knee extensions and others, were performed while seated on a chair. To increase difficulty and resistance, participants then performed; hip flexions, lateral leg raises, and repetitions of other exercises while standing upright behind the chair and holding the back of the chair for stability.

Resistance bands were used to further strengthen the upper and lower body. Lower body exercises consisted of leg extensions, hip flexions, and more. Upper body exercises included double-arm pull downs, bicep curls, and others.

Balance and gait training exercises included standing on one leg and multidirectional weight shifts. Participants were instructed on and practiced proper gait mechanics that focused on the maintenance of stability during walking, and increasing stride length, toe elevation of the forward limb, heel elevation of the rear limb, frequency of stepping, and arm swinging.

MFGM Supplementation

The MFGM group was provided with supplements in pill form, every 2 weeks. MFGM was purchased from Megmilk Snow Brand Co., Ltd. (Sapporo, Japan). The composition of the MFGM was 21.5% protein, 44.0% fat, 26.5% carbohydrate, 33.3% phospholipids (8.29% phosphatidylcholine, 8.56% phosphatidylethanolamine, 2.79% phosphatidylinositol, 3.31% phosphatidylserine, 8.03% sphingomyelin, and others), 6.4% ash, and 1.6% moisture. Each pill contained 167 mg of MFGM, and six pills (total 1 g) were ingested in the mornings, prior to activity. The pills were yogurt-flavored so the participants were able to chew or swallow the pill according to their preference. Participants were asked to fill out a daily diary on which they recorded whether or not they took the full amount of the supplement (if not, how much), and the time of day. These diary sheets were collected every two weeks.

Placebo

The placebo group followed the same protocol as the MFGM supplementation group; however, the contents of the pill differed. The placebo included whole milk powder instead of MFGM, and the placebo consisted of pills of similar shape, taste, and texture of the MFGM pills. Whole milk powder was purchased from Meiji Milk Products Co. Ltd (Tokyo, Japan). The composition of the milk powder was 26.3% protein, 25.2% fat, 39.5% carbohydrate, 0.286% phospholipids (0.067% phosphatidylcholine, 0.063% phosphatidylethanolamine, 0.037% phosphatidylinositol, 0.033% phosphatidylserine, 0.057% sphingomyelin, and others), 5.7% ash, and 3.3% moisture.

Data Analysis

Sample size calculations using univariate 1-factor repeated measures ANOVA to examine significant differences in means at baseline, 3 months, and 7 months. Setting the power at 0.80 and an alpha value of 0.05, the total sample size required was estimated to be 112 subjects [13]. When considering a potential attrition rate of 15% [14], 131 subjects were required.

Differences in baseline measures between the groups were measured using a one-way analysis of variance (ANOVA), and chi-square tests were performed on categorical variables. Percent changes in leg muscle mass and gait function were calculated using the formula: % change = ((post-intervention or follow-up value-baseline value)/baseline value)×100), and analysis of covariance (ANCOVA) adjusted for age and baseline frailty score was performed to determine significant differences in percent changes within the groups between baseline to post-intervention and follow-up, with values expressed as differences with 95% confidence intervals. The Scheffe post-hoc method was used when significance was found. The number of frailty criteria was converted to frailty score, with the score corresponding to the number of frailty criteria (eg: 0 for no frailty criteria, 1 for 1 frailty criteria, 2 for 2 frailty criteria, etc). The generalized estimating equation was used to compare the effects between the groups after 3- intervention and at the 7-month follow-up on the change of frailty score. The Kruskal–Wallis test was used to

evaluate the differences of the reversal of frailty status between the intervention groups. A post-hoc analysis was performed using the Mann-Whitney method.

To compare the effects of the four intervention groups on frailty after 3-months of intervention and follow-up, multiple logistic regressions were performed, and groups were compared with odds ratios and 95% confidence intervals. All analyses were performed using SPSS software, Windows version 20.0 (SPSS, Inc., Tokyo, Japan).

Results

There were 3 dropouts (1 death, 1 fracture, 1 spouse care) at the post-survey, 5 dropouts at the follow-up (1 death, 1 femoral neck fracture, 1 hospitalization, 1 moved, 1 declined motivation). The fractures, hospitalizations and deaths were not caused by intervention.

Baseline Characteristics and Group×Time Interactions

All baseline characteristics were similar between the groups for demographic, physical function, hematological parameters, and interview variables (Table 1).

The analysis of changes from baseline, post-intervention and follow-up in muscle mass, physical function and hematological parameters are shown in Table 2. Significant group×time interactions were observed for usual walking speed (P = 0.005), TUG (P<0.001), and (IGFBP3/IGF1) ×100 (P = 0.013). The changes in Ex+MFGM group were significantly greater than MFGM or Placebo group.

Frailty Criteria, Physical Function and Hematological Parameters

The frailty components revealed that weight loss was only reversed between baseline and follow-up in the Ex+MFGM and Ex+Plac groups (Table 3). Reversal of exhaustion was seen across all the groups. Low physical activity was also reversed in all groups, although only the Ex+MFGM group was able to maintain the reversal at follow-up. Slow walking speed was reversed by 42.4% in the Ex+MFGM group between baseline and follow-up, which was significantly greater than the other groups. Change in low muscle strength was not significant.

Physical function analysis revealed that walking speed increased in the Ex+MFGM group (14.7%) after the 3-month intervention, which was significantly greater than the MFGM and placebo groups ($P = 0.026$) (Table 4). TUG significantly improved from baseline to post-intervention in both the exercise groups in comparison with the MFGM and placebo groups (P<0.001). Leg muscle mass also increased within the Ex+MFGM and Ex+Plac groups, although significant differences were not seen between the groups.

As seen in Table 5, significant within-group increases in BDNF were observed from baseline to follow-up in both exercise groups. Similarly, significant within-group increases in myostatin were also seen in the Ex+MFGM group between baseline to post-intervention, and follow-up. Decreases in (IGFBP-3/IGF-1) ×100 percent change was only seen in the Ex+MFGM group, while steady increases were seen in the other groups.

Number of Frailty Criteria and Frailty Status

The mean number of frailty criteria (out of 5) significantly decreased in all four intervention groups after the 3-month intervention. However, at the 7-month follow-up, the reduction in number of frailty criteria was only able to be maintained in the two exercise groups (Fig. 2). Notably, although the baseline to follow-up change in number of frailty criteria was significant in the Ex+Plac group, the figure clearly depicts an increase in number of frailty criteria from

Table 1. Selected variable characteristics of participants at baseline by study group.

Variables *	Ex+MFGM (n = 33)	Ex+Placebo (n = 33)	MFGM (n = 32)	Placebo (n = 32)	F value†	P value
Age (yr)	81.0 ± 2.6	81.1 ± 2.8	81.0 ± 2.8	80.3 ± 3.3	0.530	0.662
Height (cm)	147.7 ± 5.4	147.8 ± 6.7	146.1 ± 5.5	144.3 ± 5.8	2.625	0.053
Body weight (kg)	46.1 ± 7.5	48.6 ± 9.0	47.1 ± 8.7	47.7 ± 8.7	0.503	0.681
Skeletal muscle mass (kg)	13.2 ± 1.5	13.8 ± 1.7	13.4 ± 1.7	13.4 ± 1.6	0.691	0.559
Leg muscle mass (kg)	10.1 ± 1.1	10.5 ± 1.3	10.1 ± 1.3	10.1 ± 1.2	0.789	0.502
Grip strength (kg)	17.1 ± 3.9	17.8 ± 2.8	17.5 ± 2.7	18.7 ± 3.2	1.523	0.212
Knee extension strength (N)	178.8 ± 55.2	179.1 ± 40.9	185.2 ± 52.1	184.7 ± 50.1	0.140	0.936
Usual walking speed (sec)	4.5 ± 0.9	4.6 ± 0.9	4.9 ± 1.2	4.7 ± 1.5	0.925	0.431
Timed up & go (sec)	7.7 ± 1.7	8.2 ± 2.0	8.8 ± 2.8	8.5 ± 3.5	1.096	0.354
BDNF (ng/ml)	6.6 ± 1.5	6.8 ± 1.4	6.9 ± 0.9	6.4 ± 1.3	1.167	0.325
Beta-2 microglobulin (mg/ml)	2.6 ± 1.0	2.5 ± 0.8	2.3 ± 0.8	2.6 ± 1.2	0.633	0.595
Myostatin (ng/ml)	54.9 ± 14.8	48.6 ± 11.7	51.6 ± 14.7	51.5 ± 15.5	0.902	0.443
(IGFBP-3/IGF-1)×100	5.4 ± 2.3	4.4 ± 2.1	3.9 ± 1.3	4.5 ± 1.7	2.602	0.056
Number of frailty criteria	3.8 ± 0.7	3.6 ± 0.7	3.7 ± 0.7	3.5 ± 0.6	1.429	0.237
Weight loss (%)	72.7	60.6	62.5	45.5		0.155
Exhaustion (%)	60.6	84.8	62.5	60.6		0.099
Low physical activity (%)	90.9	75.8	93.8	90.9		0.106
Low muscle strength (%)	69.7	72.7	65.6	63.6		0.861
Slow walking speed (%)	66.7	60.6	68.8	57.6		0.768
Number of frailty criteria (%)					6.956	0.325
Three	33.3	54.4	43.8	51.5		
Four	48.5	30.3	40.6	45.5		
Five	18.2	15.2	15.6	3.0		
Osteoporosis, yes (%)	51.5	51.5	53.1	42.4	0.936	0.817
Knee osteoarthritis, yes (%)	24.2	21.2	34.4	36.4	2.652	0.448

Note: * Data are presented as M (mean) and SD (standard deviation) for continuous variables, and percentage for categorical variables.

BDNF = brain derived neurotrophic factor, Ex = exercise group

MFGM = milk fat globule membrane

† One-way analysis of variance for continuous variables and chi-square test for categorical variables.

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post-intervention to follow-up (as the slope increased). The Ex+MFGM group on the other hand, maintained the same number of frailty criteria at follow-up as post-intervention.

