

Visceral Obesity Predicts Significant Fibrosis in Patients With Nonalcoholic Fatty Liver Disease

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Abstract: Nonalcoholic fatty liver disease (NAFLD) is associated with visceral obesity. However, the association between visceral adipose tissue (VAT) area and fibrosis in NAFLD patients has not been completely established. This study was aimed to determine the relationship between the computed tomography-measured VAT area and significant fibrosis in NAFLD patients. A total of 324 NAFLD patients and 132 controls were evaluated by liver biopsy. NAFLD was diagnosed based on histological examinations and alcohol consumption <20 g/day. The NAFLD patients showed a higher age and gender-adjusted VAT area than the control group (86.1 ± 2.3 vs 56.7 ± 3.7 , $P < 0.001$). The VAT area increased across the control, NAFLD without significant fibrosis, and NAFLD with significant fibrosis groups (54.9 ± 3.5 , 80.6 ± 2.4 , and 123.4 ± 6.4 , $P < 0.001$). This association persisted after adjusting for multiple confounders (P for trend = 0.028). A multivariate regression analysis demonstrated the VAT area was independently associated with NAFLD with significant fibrosis (F2–F4) (odds ratio [OR] 1.21 95% confidence interval [CI] 1.07–1.37 per 10 cm^2 increase of VAT area; OR 2.62 [per 1 – standard deviation (SD)] 95% CI 1.41–4.86). Moreover, a multivariate logistic regression analysis revealed the VAT area was independently associated with nonalcoholic steatohepatitis (NASH) in NAFLD (OR 1.17 95% CI 1.05–1.32 per 10 cm^2 increase of VAT area; OR 2.21 [per 1 – SD] 95% CI 1.25–3.89).

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Increased VAT area is independently associated with NASH or significant fibrosis and VAT might be a central target for lifestyle modifications in NAFLD patients.

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Abbreviations: ALT = alanine aminotransferase, BMI = body mass index, CI = confidence interval, CT = computed tomography, HDL = high-density lipoprotein, NAFLD = nonalcoholic fatty liver disease, NASH = nonalcoholic steatohepatitis, OR = odds ratio, SAT = subcutaneous adipose tissue, SD = standard deviation, VAT = visceral adipose tissue.

INTRODUCTION

During the past decade, obesity has become epidemic in industrialized countries and is increasingly common in developing countries worldwide. In parallel with the prevalence of obesity, nonalcoholic fatty liver disease (NAFLD) is now becoming one of major etiologies of chronic liver diseases.^{1–3} Several previous studies have demonstrated a potential role of NAFLD in the occurrence of cardiovascular disease and all-cause mortality.^{4,5}

The category of NAFLD includes simple steatosis, nonalcoholic steatohepatitis (NASH), advanced fibrosis, cirrhosis, and hepatocellular carcinoma.^{2,3,6} Simple steatosis patients usually have a good prognosis, whereas NASH patients may experience aggravation of fibrosis leading to liver cirrhosis and liver cirrhosis-associated problems as well as hepatocellular carcinoma.⁷ A recent United States population-based study reported that NAFLD patients with advanced fibrosis have a higher mortality risk, and mortality increases as fibrosis advances.⁵ Therefore, distinguishing between NAFLD with or without significant fibrosis is clinically important for determining the prognosis. Approximately 5% and 15% of patients with NAFLD and NASH, respectively, develop progressive fibrosis and, ultimately, cirrhosis.^{8,9} The progression of NASH and advanced fibrosis is associated with higher insulin resistance, high body mass index (BMI), and significant weight gain.⁹ These factors are closely related to visceral obesity. The serum concentrations of adipokines, derived from visceral fat and the proinflammatory cytokine interleukin-6 in overweight patients, are closely linked with insulin resistance and NAFLD progression.¹⁰

