Table 3. Details of Incident Fractures by Cohort

Cohort[a]	Person-years	Incident fracture				
		Osteoporotic	Hip	Distal forearm	Tibia/fibula	Humerus/elbow
AHS	6,928	78	25	32	–	14
APOSS	34,588	236	7	113	–	47
CaMos	38,016	618	90	220	18	109
DOES	9,892	339	94	100	25	48
ECOSAP	14,811	282	52	108	–	49
EPIC-Norfolk	47,973	172	82	73	–	–
EPIDOS	25,714	1,056	311	312	–	237
EVOS/EPOS	20,945	520	30	153	36	43
GBG I	9,191	255	198	–	–	–
GBG II	87,577	887	116	443	31	98
GOS	7,315	143	32	34	9	15
Manitoba	232,076	2,855	536	1,070	–	770
Miyama	3,423	51	7	11	1	5
MsOs HK	6,975	96	21	43	–	8
OFELY	7,290	132	20	50	1	17
OPUS	12,019	113	13	68	–	28
OSTPRE	30,568	259	8	192	–	24
PERF	38,991	561	58	353	–	78
Rochester	5,318	219	42	39	16	20
Rotterdam	23,977	550	156	221	37	84
SEMOF	19,639	534	80	184	20	104
Sheffield	8,235	292	91	106	14	37
SOF	115,810	3,211	1,269	967	159	735
THIN	852,566	8,343	1,953	–	–	–
WHI	596,434	8,478	1,166	3,318	1,553	1,385
Totals	2,256,271	30,280	6,457	8,210	1,920	3,955
Age at fracture (years), mean (SD)		72.7 (10.4)	79.5 (8.8)	71.0 (9.6)	69.6 (8.5)	73.6 (9.7)

– = site of fracture not given.

[a]The cohort abbreviations are defined in detail in the Cohorts studied section of Subjects and Methods, and are defined in brief in the footnotes for Table 1.

women were overweight or obese (56%), with 22.1% being obese (Table 4). Approximately 7700 women (1.9%) were underweight. There was a weak but significant negative correlation between age and BMI ($p < 0.001$; $r = -0.01$; 95% CI, −0.01 to −0.01). For example, in women aged 55 to 59 years, 1.3% of women were underweight and the proportion increased progressively with age, so that 5.8% of women aged 85 to 89 years were underweight. Conversely, the prevalence of obesity decreased with age from 25.3% in the age group 55 to 59 years to 10.9% between the ages of 85 and 89 years. There was a significant positive correlation between BMI and BMD ($p < 0.001$; $r = 0.33$; 95% CI, 0.32–0.33). In underweight women, the mean BMD femoral neck *Z*-score was −0.89 and for the obese II category it was 0.67 (Table 4).

BMI and risk of fracture

A total of 30,280 osteoporotic fractures were reported during follow-up (Table 3). A minority (19%) of all osteoporotic fractures occurred in obese women (Table 5) and the observed number was lower than expected (5798 versus 6691, respectively) if BMI was assumed to exert no influence on fracture risk. Thus obesity was a protective factor for osteoporotic fractures as a whole. Similar results were found when hip fracture or distal forearm

Table 4. Baseline Characteristics by BMI Category

	Underweight (BMI <18.5)	Normal (BMI 18.5–24.9)	Overweight (BMI 25.0–29.9)	Obese I (BMI 30.0–34.9)	Obese II (BMI ≥35.0)
Subjects (*n*)	7,699	166,087	136,873	58,919	29,032
Age (years)	65.7 (14.0)	62.2 (11.6)	63.6 (10.7)	63.2 (10.1)	61.2 (9.3)
BMI (kg/m^2)	17.2 (1.3)	22.5 (1.6)	27.2 (1.4)	32.0 (1.4)	39.3 (4.5)
Femoral neck BMD (*Z*-score)	−0.89 (0.97)	−0.25 (0.93)	0.12 (0.94)	0.41 (0.96)	0.67 (1.0)
Subjects with BMD values (*n*)	2,309	46,796	37,741	15,051	6,370

Values are mean (SD).

BMI = body mass index (kg/m^2); BMD = bone mineral density.

Table 5. Number of Fractures According to Fracture Outcome and Category of Baseline BMI

	BMI categories[a]					Obese versus non-obese		
Fracture outcome	Underweight (1.9%)	Normal (41.7%)	Overweight (34.3%)	Obese I (14.8%)	Obese II (7.3%)	HR	95% CI	*p*
Osteoporotic	806 (575)	13,293 (12,627)	10,383 (10,386)	4119 (4481)	1679 (2210)	0.85	0.82–0.88	<0.001
Hip	320 (123)	3257 (2693)	2062 (2215)	628 (956)	190 (471)	0.63	0.59–0.68	<0.001
Distal forearm	126 (150)	3424 (3424)	2990 (2816)	1202 (1215)	468 (599)	0.81	0.76–0.86	<0.001
Tibia/fibula	10 (36)	608 (801)	704 (659)	361 (284)	237 (140)	1.04	0.94–1.14	>0.30
Humerus/elbow	76 (75)	1452 (1649)	1399 (1357)	694 (585)	334 (289)	1.21	1.11–1.31	<0.001

Values are the number of fractures in each BMI category and in parentheses are the expected number of fractures according to the percentage of women in each BMI category.

BMI = body mass index; HR = hazard ratio; CI = confidence interval.

[a]BMI categories (kg/m^2): Underweight, BMI <18.5; Normal, BMI 18.5–24.9; Overweight, BMI 25.0–29.9; Obese I, BMI 30.0–34.9; Obese II, BMI ≥35.0. Percentages are the proportion of women in each BMI category.

fractures were considered individually (Table 5). In contrast, the observed incidence of lower leg fractures was not reduced, and the risk of upper arm fractures was higher than expected in obese women.

When BMI was used as a continuous variable, there was a significant association between BMI and fracture risk ($p < 0.001$). In the case of all osteoporotic fractures, the HR per unit increase of BMI was 0.98 (95% CI, 0.98–0.98) and for hip fracture it was 0.93 (95% CI, 0.92–0.94). The HR was not, however, uniform across BMI; low BMI was associated with a greater risk than would be predicted from a uniform HR and, conversely, a high BMI contributed less to fracture prevention than expected. Thus, when studying the relationship in more detail with spline functions, the function was steeper below a BMI of 25 kg/m^2 than above this value (Fig. 1). When a woman with a BMI of 15 kg/m^2 was compared with a woman with a BMI of 25 kg/m^2 using piecewise linear functions, the HR was 1.5 (95% CI, 1.4–1.6) for osteoporotic fracture and 2.9 (95% CI, 2.6–3.3) for hip fracture (Table 6). By contrast, if a woman with a BMI of 25 kg/m^2 was compared to one with a BMI of 35 kg/m^2, the HR was 0.9 (95% CI: 0.9–0.9) for osteoporotic fracture and 0.7 (95% CI = 0.6–0.8) for hip fracture.

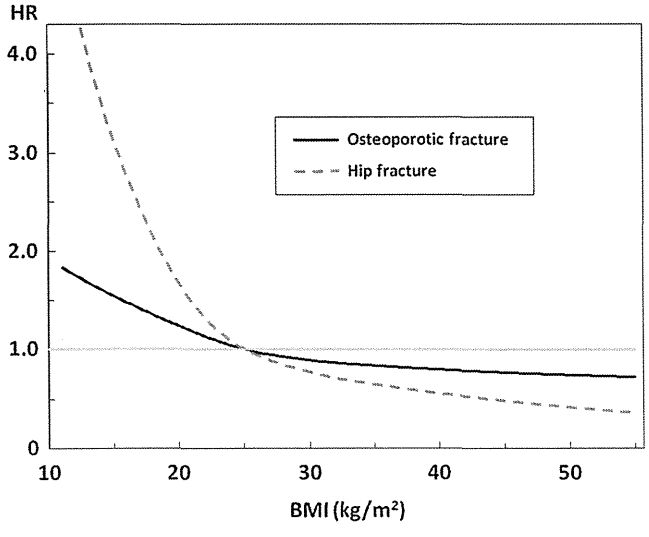

Fig. 1. Relationship between BMI and risk of fracture (HR versus BMI 25 kg/m^2) for osteoporotic fracture (solid line) and hip fracture (dashed line), adjusted for age and time since baseline. BMI = body mass index; HR = hazard ratio.

The use of BMI as a continuous variable also confirmed the different patterns between fracture sites. In the case of upper arm fractures, a BMI of 35 kg/m^2 conferred a significantly higher risk than a BMI of 25 kg/m^2, whereas a BMI of 15 kg/m^2 had a similar risk to that at 25 kg/m^2 (Table 6). The lower BMI was associated with a significant reduction in lower leg fractures, whereas the risk was similar at 25 and 35 kg/m^2 (Table 6).

Adjustment for BMD

When the association between BMI and hip fracture risk was adjusted for BMD, the association was weaker than in the absence of BMD but was still significantly negative. The HR was 0.99 per 1 kg/m^2 increase (95% CI, 0.98–0.99; $p = 0.0014$). When the relationship was examined with spline functions, the relationship was much flatter with BMD adjustment (Fig. 2) than without (Fig. 1). Not withstanding, the risk of hip fracture with low BMI was greater than the protective effect of a high BMI. Thus, a BMI of 15 kg/m^2 had an HR of 1.4 (95% CI, 1.2–1.7) compared to a BMI of 25 kg/m^2 (Table 6), but a BMI of 35 kg/m^2 conferred no greater hip protection than a BMI of 25 kg/m^2 (HR = 1.0; 95% CI, 0.9–1.2).

Interestingly, the association between BMI and osteoporotic fracture risk was weaker but inverted when adjusted for BMD, so that a higher BMI was now associated with a small but significant increase in fracture risk (HR per 1-unit increase in BMI = 1.01; 95% CI, 1.01–1.02; $p < 0.001$). For example, the HR for all osteoporotic fracture was 1.16 (95% CI, 1.09–1.23) when comparing a BMI of 35 kg/m^2 with a BMI of 25 kg/m^2; at a BMI of 15 kg/m^2, the risk was reduced. Thus, for all osteoporotic fractures a higher BMI was, if anything, a modest albeit significant risk factor following adjustment for BMD. A similar pattern was observed for distal forearm fractures. The association of high BMI with increased fracture risk following adjustment for BMD was most marked for upper arm fractures (Table 6). For lower leg fractures, fracture risk was increased and decreased at high and low BMIs, respectively, compared to 25 kg/m^2 (Table 6).

