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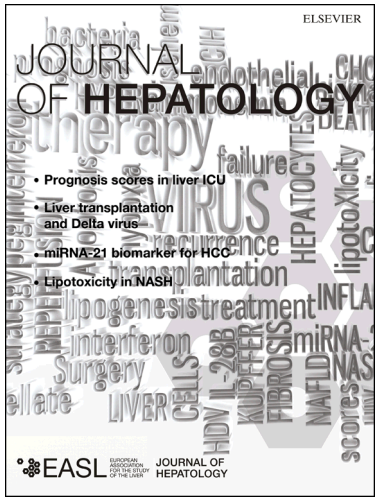

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Sarcopaenia is associated with NAFLD independently of obesity and insulin resistance: nationwide surveys (KNHANES 2008–2011)

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Abbreviations: NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; KNHANES, Korea National Health and Nutrition Examination Survey; ASM, appendicular skeletal muscle mass; SMI, skeletal muscle mass index; SD, standard deviation; BMI, body mass index; HSI, hepatic steatosis index; CNS, comprehensive NAFLD score; LFS, NAFLD liver fat score; HOMA-IR, homeostasis model assessment of insulin resistance; AST, aspartate transaminase; ALT, alanine transaminase; AOR, adjusted odds ratio.

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Authors' contributions

Study concept and design: Y.Lee, B.S.Cha.

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ABSTRACT

Background & Aims: Although sarcopaenia is associated with obesity-related comorbidities, its influence on non-alcoholic fatty liver disease (NAFLD) or steatohepatitis has not been fully determined. We aimed to investigate the direct relationship between sarcopaenia and NAFLD or steatohepatitis in the general population.

Methods: We conducted a cross-sectional study using nationally representative samples of 15,132 subjects from the Korea National Health and Nutrition Examination Surveys 2008–2011. Subjects were defined as having NAFLD when they had higher scores from previously validated NAFLD prediction models such as the hepatic steatosis index, comprehensive NAFLD score and NAFLD liver fat score. BARD and FIB-4 scores were used to define advanced fibrosis in subjects with NAFLD. The skeletal muscle index (SMI) [SMI(%)=total appendicular skeletal muscle mass (kg) / weight (kg)×100] measured by dual-energy X-ray absorptiometry was used to diagnose sarcopaenia with cut points of 32.2% for men and 25.5% for women.

Results: SMI was inversely correlated with all NAFLD predicting scores (Ps<0.001). After stratification, sarcopaenic subjects had an increased risk of NAFLD regardless of obesity (odds ratios [ORs]=1.55 to 3.02, depending on models; all Ps<0.001) or metabolic syndrome (ORs=1.63 to 4.00, all Ps<0.001). Multiple logistic regression analysis also demonstrated this independent association between sarcopaenia and NAFLD after adjusting for confounding factors related to obesity or insulin resistance (ORs=1.18 to 1.22, all Ps<0.001). Furthermore, among the NAFLD population, subjects with lower SMIs were likely to have advanced fibrosis compared with non-sarcopaenic individuals (BARD and FIB-4: ORs=1.83 and 1.69, respectively; both Ps<0.001). Compared with non-exercised subjects, individuals who

exercised regularly had a lower risk of NAFLD (P<0.001), particularly among obese people with well-preserved muscle mass.

Conclusions: Sarcopaenia is associated with increased risks of NAFLD and advanced fibrosis, independent of obesity or metabolic control.

Keywords: sarcopaenia, NAFLD, steatohepatitis, obesity

Introduction

Ageing is becoming a critical public health issue worldwide, particularly in developed societies, as the elderly population has been remarkably increasing. An annual report published by the World Health Organisation announced that an average of 22% of the population in high-income countries in 2011 were aged older than 60 years: 31% in Japan, the nation with the highest percentage, 20% overall in Europe and 19% in the United States [1]. Ageing gradually causes the decline in physical function and activity, which are linked to frailty, resulting in a substantial burden on the public health care system as well as impairment in the quality of life for individuals [2]. As one of the key components of frailty, sarcopaenia is regarded as a geriatric syndrome characterised by progressive and generalised loss of skeletal muscle mass and strength [3,4]. Recently, the concept of sarcopaenia is changing: it is now recognised not as an inevitable outcome of ageing, but as a disease condition which could be overcome [5]. Sarcopaenia is more prevalent among elderly people with obesity and exists with various comorbidities [5]. Several cardiometabolic disorders, including diabetes mellitus, metabolic syndrome, and cardiovascular disease, have been associated with sarcopaenia [3,6-8].

Non-alcoholic fatty liver disease (NAFLD) is one of the most common metabolic liver disorders with an estimated prevalence of ~30% in developed countries [9-11], and its incidence is expected to rise rapidly in the future as the rate of obesity increases, populations become aged, and sedentary lifestyles prevail. NAFLD and sarcopaenia share a similar aetiology such as insulin resistance, which may be ameliorated by the beneficial effect of exercise [12]. Among the categories of NAFLD, non-alcoholic steatohepatitis (NASH) shows a deteriorating nature of chronic liver disease and is associated with increased liver-related

mortality by elevating the risk of liver fibrosis, cirrhosis and hepatocellular carcinoma [13-15]. Advanced liver cirrhosis may induce skeletal muscle loss by reducing protein synthesis with increased protein breakdown in muscle [12].

A recent study reported a strong relationship between sarcopaenia and NAFLD, suggesting sarcopaenia as a new risk factor of NAFLD [16]. However, a substantial effect of obesity on NAFLD was not considered and adequately adjusted in this study, which assessed the relationship of NAFLD with sarcopaenic obesity rather than with sarcopaenia itself [17]. Considering the concept that insulin resistance and obesity are the common denominators between sarcopaenia and NAFLD, more robust investigation is necessary to determine the complex association of sarcopaenia with NAFLD. Therefore, we analysed data from the Korea National Health and Nutrition Examination Survey (KNHANES), which is a nationwide cross-sectional study with a nationally representative sample of Korean population annually conducted by the Korea Centre for Disease Control and Prevention to regularly assess the health and nutritional status of general civilians. The aim of this study was to investigate whether the association between sarcopaenia and NAFLD is independent of obesity or metabolic syndrome in the general population. We further evaluated the association between sarcopaenia and NASH in subjects with NAFLD.

Methods

Study participants

The KNHANES is a nationwide, population-based and cross-sectional health examination and survey regularly conducted by the Division of Chronic Disease Surveillance

of the Korea Centres for Disease Control and Prevention in the Ministry of Health and Welfare to monitor the general health and nutrition status of South Koreans, as previoulsy described in detail [18]. Similar to the National Health and Nutrition Examination Survey in the United States, each KNHANES is composed of independent datasets of participants from the general population of South Korea. All of the participants were randomly assigned from 600 randomly selected districts of cities and provinces in South Korea.

As depicted in Supplementary Fig. 1, of 37,753 participants from the KNHANES 2008–2011, we initially selected those aged ≥20 years (12,160 men and 15,911 women). Subjects with missing data for the appendicular skeletal muscle mass (ASM) were excluded (N=9,382). In addition, subjects who met the following criteria were excluded based on our protocol: (1) alcohol consumption >140 g/week for men and 70 g/week for women (N=2898); (2) positive serologic markers for hepatitis B (N=561) or hepatitis C virus (N=28); and (3) the presence of liver cirrhosis (N=42). This excluded population (N=3,557) was later used for a sensitive analysis. Finally, 15,132 participants (5,617 men and 9,515 women) were included in the analysis and were divided into four groups according to the presence of sarcopaenia and obesity. Written informed consent was secured from all of the participants before the study began, and the KNHANES was conducted following ethical approval by the Institutional Review Board of the Korea Centre for Disease Control and Prevention (No: 2008-04EXP-01-C, 2009-01CON-03-2C, 2010-02CON-21-C, 2011-02CON-06C).

Measurements of the appendicular skeletal muscle mass

As previously described [18], ASM was measured using Dual-Energy X-ray

Absorptiometry (DXA, QDR 4500A; Hologic Inc., Bedford, MA, USA). The skeletal muscle mass index (SMI) was calculated as follows: SMI (%) = total ASM (kg) / body weight (kg) × 100 [16], which was modified from the study of Janssen et al. [19]. This formula was applied based on previous evidence that placing the body weight in the denominator was the best method to minimise the effect of the strong correlation between ASM and body weight [20]. Sarcopaenia was defined as <1 standard deviation (SD) below the sex-specific average for a young reference population from the datasets of KNHANES 2008–2011 (960 men and 1240 women, aged 20–30 years) [6,20]. The cut-off points for sarcopaenia were 32.2% for men and 25.5% for women.

