Visceral Fat: A Key Mediator of Steatohepatitis in Metabolic Liver Disease

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Visceral obesity is intimately associated with metabolic disease and adverse health outcomes. However, a direct association between increasing amounts of visceral fat and end-organ inflammation and scarring has not been demonstrated. We examined the association between visceral fat and liver inflammation in patients with nonalcoholic fatty liver disease (NAFLD) to delineate the importance of visceral fat to progressive steatohepatitis and hence the inflammatory pathogenesis of the metabolic syndrome. We undertook a cross-sectional, proof of concept study in 38 consecutive adults with NAFLD at a tertiary liver clinic. All subjects had a complete physical examination, anthropometric assessment, and fasting blood tests on the day of liver biopsy. Abdominal fat volumes were assessed by magnetic resonance imaging within 2 weeks of liver biopsy. The extent of hepatic inflammation and fibrosis augmented incrementally with increases in visceral fat ($P < 0.01$). For each 1% increase in visceral fat, the odds ratio for increasing liver inflammation and fibrosis was 2.4 (confidence interval [CI]: 1.3-4.2) and 3.5 (CI: 1.7-7.1), respectively. Visceral fat remained an independent predictor of advanced steatohepatitis (odds ratio [OR] 2.1, CI: 1.1-4.2, $P = 0.05$) and fibrosis (OR 2.9, CI: 1.4-6.3, $P = 0.006$) even when controlled for insulin resistance and hepatic steatosis. Interleukin-6 (IL-6) levels, which correlated with visceral fat, also independently predicted increasing liver inflammation. Visceral fat was associated with all components of the metabolic syndrome. *Conclusion:* Visceral fat is directly associated with liver inflammation and fibrosis independent of insulin resistance and hepatic steatosis. Visceral fat should therefore be a central target for future interventions in nonalcoholic steatohepatitis and indeed all metabolic disease. (HEPATOLOGY 2008;48:449-457.)

The importance of body fat topography to metabolic disease was first recognized more than 60 years ago.[1] Subsequently, truncal obesity as measured by waist circumference and the waist–hip ratio has been the focal point of most definitions of the metabolic syndrome.[2,3] More recently, it has become apparent that it is the *visceral* component of the measured abdominal fat that is most intimately associated with metabolic disease and adverse outcomes.[4,5] Indirect evidence of the inflammatory output of visceral fat has been highlighted by the demonstration of increased levels of circulating cytokines and acute phase reactants in patients with visceral adiposity.[6,7] Similarly, there is evidence of a link between visceral fat and disease-related endpoints such as myocardial infarction, stroke, and overall mortality.[4,8] What is yet to be conclusively demonstrated is direct histological evidence of increasing tissue inflammation and of a *chronic* wound healing response (as evidenced by the development of fibrosis), in association with increasing amounts of visceral fat.

The liver provides a unique opportunity to examine an end organ that has a direct communication with visceral fat and that is amenable to biopsy for the quantitation of fat, inflammation, and scarring. Nonalcoholic fatty liver disease (NAFLD), the hepatic manifestation of the metabolic syn-

Abbreviations: CI, confidence interval; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; IL-6, interleukin-6; NAFLD; nonalcoholic fatty liver disease; OR, odds ratio; sTNFRII, soluble tumor necrosis factor receptor 2; TNF-α, tumor necrosis factor alpha.

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drome,[9] is defined by the presence of macrovesicular fat in more than 5% of hepatocytes, in the absence of significant alcohol use or other secondary causes of steatosis. Thus, these patients provide an ideal opportunity to examine the importance of visceral fat to progressive nonalcoholic steatohepatitis and hence the inflammatory pathogenesis of the metabolic syndrome. In this study, we accurately measured visceral and abdominal subcutaneous fat by magnetic resonance imaging in patients with NAFLD to characterize the association between visceral fat, inflammation, and the wound healing response. In addition, this provided us with the opportunity to explore the associations between anthropometric measures, insulin resistance, and adipokines, in relation to body fat topography.

Patients and Methods

Patients and Data Collection. We performed a cross-sectional, proof of concept study on 38 consecutive adults with biopsy-proven NAFLD recruited from a tertiary liver clinic. Patients were referred for the assessment of abnormal liver tests or hepatic steatosis detected by ultrasonography. In all patients, current and past daily alcohol intake was less than 40 g per week, confirmed by at least two physicians and close family members. All subjects had a normal serum albumin level, prothrombin time, and renal function. None of the patients was using thiazolidinediones. Secondary causes of steatohepatitis and other causes of liver disease were excluded by appropriate serological and biochemical tests. The study protocol was approved by the Human Ethics Committee of the Western Sydney Area Health Service, and written informed consent was obtained.

