1-antitrypsin deficiency); (iv) human immunodeficiency virus infection; (v) previous treatment with anti-viral therapy, immunosuppressive drugs and/or regular use of steatosis-inducing drugs, evaluated by interview; (vi) therapy with medications known to affect UA metabolism, or; (vii) active intravenous drug addiction.

The study was carried out in accordance with the principles of the Declaration of Helsinki and its appendices, and with local and national laws. Approval was obtained from the hospital's Internal Review Board and its Ethics Committee, and written informed consent was obtained from all patients and controls.

Clinical and laboratory assessment

Clinical and anthropometric data were collected at the time of liver biopsy. Body mass index (BMI) was calculated on the basis of weight in kilograms and height in metres, and patients were classified as normal weight (BMI, 18.5–24.9 kg/m^2), overweight (BMI, 25–29.9), or obese (BMI ≥30). Waist circumference was measured at the midpoint between the lower border of the rib cage and the iliac crest. A diagnosis of arterial hypertension was based on the following criteria: systolic blood pressure ≥135 mmHg and/or diastolic blood pressure ≥85 mmHg (measured three times in 30 min, in the sitting position and using a brachial sphygmomanometer), or use of blood-pressure-lowering agents. A diagnosis of type 2 diabetes was based on the revised criteria of the American Diabetes Association, using a value of fasting blood glucose ≥126 mg/dL on at least two occasions.[19]

In patients with a previous diagnosis of type 2 diabetes, current therapy with insulin or oral hypoglycaemic agents was documented. Metabolic syndrome was diagnosed according to ATPIII criteria.[20]

A 12 h overnight fasting blood sample was drawn at the time of biopsy to determine serum levels of ALT, GGT, total cholesterol, HDL cholesterol, triglycerides, plasma glucose concentration, insulin and platelet count. Insulin resistance (IR) was determined by the homeostasis model assessment (HOMA) method, using the following equation:[21] insulin resistance (HOMA-IR) = fasting insulin (μU/mL) × fasting glucose (mmol/L)/22.5. HOMA-IR has been validated in comparison with the euglycaemic/hyperinsulinemic clamp technique in both diabetic and nondiabetic patients.[22] The VAI was calculated as previously described,[17] using the following gender-specific equations, where TG is triglyceride levels expressed in mmol/L and HDL is HDL-cholesterol levels expressed in mmol/L:

$$\text{Males: VAI} = \left(\frac{\text{WC}}{39.68 + (1.88 \times \text{BMI})}\right) \times \left(\frac{\text{TG}}{1.03}\right) \times \left(\frac{1.31}{\text{HDL}}\right)$$

$$\text{Female: VAI} = \left(\frac{\text{WC}}{36.58 + (1.89 \times \text{BMI})}\right) \times \left(\frac{\text{TG}}{0.81}\right) \times \left(\frac{1.52}{\text{HDL}}\right)$$

Serum TNFα (GE Healthcare Amersham TNF-α: Human, Biotrak Easy ELISA, Piscataway, USA), IL-6 (Human IL-6b ultrasensitive singleplex Bead Kit, Invitrogen Camarillo, California, USA), adiponectin (SPIbio – Bertin Pharma Human Adiponectin EIA Kit), and leptin (CAYMAN – EIA kit leptin human, Atlanta, Georgia) levels were measured in duplicate in a subgroup of patients.

Histology

Slides were coded and read by one pathologist (D.C.), who was unaware of the patient's identity and history. A minimum length of 15 mm of biopsy specimen or the presence of at least 10 complete portal tracts was required.[23] Steatosis was assessed as the percentage of hepatocytes containing fat droplets (minimum 5%), and evaluated as a continuous variable. The Kleiner classification[24] was used to compute the NAS score (from 0 to 8, on a scale including separate scores for steatosis, lobular inflammation, and hepatocellular ballooning), and to stage fibrosis from 0 to 4.