Measurements of clinical parameters and biochemical analysis

KNHANES examined participant demographics and personal and family medical history, including data on anthropometrics, smoking history, physical activity and reproductive health (e.g., early menopause and history of oestrogen replacement therapy) from standardised health questionnaires. Smoking status was categorised as never, ex- and current smoker by self-reporting. Regular exercise was defined as engaging in vigorous exercise on a regular basis (≥20 min at a time and at least three times per week) [18]. Subjects were considered as obese when the body mass index (BMI) was ≥25 kg/m^2 based on the criteria of the Asian-Pacific region [21]. Diabetes mellitus was defined based on (1) using insulin or oral hypoglycaemic agents or (2) fasting plasma glucose ≥126 mg/dl. Impaired fasting glucose was defined as a fasting plasma glucose level of 100–125 mg/dl [22]. Participants were diagnosed as hypertensive if the systolic pressure was ≥140 mmHg, if the

diastolic pressure was ≥90 mmHg, or if current antihypertensive medication was used.

After overnight fasting for at least 8 h, blood specimens collected from each subject were processed and transported in cold storage to the Central Testing Institute (Neodin Medical Institute, Seoul, Korea). All of the blood samples were analysed within 24 h after transportation. Serum 25-hydroxyvitamin D (25[OH]D) concentration was determined by radioimmunoassay (DiaSorin Inc., Stillwater, MN, USA) using a gamma counter (1470 Wizard; PerkinElmer, Turku, Finland). The serum levels of creatinine and the lipid and liver enzyme profiles were determined using a Hitachi 7600 automated chemistry analyser (Hitachi, Tokyo, Japan) using specific indicated methods. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula. The estimated glomerular filtration rate (GFR) was derived from the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [23].

Definition of hepatic steatosis and advanced fibrosis

NAFLD was defined using previous validated fatty liver prediction models [10,24,25] as follows: (1) hepatic steatosis index (HSI) [24]; (2) comprehensive NAFLD score (CNS) [10]; (3) NAFLD liver fat score (LFS) [25]. The BARD [26] and FIB-4 [27] scores were selected as a surrogate index for defining severe conditions of NAFLD (advanced fibrosis). The calculations of BARD and FIB-4 were conducted only in subjects with NAFLD defined using NAFLD prediction models. All prediction models were summarized in Supplementary table 1.

Statistical analysis

The characteristics of the study subjects were analysed according to the status of obesity and sarcopaenia using Student's *t*-test for continuous variables and χ^2 test for categorical variables. Differences in prevalence of NAFLD were assessed using χ^2 test with Bonferroni adjustments. The association between SMI and fatty liver prediction scores (HSI, CNS and LFS) was evaluated using Chi-square test after transformation of these variables into quartiles. To exclude the effect of obesity or metabolic syndrome, the study population was stratified into two groups depending on the presence of either obesity or metabolic syndrome. Differences in fatty liver prediction scores were also compared using Student's *t*-test. Multivariable logistic regression analysis was applied to determine the independent association between sarcopaenia and NAFLD after adjustment for age and sex in model 1 or age, sex, regular exercise, homeostasis model assessment of insulin resistance (HOMA-IR), smoking, and hypertension in model 2. Continuous and categorical variables were expressed as the mean ± SD and N (%), respectively. A *p*-value less than 0.05 was considered to be statistically significant. Statistical analyses were performed using SPSS version 20.0 for Windows (IBM Corp., Armonk, NY, USA).

Results

Participant characteristics according to the status of obesity and sarcopaenia

From KNHANES 2008–2011, 15,132 individuals were analysed in the present study. Baseline characteristics of the study participants are presented in Table 1 separately according to the status of obesity and sarcopaenia in order to investigate whether the association between NAFLD and sarcopaenia is independent of obesity. The prevalence of sarcopaenia showed a large discrepancy between the non-obese and obese populations (19% for non-obese vs. 53% for obese individuals; $P<0.001$). Regardless of obesity status, sarcopaenic subjects were older and had higher BMIs, fasting glucose levels and index values of insulin resistance (HOMA-IR) than non-sarcopaenic individuals. Worse lipid profiles and kidney function were observed in sarcopaenic subjects. Moreover, sarcopaenic subjects showed an increased proportion of cardiometabolic disorders, including impaired fasting glucose, diabetes, hypertension and metabolic syndrome and tended not to exercise regularly. Aspartate transaminase (AST) levels were higher in sarcopaenic subjects regardless of obesity, whereas alanine transaminase (ALT) levels were significantly elevated only in sarcopaenic subjects with BMI<25 kg/m^2.

Hepatic steatosis was significantly increased in subjects with sarcopaenia regardless of obesity and metabolic syndrome.

The estimated prevalence of NAFLD was 22 to 29% in the overall study population depending on the NAFLD prediction models (Table 2). SMI, which indicates the degree of peripheral skeletal muscle mass, showed a strong negative relationship with all indices

predicting NAFLD (all P<0.001; Fig. 1). Because obesity and insulin resistance were well-established risk factors for NAFLD as well as sarcopaenia, we examined the independent association of sarcopaenia with NAFLD after stratification with obesity or metabolic syndrome which reflects clinical condition of insulin resistance. Both non-obese and obese subjects with sarcopaenia showed a significantly increased proportion of NAFLD, irrespective of the different NAFLD prediction models (Table 1 and Fig. 2A). Depending on the prediction models applied, 9~30% (60~83%) of non-obese (obese) subjects with sarcopaenia were likely to have NAFLD (Fig. 2A, Supplementary Fig. 2A and B). Regardless of having metabolic syndrome, a higher prevalence of NAFLD was found in sarcopaenic individuals than in non-sarcopaenic subjects (Fig. 2B, Supplementary Fig. 2C and D). Particularly, among people without metabolic syndrome, a 2.3- to 3.3-fold increase in the proportion of NAFLD was observed in sarcopaenic subjects compared with non-sarcopaenic subjects (15~31% vs. 7~10%, respectively; P<0.001). With respect to NAFLD-predicting indices, subjects with sarcopaenia had significantly higher values of HSI, CNS and LFS regardless of obesity and metabolic syndrome (Fig. 3 and Supplementary Fig. 3). Furthermore, AST and ALT levels were significantly increased in individuals with sarcopaenia independent of metabolic syndrome, but only in the non-obese population (Fig. 4). Consistent with the results of the inverse relationship between SMI and NAFLD predicting indices (Fig. 1), subjects with a higher SMI tended to have lower levels of ALT and AST (All Ps<0.001, Supplementary Fig. 4).

Sarcopaenia is independently associated with non-alcoholic or alcoholic fatty liver disease and hepatic steatosis with chronic hepatitis.

To adjust for conventional confounding covariates that affect NAFLD, a multivariable logistic regression model was applied (Table 2). In a minimally adjusted model (Model 1) with age and sex, sarcopaenic subjects had a markedly higher risk for NAFLD than non-sarcopaenic subjects regardless of the NAFLD prediction models (adjusted odds ratios [AORs] ranging from 2.9 to 4.3; all Ps<0.001). This association remained significant even after the adjustment for established risk factors of NAFLD, including BMI, diabetes status, triglyceride levels and insulin resistance as shown in Model 2, although AORs were reduced further to 1.2 (all Ps<0.05).

As a sensitivity analysis, we explored whether this relationship persists in individuals with excessive alcohol consumption or underlying chronic liver disease condition (N=3557) who were initially excluded from the analysis (Supplementary Table 2). Similarly, an independent association between sarcopaenia and hepatic steatosis was observed in this specific population with different characteristics (AORs ranging from 1.1 to 1.5; all Ps<0.05).

Relationship between NAFLD and exercise according to the presence of sarcopaenia and obesity.