Pathology. Liver tissues were stained with hematoxylin-eosin, reticulin, and Gomori trichrome stains and scored by an experienced hepatopathologist (J.G.K.). All cases were scored using the method of Brunt et al.[10] Steatosis was graded from 1 to 3 (1 = 5%-33%; 2 = 34%-66%; 3 = 67%-100%), necroinflammatory activity from 0 to 3 and fibrosis stage from 0 to 4. Patients with both advanced necroinflammation (grades 2-3) and advanced fibrosis (stages 3-4) were grouped together for statistical analysis. A more precise liver fat percentage was determined by morphometric analysis of liver core tissue, stained using Gomori trichrome. Slides were examined and photographed using a Leica DMLB microscope with a Spot RT camera (Leica Microsystems, Wetzlar Germany). For each biopsy more than 30 images that covered the entire liver core at 40× power were obtained to quantitate fat. Images were then analyzed using ImageJ software (ImageJ; U.S. National Institutes of Health, Bethesda, MD[11]) and the quantity of fat determined as a percentage of the total liver core. Fat quantitated by this method has been shown to correlate highly with liver fat as determined by magnetic resonance spectroscopy and thus is reflective of larger volumes of liver tissue.[12]

Clinical and Laboratory Evaluation. A complete physical examination was performed on each subject. Anthropometric evaluation included measures of body mass index and central obesity (waist and hip circumferences and waist–hip ratio). On the morning of liver biopsy, venous blood samples were drawn after an overnight 12-hour fast to determine the levels of serum alanine aminotransferase, bilirubin, albumin, total cholesterol, triglycerides, glucose, insulin, adiponectin, leptin, interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), and soluble tumor necrosis factor receptor 2 (sTNFRII). Serum insulin was determined by a radioimmunoassay technique (Phadeseph Insulin RIA; Pharmacia and Upjohn Diagnostics AB, Uppsala, Sweden). Serum adiponectin levels were measured in duplicate by radioimmunoassay (Linco Research, St Charles, MO). Leptin, TNF-α, sTNFRII, and IL-6 were measured in duplicate using enzyme-linked immunosorbent assays (Diagnostic Systems Laboratories, Webster, TX and Quantikine ELISA; R&D Systems, Minneapolis, MN). All other biochemical tests were performed using a conventional automated analyzer within the Department of Clinical Chemistry at Westmead Hospital. Insulin resistance was calculated by the homeostasis model (HOMA-IR) using the following formula; HOMA-IR = fasting insulin (mU/L) × plasma glucose (mmol/L)/22.5.[13]

Quantitation of Abdominal Fat. Magnetic resonance examinations were performed using a Siemens Magnetom Vision 1.5T system (Siemens, Erlangen, Germany) on all patients within 2 weeks of liver biopsy. Nineteen transverse T1-weighted and T2-weighted images were acquired from L5/S1 upward with a slice thickness of 10 mm and inter-slice spacing of 2.5 mm. Visceral and subcutaneous abdominal fat volumes were quantified using a validated automated fitting routine (Hippo fat, Pisa, Italy).[14] Fat volumes were multiplied by a factor of 0.92[15] to calculate their mass in kilograms, and then divided by body weight in kilograms. This provided an accurate fat percentage for each patient and controlled for the effects of increasing body weight and obesity.

Statistical Analysis. Statistical analysis was carried out using SPSS version 15.0 (SPSS Inc., Chicago, IL). Results are reported as mean ± standard deviation. The strength of association between continuous variables was reported using Spearman rank correlations. Univariate analysis of variance was used to examine factors associated with increasing histology grades/stages because these were categorical variables with multiple end-points. Multiple ordinal regression analysis was carried out to determine

Table 1. Baseline Characteristics of Study Cohort

Characteristic	Patients (n = 38)
Steatosis	12 (32%)
Steatohepatitis	26 (68%)
Age (years)	51 (12)
Male gender	22 (58%)
BMI (kg/m2)	30 (4)
Overweight	17 (45%)
Obese	16 (42%)
Metabolic syndrome criteria* met	
Waist	26 (68%)
Blood pressure	16 (42%)
Triglycerides	25 (66%)
HDL	12 (32%)
Glucose	18 (47%)
Three or more	20 (53%)
Diabetic	9 (24%)
ALT (IU/L)	83 (43)
HOMA-IR	4.5 (2.3)
Insulin (IU/mL)	16 (8)
Visceral fat-%	2.6 (1.2)
Subcutaneous fat-%	6.1 (1.9)
Visceral/subcutaneous fat ratio	49 (34)
Total abdominal fat-%	8.7 (1.9)

Results are expressed as mean (SD) or frequency (percentage). All fat measures were expressed as a percentage of body weight.

Abbreviations: HOMA-IR, Homeostasis Model Assessment of Insulin Resistance; WHR, waist-hip ratio; BMI, body mass index.

