Sarcopenia Modifies the Associations of NAFLD With All-cause and Cardiovascular Mortality Among Elderly Individuals

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Abstract

Background: The contribution of nonalcoholic fatty liver disease (NAFLD) to all-cause and cardiovascular mortality remains controversial. Sarcopenia, a measure of muscle mass and function, may identify which persons are most at risk for adverse effects of NAFLD. We aimed to test the hypothesis that sarcopenia modifies the associations between NAFLD and all-cause and cardiovascular mortality.

Methods: A total of 2,446 elderly individuals (≥60 years) from the third National Health and Nutrition Examination Survey were enrolled. Their mortality data were linked to death certificates in the National Death Index. Sarcopenia was defined as having low skeletal muscle mass together with slow gait speed, which captures both muscle mass and muscle function. Ultrasound tests were used for the assessment of hepatic steatosis.

Results: During follow-up (median 16.8 years), 1530 elderly subjects died from any cause, of which 379 were cardiovascular-related. All-cause and cardiovascular mortality rates were 4.31 and 1.07 per 100 person-years, respectively. In a multivariate model, using participants without NAFLD and sarcopenia as the reference group, individuals with both NAFLD and sarcopenia had 1.69 times (95% CI, 1.23-2.31) and 2.17 times (95% CI, 1.33-3.54) higher risks of all-cause and cardiovascular mortality, respectively. However, NAFLD persons without sarcopenia had hazard ratios for all-cause and cardiovascular mortality similar to those of the reference group.

Conclusions: Sarcopenia modified the associations of NAFLD with all-cause and cardiovascular mortality. Sarcopenia may identify elderly adults who are at the highest risk for adverse outcomes associated with NAFLD.

Introduction

Nonalcoholic fatty liver disease (NAFLD) is associated with metabolic disorders such as hyperglycemia and dyslipidemia [1,2]. There has been a large body of epidemiological evidence showing the relationship of NAFLD with all-cause and cardiovascular mortality, but the results are contradictory [3–5]. This may be caused by the existing substantial heterogeneity in health status among patients with NAFLD. High levels of skeletal muscle mass, an aspect of health status, have a strong protective effect against mortality [6,7]. Therefore, high levels of muscle mass may attenuate or eliminate the increased mortality risk associated with NAFLD. NAFLD and sarcopenia, which is characterized by a progressive loss of skeletal muscle mass and function, share the insulin resistance background [8,9]. Moreover, sarcopenia may be involved in the pathogenesis of NAFLD by reducing energy expenditure. Increased secretion of proinflammatory cytokines in the state of NAFLD may reduce muscle protein synthesis and promote muscle protein breakdown [10,11], and thus induce sarcopenia. A clear understanding of the combined effect of NAFLD and sarcopenia on all-cause and cardiovascular mortality risk is needed to aid in risk stratification and in turn target patients at the highest risk of all-cause and cardiovascular mortality. Hence, we aimed to investigate the combined relationship of NAFLD and sarcopenia with all-cause and cardiovascular

mortality to understand whether sarcopenia modified the association of NAFLD with all-cause and cardiovascular mortality.

Methods

Study population

The National Health and Nutrition Examination Surveys (NHANES) are a series of cross-sectional health examination surveys conducted by the National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention. All mortality data from each NHANES were ascertained by the NCHS from National Death Index (NDI) death certificate records. We chose NHANES Ⅲ as the baseline since we had detailed information regarding the assessment of hepatic steatosis. Full details of the survey have been described elsewhere [12]. The relevant data were publicly available on the website https://wwwn.cdc.gov/nchs/nhanes/nhanes3/Default.aspx. Briefly, the survey followed a complex stratified, multistage probability cluster sampling design to ensure that the sample is nationally representative of the civilian, noninstitutionalized US population. Participants were interviewed at home for basic sociodemographic and health-related information. After the in-home interview, participants are invited to attend a mobile examination center, where they underwent a set of standardized physical examinations and laboratory measurements. The survey procedures were reviewed and approved by the National Center for Health Statistics ethics review board in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Informed consent was obtained from all participants and/or their legal guardians.

We restricted our analyses to individuals aged 60 years and older who completed the gallbladder ultrasound examination and had bioelectrical impedance data and gait speed measures. Our rationale for restricting our sample to individuals aged ≥60 years was that the prevalence of sarcopenia is more prevalent in this population [13]. We excluded 476 participants with alcohol consumption in amounts > 3 drinks/day for men (330) or > 2 drinks/day for women (146), 12 participants with serum hepatitis B surface antigen positivity, 48 participants with hepatitis C antibody positivity, 87 participants with iron overload (serum transferrin saturation >50%), 69 participants with body mass index (BMI) <18.5kg/m^2, 493 participants without data on Bioelectrical impedance analysis, 502 participants without data on gait speed, 539 participants with gallbladder lumen could not be adequately visualized on ultrasound, and 46 participants with ultrasound ungradable. The remaining available 2,446 participants were included in our data analysis.