To examine the impact of exercise on NAFLD in subjects with sarcopaenia, the proportion of NAFLD was assessed among individuals who did or did not exercise regularly after stratification with the status of obesity and sarcopaenia (Fig. 5 and Supplementary Fig. 5). Among obese people with preserved skeletal muscle mass, subjects who conducted exercise regularly were less likely to have NAFLD, regardless of NAFLD prediction models (46% in regularly exercised subjects vs. 55% in non-exercised subjects; P<0.001). In other

sub-groups, there was no statistical significance, although people who regularly exercised were less likely to have NAFLD.

Association of sarcopaenia with hepatic fibrosis in subjects with NAFLD

To further evaluate the relationship between sarcopaenia and steatohepatitis, non-invasive indices of hepatic fibrosis were calculated in individuals with NAFLD. The average scores of BARD and FIB-4, which both reflect the degree of fibrosis, were significantly increased in subjects with sarcopaenia compared with those in non-sarcopaenic subjects (BARD: 1.87 vs. 1.35; FIB-4: 0.99 vs. 0.85, all Ps<0.001, Fig. 6 and Supplementary Fig. 6). When the validated cut-off points to predict hepatic fibrosis were applied in this population, sarcopaenic patients were more likely to be categorised as having advanced fibrosis than non-sarcopaenic controls, regardless of the prediction methods (BARD: 60% vs. 45%, P<0.001; FIB-4: 22% vs. 14%, P<0.001).

Discussion

This nationally representative, population-based study demonstrated that subjects with sarcopaenia had a higher risk of NAFLD independent from the status of obesity as well as metabolic syndrome compared with individuals with a preserved muscle mass. Furthermore, the degree of the fatty liver condition which was determined by elevated markers of liver fibrosis was much more severe, in subjects with a lower skeletal muscle mass among individuals with NAFLD. People who exercised regularly were less likely to have NAFLD, defined by indirect measures, only under the condition of obesity and a well-preserved muscle mass. Considering previous concerns that sarcopaenic subjects tend to have a larger fat mass, a condition that may convey the confounding effect of muscle mass on NAFLD [17], we clearly established evidence that a decreased muscle mass is significantly associated not only with NAFLD but also with its severity. In addition, sarcopaenic subjects with alcoholic liver disease or chronic viral hepatitis had an increased risk of fatty liver after the adjustment for other confounding variables.

Consistent with previous findings [10,16], liver enzymes and obesity-related parameters such as BMI, waist circumference, and glycaemic and lipid profiles were inversely associated with SMI but positively related to fatty liver prediction scores (data not shown), indicating that the NAFLD scores and sarcopaenia index are interrelated. Although the pathogenic processes of developing sarcopaenia have not been fully understood, sarcopaenia and NAFLD share common pathophysiologic mechanisms and similar phenotypes. Increased insulin resistance, which is a major causative factor of NAFLD [28], can also aggravate sarcopaenia via mitochondrial dysfunction and the degradation of muscle protein by the activation of the ubiquitin-proteasome proteolytic pathway [12]. As insulin is a

strong anabolic hormone to stimulate muscle hypertrophy via intracellular Akt/mTOR pathway, impairment in insulin signalling (insulin resistance) can diminish the synthesis of muscle protein [12]. Furthermore, this condition can also result in elevation of circulating levels of free fatty acids by lipolysis from adipose tissues, which in turn leads to inhibit growth hormone/insulin growth factor-1 axis that plays a favourable role in protection against age-related muscle loss and in muscle regeneration [12,29]. Patients with chronic liver disease or cirrhosis are reported to have a lower level of insulin growth factor-1, while increased levels of various cytokines such as tumour necrosis factor-α and transforming growth factor-β, which are closely involved in sarcopaenia [30]. In addition, obesity triggers oxidative stress with chronic inflammation and the dysregulation of adipokines secreted from subcutaneous or visceral fats, which contribute to the development of ectopic fat accumulation in the liver and loss of skeletal muscle mass [12,31]. Considering that skeletal muscle is the major insulin-responsive target organ, sarcopaenia can further promote insulin resistance and reduce energy expenditures [32], which may lead to the development of NAFLD. Therefore, insulin resistance and inflammation can contribute to a complex of vicious cycles between NAFLD and sarcopaenia.

However, our findings demonstrated that sarcopaenia was associated with higher values of NAFLD markers in non-obese people or subjects without metabolic syndrome, suggesting a possibility that sarcopaenia itself is linked to NAFLD independent of insulin resistance or obesity. Skeletal muscle is considered an endocrine organ that secretes peptides called myokines that can mediate crosstalk among metabolic tissues such as the liver [33]. Among several myokines, interleukin-6 showed a protective effect on the development of NAFLD in inflammation-prone animal models [34] and irisin, an exercise-inducible myokine

[35], was inversely associated with the degree of fatty liver in obese humans [36]. Therefore, it is plausible that muscle could play a causative role for fatty liver by secreting various myokines. It is well known that exercise has a significant benefit on the improvement in hepatic steatosis regardless of weight loss [37]. We found that regular exercise was associated with a lower levels of NAFLD-predicting markers only in obese subjects without sarcopaenia, suggesting that a preserved muscle mass may be required to exert benefits towards the amelioration of fatty liver such as exercise-induced release of healthy myokines. Further study should be necessary to support this hypothesis.

Histologically, fatty liver is defined as the accumulation of excessive fat >5% of the liver weight. Although a liver biopsy is currently the gold standard for the diagnosis of hepatic steatosis and its severity, imaging modalities are widely used in clinical practice instead due to the invasive nature of biopsy. However, radiological modalities such as ultrasonography or computed tomography are rather inaccurate because milder degrees of steatosis (<33% of fat in hepatocytes) cannot be fully detected [38]. Furthermore, without biopsy, NASH or advanced fibrosis is hardly distinguished using imaging assessments as well as ALT levels. To overcome this limitation, several non-invasive fibrosis scoring models were developed and validated [10,26,39]. In the present study, we applied several well-validated scoring systems to detect NAFLD in the general population or advanced fibrosis in patients with NAFLD. Among the many models for predicting NAFLD, three risk models—HSI, CNS, and LFS—were selected based on their high areas under the curve (0.86, 0.89, 0.82, respectively) to detect NAFLD in Korean subjects [10]. We used BARD and FIB-4 scores, which are validated in patients with biopsy-proven NAFLD [26,40] and have clinically significant implications in NAFLD-related outcomes [41,42]. Interestingly, our data

demonstrated that subjects with sarcopaenia had a higher level of liver fibrosis markers regardless of the NASH predicting model. However, extra caution should be needed in interpreting these results, due to possible bias from applying indirect markers of NAFLD or NASH.

The current study had several strengths. First, our robust investigation provided strong evidence of a close relationship between sarcopaenia and NAFLD without the involvement of either obesity or insulin resistance. Because NAFLD is highly affected by obesity, it is indispensable to distinguish the impact of sarcopaenia alone from sarcopaenic obesity on fatty liver. In addition, stratification for obesity was necessary because the prevalence of sarcopaenia was markedly different between obese and non-obese subjects. Second, our sensitivity analyses showed that sarcopaenic subjects with alcoholic liver disease or chronic viral hepatitis had an increased risk of fatty liver, suggesting the extensive role of sarcopaenia on other chronic liver diseases. Furthermore, this was a large population-based analysis using well-examined national data, which strengthens the statistical reliability of the results and generalisability of the data. Finally, for the first time, we focused on the association of low muscle mass with advanced fibrosis as reflected by several indices in subjects with NAFLD. According to the two-hit NAFLD/NASH model, key elements for triggering steatohepatitis or fibrosis are underlying oxidative stress and chronic inflammation [43], which can also deteriorate the degradation of muscle mass [12].

However, the current study has some limitations, which should be complemented by further investigation. First, due to the unavailable data of hepatic imaging or biopsy, we used operational criteria for defining NAFLD or advanced fibrosis based on several predicting models that have been well validated [10,44-46]. Second, this cross-sectional study design

did not allow us to make solid conclusions regarding the causal relationships between sarcopaenia and NAFLD or NASH. Finally, we applied the measurements of the skeletal muscle mass only to define sarcopaenia and did not evaluate muscle function, although a consensus of standardised diagnostic criteria for sarcopaenia has not been entirely established.