Fig. 3 shows that the percentage of non-frail people was significantly higher in the Ex+MFGM (57.6%) than in the MFGM (28.1%) or placebo (30.3%) groups at post-intervention ($\chi^2 = 8.827$, P = 0.032), and at the follow-up was also significantly greater in the Ex+MFGM (45.5%) and Ex+Plac (39.4%) groups compared with the placebo (15.2%) group ($\chi^2 = 8.607$, P = 0.035).

Intervention Type on Frailty Status Reversal

The multiple logistic regression analysis (Table 6) revealed that the Ex+MFGM group had a significant effect on frailty (OR = 3.12, 95%CI = 1.13–8.60) after the 3-month intervention and follow-up improvement of frailty were observed in Ex+Plac (OR = 3.64, 95%CI = 1.12–11.85) and Ex+MFGM (OR = 4.67, 95%CI = 1.45–15.08).

Table 2. Comparison of muscle mass, physical function, and blood component variables among groups after 3-month intervention and follow-up.

Variables*	Group†	Baseline	Post intervention	Follow-up	GEE‡ (G×T) (P-value)	Post hoc analysis# (P<0.05)
Appendicular skeletal muscle mass (kg)	Ex+MFGM	13.20 ± 1.50	13.51 ± 1.61	13.64 ± 1.69	$F = 0.956$ (0.416)	
	Ex+Placebo	13.86 ± 1.81	14.04 ± 1.77	14.31 ± 2.08		
	MFGM	13.20 ± 1.79	13.27 ± 1.63	13.54 ± 1.76		
	Placebo	13.44 ± 1.74	13.55 ± 1.67	13.70 ± 1.75		
Leg muscle mass (kg)	Ex+MFGM	10.08 ± 1.17	10.30 ± 1.21	10.41 ± 1.36	$F = 1.863$ (0.140)	
	Ex+Placebo	10.57 ± 1.34	10.34 ± 2.40	10.93 ± 1.68		
	MFGM	9.99 ± 1.28	9.23 ± 3.01	10.23 ± 1.37		
	Placebo	10.18 ± 1.33	10.28 ± 1.30	10.39 ± 1.38		
Grip strength (kg)	Ex+MFGM	17.19 ± 3.79	17.83 ± 4.05	17.00 ± 3.88	$F = 0.804$ (0.495)	
	Ex+Placebo	17.94 ± 3.00	18.36 ± 3.28	17.75 ± 2.90		
	MFGM	17.81 ± 2.35	18.37 ± 1.92	16.75 ± 2.24		
	Placebo	18.92 ± 3.38	19.18 ± 3.50	18.08 ± 2.92		
Knee extension strength (Nm)	Ex+MFGM	187.72 ± 49.68	191.52 ± 54.81	178.72 ± 45.92	$F = 2.663$ (0.053)	
	Ex+Placebo	179.64 ± 40.66	188.45 ± 47.82	190.32 ± 46.20		
	MFGM	188.68 ± 56.89	186.42 ± 60.47	181.26 ± 51.38		
	Placebo	192.05 ± 50.09	194.32 ± 54.14	199.95 ± 52.65		
Usual walking speed (sec)	Ex+MFGM	1.15 ± 0.16	1.25 ± 0.24	1.23 ± 0.21	$F = 4.592$ (0.005)	Ex+MFGM>MFGM
	Ex+Placebo	1.17 ± 0.21	1.26 ± 0.27	1.21 ± 0.22		
	MFGM	1.10 ± 0.22	1.08 ± 0.23	1.11 ± 0.20		
	Placebo	1.18 ± 0.24	1.13 ± 0.22	1.18 ± 0.23		
Timed up & go (sec)	Ex+MFGM	9.63 ± 2.15	7.98 ± 1.44	6.93 ± 1.61	$F = 9.763$ (<0.001)	Ex+MFGM, Ex+P> MFGM, P
	Ex+Placebo	9.89 ± 2.27	7.87 ± 1.83	7.04 ± 1.45		
	MFGM	10.77 ± 2.58	10.53 ± 2.77	7.76 ± 1.52		
	Placebo	10.44 ± 3.79	10.00 ± 4.32	7.99 ± 3.79		
BDNF (ng/ml)	Ex+MFGM	6.60 ± 1.54	7.18 ± 1.09	7.68 ± 1.17	$F = 1.041$ (0.379)	
	Ex+Placebo	6.37 ± 1.44	7.07 ± 1.01	7.03 ± 1.66		
	MFGM	6.97 ± 0.94	7.11 ± 1.05	7.39 ± 1.47		
	Placebo	6.10 ± 1.47	6.36 ± 1.31	6.52 ± 1.33		
Beta 2 microglobulin (mg/L)	Ex+MFGM	2.67 ± 1.14	2.28 ± 0.88	2.56 ± 0.52	$F = 0.813$ (0.490)	
	Ex+Placebo	2.60 ± 0.83	2.53 ± 1.32	2.73 ± 0.39		
	MFGM	2.18 ± 0.65	1.85 ± 0.48	2.46 ± 0.57		
	Placebo	2.20 ± 0.50	2.09 ± 0.59	2.49 ± 0.39		
Myostatin (ng/ml)	Ex+MFGM	54.48 ± 14.92	45.75 ± 16.02	46.39 ± 10.07	$F = 2.170$ (0.097)	
	Ex+Placebo	49.39 ± 11.63	46.48 ± 18.11	49.29 ± 12.57		
	MFGM	50.42 ± 14.82	46.84 ± 16.77	48.52 ± 12.08		
	Placebo	51.13 ± 16.02	48.64 ± 17.55	49.59 ± 13.75		
(IGFBP3/IGF1)×100	Ex+MFGM	5.50 ± 2.28	5.02 ± 1.96	4.63 ± 1.89	$F = 3.835$ (0.013)	Ex+MFGM>P
	Ex+Placebo	4.18 ± 1.46	4.90 ± 2.46	5.36 ± 1.73		
	MFGM	3.97 ± 1.36	4.11 ± 1.62	4.24 ± 1.51		
	Placebo	4.65 ± 1.72	5.38 ± 1.93	5.20 ± 1.91		
Growth hormone	Ex+MFGM	0.68 ± 0.56	1.13 ± 0.71	1.07 ± 0.46	$F = 0.301$	

(*Continued*)

Table 2. (*Continued*)

Variables*	Group†	Baseline	Post intervention	Follow-up	GEE‡ (G×T) (P-value)	Post hoc analysis# (P<0.05)
(ng/ml)	Ex+Placebo	0.52 ± 0.48	0.94 ± 0.87	0.97 ± 0.59	(0.825)	
	MFGM	0.83 ± 0.74	1.24 ± 0.90	1.18 ± 0.60		
	Placebo	0.72 ± 0.64	1.04 ± 0.88	1.09 ± 0.70		

Note: * Data are presented as mean and standard deviation

† Ex = exercise group

MFGM = milk fat globule membrane

BDNF = brain-derived neurotrophic factor

IGF = insulin-like growth factor

IGFBP = insulin-like growth factor binding protein

P = placebo.

‡ GEE = *generalized estimating equation*, G = *group*, T = *time*.

♯ A post hoc analysis was performed using the Scheffe method (P<0.05).

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Table 3. Effects of the intervention on each frailty criteria between baseline, post-intervention and follow-up.

Frailty criteria †	Group				P-value ‡	Post hoc analysis #
	Ex +MFGM (n = 33)	Ex +Placebo (n = 33)	MFGM (n = 32)	Placebo (n = 32)		(P<0.05)
Weight loss						
Reversal rate from baseline to post-intervention	0.0	-12.1	-18.7	-30.3 *	0.007	Ex+MFGM<MFGM, P
Reversal rate from baseline to follow-up	39.4 *	33.3 *	15.6	-6.1	0.005	Ex+MFGM>MFGM, P; Ex+P>P
Exhaustion						
Reversal rate from baseline to post-intervention	30.3 *	69.7 *	18.7	30.3 *	<0.001	Ex+P>Ex+MFGM, MFGM, P
Reversal rate from baseline to follow-up	33.3 *	42.4 *	25.0 *	-6.1	0.007	Ex+MFGM, Ex+P, MFGM>P
Low physical activity						
Reversal rate from baseline to post-intervention	54.5 *	57.6 *	40.6 *	30.3 *	0.096	
Reversal rate from baseline to follow-up	36.4 *	9.1	9.4	9.1	0.004	Ex+MFGM>Ex+P, MFGM, P
Low muscle strength						
Reversal rate from baseline to post-intervention	6.1	3.0	-12.5	6.1	0.495	
Reversal rate from baseline to follow-up	3.0	-3.1	-9.4	-9.1	0.536	
Slow walking speed						
Reversal rate from baseline to post-intervention	18.2	9.1	-12.5	-3.0	0.247	
Reversal rate from baseline to follow-up	42.4 *	18.2	15.6	0.0	<0.001	Ex+MFGM>Ex+P, MFGM>P

† All data of change (baseline to post-intervention; baseline to follow-up) presented as individual mean percent change.

Reversal rate signifies reversal of each frailty criteria, i.e presented with the criteria at baseline but not at post intervention or follow-up.

* McNemar test P<0.05: within-group percent change between baseline and post-intervention, and baseline and follow-up.

‡ P-values were calculated using Kruskal Wallis for continuous variables.

Post-hoc analysis was assessed using Mann-Whitney test for continuous variables (P<0.05).

Ex = exercise

MFGM = milk fat globule membrane

P = placebo.

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Table 4. Effects of the intervention on each muscle mass and physical function between baseline, post-intervention and follow-up.