There has been increasing interest in recent years in the role of visceral adipose tissue (VAT) in NAFLD.¹¹ Studies have demonstrated that VAT, which was originally considered a passive depot for energy storage, is an active endocrine tissue that releases many peptides and hormones that regulate metabolism, inflammation, and immunity, thus participating in the pathogenesis of NAFLD.^{12,13} VAT was reported to be directly associated with hepatic fibrosis in a biopsy-based study with a

small sample.¹⁴ Using the waist circumference and visceral adiposity index as surrogate markers of abdominal obesity, major risk factors for advanced fibrosis have shown inconsistent results.^{11,15} The waist circumference could not sufficiently discriminate between visceral and subcutaneous fat compartments and showed stronger associations with subcutaneous adipose tissue (SAT) than with VAT.¹⁶ To overcome this problem, a visceral adiposity index using anthropometric (BMI and waist circumference) and metabolic (triglycerides and high-density lipoprotein [HDL] cholesterol) parameters was developed. However, this model has not been sufficiently validated. Moreover, in one study, the visceral adiposity index was shown to not be a stronger predictive factor than the waist circumference, reflecting the limitations of this formula.¹⁵ There have been 2 conflicting studies concerning visceral fat mass estimated indirectly by the visceral adiposity index. Their conflicting results reflect the complex relationship between visceral obesity and histological features in patients with NAFLD.^{11,15} The development of imaging technologies has facilitated remarkable advances in the direct measurement of the VAT area.¹⁷ In light of these facts, the association between the VAT area measured directly by computed tomography (CT) scanning and histological features in patients with NAFLD should be examined.

In this study, we aimed to demonstrate the relationship between the CT-measured VAT area and significant fibrosis and NASH in patients with NAFLD.

PATIENTS AND METHODS

Patients

We retrospectively enrolled 534 consecutive clinically suspected NAFLD patients who had elevated alanine aminotransferase levels in the absence of alcohol or other chronic liver disease or who had clinical features commonly associated with NAFLD, including obesity, type 2 diabetes mellitus, dyslipidemia, or metabolic syndrome.^{5,18} They underwent liver biopsy for the evaluation of elevated transaminase levels after exclusion of chronic viral hepatitis, excessive alcohol consumption, and other liver diseases, etc. at affiliated hospitals of Seoul National University Hospital, Seoul, Korea between July 2000 and August 2014. NAFLD was diagnosed based on typical histological findings.^{19,20} Normal controls who had no or minimal steatosis (<5%), no lobular/portal inflammation, and no significant fibrosis (less than portal expansion) were selected based on histological findings. CT scans were performed to exclude cirrhosis, hepatocellular carcinoma, and other liver diseases in NAFLD patients and to estimate liver volume in the living liver donors. Patients with the subsequent conditions were excluded: concurrent liver disease like chronic viral hepatitis (hepatitis B virus and hepatitis C virus using HBsAg, anti-HCV), biliary obstruction, drug-induced liver disease, primary biliary cirrhosis, autoimmune hepatitis, primary sclerosing cholangitis, hemochromatosis, Wilson's disease, or α 1-antitrypsin deficiency. Patients who drank alcohol more than 20 g/day were also excluded. Additionally, patients who had any of the following conditions were excluded: no available CT images; previous or current malignancy; an accompanying serious medical illness like active infection, congestive heart failure, chronic kidney disease, or hematological disease. Pediatric patients were excluded because the unsteady patterns of fibrosis were observed in children.²¹ The total number of enrolled subjects consisted of 324 NAFLD patients and 132 normal controls (Fig. 1). This study was

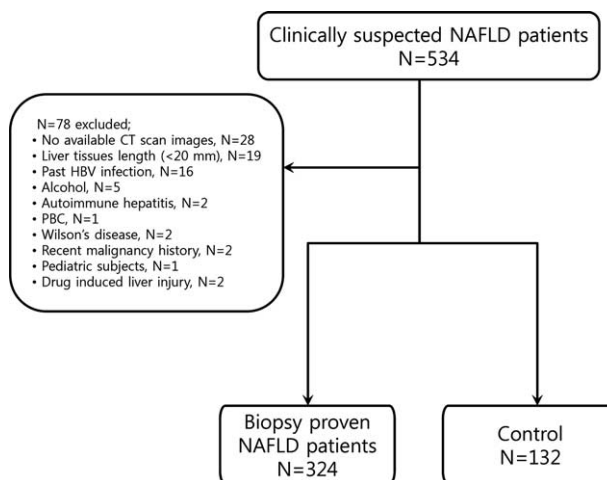


FIGURE 1. Flowchart of patient selection. After the exclusion of 78 patients who had any evidence of chronic liver disease or who met the exclusion criteria, among 534 patients, 456 patients (324 nonalcoholic fatty liver disease [NAFLD] vs 132 controls) were finally enrolled and analyzed in this study.

reviewed and approved by the Institutional Review Board of Seoul National University Hospital (IRB No H-1304-074-482).

