

# Association of serum creatinine-to-cystatin C ratio with skeletal muscle mass and strength in nonalcoholic fatty liver disease in the Iwaki Health Promotion Project

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We evaluated the feasibility of using serum creatinine-to-cystatin C ratio in the assessments of muscle mass and strength in nonalcoholic fatty liver disease. In a community-based cross-sectional study, skeletal muscle mass and handgrip strength were assessed in 641 Japanese adults. Low skeletal muscle mass index and low handgrip strength were defined as indicated in the sarcopenia diagnostic criteria of the Japan Society of Hepatology. Nonalcoholic fatty liver disease was defined as fatty liver on ultrasonography in the absence of other causes of steatosis. The creatinine-to-cystatin C ratio was useful for identifying the participants with low skeletal muscle mass index, with an area under the receiver-operating characteristic curve of 0.84 [95% confidence interval (CI), 0.77–0.91] in men and 0.72 in women (95% CI, 0.65–0.78), and those with low handgrip strength, with an area under the receiver-operating characteristic curve of 0.96 (95% CI, 0.93–0.99) in men and 0.79 (95% CI, 0.66–0.92) in women. Moreover, the creatinine-to-cystatin C ratio correlated with skeletal muscle mass index ( $r=0.511$ ,  $p<0.001$ ) and handgrip strength ( $r=0.657$ ,  $p<0.001$ ), whereas it did not correlate with exacerbation of hepatic steatosis. In this study, creatinine-to-cystatin C ratio correlated with muscle mass and strength in nonalcoholic fatty liver disease regardless of hepatic steatosis.

**Key Words:** creatinine, cystatin C, nonalcoholic fatty liver disease (NAFLD), skeletal muscle mass index (SMI), muscle strength

Nonalcoholic fatty liver disease (NAFLD) is one of the most common liver disorders worldwide, affecting approximately 25% of adults.<sup>(1,2)</sup> It progresses to nonalcoholic steatohepatitis, which can develop into liver cirrhosis, liver failure, and hepatocellular carcinoma (HCC).<sup>(3)</sup> Various factors are involved in the pathogenesis of NAFLD, such as lifestyle, nutrition, genetic profile, obesity, and metabolic syndrome.<sup>(1,4)</sup> Recent studies have shown that sarcopenia, which is characterized by loss of skeletal muscle mass and strength,<sup>(5,6)</sup> is a risk factor for NAFLD<sup>(7–9)</sup> and a prognostic factor for liver cirrhosis and HCC.<sup>(10–12)</sup> Thus, early detection of sarcopenia is of primary importance in patients with NAFLD. To assess skeletal muscle mass, bioelectrical impedance analysis (BIA) and body imaging modalities such as computed tomography (CT), dual energy X-ray absorptiometry (DXA), and magnetic resonance imaging (MRI) are recommended.<sup>(5)</sup> In

clinical practice, however, these techniques are often not used owing to their high cost and unavailability. Therefore, to facilitate screening of sarcopenia in patients with NAFLD, more inexpensive and accessible approaches are necessary to assess skeletal muscle mass and strength.

Both creatinine (Cre) and cystatin C (CysC) are excreted from the kidneys in the same manner, and their serum levels are frequently used for evaluation of renal function. Although serum Cre is generated from muscle catabolism, serum CysC is excreted by all nucleated cells and is thus not affected by muscle mass. Consequently, the difference between Cre and CysC reflects the muscle mass of the body. In fact, the serum Cre/CysC ratio indicates residual muscle mass and has been suggested as a surrogate marker of sarcopenia.<sup>(13–15)</sup> However, the association between Cre/CysC ratio and skeletal muscle mass and muscle strength in NAFLD remains unclear. Therefore, in the present study, we assessed the association of serum Cre/CysC ratio with both skeletal muscle mass and muscle strength in a Japanese community population with NAFLD.

## Materials and Methods

**Participants and study design.** This cross-sectional study is a secondary analysis of data from the Iwaki Health Promotion Project, which is an ongoing community-based health promotion study of Japanese people aged  $\geq 20$  years that was designed to prevent lifestyle-related diseases and prolong the lifespan of the population. This program has been conducted annually since 2005 with approximately 1,000 participants in the Iwaki region of Hirosaki City in Aomori Prefecture in northern Japan.<sup>(16–19)</sup> All the study subjects participated voluntarily in response to a public announcement, and approximately 600 data points were collected from each participant, including their demographics, medical history, lifestyle data, and microbiota and blood chemical analysis data. To date, we have shown the association of NAFLD with adipokines, microbiota, and changed amino acids in this health promotion study.<sup>(20–22)</sup> Our research on the association of NAFLD with skeletal muscle mass and strength is one part of this project. In 2016, 1,148 individuals were enrolled in this

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**Table 1.** Demographic and clinical characteristics of the participants