Table 6. HRs for Fracture and 95% CIs Comparing a BMI of 25 kg/m^2 With BMIs of 15 kg/m^2 and 35 kg/m^2, Respectively, According to Different Fracture Outcomes

	Not adjusted for BMD		Adjusted for BMD	
Fracture outcome	BMI 15 versus 25	BMI 35 versus 25	BMI 15 versus 25	BMI 35 versus 25
Osteoporotic	1.54 (1.44–1.64)	0.87 (0.85–0.90)	0.89 (0.80–0.99)	1.16 (1.09–1.23)
Hip	2.88 (2.56–3.25)	0.68 (0.62–0.75)	1.41 (1.16–1.72)	0.99 (0.86–1.15)
Distal forearm	1.05 (0.91–1.20)	0.76 (0.71–0.81)	0.72 (0.60–0.86)	0.97 (0.87–1.07)
Tibia/fibula	0.64 (0.45–0.89)	1.03 (0.94–1.14)	0.34 (0.16–0.74)	1.14 (0.87–1.49)
Humerus/elbow	1.13 (0.92–1.37)	1.18 (1.04–1.27)	0.70 (0.54–0.90)	1.60 (1.42–1.80)

Values are HR (95% CI), adjusted for age and time since baseline.
HR = hazard ratio; CI = confidence interval; BMI = body mass index; BMD = bone mineral density.

Interactions with BMI

There was a significant interaction between age and BMI for osteoporotic fracture ($p < 0.001$). This age interaction was significant both below and above a BMI of 25 kg/m^2 ($p = 0.042$ and $p < 0.001$, respectively). Thus, when BMI was set at 15 kg/m^2 and compared with a BMI of 25 kg/m^2 using piecewise linear functions, the HR was 1.4 at the age of 50 years and 1.7 at the age of 80 years, suggesting that low BMI was a stronger risk factor for osteoporotic fractures in elderly women. The same age-BMI interaction was true for BMI greater than 25 kg/m^2, in that high BMI was a stronger protective factor for elderly women. A significant interaction between age and BMI was seen for hip fracture below a BMI of 25 kg/m^2 ($p < 0.001$), but not for BMI above 25 kg/m^2 ($p = 0.058$). Thus, when BMI, set at 15 kg/m^2, was compared with a BMI of 25 kg/m^2 using piecewise linear functions, the HR was 9.2 at the age of 50 years and 3.1 at the age of 80 years, indicating that low BMI was a stronger risk factor for hip fracture in younger women than in elderly women.

Because there was a significant correlation between BMD and BMI, and BMD affected the relationship between BMI and the risk of fracture, the interaction between BMI and BMD was investigated with both linear and cubic models. No such interactions were found, indicating that the correlation between BMI and fracture risk did not change for different values of BMD. There were also no significant interactions between BMI and time since baseline; ie, the predictive value of BMI did not change with time ($p > 0.20$ for both osteoporotic and hip fracture outcomes).

When women allocated to treatments for osteoporosis in the WHI cohort were included, the results were similar. So, too, were the results when the analysis was confined to population-based cohorts.

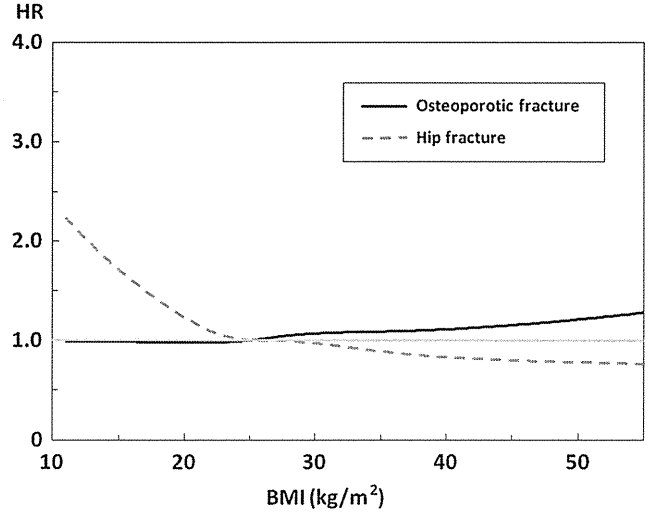

Fig. 2. Relationship between BMI and risk of fracture (HR versus BMI 25 kg/m^2) for osteoporotic fracture (solid line) and hip fracture (dashed line), adjusted for age, time since baseline, and BMD. BMI = body mass index; HR = hazard ratio; BMD = bone mineral density.

Discussion

The principal finding of the present meta-analysis of predominantly prospective population-based cohorts of women is the significant association between BMI at baseline and future osteoporotic fracture, in that a low BMI was a significant risk factor for all osteoporotic fractures, including hip and forearm fractures. These finding are very consistent with an earlier but smaller meta-analysis,[11] though it should be acknowledged that 11% of the women over a shorter time appeared in both meta-analyses. As previously reported in that study, a high BMI was a protective risk factor for osteoporotic fracture, including hip fracture, but a high BMI was weaker as a protective factor than low BMI was as a risk factor. An important conclusion is that obesity itself is not a risk factor for osteoporotic fracture, hip fracture, or forearm fracture. As also seen in the earlier analysis,[11] the association between BMI and fracture risk was dependent on BMD. In the subset of women in whom femoral neck BMD was measured, the association of BMI with hip fracture risk was attenuated and was not evident for all osteoporotic fractures combined. It should be noted that the HRs with and without adjustment for BMD are not strictly comparable; a minority of women (27%) had a BMD test and there was a significant cohort bias in the proportion of women with a BMD test. With this caveat, the results are consistent with the earlier meta-analysis.

Our results also suggest that the association between BMI and risk of future fracture is site-specific. Whereas low BMI was a risk factor for all osteoporotic fractures, a low BMI was a protective factor for lower leg fracture. In this regard, several of the cohorts did not adequately distinguish fractures of the lower leg that are associated with low BMD (eg, proximal tibial fractures) from ankle fractures which are not regarded as being associated with osteoporosis.[52] Exclusion of these cohorts from the analysis still

showed a similar pattern of association of lower leg fractures with BMI (data not shown). In the present study, a high BMI was a significant risk factor for humerus fractures and this persisted after adjustment for BMD. The finding is consistent with a recent short-term (1 year) prospective analysis in 832,775 Spanish women aged 50 years or more visiting general practitioners (SIDIAP),[16] in which a protective effect of obesity was found on future hip fracture and forearm fracture (relative risk [RR] = 0.49; 95% CI, 0.44–0.55, and RR = 0.83; 95% CI, 0.75–0.91, respectively), but obese women were at significantly higher risk of future proximal humeral fracture than the rest of the study population (RR = 1.28; 95% CI, 1.04–1.58). These findings are also consistent with an earlier report that obese women had a higher prevalence of a prior humeral fracture (odds ratio [OR] = 3.48; 95% CI, 0.18–6.68).[56] The reasons for the site-specific association between high BMI and humeral fracture risk are not known, though it may conceivably reflect a different pattern of falling or a greater load upon bones in the upper extremity in falls among the obese population. Moreover, a different padding effect of the soft tissues in different skeletal regions may produce diverse energy dissipation after trauma and, therefore, a different protection of the underlying bone.

Our results are at first sight at variance with the conclusions of Compston and colleagues,[15] who state that that obesity is not protective against fracture in postmenopausal women. That study, however, included a large number of non-adjudicated ankle and tibial fractures. Ankle fractures are not generally regarded as being associated with osteoporosis[51,56] and, as implied above, the accuracy of a self-reported distinction between ankle and other lower leg fractures is questionable. In their report, ankle fractures were significantly more frequent in obese compared with non-obese women. Given that the incidence of forearm, hip, pelvic, upper leg, and spine fractures was higher in underweight women than in obese women, their report is not inconsistent with our findings. Moreover, the present study also found a protective effect of low BMI for future lower leg fracture.

The question arises whether our findings have implications for the Fracture Risk Assessment Tool (FRAX®), which predicts the probability of a hip and a major fracture based on clinical risk factors such as sex, age, BMI, previous fracture, family history, glucocorticoid use, smoking, alcohol use, and secondary osteoporosis.[57] BMI is used as a continuous variable in FRAX, and BMD can be optionally entered into the model. Data from the meta-analysis of De Laet and colleagues[11] were used in the construct of FRAX. The association between BMI and the risk of hip fracture and other osteoporotic fractures in the present study is nearly identical to that described by De Laet and colleagues[11] in the absence of BMD. After adjustment for BMD, the risk of hip fracture associated with low BMI was attenuated in the same way as that described.[11] In the case of osteoporotic fractures, we have shown a slight though significant increase in risk with increasing BMI (see Table 6). This finding is consistent with the earlier meta-analysis, though the increase in risk was not statistically significant because of the smaller sample size. These considerations indicate that modifications of the FRAX algorithm are not warranted based on the present analysis; a view consistent with a recent report from the SOF study that FRAX is of value predicting fractures in obese women, particularly when used with BMD.[58]

The present study has several limitations, some of which we have discussed. These include the limited sampling frame for BMD measurements, inaccuracies in the estimate of BMD in the presence of a high fat mass, and uncertainties in the coding of some fractures. With regard to the first limitation, our results were similar when HRs not adjusted for BMD were calculated in those 27% of women in whom BMD was measured. The different settings of the cohorts are also a limitation, but that would weaken, not strengthen, an association between BMI and fracture. Conversely, the different settings increase the generalizability of our findings. The greatest limitation is that the present analysis is confined to women. Several lines of evidence suggest that the relationship between BMI and fracture risk may differ in men.[11,59]

A limitation in the understanding of possible mechanisms is that we have not been able to examine all potential confounding factors (eg, smoking, previous fracture, alcohol, comorbidities). Of possible relevance is the association of type 2 diabetes with high BMI. In a recent large clinical database in Manitoba, Canada, individuals with diabetes had a BMI approximately 3 kg/m^2 higher than those without diabetes.[60] Of particular interest, diabetes was associated with a 60% increased risk for major osteoporotic fracture when adjusted for clinical risk factors for fracture including BMI and BMD (HR = 1.61; 95% CI, 1.42–1.83). Thus, the higher risk for osteoporotic fracture for obese women (BMI 35 kg/m^2 versus 25 kg/m^2) in this report could be related in part to diabetes. Diabetic status was recorded in the present analysis for only 9% of women. In the women that had information on diabetes, the prevalence of diabetes was 3.4% in women with a normal BMI and 6.7% in obese women (data not shown). The small size of the available sample meant that we were unable to examine the impact of diabetes on the relationship between BMI and future fracture risk in more detail. The age interactions, the result with and without BMD and some of the fracture-specific findings might suggest an important role for low physical function and frailty in explaining these associations; but, as was the case for diabetes, we were unable to examine this further.

