Research Article

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Sarcopenia is an independent risk factor for non-alcoholic steatohepatitis and significant fibrosis

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See Editorial, pages 8–10

Background & Aims: We explored whether sarcopenia is associated with the histological severity of non-alcoholic fatty liver disease (NAFLD), especially non-alcoholic steatohepatitis (NASH) and significant fibrosis.

Methods: In a biopsy-proven NAFLD cohort, the appendicular skeletal muscle mass (ASM) was measured. Sarcopenia was defined as a ASM/body weight (ASM%) value beyond two standard deviations below the mean for healthy young adults.

Results: Among the entire set of 309 subjects, the prevalence of sarcopenia in subjects without NAFLD, with non-alcoholic fatty liver (NAFL), and with NASH were 8.7%, 17.9%, and 35.0%, respectively (p <0.001). ASM% was inversely correlated with the severity of fibrosis (p <0.001), and the prevalence of significant fibrosis ($\geqslant$F2) was higher in subjects with sarcopenia than in those without (45.7% *vs.* 24.7%; p <0.001). A crude analysis revealed that sarcopenia was associated with NAFLD (odds ratio [OR], 3.82; 95% confidence interval [CI], 1.58–9.25), which became insignificant after adjustment for body mass index (BMI), diabetes, and hypertension. Among NAFLD subjects, subjects with sarcopenia were more likely to have NASH than those without sarcopenia through a multivariate analysis adjusted for age, gender, BMI, hypertension, diabetes, and smoking status (OR, 2.28; 95% CI, 1.21–4.30), and this finding was obtained even after adjustment for insulin resistance (OR, 2.30; 95% CI, 1.08–4.93). Sarcopenia was also associated with significant fibrosis independent of BMI and insulin resistance (OR, 2.05; 95% CI, 1.01–4.16).

Conclusions: In this large biopsy-proven NAFLD cohort, sarcopenia was significantly associated with NASH and significant fibrosis.

Lay summary: Low muscle mass was found to be associated with histological severity in non-alcoholic fatty liver disease, and sarcopenia was significantly associated with non-alcoholic steatohepatitis and significant fibrosis, independent of obesity, inflammation, and insulin resistance.

Clinical trial number: NCT 02206841.

Keywords: Hepatic steatosis; Non-alcoholic fatty liver disease; Non-alcoholic steatohepatitis; Appendicular skeletal muscle mass; Sarcopenia; Insulin resistance; Fibrosis; Body weight; Diabetes.

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Introduction

Non-alcoholic fatty liver disease (NAFLD), defined as hepatic steatosis that is not caused by significant alcohol consumption or other causes of liver disease, is currently the most prevalent liver disease worldwide [1]. Lipid accumulation and peroxidation and associated inflammation can induce hepatocellular damage and subsequent hepatic fibrosis [2], which results in non-alcoholic steatohepatitis (NASH) [1]. NAFLD may progress to NASH, advanced fibrosis, cirrhosis, or hepatocellular carcinoma [3,4]. Because patients with NASH or advanced fibrosis have higher rates of liver-related [5,6] and non-liver-related mortality [5–7] than those with non-alcoholic fatty liver (NAFL), early identification and intervention of this high-risk group may reduce the burden associated with these diseases.

Insulin resistance is one of the main pathophysiological mechanisms underlying the development of NAFLD [1,8]. Because the skeletal muscle is the primary tissue responsible for insulin-mediated glucose disposal [9–11], low skeletal muscle mass reduces insulin-mediated glucose disposal, independent of obesity, and might explain the association between NAFLD and insulin resistance, which cannot be explained by fat mass [12]. Recent epidemiological studies have shown that sarcopenia is also associated with NAFLD and advanced fibrosis based on the detection

of non-invasive markers in an Asian population [13–15]. These previous studies defined NAFLD using validated non-invasive serum panels [13,14] or liver attenuation indices as measured by computed tomography (CT) [15]. However, liver biopsy remains the gold standard for characterizing liver histology in subjects with NAFLD [1]. A considerable number of patients with significant steatosis on biopsy are not recognized by imaging [16], and non-invasive markers for the diagnosis of NAFLD may also result in the misclassification of NASH [17] or advanced fibrosis [18].

In this prospective cohort study, we aimed to determine the association between sarcopenia and the histological severity of NAFLD. Specifically, we investigated whether the presence of sarcopenia might be associated with the risk of NASH and significant fibrosis, independent of obesity, metabolic risk factors, and insulin resistance.

Patients and methods

Subjects and measurement of clinical parameters

We prospectively enrolled this cross-sectional cohort derived from the ongoing Boramae NAFLD registry (NCT 02206841). Subjects with radiologic evidence of hepatic steatosis were eligible for study inclusion from January 2013. The eligibility criteria for this study were as follows: (i) ⩾18 years old, (ii) bright echogenic liver on ultrasound scanning (increased liver/kidney echogenicity and posterior attenuation), and (iii) unexplained high alanine aminotransferase (ALT) levels above the reference range within the past 6 months [19]. The following exclusion criteria were used: (i) hepatitis B or C virus infection, (ii) autoimmune hepatitis, (iii) drug-induced liver injury or steatosis, (iv) Wilson disease or hemochromatosis, (v) excessive alcohol consumption (male >30 g/day, female >20 g/day) [1], and (vi) diagnosis of malignancy within the past year. Of the eligible study participants, those with at least two of the following risk factors underwent liver biopsy [20]: diabetes mellitus, central obesity (waist circumference ⩾90 cm for men or ⩾80 cm for women), a high level of triglyceride (⩾150 mg/dl), a low level of high-density lipoprotein (HDL)-cholesterol (<40 mg/dl for men or <50 mg/dl for women), presence of insulin resistance, hypertension, and clinically suspected NASH or fibrosis.