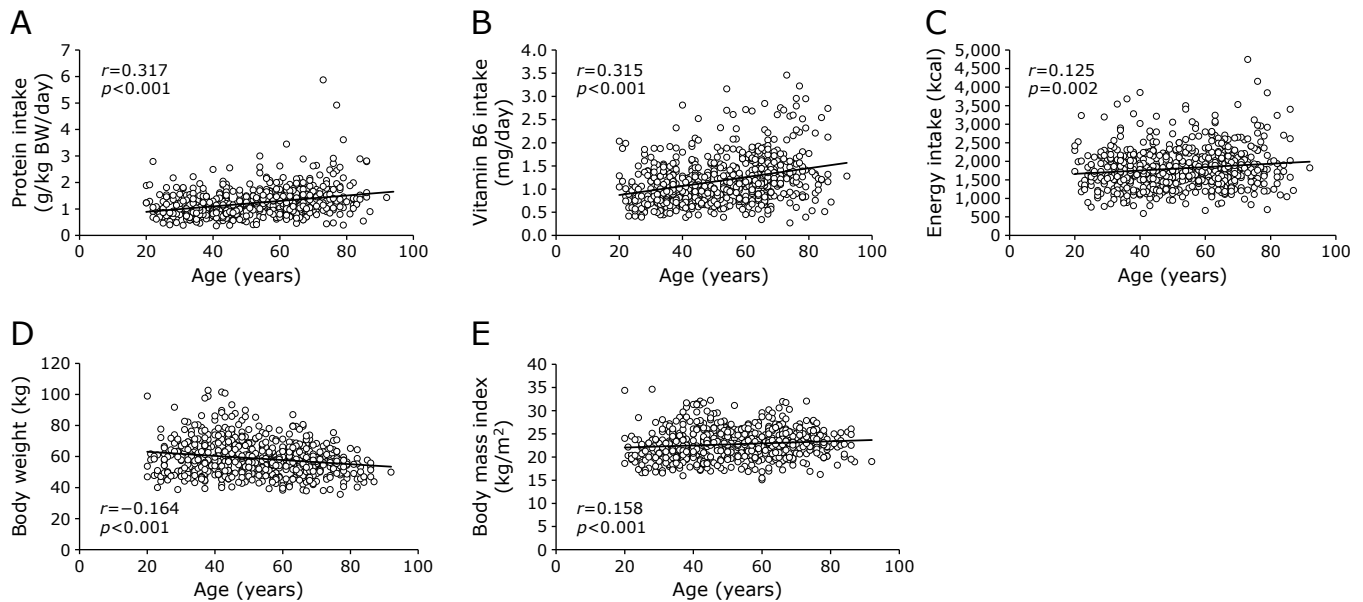
| Characteristics                       | Total (n = 641)     | Men (n = 215)       | Women (n = 426)     | p value |
|---------------------------------------|---------------------|---------------------|---------------------|---------|
| Age (years)                           | 53 (38–66)          | 47 (36–65)          | 55 (42–66)          | 0.006   |
| BW (kg)                               | 57.2 (50.6–65.3)    | 66.3 (58.7–73.8)    | 53.3 (48.2–59.2)    | <0.001  |
| BMI (kg/m <sup>2</sup> )              | 22.6 (20.3–24.8)    | 23.3 (21.6–25.4)    | 22.1 (19.8–24.4)    | <0.001  |
| BFP (%)                               | 26.3 (21.2–32.2)    | 20.8 (16.3–23.8)    | 30.4 (24.8–34.6)    | <0.001  |
| ASM (kg)                              | 16.9 (14.9–21.3)    | 23.3 (21.2–25.7)    | 15.5 (14.3–17.0)    | <0.001  |
| SMI (kg/m <sup>2</sup> )              | 6.77 (6.18–7.77)    | 8.16 (7.53–8.77)    | 6.37 (6.01–6.83)    | <0.001  |
| Handgrip strength (kg)                | 29 (25–36)          | 42 (36–46)          | 25 (23–29)          | <0.001  |
| Daily energy intake (kcal)            | 1,731 (1,449–2,122) | 1,966 (1,653–2,315) | 1,630 (1,377–1,962) | <0.001  |
| Daily protein intake (g)              | 65.5 (51.0–82.4)    | 69.6 (55.3–88.3)    | 63.7 (49.6–79.9)    | 0.002   |
| Daily protein intake (g/kg BW)        | 1.14 (0.87–1.45)    | 1.03 (0.81–1.34)    | 1.18 (0.90–1.52)    | <0.001  |
| Daily vitamin B6 intake (mg)          | 1.10 (0.82–1.42)    | 1.13 (0.87–1.53)    | 1.09 (0.82–1.37)    | 0.109   |
| Hypertension                          | 218 (34.0)          | 80 (37.2)           | 138 (32.3)          | 0.224   |
| Diabetes                              | 58 (9.0)            | 23 (10.6)           | 35 (8.2)            | 0.301   |
| Dyslipidemia                          | 277 (43.2)          | 83 (38.6)           | 194 (45.5)          | 0.094   |
| Current smoker                        | 81 (12.6)           | 51 (23.7)           | 30 (7.0)            | <0.001  |
| Habitual exerciser                    | 225 (35.1)          | 81 (37.6)           | 144 (33.8)          | 0.332   |
| Alcohol consumption (g/day)           | 0.22 (0–5.7)        | 3.5 (0–15.0)        | 0.0 (0.0–2.3)       | <0.001  |
| AST (U/L)                             | 21 (17–25)          | 23 (19–27)          | 20 (17–24)          | <0.001  |
| ALT (U/L)                             | 17 (13–23)          | 22 (17–31)          | 15 (12–20)          | <0.001  |
| GGT (U/L)                             | 20 (14–29)          | 27 (19–40)          | 17 (13–24)          | <0.001  |
| Glucose (mg/dl)                       | 87 (81–95)          | 90 (83–98)          | 87 (80–94)          | <0.001  |
| HbA1c (%)                             | 5.8 (5.5–6.0)       | 5.8 (5.5–6.0)       | 5.8 (5.6–6.0)       | 0.254   |
| Total cholesterol (mg/dl)             | 201 (179–227)       | 198 (175–218)       | 203 (180–229)       | 0.005   |
| Triglyceride (mg/dl)                  | 77 (56–108)         | 91 (66–134)         | 71 (54–99)          | <0.001  |
| BUN (mg/dl)                           | 13.7 (11.5–16.4)    | 14.4 (12.2–16.6)    | 13.4 (11.3–16.4)    | 0.008   |
| Creatinine (mg/dl)                    | 0.67 (0.59–0.79)    | 0.83 (0.74–0.90)    | 0.63 (0.57–0.69)    | <0.001  |
| Cystatin C (mg/L)                     | 0.70 (0.64–0.80)    | 0.74 (0.68–0.82)    | 0.69 (0.61–0.78)    | <0.001  |
| Uric acid (mg/dl)                     | 4.8 (4.0–5.7)       | 5.8 (5.1–6.7)       | 4.27 (0.93)         | <0.001  |
| Cre/CysC ratio                        | 0.96 (0.85–1.09)    | 1.10 (0.98–1.21)    | 0.89 (0.13)         | <0.001  |
| eGFRcre (ml/min/1.73 m <sup>2</sup> ) | 78.3 (68.8–88.1)    | 81.2 (69.8–89.6)    | 77.4 (68.4–87.3)    | 0.044   |
| eGFRcys (ml/min/1.73 m <sup>2</sup> ) | 106.8 (90.8–124.0)  | 109.7 (95.3–123.4)  | 105.5 (88.5–124.9)  | 0.428   |
| NAFLD                                 | 140 (21.8)          | 61 (28.4)           | 79 (18.5)           | 0.004   |

Data are presented as *n* (%) or median (IQR). Hypertension was defined as blood pressure  $\geq 140/90$  mmHg or use of antihypertensive medication. Diabetes was defined as a fasting serum glucose level  $\geq 126$  mg/dl, HbA1c  $\geq 6.5\%$ , use of diabetes medication, or a prior known diabetes diagnosis. Dyslipidemia was defined as total cholesterol level  $\geq 220$  mg/dl, triglyceride level  $\geq 150$  mg/dl, or use of antihyperlipidemic medication. NAFLD, nonalcoholic fatty liver disease; BW, body weight; BMI, body mass index; BFP, body fat percentage; ASM, appendicular skeletal muscle mass; SMI, skeletal muscle mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl-transpeptidase; HbA1c, hemoglobin A1c; BUN, blood urea nitrogen; Cre, creatinine; CysC, cystatin C; eGFRcre, estimated glomerular filtration rates of serum creatinine; eGFRcys, estimated glomerular filtration rate of serum cystatin C.