Clinical and Laboratory Evaluation

A complete physical examination was performed on each subject. The anthropometric evaluation included measures of BMI and waist circumference. BMI was calculated by dividing the subject's weight (kg) by the square of the subject's height (m²). BMI cut-offs for overweight (≥ 23.0 kg/m²) and obesity (≥ 25.0 kg/m²) for Asians were adopted in this study.²² Waist circumference measurements were made at the WHO recommended site of the midpoint between the lower border of the rib cage and the iliac crest,²³ and the criteria for abdominal obesity in men and women were waist circumference ≥ 90 and ≥ 80 cm, respectively, according to the Regional Office for the Western Pacific Region of the World Health Organization (WPRO) Waist Circumference criteria based on the Adult Treatment Panel III–WPRO.²² After a 12-hour overnight fast, all of the biochemical tests, including serum alanine aminotransferase, aspartate aminotransferase, gamma-glutamyltransferase, albumin, total cholesterol, HDL cholesterol, triglycerides, platelet, and glucose, were performed using a conventional automated analyzer within the Department of Clinical Chemistry at Seoul National University Hospital during the admission for liver biopsy. Subjects with fasting plasma glucose levels ≥ 126 mg/dL and/or treatment with a hypoglycemic agent or insulin were defined as having diabetes mellitus. Subjects with a systolic blood pressure ≥ 140 mmHg or a diastolic blood pressure ≥ 90 mmHg and/or previous use of antihypertensive medication were defined as having hypertension. Subjects who smoked regularly during the previous 12 months were considered current smokers.

Measurement of Adipose Tissue Areas

We used a previously described method for the VAT area measurements on cross-sectional CT images.²³ In brief, CT exams were performed using a multidetector CT system (Somatom Sensation 16, Siemens AG, Erlangen, Germany; Brilliance 64, Philips Healthcare, Best, Netherlands) with the subject in

the supine position, within 1 month of liver biopsy. The skin defined the outer boundary for the SAT region, and abdominal muscle and bone were used to trace the inner-boundary.²⁴ The VAT area was measured with commercially available CT software (Rapidia 2.8; INFINTT, Seoul, Korea) that electronically determined the adipose tissue area by setting the attenuation values for a region of interest within a range of -250 to -50 Hounsfield units.⁴ The SAT areas were obtained using the following formula: SAT = total adipose tissue – VAT.

Liver Biopsy Specimen Examination

Hematoxylin and eosin (H&E) and Masson trichrome staining were carried out using formalin-fixed, paraffin-embedded liver biopsy specimens. The histological findings were examined by well-experienced 2 pathologists who were unaware of the clinical information. Liver tissue samples with a length of <20 mm or samples that contained <11 portal tracts were excluded.²⁵ The presence of more than 5% steatosis was defined as fatty liver.¹⁹ The fibrosis stage was estimated by the 5-point scale proposed by Brunt and modified by Kleiner et al.¹⁹ as follows: F0 = absence of fibrosis, F1 = portal/perisinusoidal fibrosis, F2 = perisinusoidal and portal/periportal fibrosis, F3 = septal or bridging fibrosis, and F4 = cirrhosis.²⁶ Significant fibrosis was defined as stages F2–F4. NASH was defined as a combination of varying amounts of steatosis, lobular inflammation, and hepatocellular ballooning features by the NASH Clinical Research Network Histologic Scoring System.¹⁹

Statistical Analysis

The continuous variables are presented as the mean \pm standard deviation (SD), and the categorical variables are presented as frequencies (percentages). To assess the differences between the 3 groups (control vs NAFLD without significant fibrosis vs NAFLD with significant fibrosis), an analysis of variance was used for continuous variables, and Pearson's Chi-square test was used for categorical factors. An analysis of covariance was used to compare the adjusted VAT area means between the groups. Age, gender, hypertension, diabetes, smoking, BMI, platelet count, SAT area, total cholesterol, HDL cholesterol, and triglycerides were included as confounders to statistically control for baseline differences in these variables. The VAT area was standardized to a mean of 0 and a SD of 1. The tests for the odds ratios (OR) and significance of the differences of VAT area were performed to estimate the association between VAT and the significant fibrosis and NASH in patients with NAFLD. A multivariate logistic regression analysis was used to identify the factors independently associated with NAFLD with significant fibrosis and NASH, which was included as the dependent variable. The covariate for the multivariate logistic regression analysis was selected as the potential confounding factor based on the significance in the univariate analysis and clinically important factor. All of the analyses were conducted using SPSS version 19.0 (SPSS Inc., Chicago, IL), and P values <0.05 were considered statistically significant.