Anthropometric and biochemical measurements

As elaborated in our previous report [14], BMI was calculated as weight (in kilograms) divided by the square of height (in meters). Waist circumference (WC) was measured with a steel measuring tape just above the iliac crest to the nearest 1 mm. Blood pressure (BP) was measured using mercury sphygmomanometers. The last two readings were averaged.

Plasma glucose was measured by the modified hexokinase enzymatic assay (Cobas Mira Chemistry System; Roche Diagnostic Systems, Montclair, NJ). Total cholesterol (TC) and triglycerides were measured enzymatically. HDL-cholesterol was measured by the heparin manganese precipitation method. C-Reactive Protein (CRP) was measured using a latex-enhanced Behring nephelometry. Serum insulin was measured by radioimmunoassay using a double-antibody batch method (Pharmacia Insulin RIA kit; Pharmacia Diagnostics, Uppsala, Sweden). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated by the formula: HOMA-IR = fasting insulin (micro-international units per milliliter) × fasting glucose (millimoles per liter)/22.5.

Assessment of NAFLD

Ultrasound tests (Toshiba SSA-90A, Tustin, CA) were used for the assessment of hepatic steatosis. Archived videotapes on gallbladder ultrasounds were reviewed between 2009 and 2010 to ascertain the presence of fat within the hepatic parenchyma. The diagnosis of fatty liver was based on the following five criteria [15]: the brightness of liver parenchymal, presence of liver to kidney contrast, presence of echogenic walls within the small intrahepatic vessels, presence of deep beam attenuation, and definition of gallbladder walls, which were also described in our previous report [14]. NAFLD was initially categorized as a 4-level classification (none, mild, moderate, or severe) and then recorded as a 2-level classification (none to mild or moderate to severe), which was the classification used for the current analysis. For the two-level hepatic steatosis categorization, the intrarater and interrater k statistics were 0.77 (95% confidence interval [CI] 0.73-0.82) and 0.70 (0.64-0.76), respectively.

Definition of sarcopenia

Bioelectrical impedance analysis (BIA) was assessed using a Valhalla 1990B Bio-Resistance Body Composition Analyzer (Valhalla Scientific, San Diego, CA, USA). All subjects fasted for a minimum of 6 h. A single tetrapolar measurement of resistance was taken between the right wrist and ankle while lying in the supine position. Skeletal muscle mass was calculated using a validated formula [16]: Skeletal muscle mass (kg) = [(height ^{2}/ BIA-resistance × 0.401) + (gender × 3.825) + (age × -0.071)] + 5.102, where height is expressed in cm, BIA-resistance is expressed in ohms, gender is equal to 1 for men and 0 for women, and age is expressed in years. The skeletal muscle index (SMI, kg/m^2) is the skeletal muscle mass (kg) indexed for height^2 (in meters). Low muscle mass is defined as SMI <10.76 kg/m^2 for men and < 6.75 kg/m^2 for women[17]. These SMI thresholds are based on disability risk and are recommended to identify sarcopenia [17].

It has demonstrated that slow gait speed reflect functional capacity and subclinical health impairment in the elderly [18-20]. A timed 8-foot walk test was performed twice in NHANES Ⅲ. The participant was asked to walk at their usual pace. The faster of the two trials were used in this analysis. Timing began when the participant's first foot stepped over the starting line and ended when one of the feet crossed over the finish line. The 8-foot speed was converted to a 4-meter equivalent by adopting an established formula

[21]. Slow gait speed is defined as a gait speed ≤ 0.8 m/s [17]. This cutoff is associated with adverse health outcomes [22].

According to the criteria defined on Sarcopenia in the elderly, participants with slow gait speed, a measure of muscle function, together with low muscle mass were classified as having sarcopenia [17].

Mortality Data

Mortality data including causes of death were available from the date of NHANES Ⅲ survey participation (1988-1994) through December 31, 2011, using a probabilistic match that linked NHANES Ⅲ participants with NDI death certificate records. The NCHS indicated that 96.1 % of deceased individuals and 99.4 % of living individuals were correctly classified using this matching methodology [23]. Causes of death for those dying prior to 1998 were determined according to the 9th revision of the International Statistical Classification of Disease, Injuries and Causes of Death (ICD-9) guidelines. After 1998, they were determined by the 10th revision (ICD-10) guidelines [24]. The present study focused on all-cause mortality and cardiovascular mortality (ICD-10 I00-09, I11, I13, I20-51, and I60-69).

Statistical analysis

Complex survey procedures in SAS 9.2 (SAS Institute, Inc., Cary, NC) were performed for all analyses. Sample weights were incorporated to produce nationally representative estimates. Participants were divided into 4 mutually exclusive groups based on the cross-classification of NAFLD (with and without NAFLD) and sarcopenia status (with and without sarcopenia). Continuous variables were presented as means ± standard errors (SE). Logarithmic transformation was performed where needed. Categorical variables were presented as percentages. ANOVA was applied to compare differences in means between groups. A Chi-square test was performed to assess differences in proportions between groups. Follow-up time was from the date of the NHANES Ⅲ examination to the date of death or December 31, 2011, whichever came first. Cox proportional hazards models were used to examine the independent effects of NAFLD and sarcopenia on mortality. Proportional hazard assumption was adjusted for all potential mortality predictors. Cox proportional hazards models were used to examine the joint associations of NAFLD and sarcopenia with mortality risk to determine whether the associations of NAFLD with mortality risk was modified by sarcopenia status. Significance was accepted at a two-tailed $P < 0.05$.