Statistics

Continuous variables were summarised as mean ± s.d., and categorical variables as frequency and percentage. The Student's *t*-test and Chi-squared test were used when appropriate. Multiple linear regression analysis was done to identify independent predictors of VAI as the continuous dependent variable. As candidate risk factors, we selected age, gender, BMI, WC, baseline ALT, platelet count levels, triglycerides, total and HDL cholesterol, blood glucose, insulin, HOMA score, adipocytokines (in patients for whom these data were available), diabetes, arterial hypertension, metabolic syndrome, steatosis, lobular inflammation, ballooning, NAS score, and fibrosis.

Multiple logistic regression models were used to assess the relationship of NAS score and fibrosis to the demographic, metabolic and histological characteristics of patients. In the first model, the dependent variable was NAS score, coded as 1 = NAS ≥5 vs. 0 = NAS <5. In the second model, the dependent variable was significant fibrosis coded as 1 = present (F2–F4) vs. 0 = absent (F0–F1). As candidate risk factors, we selected the same independent variables included in the linear model, and added the VAI score as an additional independent variable.

Variables associated with the dependent variable on univariate analysis (probability threshold, $P \leq 0.10$) were included in the multivariate regression models. To avoid the effect of colinearity, diabetes, IR, HOMA score, blood glucose levels and insulin levels, as well as waist circumference, BMI, HDL cholesterol, triglycerides, metabolic syndrome and VAI score, or the NAS score and its components, were not included in the same multivariate model. Regression analyses were done using Proc Logistic, Proc Reg and subroutine in SAS (SAS Institute, Inc., Cary, NC, USA).[25]

RESULTS

Patient characteristics and histology

The baseline characteristics of the 142 patients are shown in Table 1. The majority of our patients were in the overweight to obesity range, and nearly a quarter were hypertensive. Diabetes was present in about 15% of patients, and metabolic syndrome was diagnosed in 30% of patients. Using the Kleiner criteria (24), at liver biopsy 65% of NAFLD were classified as NASH, 9% as non-NASH, and 26% were indeterminate. One patient in three had steatosis of >66%, and half the number of patients had fibrosis ≥2.

In the 111 patients whose serum samples were available, and comparable to the entire population (76 male patients; mean age 45.6 ± 12.5 years; mean HOMA 4.35 ± 3.54; mean VAI 2.33 ± 1.50 mg/dL; 66% with

Table 1 | Baseline demographic, laboratory, metabolic and histological characteristics of 142 patients with non-alcoholic fatty liver disease

Variable	Non-alcoholic fatty liver disease (n = 142)
Mean Age – years	45.4 ± 13.0
Gender	
Male vs. female	95 (66.9)/47 (33.1)
Mean Body Mass Index – kg/m^2	30.1 ± 4.7
Body Mass Index – kg/m^2	
<25	16 (11.3)
25–29.9	65 (45.8)
≥30	61 (42.9)
Waist circumference (cm)	100.7 ± 13.1
Arterial hypertension	
Absent/present	112 (78.9)/30 (21.1)
Type 2 diabetes	
Absent/present	119 (83.8)/23 (16.2)
Metabolic syndrome	
Absent/present	98 (60.1)/44 (30.9)
Alanine aminotransferase – IU/L	77.5 ± 49.0
Platelet Count – 10^3 × mmc	220.4 ± 62.2
γ-glutamil transferase – IU/L	117.0 ± 229.8
Cholesterol – mg/dL	204.4 ± 47.6
HDL Cholesterol – mg/dL	48.1 ± 15.8
LDL Cholesterol – mg/dL	126.0 ± 39.8
Triglycerides – mg/dL	151.6 ± 73.9
Blood glucose – mg/dL	98.9 ± 28.8
Insulin – μU/mL	17.7 ± 10.5
HOMA	4.52 ± 3.77
VAI	2.41 ± 1.55
Leptin ng/mL*	15.35 ± 14.95
Adiponectin μg/mL*	5.47 ± 2.16
Tumour necrosis factor-α pg/mL*	7.20 ± 3.90
Interleukin-6 fg/mL*	2.44 ± 1.67
Histology at biopsy	
Steatosis as continuous variable (percent of total cells)	46.3 ± 25.4
Steatosis grade	
1 (5–33%)	50 (35.2)
2 (>33–66%)	45 (31.7)
3 (>66%)	47 (33.1)
Hepatocellular ballooning	
0	8 (5.6)
1	52 (36.6)
2	82 (57.8)
Lobular inflammation	
0	10 (7)
1	66 (46.5)
2	61 (43)
3	5(3.5)
NAFLD activity score (NAS)	
0–2	13 (9.1)
3–4	37 (26)
5–8	92 (64.9)
Stage of fibrosis	
0	32 (22.5)
1	39 (27.5)
2	34 (23.9)
3	29(20.5)
4	8 (5.6)