In conclusion, this nationwide survey of a representative sample of the Korean population demonstrated that sarcopaenia was significantly associated not only with higher values of NAFLD-predicting markers but also with severe conditions of hepatic steatosis independent from condition of obesity or insulin resistance. Considering the worldwide increase of ageing and obesity societies, prospective studies are warranted to elucidate the complex causal relationship between a low skeletal muscle mass and NAFLD or NASH.

Disclosure of potential conflicts of interest

All authors state that they have no conflicts of interest.

Acknowledgments

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Figure legends

Fig. 1. The association of the skeletal muscle index by quartiles with different fatty liver scores by quartiles. (A) Hepatic steatosis index (HSI), (B) Comprehensive NAFLD score (CNS), (C) Liver fat score (LFS).

Fig. 2. The difference in the prevalence of NAFLD according to the sarcopaenic status after stratification for obesity or metabolic syndrome. (A) The prevalence of NAFLD by status of sarcopaenia and obesity. or metabolic syndrome, (B) The prevalence of NAFLD by status of sarcopaenia and metabolic syndrome.

Fig. 3. The difference in the hepatic steatosis index according to the sarcopaenic status after stratification for obesity or metabolic syndrome. (A) The hepatic steatosis index by status of sarcopaenia and obesity. or metabolic syndrome, (B) The hepatic steatosis index by status of sarcopaenia and metabolic syndrome.

Fig. 4. The difference in serum levels of transaminases according to the sarcopaenic status after stratification for obesity or metabolic syndrome. (A and B) Serum levels of ALT, (C and D) Serum levels of AST. The data are presented as the mean ± SE. *P<0.001, **P<0.01, and ***P<0.05. MS, metabolic syndrome.

Fig. 5. The difference in the prevalence of NAFLD according to the status of sarcopaenia and

exercise after stratification for obesity.

Fig. 6. The association between hepatic fibrosis and sarcopaenia in subjects with NAFLD. The difference in the BARD (A) and FIB-4 (B) scores according to the sarcopaenic status. The data are presented as the mean ± SD. The proportion of subjects with hepatic fibrosis as defined by BARD (C) or FIB-4 (D) scores according to the sarcopaenic status.

Table 1. Characteristics of the study participants according to the status of obesity and sarcopaenia

Table 2. Adjusted odds ratios (AORs) with 95% confidence intervals (CIs) of non-alcoholic fatty liver disease (NAFLD) assessed by different predictive models

References

[1] World Health Organization. World health statistics 2013 2013.

[2] Villareal DT, Chode S, Parimi N, Sinacore DR, Hilton T, Armamento-Villareal R, et al. Weight loss, exercise, or both and physical function in obese older adults. N Engl J Med 2011;364:1218-1229.

[3] Cruz-Jentoft AJ, Landi F, Topinkova E, Michel JP. Understanding sarcopenia as a geriatric syndrome. Curr Opin Clin Nutr Metab Care 2010;13:1-7.

[4] Clegg A, Young J, Iliffe S, Rikkert MO, Rockwood K. Frailty in elderly people. Lancet 2013;381:752-762.

[5] The Lancet Diabetes E. Sarcopenia: a fate worth challenging. Lancet Diabetes Endocrinol 2014;2:183.

[6] Lim S, Kim JH, Yoon JW, Kang SM, Choi SH, Park YJ, et al. Sarcopenic obesity: prevalence and association with metabolic syndrome in the Korean Longitudinal Study on Health and Aging (KLoSHA). Diabetes care 2010;33:1652-1654.

[7] Stephen WC, Janssen I. Sarcopenic-obesity and cardiovascular disease risk in the elderly. J Nutr Health Aging 2009;13:460-466.

[8] Kim TN, Park MS, Yang SJ, Yoo HJ, Kang HJ, Song W, et al. Prevalence and determinant factors of sarcopenia in patients with type 2 diabetes: the Korean Sarcopenic Obesity Study (KSOS). Diabetes Care 2010;33:1497-1499.

[9] Vernon G, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. Aliment Pharmacol Ther 2011;34:274-285.

[10] Lee YH, Bang H, Park YM, Bae JC, Lee BW, Kang ES, et al. Non-laboratory-based self-assessment screening score for non-alcoholic fatty liver disease: development, validation and comparison with other scores. PLoS One 2014;9:e107584.

[11] Fan JG, Zhu J, Li XJ, Chen L, Li L, Dai F, et al. Prevalence of and risk factors for fatty liver

in a general population of Shanghai, China. J Hepatol 2005;43:508-514.

[12] Kalyani RR, Corriere M, Ferrucci L. Age-related and disease-related muscle loss: the effect of diabetes, obesity, and other diseases. Lancet Diabetes Endocrinol 2014;2:819-829.

[13] Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. Gastroenterology 1999;116:1413-1419.

[14] Ekstedt M, Franzen LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, et al. Long-term follow-up of patients with NAFLD and elevated liver enzymes. Hepatology 2006;44:865-873.

[15] Argo CK, Northup PG, Al-Osaimi AM, Caldwell SH. Systematic review of risk factors for fibrosis progression in non-alcoholic steatohepatitis. J Hepatol 2009;51:371-379.

[16] Hong HC, Hwang SY, Choi HY, Yoo HJ, Seo JA, Kim SG, et al. Relationship between sarcopenia and nonalcoholic fatty liver disease: the Korean Sarcopenic Obesity Study. Hepatology 2014;59:1772-1778.

[17] Kim SW, Jung HW. Which one is associated with nonalcoholic fatty liver disease?: Small muscle mass or large fat mass. Hepatology 2014.

[18] Lee YH, Kim JE, Roh YH, Choi HR, Rhee Y, Kang DR, et al. The Combination of Vitamin D Deficiency and Mild to Moderate Chronic Kidney Disease Is Associated With Low Bone Mineral Density and Deteriorated Femoral Microarchitecture: Results From the KNHANES 2008-2011. J Clin Endocrinol Metab 2014;99:3879-3888.

[19] Janssen I, Heymsfield SB, Ross R. Low relative skeletal muscle mass (sarcopenia) in older persons is associated with functional impairment and physical disability. J Am Geriatr Soc 2002;50:889-896.

[20] Lee SG, Lee YH, Kim KJ, Lee W, Kwon OH, Kim JH. Additive association of vitamin D insufficiency and sarcopenia with low femoral bone mineral density in noninstitutionalized elderly population: the Korea National Health and Nutrition Examination Surveys 2009-2010.

Osteoporos Int 2013.

[21] Oh SW. Obesity and metabolic syndrome in Korea. Diabetes Metab J 2011;35:561-566.

[22] American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2010;33 Suppl 1:S62-69.

[23] Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med 2009;150:604-612.

[24] Lee J, Kim D, Kim HJ, Lee C, Yang JI, Kim W, et al. Hepatic steatosis index: a simple screening tool reflecting nonalcoholic fatty liver disease. Dig Liver Dis 2010;42:503-508.

[25] Kotronen A, Peltonen M, Hakkarainen A, Sevastianova K, Bergholm R, Johansson LM, et al. Prediction of non-alcoholic fatty liver disease and liver fat using metabolic and genetic factors. Gastroenterology 2009;137:865-872.

[26] Harrison SA, Oliver D, Arnold HL, Gogia S, Neuschwander-Tetri BA. Development and validation of a simple NAFLD clinical scoring system for identifying patients without advanced disease. Gut 2008;57:1441-1447.

[27] Vallet-Pichard A, Mallet V, Nalpas B, Verkarre V, Nalpas A, Dhalluin-Venier V, et al. FIB-4: an inexpensive and accurate marker of fibrosis in HCV infection. comparison with liver biopsy and fibrotest. Hepatology 2007;46:32-36.

[28] Utzschneider KM, Kahn SE. Review: The role of insulin resistance in nonalcoholic fatty liver disease. J Clin Endocrinol Metab 2006;91:4753-4761.

[29] Bonaldo P, Sandri M. Cellular and molecular mechanisms of muscle atrophy. Dis Model Mech 2013;6:25-39.

[30] Tessari P. Protein metabolism in liver cirrhosis: from albumin to muscle myofibrils. Curr Opin Clin Nutr Metab Care 2003;6:79-85.

[31] Anderson EJ, Lustig ME, Boyle KE, Woodlief TL, Kane DA, Lin CT, et al. Mitochondrial H2O2 emission and cellular redox state link excess fat intake to insulin resistance in both rodents and humans. J Clin Invest 2009;119:573-581.