Variable	Group				P-value ‡	Post hoc analysis #
	Ex+MFGM	Ex+Placebo	MFGM	Placebo		
	(n = 33)	(n = 33)	(n = 32)	(n = 32)		(P<0.05)
Leg muscle mass						
Change from baseline to post-intervention†	2.4 ± 0.5	1.5 ± 0.5	1.2 ± 2.5	1.1 ± 3.0	0.565	
(95% CI for difference)	(1.3 to 3.5)	(0.3 to 2.3)	(0.2 to 2.2)	(-0.1 to 2.2)		
Change from baseline to follow-up†	3.2 ± 1.1	3.2 ± 1.0	2.5 ± 1.1	2.1 ± 1.0	0.834	
(95% CI for difference)	(1.1 to 5.4)	(1.2 to 5.3)	(0.29 to 4.7)	(-0.04 to 4.3)		
Grip strength						
Change from baseline to post-intervention†	3.9 ± 1.6	2.8 ± 1.6	3.7 ± 2.2	1.4 ± 1.2	0.701	
(95% CI for difference)	(0.5 to 7.2)	(-0.5 to 6.0)	(-0.9 to 8.3)	(-1.1 to 3.8)		
Change from baseline to follow-up†	-0.2 ± 2.6	-0.4 ± 1.8	-5.4 ± 1.9	-3.4 ± 2.5	0.330	
(95% CI for difference)	(-5.6 to 5.1)	(-4.2 to 3.3)	(-9.3 to -1.5)	(-8.6 to 1.8)		
Usual walking speed						
Change from baseline to post-intervention†	14.7 ± 4.1	9.6 ± 3.4	2.1 ± 1.9	3.6 ± 2.7	0.026	Ex+MFGM>MFGM, P
(95% CI for difference)	(6.4 to 23.1)	(2.7 to 16.4)	(-1.8 to 5.9)	(-1.9 to 9.1)		
Change from baseline to follow-up†	14.8 ± 3.2	5.3 ± 2.5	7.1 ± 2.9	6.7 ± 2.4	0.070	
(95% CI for difference)	(8.2 to 21.4)	(0.2 to 10.5)	(1.1 to 13.1)	(1.8 to 11.5)		
Timed up & go						
Change from baseline to post-intervention†	-14.1 ± 2.0	-18.5 ± 2.1	-6.1 ± 2.6	-3.0 ± 2.6	<0.001	Ex+MFGM, Ex+P>MFGM, P
(95% CI for difference)	(-13.8 to -9.9)	(-22.9 to -14.0)	(-11.6 to -0.7)	(-8.3 to 2.3)		
Change from baseline to follow-up†	-6.5 ± 2.1	-10.2 ± 2.5	-4.6 ± 3.5	-4.9 ± 2.2	0.394	
(95% CI for difference)	(-10.8 to -2.3)	(-15.3 to -5.0)	(-11.8 to 2.5)	(-9.3 to -0.4)		

†All data of change (baseline to post-intervention
baseline to follow-up) presented as mean percent change ± standard error, with 95% confidence intervals (CI)
MFGM = milk fat globule membrane
Ex = exercise.
All mean changes calculated by analysis of covariance adjusted for baseline age and frailty score.
‡ P-values were calculated using ANCOVA for continuous variables.
# Post-hoc analysis was assessed using the Scheffe method for continuous variables (P<0.05).

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Discussion

Main Findings

The results of this study showed that the 3-month exercise and nutrition supplementation program had an effect on frailty status improvement after the intervention and follow-up. In particular, the frailty components revealed that exhaustion, low physical activity, and slow walking speed were reversed, but low muscle strength did not significantly change. The percentage of non-frail people was significantly higher in the Ex+MFGM (57.6%) than in the MFGM (28.1%) or placebo (30.3%) groups at post-intervention ($\chi^2 = 8.827$, P = 0.032). Similarly at the follow-up, the percentage of non-frail people was significantly greater in the Ex+MFGM (45.5%) and Ex+Plac (39.4%) groups compared with the placebo (15.2%) group ($\chi^2 = 8.607$, P = 0.035). The Ex+MFGM group was over 4 times more likely to reverse frailty than the placebo group, and the Ex+Plac group also had a high likelihood of reversing frailty with a significant OR of 3.64 in reference to the placebo group.

Table 5. Effects of the intervention on blood components between baseline, post-intervention and follow-up.

Variable	Group				P-value ‡	Post hoc analysis #
	Ex+MFGM	Ex+Placebo	MFGM	Placebo		
	(n = 33)	(n = 33)	(n = 32)	(n = 32)		(P<0.05)
BDNF						
Change from baseline to post-intervention†	12.1 ± 5.5	13.7 ± 4.5	2.2 ± 2.1	5.0 ± 2.9	0.141	
(95% CI for difference)	(0.9 to 23.3)	(4.4 to 23.1)	(-2.1 to 6.5)	(-1.0 to 11.0)		
Change from baseline to follow-up†	23.9 ± 7.7	17.1 ± 10.0	6.9 ± 4.5	14.8 ± 9.4	0.542	
(95% CI for difference)	(8.1 to 39.7)	(3.6 to 37.9)	(-2.4 to 16.2)	(-4.6 to 34.2)		
Myostatin						
Change from baseline to post-intervention†	-17.4 ± 2.7	-6.9 ± 4.3	-7.9 ± 3.7	-3.0 ± 5.0	0.083	
(95% CI for difference)	(-23.0 to -11.8)	(-15.9 to 2.2)	(-15.7 to -0.2)	(-13.5 to 7.5)		
Change from baseline to follow-up†	-11.1 ± 4.2	1.6 ± 4.5	-2.2 ± 5.6	3.0 ± 6.6	0.211	
(95% CI for difference)	(-19.8 to -2.4)	(-7.8 to 10.9)	(-13.9 to 9.5)	(-10.7 to 16.7)		
IGFBP-3/IGF-1						
Change from baseline to post-intervention†	-5.3 ± 3.7	20.9 ± 10.1	8.3 ± 7.9	20.7 ± 7.8	0.051	
(95% CI for difference)	(-13.0 to 2.3)	(0.2 to 41.6)	(-8.1 to 24.7)	(4.6 to 36.9)		
Change from baseline to follow-up†	-7.7 ± 8.3	40.1 ± 14.2	18.8 ± 14.1	22.7 ± 10.8	0.036	Ex+MFGM<Ex+P
(95% CI for difference)	(-24.8 to 9.4)	(10.6 to 69.6)	(-10.7 to 48.3)	(0.3 to 45.1)		

† All data of change are presented as mean percent change ± standard error, with 95% confidence intervals (CI)

All mean changes calculated by analysis of covariance (ANCOVA) are adjusted for baseline age and frailty score.

MFGM = *milk fat globule membrane*

P = placebo; BDNF = brain-derived neurotrophic factor; IGF-1 = insulin-like growth factor 1

IGFBP-3 = insulin-like growth factor binding protein 3.

‡ *P-values were calculated using ANCOVA for continuous variables.*

Post-hoc analysis was assessed using the Scheffe method for continuous variables (P<0.05).

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Implications for Frailty Reversal by Exercise and Nutrition Supplementation

Many trials have focused on resistance exercise or nutrition supplementation as treatments to reverse frailty, but the results of these previous trials have not always been consistent. Cameron et al, reported that a multifactorial interdisciplinary intervention can successfully treated frailty status [15]. Furthermore, these results were confirmed, that a 3-month exercise and nutrition intervention resulted in short-term frailty status improvement and long-term effect on bone mineral density and serum vitamin D among Taiwanese community-dwelling elders [14]. Another previous trial showed that although no significant improvements were observed, the intervention group tended to have a better outcome in improving frailty [16]. Poor compliance with the intervention program seemed to be the main reasons for unfavorable outcomes. In the current study on frail elderly women, the Ex+MFGM and exercise groups significantly reduced the number of participants who classified as frail. However the combination of Ex+MFGM may be more beneficial as this group was able to reverse weight loss, low physical activity, and slow walking speed in comparison to all the other groups. The post-intervention OR for frailty reversal was 3.12 in the Ex+MFGM group, which was greater than the 2.44 OR in the Ex+Plac group and 0.90 in the MFGM group. Both the Ex+MFGM (OR = 4.67) and the Ex+Plac (OR = 3.64) groups had significant ORs for the reversal of frailty at follow-up, with the Ex+MFGM

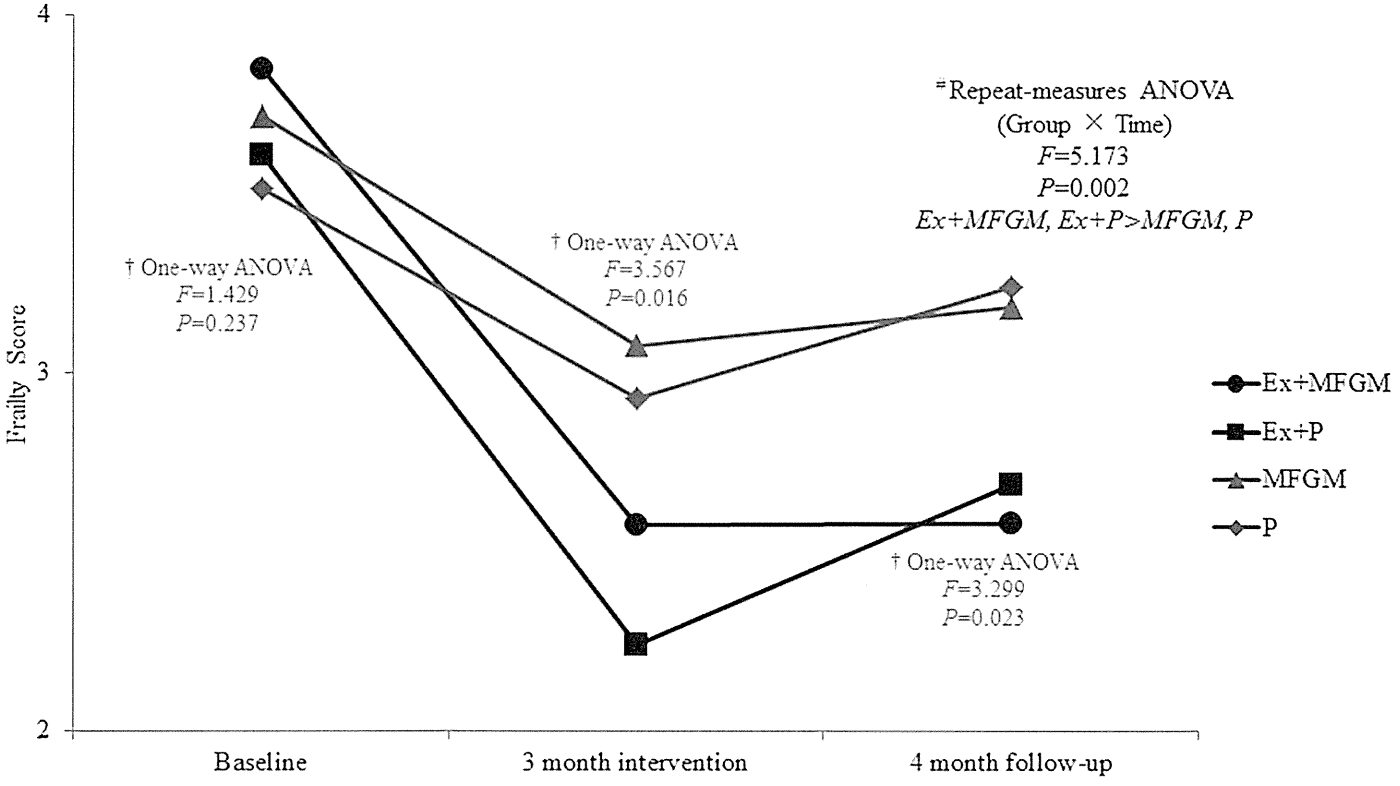

Fig 2. Changes of frailty score between baseline, post-intervention and follow-up. ANOVA = analysis of variance.