With these caveats, we conclude that low BMI remains an important clinical risk factor for hip and all osteoporotic fractures combined and that obesity in women is associated with a significant, albeit modest, reduction in fracture risk. In contrast, obese postmenopausal women appear to be at higher risk for humeral fractures than those with normal BMI. Moreover, after adjustment for BMD there is a slight increase in osteoporotic fracture risk with increasing BMI.

Disclosures

All authors state that they have no conflicts of interest.

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Authors' roles: Study design: HJ, AO, JAK, and EMC. Study conduct: HJ, AO, JAK, and EMC. Data collection: RDC, CC, SRC, ADP, JAE, SF, CCG, DG, DH, KTK, MAK, HK, AZL, EL, WBL, DM, LJM, TWON, JAP, MCZ, FR, JCP, DMR, TvS, and NY. Data analysis: HJ. Data interpretation: HJ, AO, JAK, and EMC. Drafting manuscript: HJ, JAK, and EMC. Revising manuscript content: HJ, AO, JAK, EMC, RDC, CC, SRC, ADP, JAE, SF, CCG, DG, DH, KTK, MAK, HK, AZL, EL, WBL, DM, LJM, TWON, JAP, MCZ, FR, JCP, DMR, TvS, and NY. Approving final version of manuscript: HJ, AO, JAK, EMC, RDC, CC, SRC, ADP, JAE, SF, DG, CCG, DH, KTK, MAK, HK, AZL, EL, WBL, DM, LJM, TWON, JAP, MCZ, FR, JCP, DMR, TvS, and NY. HJ takes responsibility for the integrity of the data analysis.

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A Randomized Controlled Trial of Multicomponent Exercise in Older Adults with Mild Cognitive Impairment

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Abstract

Background: To examine the effect of multicomponent exercise program on memory function in older adults with mild cognitive impairment (MCI), and identify biomarkers associated with improvement of cognitive functions.

Methodology/Principal Findings: Subjects were 100 older adults (mean age, 75 years) with MCI. The subjects were classified to an amnestic MCI group (n = 50) with neuroimaging measures, and other MCI group (n = 50) before the randomization. Subjects in each group were randomized to either a multicomponent exercise or an education control group using a ratio of 1:1. The exercise group exercised for 90 min/d, 2 d/wk, 40 times for 6 months. The exercise program was conducted under multitask conditions to stimulate attention and memory. The control group attended two education classes. A repeated-measures ANOVA revealed that no group × time interactions on the cognitive tests and brain atrophy in MCI patients. A sub-analysis of amnestic MCI patients for group × time interactions revealed that the exercise group exhibited significantly better Mini-Mental State Examination ($p = .04$) and logical memory scores ($p = .04$), and reducing whole brain cortical atrophy ($p < .05$) compared to the control group. Low total cholesterol levels before the intervention were associated with an improvement of logical memory scores ($p < .05$), and a higher level of brain-derived neurotrophic factor was significantly related to improved ADAS-cog scores ($p < .05$).

Conclusions/Significance: The results suggested that an exercise intervention is beneficial for improving logical memory and maintaining general cognitive function and reducing whole brain cortical atrophy in older adults with amnestic MCI. Low total cholesterol and higher brain-derived neurotrophic factor may predict improvement of cognitive functions in older adults with MCI. Further studies are required to determine the positive effects of exercise on cognitive function in older adults with MCI.

Trial Registration: UMIN-CTR UMIN000003662

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Introduction

Alzheimer's disease (AD) places a considerable and increasing burden on patients, caregivers and society. The number of older adults living with AD is predicted to increase from the current 26.6 million to 106.2 million by 2050 globally. [1] The current standard of care for mild to moderate AD involves treatment with acetylcholinesterase inhibitors to improve cognitive function. The *N*-methyl-d-aspartate antagonist memantine has also been reported to improve cognitive function in patients with moderate to severe AD. [2] While these drugs improve the symptoms of AD, they do not have substantial disease-modifying effects. [3] Thus, attempts have been made to identify individuals at increased risk of AD, and to test interventions that might delay the progression of prodromal symptoms of dementia.

An association has been proposed between regular participation in physical activity, especially aerobic exercise, and a variety of cognitive benefits. [4,5,6,7,8] Several meta-analyses have reported that physical activity is associated with improvements in attention, processing speed, and executive function in older adults with and without cognitive impairments. [9,10,11] However, these studies produced some inconsistent findings, with some reporting

cognitive gains in memory function [10,11] and other study reporting equivocal results. [9]

Evidence from neuropsychological and neuroimaging studies has suggested that mild cognitive impairment (MCI) represents a clinical prodrome to degenerative dementias such as AD. [12] For example, a population-based study in Sweden reported that the relative risks of progression to dementia in a 3-year follow-up in subjects with mild, moderate, and severe cognitive impairment (without dementia), were 3.6, 5.4, and 7.0, respectively. [13] However, of the individuals with MCI, 11% remained stable, and 25% exhibited an improvement in cognitive function between baseline and follow-up observation. [13] This variation in MCI populations should be examined to facilitate the development of interventions for inhibiting the progression of dementia. Several randomized controlled trials (RCTs) have been conducted to investigate the effects of exercise or physical activity on cognitive function in older adults with MCI. [4,5,6,7,8] These studies have revealed the effects of exercise or physical activity on cognitive function, including executive function, in older adults with MCI. However, the effect of exercise on memory function in this population remains unclear.

The precise neurobiological mechanism for the improvement of cognitive functions remains unknown, however a large number of rodent studies suggest a central role of certain molecules such as brain-derived neurotrophic factor (BDNF), insulin-like growth factor (IGF-1), and vascular endothelial growth factor (VEGF). The molecules have been shown to facilitate neurogenesis in the hippocampus, promote synaptic plasticity in the hippocampus and cerebral cortex, and angiogenesis and enhance growth and protection of neurovasculature. [14,15] In fact, some neuroimaging studies of human subjects revealed that aerobic exercise increased hippocampal volume, [16] and gray and white matter regions including the cingulate cortex, supplementary motor cortex, inferior frontal gyrus, and superior temporal gyrus. [17]

The present randomized trial was designed to test whether a 6-month supervised multicomponent exercise program could reduce the rate of cognitive decline, especially in memory function, and reduce the rate of brain volume decline among older adults with MCI. The multicomponent exercise program included aerobic exercise, muscle strength training, and postural balance retraining, because previous reviews suggested that combined aerobic exercise and strength training interventions improved attention and working memory to a greater extent than aerobic exercise alone. [11,18] We explored the biomarkers for identifying improvement of cognitive functions. Serum total cholesterol (T-cho), hemoglobin A1c (HbA1c), BDNF, and VEGF levels at baseline were used as potential predictors.

Methods

CONSORT checklist and the protocol for this trial is available as supporting information; see **Checklist S1** and **Protocol S1**.

Participants

Subjects in this study were recruited from two volunteer databases (n = 1,543), which included elderly individuals (65 years and over) selected either by random sampling or when they attended a medical check-up in Obu, Japan. Inclusion criteria specified that prospective participants were community-dwelling individuals aged 65 years and over. A total of 528 prospective participants with a Clinical Dementia Rating (CDR) of 0.5, or who complained of memory impairment, were recruited in the first round of eligibility assessments. Of these, 135 subjects satisfied the requirements of the second round of eligibility assessments, which included neuropsychological tests, which included language and memory tests, attention and executive function tests, clinical diagnosis, activities of daily living (ADL), educational level, and magnetic resonance imaging. Thirty-five subjects were excluded, meaning that a total of 100 subjects took part in the study (mean age, 75.4±7.1 years; 65–95 years, men n = 55, 51%). All subjects met the definition of MCI as per the Petersen criteria. [19] All MCI subjects had objective impairments in either episodic memory and/or executive functioning at least 1.5 standard deviations below the age-adjusted mean for at least one of the neuropsychological tests. Final classification of subjects was based on the above factors and consensus of a team of neuroscientists. Exclusion criteria included a CDR = 0, or a CDR of 1–3, a history of neurological, psychiatric, or cardiac disorders or other severe health issues, use of donepezil, impairment in basic activities of daily living (ADL), and participation in other research projects. Subjects were classified to an amnestic MCI group (aMCI) (n = 50) with neuroimaging measures, and other MCI group (n = 50) before the randomization. Then, the subjects in each group were randomly assigned to either a multicomponent exercise or an education control group using a ratio of 1:1. Participant characteristics at the beginning of the study are shown in **Table 1**. We confirmed that there were no significant differences in demographic characteristics, physical performance, or instrumental ADL levels between the exercise and control groups. Fifty subjects with aMCI (mean age, 76.0±7.1 years; 65–92 years, men n = 27, 54%) were selected from among the subjects to participate in a sub-analysis. All subjects included the aMCI group agreed to measure functional neuroimaging tests. This sub-analysis was limited to aMCI patients because aMCI is most likely to progress to AD.[20] Objective memory impairment to determine aMCI was defined as a lower memory score on the Wechsler Memory Scale-Revised (WMS-R) Logical Memory II. [21]

Ethics

The Ethics Committee of the National Center for Geriatrics and Gerontology approved the study protocol. The purpose, nature, and potential risks of the experiments were fully explained to the subjects, and all subjects gave written, informed consent before participating in the study. The subjects had the capacity to consent because they maintained general cognitive function and daily activities.