[32] Kim TN, Yang SJ, Yoo HJ, Lim KI, Kang HJ, Song W, et al. Prevalence of sarcopenia and sarcopenic obesity in Korean adults: the Korean sarcopenic obesity study. Int J Obes (Lond) 2009;33:885-892.

[33] Pedersen BK, Febbraio MA. Muscles, exercise and obesity: skeletal muscle as a secretory organ. Nat Rev Endocrinol 2012;8:457-465.

[34] Miller AM, Wang H, Bertola A, Park O, Horiguchi N, Ki SH, et al. Inflammation-associated interleukin-6/signal transducer and activator of transcription 3 activation ameliorates alcoholic and nonalcoholic fatty liver diseases in interleukin-10-deficient mice. Hepatology 2011;54:846-856.

[35] Bostrom P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, et al. A PGC1-alpha-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature 2012;481:463-468.

[36] Zhang HJ, Zhang XF, Ma ZM, Pan LL, Chen Z, Han HW, et al. Irisin is inversely associated with intrahepatic triglyceride contents in obese adults. J Hepatol 2013;59:557-562.

[37] Thoma C, Day CP, Trenell MI. Lifestyle interventions for the treatment of non-alcoholic fatty liver disease in adults: a systematic review. J Hepatol 2012;56:255-266.

[38] Saadeh S, Younossi ZM, Remer EM, Gramlich T, Ong JP, Hurley M, et al. The utility of radiological imaging in nonalcoholic fatty liver disease. Gastroenterology 2002;123:745-750.

[39] Dyson JK, Anstee QM, McPherson S. Non-alcoholic fatty liver disease: a practical approach to diagnosis and staging. Frontline Gastroenterol 2014;5:211-218.

[40] Sumida Y, Yoneda M, Hyogo H, Itoh Y, Ono M, Fujii H, et al. Validation of the FIB4 index in a Japanese nonalcoholic fatty liver disease population. BMC Gastroenterol 2012;12:2.

[41] Angulo P, Bugianesi E, Bjornsson ES, Charatcharoenwitthaya P, Mills PR, Barrera F, et al. Simple noninvasive systems predict long-term outcomes of patients with nonalcoholic fatty liver disease. Gastroenterology 2013;145:782-789 e784.

[42] Kim D, Kim WR, Kim HJ, Therneau TM. Association between noninvasive fibrosis markers

and mortality among adults with nonalcoholic fatty liver disease in the United States. Hepatology 2013;57:1357-1365.

[43] Day CP, James OF. Steatohepatitis: a tale of two "hits"? Gastroenterology 1998;114:842-845.

[44] Kahl S, Strassburger K, Nowotny B, Livingstone R, Kluppelholz B, Kessel K, et al. Comparison of liver fat indices for the diagnosis of hepatic steatosis and insulin resistance. PLoS One 2014;9:e94059.

[45] Ruffillo G, Fassio E, Alvarez E, Landeira G, Longo C, Dominguez N, et al. Comparison of NAFLD fibrosis score and BARD score in predicting fibrosis in nonalcoholic fatty liver disease. J Hepatol 2011;54:160-163.

[46] Kim BK, Kim do Y, Park JY, Ahn SH, Chon CY, Kim JK, et al. Validation of FIB-4 and comparison with other simple noninvasive indices for predicting liver fibrosis and cirrhosis in hepatitis B virus-infected patients. Liver Int 2010;30:546-553.