Results

There was no difference in SMI or most of the studied risk factors between subjects who had and did not have gait speed data (supplementary Table S1).

During follow-up (median 16.8 years), 1,530 elderly subjects died from any cause, of which 379 were cardiovascular-related. All-cause and cardiovascular mortality rates were 4.31 and 1.07 per 100 person-years, respectively.

BMI, WC, systolic BP, plasma glucose, HbA1c, triglycerides, and HOMA-IR were significantly higher, whereas HDL-C was lower in NAFLD individuals than counterparts without NAFLD (Table 1). Compared with individuals without sarcopenia, sarcopenic counterparts were more likely to be older, less educated. HOMA-IR and CRP were significantly higher, whereas BMI and gait speed were lower in sarcopenic individuals than counterparts without sarcopenia (Table 1).

In these elderly participants, the effects of NAFLD and sarcopenia on all-cause and cardiovascular mortality in multivariate-adjusted models were presented in Table 2 and Table 3, respectively. NAFLD patients had hazard ratios (HR) for all-cause and cardiovascular mortality similar to those without NAFLD (Table 2). Sarcopenia carried a greater risk of all-cause (HR 1.47, 95% CI, 1.20–1.80) and cardiovascular (HR 1.73, 95% CI, 1.20-2.48) mortality. Adjustment for NAFLD, chronic conditions, CRP, and HOMA-IR did not markedly change these associations (Table 3).

SMI showed a strong negative relationship with FIB-4 [25], a noninvasive marker of severity of hepatic fibrosis in subjects with NAFLD (supplementary Fig. S1), which was calculated by the following formula: FIB-4 = (age [years]*AST [U/L])/(PLT [10^9/L] *(ALT [U/L])$^{1/2}$). When other indicators of the severity of hepatic fibrosis such as NFS [26] and APRI [27] were used, results were remarkably the same (data not shown). Table 4 listed the joint associations of NAFLD and sarcopenia with all-cause and cardiovascular mortality. Compared with the reference group (those without NAFLD and sarcopenia), the sex-, age-, ethnicity-, and education-adjusted HR of all-cause mortality was 1.35 (1.06-1.70) for participants with exclusive sarcopenia, 0.97 (0.81-1.15) for participants with exclusive NAFLD, and 1.91 (1.39-2.64) for participants with both NAFLD and sarcopenia. The association of the combined NAFLD and sarcopenia with increased all-cause mortality risk persisted after additional adjustment for BMI, smoking and drinking habits, hypertension, and diabetes status (Model 2). Further adjustment for TC, triglyceride, and HDL-cholesterol did not change the association (Model 3). The addition of inflammation (CRP) did not significantly reduce the HR (Model 4). Since insulin resistance predicted increased mortality risk, we performed an additional adjustment for HOMA-IR, and a similar association was observed (Model 5). Because chronic conditions such as stroke, coronary heart disease, and chronic obstructive pulmonary disease were closely associated with all-cause mortality, a multivariate analysis adjusted for these chronic conditions showed that the significant association remained (Model 6). The combined association of NAFLD and sarcopenia with cardiovascular mortality showed similar patterns (Table 4). The interactions between NAFLD and sarcopenia on all-cause and cardiovascular mortality showed statistical significance, indicating that the associations of NAFLD with all-cause and cardiovascular mortality (all P values <0.05) differed by sarcopenia status. Since fat mass to fat-free mass ratio (FM/FFM) can provide a robust measure of body composition changes [28]. Sarcopenia was also defined as slow gait speed together with increased FM/FFM, which was defined as FM/FFM≥3/4 percentile. We further analyzed the relationship between FM/FFM-defined sarcopenia and all-cause mortality risk (Supplementary Table S2). We found a consistent role of sarcopenia on all-cause mortality, regardless of the definitions used to define sarcopenia (Table 4 and Supplementary Table S2).

Considering that diabetes, hypertension, and inflammation were well-established risk factors for all-cause and cardiovascular mortality, we explored the combined association of NAFLD and sarcopenia with all-cause and cardiovascular mortality after stratification by these conditions. NAFLD patients with sarcopenia conferred increased risks of all-cause (Figure 1) and cardiovascular (Figure 2) mortality, irrespective of the status of diabetes, hypertension, or inflammation. To take into account the potential confounder of age, we stratified the study population by age group (60-69 and ≥ 70 years). NAFLD patients with sarcopenia in both age groups had significantly higher risks of all-cause and cardiovascular mortality. Further, sarcopenia was associated with an equal or higher risk of cardiovascular mortality among individuals aged ≥70 years, indicating that sarcopenia was associated with cardiovascular mortality independent of age.