This study was conducted in accordance with the provisions of the Declaration of Helsinki for the participation of human subjects in research and was approved by the Institutional Review Board of Boramae Medical Center (IRB No. 16-2013-45). Written informed consent was obtained from each subject in the study cohort.

Anthropometric measurements were recorded by a well-trained examiner according to a consistent protocol. Weight was measured to the nearest 0.1 kg, height was measured to the nearest 0.1 cm, and body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). Waist circumference was measured at the end of normal expiration, measuring at the mid-point between the highest point of the iliac crest and the last floating rib to the nearest 0.1 cm [21]. Venous blood samples were drawn at the time of biopsy after a 12 h overnight fast, and plasma was separated immediately via centrifugation. The plasma glucose and lipid concentrations were measured enzymatically using the Hitachi Automatic Analyzer B2400 (Hitachi, Tokyo, Japan). Fasting insulin levels were measured using immunoradiometric assays (DIAsource ImmunoAssays, Nivelles, Belgium). Insulin resistance was evaluated using the homeostasis model assessment of insulin resistance (HOMA-IR), as described previously [22].

Diabetes mellitus was defined as fasting plasma glucose levels of ⩾126 mg/dl, HbA1c levels of ⩾6.5% and/or treatment with anti-diabetic medication at the time of the survey [23]. Hypertension was defined as systolic blood pressure ⩾140 mmHg, diastolic blood pressure ⩾90 mmHg and/or the current use of anti-hypertensive medication. Smokers were defined as those who had smoked at least one cigarette per day during the previous year.

Definition of sarcopenia

Bioelectrical impedance analysis (BIA) was performed using the InBody 330 body composition analyzer (InBody, Seoul, Korea), which provides impedance for each segment, including the four limbs and the trunk, by performing multi-frequency measurements to estimate the appendicular skeletal muscle mass (ASM) [24]. In this study, the ASM, calculated as the sum of the lean muscle mass in the bilateral upper and lower limbs, was divided by body weight (kg) and expressed as a percentage (ASM/weight, ASM%). This measurement was modified from the study of Janssen *et al.*, [25]. Sarcopenia (sarcopenia$_{_Wt}$) was defined as an ASM% beyond two standard deviations (SDs) below the gender-specific mean for healthy young adults according to nationwide health examinations of the Korean population (ASM% <29.0 in men or <22.9 in women was considered to indicate sarcopenia) [26–28]. For a sensitivity analysis, we adopted a different definition for sarcopenia (sarcopenia$_{_BMI}$) developed by the National Institutes of Health (NIH) Sarcopenia Project [29]. Using this ASM-to-BMI ratio (ASM/BMI), sarcopenia was defined as <0.789 in men or <0.512 in women.

Liver histology

Liver specimens were obtained using 16 G disposable needles, fixed in 4% formalin, and embedded in paraffin. Adequate specimens were required to be at least 20 mm in length, and sections (3 mm thick) were stained with hematoxylin-eosin and Masson's trichrome. Control liver tissues were collected from subjects who underwent liver biopsy in a pre-evaluation for donor liver transplantation or in a characterization of solid liver masses that were suspected to be hepatic adenoma or focal nodular hyperplasia based on radiological results without any evidence of hepatic steatosis.

All liver biopsies were assessed and reviewed by a single experienced liver pathologist (J.H.K.) [30]. NAFLD was defined as the presence of ⩾5% macrovesicular steatosis. NASH was diagnosed based on an overall pattern of histological hepatic injury consisting of macrovesicular steatosis, inflammation, or hepatocellular ballooning according to Brunt *et al.*'s criteria [30,31]. To determine the association between ASM and the severity of each histological feature, we graded steatosis, lobular inflammation, and hepatocellular ballooning according to the NAFLD activity score [32]. Fibrosis was assessed according to a 5-point scale proposed by Brunt and modified by Kleiner *et al.*: F0, absence of fibrosis; F1, perisinusoidal or periportal fibrosis; F2, perisinusoidal and portal/periportal fibrosis; F3, bridging fibrosis; and F4, cirrhosis [32]. Significant fibrosis was defined as F2–F4.

Measurements of liver stiffness

Transient elastography (TE) using FibroScan® (Echosens, Paris, France) provides the Young's modulus (kPa) for measuring liver stiffness, and the clinical usefulness of this measure was previously reported [33]. TE was performed after fasting for at least 2 h within 1 month of percutaneous liver biopsy. Liver stiffness was measured by a well-trained radiologic technician (with experience consisting of more than 1000 cases of TE) blinded to the clinical, laboratory, and histologic details of the subjects at the time of the procedure.

Statistical analysis

The statistical significance of differences between groups was evaluated using the independent *t* test, the Mann-Whitney *U* test, analysis of variance (ANOVA) or the Kruskal-Wallis test for continuous variables and the chi-square test for categorical variables. Post hoc analysis of the ANOVA results was performed using the Bonferroni method. Spearman's correlation analysis was performed to assess the relationship between ASM% and histological parameters, and the linear-by-linear association test was used to identify trends in histological severity according to sarcopenia status.

To investigate the independent determining factors for the presence of NASH or significant fibrosis, a binary logistic regression model adjusted for covariates was generated. Significance was defined as p <0.05. All statistical analyses were conducted using IBM SPSS Statistics software ver. 20.0 (IBM Inc., Armonk, NY, USA).