Fig 1

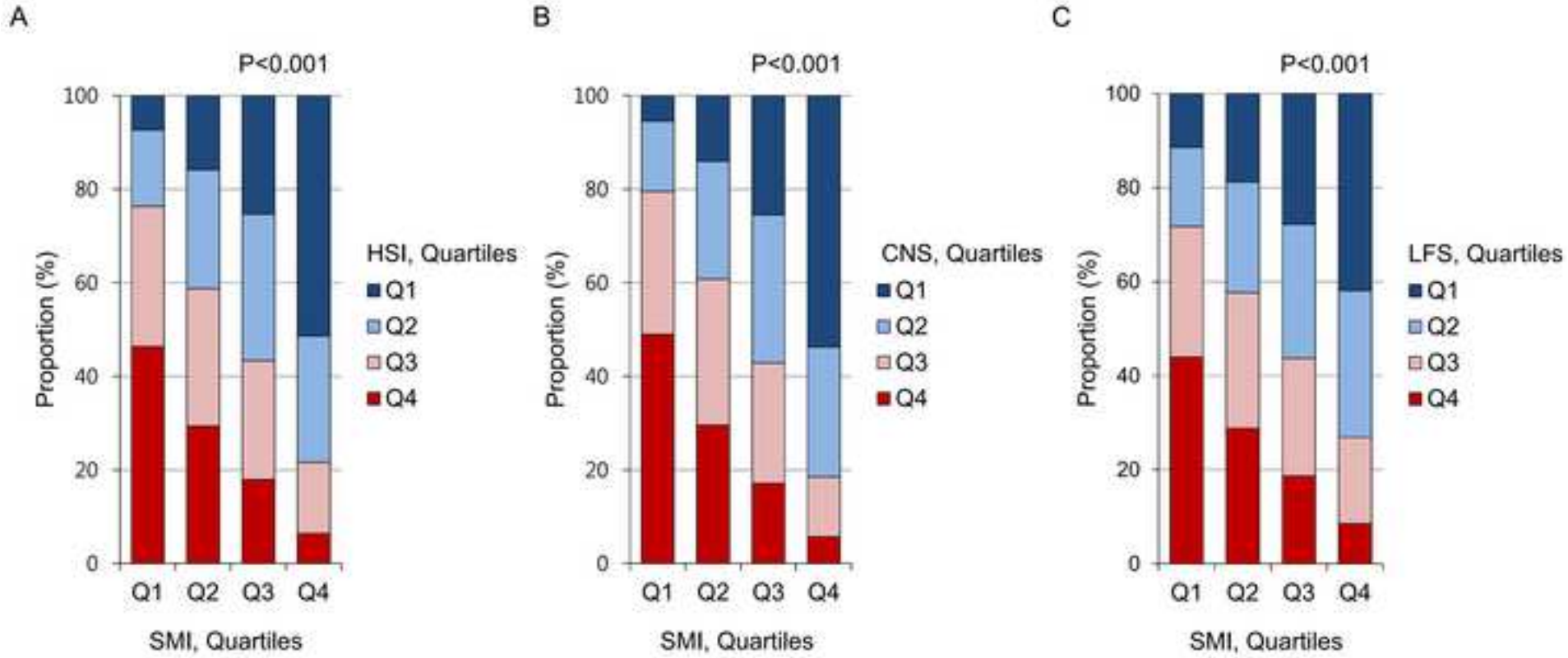

Fig 2

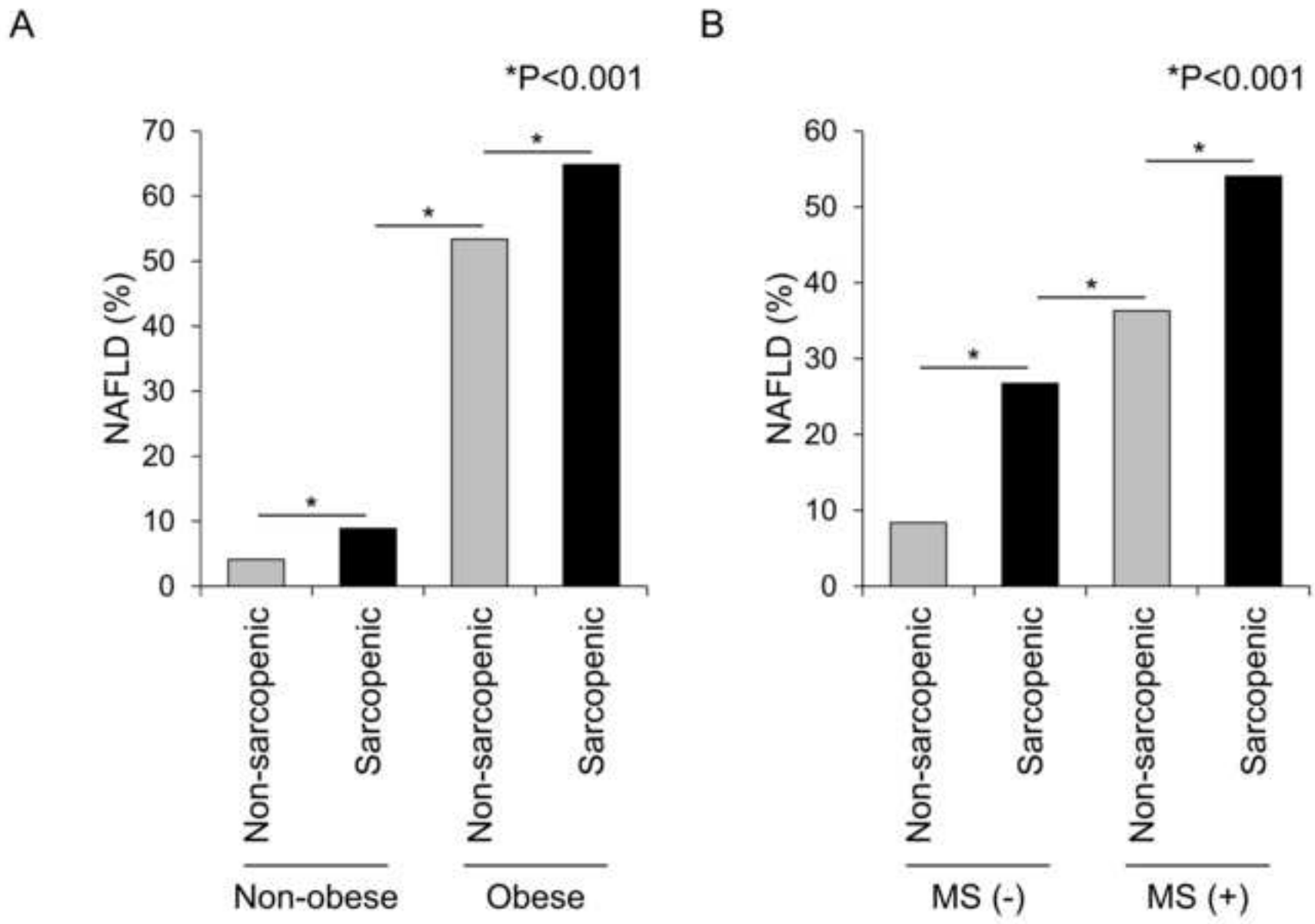

Fig 3

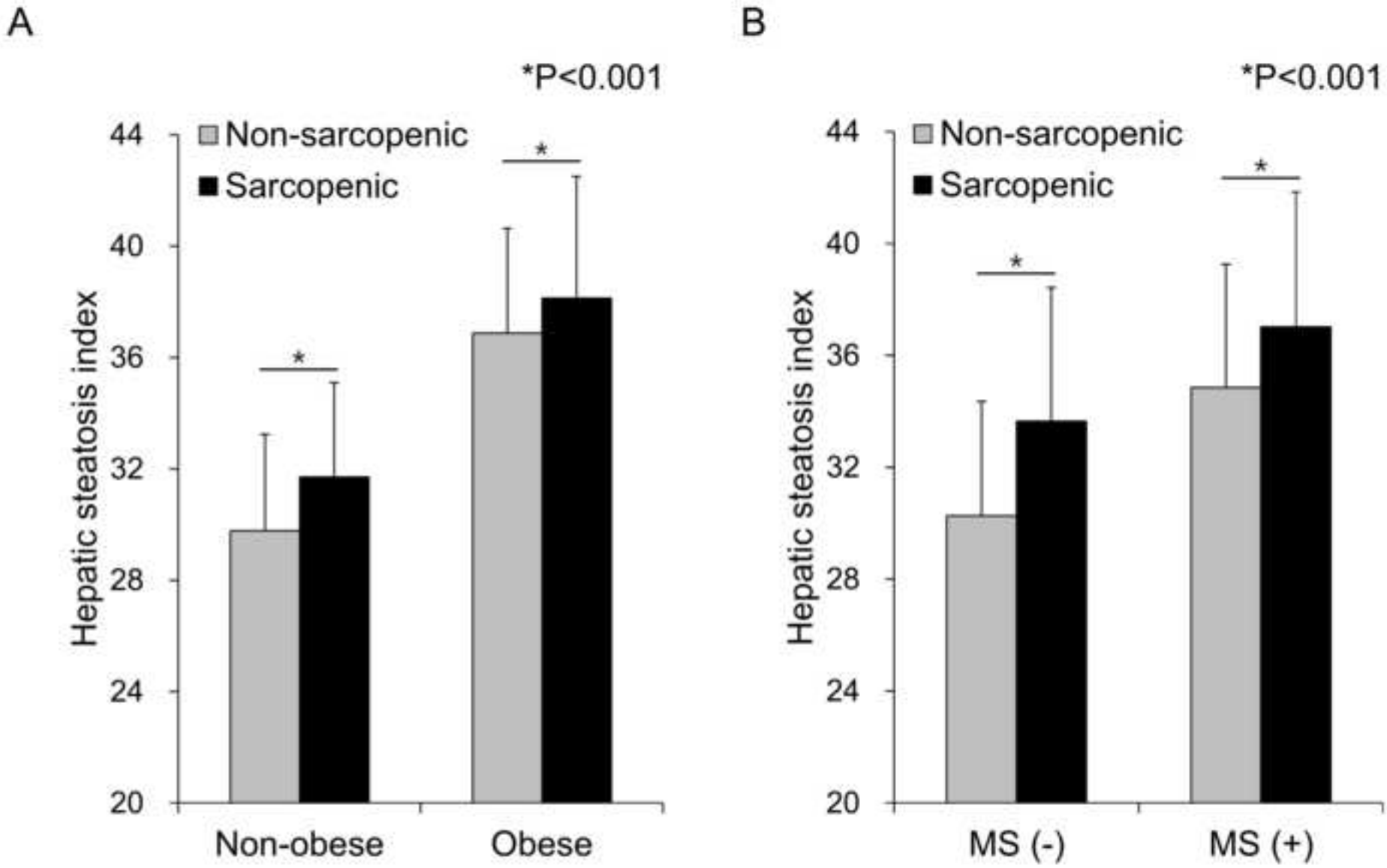

Fig 4

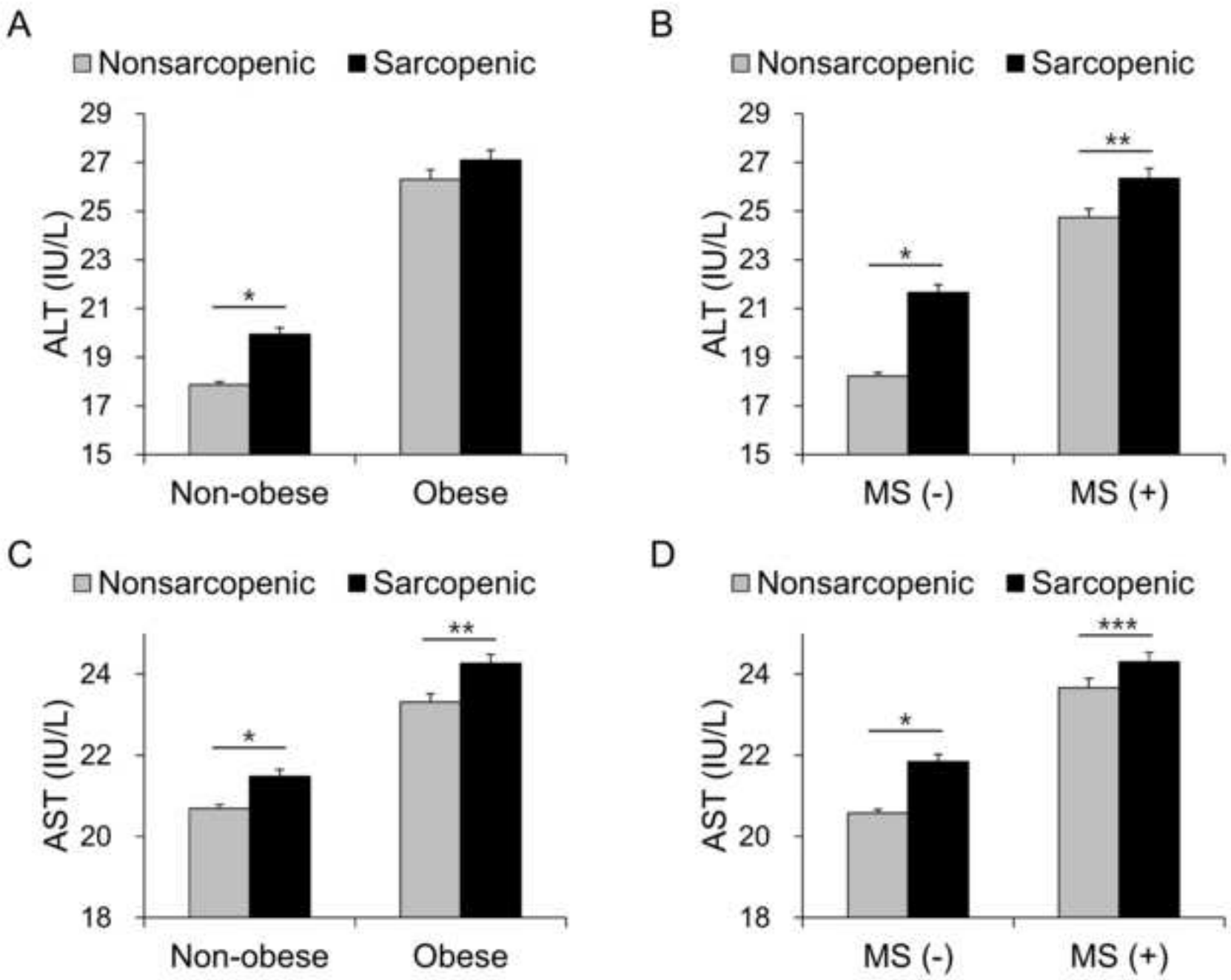

Fig 5

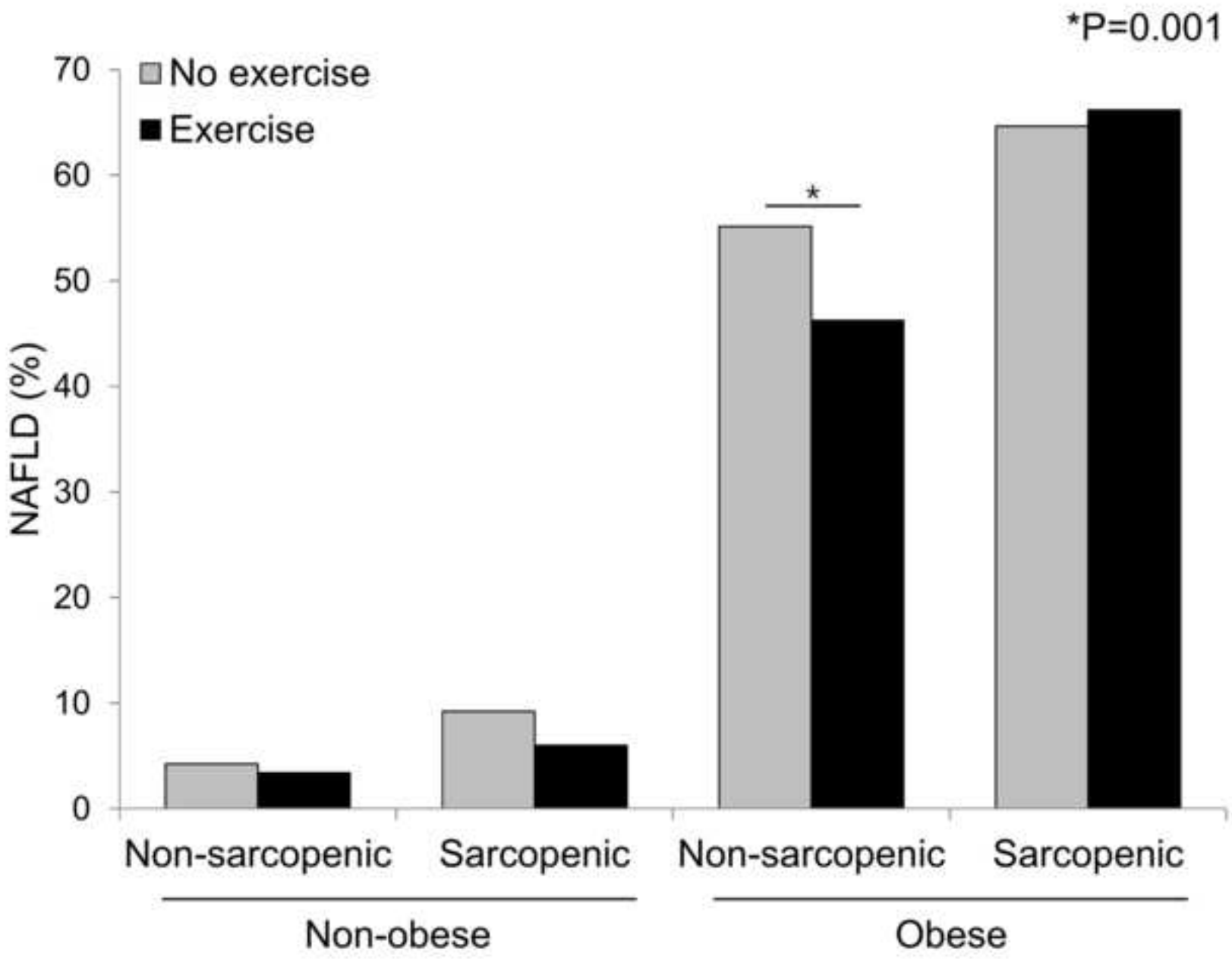

Fig 6

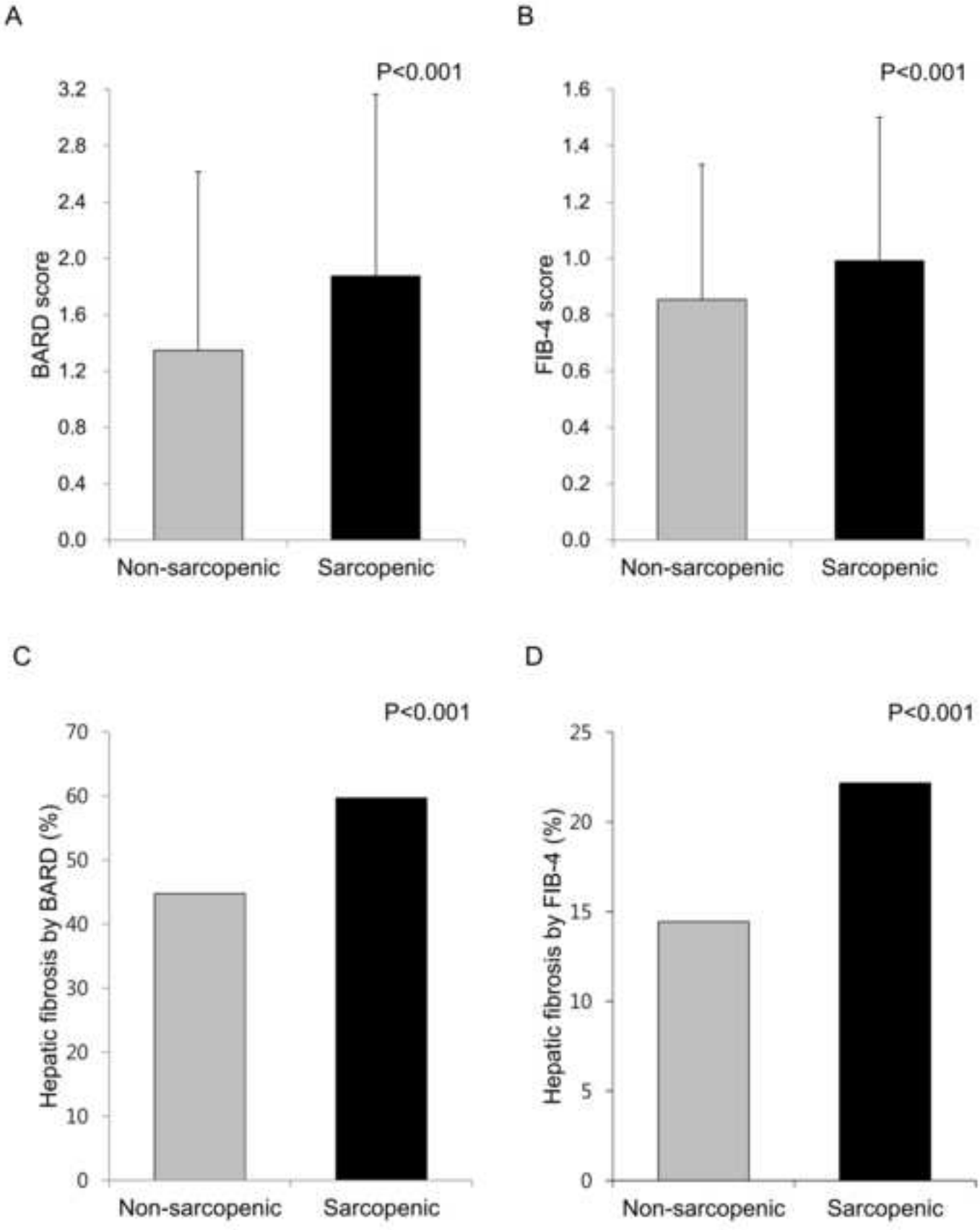

Table 1. Characteristics of the study participants according to the status of obesity and sarcopaenia

	Non-obese (N=10479)			Obese (N=4653)		
	Non-sarcopenic (N=8475)	Sarcopenic (N=2004)	P	Non-sarcopenic (N=2198)	Sarcopenic (N=2455)	P
Age (years)	47.9 ± 16.8	56.7 ± 16.3	<0.001	49.1 ± 14.3	56.0 ± 15.1	<0.001
Sex (female), N(%)	5469 (65)	1307 (65)	0.562	1160 (53)	1579 (64)	<0.001
BMI (kg/m^2)	21.6 ± 2.1	22.8 ± 1.6	<0.001	26.9 ± 1.8	27.9 ± 2.5	<0.001
Systolic blood pressure (mmHg)	114.1 ± 17.3	120.4 ± 18.5	<0.001	120.8 ± 16.3	124.9 ± 17.5	<0.001
Diastolic blood pressure (mmHg)	72.3 ± 10.0	74.2 ± 10.3	<0.001	77.5 ± 10.4	78.1 ± 10.1	0.061
ASM (kg)	17.7 ± 4.3	15.4 ± 3.6	<0.001	22.1 ± 5.1	18.6 ± 4.7	<0.001
SMI (%)	31.1 ± 4.1	26.5 ± 3.3	<0.001	30.5 ± 3.9	26.0 ± 3.5	<0.001
ASM/Ht^2 (kg/m^2)	6.7 ± 1.0	6.0 ± .9	<0.001	8.2 ± 1.1	7.2 ± 1.1	<0.001
Biochemistry						
Fasting glucose (mg/dL)	94.0 ± 19.8	99.7 ± 25.3	<0.001	100.7 ± 23.8	105.1 ± 27.6	<0.001
Fasting insulin (μIU/mL)	8.8 ± 4.1	10.1 ± 6.8	<0.001	11.6 ± 5.1	12.8 ± 6.8	<0.001
HOMA-IR	2.1 ± 1.2	2.5 ± 3.1	<0.001	2.9 ± 1.7	3.4 ± 2.9	<0.001
Total cholesterol (mg/dl)	182.8 ± 34.0	193.3 ± 36.4	<0.001	193.5 ± 36.1	198.6 ± 37.2	<0.001