Discussion

This nationally representative, the elderly population-based study revealed the modulating effect of sarcopenia on the association of NAFLD with all-cause and cardiovascular mortality. Specifically, among elderly individuals, NAFLD was associated with increased risks of all-cause and cardiovascular mortality only among sarcopenic subjects. In contrast, NAFLD was a benign condition for all-cause and cardiovascular mortality among individuals without sarcopenia. To our knowledge, this is the first study to clarify the impact of sarcopenia on the associations between NAFLD and all-cause and cardiovascular mortality. This study further demonstrated that the magnitude of all-cause and cardiovascular mortality risk contributed by sarcopenia appeared to be much greater than the risk imparted by NAFLD. In this study, combined analyses broaden our understanding of risk factors' relative influence on all-cause and cardiovascular mortality.

Investigations that disregarded the potential effect of sarcopenia on mortality concluded that NAFLD was associated with an increased mortality risk [4]. In contrast, other studies reported that mortality risk was not statistically different between subjects with and without NAFLD [5,29,30]. These conflicting data may be attributed, at least in part, to the inability to account for sarcopenia as a potential effect modifier. To date, investigations into sarcopenia as an effect modifier of the association of NAFLD with mortality are sparse. The present study reported that a preserved muscle mass in NAFLD patients may be required to exert benefits towards a reduction in mortality risk. Therefore, sarcopenia may, in part, help explain why some NAFLD individuals have an unfavorable long-term prognosis. Contrary to the benign prognosis of simple steatosis, NAFLD with advanced fibrosis correlates with increased all-cause and cardiovascular mortality [31,32]. A recent investigation provided strong evidence of a close relationship between sarcopenia and liver fibrosis independently of obesity, insulin resistance, and liver enzyme levels among NAFLD subjects [33]. Our results also evidenced the negative relationship between SMI and the severity of hepatic fibrosis. Accordingly, sarcopenia may be a surrogate marker of fibrosis in the state of NAFLD. Hence, our findings suggest the necessity for an active assessment of sarcopenia status in NAFLD subjects.

Several possible theories may serve to explain the sarcopenia-mediated alterations in the association between NAFLD and mortality risk. Compared with non-sarcopenic NAFLD subjects, sarcopenic NAFLD

persons may be more insulin resistant and have lower levels of physical activity, and a higher pro-inflammatory state, all of which are associated with an increased mortality risk.

Our finding that NAFLD is associated with increased mortality risk among sarcopenic subjects is of particular clinical importance. In the United States, NAFLD represents one of the most frequent causes of chronic liver disease and the most common indication for liver transplantation [34]. Worse is that sarcopenia predicts negative preoperative and postoperative outcomes in liver transplant patients [35]. No approved pharmacotherapies for NAFLD are currently available. Considering that resistance training is a potent therapy that improves sarcopenia [36], increasing the skeletal muscle mass through resistance exercise may be a promising potential treatment option for NAFLD. Furthermore, resistance training may be effective in reducing the incidence of NAFLD [37]. Awareness of the accompanying sarcopenia in NAFLD persons when deciding the optimal time for commencing therapeutic interventions is undoubtedly necessary to prevent disease progression and improve the negative long-term outcomes in NAFLD patients.

Information on the relative influence of NAFLD and sarcopenia on mortality risk is limited. We observed a significantly increased mortality risk associated with sarcopenia compared with no increase in the risk associated with NAFLD. Further, we noted that NAFLD was associated with increased risks of all-cause and cardiovascular mortality only in the presence of sarcopenia. Taken together, the magnitude of association with muscle mass is much greater than with adiposity. Our results provide evidence that resistance training and nutritional treatment for sarcopenia may have a greater effect on the reduction of mortality risk than weight reduction. It has been postulated that muscle mass can affect insulin metabolism by releasing healthy myokines such as irisin [38]. A preserved muscle mass may therefore be a key mechanism to increase the secretion of the favorable myokines and subsequently reduce the mortality risk. Further studies are needed to clarify the protective effects of sarcopenia management on the amelioration of NAFLD-associated mortality.

Our study further demonstrated that NAFLD combined with sarcopenia presented the greatest all-cause and cardiovascular mortality risk. However, the mortality risk associated with the combined NAFLD and sarcopenia did not exceed the sum of their individual risk, suggesting that NAFLD and sarcopenia have an interactive rather than additive effect on mortality. Although NAFLD and sarcopenia are viewed as 2 independent variables, they may be interacting with each other and contributing to the same causal pathway leading to all-cause and cardiovascular mortality.

This study has several strengths. First, a population-based analysis using well-examined nationwide data ensures the statistical reliability of our results and generalizability of the data. Second, our study provides solid evidence of an independent association of combined NAFLD and sarcopenia with mortality after adjusting for a variety of important confounders including insulin resistance, inflammatory markers, and comorbid conditions. Third, we applied the measurements of skeletal muscle mass and gait speed, which capture both muscle mass and muscle function, to define sarcopenia. Evidence showed that combining muscle mass with gait speed could better assess health status compared to either measure alone [39].