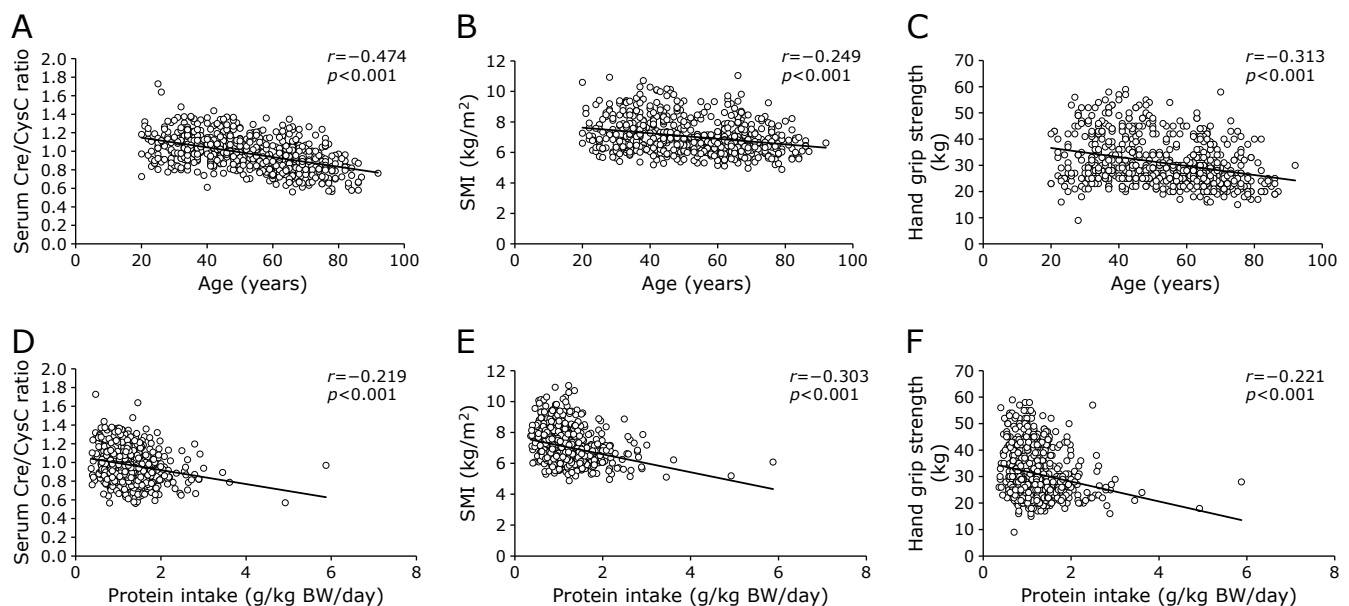
project. Of these individuals, all those who did not have complete clinical data ( $n = 273$ ), tested positive for hepatitis B surface antigen or anti-hepatitis C virus antibody ( $n = 43$ ), and/or had excessive alcohol consumption [daily alcohol intake  $>30$  g/day for men ( $n = 149$ ) and  $>20$  g/day for women ( $n = 42$ )] were excluded. Ultimately, 641 subjects (215 men and 426 women) were included in the present study. This study was approved by the ethics committee of the Hirosaki University School of Medicine, and written informed consent was obtained from all the participants.

**Clinical and laboratory measurements.** The following clinical characteristics were measured: height, body weight (BW), and body composition. Body mass index (BMI) was calculated as BW divided by height squared and expressed in kilograms per meter squared. Body fat percentage (BFP) and appendicular skeletal muscle mass (ASM) were measured with a BIA body composition analyzer (MC-190; Tanita Corp., Tokyo, Japan). The skeletal muscle mass index (SMI) was calculated as the ASM divided by height squared (ASM/height<sup>2</sup>, kg/m<sup>2</sup>).<sup>(23,24)</sup>

Low SMI and low handgrip strength were defined as indicated in the sarcopenia diagnostic criteria of the Japan Society of Hepatology.<sup>(25)</sup> Basically, low SMI was defined as  $<7.0$  kg/m<sup>2</sup> for men and  $<5.7$  kg/m<sup>2</sup> for women. Muscle strength was measured with a handheld dynamometer with the subject in a standing position. Handgrip strength was recorded two times for each hand, and the highest value was used for the analysis. Low handgrip strength was defined as  $<26$  kg for men and  $<18$  kg for women. Usual dietary intakes were assessed by using a brief-type self-administered diet history questionnaire (BDHQ), which is commonly used in assessing dietary habits in Japan.<sup>(26)</sup> Smoking and exercise habits were determined from responses to a questionnaire. Diabetes was defined as a fasting serum glucose level  $\geq 126$  mg/dl, HbA1c  $\geq 6.5\%$ , use of diabetes medication, or a prior known diabetes diagnosis. Dyslipidemia was defined as a total cholesterol level  $\geq 220$  mg/dl, triglyceride level  $\geq 150$  mg/dl, or use of antihyperlipidemic medication. Hypertension was defined as blood pressure  $\geq 140/90$  mm Hg or use of antihypertensive medication.



**Fig. 1.** Relationship of age with nutrition intake and body composition. The correlation of age with protein intake (A), vitamin B6 intake (B), body weight (C), energy intake (D), and body mass index (E).



**Fig. 2.** Association with age or protein intake among serum Cre/CysC ratio, SMI, and handgrip strength. The correlations of age with serum Cre/CysC ratio (A), SMI (B), and handgrip strength (C). The correlations of protein intake with serum Cre/CysC ratio (D), SMI (E), and handgrip strength (F). The Spearman correlation coefficient was used for the statistical evaluation. SMI, skeletal muscle mass index; Cre/CysC, creatinine-to-cystatin C.

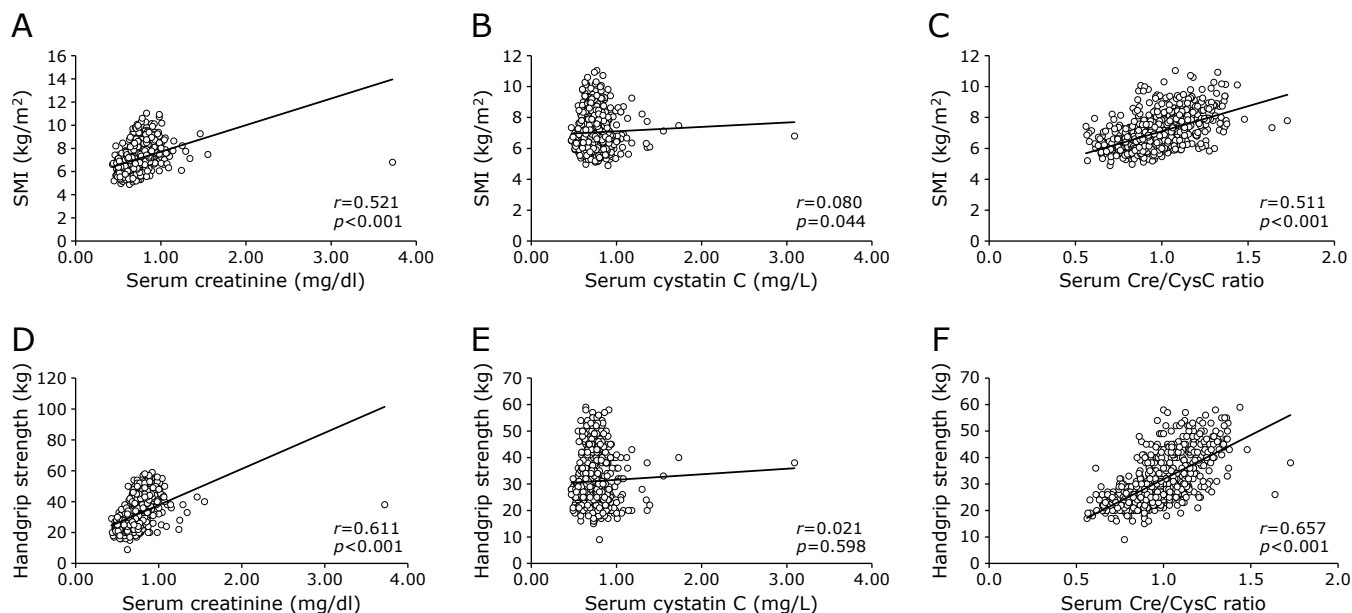
**Table 2.** Number of habitual exercisers by age

|                    | 20–39 years (n = 165) | 40–64 years (n = 298) | ≥65 years (n = 178) | p value for trend |
|--------------------|-----------------------|-----------------------|---------------------|-------------------|
| Habitual exerciser | 44 (26.6)             | 89 (29.8)             | 92 (51.6)           | <0.001            |

Data are presented as n (%).