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group having a greater OR. The OR for MFGM however, was not significant (OR = 1.87). The adjusted ORs for frailty reversal at both post-intervention and follow-up were greater in the Ex +MFGM group than the Ex+Plac and MFGM only groups.

While some studies have focused on frailty status improvement, others placed importance on improving mobility and function. One previous pilot study reported that a home-based older people's exercise program showed evidence that exercise can reduce the deterioration of mobility in frail older people [17]. Another study demonstrated that resistance training improved muscle strength and size in frail elderly people [18]. These changes were accompanied by improvement in mobility and an increased level of spontaneous physical activity. Multinutrient supplementation did not have any independent or additive effects on the outcomes. Results from clinical trials suggest the use of nutritional consultation as a component in frailty interventions, however not as a stand-alone intervention. One previous study showed that diet and exercise was more effective than diet or exercise alone in improving frailty indicators among 93 obese and frail older adults [19].

Some components of the frailty definition are self-reported while others, like walking speed, are performance based. Performance-based measure should reduce observer bias. Many previous studies have indicated that mobility is a major item in many frailty definitions and appears to predict incident frailty [20]. Mobility is significantly associated with transitions in frailty status [21], and may even be considered as a single-item frailty screening tool [22]. In this study, we analyzed walking ability in frail women in more detail.

Subgroup analysis of the frailty components showed that usual walking speed increased in the Ex+MFGM group by 0.25±0.07 m/s (mean percent change 27.0%, 95% CI for difference =

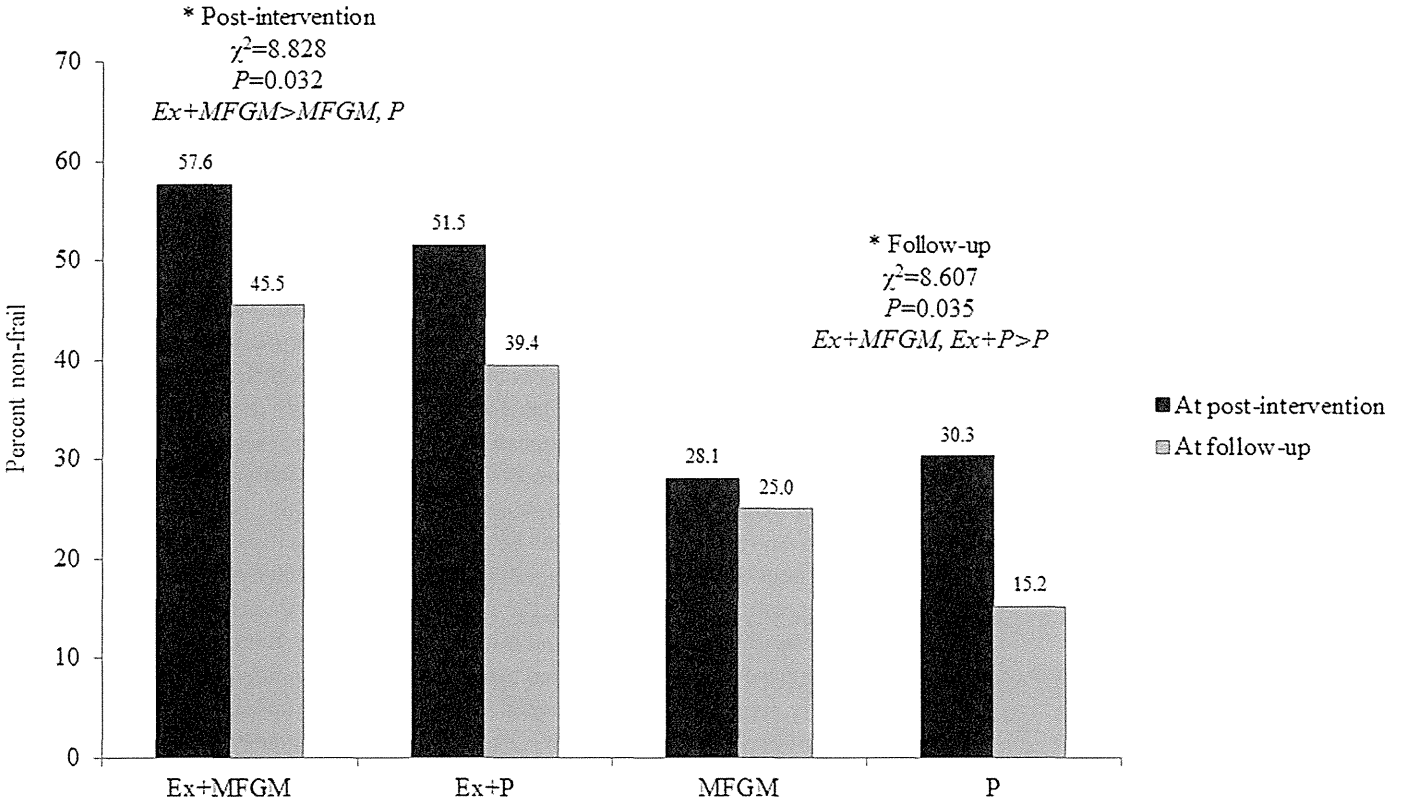

Fig 3. Reversal rates of frailty. Black bar signifies baseline to post-intervention and gray bar signifies baseline and follow-up. Ex = exercise; MFGM = milk fat globule membrane; P = placebo.

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9.69 to 44.26) at post intervention and 0.22±0.05 m/s (mean percent change 23.9%, 95% CI for difference = 13.04 to 34.82) at follow-up, and in the Ex+Plac group by 0.21±0.05 m/s (mean percent change 22.5%, 95% CI for difference = 9.89 to 35.19) after the three-month intervention and 0.09±0.06 m/s (mean percent change 11.0%, 95% CI for difference = -5.6 to 27.7) after the follow-up. However, the changes were not significant in the MFGM and Placebo groups. The improvements in walking speed observed in both exercise groups are clinically significant, as the Society on Sarcopenia, Cachexia, and Wasting Disease stated that an improvement in gait speed of at least 0.1 m/s can be considered as such [23]. Exercise alone or combined exercise and MFGM supplementation was effective for improving walking ability in frail women

Table 6. Adjusted Odds Ratios with 95% Confidence Intervals for frailty at post-intervention and follow-up by intention to treat analysis.

	Adjusted Odds Ratio (95% Confidence Interval)		
Dependent Variable*	**MFGM**	**Exercise+Placebo**	**Exercise + MFGM**
Frailty reversal at post-intervention	0.90 (0.31–2.62)	2.44 (0.89–6.70)	3.12 (1.13–8.60)
Frailty reversal at follow-up	1.87 (0.54–6.47)	3.64 (1.12–11.85)	4.67 (1.45–15.08)

Reference: placebo group

* 0 = frailty, 1 = no frailty

MFGM = milk fat globule membrane.

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with walking speed less than 1.0 m/s. Further analysis showed that the change in walking speed among participants with speeds less than 1.0 m/s, based on the frailty criteria, was 0.13 m/s at post-intervention and 0.12 m/s at follow-up, and in those with walking speeds greater than 1.0 m/s was 0.05m/s at post-intervention and 0.06 m/s at follow-up. Greater improvements were observed in participants with slow walking speed, and smaller improvements were seen in those with faster walking speeds. The improvements in physical activity and exhaustion seen may have possibly been related to the improvements in walking ability.

Researchers have suggested that grip strength testing is likely to be increasingly used in clinical setting, for example in the assessment of sarcopenia, frailty and undernutrition in hospitalized older people [24,25]. In the current study, statistically significant changes in grip strength were not observed in the intervention groups. This may be due to the content of the exercise program. Grip strength is a measure of upper body strength; however the focus of the exercise program was placed on the lower body. Therefore since grip strength as an outcome variable may not have been a particularly suitable measure of strength of the exercise intervention conducted, lower extremity strength measures should also be used in future studies.

Unintentional weight loss is one criterion included in the definition of frailty based on Fried's phenotype; however, its use may not be appropriate in evaluating the effects of interventions on frailty status. During the three month intervention in this study, the change in weight was -3.0 ~ 2.0 kg, and -3.1 ~ 3.5 kg during the follow-up. Whether these changes were unintentional or due to the exercise are unclear and further research is necessary.

Although the Fried frailty phenotype has been validated and modified for use in several published reports, cognitive and psychological factors, which have known association with functional decline and disability, were not included in the frailty phenotype [26]. Several clinical studies show that blood brain-derived neurotrophic factor (BDNF) levels are reduced in Alzheimer's disease [27], mild cognitive impairment [26], and major depressive disorder [28]. In the current study, changes in BDNF levels in the Ex+MFGM and Ex+Plac groups between baseline and follow-up were 23.9% and 17.1%, respectively. These results were significant within each group, hence exercise increased BDNF levels, which is in agreement with previous findings [29,30]. Although depression and cognitive function were not investigated, exercise alone or in congruence with nutrition intervention may potentially be beneficial for the improvement of depression and cognitive function. Further research is necessary.

Myostatin is a significant negative regulator of skeletal muscle development and size [31]. Some studies showed decreases in myostatin protein levels in postmenopausal women and middle-aged men after aerobic exercise [32,33]. However, the effects of modest exercise and nutrition supplementation on myostatin in skeletal muscle remain largely unaddressed in elderly adults. The within group decreases in myostatin from baseline to post-intervention, and follow-up in the Ex+MFGM group was 17.4%, and 11.1%, respectively. Although the decreases obtained in this study were less than those reported by Ryan et al [33], who conducted a 6 month, 3 days per week intervention, the Ex+MFGM intervention significantly decreased myostatin in community-dwelling elderly women.