IU, international units; HOMA, homeostasis model assessment; HDL, high density lipoprotein; LDL, low density lipoprotein; VAI, visceral adiposity index.

Data are given as mean ± standard deviation or as number of case (%).

* Data are relative to 111 patients.

NAS ≥ 5; and 47% with fibrosis ≥2), serum levels of TNFα, IL-6, adiponectin and leptin were measured. The mean TNFα serum level was 7.2 ± 3.9 pg/mL (range 1–16.4), the mean IL-6 serum level was 2.44 ± 1.67 fg/mL (range 0.13–8.29), the mean adiponectin serum level was 5.47 ± 2.16 μg/mL (range 2–13.6), and the mean leptin serum level was 15.35 ± 14.95 ng/mL (range 0.2–73.2).

Factors associated with VAI score

The mean VAI was 2.41. Older age (P = 0.03), male gender (P = 0.03), high blood glucose (P = 0.01), high insulin (P < 0.001), high HOMA score (P < 0.001), presence of metabolic syndrome (P < 0.001), hepatocellular ballooning (P = 0.03), lobular inflammation (P = 0.001), NAS score (P = 0.001) and fibrosis (P < 0.001), were associated with higher VAI in NAFLD, although only higher HOMA (P = 0.04), and fibrosis (P = 0.04) were independent factors on multiple linear regression analysis (Table 2).

Table 2 | Univariate and multivariate analysis of factors associated with visceral adiposity index as continuous variable in 142 patients with non-alcoholic fatty liver disease

	Univariate Analysis			Multivariate Analysis		
Variable	B	S.E.	*P* value	*β*	S.E.	*P* value
Mean Age – years	0.178	0.010	0.03	0.041	0.010	0.63
Male Gender	0.173	0.274	0.03	0.071	0.274	0.39
Mean Body Mass Index – kg/m^2	0.183	0.027	0.02	–		
Waist Circumference (cm)	0.230	0.010	0.006	–		
Alanine Aminotransferase – IU/L	0.139	0.003	0.10	–		
γ-glutamyl transferase	0.071	0.001	0.39	–		
Cholesterol – mg/dL	0.370	0.003	<0.001	–		
HDL cholesterol – mg/dL	−0.486	0.007	<0.001	–		
LDL cholesterol – mg/dL	0.303	0.003	<0.001	–		
Triglycerides – mg/dL	0.896	0.001	<0.001	–		
Platelet count – $10^3 \times$ mmc	−0.101	0.002	0.37	–		
Blood glucose – mg/dL	0.209	0.004	0.01	–		
Insulin – μU/mL	0.344	0.012	<0.001	–		
HOMA	0.309	0.033	<0.001	0.134	0.037	0.04
Diabetes	0.118	0.352	0.16	–		
Arterial hypertension	0.137	0.317	0.10	–		
Metabolic syndrome	0.651	0.215	<0.001	–		
Histology at biopsy						
Steatosis as continuous variable	0.082	0.005	0.33	–		
Steatosis grade	0.061	0.158	0.47	–		
Hepatocellular ballooning	0.179	0.202	0.03	–		
Lobular inflammation	0.279	0.186	0.001	–		
NAFLD activity score (NAS)	0.206	0.084	0.001	0.047	0.091	0.60
Stage of fibrosis	0.337	0.103	<0.001	0.201	0.129	0.04

β, *β* coefficient; S.E., standard error of *β*; IU, international units; HOMA, homeostasis model assessment.