Whole blood samples were obtained after an overnight fast, and serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl-transpeptidase (GGT),

glucose, glycated hemoglobin (HbA1c), total cholesterol, triglyceride, blood urea nitrogen (BUN), Cre, CysC, and uric acid were determined (LSI Medience Corp., Tokyo, Japan). The



**Fig. 3.** Association of SMI, handgrip strength, and serum markers. The correlations of SMI with serum creatinine level (A), cystatin C level (B), and Cre/CysC ratio (C), and handgrip strength with serum creatinine level (D), cystatin C level (E), and Cre/CysC ratio (F), are shown. The Spearman correlation coefficient was used for the statistical evaluation. SMI, skeletal muscle mass index; Cre/CysC, creatinine-to-cystatin C.

estimated glomerular filtration rates (eGFR) of Cre (eGFRcre) and CysC (eGFRcys) were calculated from the serum Cre and CysC levels, respectively, using equations based on the guidelines of the Japanese Society of Nephrology for Japanese patients as follows: eGFRcre (male) =  $194 \times \text{serum Cre (mg/dl)}^{-1.094} \times \text{age (years)}^{-0.287}$ , eGFRcre (female) = eGFRcre (male)  $\times 0.739$ , eGFRcys (male) =  $(104 \times \text{serum CysC (mg/L)}^{-1.019} \times 0.996^{\text{age (years)}}) - 8$ , and eGFRcys (female) =  $(104 \times \text{serum CysC (mg/L)}^{-1.019} \times 0.996^{\text{age (years)}} \times 0.929) - 8$ . Serum Cre/CysC ratio was calculated as serum Cre (mg/dl) divided by serum CysC (mg/L).

**Assessment of NAFLD.** NAFLD was diagnosed on the basis of abdominal ultrasonography findings using a Prosound F37 (Hitachi Aloka Medical Ltd., Tokyo, Japan). Ultrasonography examinations were performed by one of five well-trained hepatology specialists, each with >5 years of experience, without detailed knowledge of the participants' data. Images were stored and reevaluated by a single hepatologist with >20 years of experience. Based on B-mode ultrasonography observation, the severity of echogenicity was graded as follows: normal, normal echogenicity; mild, slight diffuse increase in fine echoes in liver parenchyma with normal visualization of the diaphragm and intrahepatic vessel borders; moderate, moderate diffuse increase in fine echoes with slightly impaired visualization of the hepatic vessel border and diaphragm; severe, marked increase in fine echoes with poor or non-visualization of the intrahepatic vessel borders, diaphragm, and posterior right lobe of the liver.<sup>(27)</sup>

**Statistical analyses.** Categorical variables were compared using the chi-square test. Characteristics were compared between the male and female subjects, subjects with normal SMI and those with low SMI, and subjects with normal handgrip strength and those with low handgrip strength by using the Mann-Whitney *U* test. The Kruskal-Wallis test and Spearman correlation coefficient were used to calculate the correlation between protein intake and age, Cre/CysC ratio, SMI, or handgrip strength; and the correlation between hepatic steatosis and SMI, handgrip strength, or Cre/CysC ratio. Cochran-Armitage test was used to analyze the association of habitual exerciser with age. Area under the receiver-operating characteristic curve (AUROC) analyses were performed to assess the diagnostic value of serum

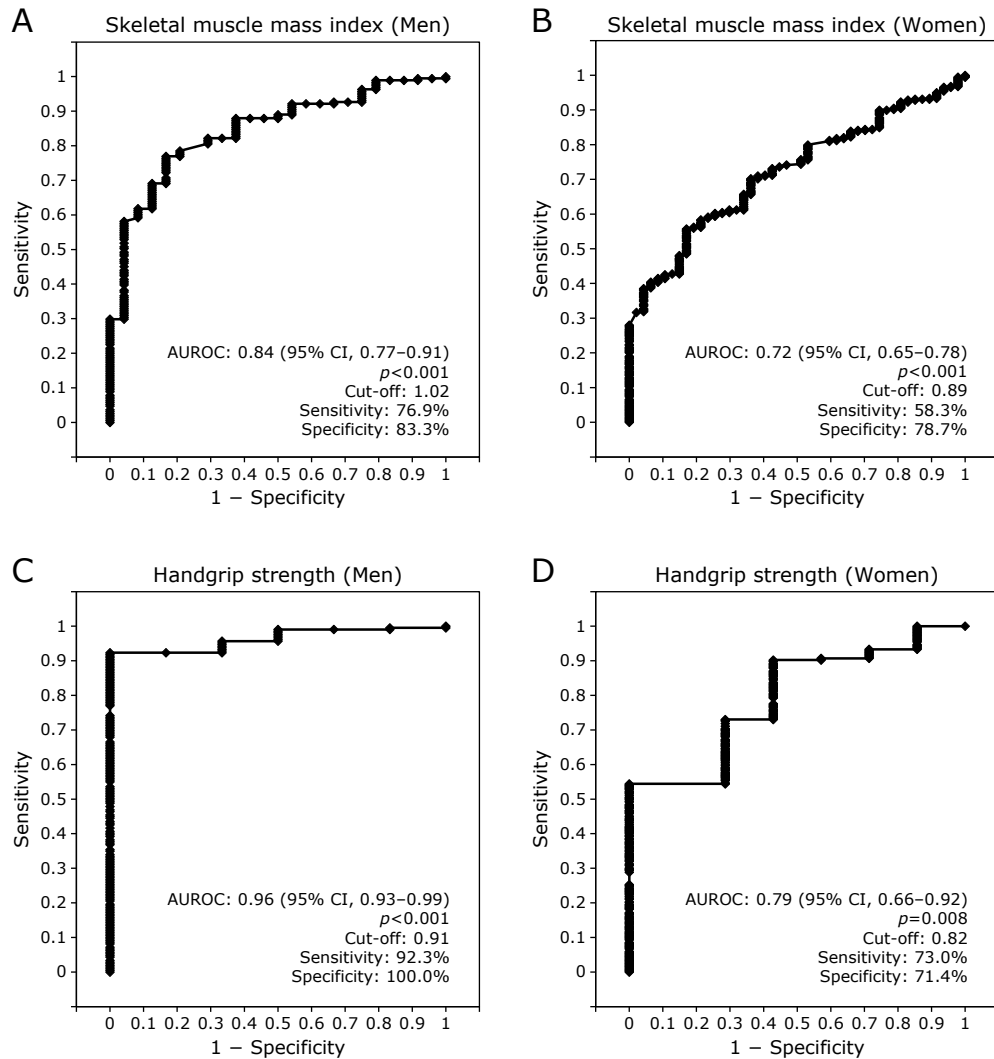
Cre/CysC ratio for low muscle mass and strength. The sensitivity and specificity were calculated at an optimal cutoff point. All the statistical analyses of the collected data were performed using the Excel statistical software package for Macintosh (Excel-Toukei 2016; Esumi Co., Ltd., Tokyo, Japan). Differences were considered significant with *p* values of <0.05.

## Results

**Characteristics of the participants.** Table 1 summarizes the characteristics and sex distribution of 215 men (61 NAFLD cases, 28.4%) and 426 women (79 NAFLD cases, 18.5%), with median ages of 47 and 55 years, respectively. Among the 641 participants, 218 participants (34.0%) had hypertension, 58 (9.0%) had diabetes, 277 (43.2%) had dyslipidemia, 81 (12.6%) were current smokers, and 225 (35.1%) were habitual exercisers. None of the participants had signs of chronic liver disease or liver cirrhosis as evaluated with ultrasonography. The women had lower BW, BMI, ASM, SMI, handgrip strength, daily energy and protein intake, and serum levels of AST, ALT, GGT, glucose, triglyceride, and uric acid, and higher BFP and serum total cholesterol levels than the men. There was no difference in daily vitamin B6 intake between men and women. In addition, the serum levels of Cre and CysC, eGFRcre, and serum Cre/CysC ratios were significantly lower in the women, whereas no significant difference in eGFRcys was found between the men and women.

