ORIGINAL ARTICLE

Journal of Cachexia, Sarcopenia and Muscle 2023; **14**: 2253–2263
Published online 10 August 2023 in Wiley Online Library (wileyonlinelibrary.com) **DOI:** 10.1002/jcsm.13309

Association between the 110-kDa C-terminal agrin fragment and skeletal muscle decline among community-dwelling older women

Kuniyasu Kamiya[1]*, Takahiro Tachiki[2], Yuho Sato[3], Katsuyasu Kouda[4], Etsuko Kajita[2], Junko Tamaki[1], Sadanobu Kagamimori[5] & Masayuki Iki[6]

[1]*Department of Hygiene and Public Health, Faculty of Medicine, Osaka Medical and Pharmaceutical University, Takatsuki, Japan;* [2]*Faculty of Nursing, Chukyo Gakuin University, Mizunami, Japan;* [3]*Department of Human Life, Jin-ai University, Echizen, Japan;* [4]*Department of Hygiene and Public Health, Kansai Medical University, Hirakata, Japan;* [5]*University of Toyama, Toyama, Japan;* [6]*Department of Public Health, Kindai University Faculty of Medicine, Osaka-Sayama, Japan*

Abstract

Background C-terminal agrin fragment (CAF) is a biomarker for neuromuscular junction degradation. This study aimed to investigate whether 110-kDa CAF (CAF110) was associated with the presence and incidence of low muscle mass and strength.

Methods This cross-sectional retrospective cohort study comprised women aged ≥65 years. We measured muscle mass using a dual-energy X-ray absorptiometry scanner, hand-grip strength, and blood sampling between 2011 and 2012. A follow-up study with the same measurements was conducted between 2015 and 2017. Low muscle mass and strength were defined as an appendicular skeletal muscle mass index <5.4 kg/m^2 and hand-grip strength <18 kg, respectively. The CAF110 level was measured using enzyme-linked immunosorbent assay kits.

Results In total, 515 women (74.3 ± 6.3 years) were included in this cross-sectional analysis. Of these, 101 (19.6%) and 128 (24.9%) women presented with low muscle mass and strength, respectively. For low muscle mass, the odds ratios (ORs) of the middle and highest CAF110 tertile groups, compared with the lowest group, were 1.93 (95% confidence interval: 1.09–3.43; $P = 0.024$) and 2.15 (1.22–3.80; $P = 0.008$), respectively. After adjusting for age, the ORs remained significant: 1.98 (1.11–3.52; $P = 0.020$) and 2.27 (1.28–4.03; $P = 0.005$), respectively. Low muscle strength ORs of all the CAF110 tertile groups were not significant. In the longitudinal analysis, 292 and 289 women were assessed for incidents of low muscle mass and strength, respectively. Of those, 34 (11.6%) and 20 (6.9%) women exhibited low muscle mass and strength, respectively. For incident low muscle mass, the crude OR of the CAF110 ≥ the median value group was marginally higher than that of the CAF110 < median value group (median [interquartile range]: 1.98 [0.94–4.17] ($P = 0.072$). After adjusting for age and baseline muscle mass, the OR was 2.22 [0.97–5.06] ($P = 0.058$). All low muscle strength ORs of the median categories of CAF110 were not significant.

Conclusions CAF110 was not associated with low muscle strength. However, CAF110 may be a potential marker for the incidence of low muscle mass.

Keywords Aging; Agrin; Biomarker; Neuromuscular junction

Received: 8 December 2022; Revised: 14 June 2023; Accepted: 11 July 2023
**Correspondence to: Kuniyasu Kamiya, Basic Medical Sciences Region, Kobe City College of Nursing 3-4, Gakuen-nishi-machi, Nishi-ku, Kobe 651-2103, Japan.*
Email: kuniyasu-kamiya@kobe-ccn.ac.jp

© 2023 The Authors. Journal of Cachexia, Sarcopenia and Muscle published by Wiley Periodicals LLC.
This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

Introduction

Age-related muscle loss, termed 'sarcopenia', is affected by various factors such as abnormalities in muscle protein turnover, endocrine system, or growth factors.[1] According to the revised definition by the European Working Group on Sarcopenia in Older People,[2] sarcopenia is probable when low muscle strength is detected. A sarcopenia diagnosis is confirmed by the presence of low muscle quantity or quality.[2] Nervous system deterioration is thought to be the major mechanism underlying the development and acceleration of sarcopenia.[1,3] Moreover, neuromuscular junction (NMJ) degeneration, which is characterized by the degradation of several components such as the regression of motor neurons or fragmentation of acetylcholine receptor (AChR) clusters on the postsynaptic membrane, contributes to skeletal muscle loss.[4,5] In an animal study, fragmentation of AChR clusters was specifically observed in skeletal muscles that showed the pathological state of sarcopenia.[6] Another animal study focusing on NMJ morphology and myofibre profiles reported that NMJ degeneration may precede incident sarcopenia.[7] Therefore, a biomarker for NMJ degeneration could help predict sarcopenia.

Agrin, a heparin sulphate proteoglycan synthesized in motor neurons, has an important role in the initial formation and maintenance of the NMJ through AChR aggregation.[8] Agrin is fragmented by neurotrypsin at the NMJ.[9] In mice, the overexpression of neurotrypsin increases agrin fragments[10] and causes NMJ degeneration, muscle atrophy, and sarcopenic alteration of myofibre profiles.[10,11] In human serum, the C-terminal agrin fragment (CAF), particularly the 110-kDa CAF (CAF110) and 22-kDa CAF (CAF22), can be detected.[12] A previous study involving a 10 day bed rest in young men demonstrated NMJ instability, decreased muscle mass and strength, and an increased blood level of CAF22.[13] Several cross-sectional studies report that increased levels of CAF22 are associated with low muscle mass,[14–16] low muscle strength,[15] or prevalent sarcopenia[12,15,16] among community-dwelling older people. However, another cross-sectional study[17] did not find a significant association among older patients with hip fractures. CAF22 is filtered by the kidney; therefore, the serum level of CAF22 is affected by renal function.[18] Additionally, the CAF22 serum level has a moderate correlation with serum creatinine levels among patients with heart failure.[19] In contrast, CAF110 is not filtered by the kidney,[18] which means that its serum levels may be a more accurate measure of NMJ degeneration. To the best of our knowledge, only one study has reported an association between increased CAF110 levels and low muscle mass or strength.[20] Additionally, no study has investigated whether a particular CAF level is predictive of the future incidence of low muscle mass or strength.

Therefore, this study aimed to examine whether (i) CAF110 would be cross-sectionally associated with the presence of low muscle mass and weakness and (ii) CAF110 would be longitudinally associated with the future incidence of low muscle mass and weakness among community-dwelling older women. We hypothesized that CAF110 would be associated with the presence or future incidence of low muscle mass and strength.