In the 111 patients assessed for adipocytokines, VAI was linked to older age ($P = 0.01$), high blood glucose ($P = 0.08$), high insulin ($P = 0.009$), high HOMA ($P = 0.002$), low adiponectin ($P = 0.002$), metabolic syndrome ($P < 0.001$), lobular inflammation ($P = 0.003$), NAS score ($P = 0.08$) and fibrosis ($P = <0.001$), although only lower adiponectin level (β −0.285; S.E. 0.062; $P = 0.002$) and fibrosis (β 0.240; S.E. 0.129; $P = 0.02$) were independent factors on multiple linear regression analysis. No significant associations were found between VAI and TNFα, IL-6 and leptin levels.

Characteristics of the entire population according to VAI quintiles are shown in Table S1. Figure 1 shows serum adiponectin distribution according to quintiles of VAI.

Factors associated with histological features

The univariate and multivariate comparisons of variables between patients with and without significant fibrosis (F2-F4) are reported in Table 3. Multivariate logistic regression analysis showed that the following features were independently linked to significant fibrosis (F2–F4): higher HOMA (OR 1.149, 95% CI 1.003–1.163, $P = 0.04$), higher VAI (OR 1.446, 95% CI 1.023–2.043, $P = 0.03$), hepatocellular ballooning (OR 2.884, 95% CI 1.231–6.757, $P = 0.01$) and lobular inflammation (OR 3.777, 95% CI 1.771–8.051, $P = 0.001$). Figure 2a shows the distribution of VAI according to significant fibrosis.

The ROC curve analysis identified a VAI of >2.10 (sensitivity 69%, specificity 70%; *AUC* 0.715) and a HOMA score of > 3.40 (sensitivity 68%, specificity 66%; *AUC*

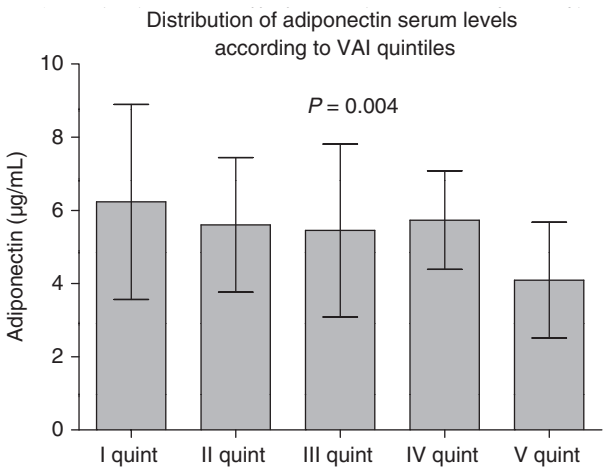

Figure 1 | Distribution of adiponectin serum levels according to VAI quintiles.

sis. It is noteworthy that by combining these two non-invasive classes of variables, the prevalence of significant fibrosis progressively increased from patients in the best class (VAI ≤ 2.1 and HOMA ≤ 3.4, significant fibrosis in 12/46, 26%), to those with only one positive predictor (VAI ≤ 2.1 and HOMA > 3.4, significant fibrosis in 10/26, 38%; VAI > 2.1 and HOMA ≤ 3.4, significant fibrosis in 11/24, 46%), and further to those in the worst class (VAI > 2.1 and HOMA > 3.4, significant fibrosis in 38/46, 83%) (Figure 2b). The positive predictive value of both VAI > 2.1 and HOMA > 3.4 for the diagnosis of significant fibrosis was 83%, whereas the negative predictive value of both VAI ≤ 2.1 and HOMA ≤ 3.4 for the exclusion of significant fibrosis was 74%.

Considering adipocytokine levels, no association was found between significant fibrosis and TNFα (7.03 ± 3.99 vs. 7.51 ± 4.02; $P = 0.52$), IL-6 (2.23 ± 1.83 vs. 2.69 ± 1.43; $P = 0.14$), adiponectin (5.80 ± 2.40 vs. 5.03 ± 1.69; $P = 0.06$) and leptin (12.81 ± 15.58 vs. 18.23 ± 13.80; $P = 0.06$) levels.