RESULTS

Patient Characteristics

A total of 456 patients (299 males and 157 females) met the inclusion criteria for this study; the mean age was 34.8 ± 14.1 years, the mean BMI was 25.7 ± 6.0 , the mean VAT was

77.6 ± 50.7 cm², and the mean SAT was 144.8 ± 77.3 cm². A total of 132 patients had no evidence of histological NAFLD (control group), 277 patients had histologically diagnosed NAFLD without significant fibrosis (F0, F1), and 47 patients had histologically diagnosed NAFLD with significant fibrosis (F2–F4). The fibrosis stages were determined in all 456 patients; 395 patients were at F0, 14 at F1, 27 at F2, 6 at F3, and 14 at F4. NASH was diagnosed in 63 patients. There was no evidence of hepatic decompensation in the included patients. The characteristics of the study populations and the other histological parameters including steatosis, lobular inflammation, ballooning, and NAFLD Activity Score are summarized in Table 1. Among the overall group comparisons, statistically significant differences were observed in the mean age; BMI, and waist circumference according to the WHO guideline for Asian population;²² the mean serum levels of total cholesterol, HDL cholesterol, triglyceride, gamma-glutamyl-transferase, and platelet count; the mean adipose tissue area; and histological parameters. The prevalence of hypertension and diabetes was higher in the subjects with significant fibrosis than in the subjects without significant fibrosis.

Association of VAT area With NAFLD status

The VAT areas were significantly higher in patients with NAFLD than in those without NAFLD (89.4 ± 50.1 vs 48.4 ± 39.4 , $P < 0.001$, Figure 2A). After adjustment for age and gender, the VAT area increased in the NAFLD groups compared to the control (86.1 ± 2.3 vs 56.7 ± 3.7 , $P < 0.001$). The VAT areas were found to be markedly higher in the patients with established NASH compared with the patients with simple steatosis or those with normal biopsy (133.1 ± 46.1 vs 78.9 ± 45.1 and 48.4 ± 39.4 , respectively; $P < 0.001$ for all, Figure 2B). The patients with NAFLD with significant fibrosis had significantly higher VAT areas than those with NAFLD without significant fibrosis or the controls (145.1 ± 47.2 vs 80.0 ± 44.1 and 48.4 ± 39.4 , respectively, $P < 0.001$ for all, Figure 2C).

The VAT area remained significantly associated with the severity of NAFLD status after adjusting for age and gender. The patients with NAFLD with significant fibrosis had higher age and gender-adjusted VAT areas than the patients with NAFLD without significant fibrosis and the control group (123.4 ± 6.4 vs 80.6 ± 2.4 and 54.9 ± 3.5 , respectively, $P < 0.001$, Table 2). Similarly, the VAT area increased in NAFLD with significant fibrosis than NAFLD without significant fibrosis, and the control after adjusting for age, gender, BMI, diabetes, hypertension, platelet count, smoking status, SAT area, total cholesterol, HDL-cholesterol, and triglycerides (91.4 ± 5.7 , 76.5 ± 1.9 , and 72.9 ± 3.0 , $P = 0.028$, Table 2).

Association Between VAT Area and Significant Fibrosis and NASH in Patients With NAFLD by Multivariate Logistic Regression Analysis

To investigate whether visceral obesity is independently associated with significant fibrosis and NASH in patients with NAFLD, multivariate logistic regressions were analyzed using confounders. The multivariate logistic regression analysis demonstrated that the VAT area was independently associated with NAFLD with significant fibrosis (F2–F4) (OR 1.21, 95% confidence interval [CI] 1.07–1.37 per 10 cm² increase of VAT area; OR 2.56 [per 1 – SD] 95% CI 1.38–4.75) (Table 3). This association persisted after adjusting for SAT area (OR 2.62 [per 1 – SD] 95% CI 1.41–4.86).

