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

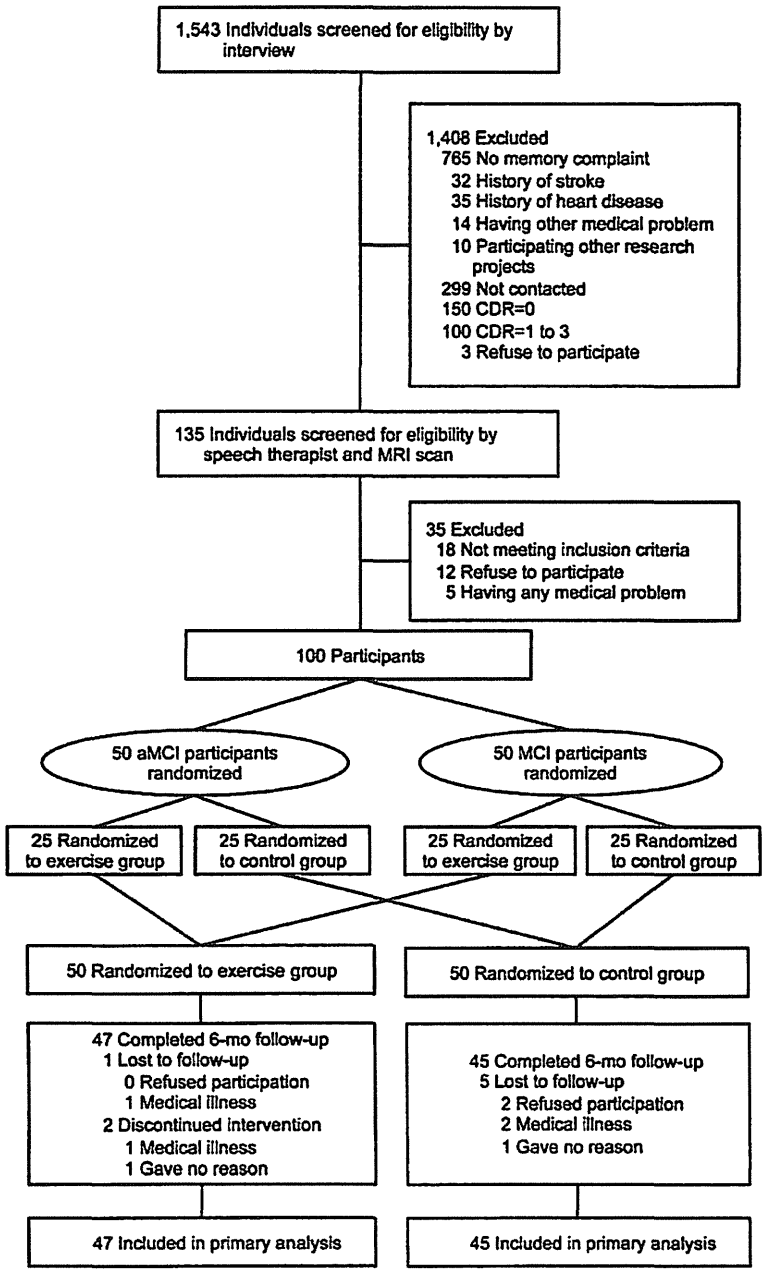

Figure 1. Subject flow diagram from initial contact through to study completion.
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early marker for dementia, [31,32] and dual-task-related changes in performance were greater in subjects with MCI compared with cognitively normal age-matched controls. [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]