The univariate and multivariate comparisons of variables between patients with NAS score ≥5 and those with a NAS score of <5 are reported in Table S2. Multivariate logistic regression analysis showed that the following features were independently linked to a NAS score ≥5: higher ALT levels (OR 1.011, 95% CI 1.001–1.022, $P = 0.02$), and higher HOMA (OR 1.187, 95% CI 1.005–1.402, $P = 0.04$).

DISCUSSION

In a cohort of patients with a histological diagnosis of NAFLD, we found that VAI is an expression of adipose tissue dysfunction. In addition, we found that this index was associated not only with IR, but, together with IR, was also independently correlated with the severity of liver fibrosis.

Data in the literature suggest that VAI appears able to indirectly indicate both fat distribution and function in non-obese, healthy patients and in primary care patients.[17] Therefore, this index reflects other nonclassic cardiometabolic risk factors, such as altered production of adipocytokines/cytokines, increased lipolysis and plasma free fatty acids, which are not signified by BMI, WC, TG and HDL separately.[17]

In this study we confirmed the association between VAI and IR in NAFLD patients, previously reported also in healthy patients,[17] in primary care patients,[17] and in G1 CHC patients,[18] and have speculated on the ability of adipose tissue to directly participate in IR pathogenesis. This is, to the best of our knowledge, the first reported finding of a linear, independent association between a higher VAI and lower adiponectin levels. This data is of particular interest because this is the first study to evaluate the potential association between VAI and adipocytokine imbalance as an expression of adipose tissue dysfunction. Interestingly, we also confirmed the hypothesis that VAI is an expression not only of fat distribution, but also of adipose tissue dysfunction, demonstrated by the inverse relation between VAI and adiponectin levels observed in our NAFLD patients. In fact, it is well known that inflamed adipose tissue leads to different changes in the systemic adipocytokine environment, also characterised by a lower adiponectin production.[7]

The assessment of liver fibrosis is crucial for the prognostic evaluation of NAFLD patients, particularly in those without advanced disease, but at risk of developing cirrhosis, like those with significant fibrosis. In this line different studies evaluated factor directly or indirectly affecting liver fibrosis severity in NAFLD,[26] and other studies focused on the development of non-invasive tools to predict the stage of fibrosis in these patients.[27, 28] In particular it is possible to discriminate between direct or indirect serum markers, aimed at predicting fibrosis stage using clinical features and parameters measurable in serum,[27, 28] and methodologies derived from elaboration of parameters obtainable with the current liver imaging techniques [ultrasound, computed tomography (CT) scan, magnetic resonance] or to the innovative use of principles of physics like fibroscan.[27–29]

A novel finding of this study is that significant fibrosis is independently associated not only with IR and lobular

Table 3 | Univariate and multivariate analysis of risk factors associated with significant fibrosis (F2–F4) in 142 patients with non-alcoholic fatty liver disease, by logistic regression analysis