Results

Clinical characteristics according to the spectrum of NAFLD

Among the total of 309 subjects (mean age, 53 ± 14 years; men, 46.9%), 123 (men, 42.3%) and 117 subjects (men, 55.6%) were classified as biopsy-proven NASH and NAFL, respectively; thus,

Table 1. Characteristics of study participants according to NAFLD status.

	No NAFLD (n = 69)	NAFL (n = 117)	NASH (n = 123)	*p* value
Age, yr	52.0 ± 13.7	53.3 ± 12.9	53.4 ± 15.7	0.786
Male, N (%)	28 (40.6)	65 (55.6)	52 (42.3)	0.058
BMI, kg/m^2	24.40 ± 2.66	26.72 ± 3.06	28.10 ± 3.91	<0.001
WC, cm	85.85 ± 8.25	92.07 ± 7.69	95.87 ± 10.30	<0.001
ASM, kg	18.71 ± 4.77	20.37 ± 5.19	19.61 ± 5.46	0.109
ASM/BMI, m^2	0.770 ± 0.189	0.764 ± 0.182	0.700 ± 0.177	0.008
Sarcopenia$_{_BMI}$, N (%)	6 (8.7)	17 (14.5)	33 (26.8)	0.003
ASM%	28.81 ± 4.55	28.29 ± 3.91	26.59 ± 3.76	<0.001
Sarcopenia$_{_Wt}$, N (%)	6 (8.7)	21 (17.9)	43 (35.0)	<0.001
Smoking, N (%)	16 (23.2)	28 (23.9)	26 (21.1)	0.869
Hypertension, N (%)	21 (30.4)	39 (33.3)	58 (47.2)	0.029
Diabetes, N (%)	7 (10.1)	43 (36.8)	52 (42.3)	<0.001
SBP, mmHg	121.6 ± 13.7	125.3 ± 13.1	128.3 ± 17.7	0.014
DBP, mmHg	75.7 ± 92	78.3 ± 10.9	78.3 ± 12.1	0.241
Total cholesterol, mg/dl	186.4 ± 37.1	182.0 ± 42.1	187.8 ± 38.5	0.518
HDL cholesterol, mg/dl	57.1 ± 13.4	46.2 ± 12.8	45.3 ± 11.2	<0.001
Triglycerides, mg/dl	86.0 (71.5, 131.5)	142.0 (105.0, 202.0)	139.0 (101.0, 190.0)	<0.001
ALT, IU/L	19.0 (13.0, 34.0)	29.0 (21.5, 48.0)	69.0 (36.0, 113.0)	<0.001
AST, IU/L	23.0 (19.0, 32.5)	28.0 (22.0, 36.0)	58.0 (35.0, 80.0)	<0.001
GGT, IU/L	35.0 (14.0, 76.0)	31.0 (20.0, 54.0)	62.0 (36.0, 99.0)	<0.001
Albumin, g/dl	4.16 ± 0.33	4.21 ± 0.36	4.23 ± 0.28	0.286
Platelet, x10^9/L	229.7 ± 56.9	231.5 ± 65.9	220.4 ± 60.8	0.345
Glucose, mg/dl	99.0 (93.5, 110.0)	105.0 (96.0, 121.5)	108.0 (98.0, 127.0)	0.003
Insulin, μIU/ml	7.7 (6.5, 9.5)	11.0 (8.2, 13.4)	14.7 (10.9, 22.0)	<0.001
HOMA-IR	1.96 (1.53, 2.53)	2.74 (2.15, 4.02)	4.16 (2.89, 6.50)	<0.001
hsCRP	0.05 (0.03, 0.14)	0.09 (0.06, 0.17)	0.15 (0.08, 0.35)	<0.001

The data are expressed as the means ± standard deviations or medians (interquartile ranges).
NAFLD, non-alcoholic fatty liver disease; NAFL, non-alcoholic fatty liver; NASH, non-alcoholic steatohepatitis; BMI, body mass index; WC, waist circumference; ASM, appendicular skeletal muscle mass; ASM/BMI, ASM-to-BMI ratio; ASM%, appendicular skeletal muscle mass divided by body weight; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; HOMA-IR, homeostasis model assessment of insulin resistance; hsCRP, high sensitivity C-reactive protein.

51.3% of all NAFLD patients were diagnosed with NASH. The baseline characteristics of the study population are shown in Table 1. There were noticeable differences in clinical and anthropometric characteristics according to the spectrum of NAFLD: BMI, waist circumference, the prevalence of diabetes mellitus and hypertension, serum ALT, aspartate aminotransferase (AST), and gamma-glutamyl transferase (GGT) levels, and HOMA-IR displayed a linear correlation with the severity of NAFLD, with the direction indicating that NASH reflects a poorer health status (Table 1). As NAFLD severity increased, the high sensitivity C-reactive protein (hsCRP) level also increased significantly (p <0.001).

Although there was no significant difference in ASM according to NAFLD severity, significant differences in ASM/BMI and ASM% according to NAFLD severity were observed (p = 0.008 and p <0.001, respectively; Table 1). Subjects with NASH showed a significantly lower ASM% compared to those without NAFLD or those with NAFL (p = 0.001 and p = 0.004, respectively; Fig. 1A).