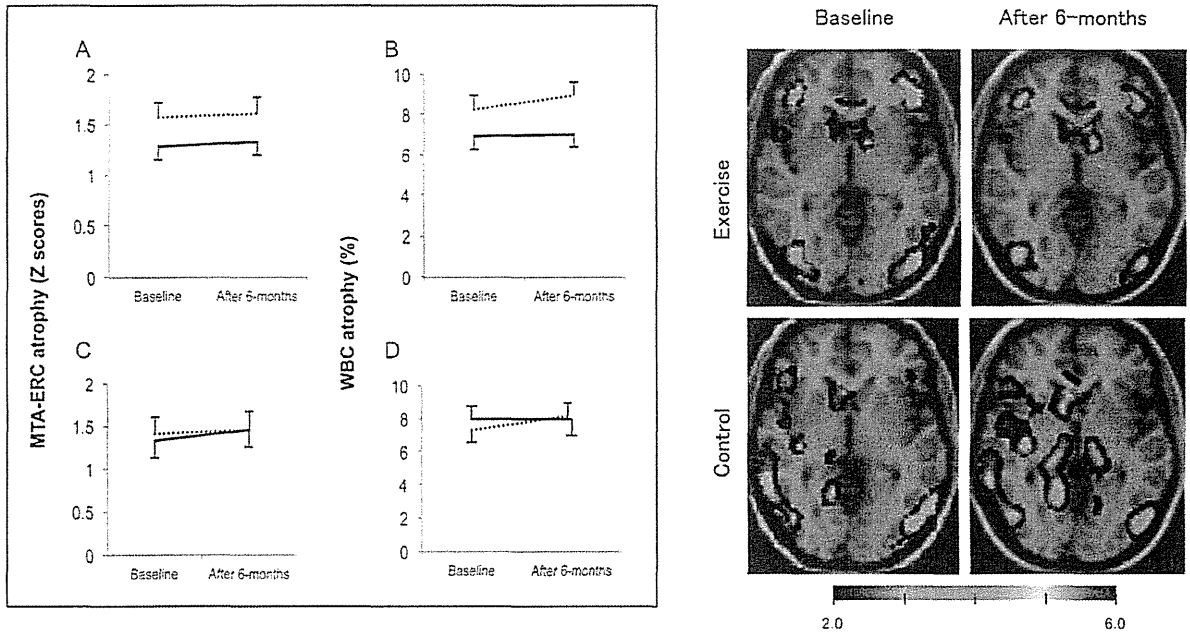

Figure 2. Change in MTA-ERC and WBC volumes in response to the 6-month intervention. Abbreviations: MTA-ERC, medial temporal areas including the entorhinal cortex; WBC, whole brain cortices. Left panel shows change in MTA-ERC and WBC volumes before and after the 6-month intervention. *Solid and dashed 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.