Variable	No significant fibrosis (F0–F1) *n* = 71	Significant fibrosis (F2–F4) *n* = 71	Univariate analysis *P* value	Multivariate analysis OR (95% CI)	*P* value
Age – years	41.7 ± 10.8	49.2 ± 14.0	0.001	1.017 (0.983–1.053)	0.33
Gender					
Male vs. Female	55/16	40/31	0.007	1.117 (0.448–2.787)	0.81
Body Mass Index – kg/m^2	28.9 ± 5.0	31.4 ± 4.1	0.002	–	
Waist circumference-cm	98.1 ± 13.2	103.4 ± 12.5	0.01	–	
Alanine Aminotransferase – IU/L	77.5 ± 48.5	77.5 ± 49.8	0.99	–	
Platelet count – 10^3 × mmc	223.8 ± 53.2	217.0 ± 70.2	0.52	–	
γ-glutamyl transferase – IU/L	127.2 ± 288.9	106.7 ± 150.8	0.59	–	
Cholesterol – mg/dL	205.6 ± 43.6	203.2 ± 51.6	0.75	–	
HDL cholesterol – mg/dL	52.1 ± 17.7	44.0 ± 12.5	0.002	–	
LDL cholesterol – mg/dL	126.6 ± 34.8	125.4 ± 44.5	0.86	–	
Triglycerides – mg/dL	134.9 ± 69.6	168.7 ± 74.5	0.006	–	
Blood glucose – mg/dL	94.1 ± 24.2	103.7 ± 32.2	0.04	–	
Insulin – μU/mL	13.7 ± 7.6	21.7 ± 11.4	<0.001	–	
HOMA	3.31 ± 2.90	5.74 ± 4.15	<0.001	1.149 (1.003–1.316)	0.04
Arterial hypertension					
Absent/present	60/11	52/19	0.10	–	
Type 2 diabetes					
Absent/present	65/6	54/17	0.01	–	
Metabolic syndrome					
Absent/present	59/12	39/32	<0.001	–	
VAI	1.86 ± 1.21	2.96 ± 1.66	<0.001	1.446 (1.023–2.043)	0.03
Histology at biopsy					
Steatosis as continuous variable	40.5 ± 27.0	52.1 ± 22.4	0.006	1.010 (0.992–1.029)	0.28
Steatosis grade					
1/2/3	31/21/18	18/24/29	0.01	–	
Hepatocellular ballooning					
0/1/2	8/33/30	0/29/52	<0.001	2.884 (1.231–6.757)	0.01
Lobular inflammation					
0/1/2/3	10/43/18/0	0/23/43/5	<0.001	3.777 (1.771–8.051)	0.001
NAFLD activity score (NAS)					
0–2/3–4/5–8	12/28/31	1/9/61	<0.001	–	

IU, international units; HOMA, homeostasis model assessment; HDL, high density lipoprotein; LDL, low density lipoprotein; VAI, visceral adiposity index.

Data are given as mean ± standard deviation or as number of case (%).

inflammation, two well-known risk factors for fibrosis in NAFLD,[4–6] but also with VAI. Other studies have found a direct association between visceral adipose tissue, evaluated by magnetic resonance, and severity of fibrosis.[11] Similarly, a recent Asian prospective study identified visceral obesity, evaluated by WC, as a predictor of fibrosis progression in NAFLD patients.[30] However, these studies did not correct for the presence of IR and its effect on