HDL cholesterol (mg/dl)	49.6 ± 11.1	46.8 ± 10.7	<0.001	43.5 ± 9.3	44.1 ± 9.7	0.040
Triglycerides (mg/dl)*	109.1 ± 79.2	134.3 ± 85.9	<0.001	155.9 ± 107.2	159.4 ± 95.0	0.002
LDL cholesterol (mg/dl)	110.9 ± 29.7	119.8 ± 32.6	<0.001	119.9 ± 31.6	124.2 ± 33.6	<0.001
AST (IU/L)	20.7 ± 9.2	21.5 ± 7.5	0.004	23.3 ± 10.0	24.3 ± 10.9	0.002
ALT (IU/L)	17.9 ± 11.5	19.9 ± 12.1	<0.001	26.3 ± 18.7	27.1 ± 20.0	0.159
Creatinine (mg/dl)	0.8 ± 0.2	0.8 ± 0.3	0.712	0.9 ± 0.2	0.8 ± 0.3	<0.001
eGFR (mL/min/1.73m^2)	96.8 ± 17.3	92.1 ± 19.2	<0.001	92.6 ± 16.3	90.1 ± 18.5	<0.001
25(OH)D (ng/ml)	18.4 ± 7.1	17.4 ± 6.7	<0.001	19.3 ± 6.8	17.9 ± 6.5	<0.001
Clinical parameters						

IFG/diabetes, N(%)	1270/530 (15/6)	415/291 (21/15)	<0.001	560/301 (25/14)	652/463 (27/19)	<0.001
Hypertension, N(%)	1691 (20)	759 (38)	<0.001	773 (35)	1233 (50)	<0.001
Metabolic syndrome, N(%)	1184 (14)	628 (31)	<0.001	1074 (49)	1489 (61)	<0.001
Smoking (Past/Current), N(%)	868/1406 (10/17)	225/251 (11/13)	<0.001	277/435 (13/20)	236/348 (10/14)	<0.001
Regular exercise, N(%)	1237 (15)	201 (10)	<0.001	441 (20)	328 (13)	<0.001
Residence (Metro/City/Rural), %	45/34/21	43/36/21	0.093	44/33/23	44/34/22	0.636
Hepatic steatosis						
assessed by HSI, N(%)	349 (4)	178 (9)	<0.001	1173 (53)	1592 (65)	<0.001
assessed by CNS, N(%)	732 (10)	419 (25)	<0.001	1391 (72)	1728 (83)	<0.001
assessed by LFS, N(%)	1153 (14)	609 (30)	<0.001	1098 (50)	1490 (61)	<0.001

Data for continuous variables were expressed as mean ± SD. *Log transformed.