Interventions

The six-month, multicomponent exercise program included biweekly 90-minute sessions involving aerobic exercise, muscle strength training, postural balance retraining, and dual-task training. In addition, the exercise program included a focus on promoting exercise and behavior change. Two trained physiotherapists involved in geriatric rehabilitation conducted each intervention. Each exercise class contained 16–17 participants, and each supervised session began with a 10-min warm-up period and stretching exercise, followed by 20 min of muscle strength exercise. The subjects then practiced aerobic exercise, postural balance retraining, and dual-task training for 60 min. In the aerobic exercise and postural balance retraining, subjects underwent circuit training, including stair stepping, endurance walking, and walking on balance boards. The mean intensity of the aerobic exercise was approximately 60% of maximum heart rate which was similar to the intensity used in previous studies. [4,6] Eleven of the 40 classes during the six-month intervention period included approximately 20–30 minutes of consecutive outdoor walking. In the dual-task training sessions, subjects performed concurrent cognitive tasks during exercise. For example, the subjects in the

Table 1. Characteristics of the subjects.

	All subjects		aMCI subjects	
	Exercise (n = 50)	Control (n = 50)	Exercise (n = 25)	Control (n = 25)
Age, mean (SD), y	74.8 (7.4)	75.8 (6.1)	75.3 (7.5)	76.8 (6.8)
Men, No. (%)	25 (50.0)	26 (52.0)	13 (52.0)	14 (56.0)
Educational level, mean (SD), y	10.9 (2.8)	10.4 (2.4)	11.1 (2.4)	10.8 (2.7)
Diagnosis, No. (%)				
Hypertension (3*, 1†)	23 (46.9)	22 (45.8)	13 (52.0)	11 (45.8)
Heart disease (4*, 1†)	5 (10.2)	1 (2.1)	2 (8.0)	0 (0)
Diabetes Mellitus	8 (16.0)	3 (6.0)	5 (20.0)	3 (12.0)
Medication, 3 and over (2*, 1†)	22 (44.0)	19 (39.6)	10 (40.0)	11 (45.8)
Blood pressure, mmHg				
Systolic, mean (SD)	144.6 (21.6)	142.4 (19.4)	152.2 (21.0)	143.7 (21.3)
Diastolic, mean (SD)	74.6 (11.7)	75.1 (11.2)	77.3 (11.1)	74.3 (10.1)
Blood test				
Total cholesterol, mean (SD), mg/dL	211.7 (36.2)	200.5 (34.5)	212.6 (36.9)	202.8 (32.2)
HbA1c, mean (SD), %	5.6 (0.8)	5.4 (0.5)	5.6 (0.6)	5.4 (0.5)
BDNF, mean (SD), ng/mL	12.1 (10.0)	13.5 (10.4)	11.9 (11.3)	14.4 (12.2)
VEGF, mean (SD), pg/mL	97.6 (19.7)	103.5 (22.2)	95.9 (18.4)	96.7 (15.4)
Physical performances				
Grip strength, mean (SD), kg	24.7 (8.1)	23.5 (7.3)	25.2 (7.3)	23.1 (8.4)
One legged standing, mean (SD), s	34.6 (24.6)	31.2 (23.9)	34.0 (25.1)	29.3 (23.6)
Timed up & go, mean (SD), s	8.8 (2.5)	9.2 (2.1)	9.0 (2.2)	9.1 (2.0)
IADL subscale of TMIG index, mean (SD), score	4.8 (0.9)	4.9 (0.3)	5.0 (0.2)	4.9 (0.3)
GDS, mean (SD), score	3.8 (3.1)	3.3 (2.8)	3.0 (2.1)	2.6 (2.0)
Cognitive functions, score				
MMSE, mean (SD)	26.8 (2.3)	26.3 (2.7)	26.8 (1.8)	26.6 (1.6)
ADAS-cog, mean (SD)	6.0 (2.8)	6.5 (2.8)	6.3 (2.2)	6.8 (2.2)
WMS-LM I, mean (SD)	14.6 (6.9)	13.8 (7.4)	12.5 (5.9)	12.0 (4.9)
WMS-LM II, mean (SD)	10.5 (7.4)	9.4 (7.4)	8.2 (5.4)	6.9 (5.0)
Clinical subtype, No. (%)				
Amnestic MCI	34 (68.0)	37 (74.0)		
Non-amnestic MCI	16 (32.0)	13 (26.0)		
VSRAD				
MTA-ERC atrophy, mean (SD) (1*)	1.3 (0.9)	1.5 (1.0)	1.4 (1.0)	1.4 (1.0)
WBC atrophy, mean (SD) (1*)	7.3 (4.7)	8.3 (4.6)	7.9 (3.9)	7.4 (3.3)

Abbreviations: IADL subscale of TMIG index, instrumental activities of daily living subscale of Tokyo Metropolitan Institute of Gerontology index; GDS, Geriatric Depression Scale; MMSE, Mini-Mental State Examination; ADAS-cog, Alzheimer's Disease Assessment Scale-cognitive subscale; WMS, Wechsler Memory Scale; MCI, mild cognitive impairment. *missing value in all subjects. †missing value in the aMCI subjects.

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exercise group were asked to walk while inventing their own poem, as aerobic exercise. In the ladder training exercise, subjects learned to step in consecutive square segments, and were instructed to step as quickly and accurately as possible. Before and after each session of the program, physiotherapists conducted a health check of each subject. The physiotherapists and a well-trained instructor implemented risk management for accidents and other adverse events during the program. The subjects were instructed to carry out daily home-based muscle strength exercises and walking, which were self-monitored using a booklet and pedometer based on the concept of promoting exercise and behavior change. Attendance at each session was recorded and a transportation service was provided for participants, if necessary, to help subjects maintain their participation in the program.

Subjects in the education control group attended two education classes about health promotion during the 6-month study period. The class provided information regarding healthy diet, oral care, prevention of urinary incontinence, and health checks. However, the group did not receive specific information regarding exercise, physical activity, or cognitive health.

Outcomes

Cognitive Functions. The Mini-Mental State Examination (MMSE) [22] and Alzheimer's Disease Assessment Scale-Cognitive subscale (ADAS-cog) [23] were used to assess general cognitive function.

Modified versions of the logical memory subtest from the WMS-R [21] was used to assess memory function. In the WMS-R, two short stories (Story A and B) were read aloud to the subject, who was then instructed to recall details of the stories immediately (LM I, immediate recall) and after 30 min (LM II, delayed recall; each total recall score = 50). [21]

MRI. MRI was performed with a 1.5-T system (Magnetom Avanto, Siemens, Germany). Three-dimensional volumetric acquisition with a T1-weighted gradient echo sequence was then used to produce a gapless series of thin sagittal sections using a magnetization preparation rapid-acquisition gradient-echo sequence (repetition time, 1700 ms; echo time, 4.0 ms; flip angle 15°, acquisition matrix 256×256, 1.3-mm slice thickness).

In analysis of brain volume, we used the voxel-based specific regional analysis system for Alzheimer's disease (VSRAD), which enables the examination of atrophy of the bilateral medial temporal areas including the entorhinal cortex (MTA-ERC) using voxel-based morphometry. [24] The VSRAD has been shown to achieve high accuracy (87.8%) in discriminating patients in the very early stages of AD with MCI from normal control subjects using Z scores. [24] A previous VSRAD study reported that atrophy of the MTA-ERC exhibited a clear functional relationship with blood flow changes in the hippocampus, thalamus and temporal lobe, which were suggested to be closely related to inter-regional anatomical and physiological connections. [25]

Acquired MRI images were formatted to gapless, transaxial images, followed by extraction of the gray matter images using SPM2. Anatomical standardization was used to fit each individual brain to standard template MRIs in the common coordinate system of the MNI T1 MRI template. [26] The segmented gray matter images were then subjected to affine and nonlinear standardization using a template of prior gray matter. The anatomically standardized gray matter images were then smoothed again using an isotropic Gaussian kernel 12 mm in full width at half maximum, to determine the partial volume effect and create a spectrum of gray matter intensities. Gray matter intensities were equivalent to the weighted average of gray matter voxels located in the volume fixed by the smoothing kernel. Regional intensity was considered equivalent to gray matter concentration. We compared the gray matter image of each patient with the mean and standard deviation (SD) of gray matter images of healthy volunteers using voxel-by-voxel Z score analysis. In the final step, the Z score was calculated according to the following equation: (Z score = ((control mean)−(individual value))/control SD). The Z score thus reflected the degree of atrophy in bilateral MTA-ERC. Higher Z scores indicated clearer MTA-ERC atrophy. VSRAD also automatically measured the degree of atrophy in the whole brain cortices (WBC), including the hippocampus: if the Z-score was more than 2.0 within a voxel, the area was considered to exhibit atrophy. [24] Thus, the proportion of atrophic area in the whole brain (%) was measured in the following way: 100×([the number of voxels with Z-score≥2.0]/[the number of whole brain voxels]).

Biochemical measures3. T-cho, HbA1c, BDNF, VEGF receptor 1 (VEGFR1) were used as biomarkers. Blood samples were collected between 11 am and 4 pm in a non-fasting state. The blood samples were kept at room temperature for 30 min to allow for clotting, after which the samples were centrifuged for 15 min. Serum was then harvested and stored at −25 °C until analysis. Analyses were carried out centrally in one laboratory (Special Reference Laboratories, Tokyo, Japan). BDNF and VEGFR1 were measured with the Quantikine Human kit (R&D systems, Inc. Minneapolis, MN, USA). Coefficients of variation (CVs) of BDNF in intra-assay and inter-assay precision were 2.6–3.2 and 5.5–9.8, respectively. Those of VEGFR1 were 3.8–6.2 for intra-assay and 7.6–11.3 inter-assay precision.

Sample size

Since participants were selected on the basis of memory impairments, memory was considered the most important cognitive outcome in our study. Therefore, sample size calculations were based on AVLT data. [27] A previous study reported that a sample of 34 participants per group to detect a clinically relevant effect, with 80% power. [6] To allow for a dropout of 25%, the final sample size was 85 participants.