Metabolic syndrome as defined by APTIII criteria; Waist > 102 cm in males or >88 cm in females, BP >130/85 or treatment, TG >1.69 mmol/L, HDL <1.0 mmol/L in males or <1.29 mmol/L in females, fasting glucose >6.1 mmol/L or treatment.

which factors that were significant on analysis of variance remained independent predictors when adjusted for clinically relevant confounders. Student *t* tests were used to compare means of continuous variables, and binary logistic regression was then used to compare multiple variables to see which remained significant.

Results

The baseline characteristics of the 38 patients studied are presented in Table 1. The mean age was 51 years, and most were male (58%) and either overweight or obese (87%). Nine patients had diabetes, and 53% met revised Adult Treatment Panel III (APTIII) criteria[3] for the metabolic syndrome. When expressed as a percentage of body weight, the mean values for visceral fat, subcutaneous fat, and total abdominal fat were 2.6 %, 6.1%, and 8.7%, respectively. The mean ratio of visceral to subcutaneous fat was 49%.

Table 3. Association Between Visceral Fat and Metabolic Syndrome (MetS)

	Visceral Fat-%	Hepatic Fat %
Number of Metabolic Syndrome† criteria		
0-1 (*n* = 7)	1.9 (1.0)	7.8 (9.6)
2-3 (*n* = 23)	2.4 (0.9)	9.2 (7.1)
4-5 (*n* = 8)	3.6 (1.4)	11.9 (5.5)
P values*	0.007	0.53
Metabolic Syndrome Diagnosis (≥3 criteria)		
No (*n* = 18)	2.1 (0.8)	8.5 (7.8)
Yes (*n* = 20)	2.9 (1.4)	10.3 (6.8)
P values*	0.04	0.42

All values expressed as mean (SD).

Abbreviation: Visceral fat-%, visceral fat as a percentage of body weight.

†Metabolic syndrome as defined by APTIII criteria; Waist > 102 cm in males or >88 cm in females, BP >130/85 or treatment, TG >1.69 mmol/L, HDL < 1.0 mmol/L in males or <1.29 mmol/L in females, fasting glucose >6.1 mmol/L or treatment.

**P* values for analysis of variance (ANOVA) or independent sample *t* tests.

The correlates of abdominal fat compartments in comparison with those for liver fat are presented in Table 2. Visceral fat was positively correlated with insulin resistance, serum triglycerides, and low levels of high-density lipoprotein (HDL) ($P < 0.05$ for all). Visceral fat also correlated significantly with IL-6 levels, but not with any of the other measured adipokines, including adiponectin, leptin, sTNFRII, and TNF-α. Increasing visceral fat was intimately related to increasing incidence of components of the metabolic syndrome as defined by APTIII criteria and was also significantly higher in those who met diagnostic criteria for the metabolic syndrome compared with those with two components or less (Table 3).

Liver fat had significant correlations with metabolic variables such as waist circumference, triglycerides, and

Table 2. Rank Correlations Between Abdominal Adipose Tissue Compartments, Liver Fat and Other Key Metabolic Variables

	HOMA-IR	BMI (kg/m2)	WHR	Adiponectin (μg/mL)	Leptin (ng/mL)	TNFRII (μg/mL)	TNF-α (pg/mL)	IL-6 (pg/mL)	Waist (cm)	TG (mmol)	HDL (mmol)	Glucose (mmol)	Hepatic Fat
Visceral fat	0.49**	0.28	0.60**	−0.21	−0.01	0.12	0.18	0.32*	0.40*	0.33*	−0.31*	0.31*	0.22
Subcutaneous fat	−0.15	0.25	−0.04	0.58**	0.68**	0 .18	0.18	0.33*	0.29	−0.40*	0.34*	0.01	−0.03
Visceral/subcutaneous fat ratio	0.41*	0.04	0.44**	−0.46**	−0.38*	−0.08	−0.04	−0.03	0.11	0.41*	−0.37*	0.19	0.17
Total abdominal fat	0.14	0.40*	0.35*	0.44**	0.59**	0.19	0.28	0.51**	0.51**	−0.12	0.15	0.22	0.03
Hepatic fat	0.25	0.29	0.35*	−0.15	0.22	0.13	0.13	0.32*	0.34*	0.12	−0.31*	0.31*	–

All values expressed as r correlation coefficient (*P*–value). **P* value < 0.05; ***P* value < 0.01.

All fat measures were expressed as a percentage of body weight.

Abbreviations: HOMA-IR, Homeostasis Model Assessment of Insulin Resistance; WHR; waist-hip ratio; BMI, body mass index; TNFRII, tumor necrosis factor receptor 2; TG, triglycerides; HDL, high-density lipoprotein.