The insulin-like growth factor (IGF-1) pathway is thought to play major roles in exercise induced muscle hypertrophy and maintenance of muscle, and several groups have explored the effects of resistance training on IGF-1 in older persons, although the results have been inconclusive [34–36]. Several studies have shown that resistance training does not alter IGF-1 or IGFBP-3 in elderly subjects [37,38]. In contrast, Parkhouse et al reported that resistance training increased IGF-1 in older women who had low bone mineral density, and the IGFBP-3/IGF-1 ratios significantly decreased from resistance training [39]. The authors suggested that potentially more IGF-1 were bound to IGFBP-3, possibly contributing to the significant strength gains observed with resistance training in the elderly population. In the current study, IGFBP-

3/IGF-1 ratios only decreased in the Ex+MFGM group, while all the other groups showed increases.

The effects of MFGM alone on frailty reversal, physical function, and biomarkers were minimal in this study. However, MFGM alone did show reversal in exhaustion and low physical activity, although this may be attributed to patient-provider interaction where simply participating in this trial motivated participants in both the MFGM and placebo groups to live healthier lifestyles by increasing their daily physical activity. However, Haramizu et al suggested that the phospholipid and sphingolipids in MFGM together with exercise can improve muscle function deficits through neuromuscular development, as well as neuromuscular junction formation [10]. The authors suggest that the increase in plasma adiponectin observed could have contributed to the greater muscle force and whole-body energy expenditure with exercise and MFGM supplementation in mice. Furthermore, the combination of exercise and MFGM increased the levels of MRNA expression for MyoD and myogenin, which may help to improve NMJ formation, hence improving contractile function of the muscles. This data shows that leg muscle mass and walking speed increase after the intervention in the combine group (Tables 4 and 5), and myostatin levels improved with the intake of MFGM. However, statistically significant additive effects of MFGM with exercise were not observed.

Strengths and Limitations

Strength of this study is in the randomized controlled trial design, and also that it is a double blind and placebo-controlled trial. This study is also the first to explore the effects of an exercise and nutrition intervention on frailty status as well three different aspects: body composition, functional fitness, and hematological parameters.

This study has several limitations. This study focused on elderly women; hence the results of this study cannot be generalized for elderly men. Typically, based on Fried's criteria for frailty, walking speed and grip strength are stratified by height and BMI, respectively. The height cut point used by Fried was 159 cm for women, however only 29 (1.6%) out of 1,835 women were taller than 159 cm, in this Japanese population. Similarly, only 57 (3.1%) people out of the 1,835 had a BMI of greater than 29 kg/m^2, which was the highest quartile cut point used by Fried. Therefore, the cut points used for an American population may be difficult for use in a Japanese population. Further research and analysis of frailty with these cut points in a larger sample of Japanese people is necessary. Future research should also look to follow the participants for a longer follow-up period. Furthermore, readers should be aware of the placebo effects and bias as only the nutritional supplementation could be blinded and exercise could not.

Finally, while statistically significant additive effects of MFGM with exercise could not be confirmed in this population, this data suggested that the nutritional supplementation may be beneficial for the improvement of frailty in elderly women. Generalization of these findings requires the consideration that only 39.6% of all the potential participants who were defined as frail participated in the intervention. The 60.4% of non-participants (or those excluded) had greater mobility impairments. The results of this study should be interpreted with consideration of such selection bias. Further investigation in larger samples is necessary.

Conclusion

This study found that Ex+MFGM showed greater odds of frailty reversal, suggesting that this nutritional supplementation may perhaps be beneficial for the improvement of frailty in elderly women. However, statistically significant additive effects of MFGM with exercise could not be confirmed in this population. Further analysis showed that Ex+MFGM significantly reversed

four of the five components in Fried's frailty phenotype, where improvements in walking ability due to the exercise may have played a major role. The improvements in muscle mass and hematological parameters may have been mediating factors in the improvement in walking ability, possibly leading to the reversal of frailty status, however further research on a larger sample is necessary.

Supporting Information

S1 CONSORT Checklist.
(DOC)

S1 Protocol.
(DOCX)

Acknowledgments

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Author Contributions

Conceived and designed the experiments: HK HY. Performed the experiments: HK MK NK EH HY. Analyzed the data: HK NO AS TH HY TS. Contributed reagents/materials/analysis tools: HK NO AS TH TS. Wrote the paper: HK NO AS TH EH.

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Original Study

Incidence and Predictors of Sarcopenia Onset in Community-Dwelling Elderly Japanese Women: 4-Year Follow-Up Study

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Keywords:
Sarcopenia
incidence
predictor
elderly women

A B S T R A C T

Objectives: Several studies have explored the prevalence and risk factors of sarcopenia, but they have been based on cross-sectional data. The objective of this study was to determine the incidence and predictors of the onset of sarcopenia over 4 years in community-dwelling elderly women.
Design: Four-year longitudinal follow-up study.
Setting: Urban community in Tokyo, Japan.
Participants: A total of 538 nonsarcopenic women older than 75 years.
Measurements: Body composition was determined by bioelectrical impedance analysis. Functional fitness measurements, including grip strength, usual walking speed, timed up and go (TUG), and interview surveys were conducted at baseline and 4-year follow-up. Blood samples were obtained to analyze serum albumin and hemoglobin A1c, and kidney function was analyzed using serum creatinine and cystatin C. Sarcopenia was defined based on the criteria suggested by the European Working Group on Sarcopenia in Older People, and the development of all stages, that is, presarcopenia, sarcopenia, and severe sarcopenia as well as the components of sarcopenia skeletal muscle index (SMI), grip strength, and walking speed, were analyzed.
Results: The incidence of total sarcopenia was 39.6% (presarcopenia 23.8%, sarcopenia 11.2%, severe sarcopenia 4.6%). Older age was significantly predictive of the development of presarcopenia and severe sarcopenia. Body mass index (BMI) lower than 21.0 kg/m^2 was significantly predictive of the development of all stages of sarcopenia, as well as declines in SMI, grip strength, and walking speed. Slow TUG was a predictor of the development of presarcopenia and severe sarcopenia. Increased calf circumference showed protective effects from the development of all stages of sarcopenia. Greater albumin levels also showed lower risk of declines in SMI, walking speed, and development of presarcopenia. Cystatin C was positively associated with the development of severe sarcopenia (odds ratio 1.83, 95% confidence interval 1.08–3.12). Heart disease and hyperlipidemia history were associated with presarcopenia and sarcopenia, respectively.
Conclusion: Age, BMI, calf circumference, and TUG were consistent predictors of the various stages and components of sarcopenia. The data also suggest that cystatin C was associated with higher odds of incident severe sarcopenia, and further study into kidney function and onset of sarcopenia in large populations is needed.

Sarcopenia, the age-associated decline in muscle mass, is a prevalent condition associated with functional decline, falls, disability, morbidity, and mortality among the elderly population.[1,2] Several diagnostic criteria have been suggested since the term sarcopenia was coined by Rosenberg in 1989,[3] although a consensus is yet to be reached. The European Working Group on Sarcopenia

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in Older People (EWGSOP) published a set of criteria defining sarcopenia based on muscle mass, muscle strength, and physical performance. The EWGSOP also suggested conceptual stages, in which sarcopenia is characterized by low muscle mass and either low muscle strength or low physical performance. Severe sarcopenia includes declines in all 3 criteria (low muscle mass, strength, and physical performance).

Previous studies have reported age, low body mass index (BMI), underweight, and minimal physical activity as risk factors of sarcopenia, based on cross-sectional data.[4–6] Interestingly, although causality was not determined, declines in kidney function have cross-sectionally been associated with loss of lean muscle mass, mobility disability, reduced gait speed, and poor physical function.[7–9] There also have been previous findings suggesting that sarcopenia is common among community-dwelling adults with chronic kidney disease,[10] but minimal information is available on whether kidney function measured using cystatin C may be a predictor of sarcopenia. Longitudinal data are necessary to investigate the predictors associated with the onset of sarcopenia, and to confirm or add to the existing conclusions based on cross-sectional results. Furthermore, few studies, if any, have investigated the onset of sarcopenia longitudinally.

Understanding the predictors of sarcopenia onset would provide insight into possible preventive measures as well as identify individuals at risk. Therefore, the purpose of this study was to investigate the predictors of the onset of sarcopenia in community-dwelling elderly Japanese women.

Methods

Subjects

There were 19,900 people living in the Itabashi ward as of April 1, 2008, and 10,948 (55.0%) lived in the Southeastern area of the ward. To maintain a representation of this larger population, a letter inviting people older than 75 years to participate in a comprehensive geriatric health examination survey was sent to these 10,948 community-dwelling people. There were 1670 (15.3%) respondents willing to participate. Among them, 1289 (77.2%) elderly women participated in the survey conducted at the Tokyo Metropolitan Institute of Gerontology (TMIG). Of these women, 575 (44.6%) were present on-site at the time of the follow-up survey in 2012 (Figure 1). For the purposes of this study, the women who were defined as sarcopenic in 2008 were excluded. Of 1082 nonsarcopenic women in 2008, 554 women participated only in the postal interview survey for the follow-up in 2012, and because all body composition, muscle strength, and blood component data could not be obtained, these people were excluded from the analysis. Any missing data or dropouts between 2008 and 2012 were excluded from the analysis.

Based on the EWGSOP definition,[11] presarcopenia was defined as reduced muscle mass (skeletal muscle index [SMI] mass/height^2 <6.42 kg/m^2),[12] sarcopenia was defined as reduced muscle mass (SMI mass/height^2 <6.42 kg/m^2)[12] and either reduced muscle strength (the cutoff for grip strength was adjusted for BMI; elderly women with BMI ≤23.0 had a grip strength cutoff of ≤17 kg; BMI between 23.1 and 26.0 cutoff was ≤17.3 kg; BMI between 26.1 and 29.0 cutoff was ≤18 kg; and BMI >29.0 cutoff was 21.0 kg)[13] or performance (usual walking speed <1.0 m/s),[14] and severe sarcopenia was defined as the presence of all 3 categories, that is, reduced muscle mass, strength, and performance.