**Association of protein intake with Cre/Cys ratio, SMI, and handgrip strength.** Daily protein and vitamin B6 intake were increased with aging ( $r = 0.317$ ,  $p < 0.001$  and  $r = 0.315$ ,  $p < 0.001$ , respectively; Fig. 1A and B), while little correlations were found between age and energy intake, BW, or BMI (Fig. 1C–E).

On the other hand, serum Cre/Cys ratio, SMI, and handgrip strength were decreased with aging ( $r = -0.474$ ,  $r = -0.249$ , and  $r = -0.313$ , respectively, all  $p < 0.001$ ; Fig. 2A–C). Serum Cre/Cys ratio, SMI, and handgrip strength had a weak negative correlation ( $r = -0.219$ ,  $r = -0.303$ , and  $r = -0.221$ , respectively, all  $p < 0.001$ ; Fig. 2D–F). The number of habitual exercisers was increased with advancing years (Table 2).



**Fig. 4.** AUROCs of serum Cre/CysC ratio for the prediction of low SMI and handgrip strength. The SMI values of the men (A) and women (B), and handgrip strengths of the men (C) and women (D), are shown.

**Association of Cre/Cys ratio with SMI and handgrip strength.** Serum creatinine level and Cre/CysC ratio both positively correlated with SMI ( $r = 0.521$ ,  $p < 0.001$  and  $r = 0.511$ ,  $p < 0.001$ , respectively; Fig. 3A and C) and handgrip strength ( $r = 0.611$ ,  $p < 0.001$  and  $r = 0.657$ ,  $p < 0.001$ , respectively; Fig. 3D and F), while serum cystatin C level did not significantly correlate with either SMI or handgrip strength (Fig. 3B and E).

When Cre/CysC ratio was estimated using an AUROC analysis, Cre/CysC ratio presented an acceptable AUROC for predicting normal and low SMI (Fig. 4A and B), and normal and low handgrip strength (Fig. 4C and D), in both the men and women.

Correlation analysis revealed that Cre/CysC ratio positively correlated with eGFR<sub>cre</sub> ( $r = 0.551$ ,  $p < 0.001$ ) and negatively correlated with age ( $r = -0.474$ ,  $p < 0.001$ ) and BFP ( $r = -0.460$ ,  $p < 0.001$ ), but it revealed no correlation between Cre/CysC ratio and NAFLD (Table 3).

**Severity of hepatic steatosis and Cre/Cys ratio, SMI, or handgrip strength.** Table 4 shows the data stratified by hepatic steatosis. The distribution of hepatic steatosis was 78.1% for normal, 12.8% for mild steatosis, 7.5% for moderate steatosis, and 1.6% for severe steatosis. The proportion of women tended to decrease with advanced hepatic steatosis. The

**Table 3.** Correlation between serum Cre/CysC ratio and selected variables

| Variables                                         | <i>r</i> | <i>p</i> value |
|---------------------------------------------------|----------|----------------|
| eGFR <sub>cre</sub> (ml/min/1.73 m <sup>2</sup> ) | 0.551    | <0.001         |
| Age (years)                                       | -0.474   | <0.001         |
| BFP (%)                                           | -0.46    | <0.001         |

eGFR, estimated glomerular filtration rate; BFP, body fat percentage.

participants with advanced hepatic steatosis had higher BMI, BFP, ASM, and daily protein intake, and higher prevalence rates of hypertension, diabetes, and dyslipidemia. The serum levels of AST, ALT, GGT, glucose, HbA1c, total cholesterol, and triglyceride increased with the severity of hepatic steatosis. The daily vitamin B6 intake, serum Cre/CysC ratio, eGFR<sub>cre</sub>, and eGFR<sub>cre</sub> did not correlate with the severity of hepatic steatosis.

Table 5 shows a comparison of the characteristics of the participants with low SMI or handgrip strength and those with normal values. Of the 641 participants, 71 (11.0%) had low SMI and 13 (2.0%) had low handgrip strength. The participants with low SMI or handgrip strength were both older and had higher



**Table 4.** Clinical characteristics of participants, stratified by steatosis severity

| Characteristics                       | Hepatic steatosis  |                    |                   |                    | p value for trend |
|---------------------------------------|--------------------|--------------------|-------------------|--------------------|-------------------|
|                                       | Normal (n = 501)   | Mild (n = 82)      | Moderate (n = 48) | Severe (n = 10)    |                   |
| Age (years)                           | 52 (37–66)         | 54 (43–66)         | 59 (45–66)        | 49 (42–52)         | 0.253             |
| Women                                 | 347 (69.2)         | 46 (56.0)          | 28 (58.3)         | 5 (50.0)           | 0.039             |
| BMI (kg/m <sup>2</sup> )              | 21.9 (19.8–23.6)   | 24.7 (23.1–26.0)   | 25.8 (24.1–27.5)  | 30.6 (25.7–32.2)   | <0.001            |
| BFP (%)                               | 25.7 (20.5–31.2)   | 28.7 (23.1–34.1)   | 32.5 (25.5–36.4)  | 35.9 (32.2–43.9)   | <0.001            |
| ASM (kg)                              | 16.4 (14.8–20.0)   | 18.4 (16.3–23.3)   | 18.3 (16.2–25.7)  | 22.1 (18.7–28.9)   | <0.001            |
| SMI (kg/m <sup>2</sup> )              | 6.64 (6.09–7.49)   | 7.31 (6.61–8.37)   | 7.35 (6.70–8.88)  | 8.13 (7.26–10.00)  | <0.001            |
| Handgrip strength (kg)                | 28 (24–35)         | 30 (26–43)         | 31.5 (26–42)      | 35 (25–46)         | <0.001            |
| Daily protein intake (g/kg BW)        | 1.16 (0.90–1.49)   | 1.05 (0.80–1.36)   | 1.01 (0.79–1.41)  | 0.91 (0.63–1.06)   | 0.002             |
| Daily vitamin B6 intake (mg)          | 1.09 (0.83–1.38)   | 1.15 (0.80–1.44)   | 1.17 (0.88–1.63)  | 0.97 (0.64–1.47)   | 0.594             |
| Hypertension                          | 152 (30.3)         | 35 (42.6)          | 25 (52.0)         | 6 (60.0)           | 0.001             |
| Diabetes                              | 36 (7.1)           | 7 (8.5)            | 13 (27.0)         | 2 (20.0)           | <0.001            |
| Dyslipidemia                          | 194 (38.7)         | 45 (54.8)          | 31 (64.5)         | 7 (70.0)           | <0.001            |
| Current smoker                        | 64 (12.7)          | 7 (8.5)            | 8 (16.6)          | 2 (20.0)           | 0.484             |
| Habitual exerciser                    | 176 (35.1)         | 34 (41.4)          | 13 (27.0)         | 2 (20.0)           | 0.282             |
| Alcohol consumption (g/day)           | 0.23 (0.00–5.61)   | 0.00 (0.00–4.77)   | 0.05 (0.00–10.03) | 0.00 (0.00–0.00)   | 0.657             |
| AST (U/L)                             | 20 (17–24)         | 21 (18–25)         | 27 (22–33)        | 26 (21–30)         | <0.001            |
| ALT (U/L)                             | 15 (12–20)         | 21 (17–30)         | 35 (23–44)        | 36 (31–65)         | <0.001            |
| GGT (U/L)                             | 18 (14–26)         | 24 (17–33)         | 34 (25–44)        | 53 (38–77)         | <0.001            |
| Glucose (mg/dl)                       | 86 (80–94)         | 91 (85–99)         | 95 (86–106)       | 93 (91–119)        | <0.001            |
| HbA1c (%)                             | 5.7 (5.5–5.9)      | 5.9 (5.6–6.2)      | 6.0 (5.8–6.5)     | 5.9 (5.9–6.1)      | <0.001            |
| Total cholesterol (mg/dl)             | 199 (177–223)      | 209 (187–228)      | 209 (197–243)     | 217 (186–236)      | 0.001             |
| Triglyceride (mg/dl)                  | 70 (53–98)         | 96 (73–143)        | 114 (93–158)      | 157 (104–242)      | <0.001            |
| BUN (mg/dl)                           | 13.7 (11.4–16.4)   | 13.8 (11.9–16.2)   | 14.3 (12.3–16.3)  | 12.6 (11.7–13.6)   | 0.771             |
| Creatinine (mg/dl)                    | 0.67 (0.59–0.77)   | 0.72 (0.64–0.81)   | 0.71 (0.64–0.81)  | 0.68 (0.60–0.78)   | 0.01              |
| Cystatin C (mg/L)                     | 0.70 (0.63–0.79)   | 0.71 (0.64–0.81)   | 0.75 (0.68–0.83)  | 0.74 (0.66–0.82)   | 0.056             |
| Uric acid (mg/dl)                     | 4.6 (3.9–5.5)      | 5.2 (4.4–6.3)      | 5.8 (4.7–6.9)     | 6.6 (5.7–6.9)      | <0.001            |
| Cre/CysC ratio                        | 0.96 (0.84–1.08)   | 1.00 (0.88–1.12)   | 0.96 (0.85–1.07)  | 0.84 (0.78–1.18)   | 0.383             |
| eGFRcre (ml/min/1.73 m <sup>2</sup> ) | 78.6 (69.2–88.5)   | 77.4 (67.5–84.8)   | 77.5 (68.7–86.0)  | 85.5 (76.2–95.0)   | 0.443             |
| eGFRcys (ml/min/1.73 m <sup>2</sup> ) | 108.2 (90.8–124.4) | 102.7 (93.1–125.2) | 96.2 (90.1–116.8) | 106.3 (90.3–123.1) | 0.127             |