Table 4. Comparison of Various Predictors for Severity of Liver Histology

	Visceral Fat %	Subcutaneous Fat %	Visceral/Subcutaneous Fat Ratio	Total Abdominal Fat %	HOMA-IR	WHR	IL-6 (pg/mL)
Diagnosis							
Steatosis (*n* = 12)	2.0 (0.9)	5.8 (2.3)	42 (25)	7.8 (1.9)	3.3 (1.8)	0.96 (0.1)	2.2 (1.0)
NASH (*n* = 26)	2.8 (1.2)	6.2 (1.8)	52 (37)	9.1 (1.8)	4.9 (2.3)	1.02 (0.1)	3.9 (2.5)
***P* values†**	0.03	0.57	0.32	0.06	0.03	0.03	0.03
Steatosis Grade							
1 (*n* = 16)	2.3 (1.2)	6.4 (2.3)	42.6 (26)	8.7 (2.4)	3.7 (2.7)	0.99 (0.1)	3.2 (2.6)
2 (*n* = 15)	2.8 (1.4)	6.0 (1.8)	56.2 (46)	8.7 (1.5)	5.1 (1.6)	1.01 (0.1)	3.3 (1.6)
3 (*n* = 7)	2.6 (0.7)	5.8 (1.2)	47.1 (15)	8.4 (1.3)	4.8 (2.6)	1.05 (0.1)	3.8 (3.0)
P* values	0.58	0.78	0.54	0.94	0.25	0.08	0.81
Necroinflammatory Grade							
0 (*n* = 12)	2.0 (1.0)	5.8 (2.3)	42 (25)	7.8 (1.9)	3.3 (1.8)	0.97 (0.1)	2.2 (1.0)
1 (*n* = 17)	2.5 (1.0)	6.3 (1.5)	43 (22)	8.8 (1.5)	4.7 (2.3)	1.02 (0.1)	3.4 (1.8)
2-3 (*n* = 9)	3.5 (1.4)	6.1 (2.3)	70 (53)	9.6 (2.2)	5.5 (2.4)	1.03 (0.1)	4.8 (3.4)
P* values	0.01	0.78	0.09	0.17	0.03	0.05	0.03
Fibrosis Stage							
0 (*n* = 11)	1.9 (0.8)	6.0 (2.5)	38 (24)	7.9 (2.1)	2.9 (1.4)	0.95 (0.1)	2.1 (0.9)
1 (*n* = 12)	2.3 (0.9)	6.2 (1.3)	39 (18)	8.5 (1.5)	4.5 (2.5)	1.01 (0.1)	3.0 (1.4)
2 (*n* = 6)	2.7 (0.7)	6.2 (1.7)	46 (18)	8.9 (1.4)	5.7 (2.0)	1.05 (0.1)	4.1 (2.2)
3-4 (*n* = 9)	3.7 (1.4)	5.9 (2.3)	77 (52)	9.6 (2.1)	5.5 (2.4)	1.04 (0.1)	4.8 (3.4)
P* values	0.002	0.98	0.03	0.23	0.03	0.01	0.04

All values expressed as mean (SD). All fat measures were expressed as a percentage of body weight. Abbreviations: HOMA-IR, Homeostasis Model Assessment of Insulin Resistance; WHR, waist-hip ratio.

†*P* values for independent variable t tests; **P* values for analysis of variance (ANOVA).

low HDL ($P < 0.05$), but the association was weaker than that of visceral fat, and there was no correlation between increasing liver fat with increasing number of either metabolic syndrome criteria or of metabolic syndrome diagnosis (Table 3). There was no significant correlation between the extent of hepatic fat and any of the measured fat compartments, including visceral fat. Subcutaneous fat had strong positive correlations with both leptin and adiponectin ($P < 0.001$ for both) and was also associated with increasing IL-6 ($P < 0.05$). Subcutaneous fat appeared metabolically protective because of its correlation with decreasing levels of triglycerides and increasing HDL ($P < 0.05$). The visceral/subcutaneous fat ratio had a moderate negative correlation with adiponectin and leptin but otherwise shared similar associations to visceral fat.