We acknowledge several limitations. First, results may be biased when using skeletal muscle mass and walking speed, which may vary over time, assessed from a single time point. Second, we classified skeletal muscle mass and gait speed into 2 levels to maximize statistical power. However, there is no apparent threshold in graded associations between these two measures and mortality. Third, since the sample is based on a cohort of participants aged ≥60 years, extrapolating results to younger adults should be done cautiously. Lastly, the cross-sectional study design makes it difficult to infer the causality or temporality between NAFLD/sarcopenia and risks of all-cause and cardiovascular mortality.

Conclusion

NAFLD is associated with an increased all-cause and cardiovascular mortality risk in sarcopenic adults. The association of NAFLD and mortality is less clear in adults without sarcopenia. These findings underscore the critical importance of sarcopenia as a determinant of mortality in NAFLD persons. The magnitude of the association with mortality risk was much greater for sarcopenia than for NAFLD.

Abbreviations

NAFLD: nonalcoholic fatty liver disease, BMI: body mass index, WC: waist circumference, BP: blood pressure, TC: total cholesterol, CRP: C-reactive protein, HOMA-IR: homeostasis model assessment of insulin resistance, BIA: bioelectrical impedance analysis, SMI: skeletal muscle index, HR: hazard ratio, FM:fat mass, FFM: fat-free mass, ICD: Injuries and Causes of Death, NHANES: The National Health and Nutrition Examination Surveys, NCHS: The National Center for Health Statistics, NDI: National Death Index.

Declarations

Conflict of interests

The authors declare that there is no conflict of interest.

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Authors' contributions

XXS conceived the study design, wrote the first draft of the manuscript,

analyzed the data, contributed to interpretation of results, commented on

drafts, and approved the final version. ZLL, FQC and TTD contributed to

interpretation of results, commented on drafts, and approved the final

version. XXS and TTD are the guarantors of this work, and had full access to

all the data in the study and takes responsibility for the integrity of the data

and the accuracy of the data analysis. All authors read and approved the

final manuscript.

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Tables

Table 1 Characteristics of the study participants according to the presence of nonalcoholic fatty liver disease (NAFLD) or sarcopenia. Data are presented as means ± standard errors or percent.

	Without NAFLD	With NAFLD	P (NAFLD vs without NAFLD)	Without sarcopenia	With sarcopenia	P (Sarcopenia vs without Sarcopenia
N	1732	714		2022	444	
Age, years	66.6±0.1	66.6±0.2	0.711	66.4±0.2	67.9±0.4	0.0008
Men, %	41.4	45.0	0.175	40.8	56.6	<0.001
Race ethnicity, %						
Non-Hispanic white	82.9	86. 0	0.839	84.4	80.0	0.143
Non-Hispanic black	8.8	6.2	0.004	7.5	11.4	0.053
Mexican American	2.3	3.4	0.028	2.5	3.4	0.071
Smoking, %	58.5	60.8	0.518	56.7	74.4	<0.001
Education, years	11.6±0.2	11.1±0.2	0.002	11.6±0.2	10.4±0.3	0.0002
Body mass index, kg/m^2	26.5±0.2	30.0±0.3	<0.001	27.8±0.2	25.6±0.3	<0.001
Waist circumference, cm	95.2±0.5	104.8±0.6	<0.001	98.2±0.4	96.5±0.9	0.106
Systolic blood pressure, mmHg	134.5±0.6	140.0±0.9	<0.001	135.8±0.6	137.6±1.5	0.322
Diastolic blood pressure, mmHg	74.8±0.3	75.3±0.5	0.299	74.9±0.3	74.8±0.8	0.860
Plasma glucose, mmol/l	5.6±0.1	6.5±0.1	<0.001	5.9±0.1	5.9±0.1	0.703
HbA1c, %	5.7±0.0	6.2±0.1	<0.001	5.8±0.0	5.8±0.1	0.643
Total cholesterol, mmol/l	5.8±0.0	5.9±0.1	0.181	5.9±0.0	5.8±0.1	0.706
Ln Triglycerides, mmol/l	0.4±0.0	0.8±0.0	<0.001	0.5±0.0	0.5±0.0	0.535
HDL-cholesterol,	1.4±0.0	1.2±0.0	<0.001	1.3±0.0	1.4±0.0	0.173

mmol/l						
LDL-cholesterol, mmol/l	3.7 ±0.1	3.6±0.1	0.218	3.7±0.0	3.6±0.1	0.430
C-reactive protein, mg/l	1.0±0.1	1.0±0.1	0.932	1.0±0.1	1.3±0.1	0.037
Ln HOMA-IR	0.8±0.0	1.4±0.1	<0.001	0.9±0.1	1.2±0.0	0.042
Gait speed, m/s	0.9±0.0	0.9±0.0	0.344	0.9±0.0	0.7±0.0	<0.001
Skeletal muscle index, kg/m^2	8.8±0.1	8.2±0.1	<0.001	8.5±0.1	8.0±0.1	0.003