Data are presented as *n* (%) or median (IQR). NAFLD, nonalcoholic fatty liver disease; BMI, body mass index; BFP, body fat percentage; ASM, appendicular skeletal muscle mass; SMI, skeletal muscle mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl-transpeptidase; HbA1c, hemoglobin A1c; BUN, blood urea nitrogen; Cre, creatinine; CysC, cystatin C; eGFRcre, estimated glomerular filtration rates of serum creatinine; eGFRcys, estimated glomerular filtration rate of serum cystatin C.

serum CysC levels and lower Cre/CysC ratio and eGFRcys. The participants with low SMI had a lower prevalence of NAFLD, lower BMI, lower handgrip strength, and lower serum creatinine levels than those with normal SMI, while those with low handgrip strength had a higher prevalence of dyslipidemia.

SMI increased with the severity of hepatic steatosis in both the men and women. On the other hand, handgrip strength and Cre/CysC ratio were not associated with the severity of hepatic steatosis in either the men or women (Fig. 5).

We also analyzed the relationship between Cre/CysC ratio, SMI, and handgrip strength, which was stratified by the severity of hepatic steatosis. Cre/CysC ratio positively correlated with both SMI and handgrip strength at all degrees of severity of hepatic steatosis (Fig. 6).

## Discussion

This study showed that serum Cre/CysC ratio correlated both with SMI and handgrip strength regardless of severity of hepatic steatosis in NAFLD.

Recent studies have revealed that serum Cre/CysC ratio correlates with skeletal muscle mass and may be a screening marker for sarcopenia.<sup>(13–15)</sup> Furthermore, Cre/CysC ratio correlated with

muscle strength<sup>(28)</sup> and predicted prognostic outcomes in various diseases, including hepatocellular carcinoma.<sup>(29–33)</sup> However, to the best of our knowledge, the association between Cre/CysC ratio and sarcopenia in NAFLD has never been reported. Thus, our study is the first to show a positive relationship between Cre/CysC ratio and both muscle mass and muscle strength in NAFLD.

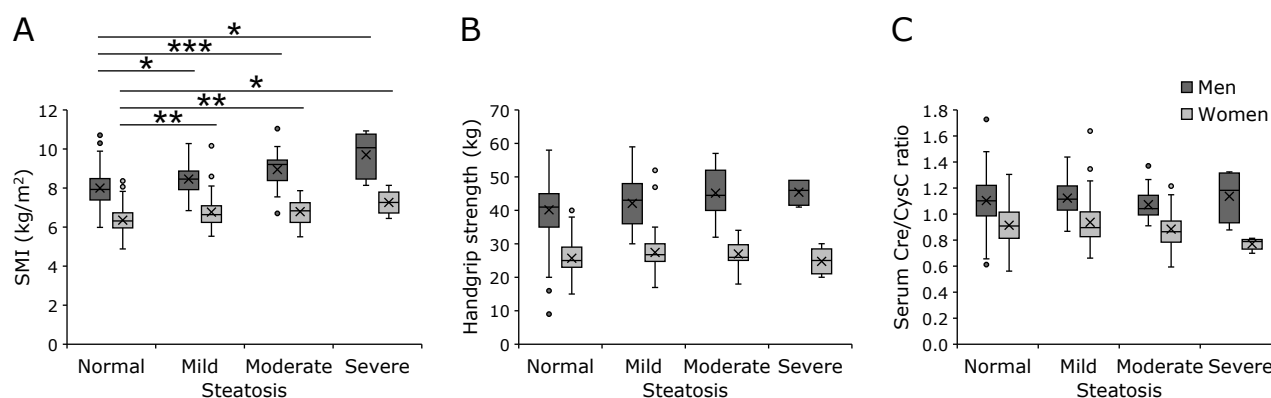
Serum Cre/CysC ratio could distinguish the participants with low muscle mass and strength from those with normal muscle mass and strength in both the men and women in our study. Moreover, we observed that Cre/CysC ratio was positively associated with eGFRcys and negatively associated with age and BFP, as was shown in previous reports.<sup>(13,34,35)</sup> Namely, loss of muscle mass evaluated with SMI was associated with aging, renal dysfunction, and obesity. These factors exactly matched the factors responsible for sarcopenia, which involves multifactorial processes, including aging- and disease-related sarcopenia, and sarcopenic obesity.<sup>(36,37)</sup> Therefore, the decrement of serum Cre/CysC ratio might reflect the progression of sarcopenia.