Clinical characteristics according to sarcopenia status

The prevalences of sarcopenia$_{_Wt}$ were 8.7%, 17.9% and 35.0% in the no NAFLD, NAFL, and NASH groups, respectively (p <0.001; Table 1 and Fig. 1B). This trend was consistent with the prevalence of sarcopenia using an alternative definition, sarcopenia$_{_BMI}$ (p = 0.003; Table 1 and Fig. 1C). As expected, subjects with sarcopenia were more metabolically unfavorable than those without sarcopenia. Subjects with sarcopenia$_{_Wt}$ showed a higher BMI, waist circumference, serum AST, GGT, and hsCRP levels, and HOMA-IR than those without sarcopenia (Table 2). The ASM% showed a significant inverse correlation with the hsCRP level (Spearman's ρ = −0.260, p <0.001; Supplementary Fig. 1).

Liver histology according to ASM

Subjects with sarcopenia$_{_Wt}$ had more severe histological grades of steatosis and hepatocellular ballooning (p for trend = 0.005 and <0.001, respectively) and a higher fibrosis stage (p for trend <0.001) than those without sarcopenia$_{_Wt}$ (Table 3). The prevalence of significant fibrosis (⩾F2) was significantly higher among those with sarcopenia (45.7%) than among those without sarcopenia (24.7%) (p <0.001). ASM% inversely correlated with the severity of lobular inflammation (Spearman's ρ = −0.147, p = 0.010), hepatocellular ballooning (Spearman's ρ = −0.163, p = 0.004), and fibrosis (Spearman's ρ = −0.223, p <0.001) but not steatosis (Spearman's ρ = −0.096, p = 0.093; Figs. 2A–D). Subjects with significant fibrosis (⩾F2) displayed a significantly lower ASM% than those without significant fibrosis (<F2) (p = 0.001; Fig. 2E).

To confirm the inverse association between fibrosis severity and ASM%, we further analysed the association between ASM%

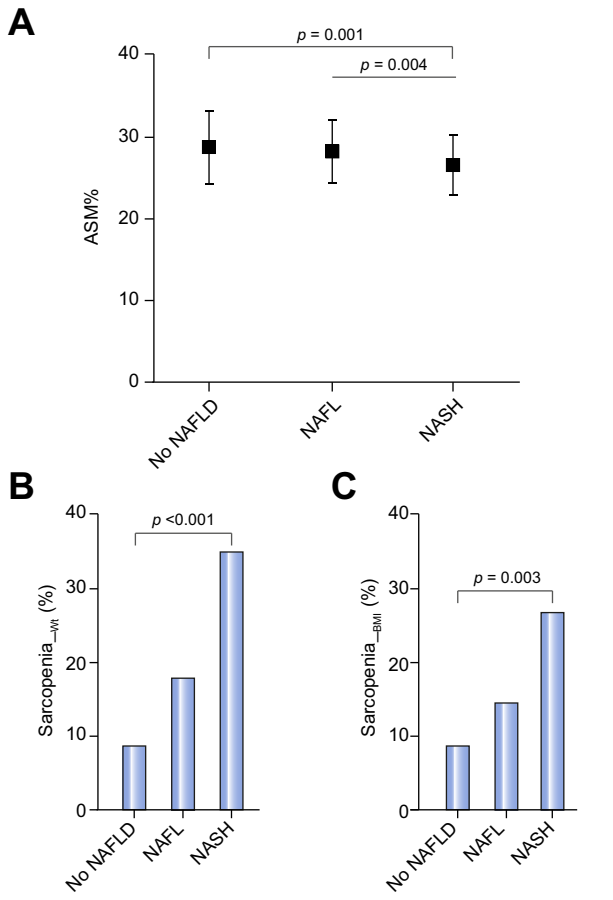

Fig. 1. Appendicular skeletal muscle mass relative to body weight (ASM%) and the prevalence of sarcopenia according to the histological classification of NAFLD. Mean ASM% significantly decreased as the histological severity of NAFLD increased (A). Subjects with NASH showed a significantly lower ASM% than subjects without NAFLD or with NAFL ($p = 0.001$ and $p = 0.004$, respectively from post hoc analysis [ANOVA; $p < 0.001$]). The prevalence of sarcopenia, defined according to a cut-off value obtained from Korean population data (B; sarcopenia_Wt) or from multi-ethnic population data (C; sarcopenia_BMI), increased as the histological severity of NAFLD increased. The bars represent standard deviations. ASM%, appendicular skeletal muscle mass/body weight; NAFLD, non-alcoholic fatty liver disease; NAFL, non-alcoholic fatty liver; NASH, non-alcoholic steatohepatitis.

and liver stiffness as assessed by TE. The sarcopenic index (ASM%) showed a significant inverse correlation with liver stiffness (Spearman's $\rho = -0.250$, $p = 0.002$; Supplementary Fig. 2).