Methods

Study design and participants

In the present study, cross-sectional and longitudinal analyses were conducted using previously described data from the Japanese Population-based Osteoporosis (JPOS) cohort study.[21] Briefly, in 1996, women aged 15–79 years were randomly selected from 5-year age groups, using resident registrations in seven municipalities across Japan. They were invited to complete a survey. In this cohort study, women who completed the survey in 1996 were followed up in 1999, 2002, 2006, 2011/2012, and 2015–2017. Body composition measurements started in the 2011/2012 follow-up study; therefore, the baseline and follow-up of the present study were surveys conducted in 2011/2012 and 2015–2017, respectively. The surveys were conducted in four municipalities. For the cross-sectional analysis, we included women aged ≥65 years who had completed blood sampling, body composition, hand-grip strength, and walking speed measurements at baseline. We excluded women with a history of stroke or an estimated glomerular filtration rate (eGFR) < 30 mL/min/1.73 m^2. For the longitudinal analysis, we included women who were evaluated in the cross-sectional analysis who also completed body composition, hand-grip strength, and walking speed measurements at follow-up. To analyse the future incidence of low muscle mass and strength, women with low muscle mass and strength at baseline were accordingly excluded. All participants provided written informed consent before participating in each survey. The study protocol was approved by the Ethics Committee of Kindai University Faculty of Medicine (Osaka-Sayama, Japan; approval no. 29-112). It conforms to the principles embodied in the Declaration of Helsinki.[22]

Measurements of muscle mass and strength

Body composition measurements were performed using a single dual-energy X-ray absorptiometry (DXA) scanner (QDR-4500A; Hologic, Bedford, MA, USA). A single experienced radiologic technologist performed all scans. The lean soft tissue mass (LSTM) was divided into the head, arms, legs, and trunk. The LSTM of the arms and legs were isolated from the trunk LSTM by using methods described in previous studies.[23,24] In the anterior images, the arms were delineated

at the vertical shoulder line bisecting the heads of the humerus at the glenoid fossa. The legs were separated by two angled lines, which comprised a pelvic triangle with a horizontal line at the crest of the ilium and bisected the two femoral necks. Appendicular skeletal muscle mass (kg) was calculated as the sum of the arm and leg LSTM. Appendicular skeletal muscle mass index (ASMI) was calculated as the appendicular skeletal muscle mass (kg) divided by the height squared (m^2). The in vivo reproducibility, represented by the coefficients of variation (CVs) of the LSTM and appendicular skeletal muscle mass, was 1.1% and 1.4%, respectively.[21]

Hand-grip strength (kg) was measured using a digital hand-grip strength dynamometer (TKK-5101; Takei Kagaku, Tokyo, Japan). The grip width was set as one-half the length from the base of the thumb to the end of the index finger. Participants stood with their shoulder, elbow, and wrist in a neutral position. The participants were told to hold the dynamometer at maximum effort for several seconds. The measurements were conducted twice for each hand with an interval of several seconds. A maximum value (in 0.1 kg) of all measurements was used in the present study.[25] Reproducibility, represented by the CV of hand-grip strength, was 3.6%.

Based on the Asian Working Group for Sarcopenia criteria,[26] low muscle mass and strength were defined as an ASMI <5.4 kg/m^2 and hand-grip strength <18 kg, respectively, for women.

C-terminal agrin fragment measurements

Serum levels of CAF110 were obtained in duplicate using commercial sandwich-type enzyme-linked immunosorbent assay (ELISA) kits (Human CAF-110KD ELISA Kit; Glory Science Co., Ltd., Shanghai, China) and a spectrophotometric microplate reader (Model 680; Bio-Rad, Hercules, CA, USA). The ELISA kit combines CAF110 antibody with labelled horseradish peroxidase to form an antibody–antigen–enzyme–antibody complex and uses tetramethylbenzidine as the colorimetric substrate. Intra- and inter-assay precisions, represented by CVs, were 4.5% and 8.6%, respectively.

Other variables

Information on disease history was obtained via a survey questionnaire, and details were confirmed via interviews conducted by trained public health nurses. Venous blood samples were drawn without controlling meals. Haemoglobin A1c (HbA1c) values were estimated as National Glycohemoglobin Standardization Program equivalent values. Serum samples were obtained from whole blood samples by centrifugation at 3000× *g* for 10 min. Serum levels of total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and creatinine were measured. The residual serum samples were stored at −80°C. The eGFR was calculated using the formula recommended by the Japanese Society of Nephrology,[27] which includes age and serum creatinine levels.

Body height and weight were measured using an automatic scale (TK-11868h; Takei Kagaku). Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2). Energy intake was estimated using the 28-item Food Frequency Questionnaire (FFQ).[28] The details were confirmed via interviews with trained dietitians. The Spearman's correlation between the FFQ and energy intake diet record methods was 0.471.[28] The CV of repeated measurements from the FFQ for energy intake was 9.6%.[28] Upon measuring the 10 m maximal walking speed, participants were requested to walk on a flat straight surface at their maximal pace for 14 m, of which, the middle 10 m mark was recorded by an automatic timer (TKK340a; Takei Kagaku). The timer automatically started or stopped as the participants walked past the mark. The measurement was completed twice. Between the measurements, participants were told to walk back to the start position at their usual speed. The mean speed of two measurements was used in the analysis.

To measure the standing balance function, participants were requested to hold the semi-tandem, tandem, and one-leg standing positions for 60 s, measured using a stopwatch. In the situation that a participant refused to comply or held a position for less than 30 s, the time was noted in seconds and no further position with a higher degree of difficulty was attempted. The one-leg stand was measured twice if the time of the first trial was less than 30 s. To measure the timed up and go (TUG) time, participants were requested to stand up from an armchair, walk 3 m, turn, walk back to the chair, and sit down as fast as possible. The time from standing up to sitting down was recorded by an automatic timer (TKK5804; Takei Kagaku).