The relationship between the increasing hepatic steatosis, inflammation, and fibrosis and the distribution of adipose tissue and its correlates were analyzed using univariate (Table 4) and multivariate models (Table 5). The extent of liver inflammation ($P = 0.01$) and fibrosis ($P = 0.002$) increased significantly with increases in visceral fat (Table 4; Fig. 1). For every 1% increase in visceral fat, the odds ratio for increasing necroinflammatory grade was 2.4 (confidence interval [CI]: 1.25-4.53, unadjusted), whereas the association with increasing fibrosis was even stronger with an unadjusted OR of 3.5 (CI: 1.7-7.1, $P = 0.01$). Whereas visceral fat did not correlate with steatosis grade or with hepatic fat percentage as measured by morphometry, there was a strong correlation between increasing hepatic fat and increasing inflammatory grade and fibrosis stage ($P < 0.05$). The amount of subcutaneous fat and total abdominal fat were not predictive of increasing steatosis, inflammation, or fibrosis. Visceral/subcutaneous fat ratio had a weak association with increasing fibrosis stage alone (odds ratio [OR], 1.03; $P = 0.01$). Insulin resistance as measured by HOMA-IR significantly increased with increasing inflammatory grade (OR 1.3, CI: 1.0-1.8, $P = 0.03$) and fibrosis stage (OR 1.5, CI 1.1-2.0, $P = 0.05$), but not increasing hepatic steatosis. Increasing age was associated with increased fibrosis stage with an OR of 1.5 (CI 1.1-1.9, $P = 0.006$) for every additional 5 years of life. Metabolic syndrome diagnosis was associated with increasing fibrosis ($P = 0.05$), but not inflammation ($P = 0.09$) or steatosis. The waist–hip ratio was the best anthropomorphic predictor of worsening liver histology with significant but modest increases with increasing fibrosis ($P = 0.01$) and inflammation ($P = 0.05$), but not with hepatic steatosis ($P = 0.08$). In contrast to the other adipokines, which were not useful, increasing IL-6 was significantly associated with both increasing inflammation (OR 1.5; CI 1.1-2.1, $P = 0.01$) and fibrosis (OR 1.4; CI 1.0-2.1, $P = 0.05$).

To investigate whether visceral fat independently predicted increasing necroinflammation and fibrosis, multiple ordinal regression models were created using input variables of visceral fat, HOMA-IR, IL-6, age, metabolic

Table 5. Univariate and Multivariate Analysis of Visceral Fat as a Predictor of Increasing Grades of Fibrosis and Inflammation. Adjusted Models Were Analyzed by Ordinal Regression

	Unadjusted		Adjusted for HOMA-IR and Hepatic Fat		Best Fitting Model	
Factor	Odds Ratio (95% CI)	*P* Value	Odds Ratio (95% CI)	*P* Value	Odds Ratio (95% CI)	*P* Value
Inflammation						
Visceral fat (per 1% increase)	2.4 (1.3-4.2)	0.008	2.1 (1.1-4.2)	0.05	2.1 (1.1-4.1)	0.03
IL-6 (per 1pg/mL increase)	1.5 (1.1-2.1)	0.01	1.4 (0.9-1.9)	0.07	1.4 (1.0-2.0)	0.05
Hepatic fat (per 5% increase)	1.6 (1.0-2.5)	0.04	–	–	–	NS
HOMA-IR (per 1 unit increase)	1.3 (1.0-1.8)	0.03	–	–	–	NS
Metabolic syndrome†	1.0 (0.9-2.2)	0.09	–	NS	–	NS
Fibrosis						
Visceral fat (per 1% increase)	3.5 (1.7-7.1)	< 0.001	2.9 (1.4-6.3)	0.006	2.8 (1.3-6.0)	0.008
Hepatic fat (per 5% increase)	1.6 (1.1-2.5)	0.03	–	–	1.8 (1.1-2.9)	0.02
Age (per 5 year increase)	1.5 (1.1-1.9)	0.006	1.5 (1.1-2.0)	0.009	1.4 (1.0-2.0)	0.03
HOMA-IR (per 1 unit increase)	1.5 (1.1-2.0)	0.007	–	–	–	NS
IL-6 (per 1 pg/mL increase)	1.4 (1.0-2.1)	0.05	1.4 (1.0-2.0)	0.05	–	NS
Metabolic syndrome	1.2 (1.0-2.4)	0.05	–	NS	–	NS

Hepatic fat as measured by morphometry, visceral fat expressed as a percentage of body weight. Abbreviation: HOMA-IR, Homeostasis Model Assessment of Insulin Resistance. †Metabolic syndrome as defined by APTIII criteria; Waist > 102 cm in males or >88 cm in females, BP >130/85 or treatment, TG >1.69 mmol/L, HDL <1.0 mmol/L in males or <1.29 mmol/L in females, fasting glucose >6.1 mmol/L or treatment.

syndrome diagnosis, and liver fat by morphometry (Table 5). Factors such as the waist–hip ratio and the visceral/subcutaneous fat ratio were excluded because they are surrogate markers of the extent of visceral fat, but with substantially reduced predictive power. In the first model, all factors were adjusted for HOMA-IR and hepatic fat. This allowed us to determine whether visceral fat was simply a marker of hepatic steatosis and insulin resistance, or whether it had a direct and independent association with liver inflammation and fibrosis. We then created a best fitting model using backward stepwise elimination of variables. Visceral fat was the only factor that was a significant independent predictor of both increasing necroinflammation and fibrosis in all models with little change in effect size or confidence intervals. The effect of visceral fat was also independent of metabolic syndrome diagnosis, implying that it is the active mediator, rather than simply a marker of the condition. For every 1% increase in visceral fat there was an OR of 2.1 for increasing necroinflammatory grade (CI: 1.1-4.1, $P = 0.03$) and an OR of 2.8 for increasing fibrosis stage. (CI: 1.3-6.0, $P = 0.008$). IL-6 levels were independently associated with increasing inflammation (OR 1.4; CI 1.0-2.0, $P = 0.05$), but these effects were attenuated by insulin resistance and were not independent predictors of fibrosis. In contrast, both hepatic fat content and increasing age were associated with increasing fibrosis but not inflammation. Insulin resistance was not an independent predictor in either of the models.