Randomization–Sequence generation

Subjects were randomly assigned after completion of baseline assessments. Subjects were classified to an amnestic MCI group (n = 50) with neuroimaging measures, and other MCI group (n = 50) before the randomization. The subjects in each group were randomized to either a multicomponent exercise or an education control group using a ratio of 1:1. The subjects were further randomized and dichotomized into two groups, an amnestic MCI group (n = 50) with neuroimaging measures, and other MCI group (n = 50).

Randomization–Implementation and concealment

After the baseline assessment, subjects were randomized using the option "random sample of cases" in IBM SPSS statistics software (Version 19; SPSS Inc., Chicago, IL, USA). A researcher who was not aware of the aims of the study performed the randomization procedure.

Blinding

Study personnel involved in the collection of outcome measures were blinded to the randomization assignment. Several trained speech therapists blinded to group status conducted the cognitive tests, and one speech therapist recalculated all of the results.

Statistical methods

Statistical analysis was performed using IBM SPSS statistics software. For the baseline comparisons between exercise and control groups for all subjects, and for the amnestic MCI (aMCI) sub-analysis, Pearson's method, together with Chi square analysis with Fisher's exact test was used to investigate the categorical data. Kolmogorov-Smirnov tests confirmed that all continuous variables followed a normal distribution. Basic characteristics of patients were compared between the two groups using *t*-tests.

A general linear model for repeated-measures analysis of variance (ANOVA) was used to determine the group difference for the cognitive tests and VSRAD measurements. Two time points were treated as the within-subjects factor (effect over time) and the differences between the exercise and control groups were treated as the between-subjects factor. When the repeated-measures ANOVA indicated that the group × time interaction was significant, tests of simple main effects were performed to determine which group or groups differed significantly across the intervention period. Alpha level of the post-hoc analyses were adjusted for the Bonferroni method, i.e. corrected alpha = .025.

Multiple logistic regression models were used to determine the predictors of improvements in cognitive function. Dependent variables were the cognitive tests which showed significant improvements in the comparison between before and after the intervention of all subjects. Based on the results from the cognitive tests, the subjects were dichotomized into two categories; the subjects who improved their cognitive test scores (improvement

group) and the subjects who showed no improvement, or who exhibited a deterioration in their cognitive test scores (no improvement group). Biochemical variables at baseline measurements were treated as independent variables. Covariates such as age, sex, educational level, and the intervention group were included in the logistic model.

The univariate analyses and repeated-measures ANOVA were performed with all subjects grouped together as well as with a subgroup that was limited to older adults with aMCI. The logistic regression analysis was performed to determine the predictors of improvement of cognitive functions in all subjects. All statistical significance tests were two-sided, and an alpha-level of .05 was considered statistically significant.

Results

Participant flow

Figure 1 shows the flow of participants from the time of screening to study completion at 6 months. Ninety-two (exercise group, n = 47) subjects completed the 6-month follow-up. Of the 50 aMCI subjects, 47 (94%) completed the 6-month follow-up. Two of the remaining 47 subjects in the exercise group (one male, one female) missed all exercise programs, but completed the examinations before and after the intervention. The two subjects were included in the following analyses. Mean adherence to the exercise program, including the remaining 47 subjects, was 85.9%, and 38 subjects (80.9%) in the exercise group attended our intervention program with greater than 80% adherence.

Baseline data

There were no significant differences in baseline characteristics between all subjects grouped together and the aMCI group alone (**Table 1**).

Participants analyzed

Our primary analysis of cognitive function included all patients who remained at the end of the study (total n = 92; exercise group, n = 47; control group, n = 45). A total of 90 subjects (exercise group, n = 46; control group, n = 44) completed MRI scanning. When the analyses were limited to subjects with aMCI, the exercise and control groups included 24 and 23 subjects in assessments of cognitive function and MRI, respectively.

Outcomes in all MCI subjects

Table 2 shows changes in cognitive scores over the 6-month period by group. There were main effects of time in ADAS-cog ($p = .01$), WMS-LM I ($p < .01$), WMS-LM II ($p < .01$), and WBC atrophy level ($p = .03$), although no main effects of group and group × time interactions were detected on the cognitive tests and brain atrophy (**Table 2**).

Outcomes in aMCI subjects

When the analyses were limited to subjects with aMCI, the repeated-measures ANOVA for MMSE showed a significant effect of group ($p = .03$) and there was a group × time interaction in MMSE ($p = .04$) indicating benefit of the exercise over time. Tests of simple main effects revealed that the control group decreased in MMSE score ($p = .015$) after intervention. A repeated-measures ANOVA showed a significant effect of time ($p < .01$) and group × time interaction ($p = .04$) in WMS-LM I. Tests of simple main effects showed that the exercise group exhibited better WMS-LM I ($p < .01$) scores compared to baseline, but not in the control group. The repeated-measures ANOVA for WMS-LM II ($p < .01$) and MTA-ERC atrophy ($p = .03$) showed a significant effect of time. However, there were no main effects of group and no group × time interactions. A repeated-measures ANOVA showed a significant group × time interaction ($p < .05$) in WBC atrophy level. There were no main effects of group or time. Tests of simple main effects revealed that the subjects in the control group showed increased WBC atrophy ($p = .01$) after intervention, compared with their baseline scores (**Table 2, Figure 2**).

Relationships between cognitive functions and biomarkers

Paired *t*-tests revealed significant improvements in ADAS-cog ($p = .01$), WMS-LM I ($p < .01$), and WMS-LM II scores ($p < .01$) after the intervention. Multiple logistic regression analysis revealed that low T-cho level before the intervention was associated with improvement in WMS-LM I (odds ratio (OR) 0.98, 95% confidence interval (95% CI) 0.96–1.00, $p = .02$). Higher BDNF level at baseline was significantly related to improvements in ADAS-cog (OR 1.07, 95% CI 1.02–1.13, $p = .01$) independent of age, sex, educational level, and intervention (**Table 3**).

Adverse events

Four subjects (exercise group, n = 2; control group, n = 2) experienced adverse events (hospitalization for illness). Falls (as a type of minor adverse event) over a 6-month period were reported by 23/90 (26%) of subjects, with no significant differences among groups. There were no other adverse events during exercise intervention for 6-months.

Discussion

Evidence of exercise on cognitive function

Older adults with MCI have been found to exhibit greater decreases in memory function than in other cognitive functions, relative to healthy older adults. [28] The enhancement of cognitive function, especially memory function, in individuals with MCI may play a crucial role in preventing the progression from MCI to AD in older adults. Klusmann et al. reported significant effects of a multifaceted exercise program on cognitive function, finding that a 6-month exercise program resulted in improvements in delayed story recall. [29] However, their sample consisted of healthy, well-functioning females without any signs of cognitive impairment. In addition, previous studies reported that aerobic exercise or other physical activity can increase executive function in older adults with cognitive impairments, but the effects of exercise on memory function in this population remain unclear. [4,5,6,7,8] To our knowledge, this is the first study to demonstrate an improvement in logical memory following multicomponent exercise training among older adults with aMCI. The exercise group showed significant differences not only in WMS-LM I scores, but also in MMSE scores compared to the control group in aMCI populations. Our intervention study extends the results of previous studies with healthy samples, indicating the potential for an increase in memory performance and maintenance of general cognitive function in subjects exhibiting signs of cognitive decline.

A meta-analysis of aerobic exercise and neurocognitive performance demonstrated that interventions combining aerobic exercise and strength training, similar to our program, improved attention, processing speed and working memory to a greater extent than aerobic exercise alone. [11] However, the mechanism underlying this improvement remains unclear. A previous study reported that subjects with MCI improved their episodic memory performance when they were exposed to a multifactorial cognitive intervention program that included dual-task attentional and memory training. [30] Dual-task deficit is recognized as a potential