Table 2 Hazzard ratios (with 95% confidence intervals) for nonalcoholic fatty liver disease -related risk of all-cause and cardiovascular mortality

	Without NAFLD	With NAFLD
All-cause mortality		
Model 1	1	1.0 (0.85-1.17)
Model 2	1	0.85 (0.72-1.10)
Model 3	1	0.85 (0.71-1.20)
Model 4	1	0.85 (0.72-1.01)
Model 5	1	0.83 (0.70-0.98)
Model 6	1	0.89 (0.75-1.05)
Model 7	1	0.90 (0.76-1.06)
Cardiovascular mortality		
Model 1	1	1.16 (0.86-1.57)
Model 2	1	0.98 (0.72-1.33)
Model 3	1	0.91 (0.67-1.24)
Model 4	1	0.91 (0.67-1.24)
Model 5	1	0.88 (0.65-1.21)
Model 6	1	1.0 (0.73-1.37)
Model 7	1	1.02 (0.75-1.39)

Model 1 was adjusted for age, gender, race ethnicity, and education level

Model 2 was adjusted for smoking and drinking status, body mass index, hypertension, and diabetes in addition to the factors included in model 1.

Model 3 was adjusted for total cholesterol, triglyceride, and HDL-cholesterol in addition to the factors included in model 2.

Model 4 was adjusted for C-reactive protein in addition to the factors included in model 3.

Model 5 was adjusted for HOMA-IR in addition to the factors included in model 3.

Model 6 was adjusted for comorbid conditions such as stoke, coronary heart disease, and chronic obstructive pulmonary disease in addition to the factors included in model 3.

Model 7 was adjusted for all the variables in model 6 plus sarcopenia status.

Table 3 Hazzard ratios (with 95% confidence intervals) for sarcopenia-related risk of all-cause and cardiovascular mortality

	Without sarcopenia	With sarcopenia
All-cause mortality		
Model 1	1	1.47 (1.20-1.80)
Model 2	1	1.46 (1.20-1.78)
Model 3	1	1.49 (1.23-1.81)
Model 4	1	1.47 (1.22-1.79)
Model 5	1	1.51 (1.25-1.83)
Model 6	1	1.45 (1.19-1.70)
Model 7	1	1.44 (1.18-1.75)
Cardiovascular mortality		
Model 1	1	1.73 (1.20-2.48)
Model 2	1	1.72 (1.19-2.49)
Model 3	1	1.87 (1.29-2.72)
Model 4	1	1.81 (1.24-2.63)
Model 5	1	1.92 (1.32-2.78)
Model 6	1	1.78 (1.24-2.55)
Model 7	1	1.78 (1.24-2.56)

Model 1 was adjusted for age, gender, race ethnicity, and education level

Model 2 was adjusted for smoking and drinking status, body mass index, hypertension, and diabetes in addition to the factors included in model 1.

Model 3 was adjusted for total cholesterol, triglyceride, and HDL-cholesterol in addition to the factors included in model 2.

Model 4 was adjusted for C-reactive protein in addition to the factors included in model 3.

Model 5 was adjusted for HOMA-IR in addition to the factors included in model 3.

Model 6 was adjusted for comorbid conditions such as stoke, coronary heart disease, and chronic obstructive pulmonary disease in addition to the factors included in model 3.

Model 7 was adjusted for all the variables in model 6 plus nonalcoholic fatty liver disease status.

Table 4 All-cause and cardiovascular mortality during 16.8 years of follow-up according to combinations of nonalcoholic fatty liver disease (NAFLD) and sarcopenia status

	Without NAFLD		NAFLD		Interaction
	Without sarcopenia	Sarcopenia	Without sarcopenia	Sarcopenia	
All-cause mortality					
Model 1	1	1.35 (1.06-1.70)	0.97 (0.81-1.15)	1.91 (1.39-2.64)	0.056
Model 2	1	1.30 (1.03-1.64)	0.80 (0.67-0.95)	1.64 (1.21-2.22)	0.025
Model 3	1	1.32 (1.0-1.65)	0.80 (0.67-0.96)	1.67 (1.21-2.30)	0.026
Model 4	1	1.30 (1.05-1.65)	0.81 (0.67-0.97)	1.62 (1.17-2.24)	0.02
Model 5	1	1.35 (1.07-1.69)	0.78 (0.65-0.94)	1.62 (1.17-2.24)	0.034
Model 6	1	1.29 (1.03-1.62)	0.84 (0.70-1.01)	1.69 (1.23-2.31)	0.03
Cardiovascular mortality					
Model 1	1	1.58 (1.01-2.48)	1.12 (0.80-1.5)	2.62 (1.53-4.48)	0.029
Model 2	1	1.51 (0.95-2.38)	0.91 (0.64-1.29)	2.22 (1.33-3.70)	0.017
Model 3	1	1.65 (1.05-2.61)	0.86 (0.61-1.23)	2.22 (1.29-3.80)	0.022
Model 4	1	1.62 (1.02-2.56)	0.87 (0.60-1.23)	2.0 (1.20-3.58)	0.029
Model 5	1	1.70 (1.08-2.70)	0.84 (0.59-1.19)	2.16 (1.26-3.68)	0.026
Model 6	1	1.63 (1.04-2.55)	0.96 (0.60-1.37)	2.17 (1.33-3.54)	0.033

Model 1 was adjusted for age, gender, race ethnicity, and education level

Model 2 was adjusted for smoking and drinking status, body mass index, hypertension, and diabetes in addition to the factors included in model 1.