In our study, SMI was increased with aggravation of hepatic steatosis in both men and women. On the other hand, handgrip strength was not associated with severity of hepatic steatosis. Although an association between sarcopenia and NAFLD has

**Table 5.** Clinical characteristics of the participants with normal or low SMI and handgrip strength

| Characteristics                       | SMI                |                   |         | Handgrip strength  |                  |         |
|---------------------------------------|--------------------|-------------------|---------|--------------------|------------------|---------|
|                                       | Normal (n = 570)   | Low (n = 71)      | p value | Normal (n = 628)   | Low (n = 13)     | p value |
| Age (years)                           | 50 (38–64)         | 65 (53–76)        | <0.001  | 52 (38–65)         | 75 (66–79)       | 0.002   |
| Women                                 | 379 (43.5)         | 47 (66.1)         | 0.961   | 419 (66.7)         | 7 (53.8)         | 0.331   |
| NAFLD                                 | 135 (23.7)         | 5 (7.0)           | 0.001   | 139 (22.1)         | 1 (7.7)          | 0.212   |
| BMI (kg/m <sup>2</sup> )              | 22.9 (20.7–25.1)   | 19.8 (18.1–21.6)  | <0.001  | 22.6 (20.3–24.8)   | 22.5 (20.1–25.0) | 0.801   |
| BFP (%)                               | 26.5 (21.5–32.8)   | 24.7 (18.9–28.4)  | 0.002   | 26.2 (21.2–32.1)   | 31.8 (23.2–36.2) | 0.146   |
| ASM (kg)                              | 17.1 (15.3–21.9)   | 14.0 (12.9–16.3)  | <0.001  | 17.0 (15.0–21.3)   | 14.8 (13.8–15.9) | 0.006   |
| SMI (kg/m <sup>2</sup> )              | 6.87 (6.29–7.86)   | 5.63 (5.46–6.64)  | <0.001  | 6.77 (6.18–7.79)   | 6.81 (6.60–6.90) | 0.538   |
| Handgrip strength (kg)                | 29 (25–38)         | 25 (22–29)        | <0.001  | 29 (25–37)         | 17 (16–20)       | <0.001  |
| Hypertension                          | 189 (33.1)         | 29 (40.8)         | 0.197   | 211 (33.5)         | 7 (53.8)         | 0.127   |
| Diabetes                              | 51 (8.9)           | 7 (9.8)           | 0.801   | 58 (9.2)           | 0 (0.0)          | 0.251   |
| Dyslipidemia                          | 242 (42.4)         | 35 (49.2)         | 0.273   | 267 (42.5)         | 10 (76.9)        | 0.013   |
| Current smoker                        | 77 (13.5)          | 4 (5.6)           | 0.06    | 80 (12.7)          | 1 (7.6)          | 0.588   |
| Habitual exerciser                    | 196 (34.3)         | 29 (40.8)         | 0.282   | 219 (34.8)         | 6 (46.1)         | 0.399   |
| Alcohol (g/day)                       | 0.23 (0.00–6.85)   | 0.00 (0.00–2.81)  | 0.019   | 0.22 (0.00–5.85)   | 0.00 (0.00–0.00) | 0.067   |
| AST (U/L)                             | 20 (17–25)         | 22 (19–25)        | 0.166   | 21 (17–25)         | 21 (18–24)       | 0.957   |
| ALT (U/L)                             | 17 (13–24)         | 15 (12–19)        | 0.012   | 17 (13–23)         | 14 (11–28)       | 0.06    |
| GGT (U/L)                             | 20 (15–30)         | 18 (13–23)        | 0.018   | 20 (14–30)         | 18 (15–21)       | 0.425   |
| Glucose (mg/dl)                       | 87 (81–95)         | 88 (82–94)        | 0.974   | 87 (81–95)         | 89 (85–92)       | 0.694   |
| HbA1c (%)                             | 5.8 (5.5–6.0)      | 5.8 (5.6–6.0)     | 0.25    | 5.8 (5.5–6.0)      | 5.8 (5.7–6.0)    | 0.597   |
| TC (mg/dl)                            | 200 (177–226)      | 208 (192–236)     | 0.018   | 201 (179–227)      | 216 (186–227)    | 0.219   |
| TG (mg/dl)                            | 78 (57–110)        | 68 (52–96)        | 0.034   | 77 (56–108)        | 77 (56–105)      | 0.894   |
| BUN (mg/dl)                           | 13.5 (11.4–16.3)   | 14.8 (12.5–18.1)  | 0.009   | 13.7 (11.5–16.4)   | 17.9 (13.3–20.9) | 0.063   |
| Cre (mg/dl)                           | 0.68 (0.60–0.80)   | 0.66 (0.55–0.72)  | 0.033   | 0.67 (0.60–0.80)   | 0.63 (0.59–0.66) | 0.137   |
| CysC (mg/L)                           | 0.70 (0.63–0.78)   | 0.77 (0.67–0.88)  | 0.001   | 0.70 (0.64–0.79)   | 0.80 (0.73–0.90) | 0.006   |
| UA (mg/dl)                            | 4.8 (4.0–5.8)      | 4.5 (3.7–5.4)     | 0.054   | 4.7 (4.0–5.7)      | 5.1 (3.9–5.4)    | 0.656   |
| Cre/CysC ratio                        | 0.98 (0.86–1.11)   | 0.85 (0.78–0.94)  | <0.001  | 0.96 (0.85–1.09)   | 0.82 (0.72–0.89) | <0.001  |
| eGFRcre (ml/min/1.73 m <sup>2</sup> ) | 78.2 (69.4–87.6)   | 79.5 (64.5–90.7)  | 0.903   | 78.3 (69.0–87.9)   | 75.0 (67.8–97.2) | 0.781   |
| eGFRcys (ml/min/1.73 m <sup>2</sup> ) | 108.8 (93.5–124.9) | 94.9 (76.7–110.7) | <0.001  | 107.4 (91.6–124.3) | 88.7 (71.8–93.7) | 0.002   |