Statistical analysis

CAF110 levels, standing balance function times, and TUG times were non-normally distributed. The other interval variables tested were normally distributed. More than one-half of the participants completed the semi-tandem and tandem standing for the maximum duration (60 s); therefore, we did not conduct subsequent tests on these variables. CAF110, one-leg stand time, and TUG time were categorized by tertile and median values in the cross-sectional and longitudinal analyses, respectively. In the cross-sectional analysis, the ASMI, hand-grip strength, and prevalence of low muscle mass or strength comparisons among the CAF110 tertile groups were conducted using the analysis of variance or χ^2, as appropriate. Subsequent pairwise multiple comparisons of the *post hoc* tests were conducted after a significant *P*-value was obtained in the analysis of variance or χ^2 tests. A comparison

of the characteristics among the three groups was conducted using the Cuzick test for continuous variables or the Cochran–Armitage test for prevalence. Characteristics between the two groups were compared using the unpaired *t*-test for normally distributed variables, Mann–Whitney *U* test for non-normally distributed variables, or the χ^2 test for prevalence. Spearman's rank correlations were performed to examine the correlation between CAF110 levels with age and eGFR levels. The association between CAF110 levels with the presence or future incidence of low muscle mass or strength was analysed using logistic regression models with the lowest category used as the reference group and represented by odds ratios (ORs). In the cross-sectional analysis, the models were adjusted for age. In the longitudinal analysis of incident low muscle mass, the models were adjusted for age and ASMI at baseline. In the analysis with incident low muscle strength, models were adjusted for age, hand-grip strength, and BMI at baseline. Analyses were conducted using Stata version 17 (StataCorp, College Station, TX, USA). $P < 0.05$ and $P < 0.1$ were indicative of statistical significance and tendency, respectively.

Results

Cross-sectional analysis

Five hundred and forty-six women completed the survey in 2011/2012. After excluding 23 women with a history of stroke and eight women with an eGFR <30 mL/min/1.73 m^2, 515 women were enrolled in the cross-sectional study (*Figure 1*). Comparisons of the characteristics among the CAF110 level tertile groups are shown in Table 1. Women in the higher CAF110 level category were younger, had lower HDL cholesterol levels, marginally longer one-leg stand times, were less likely to have a history of hypertension, and had a higher prevalence of low muscle mass. At baseline, 101 (19.6%) and 128 (24.9%) women presented with low muscle mass and strength, respectively; of these, 45 (8.7%) women presented with both characteristics. The prevalence of low muscle mass, rather than low muscle strength, was significantly different among tertile groups (P = 0.020 and P = 0.857, respectively), based on the χ^2 test. In the *post hoc* test, only the lowest and highest tertile groups had significantly different muscle masses.

The results of the logistic regression analysis in the cross-sectional study are depicted in *Figure 2*. Regarding low muscle mass, the crude ORs for the middle and highest CAF110 tertile groups were higher than the OR of the lowest tertile group [OR (95% confidence interval): 1.93 (1.09–3.43) (P = 0.024) and 2.15 (1.22–3.80) (P = 0.008), respectively]. After adjusting for age, the ORs remained significant [1.98 (1.11–3.52) (P = 0.020) and 2.27 (1.28–4.03) (P = 0.005), respectively]. For low muscle strength, the ORs in all CAF110 tertile groups were not significant; the crude ORs were 1.03 (0.63–1.69) and 1.14 (0.70–1.86) and the age-adjusted ORs were 1.10 (0.66–1.83) and 1.33 (0.80–2.21), respectively. The ORs of other variables are presented in *Table 2*.

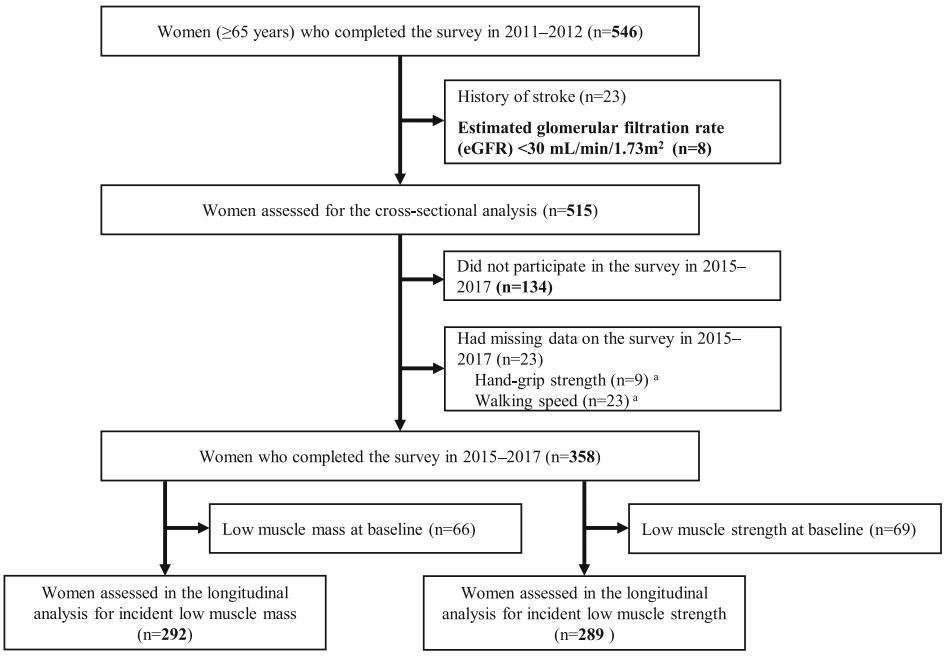

Figure 1 Flowchart of the participants. [a]The participants had missing data on hand-grip strength and walking speed.

Table 1 Comparison of the characteristics of the participants, categorised by the CAF110 tertile group, in the cross-sectional analysis

	CAF110 tertile group			
	<43.4 pg/mL (n = 172)	43.4–53.0 pg/mL (*n* = 172)	>53.0 pg/mL (*n* = 171)	*P* for trend
Age, years	75.0 (6.5)	74.4 (6.3)	73.4 (6.2)	0.026*
BMI, kg/m^2	23.0 (3.7)	23.0 (3.5)	22.9 (3.5)	0.835
eGFR, mL/min/1.73 m^2	71.3 (16.5)	70.2 (14.8)	72.6 (15.3)	0.438
HbA1c, %	5.8 (0.5)	5.8 (0.5)	5.8 (0.7)	0.667
Total cholesterol, mg/dL	213 (63)	210 (43)	207 (57)	0.386
HDL cholesterol, mg/dL	62 (14)	61 (14)	59 (15)	0.037*
LDL cholesterol, mg/dL	120 (28)	123 (27)	123 (28)	0.361
History of ischaemic heart disease	5.2%	4.7%	7.0%	0.474
History of hypertension	57.6%	56.4%	44.4%	0.015*
History of dyslipidemia	37.2%	34.9%	32.7%	0.386
History of diabetes mellitus	9.9%	9.3%	9.4%	0.868
Energy intake, kcal/day	1,626 (361)	1,639 (303)	1,590 (331)	0.291
Maximal walking speed, m/s	1.60 (0.40)	1.59 (0.37)	1.66 (0.39)	0.266
One-leg stand time, s	10.8 [0–34.6]	10.8 [0–36.4]	19.8 [1.0–45.6]	0.057
Timed up and go time, s	7.06 [5.97–8.72]	7.05 [6.21–8.28]	6.98 [6.27–8.20]	0.911
ASMI, kg/m^2	5.95 (0.62)	5.89 (0.56)	5.84 (0.60)	0.154
Low muscle mass[a]	12.8%	22.1%	24.0%	0.009**
Hand-grip strength, kg	21.1 (4.9)	20.9 (4.2)	20.7 (4.3)	0.282
Low muscle strength[b]	23.8%	24.4%	26.3%	0.596

Data are presented as the mean (standard deviation), median [interquartile range], or prevalence.
ASMI, appendicular skeletal muscle mass index; BMI, body mass index; CAF, C-terminal agrin fragment; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
[a]Low muscle mass, defined as ASMI <5.4 kg/m^2.
[b]Low muscle strength, defined as a hand-grip strength <18 kg.
*$P < 0.05$,
**$P < 0.01$.