Model 3 was adjusted for total cholesterol, triglyceride, and HDL-cholesterol in addition to the factors included in model 2.

Model 4 was adjusted for C-reactive protein in addition to the factors included in model 3.

Model 5 was adjusted for HOMA-IR in addition to the factors included in model 3.

Model 6 was adjusted for comorbid conditions such as stoke, coronary heart disease, and chronic obstructive pulmonary disease in addition to the factors included in model 3.

Figures

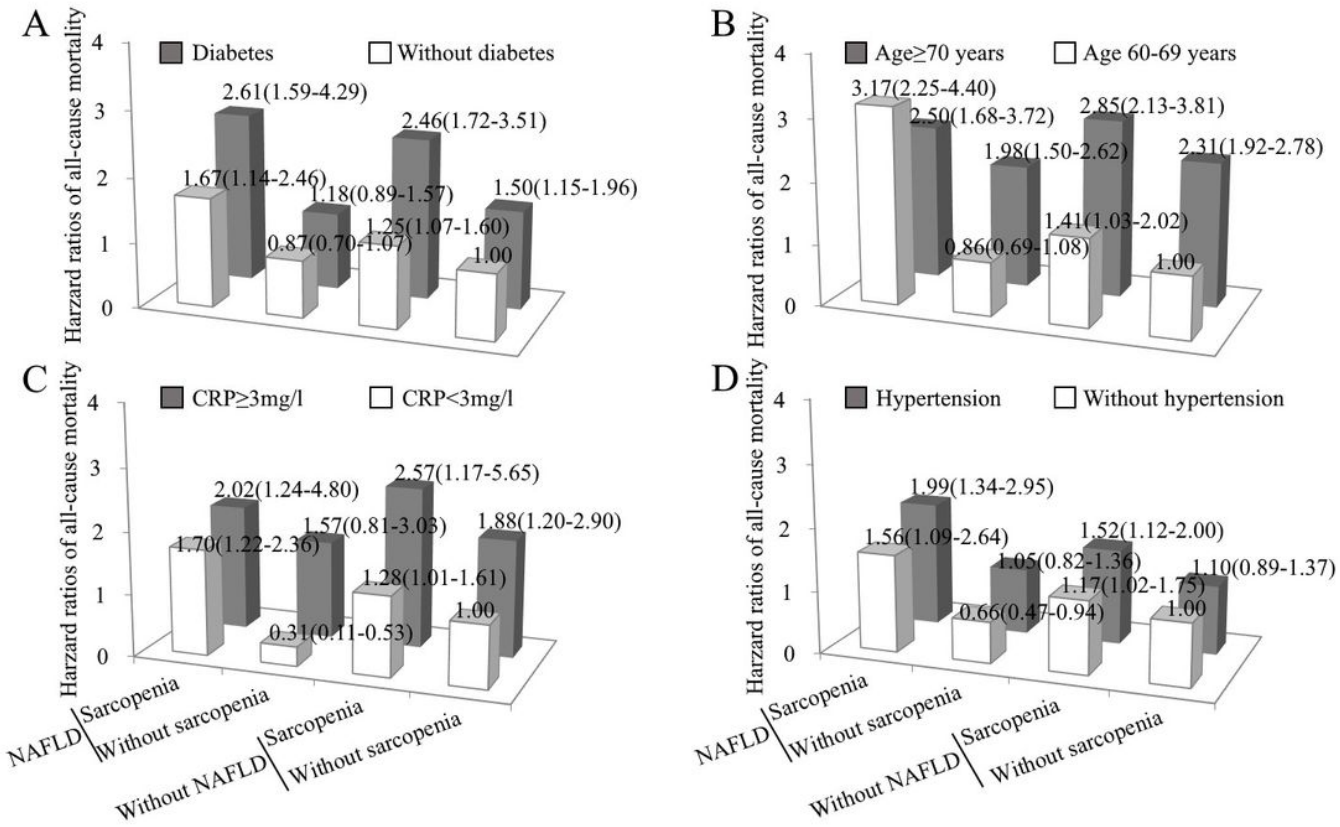

Figure 1

Joint effects of nonalcoholic fatty liver disease (NAFLD) and sarcopenia on all-cause mortality after stratification for diabetes, hypertension, inflammation or age status. All-cause mortality risk was significantly higher in sarcopenic subjects with NAFLD regardless of the status of diabetes, hypertension, inflammation and age. Hazzard ratios (95% confidence intervals) of all-cause mortality were adjusted for age, gender, race ethnicity, education level, smoking and drinking status, body mass index, total cholesterol, triglyceride, HDL-cholesterol, and comorbid conditions such as stoke, coronary heart disease, and chronic obstructive pulmonary disease.