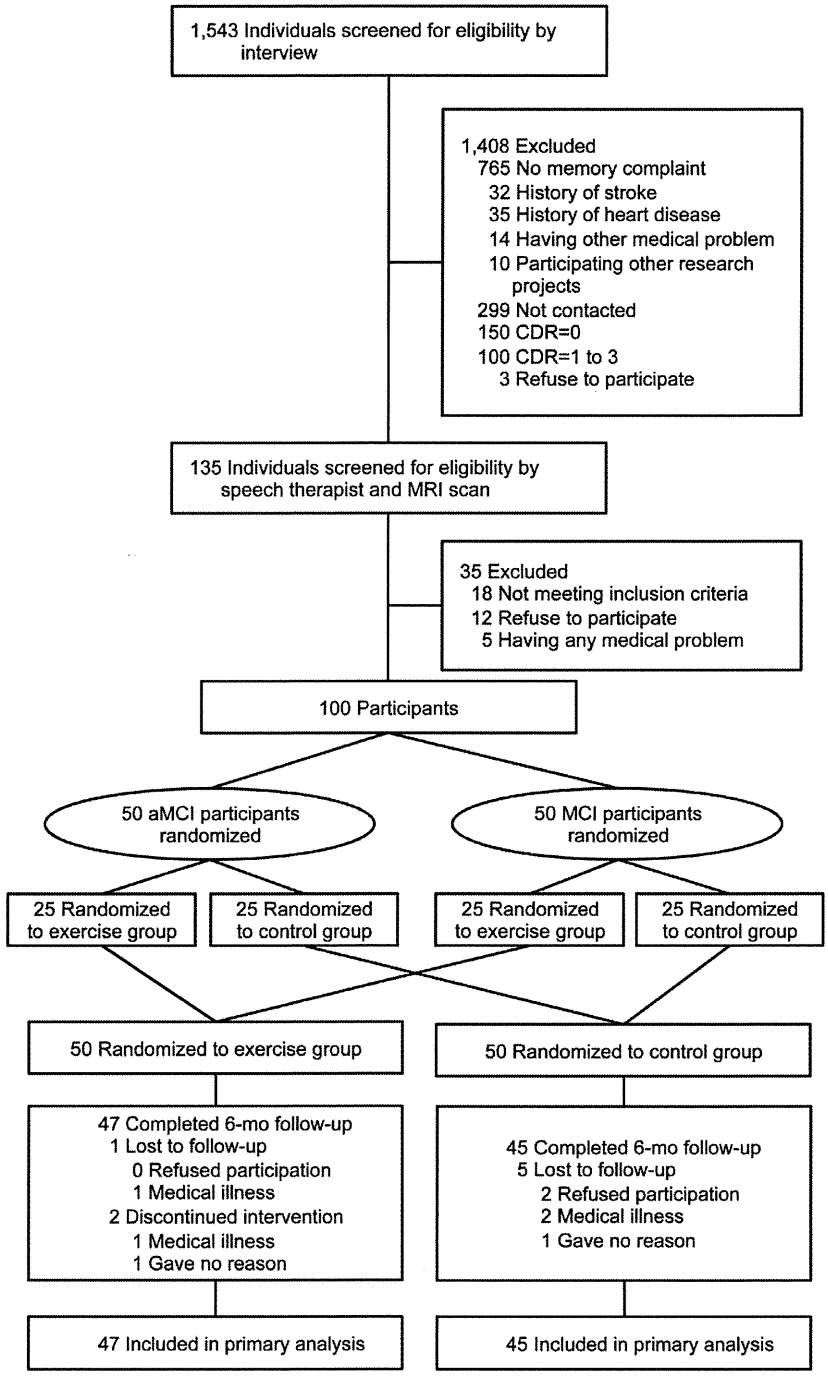

Figure 1. Subject flow diagram from initial contact through to study completion.
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early marker for dementia, [31,32] and dual-task-related changes in performance were greater in subjects with MCI compared with cognitively normal age-matched controls. [33,34] Our multicomponent program involved changes in cognitive load using dual-task stimulation and learning tasks. We believe that dual-task training may have a greater effect on various cognitive functions, for example, general and memory functions, than interventions that only focus on aerobic exercise. [7,10] However, the results from the present study do not provide direct evidence for the positive effect of dual-task training. Future studies are required to investigate the effects of dual-task training on cognitive function in the older adults with MCI.

Lautenschlager et al. reported that physical activity and behavioral intervention improved general cognition among adults with MCI. [4] The multicomponent exercise training in the current study also included encouragement for subjects to engage in more physical activity. Our results further support the notion that training involving physical activity can have a beneficial effect

Table 2. Comparison of Cognitive Function between the Exercise and Control Group.

	All subjects (n = 100)						aMCI subjects (n = 50)					
	Mean Difference From Baseline (95% CI) in All Subjects		P Value ANOVA for Repeated Measures			ES	Mean Difference From Baseline (95% CI) in aMCI Group		P Value ANOVA for Repeated Measures			ES
	Exercise Group (n = 47)	Control Group (n = 45)	Group	Time	Group × time interaction	r	Exercise Group (n = 24)	Control Group (n = 23)	Group	Time	Group × time interaction	r
MMSE	0.2 (−0.5, 0.9)	−0.3 (−1.1, 0.4)	0.18	0.79	0.32	0.11	0.3 (−0.8, 1.3)	−1.4 (−2.5, −0.3)	0.03	0.14	.04[b]	0.31
ADAS-cog	−0.8 (−1.4, −0.2)	−0.2 (−0.8, 0.4)	0.17	0.01	.16 (1)[c]	0.15	−1.2 (−2.1, −0.3)	−0.1 (−1.0, 0.8)	0.1	0.06	0.1	0.24
WMS-LM I	2.8 (1.4, 4.2)	1.0 (−0.5, 2.4)	0.29	<.01	0.08	0.19	3.8 (1.6, 5.9)	0.5 (−1.6, 2.7)	0.14	<.01	.04[a]	0.31
WMS-LM II	3.4 (2.0, 4.8)	1.9 (0.4, 3.4)[b]	0.28	<.01	0.15	0.15	3.8 (1.8, 5.7)	2.1 (0.1, 4.2)	0.11	<.01	0.26	0.17
MTA-ERC	0 (−0, 0.1)	0 (0, 0.1)	0.18	0.08	0.89	0.02	0.1 (0, 0.2)	0 (−0.1, 0.1)	0.91	0.03	0.27	0.17
WBC	0.1 (−0.4, 0.7)	0.7 (0.1, 1.2)	0.08	0.03	0.16	0.15	−0.1 (−0.8, 0.6)	0.9 (0.2, 1.6)	0.86	0.08	<.05[b]	0.29

Abbreviations: MMSE, Mini-Mental State Examination; ADAS-cog, Alzheimer's Disease Assessment Scale-cognitive subscale; WMS, Wechsler Memory Scale; MTA-ERC, medial temporal areas including the entorhinal cortex; WBC, whole brain cortices; ES, effect size.

[a]$p<.025$; significant differences before versus after intervention in the exercise group

[b]$p<.025$; significant differences before versus after intervention in the control group

[c]Missing value

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not only on memory function, but also on general cognitive function in people with aMCI. General cognitive function can be used to discriminate between people who progress to AD and those who do not. [35] Improvements of memory function and maintenance of general cognitive function suggest that multicomponent exercise can help prevent progression from MCI to AD. However, despite significant interactions, the effect sizes in general cognitive function and logical memory were small. Moreover, these interactions would not become significant if the p-values were adjusted for multiple comparisons. Further studies are required to determine the positive effects of exercise on cognitive function in older adults with MCI.

Relationship between exercise and brain atrophy

It is well established that structures in the medial temporal lobe, particularly the hippocampus and ERC, are essential for normal memory function. There is an emerging literature describing baseline structural MRI correlates of cognitive impairment in elderly adults with mild cognitive impairment (MCI) and Alzheimer's disease (AD). Some studies have identified relationships between aerobic exercise and increased brain volume [16,17] and functional connectivity between parts of the frontal, posterior, and temporal cortices [36] in healthy older adults. For example, Erickson et al. found that the hippocampus remains plastic in late adulthood and that a 1-year period of aerobic exercise was sufficient for enhancing volume. [16] Our 6-month multicomponent exercise program with MCI subjects revealed that exercise did not have a significant group × time interaction on MTA-ERC scores or WBC atrophy compared to the control group. However, there was significant group × time interaction in WBC atrophy level, when tested in a sub-analysis restricted to aMCI subjects. Post-hoc analyses revealed that the control group exhibited increased WBC atrophy after intervention, compared with their baseline scores. These results suggest that older adults with aMCI may exhibit high levels of plasticity in WBC atrophy. Further study is needed to establish our findings using large samples and detailed neuroimaging analysis.

Predictors of increasing of cognitive function

In the relationships between cognitive function and biochemical measures, low T-cho and high BDNF serum levels at baseline were associated with increased memory and general cognitive function in the MCI subjects, respectively. Serum lipoprotein levels may be a common and potentially modifiable risk factor for AD. [37] For example, a prospective study reported that lower serum levels of LDL and T-cho were associated with better cognitive performance and a lower risk of cognitive impairment in 1,037 women with cardiovascular disease. [38] Our finding extends knowledge about the relationships between T-cho and cognitive function to older adults with MCI. Animal studies have revealed that the structure and function of the hippocampus, a brain region critical for certain forms of cognition, is adversely affected by hyperlipidemia. (e.g. [39]) Abnormal lipid metabolism may be undesirable status for improvement cognitive functions, especially memory. Exercise is also a valid and feasible way to manage lipoprotein levels and regular activity may be potential strategies for preventing cognitive decline in elderly individuals. [40]

One of the main determinants of cell size is cell growth, which is modulated by certain growth factors, such as BDNF. The levels of BDNF-associated gene expression have been found to increase with physical activity. [14] BDNF expression has also been suggested to play a role in learning and synaptic plasticity. [41]