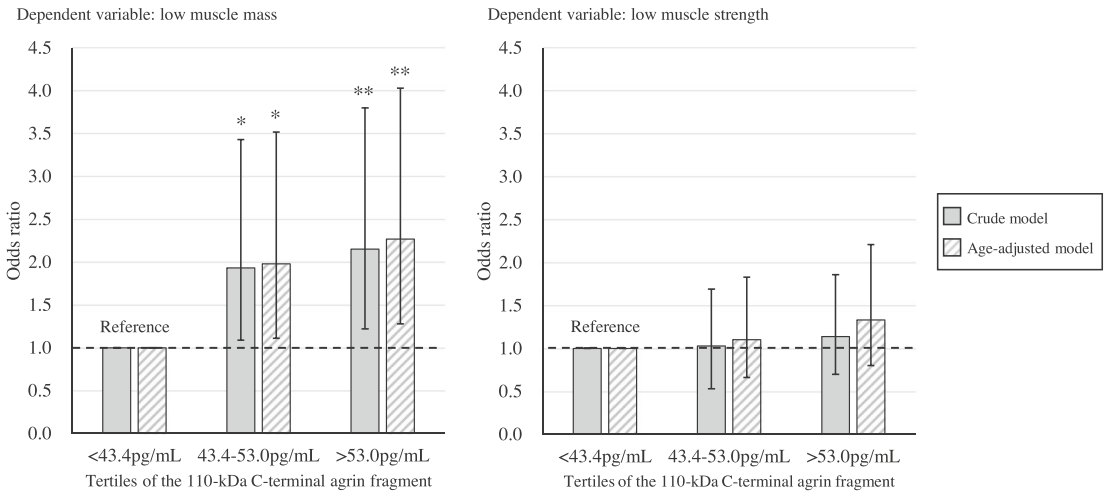

Figure 2 The odds ratios of the CAF110 tertile groups for low muscle mass and low muscle strength in the cross-sectional analysis. *$P < 0.05$ and **$P < 0.01$.

Low muscle mass was significantly associated with older age, lower BMI, lower HbA1c, absence of hypertension, dyslipidaemia, diabetes mellitus, lower hand-grip strength, and marginally higher HDL cholesterol level. Low hand-grip strength was significantly associated with older age, lower BMI, lower energy intake, slower walking speed, lower ASMI, shorter one-leg stand time, and longer TUG time, and it was marginally associated with lower total cholesterol level, HDL cholesterol level, and history of ischaemic heart disease. At baseline, the CAF110 level was negatively correlated with age ($\rho = -0.111$, $P = 0.009$), but not correlated with eGFR ($P = 0.381$).

Longitudinal analysis

Among 515 participants enrolled in this cross-sectional study, only 358 women completed follow-up assessments, whereas

Table 2 The crude odds ratios for low muscle mass and low muscle strength in the cross-sectional analysis

	Crude OR	*P*-value
Dependent variable: low muscle mass		
Age (per 5-year increase)	1.14 (0.96–1.35)	0.124
BMI (per 1SD decrease)	5.98 (3.94–9.06)	<0.001**
eGFR (per 1SD decrease)	0.86 (0.70–1.07)	0.180
HbA1c (per 1SD increase)	0.70 (0.52–0.93)	0.016*
Total cholesterol (per 1SD increase)	0.94 (0.75–1.19)	0.615
HDL cholesterol (per 1SD increase)	1.20 (0.97–1.49)	0.094
LDL cholesterol (per 1SD increase)	0.96 (0.77–1.20)	0.720
History of ischaemic heart disease	1.61 (0.69–3.75)	0.269
History of hypertension	0.57 (0.37–0.88)	0.012*
History of dyslipidemia	0.52 (0.31–0.85)	0.009**
History of diabetes mellitus	0.34 (0.12–0.96)	0.042*
Energy intake (per 1SD decrease)	1.10 (0.88–1.39)	0.398
Maximal walking speed (per 1SD decrease)	1.02 (0.83–1.28)	0.798
Tertile group of one-leg stand time		
>28.45 s	1	
3.45–28.45 s	0.53 (0.30–0.95)	0.033*
<3.45 s	1.17 (0.71–1.94)	0.541
Tertile group of timed up and go time		
<6.4 s	1	
6.4–7.8 s	1.27 (0.76–2.14)	0.365
>7.8 s	0.87 (0.50–1.51)	0.628
Hand-grip strength (per 1SD decrease)	1.73 (1.37–2.18)	<0.001**
Dependent variable: low hand-grip strength		
Age (per 5-year increase)	1.55 (1.32–1.82)	<0.001**
BMI (per 1SD decrease)	1.28 (1.04–1.59)	0.023*
eGFR (per 1SD decrease)	0.89 (0.73–1.08)	0.227
HbA1c (per 1SD increase)	0.82 (0.64–1.04)	0.100
Total cholesterol (per 1SD increase)	0.81 (0.64–1.02)	0.075
HDL cholesterol (per 1SD increase)	0.83 (0.68–1.03)	0.085
LDL cholesterol (per 1SD increase)	0.90 (0.73–1.10)	0.285
History of ischaemic heart disease	1.92 (0.88–4.20)	0.098
History of hypertension	1.25 (0.84–1.88)	0.271
History of dyslipidemia	0.73 (0.47–1.12)	0.151
History of diabetes mellitus	0.86 (0.43–1.74)	0.682
Energy intake (per 1SD decrease)	1.56 (1.23–1.97)	<0.001**
Maximal walking speed (per 1SD decrease)	2.41 (1.90–3.07)	<0.001**
Tertile group of one-leg stand time		
>28.45 s	1	
3.45–28.45 s	1.56 (0.89–2.73)	0.122
<3.45 s	3.73 (2.21–6.29)	<0.001**
Tertile group of timed up and go time		
<6.4 s	1	
6.4–7.8 s	3.04 (1.63–5.67)	<0.001**
>7.8 s	7.18 (3.95–13.05)	<0.001**
ASMI (per 1SD decrease)	1.74 (1.39–2.18)	<0.001**