TABLE 1. Characteristics of Study Participants*

| | Control (n = 132) | NAFLD Without Significant Fibrosis (n = 277) | NAFLD With Significant Fibrosis (n = 47) | P Value |
|--------------------------------------|----------------------|---|---|---------|
| Age, years | 29.9 ± 9.7 | 34.2 ± 12.6 | 52.4 ± 19.1 | <0.001 |
| Gender (male, %) | 92 (69.7) | 188 (67.9) | 19 (40.4) | 0.001 |
| BMI, kg/m ² | 22.26 ± 2.80 | 24.59 ± 3.56 | 28.69 ± 4.34 | <0.001 |
| BMI ≥25.0, kg/m ² , % | 21 (15.9) | 112 (40.4) | 36 (76.6) | |
| BMI 23.0–24.9, kg/m ² , % | 25 (18.9) | 75 (27.1) | 8 (17.0) | |
| BMI <23.0, kg/m ² , % | 86 (65.2) | 90 (32.5) | 3 (6.4) | |
| WC, cm | 73.88 ± 8.26 | 81.41 ± 8.95 | 95.59 ± 10.81 | <0.001 |
| ≥90 cm (male), ≥80 cm (female), % | 14 (10.6) | 91 (32.9) | 40 (85.1) | |
| Smoking, % | 40 (30.3) | 74 (26.7) | 9 (19.1) | 0.331 |
| Hypertension, % | 21 (15.9) | 60 (21.7) | 27 (57.4) | <0.001 |
| Diabetes, % | 20 (15.2) | 53 (19.1) | 24 (51.1) | <0.001 |
| SBP, mm Hg | 122.5 ± 13.3 | 123.8 ± 14.4 | 125.7 ± 15.6 | 0.405 |
| DBP, mm Hg | 73.6 ± 10.1 | 75.5 ± 11.6 | 74.9 ± 11.3 | 0.277 |
| Total cholesterol, mg/dL | 145.4 ± 36.7 | 163.0 ± 44.2 | 181.1 ± 43.5 | <0.001 |
| HDL cholesterol, mg/dL | 52.1 ± 12.3 | 48.8 ± 13.9 | 44.1 ± 10.6 | 0.002 |
| Triglycerides, mg/dL | 91.6 ± 53.0 | 120.1 ± 82.6 | 184.3 ± 172.3 | <0.001 |
| ALT, IU/L | 71.3 ± 74.0 | 72.7 ± 81.9 | 79.6 ± 11.1 | 0.822 |
| AST, IU/L | 61.0 ± 57.1 | 60.0 ± 65.9 | 63.4 ± 43.1 | 0.936 |
| GGT, IU/L | 47.8 ± 77.7 | 56.2 ± 119.1 | 128.2 ± 185.0 | <0.001 |
| Albumin, g/dL | 3.81 ± 0.50 | 4.11 ± 2.65 | 4.15 ± 0.50 | 0.366 |
| Platelet, ×10 ⁹ /L | 218.4 ± 66.8 | 241.0 ± 70.9 | 220.3 ± 75.2 | 0.005 |
| Glucose, mg/dL | 106.4 ± 21.9 | 108.4 ± 27.6 | 117.6 ± 34.4 | 0.055 |
| TAT area, cm ² | 145.8 ± 84.6 | 233.6 ± 96.5 | 370.9 ± 119.3 | <0.001 |
| VAT area, cm ² | 48.4 ± 39.4 | 80.0 ± 44.1 | 145.1 ± 47.2 | <0.001 |
| SAT area, cm ² | 97.3 ± 55.6 | 153.7 ± 67.8 | 225.7 ± 95.3 | <0.001 |
| Steatosis score | 0 | 2.00 ± 0.82 | 2.09 ± 0.81 | <0.001 |
| Lobular inflammation score | 0.81 ± 0.40 | 1.00 ± 0.00 | 1.18 ± 0.39 | 0.003 |
| Ballooning score | 0.38 ± 0.50 | 0.75 ± 0.45 | 1.12 ± 0.42 | <0.001 |
| NAFLD activity score | 1.19 ± 0.75 | 3.75 ± 1.13 | 4.39 ± 1.00 | <0.001 |

ALT = alanine aminotransferase, AST = aspartate aminotransferase, BMI = body mass index, DBP = diastolic blood pressure, GGT = gamma-glutamyltransferase, HDL = high-density lipoprotein, NAFLD = nonalcoholic fatty liver disease, SAT = subcutaneous adipose tissue, SBP = systolic blood pressure, TAT = total adipose tissue, VAT = visceral adipose tissue, WC = waist circumference.

* Mean ± standard deviation.

For every 10 cm² increase in the VAT area, there was an OR of 1.18 for NASH (95% CI 1.05–1.32, $P = 0.005$) after adjustment for confounders including age, gender, BMI, platelet count, smoking, hypertension, and diabetes (Table 4). After further adjusting SAT area, the OR for VAT area per 10 cm² increase was 1.17 (95% CI 1.05–1.32, $P = 0.006$). These data might suggest that the VAT area is an independent risk factor for the presence of significant fibrosis and NASH among patients with NAFLD.