References

1. Brookmeyer R, Johnson E, Ziegler-Graham K, Arrighi HM (2007) Forecasting the global burden of Alzheimer's disease. Alzheimers Dement 3: 186–191.
2. Emre M, Mecocci P, Stender K (2008) Pooled analyses on cognitive effects of memantine in patients with moderate to severe Alzheimer's disease. Journal of Alzheimer's disease: JAD 14: 193–199.
3. Citron M (2010) Alzheimer's disease: strategies for disease modification. Nat Rev Drug Discov 9: 387–398.
4. Lautenschlager NT, Cox KL, Flicker L, Foster JK, van Bockxmeer FM, et al. (2008) Effect of physical activity on cognitive function in older adults at risk for Alzheimer disease: a randomized trial. JAMA 300: 1027–1037.
5. Scherder EJ, Van Paasschen J, Deijen JB, Van Der Knokke S, Orlebeke JF, et al. (2005) Physical activity and executive functions in the elderly with mild cognitive impairment. Aging Ment Health 9: 272–280.
6. van Uffelen JG, Chinapaw MJ, van Mechelen W, Hopman-Rock M (2008) Walking or vitamin B for cognition in older adults with mild cognitive impairment? A randomised controlled trial. Br J Sports Med 42: 344–351.
7. Baker LD, Frank LL, Foster-Schubert K, Green PS, Wilkinson CW, et al. (2010) Effects of aerobic exercise on mild cognitive impairment: a controlled trial. Arch Neurol 67: 71–79.
8. Lam LC, Chau RC, Wong BM, Fung AW, Lui VW, et al. (2011) Interim follow-up of a randomized controlled trial comparing Chinese style mind body (Tai Chi) and stretching exercises on cognitive function in subjects at risk of progressive cognitive decline. International journal of geriatric psychiatry 26: 733–740.
9. Angevaren M, Aufdemkampe G, Verhaar HJ, Aleman A, Vanhees L (2008) Physical activity and enhanced fitness to improve cognitive function in older people without known cognitive impairment. Cochrane Database Syst Rev: CD005381.
10. van Uffelen JG, Chin APMJ, Hopman-Rock M, van Mechelen W (2008) The effects of exercise on cognition in older adults with and without cognitive decline: a systematic review. Clin J Sport Med 18: 486–500.
11. Smith PJ, Blumenthal JA, Hoffman BM, Cooper H, Strauman TA, et al. (2010) Aerobic exercise and neurocognitive performance: a meta-analytic review of randomized controlled trials. Psychosom Med 72: 239–252.
12. Petersen RC, Doody R, Kurz A, Mohs RC, Morris JC, et al. (2001) Current concepts in mild cognitive impairment. Arch Neurol 58: 1985–1992.
13. Palmer K, Wang HX, Backman L, Winblad B, Fratiglioni L (2002) Differential evolution of cognitive impairment in nondemented older persons: results from the Kungsholmen Project. Am J Psychiatry 159: 436–442.
14. Neeper SA, Gomez-Pinilla F, Choi J, Cotman C (1995) Exercise and brain neurotrophins. Nature 373: 109.
15. Ding YH, Li J, Zhou Y, Rafols JA, Clark JC, et al. (2006) Cerebral angiogenesis and expression of angiogenic factors in aging rats after exercise. Curr Neurovasc Res 3: 15–23.
16. Erickson KI, Voss MW, Prakash RS, Basak C, Szabo A, et al. (2011) Exercise training increases size of hippocampus and improves memory. Proc Natl Acad Sci U S A 108: 3017–3022.
17. Colcombe SJ, Erickson KI, Scalf PE, Kim JS, Prakash R, et al. (2006) Aerobic exercise training increases brain volume in aging humans. J Gerontol A Biol Sci Med Sci 61: 1166–1170.
18. Colcombe S, Kramer AF (2003) Fitness effects on the cognitive function of older adults: a meta-analytic study. Psychol Sci 14: 125–130.
19. Petersen RC (2004) Mild cognitive impairment as a diagnostic entity. J Intern Med 256: 183–194.
20. Gauthier S, Reisberg B, Zaudig M, Petersen RC, Ritchie K, et al. (2006) Mild cognitive impairment. Lancet 367: 1262–1270.
21. Wechsler D, Stone C (1973) Wechsler Memory Scale manual. New York: Psychological Corp.
22. Folstein MF, Folstein SE, McHugh PR (1975) "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 12: 189–198.
23. Rosen WG, Mohs RC, Davis KL (1984) A new rating scale for Alzheimer's disease. Am J Psychiatry 141: 1356–1364.
24. Hirata Y, Matsuda H, Nemoto K, Ohnishi T, Hirao K, et al. (2005) Voxel-based morphometry to discriminate early Alzheimer's disease from controls. Neurosci Lett 382: 269–274.
25. Li X, Shimizu S, Jibiki I, Watanabe K, Kubota T (2010) Correlations between Z-scores of VSRAD and regional cerebral blood flow of SPECT in patients with Alzheimer's disease and mild cognitive impairment. Psychiatry Clin Neurosci 64: 281–292.
26. Ashburner J, Friston KJ (2000) Voxel-based morphometry the methods. Neuroimage 11: 805–821.
27. Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, et al. (1999) Mild cognitive impairment: clinical characterization and outcome. Archives of neurology 56: 303–308.
28. Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, et al. (1999) Mild cognitive impairment: clinical characterization and outcome. Arch Neurol 56: 303–308.
29. Klusmann V, Evers A, Schwarzer R, Schlattmann P, Reischies FM, et al. (2010) Complex mental and physical activity in older women and cognitive performance: a 6-month randomized controlled trial. J Gerontol A Biol Sci Med Sci 65: 680–688.
30. Belleville S, Gilbert B, Fontaine F, Gagnon L, Menard E, et al. (2006) Improvement of episodic memory in persons with mild cognitive impairment and healthy older adults: evidence from a cognitive intervention program. Dementia and geriatric cognitive disorders 22: 486–499.
31. Perry RJ, Watson P, Hodges JR (2000) The nature and staging of attention dysfunction in early (minimal and mild) Alzheimer's disease: relationship to episodic and semantic memory impairment. Neuropsychologia 38: 252–271.
32. Pettersson AF, Olsson E, Wahlund LO (2007) Effect of divided attention on gait in subjects with and without cognitive impairment. Journal of geriatric psychiatry and neurology 20: 58–62.
33. Damhauser TM, Walker Z, Stevens T, Lee L, Seal M, et al. (2005) The functional anatomy of divided attention in amnestic mild cognitive impairment. Brain: a journal of neurology 128: 1418–1427.