Data are presented as the odds ratio (95% confidence interval).
1SD, one standard deviation; ASMI, appendicular skeletal muscle mass index; BMI, body mass index; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
*$P < 0.05$,
**$P < 0.01$.

the remaining women did not participate or had missing data (*Figure 1*). Comparisons of baseline characteristics between women who completed the follow-up survey and those who did not are shown in *Table S1*. Women who did not complete the follow-up survey were older and had a higher prevalence of ischaemic heart disease and hypertension; lower energy intake, maximal walking speed, and hand-grip strength; shorter one-leg stand time; longer TUG time; and marginally lower CAF110 levels. Of the women who completed the follow-up survey, 292 women were assessed for incident low muscle mass. The median [inter-quartile range (IQR)] of the interval from baseline to follow-up was 4.0 [3.8, 5.0] years. For the incidence of low muscle strength, 289 women were assessed, and the interval from baseline to follow-up was 4.0 [3.9, 4.9] years. We identified 34 (11.6%) and 20 (6.9%) women who exhibited incident low muscle mass and strength, respectively, during the follow-up survey.

To determine low muscle mass incidence, women were categorized according to the median CAF110 level and the comparison of characteristics between the various levels is shown in *Table 3*. The women in the higher CAF110 level category had lower HDL cholesterol levels, higher LDL choles-

Table 3 Comparison of the characteristics of participants, categorised by the median CAF110 level, in the longitudinal analysis of incident low muscle mass

	Median CAF110 level		*P* for difference
	<48.5 pg/mL (*n* = 146)	≥48.5 pg/mL (*n* = 146)	
At baseline			
Age, y	73.1 (5.7)	72.2 (5.1)	0.166
BMI, kg/m^2	23.7 (3.2)	23.8 (3.2)	0.714
eGFR, mL/min/1.73 m^2	71.6 (14.7)	71.0 (13.8)	0.698
HbA1c, %	5.8 (0.6)	5.8 (0.5)	0.204
Total cholesterol, mg/dL	219 (61)	208 (56)	0.125
HDL cholesterol, mg/dL	62 (13)	59 (14)	0.049*
LDL cholesterol, mg/dL	118 (27)	127 (25)	0.005**
History of ischaemic heart disease	2.7%	4.8%	0.356
History of hypertension	58.9%	47.9%	0.061
History of dyslipidaemia	39.0%	36.3%	0.629
History of diabetes mellitus	11.0%	11.0%	>0.999
Energy intake, kcal/day	1,696 (353)	1,613 (345)	0.042*
Maximal walking speed, m/s	1.71 (0.38)	1.69 (0.40)	0.723
One leg standing time, s	17.2 [2.9–45.3]	23.0 [5.7–46.7]	0.375
Timed up and go time, s	6.7 [5.9–7.6]	6.8 [6.0–4.9]	0.362
Hand-grip strength, kg	22.4 (4.1)	21.6 (4.3)	0.094
ASMI, kg/m^2	6.09 (0.48)	6.07 (0.45)	0.629
At follow-up			
eGFR, mL/min/1.73 m^2	65.2 (15.4)	66.0 (14.0)	0.630
BMI, kg/m^2	24.1 (3.8)	24.1 (3.4)	0.852
HbA1c, %	6.0 (0.8)	5.8 (0.5)	0.075
Total cholesterol, mg/dL	212 (44)	208 (50)	0.455
HDL cholesterol, mg/dL	64 (14)	62 (15)	0.165
LDL cholesterol, mg/dL	119 (24)	120 (26)	0.729
History of ischaemic heart disease	5.5%	6.2%	0.803
History of hypertension	62.3%	53.4%	0.123
History of dyslipidaemia	40.4%	43.8%	0.553
History of diabetes mellitus	10.3%	11.0%	0.849
Energy intake, kcal/day	1709 (292)	1,665 (300)	0.201
Maximal walking speed, m/s	1.69 (0.44)	1.67 (0.38)	0.651
One leg standing time, s	18.7 [7.3–50.5]	19.5 [8.4–46.0]	0.865
Timed up and go time, s	6.7 [5.9–8.3]	6.9 [6.1–8.4]	0.434
Hand-grip strength, kg	22.9 (4.3)	21.6 (4.6)	0.016*
ASMI, kg/m^2	6.06 (0.54)	5.98 (0.56)	0.247
Low muscle mass [a]	8.2%	15.1%	0.068

Data are presented as the mean (standard deviation), median [interquartile range], or prevalence.
ASMI, appendicular skeletal muscle mass index; BMI, body mass index; CAF, C-terminal agrin fragment; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
[a]Low muscle mass, defined as ASMI <5.4 kg/m^2.
*$P < 0.05$.
**$P < 0.01$.

terol levels, lower energy intake, marginally lower prevalence of hypertension, and marginally less hand-grip strength at baseline, and they had lower hand-grip strength, marginally lower HbA1c, and marginally higher prevalence of low muscle mass at follow-up.

Regarding incident low muscle mass, the crude OR of the CAF110 ≥ median value group (i.e. 48.5 pg/mL) was marginally higher than that of the CAF110 < median value group [1.98 (95% confidence interval: 0.94–4.17), $P = 0.072$]. After adjusting for age and ASMI at baseline, the OR remained marginally higher [2.22 (0.97–5.06), $P = 0.058$]. In the logistic regression analysis of incident low muscle strength, the ORs of categories divided by CAF110 median value (47.5 pg/mL) were not significant: 1.23 (0.49–3.07) ($P = 0.655$) in the crude model and 1.47 (0.51–4.23) ($P = 0.473$) in the model adjusted for age, hand-grip strength, and BMI at baseline. The ORs of other variables at baseline are presented in *Table 4*. Low muscle mass was significantly associated with lower BMI, higher LDL cholesterol, and less hand-grip strength, and marginally associated with a shorter one-leg stand time. Less hand-grip strength was significantly associated with older age, lower BMI, slower walking speed, shorter one-leg stand time, and longer TUG time, and marginally associated with a lower eGFR level and the presence of hypertension.