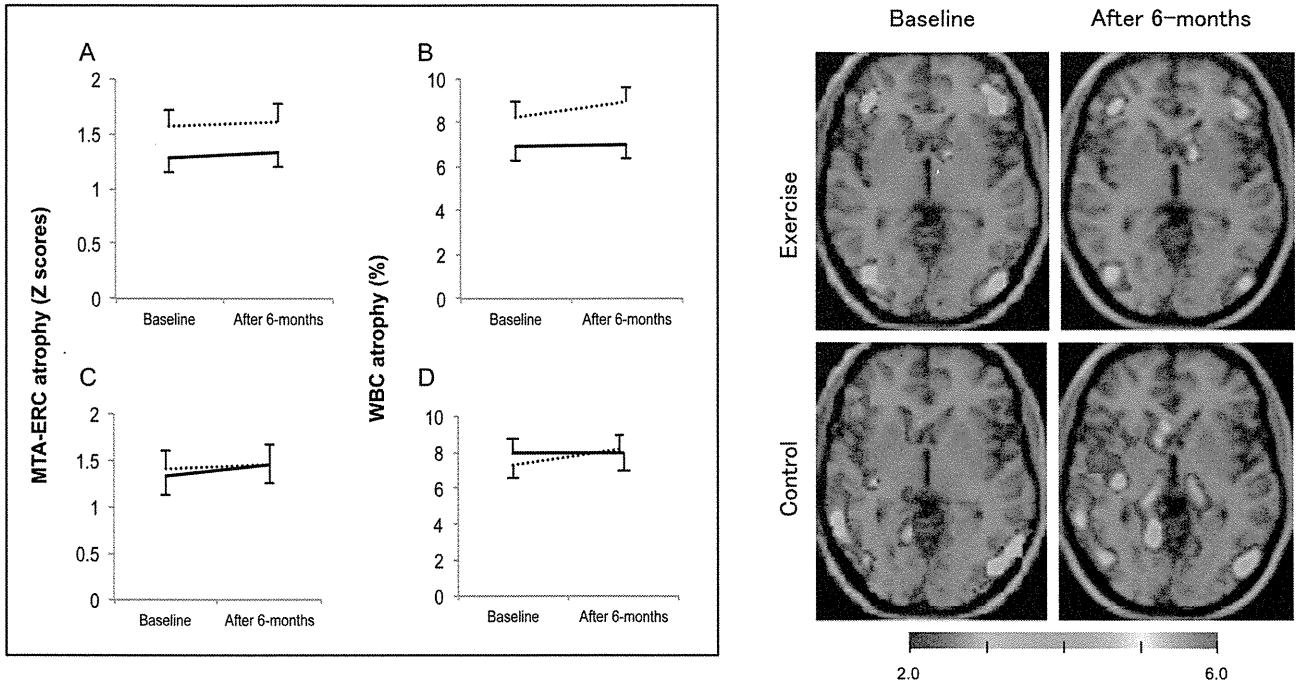

Figure 2. Change in MTA-ERC and WBC volumes in response to the 6-month intervention. Abbreviations: MTA-ERC, medial temporal areas including the entorhinal cortex; WBC, whole brain cortices. Left panel shows change in MTA-ERC and WBC volumes before and after the 6-month intervention. Solid and dashed lines indicate the exercise and control groups, respectively. Group mean differences and standard errors for MTA-ERC and WBC atrophy are shown in panels A and B, respectively, for all subjects. Panels C and D show mean differences and standard errors for MTA-ERC and WBC atrophy, respectively, for older adults with aMCI. The repeated-measures ANOVA revealed that there was a significant group × time interaction on WBC atrophy level ($p<.05$) in older adults with aMCI. Right panel shows typical images for VSRAD, indicated atrophy region, in subjects with aMCI in the exercise and control groups. The upper panel shows WBC atrophy in a man (81 years old) with aMCI who completed the 6-month exercise program. The rate of WBC atrophy decreased after the intervention (8.74% at baseline to 6.39% after the intervention). The lower panel shows WBC atrophy of a man (80 years old) with aMCI in the control group. The rate of WBC atrophy increased after the 6-month intervention period (7.19% at baseline to 10.48% after the intervention).
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The present results indicate that high serum BDNF levels have a beneficial effect on general cognitive function in older adults with MCI.

Limitations

The present study involved several limitations. The small sample size should be addressed by replication with a larger group of adults with MCI. Of the 135 potential subjects screened for eligibility in our study, 35 were excluded for not meeting inclusion criteria, refusal to participate, or medical reasons (**Figure 1**). This

Table 3. Predictors of Improvements in Cognitive Function.

	ADAS-cog	*P*	WMS-LM I	*P*	WMS-LM II	*P*
	OR (95% CI)		**OR (95% CI)**		**OR (95% CI)**	
Age, years	0.97 (0.91–1.05)	.44	0.95 (0.89–1.03)	.22	0.96 (0.90–1.04)	.34
Sex, women/men	1.00 (0.35–2.82)	1.00	0.74 (0.26–2.13)	.57	2.56 (0.85–7.66)	.09
Educational level, years	0.85 (0.70–1.04)	.11	0.93 (0.76–1.13)	.45	1.01 (0.83–1.22)	.96
Intervention, exercise group/control group	2.85 (1.10–7.37)	.03	2.27 (.90–5.72)	.08	1.98 (.77–5.12)	.16
T-cho, mg/dl	1.00 (0.98–1.02)	.96	**0.98 (0.96–1.00)**	**.02**	0.99 (0.97–1.01)	.18
HbA1c, %	0.53 (0.25–1.14)	.10	1.20 (0.57–2.53)	.64	0.61 (0.29–1.30)	.20
BDNF, ng/ml	**1.07 (1.02–1.13)**	**.01**	1.00 (0.95–1.05)	.94	1.02 (0.97–1.08)	.39
VEGFR1, pg/ml	0.99 (0.97–1.01)	.39	0.99 (0.96–1.01)	.32	1.00 (0.98–1.03)	.74

Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval; ADAS-cog, Alzheimer's Disease Assessment Scale-cognitive subscale; WMS, Wechsler Memory Scale; T cho, total cholesterol; HbA1c, hemoglobin A1c; BDNF, brain-derived neurotrophic factor (BDNF); VEGFR1, vascular endothelial growth factor receptor 1.
Missing values: ADAS-cog (n = 10), WMS-LM I (n = 9), WMS-LM II (n = 9)
doi:10.1371/journal.pone.0061483.t003

selection bias may have affected the generalizability of our findings to population-based samples. Other limitations include unknown group differences in risk factors of cognitive decline and AD, such as apolipoprotein E ε4 genotype, and inflammation, although there were no significant differences between groups in terms of hypertension, diabetes mellitus, medications, biomarkers of lipid metabolism, physical performance, instrumental ADL functioning, or depressive mood. In addition, it is possible that the improvement in the exercise group resulted from the social contact to which the intervention group was exposed. This possibility cannot be completely excluded with the present design, and should be addressed in future studies.

Conclusion

The current results indicate that a multicomponent exercise program can provide cognitive benefits for older adults with aMCI. The effects of exercise were most pronounced for logical memory and general cognitive function in older adults with aMCI. Exercise was found to maintain the atrophy levels of the whole brain cortex in older adults with aMCI. Improvement of cognitive function was associated with low T-cho and high BDNF levels at baseline. A future follow-up investigation is required to determine whether the observed effects are associated with prevention or delayed onset of AD in older adults with MCI.

Supporting Information

Checklist S1 CONSORT Checklist.
(DOC)

Protocol S1 Trial Protocol.
(DOCX)

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Author Contributions

Conceived and designed the experiments: TS H. Shimada. Performed the experiments: H. Shimada HM TD DY TK. Analyzed the data: H. Shimada. Contributed reagents/materials/analysis tools: KI H. Shimokata YW HE. Wrote the manuscript: TS H. Shimada HM TD DY. Review of manuscript: KI H. Shimokata YW HE TK.

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ORIGINAL ARTICLE: BEHAVIORAL AND SOCIAL SCIENCES

Effects of exercise and tea catechins on muscle mass, strength and walking ability in community-dwelling elderly Japanese sarcopenic women: A randomized controlled trial

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Aim: To investigate the effects of exercise and/or tea catechin supplementation on muscle mass, strength and walking ability in elderly Japanese women with sarcopenia.

Methods: A total of 128 women aged over 75 years were defined as sarcopenic and randomly assigned into four groups: exercise and tea catechin supplementation ($n = 32$), exercise ($n = 32$), tea catechin supplementation ($n = 32$) or health education ($n = 32$). The exercise group attended a 60-min comprehensive training program twice a week and the tea catechin supplementation group ingested 350 mL of a tea beverage fortified with catechin daily for 3 months. Body composition was determined by bioelectrical impedance analysis. Interview data and functional fitness measurements, such as muscle strength, balance and walking ability, were collected at baseline and after the 3-month intervention.

Results: There were significant group × time interactions observed in timed up & go ($P < 0.001$), usual walking speed ($P = 0.007$) and maximum walking speed ($P < 0.001$). The exercise + catechin group showed a significant effect (odds ratio 3.61, 95% confidence interval 1.05–13.66) for changes in the combined variables of leg muscle mass and usual walking speed compared with the health education group.

Conclusions: The combination of exercise and tea catechin supplementation had a beneficial effect on physical function measured by walking ability and muscle mass. **Geriatr Gerontol Int 2013; 13: 458–465.**

Keywords: exercise, muscle mass, physical function, sarcopenic women, tea catechin supplementation.

Introduction

It is generally accepted that aging is associated with a progressive decline of lean body mass, and muscle mass in particular. This involuntary loss of skeletal muscle mass and strength, defined as sarcopenia, has been associated with loss of independence, diminished quality of life, physical disability, increased risk for falls, mobility impairments, high healthcare burden and medical needs, and mortality in the elderly.[1,2] Hence, prevention and treatment of sarcopenia is very important and necessary for the well-being of the growing elderly population. Although there are several methods of treatment that have being researched, skeletal muscle disuse or inactivity has been considered potentially preventable with targeted interventions.[3,4] Resistance exercise is considered the cornerstone of sarcopenia[5] management, as its beneficial effects in increasing muscle mass and strength have been confirmed in previous studies.[6–8]

Furthermore, several studies have investigated the treatment effects of green tea beverages abundant in tea catechins (TC), a chemical anti-oxidant,[9–11] as a potential nutritional supplementation for elderly adults; however, the results are controversial. Previous studies have suggested that TC have many health benefits for different disorders varying from cancer to weight loss.[9,11] Research on TC, and its effects on age-related declines in functional fitness and muscle mass in humans are scarce, and the mice studies available show inconsistent evidence. One study investigated the combined effects of exercise and TC ingestion in mice, and found that concomitant TC ingestion with habitual exercise is beneficial for suppressing age-related declines in physical

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performance,[10] focusing on endurance exercise. Nevertheless, there are few, if any, randomized controlled trials on the effects of exercise and TC supplementation on basic physical function in elderly people.

The purpose of the present study was to investigate the effects of exercise and/or TC ingestion on muscle mass, strength and walking ability in sarcopenic women.

Methods

Study population

Invitation letters were mailed to 1472 people aged 75 years and older who were randomly selected from the Basic Resident Register of elderly people residing in the Itabashi ward of Tokyo, Japan. There were 1355 responses to the invitation letters, where 1094 people agreed to participate and 261 people refused or did not respond. The baseline assessment was carried out at the Tokyo Metropolitan Institute of Gerontology (TMIG) between October and November 2009, where 974 participated and 120 who had initially agreed to participate were absent.

We operationally defined sarcopenic women based on categorization into at least one of the following inclusion criteria: (i) appendicular skeletal muscle mass/height^2 less than 6.42 kg/m^2 and knee extension strength less than 1.01 Nm/kg ($n = 99$);[12,13] (ii) appendicular skeletal muscle mass/height^2 less than 6.42 kg/m^2 and usual walking speed less than 1.10 m/sec ($n = 21$);[14] (iii) body mass index (BMI) less than 22 and knee extension strength less than 1.10 Nm/kg ($n = 130$); and (iv) BMI less than 22 and usual walking speed less than 1.10 m/sec ($n = 16$). Out of 974 people, 266 (27.3 %) participants were operationally defined as sarcopenic (Fig. 1).

Participants for the interventions were recruited from 266 sarcopenic women. Exclusion criteria included: (i) severe knee or back pain; (ii) severely impaired mobility; (iii) impaired cognition (Mini-Mental State Examination [MMSE] score <24);[15] (iv) missing baseline data; and (v) unstable cardiac conditions. A total of 138 participants (51.9%) were excluded from the study based on the exclusion criteria, or declined participation. The present study protocol was approved by the Clinical Research Ethics Committee of TMIG. The intervention procedures were fully explained to all participants and written informed consent was obtained.