34. Maquet D, Lekeu F, Warzee E, Gillain S, Wojtasik V, et al. (2010) Gait analysis in elderly adult patients with mild cognitive impairment and patients with mild Alzheimer's disease: simple versus dual task: a preliminary report. Clinical physiology and functional imaging 30: 51–56.
35. Landau SM, Harvey D, Madison CM, Reiman EM, Foster NL, et al. (2010) Comparing predictors of conversion and decline in mild cognitive impairment. Neurology 75: 230–238.
36. Voss MW, Prakash RS, Erickson KI, Basak C, Chaddock L, et al. (2010) Plasticity of brain networks in a randomized intervention trial of exercise training in older adults. Frontiers in aging neuroscience 2.
37. Frisardi V, Solfrizzi V, Seripa D, Capurso C, Santamato A, et al. (2010) Metabolic-cognitive syndrome: a cross-talk between metabolic syndrome and Alzheimer's disease. Ageing research reviews 9: 399–417.
38. Yaffe K, Barrett-Connor E, Lin F, Grady D (2002) Serum lipoprotein levels, statin use, and cognitive function in older women. Archives of neurology 59: 378–384.
39. Farr SA, Yamada KA, Butterfield DA, Abdul HM, Xu L, et al. (2008) Obesity and hypertriglyceridemia produce cognitive impairment. Endocrinology 149: 2628–2636.
40. Angevaren M, Aufdemkampe G, Verhaar HJ, Aleman A, Vanhees L (2008) Physical activity and enhanced fitness to improve cognitive function in older people without known cognitive impairment. Cochrane database of systematic reviews: CD005381.
41. Kang H, Schuman EM (1995) Long-lasting neurotrophin-induced enhancement of synaptic transmission in the adult hippocampus. Science 267: 1658–1662.

中高年者の脳萎縮を抑制する日常歩行量の解明
～地域からの無作為抽出者を対象とした大規模縦断研究～

	独立行政法人国立長寿医療研究センター	幸　篤武
（共同研究者）	同	森　あさか
	同	李　成喆
	愛知淑徳大学	安藤　富士子
	独立行政法人国立長寿医療研究センター	下方　浩史

The Association between Daily Physical Activity Levels and Brain Atrophy Progression in Middle-aged and Elderly Japanese

by

Atsumu Yuki, Asaka Mori,
Lee SungChul, Hiroshi Shimokata
National Center for Geriatrics and Gerontology
Fujiko Ando
Aichi Shukutoku University

ABSTRACT

Brain structural atrophy is associated with impairment in learning function and cognitive function. The purpose of this study was to determine whether daily physical activity prevents age-related brain atrophy progression.

The subjects were 381 males and 393 females who had participated in both the baseline and follow-up examinations (mean duration, 8.2 years). Magnetic resonance imaging of the frontal and temporal lobes was performed at the time of the baseline and follow-up surveys. The number of steps of the subjects was recorded at baseline with uniaxial accelerometry sensors. Multiple logistic regression models were fit to

determine the association between number of steps variables and frontal and temporal lobe atrophy progression while controlling for possible confounders.

In males, the odds ratio of frontal lobe atrophy progression was increased by 1.480 (95% confidence interval [CI], 1.007-2.175)-fold for every 3,000 decrease in the number of steps. The odds ratio of frontal lobe atrophy progression for the fifth quintile compared to the first quintile in the number of steps was 3.651 (95% CI, 1.304-10.219). There were no significant differences between frontal lobe atrophy progression and the number of steps in females. There were also no significant differences between temporal lobe atrophy progression and the number of steps in males and females.

The results indicate that physical activity is significant predictors of frontal lobe atrophy progression over an 8-year period. Promoting participation in activities may be beneficial for attenuating age-related frontal lobe atrophy and for preventing dementia.

要　旨

地域から無作為抽出された中高年者を対象に，日常歩行量が脳萎縮進行に与える影響を検討した．

対象者は「国立長寿医療研究センター・老化に関する長期縦断疫学研究」第2次調査と8年後に実施された第6次調査の両方に参加した，50～79歳の男性381名，女性393名とした．8年間における前頭葉及び側頭葉萎縮の進行状況を，MRI画像より評価した．第2次調査時における歩行量調査を基に，脳萎縮進行を防ぐ歩行量閾値について，ロジスティック回帰分析により検討した．

男性において，歩行量が3,000歩ずつ減少した際の前頭葉萎縮進行のオッズ比は1.480（95%信頼区間，1.007－2.175）であった．また歩行量を5分位とした際の，第5分位に対する第1分位の前頭葉萎縮進行のオッズ比は3.651（95%信頼区間，1.304-10.219）であった．女性では前頭葉萎縮進行と歩行量との間に関連を認めなかった．側頭葉萎縮進行は，男女ともに歩行量との関連を認めなかった．