Discussion

In the present study, we found that higher levels of CAF110 were cross-sectionally associated with low muscle mass, but

DOI: 10.1002/jcsm.13309

Table 4 The crude odds ratios of the variables at baseline for incident low muscle mass and low muscle strength in the longitudinal analysis

	Crude OR	*P*-value
Dependent variable: incident low muscle mass		
Age (per 5-year increase)	0.92 (0.65–1.28)	0.609
BMI (per 1SD decrease)	1.88 (1.18–2.99)	0.007**
eGFR (per 1SD decrease)	0.94 (0.66–1.34)	0.723
HbA1c (per 1SD increase)	1.07 (0.76–1.50)	0.716
Total cholesterol (per 1SD increase)	1.17 (0.87–1.57)	0.308
HDL cholesterol (per 1SD increase)	1.31 (0.93–1.85)	0.126
LDL cholesterol (per 1SD increase)	1.17 (0.82–1.68)	0.377
History of ischaemic heart disease,	0.75 (0.09–6.06)	0.789
History of hypertension	0.57 (0.28–1.18)	0.131
History of dyslipidemia	2.32 (1.13–4.79)	0.022*
History of diabetes mellitus	1.48 (0.53–4.13)	0.459
Energy intake (per 1SD decrease)	0.86 (0.62–1.19)	0.362
Maximal walking speed (per 1SD decrease)	0.92 (0.64–1.32)	0.656
Lower half in one-leg stand time	0.50 (0.24–1.05)	0.069
Upper half in timed up and go test	1.00 (0.49–2.04)	>0.999
Hand-grip strength (per 1SD decrease)	1.56 (1.08–2.27)	0.018*
Dependent variable: incident low hand-grip strength		
Age (per 5-year increase)	2.85 (1.80–4.54)	<0.001**
BMI (per 1SD decrease)	0.72 (0.49–1.08)	0.112
eGFR (per 1SD decrease)	1.59 (0.98–2.60)	0.063
HbA1c (per 1SD increase)	1.14 (0.76–1.71)	0.541
Total cholesterol (per 1SD increase)	1.01 (0.65–1.58)	0.960
HDL cholesterol (per 1SD increase)	0.76 (0.47–1.24)	0.266
LDL cholesterol (per 1SD increase)	1.00 (0.63–1.57)	0.993
History of ischaemic heart disease,	3.63 (0.72–18.34)	0.120
History of hypertension	2.49 (0.93–6.69)	0.069
History of dyslipidemia	0.41 (0.13–1.26)	0.119
History of diabetes mellitus	1.04 (0.23–4.73)	0.961
Energy intake (per 1SD decrease)	1.20 (0.72–2.01)	0.475
Maximal walking speed (per 1SD decrease)	2.95 (1.77–4.91)	<0.001**
Lower half in one-leg stand time	4.41 (1.44–13.52)	0.027*
Upper half in timed up and go test	3.23 (1.14–9.15)	0.010*
ASMI (per 1SD decrease)	1.04 (0.66–1.64)	0.871

Data are presented as the odds ratio (95% confidence interval).
1SD, one standard deviation; ASMI, appendicular skeletal muscle mass index; BMI, body mass index; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
*$P < 0.05$.
**$P < 0.01$.

not with low muscle strength. We believe that this retrospective cohort study is the first to investigate the longitudinal association between blood levels of CAF and incident loss of muscle mass or strength. In the longitudinal analysis, we found that higher CAF110 levels were marginally associated with an increased risk of incident low muscle mass approximately 4 years later. Hence, CAF110 may be a useful predictor of future muscle mass loss in older women.

Although higher CAF110 levels were associated with an increased prevalence of low muscle mass, the same was not observed between CAF110 levels and low muscle strength. In previous studies by Steinbeck et al.,[19,20] higher levels of CAFs 22 and 110 and their sum (i.e. total CAF) were associated with low muscle mass and strength among patients with heart failure. However, to the best of our knowledge, no study has investigated the association between CAF110 and muscle loss among community-dwelling older adults. Higher CAF22 levels have been associated with low muscle mass among community-dwelling older adults.[12,14–16] In one of these studies,[15] a higher CAF22 level was also associated with low muscle strength; however, other studies[14,16] did not find a significant association between CAF22 and muscle strength. The contradictory findings in the association between CAF22 and muscle strength may be explained by the age differences of the participants. Studies that reported a significant association between CAF level and muscle strength consisted of older community-dwelling people (mean age >85 years),[15] compared with the age of other participants in other previous studies (mean age, 76 years[14] and 64 years[16]), as well as the present study (mean age, 75 years).

A plausible explanation for the lack of association between CAF levels and muscle strength in our study may be related to compensatory nerve sprouting. Despite the denervation of skeletal muscle due to NMJ degeneration, compensatory nerve sprouting may preserve muscle strength to some extent but decrease muscle mass.[7,29] In this situation, when blood CAF levels increase owing to NMJ degeneration, loss of muscle mass may occur, but muscle strength may remain unchanged to some extent.[14,16] In older community-dwelling people[15] or

patients with heart failure,[19,20] we speculate that CAF levels were associated with muscle strength since the further acceleration of NMJ degeneration may lead to loss of muscle strength.

In the cross-sectional analysis, participants in the higher CAF110 level tertile group were younger and had a higher frequency of low muscle mass. An unexpected finding was that the CAF110 values were negatively correlated with age. In a previous study,[12] CAF22 levels were not correlated with age among older people without sarcopenia; even among people with sarcopenia, only a slight tendency was observed between CAF22 and age. Agrin has two cleavage sites: cleavage at the alpha site produces CAF110, whereas cleavage at the beta site produces CAF22.[30] Although the mechanism controlling the amount of CAF110 secreted into the bloodstream remains unclear, we speculate that the increase in the amount of released CAF110 may likely occur in relatively younger people, thereby negatively affecting the maintenance of the NMJ structure. The cleavage of CAF110 at its beta site generates CAF22 and CAF90. CAF22, the terminal product of agrin cleavage, has a reduced capacity to induce acetylcholine receptor (AChR) aggregation.[31] In contrast, CAF110, an intermediate product of agrin cleavage, can induce AChR aggregation, similar to full-length agrin.[31]

CAF110 levels were not correlated with eGFR levels in the present study. This finding supported the presumption that the CAF110 level is not affected by kidney dysfunction.[18] Previous studies reported a significant correlation between CAF22 and kidney function among patients with heart failure[19] and that the sum of CAF22 and CAF110 also had a significant correlation with kidney function in patients with diabetes mellitus.[18] However, the correlation between CAF22 and kidney function was not found among healthy individuals in a previous report.[32] Therefore, CAF22 might be more affected by kidney function in patients with heart failure or diabetes mellitus, which may induce kidney dysfunction. In patients with these diseases, the prevalence of sarcopenia is higher than that in community-dwelling older adults.[33,34] Additionally, the loss of muscle mass or strength is a risk factor for hospitalization or disability among patients with diabetes mellitus[35] and for hospitalization or death among patients with heart failure.[33] Therefore, the prediction of sarcopenia among patients with these diseases is critical. Further studies should evaluate CAF110 levels as a predictive marker of sarcopenia in patients with diabetes and heart failure.