Sarcopenia and NASH

As shown in Table 4, in the age- and gender-adjusted analysis, sarcopenia_Wt was significantly associated with NAFLD (odds ratio [OR], 3.81; 95% confidence interval [CI], 1.57–9.25). However, this trend was attenuated and remained non-significant after further adjustment. Given that subjects with NASH have a higher risk of progression to cirrhosis and of mortality [3], we investigated whether sarcopenia could have a crucial effect on NASH independent of NAFL. Among patients with NAFLD, the presence of sarcopenia_Wt was associated with a 2.5-fold increase in the risk of NASH (OR, 2.46; 95% CI, 1.35–4.48). This association persisted after adjustment for age, gender, BMI, hypertension, diabetes, and smoking status (Model 1). The addition of the total cholesterol, triglyceride, HDL-cholesterol, and ALT levels did not significantly reduce the OR for this association (Model 2; OR, 2.59; 95% CI, 1.22–5.48). Because inflammation was closely associated with the sarcopenic index, a multivariate analysis adjusted for the hsCRP level showed that the significant association was slightly attenuated but remained (Model 3; OR, 2.58; 95% CI, 1.21–5.48). As insulin resistance plays an important role in NASH, we performed an additional adjustment for HOMA-IR, and a similar statistically significant association was observed (Model 4; OR, 2.30; 95% CI, 1.08–4.93). We then conducted a sensitivity analysis to examine the robustness of our findings. Analysis using a different cut-off parameter (sarcopenia_BMI) also revealed a significant association: the ORs for NASH were 2.37 (95% CI, 1.21–4.64), 2.15 (95% CI, 1.08–4.30), and 2.66 (95% CI, 1.18–5.98) in the age- and gender-adjusted model, Model 1, and Model 2, respectively. After further adjustment for insulin resistance, this association was attenuated and remained significant (Model 4; OR, 2.33; 95% CI, 1.02–5.34).

Stratified analyses to assess the impact of diabetes or obesity showed that the presence of sarcopenia was associated with a higher prevalence of NASH, irrespective of the status of diabetes or obesity (Supplementary Figs. 3A- and B).

Sarcopenia and significant fibrosis

Among the patients with NAFLD, those with significant fibrosis were older, had a higher prevalence of female, diabetes, and hypertension, and displayed lower serum albumin levels and platelet counts than those without significant fibrosis (Supplementary Table 1). The presence of sarcopenia_Wt was associated with the presence of significant fibrosis (OR, 2.01; 95% CI, 1.12–3.61; Table 5). This association persisted after adjustment for age, gender, BMI, hypertension, diabetes, and smoking status (Model 1; OR, 2.12; 95% CI, 1.09–4.10) and after additional adjustment for total cholesterol, triglyceride, and HDL-cholesterol levels (Model 2; OR, 2.21; 95% CI, 1.10–4.44). The significant association between sarcopenia_Wt and significant fibrosis was maintained even after the addition of HOMA-IR to the analysis (Model 3; OR, 2.05; 95% CI, 1.01–4.16). In the sensitivity analysis using sarcopenia_BMI, sarcopenia_BMI was independently associated with an increased risk of significant fibrosis based on Model 1 (OR, 2.59; 95% CI, 1.28–5.23), Model 2 (OR, 2.48; 95% CI, 1.19–5.17), and Model 3 (OR, 2.24; 95% CI, 1.06–4.73; Table 5).

Analyses after stratification based on the status of diabetes or obesity showed that the presence of sarcopenia was consistently associated with significant fibrosis, irrespective of the presence or absence of diabetes or obesity (Supplementary Fig. 3C- and D).

Discussion

In this study, the prevalence of sarcopenia was associated with biopsy-proven NASH and significant fibrosis in subjects with NAFLD. These associations persisted after further adjustment for obesity, metabolic risk factors, and insulin resistance. Skeletal muscle mass was associated with not only the histological grades of steatosis and hepatocellular ballooning but also the stage of fibrosis. Patients with sarcopenia exhibited approximately two-fold increased odds of suffering from NASH or significant fibrosis.

Recent epidemiological studies have shown that low skeletal muscle mass is associated with NAFLD [13–15]. Advanced fibro-

Table 2. Characteristics of study participants according to sarcopenia status (sarcopenia_wt).

	Total (n = 309)	No sarcopenia (n = 239)	Sarcopenia (n = 70)	*p* value
Age, yr	53.1 ± 14.2	52.5 ± 13.2	54.9 ± 17.0	0.273
Male, N (%)	145 (46.9)	114 (47.7)	31 (44.3)	0.615
BMI, kg/m^2	26.75 ± 3.62	26.0 ± 3.0	30.2 ± 4.0	<0.001
WC, cm	92.19 ± 9.68	89.6 ± 7.5	101.6 ± 9.8	<0.001
Smoking, N (%)	70 (22.7)	55 (23.0)	15 (21.4)	0.781
Hypertension, N (%)	118 (38.2)	98 (41.0)	41 (58.6)	0.009
Diabetes, N (%)	102 (33.0)	74 (31.0)	28 (40.0)	0.157
SBP, mmHg	125.7 ± 15.4	125.0 ± 14.8	127.9 ± 17.4	0.166
DBP, mmHg	77.7 ± 11.1	77.8 ± 11.0	77.2 ± 11.5	0.691
Total cholesterol, mg/dl	185.3 ± 39.6	187.5 ± 40.7	178.6 ± 35.3	0.101
HDL cholesterol, mg/dl	48.3 ± 13.2	49.0 ± 13.1	45.6 ± 12.7	0.057
Triglycerides, mg/dl	130.0 (88.5, 184.0)	133.0 (86.5, 189.0)	121.0 (89.5, 172.5)	0.643
ALT, IU/L	35.0 (22.5, 67.5)	34.0 (20.5, 65.0)	41.0 (26.0, 84.5)	0.052
AST, IU/L	33.0 (23.0, 53.0)	32.0 (22.0, 52.5)	36.0 (27.5, 64.5)	0.005
GGT, IU/L	43.0 (22.0, 77.0)	41.0 (20.3, 74.8)	53.0 (26.0, 83.5)	0.032
Albumin, g/dl	4.21 ± 0.32	4.22 ± 0.31	4.17 ± 0.37	0.319
Platelet, x10^9/L	226.7 ± 62.0	227 ± 61	227 ± 67	0.942
Glucose, mg/dl	105.0 (96.0, 121.5)	105.0 (95.5, 105.0)	105.0 (96.0, 124.5)	0.543
Insulin, μIU/ml	11.1 (8.1, 15.9)	10.6 (7.7, 14.6)	14.9 (10.4, 21.6)	<0.001
HOMA-IR	3.00 (2.13, 4.42)	2.77 (2.03, 4.29)	3.80 (2.76, 6.30)	<0.001
hsCRP	0.10 (0.05, 0.24)	0.08 (0.05, 0.17)	0.23 (0.10, 0.41)	<0.001