DISCUSSION

The principal finding of this study is the independent association of the VAT area with significant fibrosis and NASH in NAFLD patients. The VAT area showed a statistically significant correlation with the severity of NAFLD status. Moreover, we demonstrated that VAT is an independent predictor for the presence of significant fibrosis and NASH in patients with NAFLD. To our knowledge, this is the first study demonstrating the association of CT-measured VAT area with significant fibrosis and NASH in NAFLD patients.

Recently, visceral fat was reported to be directly linked to the severity of liver inflammation and fibrosis in NAFLD,

independent of insulin resistance, and steatosis.¹⁴ Visceral fat was associated with advanced liver inflammation and fibrosis independently of a diagnosis of metabolic syndrome, confirming that visceral fat is the active mediator, rather than a marker, of metabolic syndrome.¹⁴ However, a limitation of this previous study was the small number of patients involved (only 38 adults with NAFLD). There have been 2 studies that have indirectly estimated visceral fat mass using the visceral adiposity index; however, these studies reported conflicting evidence regarding the complex relationship between visceral obesity and histological features in patients with NAFLD.^{11,15} These results reflect the limitations of the indirect measurement of visceral fat mass. Therefore, our study had several superiorities over previous studies, including the large number of patients with NAFLD (324 patients) and the direct measurements of visceral fat mass using CT scanning.

Adipose tissue secretes several bioactive substances known as adipocytokines,²⁷ including adiponectin,²⁸ leptin, resistin, plasminogen activator inhibitor 1, and tumor necrosis factor α . The exact pathophysiology by which visceral fat exerts its harmful metabolic effects remains controversial; however, several mechanisms have been suggested. The portal/fatty acid

