Research Article

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

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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,