The data are expressed as the means ± standard deviations or medians (interquartile ranges).
BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; HOMA-IR, homeostasis model assessment of insulin resistance; hsCRP, high sensitivity C-reactive protein.

Table 3. Pathological characteristics according to sarcopenia status (sarcopenia_wt).

	Total (n = 309)	No sarcopenia (n = 239)	Sarcopenia (n = 70)	*p* value*
Steatosis, N (%)				
<5%	74 (23.9)	66 (27.6)	8 (11.4)	0.005
5-33%	73 (23.6)	53 (22.2)	20 (28.6)	
34-66%	86 (27.8)	70 (29.3)	16 (22.9)	
>66%	76 (24.6)	50 (20.9)	26 (37.1)	
Lobular inflammation, N (%)				
0	59 (19.1)	50 (20.9)	9 (12.9)	0.147
1	219 (70.9)	167 (69.9)	52 (74.3)	
2-3	31 (10.0)	22 (9.2)	9 (12.9)	
Ballooning, N (%)				
0	110 (35.6)	97 (40.6)	13 (18.6)	<0.001
1	188 (60.8)	136 (56.9)	52 (74.3)	
2	11 (3.6)	6 (2.5)	5 (7.1)	
Fibrosis, N (%)				
0	83 (26.9)	71 (29.7)	12 (17.1)	<0.001
1	135 (43.7)	109 (45.6)	26 (37.1)	
2	47 (15.2)	32 (13.4)	15 (21.4)	
3	23 (7.4)	16 (6.7)	7 (10.0)	
4	21 (6.8)	11 (4.6)	10 (14.3)	

*p for trends determined through linear-by-linear association test.

sis, as estimated using non-invasive fibrosis markers, is also associated with low skeletal muscle mass, independent of obesity or metabolic control [13,14]. However, these aforementioned studies defined NAFLD using non-invasive markers [13,14] or liver attenuation indices as measured by CT [15]. Non-contrast CT exhibits high performance for qualitatively diagnosing steatosis of 30% or greater; however, the diagnostic performance of non-contrast CT is not clinically acceptable for quantitative assessment of steatosis [34]. The use of non-invasive markers for the diagnosis of NAFLD may also result in the misclassification of NASH [17] or advanced fibrosis [18]: a recent study reported that the majority of patients with advanced fibrosis or cirrhosis were misclassified using the FIB-4 score [18]. Currently, no clinical tools are available to reliably distinguish NASH from NAFL. Thus, liver biopsy remains necessary for accurate diagnosis of NASH and fibrosis [1]. A relatively small-sized study with NASH patients showed that a stepwise significant decrease in muscle area from the control group to the NASH group to the cirrhosis group [35]. We confirmed this finding with a larger sample size and showed an independent association between NASH or significant fibrosis and sarcopenia after adopting different sarcopenia criteria. In the current study, among the various histological parameters associated with NASH, steatosis and hepatocellular ballooning, but not lobular inflammation, were significantly associated with ASM%. This result was in line with the finding from a previous study, which found that hepatocellular ballooning was most closely associated with insulin resistance [36,37].

Even though the data regarding NAFLD and cardiovascular disease are still debated [38], several studies have shown that NAFLD with advanced fibrosis is a significant predictor of mortality from cardiovascular diseases [6,7], as well as of liver-related events [5]. Although several pharmacotherapies including vitamin E [39], obeticholic acid [39], and glucagon-like peptide-1 analogue [40] show promising results, no approved pharmacotherapies for NASH and advanced fibrosis are currently available. The current treatment of choice is lifestyle modification,