ASM, appendicular skeletal muscle mass; Ht, height; SMI, skeletal muscle index; eGFR, estimated glomerular filtration rate; IFG, impaired fasting glucose; HSI, hepatic steatosis index; CNS, comprehensive NAFLD score; LFS, liver fat score.

Table 2. Adjusted odds ratios (AORs) with 95% confidence intervals (CIs) of non-alcoholic fatty liver disease (NAFLD) assessed by different predictive models

NAFLD (N, %)	NAFLD assessed by HSI (N=3292, 22%)			NAFLD assessed by CNS (N=4270, 28%)			NAFLD assessed by LFS (N=4360, 29%)		
Variables	AOR	95% CI	P	AOR	95% CI	P	AOR	95% CI	P
Adjusted model 1*									
Sarcopenia	**4.26**	**3.91−4.63**	**<0.001**	**4.30**	**3.95−4.69**	**<0.001**	**2.89**	**2.68−3.12**	**<0.001**
Adjusted model 2**									
Sarcopenia	**1.18**	**1.03−1.34**	**0.014**	**1.19**	**1.02−1.39**	**0.027**	**1.22**	**1.09−1.36**	**<0.001**
BMI, per 1 kg/m^2	2.53	2.43−2.62	<0.001	3.21	3.04−3.39	<0.001	1.39	1.36−1.42	<0.001
Regular exercise	0.83	0.74−0.94	0.002	0.84	0.72−0.97	0.016	0.86	0.78−0.95	0.003
Triglycerides, per 50mg/dl	1.15	1.11−1.18	<0.001	2.91	2.73−3.11	<0.001	1.69	1.63−1.75	<0.001
Glycemic status									
Normal fasting glucose	Reference			Reference			Reference		
Impaired fasting glucose	1.15	0.99−1.34	0.068	2.00	1.67−2.39	<0.001	3.08	2.75−3.45	<0.001
Diabetes	8.27	6.74−10.16	<0.001	19.34	14.59−25.64	<0.001	26.80	22.32−32.19	<0.001

*Logistic models are adjusted for age and sex.

**Logistic models are adjusted for age, sex, regular exercise, HOMA-IR, smoking, and hypertension status.