The study protocol was approved by the Clinical Research Ethics Committee of TMIG. Procedures were fully explained to all participants, and written informed consent was obtained.

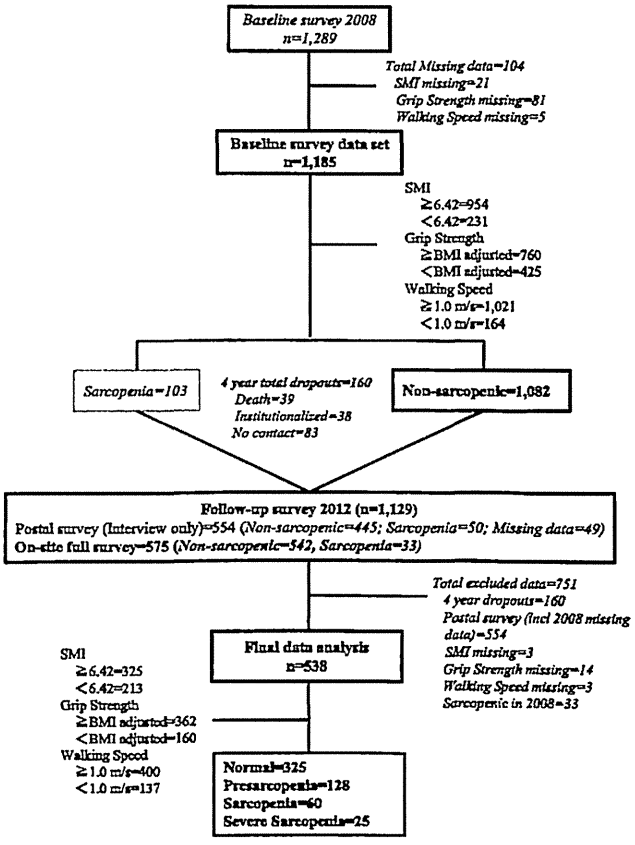

Fig. 1. Detailed flow chart of participants and dropouts over 4 years.

Diagnostic Measures for Sarcopenia

Muscle mass was measured by using a segmental multifrequency bioelectrical impedance analysis instrument that operated at frequencies of 5, 50, 250, and 550 kHz (Well-Scan 500; Elk Corp, Tokyo, Japan). Segmental muscle mass values of the legs and arms were summed to obtain appendicular skeletal muscle mass. Appendicular skeletal muscle mass (ASM) and SMI were determined by the following formula: ASM = left arm + right arm + left leg + right leg muscle, SMI = ASM/height (m) × height (m).[15] Grip strength was measured using a hand-held Smedley type dynamometer. Usual walking speed was measured on a flat, 11-m walking path, with markers at the 3-m and 8-m points. Participants were asked to walk the full 11 m, and a stopwatch was used to measure the time taken to walk 5 m between the markers. The faster of 2 trials was recorded.

Interview

Face-to-face interviews were conducted to assess pain, knee pain, falls, number of falls, fear of falling, injury and fractures, independent activities of daily living (IADL), self-rated health, and chronic conditions, such as history of heart disease, hyperlipidemia, osteoporosis, osteoarthritis (OA), and more. Participants were asked about any fall incidences in the previous 1 year, number of falls, and any injuries and fractures; a fall was defined as an event that resulted in a person coming to rest inadvertently on the ground or other lower level.[16] IADLs were measured using the Instrumental Self-Maintenance dimension of the TMIG index of competence.[17] For each of the 5 items (public transportation, shopping, food preparation, payment, handle finances), "yes" was scored as 1 and "no" as 0 (maximum

score: 5). Participants with a TMIG index Instrumental Self-Maintenance score of less than 4 were defined as having IADL disability.

Anthropometric and Physical Function Measures

Measurements of height and weight were converted to BMI. Bone mineral density (BMD) of the distal radius and ulna of the nondominant forearm was measured by dual-energy X-ray absorptiometry using a DTX-200 osteometer (Osteometer MediTech, Signal Hill, CA). Calf circumference was measured on the left leg in a seated position with the knee and ankle at right angles, feet resting on the floor. Measurements were made at the level of the widest circumference and subcutaneous tissue was not compressed. Knee extension strength was measured as the peak isometric force as the participants extended the knee with maximum power with their knee joint at 90°. A dynamometer was placed at the ankle joint to measure the force of extension. The greater measurement of 2 trials was recorded. For the timed up and go (TUG) test, time was measured in seconds as the time the participants stood up from a straight-backed chair placed against a wall, walked 3 m toward a cone as quickly and safely as possible, walked around the cone, and sat down on the chair again.[18] The faster of 2 trials was recorded. Assistive walking devices were allowed in measures of walking speed and TUG if the participant expressed concerns about walking without a device, or if the investigators suspected dangers of falling.

Blood Indicators

Blood samples were collected in a nonfasting state, in a seated position. Analyses were carried out centrally in one laboratory (Special Reference Laboratories, Tokyo, Japan). Cystatin C concentrations were measured with the sol particle homogeneous immunoassay method (Nescauto GC Cystatin C; Alfresa Pharma, Osaka, Japan).[19] The specific assays used for each measure and methods were as follows: serum albumin (bromcresol green), serum 25-hydroxyvitamin D (DiaSorin-RIA2), hemoglobin (latex agglutination), β 2-microglobulin (latex agglutination immunoassay), hematocrit (sheath flow direct current detection), and serum creatinine (enzymatic).

Covariates for Multivariate Analysis

Parameters such as muscle mass, grip strength, and walking speed used in the definition of sarcopenia were not included as covariates. In this study, covariates were classified under 3 domains in the multivariate analysis: anthropometric and fitness, blood components, and chronic conditions and lifestyle.

Anthropometric and fitness

Age, BMD, calf circumference, and TUG were analyzed as continuous variables and BMI was coded as 1 for less than 21.0 kg/m^2 and 0 for 21.0 kg/m^2 or higher.

Blood components

Albumin, 25-hydroxyvitamin D, beta 2-microglobulin, hemoglobin A1c, high-density lipoprotein (HDL) cholesterol, and cystatin C were analyzed as continuous variables.

Chronic conditions and lifestyle

The chronic conditions included in this analysis were pain, knee pain, falls, osteoporosis, heart disease, hyperlipidemia, and knee OA. Pain and knee pain were coded as 1 for yes and 0 for no, and falls was coded as 1 for yes, having fallen in the previous year and 0 for no falls. Osteoporosis, heart disease, hyperlipidemia, and knee OA were considered present for those who had been diagnosed by a physician and then coded as 1 and 0 for no symptoms.

Data Analysis

Data were presented as mean ± SD for continuous variables and percentages for categorical variables. A 1-way analysis of variance was used to compare variables collected in 2008, including anthropometric values, body composition, and functional fitness measures among the 4 groups: those who remained nonsarcopenic, and those who developed presarcopenia, sarcopenia, or severe sarcopenia in 2012. Chi-square tests were performed for comparisons in categorical variables among the 3 groups between baseline and 4-year follow-up.

Forward stepwise multiple logistic regressions were used to analyze the factors associated with the components included in the definition of sarcopenia and the onset of presarcopenia, sarcopenia, and severe sarcopenia. Model I included the anthropometric and fitness variables potentially associated with sarcopenia. Model II included the blood components on top of the anthropometric and fitness variables, and chronic conditions and lifestyle variables were added in Model III. Nonsignificant variables were forced into the models of the multiple logistic regression analyses to obtain the odds ratio (OR) and 95% confidence interval (CI). P values less than .05 were considered statistically significant. All analyses were performed using the SPSS software, Windows version 20.0 (SPSS Inc., Tokyo, Japan).

Results

Figure 1 shows the participant flow over 4 years. The total dropout rate was 58.3%. Over 4 years, 39 people died, 38 were institutionalized, and we were unable to reach 83 participants. Because body composition, strength, or blood component data could not be obtained in those who participated only in the postal interview survey (n = 554), the final analysis was performed on 538 of 575 people who participated in the full on-site survey (Figure 1). The data of all those who dropped out are outlined in the appendix (Appendix 1).

In comparing the 2008 baseline values of anthropometric, functional fitness, blood component, and chronic condition data among nonsarcopenic, presarcopenic, sarcopenic, and severe sarcopenic participants, the results showed that nonsarcopenic participants had significantly greater BMI, BMD, muscle mass, calf circumference, and were also significantly stronger than those who developed presarcopenia, sarcopenia, or severe sarcopenia (Table 1; all $P < .001$). Those with severe sarcopenia had the slowest walking speed and TUG ($P < .001$). For the blood components, the severe sarcopenia group had the lowest albumin levels ($P = .001$) and highest cystatin C levels ($P < .001$). Furthermore, a greater percentage of those who developed severe sarcopenia originally experienced knee pain ($P = .008$) and IADL disability ($P < .001$).