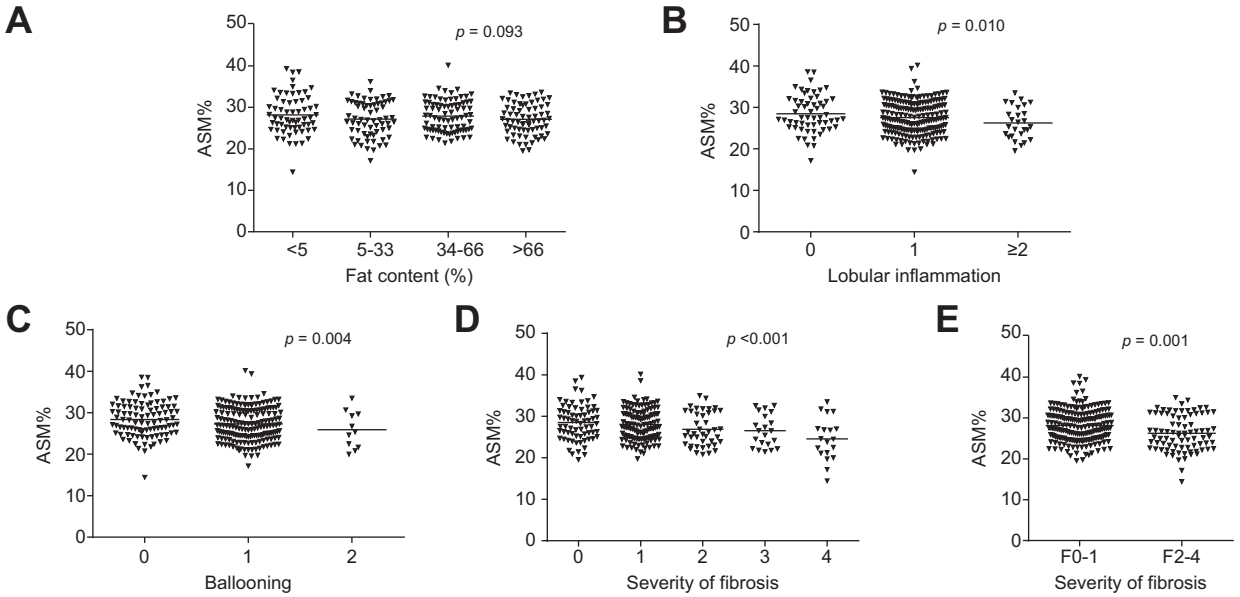

Fig. 2. Appendicular skeletal muscle mass relative to body weight (ASM%) according to histological grade (A, B, and C) and fibrosis stage (D and F). Bars represent mean values of ASM% for each histological category. ASM%, appendicular skeletal muscle mass/body weight.

Table 4. Univariate and multivariate analyses producing odds ratios of risk factors for NAFLD and NASH.

	Sarcopenia$_{-Wt}$		Sarcopenia$_{-BMI}$	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Total population (n = 309), OR for NAFLD				
Unadjusted	3.82 (1.58-9.25)	0.003	2.76 (1.13-6.75)	0.026
Age, gender adjusted	3.81 (1.57-9.25)	0.003	2.60 (1.05-6.43)	0.039
Multivariate model 1	1.78 (0.67-4.70)	0.244	1.49 (0.55-4.03)	0.428
Multivariate model 2	1.55 (0.52-4.58)	0.429	1.33 (0.45-3.97)	0.606
Multivariate model 3	1.51 (0.51-4.48)	0.459	1.33 (0.44-4.00)	0.610
Multivariate model 4	1.53 (0.50-4.65)	0.454	1.27 (0.41-3.95)	0.675
Among NAFLD (n = 240), OR for NASH				
Unadjusted	2.46 (1.35-4.48)	0.003	2.16 (1.13-4.14)	0.021
Age, gender adjusted	2.47 (1.35-4.53)	0.003	2.37 (1.21-4.64)	0.012
Multivariate model 1	2.28 (1.21-4.30)	0.011	2.15 (1.08-4.30)	0.028
Multivariate model 2	2.59 (1.22-5.48)	0.013	2.66 (1.18-5.98)	0.019
Multivariate model 3	2.58 (1.21-5.48)	0.014	2.58 (1.14-5.85)	0.023
Multivariate model 4	2.30 (1.08-4.93)	0.032	2.33 (1.02-5.34)	0.046

Sarcopenia (sarcopenia$_{-Wt}$) was defined as an ASM% beyond two standard deviations (SDs) below the gender-specific mean for healthy young adults according to nationwide health examinations of the Korean population (ASM% <29.0 in men or <22.9 in women was considered to indicate sarcopenia) [26–28]. For the sensitivity analysis, we adopted a different definition for sarcopenia (sarcopenia$_{-BMI}$) developed by the National Institutes of Health (NIH) Sarcopenia Project [29]; sarcopenia$_{-BMI}$ was defined as <0.789 in men or <0.512 in women.

Multivariate model 1 was adjusted for age, gender, body mass index, smoking, hypertension, and diabetes.

Multivariate model 2 was adjusted for the total cholesterol, triglyceride, HDL-cholesterol and ALT levels in addition to the factors included in model 1.

Multivariate model 3 was adjusted for hsCRP in addition to the factors included in model 2.

Multivariate model 4 was adjusted for HOMA-IR in addition to the factors included in model 2.

NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; OR, odds ratio; CI, confidence interval.

including weight reduction [1]. Increasing the skeletal muscle mass may be a promising potential treatment option for NAFLD [41–43]. Furthermore, resistance training is effective for reducing steatosis [41,43], independent of weight reduction, in NAFLD patients.

The importance of visceral obesity has been implicated in the pathogenesis and prognosis of NAFLD and NASH [44–46]. However, the finding that patients without visceral obesity may also develop NASH and significant fibrosis suggests that other mechanisms beyond visceral obesity might account for advanced liver damage [47]. The skeletal muscle is the primary tissue responsible for insulin-mediated glucose disposal, and the prominent role of skeletal muscle in insulin resistance has been confirmed by epidemiological [9,10] and experimental [11] studies. The finding that the association between NASH and sarcopenia was attenuated but remained significant after adjustment for HOMA-IR suggests that the association between sarcopenia and NASH might be mediated, in part, by insulin resistance rather than obesity.

Inflammation may also serve as an important link between sarcopenia and NASH. In the current study, ASM% showed a significant inverse correlation with the hsCRP level. Low muscle mass is closely associated with chronic inflammation [48]. Furthermore, subclinical inflammation and oxidative stress mediated by pro-inflammatory cytokines promote the catabolic stimulation of muscle [49], which might result in the loss of muscle mass [50,51]. Oxidative stress and chronic inflammation are also important in the development of NASH [2,52]. Our finding that the association between sarcopenia and NASH was slightly attenuated after adjustment for the hsCRP level indicates that inflammation might also partially serve as a mediator between sarcopenia and NASH.