A DXA test, which is the gold standard for measuring muscle mass, was used to measure muscle mass in the present study. However, other investigators have suggested that DXA may underestimate age-related muscle decline, compared with magnetic resonance imaging.[36] In one study,[36] the magnitude of the difference in thigh lean mass between young and older participants was smaller with DXA than with an MRI assessment. However, regarding longitudinal changes in muscle mass over 5 years, DXA and MRI show a similar percentage of decline in muscle mass.[37] Therefore, a careful interpretation is required, based on the potential underestimation of the prevalence of low muscle mass.

The present study has some limitations. First, this study had a limited number of participants. Particularly, for the longitudinal analysis, the participants were included based on their completion of the surveys in 2011/2012 and in 2015–2017. This factor may have caused selection bias and survival bias. Second, the follow-up period was restricted to approximately 4 years. Therefore, the incidence rates of low muscle mass or muscle strength were low. Third, all participants were women. The relationship between the CAF22 level and muscle mass or strength has been reported to differ between the sexes.[16] Therefore, further studies with longer follow-up periods and including both sexes are required.

The major strength of this study was that muscle mass was measured by a single experienced radiologic technologist using a single DXA scanner. Additionally, hand-grip strength was measured using a single type of equipment, according to the prescribed protocol.

In conclusion, in the present study, we found that a higher CAF110 level was associated with low muscle mass, but not with low muscle strength, among Japanese community-dwelling older women. This finding indicates that CAF110 may be a potential marker for the risk of low muscle mass.

Acknowledgements

The present study represents part of the research conducted by the Japanese Population-Based Osteoporosis Study Group (Chairman, Masayuki Iki), comprising Fumiaki Marumo (former chairman; Tokyo Medical and Dental University, Tokyo, Japan), Toshihisa Matsuzaki (former co-chairman; University of the Ryukyus, Nishihara, Japan), Hideo Yoneshima (former chairman, Shuwa General Hospital, Kasukabe, Japan), Yoshiko Kagawa (Kagawa Nutrition University, Sakado, Japan), Takashi Akiba (Tokyo Women's Medical University, Tokyo, Japan), Harumi Nishino (Toyama Pharmaceutical Association, Toyama City, Japan), Tomoharu Matsukura (Toyama Prefectural Government, Toyama, Japan), Toshio Matsumoto (University of Tokushima, Tokushima, Japan), Takashi Yamagami (Hokuriku Health Service Association, Toyama, Japan), and Jun Kitagawa (Kitasato University, Tokyo, Japan), in addition to the authors. The authors also thank the personnel of the health departments of Memuro Town, Joetsu City, Nishi-Aizu Town, and Sanuki City for their support; and Toyo Medic Co., Ltd. (Osaka, Japan), Toyukai Medical Corporation (Tokyo, Japan), and Take Medical Service (Tokyo, Japan) for their technical assistance with the surveys.

Conflict of interest

The authors declare that they have no conflict of interest.

Funding

The survey of the JPOS cohort study in 2011/2012 was supported by Grants-in-Aid for Scientific Research from Japanese Society for the Promotion of Science (grant numbers: B#23390180 [years 2011–2013]; C#23590824 [2011–2013]; #23659362 [2011–2012]; #23657176 [2011–2012]; and B#24390505 [2012–2014]), a grant-in-aid from the Japanese Dairy Association (2011), and a grant-in-aid from the University Foundation. Survey of the JPOS cohort study in 2015–2017 was supported by Grants-in-Aid for Scientific Research from the Japanese Society for the Promotion of Science (grant numbers: A#15H02526 [2015–2019]; B#15H05102 [2015–2017]; B#15H04789 [2015–2017]; #16K15384; and #16K19263 [2016–2017]), and a grant-in-aid from the Japanese Dairy Association (2015). The present study including the measurements of CAF levels was supported by Grants-in-Aid for Scientific Research from Japanese Society for the Promotion of Science (grant number: #17K15837, 2017–2018).

Online supplementary material

Additional supporting information may be found online in the Supporting Information section at the end of the article.