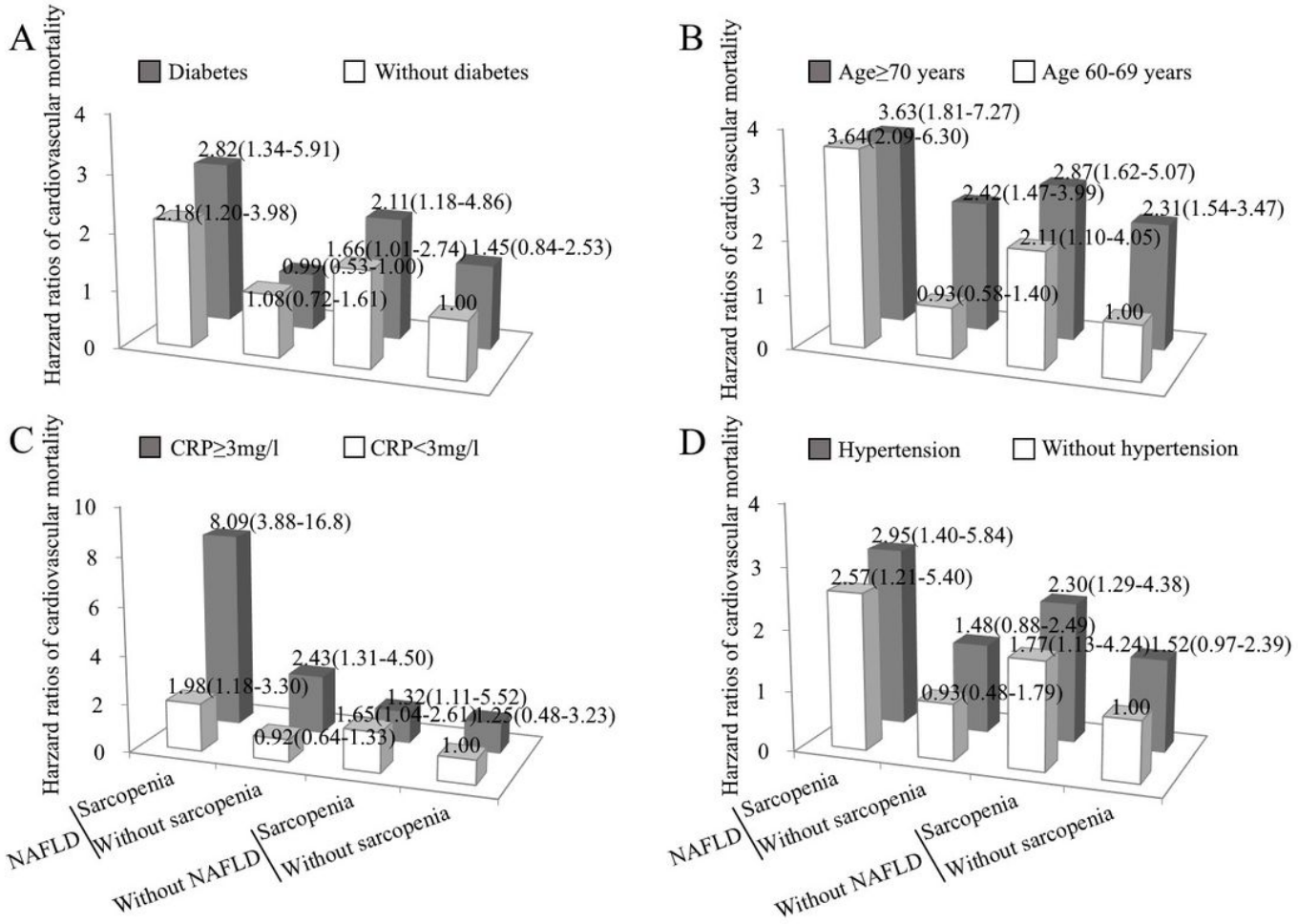

Figure 2

Joint effects of nonalcoholic fatty liver disease (NAFLD) and sarcopenia on cardiovascular mortality after stratification for diabetes, hypertension, inflammation or age. Cardiovascular mortality risk was significantly higher in sarcopenic subjects with NAFLD regardless of the status of diabetes, hypertension, inflammation and age. Hazzard ratios (95% confidence intervals) of cardiovascular mortality were adjusted for age, gender, race ethnicity, education level, smoking and drinking status, body mass index, total cholesterol, triglyceride, HDL-cholesterol, and comorbid conditions such as stoke, coronary heart disease, and chronic obstructive pulmonary disease.

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