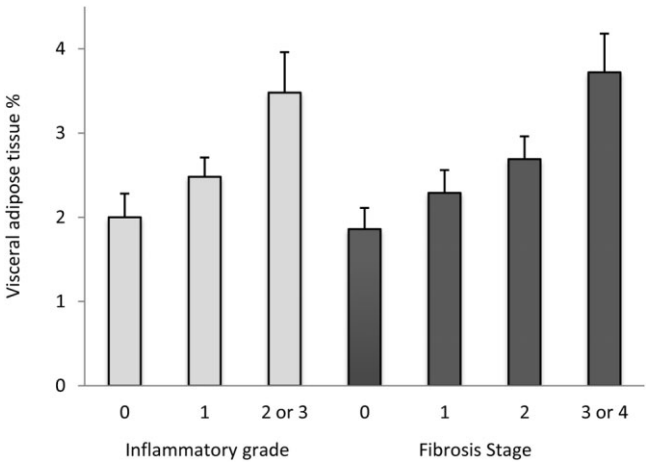

Fig. 1. Mean ± standard deviation values of visceral fat as a percentage of body weight for increasing grades of inflammation ($P = 0.01$) and fibrosis ($P = 0.002$).

To further investigate the ability of visceral fat to predict advanced steatohepatitis, we divided the cohort according to the presence of advanced necroinflammatory grade (I_{2-3}) and separately for the presence of advanced fibrosis stage (F_{3-4}). On univariate analysis, only increasing age and visceral fat were significant predictors of advanced inflammation and fibrosis, whereas the adipokines, hepatic fat content, metabolic syndrome, and other key metabolic variables were not significant. Visceral fat and patient age were analyzed in a multivariate model adjusted for HOMA-IR and hepatic fat, then a best fitting model determined by backward stepwise elimination of variables. Visceral fat was the only variable that independently predicted both advanced fibrosis and inflammation with odds ratios of 4.9 (CI: 1.5-16.7, $P = 0.009$) and 2.9 (CI: 1.1-7.6, $P = 0.03$), respectively, for

Table 6. Univariate and Multivariate Analysis of Visceral Fat as a Predictor of Advanced Inflammation and Fibrosis. Adjusted Models Were Analyzed by Binary Logistic Regression

	Unadjusted		Adjusted for HOMA-IR and Hepatic Fat		Best Fitting Model	
Factor	Odds Ratio (95% CI)	*P* Value	Odds Ratio (95% CI)	*P* Value	Odds Ratio (95% CI)	*P* Value
Advanced Inflammation (I2-3)						
Visceral fat (per 1% increase)	2.7 (1.1–6.6)	0.03	2.8 (1.01–7.6)	0.05	2.9 (1.1–7.6)	0.03
Age (per 5 year increase)	1.5 (0.98–2.2)	0.04	1.6 (1.0–2.6)	0.05	–	NS
Hepatic fat (per 5% increase)	1.5 (0.87–2.5)	0.15	–	–	–	NS
HOMA-IR (per 1 unit increase)	1.3 (0.93–1.8)	0.12	–	–	–	NS
Advanced Fibrosis (F3-4)						
Visceral fat (per 1% increase)	4.9 (1.5–16.7)	0.009	5.2 (1.4–18.6)	0.01	4.9 (1.5–16.7)	0.009
Age (per 5 year increase)	1.7 (1.06–2.8)	0.03	1.8 (1.1–3.0)	0.03	–	NS
Hepatic fat (per 5% increase)	1.2 (0.74–2.1)	0.41	–	–	–	NS
HOMA-IR (per 1 unit increase)	1.3 (0.93–1.8)	0.12	–	–	–	NS

Hepatic fat as measured by morphometry, visceral fat expressed as a percentage of body weight. Abbreviation: HOMA-IR, Homeostasis Model Assessment of Insulin Resistance.

each 1% increase in visceral fat as a fraction of body weight (Table 6).

Discussion

This study is the first to demonstrate a *direct* link between end-organ tissue inflammation and fibrosis with increasing amounts of visceral fat. Thus, in patients with NAFLD (the hepatic manifestation of the metabolic syndrome), liver necroinflammation and fibrosis increased significantly with visceral fat in a dose-dependent manner. Moreover, visceral fat remained an independent predictor of liver inflammation and fibrosis even when measures of insulin resistance, hepatic steatosis, adipokines, and increasing age were considered.