Randomized group assignment

After the baseline assessment, computer-generated random numbers were assigned to 128 participants, who were then sorted and equally divided into four groups, and any variable that identified individual information was not included in the randomization process. The groups were randomly assigned to one of the four interventions: exercise and tea catechin supplementation (Ex + TC; $n = 32$), exercise (Ex; $n = 32$), tea catechin supplementation (TC; $n = 32$) or health education (HE; $n = 32$) groups. The allocation sequence was concealed from the study coordinator, and data collection was carried out by separate physical therapy staff members who were also blind to the allocation of treatments.

Outcome measures

Data were collected at baseline and after the 3-month intervention. Measures included interview surveys, body composition assessments and physical function tests. Measurements of height and weight were used to calculate BMI (kg/m^2).

Interview survey

Each participant was interviewed face-to-face to assess the individual's history of falls, fear of falling, pain, exercise habits, urinary incontinence, frequency of going out, self-rated health and so on.

Body composition assessment

Percent body fat, lean body mass, and total and segmental muscle mass were measured using a multifrequency bioelectrical impedance analysis (BIA) instrument that operated at frequencies of 5, 50, 250 and 550 kHz (Well-Scan 500; Elk, Tokyo, Japan). The participants stood on two metallic electrodes on the scales of the BIA instrument barefoot, holding two metallic grip electrodes placed in the palm of each hand with the fingers wrapped around the handrails. Segmental muscle mass values of each leg, arm and the trunk were measured and added to obtain appendicular skeletal muscle mass and leg muscle mass.

Performance measures

The performance measures included muscular strength (grip strength, knee extension strength), walking ability (usual and maximum walking speed, and timed up & go [TUG]) and balance ability (one leg standing time with eyes open). For the TUG test, time was measured in seconds from 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.[16] Assistive walking devices were allowed in measures of walking speed and TUG only if they expressed concerns about walking without a device, or if the investigators believed there was a danger of falling. Knee extension strength was measured twice, and the higher value

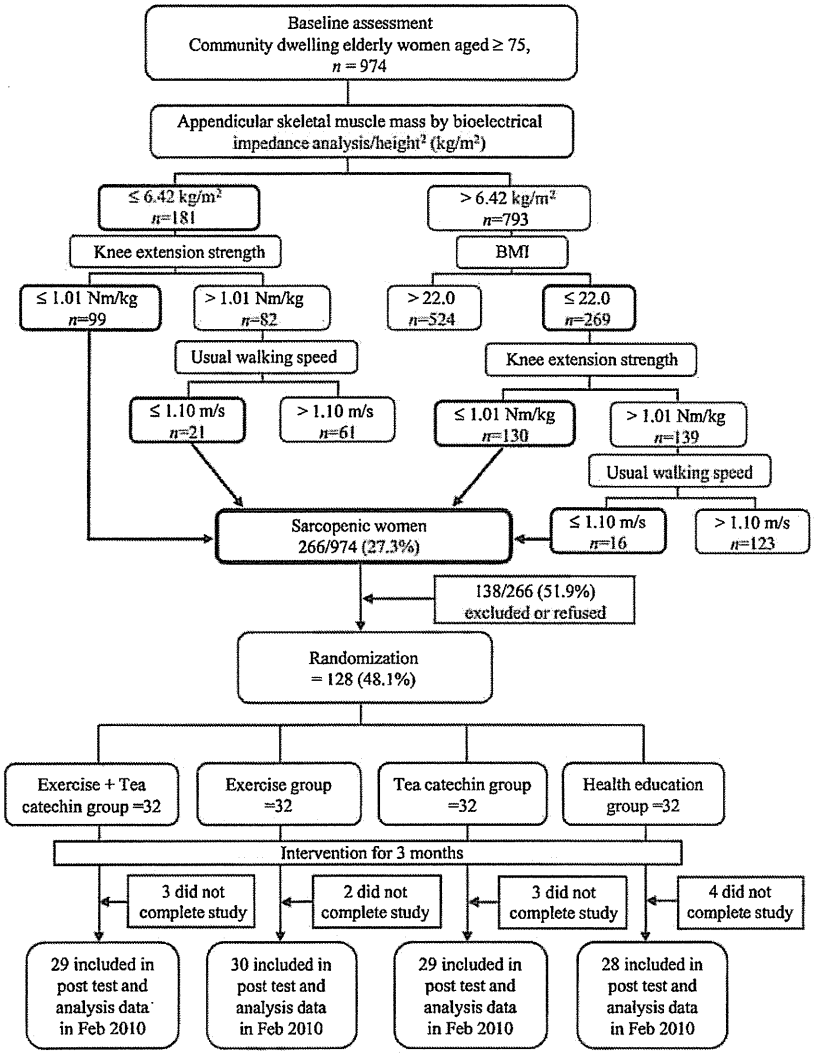

Figure 1 Algorithm for the selection of women operationally defined as sarcopenic, and flowchart of participants in the randomized controlled trial of exercise and tea catechin supplementation. BMI, body mass index.

divided by bodyweight (Nm/kg) was analyzed. Detailed procedures for the performance measures have been described previously.[17]

Intervention

Exercise

The exercise consisted of stretching, muscle strengthening, balance and gait training of moderate intensity maintained at approximately 12–14 on the Borg Rate of Perceived Exertion scale.[18] Each class was 60 min, held at the TMIG twice per week for 3-months. To ensure proper instruction to all participants, the two exercise intervention groups were divided into four subgroups, where the participants exercised together within their assigned subgroup in one of four exercise sessions offered per day.

The exercise session included a 5-min stretching warm-up of the neck, shoulders, lower back, hips, knees and ankles, 30-min of strengthening exercises, 20 min of balance and gait training, followed by a 5-min cool-down.

Muscle strength training

Strengthening exercises were carried out in a progressive sequence from the seated position, which initially provided a secure and stable position, to the standing position. Other resistance was applied through use of resistance bands for upper body strengthening and ankle weights for the lower body, as well as increasing the repetitions and sets of the exercises. Participants were initially instructed to complete up to one eight-repetition set of each type of exercise, which gradually increased to 10 repetitions, and up to two sets.

Resistance was increased on a group basis when the participants were able to execute each exercise without loss of proper execution. Each individual's ability to increase intensity was assessed by the principal investigator, the exercise instructor and assistant trainers.

To strengthen lower extremities, a fixed weight was placed on the ankle during the exercises. Weights of 0.50 kg, 0.75 kg, 1.00 kg, and 1.50 kg were prepared and used in accordance with each participant's strength level as the resistance progressively increased. Strengthening of the leg muscles focused on hip extensors and adductors, knee flexors and extensors, and ankle dorsi- and plantarflexors.

Gait and balance training

The participants practiced various walking patterns, focusing on stability maintenance during walking. Participants were taught to focus their attention on increasing toe elevation of the forward leg, heel elevation of the rear limb, and stride length through gait exercises including walking with directional changes and weight shifting. The balance training contained exercises, such as one-leg stands, tandem stand and tandem walking, for each participant to train their static, dynamic and lateral balancing ability.

Tea catechin supplementation

Bottles containing 350 mL of tea fortified with 540 mg of catechin were provided for the participants in the TC supplementation group every 2 weeks. The participants were instructed to drink one bottle per day, every day for 3 months. To monitor their TC intake accurately, the participants were asked to record the volume of tea consumed (the whole bottle, half the bottle, or about one-quarter) on record sheets that were collected every 2 weeks, along with the bottle caps of finished bottles. Participants who drank at least 54 bottles or more out of the 90 bottles (60%) were considered to have completed the supplementation intervention.

Health education

The HE group served as the control group, and the participants took a class once a month for 3 months, a total of three times. Health professionals taught topics such as cognitive function, the long-term care system and oral hygiene. No specific instructions on diet or physical activity were given, and the participants were asked to continue their regular lifestyle habits.

Data analysis

Differences in baseline measures between the groups were measured using a one-way analysis of variance (ANOVA), and χ^2-tests were carried out on categorical variables. Two-way repeated-measures ANOVA was used to analyze differences in the effect of the intervention on outcome measures between groups, and a post-hoc test was carried out where significant P-values (<0.05) were found to determine which groups were significantly different. Percentage changes in leg muscle mass and strength, and walking ability postintervention were calculated using the formula: % change = ((postintervention value – baseline value) / baseline value) × 100. To compare the effects of the four intervention groups on combined variables of leg muscle mass and functional fitness after 3 months of intervention, multiple logistic regressions were carried out. All analyses were carried out using SPSS software, Windows version 19.0 (SPSS, Tokyo, Japan) and SAS, version 9.2 for Windows (SAS Institute Japan, Tokyo, Japan).

Results

All of the baseline characteristics including age, percent body fat, muscle mass, walking speed, urinary incontinence and falls were similar between the groups (Table 1).

In comparing the pre- and postintervention changes in performance measures and body composition by two-way repeated-measures ANOVA (Table 2), there were significant group × time interactions in TUG ($F = 15.408$, $P = 0.005$), usual walking speed ($F = 4.327$, $P = 0.007$) and maximum walking speed ($F = 15.161$, $P < 0.001$), where the changes in the Ex + TC group were greater than the HE group.

Figure 2 shows the within group analyses of percent changes from pre- to postintervention. Leg muscle mass significantly increased in the Ex + TC group (2.21%, $P = 0.016$), whereas only small changes in the other groups were observed. Usual walking significantly increased in the Ex + TC group (11.36%, $P = 0.010$), a modest increase was seen in the Ex group (4.84%, $P = 0.020$), and slight decreases were observed in the TC and HE groups.

The multiple logistic regression analysis showed that the Ex + TC group had a significant effect on the combined variables of increased leg muscle mass and improved usual walking speed (OR 3.61, 95% CI 1.05–13.66; Table 3). The OR for increased leg muscle mass and knee extension strength in the Ex + TC group, although statistically non-significant, were more than twofold as great as the Ex or TC only groups.

Discussion

Although sarcopenia was originally defined as the age-related loss of muscle mass,[19] muscle strength does not depend solely on muscle mass, and the relationship