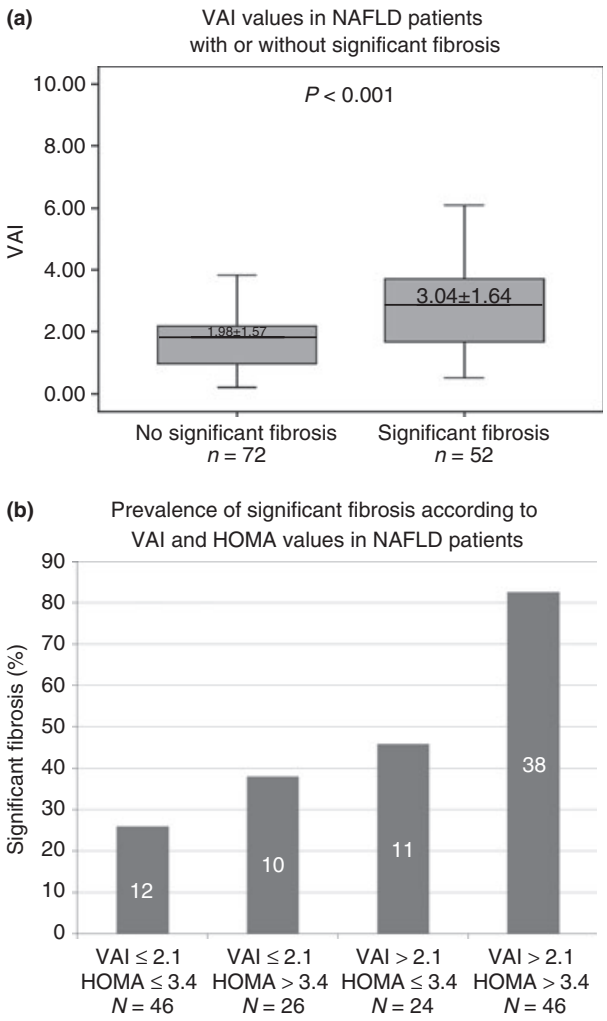

Figure 2 | (a) Distribution of VAI according to the presence or absence of significant fibrosis (F2–F4) in patients with non-alcoholic fatty liver disease. (b) Prevalence of significant fibrosis (F2–F4) according to specific patterns of predictors. VAI ≤ 2.1 and HOMA ≤ 3.4; VAI ≤ 2.1 and HOMA > 3.4; VAI > 2.1 and HOMA ≤ 3.4; VAI > 2.1 and HOMA > 3.4.

obesity. In addition, other studies have also found a link between severity of fibrosis in NAFLD and a cytokine imbalance, characterised by an increase in proinflammatory mediators and a decrease in anti-inflammatory mediators.[31–37] We have provided the first evidence in NAFLD of an independent link between liver fibrosis and an index of adipose dysfunction (VAI) that takes into account both quantitative and qualitative alterations of visceral adipose tissue, also correcting for IR. It is worth emphasising that in our study both IR and a high VAI score were independently associated with fibrosis, leading to a hypothesis on the ability of adipose tissue to interfere with fibrogenesis mechanisms not only through IR, but also through its well-known function as an endocrine organ able to modulate metabolic functions, including fibrogenesis.[7, 31]

From a clinical standpoint, a VAI at a threshold of 2.1, and HOMA at a threshold of 3.4, if combined, can identify patients at a high or a low risk of significant fibrosis. In particular, three groups of patients can be identified: one with no predictors and a rate of significant fibrosis of about 25%; a second, with at least one predictor and a rate of significant fibrosis that ranges from 38% to 45%; and a third, with all predictors, and a rate of significant fibrosis of more than 80%. These findings would seem to suggest using VAI and HOMA as a way of reducing the number of liver biopsies for staging NAFLD fibrosis, even if, clearly, furthermore studies must be done, to confirm our data in diverse patient populations and in larger cohorts of patients, before applying this method in clinical practice. In addition, it should be interesting to investigate the combination of these surrogate markers with liver elastography for a possible increase in diagnostic accuracy of the assessment of liver fibrosis. Our study might also suggest VAI as an indicator of adipose-related liver damage, though prospective studies evaluating VAI as predictor of liver disease progression, and as a new therapeutic outcome in the management of NAFLD patients, are needed.

We found no association among NAS score, expression of the activity of NAFLD and VAI, although we confirmed IR, a well-known risk factor for NASH,[4] as independently associated with a NAS score suggesting NASH diagnosis. Therefore, we posit an indirect role of VAI on liver NASH activity through reduction of insulin sensitivity.

The main limitation of this study lies in its cross-sectional nature, making it impossible to dissect the temporal relation among IR, VAI and fibrosis in NAFLD patients. A further methodological question is the potentially limited external validity of the results for different populations and settings. Our study included a cohort of Italian patients enrolled at a tertiary care centre, who may be different from the majority of cases of NAFLD in the general population. Lack of data on the serum levels of other adipocytokines, and on adipose expression of proinflammatory cytokines and adipocytokines may also have affected interpretation of the results.

In conclusion, we confirmed the hypothesis that VAI is a new index of both fat function and distribution, and observed an independent association between VAI and

significant fibrosis in NAFLD patients. These data need furthermore validation in independent, large scale studies, although they seem to suggest an evaluation of this score as a new therapeutic outcome in the management of NAFLD patients.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Characteristics of 142 patients with non-alcoholic fatty liver disease according to VAI quintiles.

Table S2. Univariate and multivariate analysis of factors associated with NAS ≥ 5 in 142 patients with non-alcoholic steatohepatitis, by logistic regression analysis.

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