The exact mechanisms by which visceral fat exerts its damaging metabolic consequences remain controversial, but a number of mechanisms have been proposed. The portal/fatty acid flux theory suggests that visceral fat, via its unique location and enhanced lipolytic activity, releases toxic free fatty acids, which are delivered in high concentrations directly to the liver. This leads to the accumulation and storage of hepatic fat and the development of hepatic insulin resistance.[16,17] In addition, dysregulation and overflow of hepatic lipid is ultimately responsible for the formation of highly atherogenic small dense low-density lipoprotein particles, and a reduction in circulating HDL.[18] At a molecular level, hepatic steatosis may itself beget inflammation through altered lipid partitioning within the hepatocyte, mitochondrial dysregulation, generation of reactive oxygen species, lipid peroxidation, and endoplasmic reticulum stress.[18] It has been suggested that this process (hepatic steatosis) alone is sufficient to set off the local and systemic inflammation that is responsible for the pathophysiology of the metabolic syndrome. Our results, however, suggest otherwise. We have shown that the only independent predictor for both increasing inflammation and fibrosis in the steatotic liver is visceral fat. These results were highly significant and independent of insulin resistance and the extent of hepatic steatosis. Of note, increasing levels of visceral fat were not associated with increasing levels of hepatic steatosis in this population with preexisting NAFLD and a high incidence of NASH. A number of studies have shown that in unselected groups, visceral fat is a strong predictor of increasing steatosis,[19,20] suggesting that visceral fat is important in the genesis of fatty liver, but thereafter increases in steatosis may occur independently. Thus, our results suggest a direct toxic effect of visceral fat that cannot be accounted for simply by excess fatty acid flux, hepatic lipid deposition, and the cascade of harmful events thereafter.

Our data suggest that the toxic properties of visceral fat (in addition to being a source of free fatty acids draining to the liver) may be directly related to its ability to synthesize, modulate, and secrete cytokines and adipokines. Much recent evidence has shown that macrophages accumulate in adipose tissue of obese individuals[21] and that this process is exaggerated in visceral fat.[22] These macrophages are responsible for the production of pro-inflammatory cytokines and the modulation of adipocyte-derived cytokines. Harmful factors such as IL-6 and TNF-α have been shown to be expressed in greater amounts in visceral than subcutaneous fat.[17] Visceral fat also has been shown to be a significant predictor for biomarkers of inflammation such as high-sensitivity C-reactive protein, fibrinogen, and plasminogen activating inhibitor-1, independent of hepatic steatosis.[23] We were unable to show significant differences in TNF-α or

sTNFRII levels with increasing visceral fat or inflammation, consistent with previous studies[24] and perhaps reflecting a more important role for IL-6.[25] In our study, IL-6 levels significantly correlated with increasing visceral fat and were also independently predictive of increasing end organ inflammation. This agrees with other work in which IL-6 strongly correlated with visceral fat and was an independent predictor of early atherosclerosis and first myocardial infarction.[26]

Adipocyte-derived factors such as adiponectin, leptin, resistin, and TNF-α are differentially expressed in visceral compared with subcutaneous fat and play important roles in the actions of these fat compartments. Adiponectin is the most highly abundant adipokine in human serum and has insulin-sensitizing and anti-inflammatory effects.[27] It is known to be reduced in obese states, particularly in visceral compared with subcutaneous fat.[28] Previous work by our group[24] has demonstrated that low adiponectin levels were associated with increases in hepatic steatosis and inflammation, independent of insulin resistance. The importance of adiponectin to inflammation and visceral fat has been demonstrated in studies of the peroxisome proliferator-activated receptor gamma (PPAR-γ) agonist pioglitazone, in which treatment resulted in increased levels of adiponectin, reductions in liver inflammation,[29] and a shift of fat distribution from visceral to subcutaneous depots.[30] Furthermore, low levels of adiponectin have been associated with increased cardiovascular risk in both cross-sectional[31] and prospective[32] studies. In our current study, there was an inverse correlation between adiponectin levels and visceral fat and visceral/subcutaneous fat ratio, but no direct correlation between adiponectin and tissue inflammation or scarring, perhaps a limitation of the small cohort size. That leptin and the other adipokines exert many of their effects in a paracrine fashion may in part explain their lack of correlation with either visceral fat or liver histology. In contrast to visceral fat, our results suggest that abdominal subcutaneous fat is not associated with histological changes in the liver and may indeed have metabolically protective properties. These included positive correlations with adiponectin, leptin, and HDL, and a negative correlation with triglyceride levels. Much of this can be explained by the differential inflammatory profile and adipokine output of subcutaneous fat as discussed previously.[17,28] Interestingly, the extent of visceral fat was a far more significant predictor of adverse outcome than the visceral/subcutaneous fat ratio. This suggests that the protective properties of subcutaneous fat are modest and overcome by the increasing extent of visceral fat, irrespective of subcutaneous fat levels.