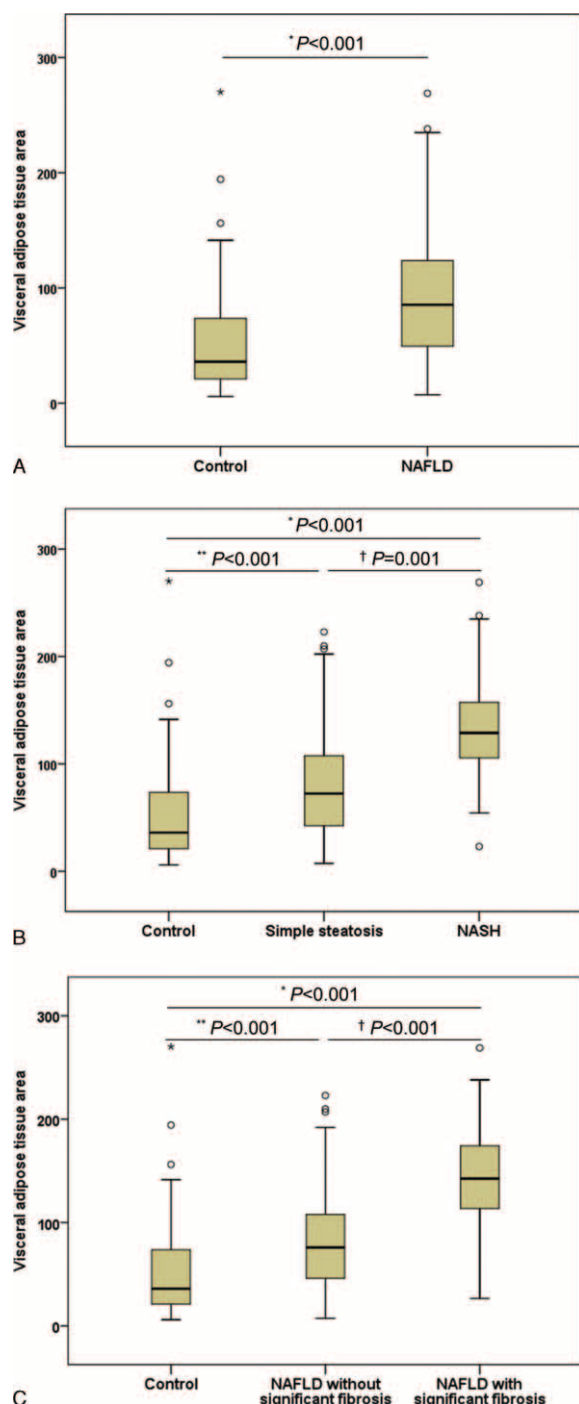


FIGURE 2. A close dependent increase in the VAT area was observed as the severity of NAFLD increased. (A) $*P < 0.001$ for control versus NAFLD; (B) $*P < 0.001$ for control versus NASH; $**P < 0.001$ for control versus simple steatosis; $†P = 0.001$ for simple steatosis versus NASH; and (C) $*P < 0.001$ for control versus NAFLD with significant fibrosis, $**P < 0.001$ for control versus NAFLD without significant fibrosis, $†P < 0.001$ for NAFLD without significant fibrosis versus NAFLD with significant fibrosis. The box plots demonstrate the interquartile range (box), median (thick line), range (thin lines), and outliers (circles) of the VAT area. NAFLD = nonalcoholic fatty liver disease, NASH = nonalcoholic steatohepatitis, VAT = visceral adipose tissue.

flux theory proposes that visceral fat, through its distinctive location and increased lipolytic activity, releases toxic free fatty acids, which are carried directly to the liver in high concentrations.¹⁴ These fatty acids lead to the development of hepatic insulin resistance through the accumulation and storage of hepatic fat.^{29,30} At the molecular level, hepatic steatosis might cause inflammation through altered lipid partitioning within hepatocytes, mitochondrial dysregulation, the generation of reactive oxygen species, lipid peroxidation, and endoplasmic reticulum stress.³¹

Although VAT is often evaluated using waist circumference measurements, as a surrogate marker of visceral adiposity, the waist circumference does not sufficiently discriminate between visceral and subcutaneous fat compartments.³² It was well known that patients with normal waist circumference may have NASH and are at risk of developing fibrosis.³³ VAT and SAT have different expression levels of adipokines.³⁴ VAT has higher expression of tumor necrosis factor α and interleukin 6,8.³⁵ Because of its insulin resistance-promoting adipokine profile, VAT might contribute more than SAT to the pathophysiology of NAFLD.³⁴ VAT was reported to be more important than SAT in the genesis of the NAFLD, and the abdominal SAT was inversely correlated with the occurrence of metabolic syndrome after adjustment for VAT.^{36,37} The development of imaging technologies such as CT and magnetic resonance imaging has facilitated remarkable advances in the field of direct measurement of the VAT area.¹⁷ Regarding the association between VAT and NAFLD, a clear positive relationship between the presence of NAFLD and increasing visceral fat has been shown.³⁸ Moreover, CT-measured visceral fat area was an independent risk factor for the presence of NASH in a small sampled study (only 30 patients with NASH and 30 control subjects).³⁹ Our study demonstrated the independent association between CT-measured VAT area and the presence of NASH using the sufficient number of patients with NASH and controls compared to the previous study.

In this study, we attempted to concentrate on NAFLD patients with significant fibrosis (F2–F4) and to separate these patients from other NAFLD patients. Because there is no established therapy for NASH other than lifestyle modifications, the diagnosis of significant fibrosis itself does not necessarily alter the therapy of the patients. The rationale for focusing on NAFLD patients with significant fibrosis (F2–F4) is that this method identifies candidates for secondary prevention and surveillance for hepatocellular carcinoma.⁴⁰

We acknowledge some limitations of this study. First, this study included only a small number of patients with significant fibrosis. However, similar to other Asian NAFLD studies, the prevalence of stage 0 to 1 fibrosis was higher than the prevalence of significant fibrosis (F2–F4).^{1,41,42} Second, because this study had a cross-sectional design, we could not evaluate the temporal cause and effect between VAT and significant fibrosis. Third, because our study was designed retrospectively, we could not take into account the relationship of insulin resistance and adipokines because the levels of insulin, adipokines, and other factors were not available. Fourth, we did not specifically measure deep SAT which was significantly increased in NASH patients.⁴³ However, although patients with NAFLD and significant fibrosis had higher SAT than those with NAFLD without significant fibrosis in univariate analysis, SAT was not an independent risk factor for significant fibrosis or NASH in multivariate analysis.