References

1. Curcio F, Ferro G, Basile C, Liguori I, Parrella P, Pirozzi F, et al. Biomarkers in sarcopenia: a multifactorial approach. *Exp Gerontol* 2016;**85**:1–8.
2. Cruz-Jentoft AJ, Bahat G, Bauer J, Boirie Y, Bruyère O, Cederholm T, et al. Sarcopenia: revised European consensus on definition and diagnosis. *Age Ageing* 2019;**48**:16–31.
3. Kwan P. Sarcopenia, a neurogenic syndrome? *J Aging Res* 2013;**2013**:791679.
4. Pratt J, De Vito G, Narici M, Boreham C. Neuromuscular junction aging: a role for biomarkers and exercise. *J Gerontol A Biol Sci Med Sci* 2021;**76**:576–585.
5. Shigemoto K, Kubo S, Mori S, Yamada S, Akiyoshi T, Miyazaki T. Muscle weakness and neuromuscular junctions in aging and disease. *Geriatr Gerontol Int* 2010;**10**:S137–S147.
6. Pannérec A, Springer M, Migliavacca E, Ireland A, Piasecki M, Karaz S, et al. A robust neuromuscular system protects rat and human skeletal muscle from sarcopenia. *Aging* 2016;**8**:712–729.
7. Deschenes MR, Roby MA, Eason MK, Harris MB. Remodeling of the neuromuscular junction precedes sarcopenia related alterations in myofibers. *Exp Gerontol* 2010;**45**:389–393.
8. Bezakova G, Rabben I, Sefland I, Fumagalli G, Lømo T. Neural agrin controls acetylcholine receptor stability in skeletal muscle fibers. *Proc Natl Acad Sci U S A* 2001;**98**:9924–9929.
9. Stephan A, Mateos JM, Kozlov SV, Cinelli P, Kistler AD, Hettwer S, et al. Neurotrypsin cleaves agrin locally at the synapse. *FASEB J* 2008;**22**:1861–1873.
10. Bütikofer L, Zurlinden A, Bolliger MF, Kunz B, Sonderegger P. Destabilization of the neuromuscular junction by proteolytic cleavage of agrin results in precocious sarcopenia. *FASEB J* 2011;**25**:4378–4393.
11. Bolliger MF, Zurlinden A, Lüscher D, Bütikofer L, Shakhova O, Francolini M, et al. Specific proteolytic cleavage of agrin regulates maturation of the neuromuscular junction. *J Cell Sci* 2010;**123**:3944–3955.
12. Hettwer S, Dahinden P, Kucsera S, Farina C, Ahmed S, Fariello R, et al. Elevated levels of a C-terminal agrin fragment identifies a new subset of sarcopenia patients. *Exp Gerontol* 2013;**48**:69–75.
13. Monti E, Reggiani C, Franchi MV, Toniolo L, Sandri M, Armani A, et al. Neuromuscular junction instability and altered intracellular calcium handling as early determinants of force loss during unloading in humans. *J Physiol* 2021;**599**:3037–3061.
14. Drey M, Sieber CC, Bauer JM, Uter W, Dahinden P, Fariello RG, et al. C-terminal agrin fragment as a potential marker for sarcopenia caused by degeneration of the neuromuscular junction. *Exp Gerontol* 2013;**48**:76–80.
15. Landi F, Calvani R, Lorenzi M, Martone AM, Tosato M, Drey M, et al. Serum levels of C-terminal agrin fragment (CAF) are associated with sarcopenia in older multimorbid community-dwellers: results from the ilSIRENTE study. *Exp Gerontol* 2016;**79**:31–36.
16. Pratt J, De Vito G, Narici M, Segurado R, Pessanha L, Dolan J, et al. Plasma C-terminal agrin fragment as an early biomarker for sarcopenia: results from the GenoFit Study. *J Gerontol A Biol Sci Med Sci* 2021;**76**:2090–2096.
17. Sánchez-Castellano C, Martín-Aragón S, Bermejo-Bescós P, Vaquero-Pinto N, Miret-Corchado C, Merello de Miguel A, et al. Biomarkers of sarcopenia in very old patients with hip fracture. *J Cachexia Sarcopenia Muscle* 2020;**11**:478–486.
18. Devetzis V, Daryadel A, Roumeliotis S, Theodoridis M, Wagner CA, Hettwer S, et al. C-terminal fragment of agrin (CAF): a novel marker for progression of kidney disease in type 2 diabetics. *PLoS ONE* 2015;**10**:e0143524.
19. Steinbeck L, Ebner N, Valentova M, Bekfani T, Elsner S, Dahinden P, et al. Detection of muscle wasting in patients with chronic heart failure using C-terminal agrin fragment: results from the Studies Investigating Co-morbidities Aggravating Heart Failure (SICA-HF). *Eur J Heart Fail* 2015;**17**:1283–1293.
20. Steinbeck L, Ebner N, Valentova M, Sandek A, Bekfani T, Doehner W, et al. C-terminal agrin fragment as a novel diagnostic marker for muscle wasting in patients with chronic heart failure: results from the Studies Investigating Co-morbidities Aggravating Heart Failure. *J Cachexia, Sarcopenia and Muscle* 2014;**5**:38.
21. Iki M, Tamaki J, Sato Y, Morita A, Ikeda Y, Kajita E, et al. Cohort profile: the Japanese Population-based Osteoporosis (JPOS) Cohort Study. *Int J Epidemiol* 2015;**44**:405–414.
22. Rickham PP. Human experimentation. Code of ethics of the World Medical Association. Declaration of Helsinki. *Br Med J* 1964;**2**:177.
23. Laskey MA. Dual-energy X-ray absorptiometry and body composition. *Nutrition* 1996;**12**:45–51.
24. Tachiki T, Kouda K, Dongmei N, Tamaki J, Iki M, Kitagawa J, et al. Muscle strength is associated with bone health independently of muscle mass in postmenopausal women: the Japanese population-based osteoporosis study. *J Bone Miner Metab* 2017;**37**:53–59.
25. Huang C, Niu K, Kobayashi Y, Guan L, Momma H, Cui Y, et al. An inverted J-shaped association of serum uric acid with muscle strength among Japanese adult men: a cross-sectional study. *BMC Musculoskelet Disord* 2013;**14**:258.

26. Chen L-K, Woo J, Assantachai P, Auyeung T-W, Chou M-Y, Iijima K, et al. Asian Working Group for Sarcopenia: 2019 Consensus Update on Sarcopenia Diagnosis and Treatment. *J Am Med Dir Assoc* 2020;**21**: 300–7.e2.
27. Matsuo S, Imai E, Horio M, Yasuda Y, Tomita K, Nitta K, et al. Revised equations for estimated GFR from serum creatinine in Japan. *Am J Kidney Dis* 2009;**53**:982–992.
28. Uenishi K, Ishida H, Nakamura K. Development of a simple food frequency questionnaire to estimate intakes of calcium and other nutrients for the prevention and management of osteoporosis. *J Nutr Sci Vitaminol (Tokyo)* 2008;**54**:25–29.
29. Aagaard P, Suetta C, Caserotti P, Magnusson SP, Kjaer M. Role of the nervous system in sarcopenia and muscle atrophy with aging: strength training as a countermeasure. *Scand J Med Sci Sports* 2010;**20**:49–64.
30. Gisler C, Lüscher D, Schätzle P, Dürr S, Baici A, Galliciotti G, et al. Zymogen activation of neurotrypsin and neurotrypsin-dependent agrin cleavage on the cell surface are enhanced by glycosaminoglycans. *Biochem J* 2013;**453**:83–100.
31. Gesemann M, Denzer AJ, Ruegg MA. Acetylcholine receptor-aggregating activity of agrin isoforms and mapping of the active site. *J Cell Biol* 1995;**128**:625–636.
32. Yu D, Li H-X, Liu Y, Ying Z-W, Guo J-J, Cao C-Y, et al. The reference intervals for serum C-terminal agrin fragment in healthy individuals and as a biomarker for renal function in kidney transplant recipients. *J Clin Lab Anal* 2017;**31**:e22059.
33. Zhang Y, Zhang J, Ni W, Yuan X, Zhang H, Li P, et al. Sarcopenia in heart failure: a systematic review and meta-analysis. *ESC Heart Fail* 2021;**8**:1007–1017.
34. Anagnostis P, Gkekas NK, Achilla C, Pananastasiou G, Taouxidou P, Mitsiou M, et al. Type 2 diabetes mellitus is associated with increased risk of sarcopenia: a systematic review and meta-analysis. *Calcif Tissue Int* 2020;**107**:453–463.
35. Liccini A, Malmstrom TK. Frailty and sarcopenia as predictors of adverse health outcomes in persons with diabetes mellitus. *J Am Med Dir Assoc* 2016;**17**:846–851.
36. Maden-Wilkinson TM, Degens H, Jones DA, McPhee JS. Comparison of MRI and DXA to measure muscle size and age-related atrophy in thigh muscles. *J Musculoskelet Neuronal Interact* 2013;**13**:320–328.
37. Cameron J, McPhee JS, Jones DA, Degens H. Five-year longitudinal changes in thigh muscle mass of septuagenarian men and women assessed with DXA and MRI. *Aging Clin Exp Res* 2020;**32**:617–624.