The analysis of the different components of the sarcopenia definition revealed that older age and BMI lower than 21.0 kg/m^2 were significantly predictive of declines across all 3 components of SMI, grip strength, and walking speed, whereas calf circumference showed significant protective effects in all components (Table 2). Greater albumin levels (OR 0.90, 95% CI 0.82–0.98) showed protective effects for decrease in SMI. History of heart disease (OR 2.05, 95% CI 1.19–3.55) and hyperlipidemia (OR 1.74, 95% CI 1.10–2.77) were significantly associated with a greater risk of SMI decline. Greater BMD (OR 0.40, 95% CI 0.17–0.91) and regular exercise habit (OR 0.30, 95% CI 0.12–0.72) had protective effects of grip strength decline. The predictors for walking speed decline included longer TUG (OR 1.28, 95% CI 1.12–1.48) and higher HDL cholesterol (OR 1.01, 95%

Table 1
Baseline Comparison Between Healthy Community-dwelling Elderly Women and Women Who Developed Sarcopenia

Variables	Nonsarcopenic (n = 325)	Onset of Presarcopenia (n = 128)	Onset of Sarcopenia (n = 60)	Onset of Severe Sarcopenia (n = 25)	*P* Value*	Post Hoc†
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD		
Age, y	78.0 ± 2.6	77.3 ± 2.0	78.5 ± 2.4	80.0 ± 2.1	<.001	SS>N, PS; S>PS
Height, cm	148.4 ± 5.1	150.1 ± 5.0	147.5 ± 5.8	145.7 ± 5.4	<.001	PS>N, S, SS
Body weight, kg	53.7 ± 7.2	46.8 ± 5.3	46.1 ± 6.8	46.8 ± 4.9	<.001	N>PS, S, SS
BMI, kg/m^2	24.4 ± 3.0	20.8 ± 2.2	21.5 ± 2.6	21.9 ± 1.7	<.001	N>PS, S, SS
BMD, g/cm^2	0.30 ± 0.06	0.28 ± 0.05	0.28 ± 0.05	0.26 ± 0.05	<.001	N>PS, S, SS
Muscle mass, kg	32.4 ± 3.1	30.1 ± 2.6	29.2 ± 3.2	28.7 ± 2.5	<.001	N>PS, S, SS
Appendicular muscle mass, kg	16.3 ± 1.7	14.9 ± 1.4	14.5 ± 1.6	14.3 ± 1.3	<.001	N>PS, S, SS
Calf circumference, cm	34.6 ± 2.5	32.1 ± 2.1	32.0 ± 2.6	31.8 ± 2.8	<.001	N>PS, S, SS
Grip strength, kg	19.5 ± 4.1	20.4 ± 3.0	18.1 ± 3.1	14.1 ± 3.4	<.001	N, PS, S>SS; PS>S
Knee extension strength, Nm	63.5 ± 16.8	62.0 ± 13.9	53.3 ± 10.0	45.1 ± 14.5	<.001	N>PS, S>SS
Usual walking speed, m/s	1.30 ± 0.24	1.43 ± 0.19	1.30 ± 0.18	0.98 ± 0.16	<.001	PS>N, S, SS; N, S>SS
Timed up and go, s	7.46 ± 2.6	6.50 ± 1.3	7.27 ± 1.9	9.96 ± 2.9	<.001	SS>N, S>PS
Creatinine, mg/dL	0.66 ± 0.16	0.62 ± 0.11	0.65 ± 0.11	0.69 ± 0.18	.018	N>PS
Albumin, g/dL	4.26 ± 0.21	4.35 ± 0.22	4.27 ± 0.22	4.21 ± 0.22	.001	PS>N, SS
25 Hydroxyvitamin D, ng/mL	21.88 ± 6.56	22.57 ± 5.99	21.88 ± 5.62	23.12 ± 7.41	.620	
β 2-microglobulin, mg/L	1.92 ± 0.53	1.18 ± 0.52	1.89 ± 0.37	2.11 ± 0.57	.047	
Cystatin C, mg/L	0.95 ± 0.21	0.87 ± 0.16	0.92 ± 0.15	1.02 ± 0.19	<.001	SS>PS; N>PS
Chronic conditions and lifestyle variables						
Falls, yes, %	16.0	15.6	16.7	20.0	.957	
Multi frequency of falls, yes, %	3.1	3.9	6.7	4.0	.604	
Pain, yes, %	63.1	52.3	63.3	72.0	.110	
Knee pain, yes, %	37.7	23.0	25.9	45.8	.008	
IADL, disability, %	1.5	0.8	3.3	16.0	<.001	
Regular exercise, yes, %	42.8	40.6	43.3	24.0	.322	
Knee osteoarthritis history, yes, %	27.2	16.4	30.0	280.0	.079	
Osteoporosis history, yes, %	23.7	32.8	35.0	24.0	.110	
Heart disease history, yes, %	16.9	23.4	25.0	24.0	.254	
Hyperlipidaemia history, yes, %	38.8	43.8	45.0	40.0	.692	

N, nonsarcopenic; PS, presarcopenia; S, sarcopenia; SS, severe sarcopenia.
*One-way analysis of variance for continuous variables and chi-square test for categorical variables.
†A post hoc analysis was performed using the Scheffe method.

CI 1.00–1.03), cystatin C levels (OR 1.34, 95% CI 1.03–1.74), and knee pain (OR 1.73, 95% CI 1.08–2.76). Greater BMD (OR 0.51, 95% CI 0.32–0.79) and albumin (OR 0.17, 95% CI 0.06–0.46) were protective against reduction in walking speed over 4 years.

The stepwise logistic regression analysis for presarcopenia revealed that older age, low BMI (<21.0 kg/m^2), and longer TUG were risk factors for the development of presarcopenia across all 3 models (Table 3). Greater calf circumference and albumin levels had

Table 2
Adjusted ORs and 95% CIs for the Predictors of Declines in Muscle Mass, Grip Strength and Walking Speed in 3 Models

Independent Variable	> Cutpoint* Reference	SMI	Grip Strength	Walking Speed
		n = 146 OR (95% CI)	n = 186 OR (95% CI)	n = 148 OR (95% CI)
Anthropometric and fitness				
Age, per 1 y	1	1.02 (0.93–1.13)†	1.32 (1.12–1.54)†	1.22 (1.12–1.32)†
BMI, <21.0 kg/m^2	1	1.86 (1.04–3.31)†	1.39 (1.13–1.72)†	1.25 (1.11–1.40)†
BMD, per 1 unit	1	1.43 (0.88–2.32)	0.40 (0.17–0.91)†	0.51 (0.32–0.79)†
Calf circumference, per 1 unit	1	0.83 (0.73–0.94)†	0.65 (0.52–0.83)†	0.81 (0.72–0.92)†
Timed up and go, per 1 unit	1	1.00 (0.89–1.13)	1.06 (0.88–1.27)	1.28 (1.12–1.48)†
Blood components				
Albumin, per 1 unit	1	0.90 (0.82–0.98)†	0.23 (0.03–1.65)	0.17 (0.06–0.46)†
25 Hydroxyvitamin D, per 1 unit	1	1.02 (0.98–1.05)	1.00 (0.94–1.06)	1.02 (0.98–1.05)
β 2-microglobulin, per 1 unit	1	2.75 (0.97–7.83)	0.71 (0.12–4.20)	1.00 (0.41–2.48)
Hemoglobin A1c, per 1 unit	1	0.80 (0.51–1.26)	0.76 (0.32–1.84)	1.39 (0.90–2.13)
HDL cholesterol	1	1.00 (0.98–1.02)	1.01 (0.98–1.05)	1.01 (1.00–1.03)†
Cystatin C, per 1 unit	1	0.08 (0.01–1.17)	1.06 (0.77–1.46)	1.34 (1.03–1.74)†
Chronic conditions and lifestyle				
Regular exercise habit, yes	1	0.80 (0.48–1.32)	0.30 (0.12–0.72)†	0.79 (0.50–1.26)
Pain, yes	1	0.85 (0.51–1.41)	0.90 (0.36–2.27)	0.76 (0.45–1.31)
Knee pain, yes	1	1.36 (0.72–2.61)	2.19 (0.89–5.45)	1.73 (1.08–2.76)†
Falls, yes	1	0.93 (0.50–1.72)	0.83 (0.36–1.95)	1.78 (0.82–3.86)
Osteoporosis, yes	1	1.66 (0.96–2.88)	1.75 (0.70–4.36)	1.14 (0.70–1.85)
Heart disease, yes	1	2.05 (1.19–3.55)†	1.32 (0.45–3.91)	1.30 (0.74–2.30)
Hyperlipidemia, yes	1	1.74 (1.10–2.77)†	0.96 (0.39–2.35)	1.00 (0.64–1.56)
Knee OA, yes	1	1.24 (0.72–2.15)	1.52 (0.53–4.39)	1.19 (0.67–2.10)

*Skeletal muscle mass index ≧6.42; grip strength greater than BMI adjusted values; usual walking speed ≧1.0 m/s.
†Statistically significant *P* values ($P < .05$) for forward stepwise multiple logistic regressions.

Table 3
Adjusted ORs and 95% CIs for the Predictors of Presarcopenia in 3 Models

Independent Variable	> Cutpoint* Reference	Model I	Model II	Model III
		OR (95% CI)	OR (95% CI)	OR (95% CI)
Anthropometric and fitness				
Age, per 1 y	1	1.13 (1.01–1.26)†	1.10 (1.00–1.22)†	1.11 (1.00–1.23)†
BMI, <21.0 kg/m^2	1	2.31 (1.19–4.49)†	6.40 (3.59–11.44)†	7.22 (4.02–12.96)†
BMD, per 1 unit	1	0.99 (0.63–1.56)	1.34 (0.87–2.06)	1.13 (0.82–2.06)
Calf circumference, per 1 unit	1	0.61 (0.54–0.69)†	0.64 (0.56–0.74)†	0.62 (0.50–0.76)†
Timed up and go, per 1 unit	1	1.29 (1.09–1.52)†	1.27 (1.09–1.49)†	1.30 (1.10–1.52)†
Blood components				
Albumin, per 1 unit	1		0.84 (0.76–0.94)†	0.85 (0.76–0.94)†
25 Hydroxyvitamin D, per 1 unit	1		0.99 (0.95–1.03)	0.99 (0.96–1.04)
β 2-microglobulin, per 1 unit	1		1.51 (0.96–2.38)	1.53 (0.98–2.42)
Hemoglobin A1c, per 1 unit	1		1.16 (0.76–1.79)	1.09 (0.68–1.74)
HDL cholesterol	1		1.00 (0.99–1.02)	1.00 (0.99–1.02)
Cystatin C, per 1 unit	1		1.17 (1.00–1.36)†	1.14 (0.97–1.33)
Chronic conditions and lifestyle				
Regular exercise habit, yes	1			0.70 (0.42–1.14)
Pain, yes	1			0.96 (0.54–1.71)
Knee pain, yes	1			0.80 (0.40–1.60)
Falls, yes	1			1.16 (0.56–2.39)
Osteoporosis, yes	1			0.69 (0.40–1.18)
Heart disease, yes	1			1.97 (1.11–3.49)†
Hyperlipidemia, yes	1			1.48 (0.91–2.41)
Knee OA, yes	1			1.21 (0.62–2.35)

*Skeletal muscle mass index ≧6.42 and grip strength greater than BMI adjusted or skeletal muscle mass index ≧6.42 and usual walking speed ≧1.0 m/s.
†Statistically significant *P* values ($P < .05$) for forward stepwise multiple logistic regressions.

protective effects against the development of presarcopenia. Cystatin C was a predictor of presarcopenia development in Model II (OR 1.17, 95% CI 1.00–1.36), and history of heart disease (OR 1.97, 95% CI 1.11–3.49) was also a predictor of presarcopenia.