Much interest has focused on the importance of hepatic steatosis to the pathogenesis of the metabolic syndrome and increased cardiometabolic risk. When compared with controls, patients with NAFLD have been shown to have higher rates of coronary, cerebrovascular, and peripheral vascular disease.[33] Although the increased risk has been shown to be independent of classical cardiovascular risk factors, one large study in diabetics found the relationship attenuated when controlled for metabolic syndrome features.[34] In our analysis, visceral fat was associated with all components of the metabolic syndrome and as the burden of metabolic syndrome features increased in a given patient, visceral fat levels rose in a highly significant manner, whereas levels of liver fat did not. Importantly, visceral fat predicted advanced liver inflammation and fibrosis independent of a diagnosis of metabolic syndrome, confirming that visceral fat is indeed the active mediator, rather than a marker of the metabolic syndrome. In large population studies, markers of increased visceral fat have been shown to be independently predictive of first myocardial infarct,[35] major coronary events,[8] cardiovascular mortality,[36] and overall mortality.[4] Thus, the cardiometabolic risk that has been demonstrated in NAFLD may in large part be attributable to underlying visceral fat.

One criticism of this study relates to the small number of patients involved and the cross-sectional nature of the data. Although this allowed analysis of associations, it makes inference of cause and effect difficult. Despite these limitations, we were able to show a very strong independent association between visceral fat and liver injury, in line with the growing body of evidence that visceral obesity is an inflammatory disorder. Conversely, longitudinal studies with invasive liver biopsies have logistic and ethical constraints and are subject to numerous interactions that make the interpretation of any findings difficult. Certainly our findings need validation in larger studies, in particular to determine specific levels of visceral fat that best correlate with an increased risk of metabolic disease.

In conclusion, we have demonstrated that visceral fat is directly associated with liver inflammation and scarring in the metabolic syndrome. Importantly, this effect was independent of levels of hepatic steatosis, patient age, and insulin resistance. As a cornerstone of the metabolic syndrome, visceral fat has long been associated with adverse cardiovascular outcomes. We have now shown that it is a key element in the genesis of nonalcoholic steatohepatitis, and thus end-organ inflammation in the metabolic syndrome (Fig. 2). Visceral obesity is probably the most important target for future interventions in metabolic disease.

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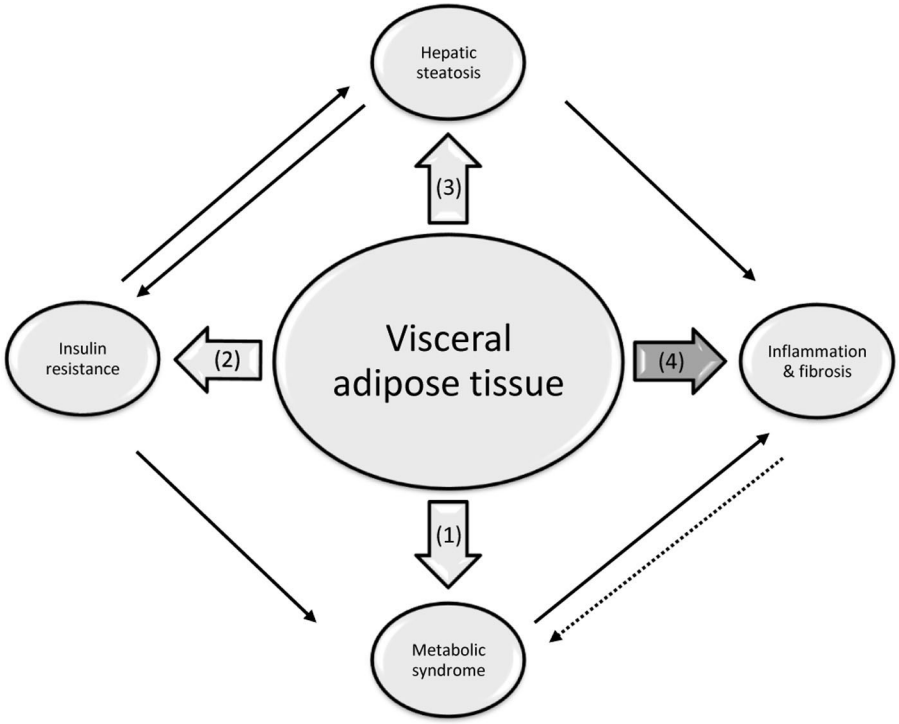

Fig. 2. The central role of visceral fat to disease. Published literature suggests that there is a close association between visceral fat and the metabolic syndrome (1), insulin resistance (2), and hepatic steatosis (3). We have demonstrated a close and direct association between visceral fat and tissue inflammation and fibrosis within the liver (4). Direct and incremental end-organ inflammation and scarring associated with increasing visceral fat likely represents an important pathophysiological basis for the association between visceral fat, atherosclerosis, glomerulosclerosis, and clinical endpoints such as myocardial infarction.

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