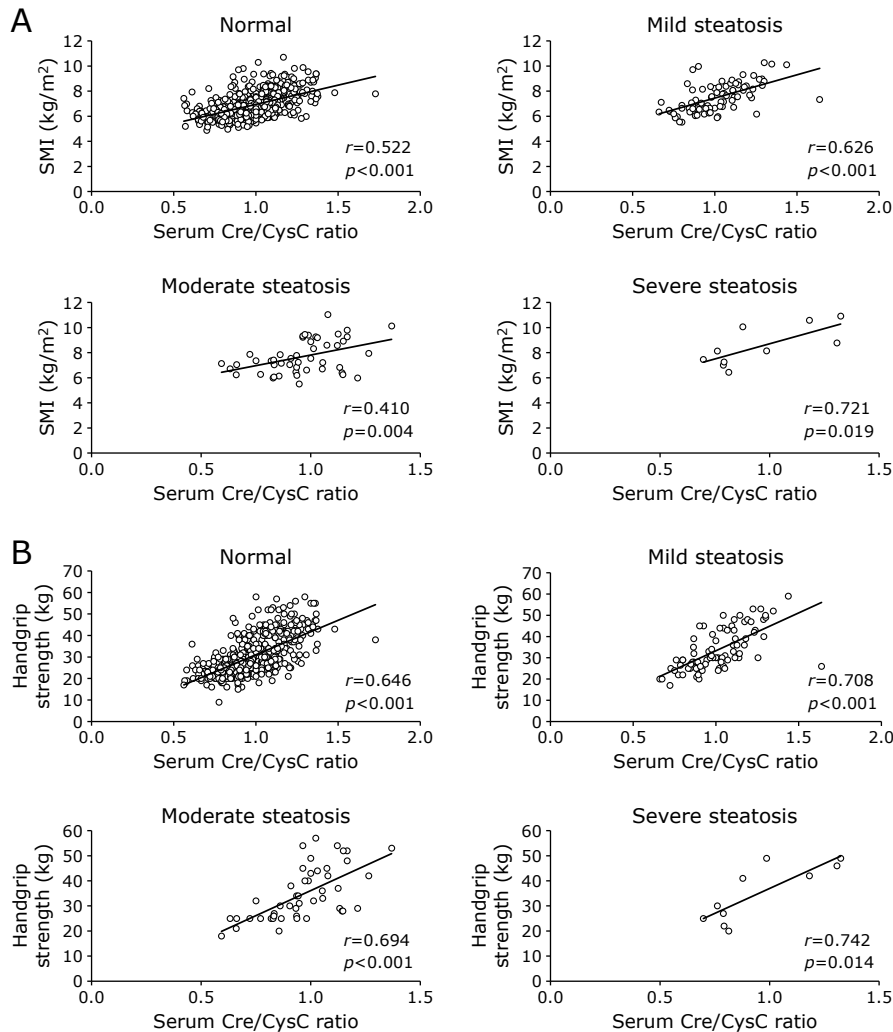
Data are presented as *n* (%) or median (IQR). Low SMI was defined as SMI <7.0 kg/m<sup>2</sup> for men and <5.7 kg/m<sup>2</sup> for women. Low handgrip strength was defined as handgrip strength <26 kg for men and <18 kg for women. NAFLD, nonalcoholic fatty liver disease; BMI, body mass index; BFP, body fat percentage; ASM, appendicular skeletal muscle mass; SMI, skeletal muscle mass index; AST, aspartate aminotransferase; ALT, alanine amino-transferase; GGT, gamma-glutamyl-transpeptidase; HbA1c, hemoglobin A1c; BUN, blood urea nitrogen; Cre, creatinine; CysC, cystatin C; eGFRcre, estimated glomerular filtration rates of serum creatinine; eGFRcys, estimated glomerular filtration rate of serum cystatin C.



**Fig. 5.** Changes in SMI, handgrip strength, and Cre/CysC ratio according to the severity of hepatic steatosis in the men and women. (A) SMI. (B) Handgrip strength. (C) Serum Cre/CysC ratio. The Kruskal–Wallis test and Spearman correlation coefficient were used for the statistical evaluation (\**p*<0.05, \*\**p*<0.01, \*\*\**p*<0.001). SMI, skeletal muscle mass index; Cre/CysC, creatinine-to-cystatin C.

been reported, the causal relationship is still unclear.<sup>(38)</sup> On the contrary, sarcopenia has been reported to be unrelated to hepatic steatosis, whereas sarcopenia is related to liver fibrosis.<sup>(39)</sup> These

different results regarding the association between sarcopenia and hepatic steatosis may be due to racial differences and how muscle mass is evaluated, for example, by using BIA, DXA, CT,



**Fig. 6.** Association between Cre/CysC ratio, SMI, and handgrip strength according to the degree of hepatic steatosis. (A) Correlations between SMI and Cre/CysC ratio in normal, mild, moderate, and severe hepatic steatosis. (B) Correlations between handgrip strength and Cre/CysC ratio in normal, mild, moderate, and severe hepatic steatosis. The Spearman correlation coefficient was used for the statistical evaluation. SMI, skeletal muscle mass index; Cre/CysC, creatinine-to-cystatin C.

MRI, and SMI. In previous reports, SMI was used after adjusting the ASM according to body weight, height squared, or BMI; however, the definition of SMI has been contentious with respect to which factor is most appropriate for adjustment of ASM.<sup>(40)</sup> That is, the association between sarcopenia and hepatic steatosis remains controversial, and further prospective studies are necessary with a unified evaluation method.

Dietary nutrients and exercise contribute to modifiable risk factors for sarcopenia.<sup>(41,42)</sup> Among the dietary intakes of nutrients, protein is the most important nutrient for attenuating the progression of muscle loss in the older population.<sup>(43,44)</sup> In our study, Cre/CysC ratio, SMI, and handgrip strength decreased with advancing age, even though older people had taken dietary protein and exercised more than younger people had. Consequently, Cre/CysC ratio, SMI, and handgrip strength were inversely correlated with dietary protein intake in our study. Moreover, recent studies showed that intake of vitamin B6 had beneficial effects on sarcopenia and NAFLD,<sup>(45,46)</sup> but intake of vitamin B6 was not correlated with NAFLD. Therefore, our results suggest that aging itself might have some influence on sarcopenia, not just by dietary intake of protein and vitamin B6 and physical activity.

This study had several limitations. First, because this study was limited by its cross-sectional design, we could not determine whether the alteration of serum Cre/CysC ratio was a risk factor of future onset of sarcopenia in NAFLD. Thus, longitudinal studies must be considered to investigate the causal relationship between sarcopenia in NAFLD and serum Cre/CysC ratio. Second, the diagnosis of NAFLD was based on ultrasonography examination findings without liver biopsy due to the invasive nature of biopsy. Instead, a common ultrasonographic definition of fatty liver has been established and used as a noninvasive modality.<sup>(47)</sup> Although the sensitivity to detect hepatic steatosis using ultrasonography is low,<sup>(48)</sup> ultrasonography is easily available.<sup>(49)</sup> Third, our survey was limited to Japanese subjects; hence, possible ethnic differences were not considered. Fourth, our study was a health promotion study, which differs from an ordinary health checkup survey; thus, the subjects who participated in our study were interested in their health and may have been healthier than the general population, resulting in a possible selection bias.

In conclusion, serum Cre/CysC ratio was associated with muscle mass and strength in NAFLD regardless of severity of hepatic steatosis. In addition, serum Cre/CysC ratio provided



cutoff values for low muscle mass and handgrip strength. This association between serum Cre/CysC ratio and sarcopenia may be useful for the evaluation of muscle mass and strength in NAFLD in a clinical setting.

## Author Contributions

KM: conceptualization, investigation, data curation, writing - original draft, writing - review and editing. TE: validation, investigation. NS: investigation. GI: investigation. MK: investigation. TH: investigation. CI: investigation. KS: investigation, data curation. MA: investigation, data curation. YS: investigation, data curation. TM: investigation, SN: project administration, funding acquisition. MM: formal analysis. HS: investigation. SF: supervision.

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## Abbreviations

|     |                                   |
|-----|-----------------------------------|
| ALT | alanine aminotransferase          |
| ASM | appendicular skeletal muscle mass |
| AST | aspartate aminotransferase        |

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|       |                                                        |
|-------|--------------------------------------------------------|
| AUROC | area under the receiver-operating characteristic curve |
| BFP   | body fat percentage                                    |
| BIA   | bioelectrical impedance analysis                       |
| BMI   | body mass index                                        |
| BUN   | blood urea nitrogen                                    |
| BW    | body weight                                            |
| CI    | confidence interval                                    |
| Cre   | creatinine                                             |
| CT    | computed tomography                                    |
| CysC  | cystatin C                                             |
| DXA   | dual energy X-ray absorptiometry                       |
| eGFR  | estimated glomerular filtration rate                   |
| GGT   | gamma-glutamyl-transpeptidase                          |
| HbA1c | glycated hemoglobin                                    |
| HCC   | hepatocellular carcinoma                               |
| MRI   | magnetic resonance imaging                             |
| NAFLD | nonalcoholic fatty liver disease                       |
| SMI   | skeletal muscle mass index                             |

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## Conflict of Interest

No potential conflicts of interest were disclosed.

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