It is acknowledged that a progressive decrease in muscle mass and an increase in fat mass, particularly that of the visceral component, are common body compositional changes associated

Table 5. Univariate and multivariate analyses producing odds ratio of risk factors for significant fibrosis (F2–F4) among the patients with NAFLD.

	Sarcopenia$_{-Wt}$		Sarcopenia$_{-BMI}$	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Unadjusted	2.01 (1.12-3.61)	0.019	2.86 (1.49-5.35)	0.001
Age, gender adjusted	2.05 (1.11-3.77)	0.022	2.49 (1.28-4.86)	0.007
Multivariate model 1	2.12 (1.09-4.10)	0.026	2.59 (1.28-5.23)	0.008
Multivariate model 2	2.21 (1.10-4.44)	0.026	2.48 (1.19-5.17)	0.016
Multivariate model 3	2.05 (1.01-4.16)	0.048	2.24 (1.06-4.73)	0.034

Sarcopenia (sarcopenia$_{-Wt}$) was defined as an ASM% beyond two standard deviations (SDs) below the gender-specific mean for healthy young adults according to nationwide health examinations of the Korean population (ASM% <29.0 in men or <22.9 in women was considered to indicate sarcopenia) [26–28]. For the sensitivity analysis, we adopted a different definition for sarcopenia (sarcopenia$_{-BMI}$) developed by the National Institutes of Health (NIH) Sarcopenia Project [29]; sarcopenia$_{-BMI}$ was defined as <0.789 in men or <0.512 in women.
Multivariate model 1 was adjusted for age, gender, body mass index, smoking, hypertension, and diabetes.
Multivariate model 2 was adjusted for the triglyceride, platelet, and albumin levels in addition to the factors included in model 1.
Multivariate model 3 was adjusted for HOMA-IR in addition to the factors included in model 2.
NAFLD, non-alcoholic fatty liver disease; OR, odds ratio; CI, confidence interval.

with aging [53,54]. In the elderly population, sarcopenia has been closely related to many clinical consequences, including metabolic impairment [9], increased cardiovascular risk [26], and mortality [55]. Furthermore, compared with sarcopenia in young adults, sarcopenia in the elderly population is more strongly associated with a susceptibility to diabetes [56]. Because sarcopenia is closely interconnected with diabetes and obesity, our study sheds light on the impact of sarcopenia on significant fibrosis and NASH independently of diabetes and obesity. Given that people are living longer in most developed nations, it is of paramount importance to manage age-related sarcopenia because we may expect an increase in the prevalence of NASH as well as of diabetes accompanying increased aging.

The first strength of this study was that we investigated the association between sarcopenia and the histological severity of NAFLD in a prospective cohort. The second strength is that the histological diagnoses of NASH and significant fibrosis were reviewed and established by a single pathologist who specialized in liver pathology. The third strength is the confirmation of the association between sarcopenia and the histological severity of NAFLD with adjustment for a variety of clinical confounders including insulin resistance and inflammatory markers. Furthermore, we successfully supported the association between the histological severity of fibrosis and ASM% through a measurement of liver stiffness.

The main limitation of this study is that the results regarding the causality of the observed relationships should be interpreted with caution due to the cross-sectional nature of the study design. Second, we estimated the skeletal muscle mass using BIA. Imaging using CT, magnetic resonance imaging and dual-energy X-ray absorptiometry (DXA) have been used for *in vivo* measurements of the skeletal muscle mass in humans. Although BIA has been reported to provide an accurate estimate of DXA-derived ASM in a population with various clinical disorders [24,57], there might be a difference between fat-free mass measured by BIA and muscle mass according to the body water content [58]. Furthermore, BIA cannot provide information on muscle quality. Third, HOMA-IR was used to determine insulin resistance in the current study. Although HOMA-IR is an indirect measure of insulin resistance, this index has been validated using the hyperinsulinemic-euglycemic clamp technique [59,60] and is thus currently used in various epidemiologic studies. Finally, because this study included only subjects of East Asian ethnicity, the conclusions might not be generalizable to other ethnic populations.

In conclusion, sarcopenia was associated with a roughly two-fold increased risk of NASH and significant fibrosis in NAFLD patients. In particular, sarcopenia was a risk factor for biopsy-proven NASH and significant fibrosis, independent of obesity and insulin resistance. Given the significant association between low skeletal muscle mass and both NASH and significant fibrosis, increasing muscle mass, especially ASM, might be a new central strategy for the prevention and management of NASH and significant fibrosis. Nevertheless, additional prospective longitudinal studies are still warranted to confirm our findings and to further elucidate the causal relationship between sarcopenia and the development of NASH.

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Conflict of interest

The authors declare that they do not have anything to disclose regarding any funding or conflict of interest with respect to this manuscript.

Authors' contributions

Sae Kyung Joo, Byeong Gwan Kim, Kook Lae Lee, and Won Kim contributed resources/designed research; Jung Ho Kim and Mee Soo Chang performed the experiments; Bo Kyung Koo, Donghee Kim, and Won Kim analysed the data; Bo Kyung Koo, Donghee Kim, and Won Kim wrote the manuscript; and Donghee Kim and Won Kim are the guarantors of this work and, as such, had full access to all of the study data and take responsibility for the integrity of the data and the accuracy of the data analyses.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhep.2016.08.019.

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Author names in bold designate shared co-first authorship.

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