The analysis for sarcopenia predictors revealed that low BMI was a significant predictor of the development of sarcopenia, whereas greater calf circumference protected against sarcopenia development across all 3 models (Table 4). Hyperlipidemia (OR 1.94, 95% CI 1.02–3.69) was a significant predictor of sarcopenia.

Older age and longer TUG were significant predictors of severe sarcopenia development, and greater calf circumference showed a protective effect for the development of severe sarcopenia (Table 5). Low BMI was a predictor of severe sarcopenia only in Models II (OR 1.54, 95% CI 1.33–1.78) and III (OR 1.45, 95% CI 1.17–1.81). Higher BMD was a protective variable of severe sarcopenia only in Model III (OR 0.21, 95% CI 0.06–0.82). Furthermore, higher cystatin C levels (OR 1.83, 95% CI 1.08–3.12) was predictive of severe sarcopenia development.

Discussion

The results showed that low BMI was consistently predictive of presarcopenia, sarcopenia, and severe sarcopenia, as well as the components of the definition (ie, declines in SMI, grip strength, and

Table 4
Adjusted ORs and 95% CIs for the Predictors of Sarcopenia in 3 Models

Independent Variable	> Cutpoint* Reference	Model I	Model II	Model III
		OR (95% CI)	OR (95% CI)	OR (95% CI)
Anthropometric and fitness				
Age, per 1 y	1	1.09 (0.96–1.22)	1.07 (0.95–1.21)	1.08 (0.94–1.23)
BMI, <21.0 kg/m^2	1	1.55 (1.35–1.79)†	1.54 (1.33–1.78)†	1.57 (1.35–1.83)†
BMD, per 1 unit	1	1.10 (0.61–1.99)	1.05 (0.57–1.94)	1.10 (0.55–2.19)
Calf circumference, per 1 unit	1	0.82 (0.69–0.98)†	0.81 (0.68–0.97)†	0.83 (0.69–0.98)†
Timed up and go, per 1 unit	1	1.03 (0.89–1.19)	1.03 (0.89–1.19)	1.02 (0.87–1.20)
Blood components				
Albumin, per 1 unit	1		1.04 (0.88–1.22)	1.06 (0.88–1.27)
25 Hydroxyvitamin D, per 1 unit	1		1.00 (0.96–1.05)	1.01 (0.96–1.06)
β 2-microglobulin, per 1 unit	1		1.16 (0.31–4.43)	1.08 (0.28–4.26)
Hemoglobin A1c, per 1 unit	1		0.96 (0.52–1.76)	0.85 (0.45–1.59)
HDL cholesterol	1		1.00 (0.98–1.02)	1.00 (0.98–1.03)
Cystatin C, per 1 unit	1		1.01 (0.71–1.44)	1.01 (0.71–1.45)
Chronic conditions and lifestyle				
Regular exercise habit, yes	1			1.08 (0.53–2.21)
Pain, yes	1			0.70 (0.31–1.55)
Knee pain, yes	1			1.56 (0.60–4.08)
Falls, yes	1			0.87 (0.37–2.06)
Osteoporosis, yes	1			1.52 (0.73–3.15)
Heart disease, yes	1			1.00 (0.71–3.52)
Hyperlipidemia, yes	1			1.94 (1.02–3.69)†
Knee OA, yes	1			2.11 (0.93–4.76)

*Skeletal muscle mass index ≧6.42 and grip strength greater than BMI adjusted or skeletal muscle mass index ≧6.42 and usual walking speed ≧1.0 m/s.
†Statistically significant *P* values ($P < .05$) for forward stepwise multiple logistic regressions.

Table 5
Adjusted ORs and 95% CIs for the Predictors of Severe Sarcopenia in 3 Models

Independent Variable	> Cutpoint* Reference	Model I	Model II	Model III
		OR (95% CI)	OR (95% CI)	OR (95% CI)
Anthropometric and fitness				
Age, per 1 y	1	1.27 (1.06–1.52)†	1.27 (1.05–1.52)†	1.25 (1.02–1.52)†
BMI, <21.0 kg/m^2	1	1.36 (0.32–5.74)	1.54 (1.33–1.78)†	1.45 (1.17–1.81)†
BMD, per 1 unit	1	0.75 (0.30–1.89)	0.58 (0.22–1.54)	0.21 (0.06–0.82)†
Calf circumference, per 1 unit	1	0.62 (0.49–0.78)†	0.62 (0.48–0.78)†	0.69 (0.49–0.96)†
Timed up and go, per 1 unit	1	1.21 (1.07–1.37)†	1.21 (1.06–1.36)†	1.42 (1.16–1.74)†
Blood components				
Albumin, per 1 unit	1		0.86 (0.66–1.12)	0.85 (0.64–1.14)
25 Hydroxyvitamin D, per 1 unit	1		0.99 (0.95–1.05)	0.94 (0.88–1.01)
β 2-microglobulin, per 1 unit	1		0.53 (0.09–3.06)	0.25 (0.04–1.78)
Hemoglobin A1c, per 1 unit	1		0.45 (0.14–1.45)	0.55 (0.06–4.84)
HDL cholesterol	1		1.00 (0.97–1.04)	1.00 (0.96–1.04)
Cystatin C, per 1 unit	1		1.37 (0.86–2.18)	1.83 (1.08–3.12)†
Chronic conditions and lifestyle				
Regular exercise habit, yes	1			0.59 (0.18–1.92)
Pain, yes	1			0.46 (0.09–2.31)
Knee pain, yes	1			0.99 (0.22–4.35)
Falls, yes	1			1.05 (0.22–4.91)
Osteoporosis, yes	1			1.21 (0.33–4.41)
Heart disease, yes	1			0.91 (0.26–3.15)
Hyperlipidemia, yes	1			1.46 (0.43–4.94)
Knee OA, yes	1			1.52 (0.39–5.88)

*Skeletal muscle mass index ≧6.42 and grip strength greater than BMI adjusted values and usual walking speed ≧1.0 m/s.
†Statistically significant *P* values ($P < .05$) for forward stepwise multiple logistic regressions.

walking speed). Greater calf circumference consistently predicted lower risk of developing all stages of sarcopenia. Slow TUG was also a consistent predictor of presarcopenia and severe sarcopenia. Cystatin C was a significant predictor of severe sarcopenia alone. These results support previous cross-sectional research that has shown that factors such as age, BMI, physical activity,[4,6] and leg strength[20] are associated with sarcopenia. Although the current study confirmed these findings with longitudinal data, there were several interesting findings that have not previously been observed.

A primary finding of this study was that a higher level of cystatin C was associated with a greater risk of severe sarcopenia onset. Sarcopenia has been reported to be common among community-dwelling older adults with chronic kidney disease,[10] and there have been studies showing significant relationships between cystatin C and physical function measures, mobility in particular, among the elderly; and sarcopenia has been seen in patients with end-stage renal disease.[21,22] Interestingly, the results of our study revealed that cystatin C was a predictor of severe sarcopenia but not sarcopenia. As these results suggest, sarcopenia is a complex systemic condition. One previous study suggested that a heightened inflammatory state could explain the association between chronic kidney disease and the development of functional impairment,[23] as higher levels of inflammatory markers were associated with declines in physical function and muscle mass as well as strength.[24,25] Although causality could not be determined in this study, there is a significant association between kidney dysfunction and severe sarcopenia. Cystatin C has been associated in previous research with declines in muscle mass, strength, and walking ability, separately. The results of the current study suggest that cystatin C may be a common mediating factor involved in muscle mass, strength, and walking ability, as associations were observed between cystatin C and the combination of all 3 factors. Perhaps the improvement of cystatin C could positively affect sarcopenia status. In a clinical setting, clinical practitioners who observe high cystatin C levels may also want to look closely for severe sarcopenia, and suggest ways to improve physical function as well as improvements in kidney function. Further research is needed to determine not only the mechanism of this relationship, but also interventions that may improve the quality of life of elderly people with kidney dysfunction and sarcopenia.

The relationship between BMD and physical performance is still unclear. There is conflicting and inconsistent evidence from various research studies, with some supporting the hypothesis that physical performance has no association with BMD, and other studies showing positive associations of BMD and lower extremity measures fitness.[26] A previous report suggested that hip BMD was a risk factor for sarcopenia in elderly men.[4] Similarly, in our study we found that higher BMD had protective effects against severe sarcopenia as well as grip strength and walking speed. This association was, however, seen only in severe sarcopenia and not sarcopenia alone. One recent study suggested that the treatment of sarcopenia may be increasingly important for the prevention of fractures.[27] Based on our findings and those of previous studies, perhaps clinical practitioners should suggest treatment options to improve sarcopenia as well as BMD or osteoporosis.

BMI may be a predictor of skeletal muscle mass for women.[20] Recently, one study indicated that nursing home residents who had a BMI higher than 21.0 kg/m^2 had lower risk of being sarcopenic, relative to those with BMI less than 21.0 kg/m^2.[6] Our data also showed that a BMI below 21.0 kg/m^2 significantly increased the risk of developing sarcopenia and severe sarcopenia.

Although research has suggested that calf circumference could be used to assess muscle mass,[28,29] one report indicated that calf circumference could not be used to predict sarcopenia, but may provide valuable information on muscle-related disability and physical function.[28] Recently, calf circumference has been positively related to lower frailty index and higher functional performance.[29] The authors suggested that calf circumference is a valuable tool for clinicians, and seems relevant for the screening of sarcopenia. Needless to say, the literature regarding calf circumference and sarcopenia is inconsistent. Participants in our study who had greater calf circumference had a significantly lower risk of developing all stages of sarcopenia over 4 years. Such results support the positive findings that calf circumference is indeed a simple, valuable measurement for predicting the risk of developing sarcopenia.

TUG was developed to predict falls in older people, and is also a commonly used assessment for physical function and balance.[30] Recently, TUG was found to be significantly associated with relative muscle mass and knee extension torque, and suggested that should