The CT-measured VAT area was found to be an independent risk factor for NAFLD with significant fibrosis or NASH in

TABLE 2. Visceral Adipose Tissue Area in Control, NAFLD Without Significant Fibrosis, and NAFLD With Significant Fibrosis (F2–F4) Groups

| | Control (n = 132) | NAFLD Without Significant Fibrosis (n = 277) | NAFLD With Significant Fibrosis (n = 47) | P Value |
|------------------------------------|----------------------|---|---|---------|
| VAT area, cm ² | 48.4 ± 39.4 | 80.0 ± 44.1 | 145.1 ± 47.2 | <0.001 |
| VAT area, adjusted age and gender | 54.9 ± 3.5 | 80.6 ± 2.4 | 123.4 ± 6.4 | <0.001 |
| VAT area, adjusted for the model 1 | 68.1 ± 3.1 | 78.6 ± 2.0 | 97.9 ± 5.7 | <0.001 |
| VAT area, adjusted for the model 2 | 72.0 ± 3.1 | 77.5 ± 1.9 | 93.7 ± 5.5 | 0.007 |
| VAT area, adjusted for the model 3 | 72.9 ± 3.0 | 76.5 ± 1.9 | 91.4 ± 5.7 | 0.028 |

Data are presented as mean ± standard deviation or standard error. The multivariate model 1 was adjusted for age, gender, body mass index, diabetes, hypertension, platelet count, and smoking. The multivariate model 2 was adjusted for subcutaneous adipose tissue area in addition to model 1. The multivariate model 3 was adjusted for total cholesterol, HDL cholesterol, and triglycerides in addition to model 2. NAFLD = nonalcoholic fatty liver disease, VAT = visceral adipose tissue.

TABLE 3. Univariate and Multivariate Odds Ratio of Risk Factors for NAFLD With Significant Fibrosis (F2–F4)

| | Univariate Model | | Multivariate Model 1 | | Multivariate Model 2 | |
|------------------------------|--|---------|----------------------|---------|----------------------|---------|
| | OR (95% CI) | P Value | OR (95% CI) | P Value | OR (95% CI) | P Value |
| Among NAFLD | Per 10 cm ² increase of VAT | | | | | |
| No fibrosis (F0, F1) | 1 | | 1 | | 1 | |
| Significant fibrosis (F2–F4) | 1.28 (1.17–1.41) | <0.001 | 1.21 (1.07–1.37) | 0.003 | 1.21 (1.07–1.37) | 0.002 |
| | Per 1 – SD of VAT | | | | | |
| No fibrosis (F0, F1) | 1 | | 1 | | 1 | |
| Significant fibrosis (F2–F4) | 3.44 (2.14–5.54) | <0.001 | 2.56 (1.38–4.75) | 0.003 | 2.62 (1.41–4.86) | 0.002 |

The multivariate model 1 was adjusted for age, gender, body mass index, platelet count, smoking, hypertension, and diabetes. The multivariate model 2 was adjusted for subcutaneous adipose tissue area in addition to model 1. CI = confidence interval, NAFLD = nonalcoholic fatty liver disease, OR = odds ratio, SD = standard deviation, VAT = visceral adipose tissue.

TABLE 4. Univariate and Multivariate Odds Ratio of Risk Factors for NASH

| | Univariate Model | | Multivariate Model 1 | | Multivariate Model 2 | |
|---------------|--|---------|----------------------|---------|----------------------|---------|
| | OR (95% CI) | P Value | OR (95% CI) | P Value | OR (95% CI) | P Value |
| Among NAFLD | Per 10 cm ² increase of VAT | | | | | |
| No NASH | 1 | | 1 | | 1 | |
| NASH (n = 63) | 1.22 (1.13–1.32) | <0.001 | 1.18 (1.05–1.32) | 0.005 | 1.17 (1.05–1.32) | 0.006 |
| | Per 1 – SD of VAT | | | | | |
| No NASH | 1 | | 1 | | 1 | |
| NASH | 2.69 (1.85–3.92) | <0.001 | 2.23 (1.27–3.89) | 0.005 | 2.21 (1.25–3.89) | 0.006 |

The multivariate model 1 was adjusted for age, gender, body mass index, platelet count, smoking, hypertension, and diabetes. The multivariate model 2 was adjusted for subcutaneous adipose tissue area in addition to model 1. CI = confidence interval, NAFLD = nonalcoholic fatty liver disease, NASH = nonalcoholic steatohepatitis, OR = odds ratio, SD = standard deviation, VAT = visceral adipose tissue.

NAFLD patients. These relationships were independent of other metabolic risk factors. Visceral obesity is probably the most important target for future interventions in NAFLD patients. A future larger-scale prospective study involving more patients with significant fibrosis is needed.

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