中高年男性では，前頭葉萎縮進行を予防するために，一日あたり5,800歩以上の歩行量を維持する必要性が示唆された．

緒　言

アルツハイマー病では脳の構造的な萎縮が顕著におこり，認知機能や学習機能に障害をきたす[13]．脳萎縮は加齢によっても進行し，ヒトの脳灰白質量は20歳代から70歳代にかけて，約15%減少することが報告されている[25]．一般高齢者を対象とした6年間の追跡調査では，脳萎縮の進行状態と認知機能レベルは強い関連を示すことが報告されており[20]，脳萎縮を予防することで認知機能の低下や障害の予防に繋がる可能性が示唆されている．

近年では，有酸素運動が神経新生を促進し，脳量の増加，保持に働くことが示されている[8]．高齢者では6ヶ月間の有酸素運動トレーニングによって前頭葉，側頭葉，海馬の脳量が増加したことが報告されている[5]．また，速歩を用いた有酸素性トレーニングは海馬の萎縮を改善するなど[10]，有酸素運動による脳萎縮の予防的効果が示されている．

脳量と有酸素運動の関連性が示される一方で，日常生活における身体活動と脳量の関連については不明な点が多い．中高年者では，身体活動量と有酸素能は相関することが報告されており[1, 4]，日常の身体活動量を高く保つことが脳萎縮の予防へと繋がるものと考えられる．実際に横断研究において，身体活動量と脳量は関連することが報告されているが[3, 11]，日常の身体活動量と脳量及び脳萎縮との関連を検討した縦断研究は見当たらない．近年では，身体活動量の多い高齢者では加齢による認知機能低下のリスクが低いことが，縦断研究によって示されている[21]．日常身体活動による脳萎縮抑制へと繋がる知見が得られれば，身体活動が認知機能低下を抑制することを裏付ける根拠となり，認知機能低下の予防を目的とした身体活動を推奨するためのエビデンスとなると考えられる．

そこで本研究は，無作為抽出された地域在住の中高年者を対象とする約8年間の追跡データを用い，日常歩行量と加齢による脳萎縮進行の関連について検討を行い，脳萎縮進行を抑制する歩行量閾値を解明することで，認知機能低下の予防を目的とした運動処方のエビデンスを作成することを目的とした．

1．方　法

1．1　地域住民におけるデータの収集

本研究は「国立長寿医療研究センター・老化に関する長期縦断疫学研究（NILS-LSA）」の参加者のデータを用いて行われた．NILS-LSAの参加者は長寿医療研究センター周辺の，観察開始時年齢が40歳から79歳までの地域住民約2,300名であり，住民台帳から年齢・性別に層化した無作為抽出によって選定された[22]．選定された者を説明会に招き，調査の目的や方法などを十分に説明し，書面による同意を得た上で調査は実施された．またNILS-LSAは，国立長寿医療研究センター倫理委員会での研究実施の承認を受けた上で実施された．

1．2　対象者

対象者は，ベースラインとしたNILS-LSAの第2次調査（2000年4月から2002年5月まで）を完了した参加者1,545名（男性715名，女性830名）中，その約8年後に実施された第6次調査（2008年7月から2010年7月まで）にも参加した男性514名，女性566名とした．そのうち，脳萎縮の保有率と進行率が他の年代と比較して特に少ない40歳代と，参加人数の少ない80歳代は解析の対象から除外した．また，パーキンソン病既往歴，認知症既往歴，開頭手術歴を有する者についても解析の対象から除外し，最終的な解析の対象は男性381名，女性393名とした．解析対象者のうち，第2次調査時において脳萎縮がグレード4（重度）に該当する者は含まれていなかった．

1．3　頭部核磁気共鳴画像法（MRI）検査

第2次調査時とその8年後に実施された第6次調査時において，頭部MRI検査（Visart 1.5T, 東芝）を実施した．頭部MRI検査はRepetition time = 500msec，Echo time = 15msec，Slice thickness = 8mm，Slice gap = 1.5mm，Matrix = 256×256 の条件でスキャンを行い，眼窩耳孔線に対し平行となるT1強調画像14枚を得た．

各調査時において得られた頭部MRI画像を基に，前頭葉及び側頭葉についてそれぞれ，萎縮を4段階（1 無し；2 軽度；3 中等度；4 重度）に分類した（図1）[16, 23]．さらに第2次調査時と第6次調査時の萎縮グレードを比較し，第6次調査時の萎縮グレードが第2次調査時のものと比較して高い群を「萎縮進行あり群」として，それ以外の場合を「萎縮進行なし群」として分類した．

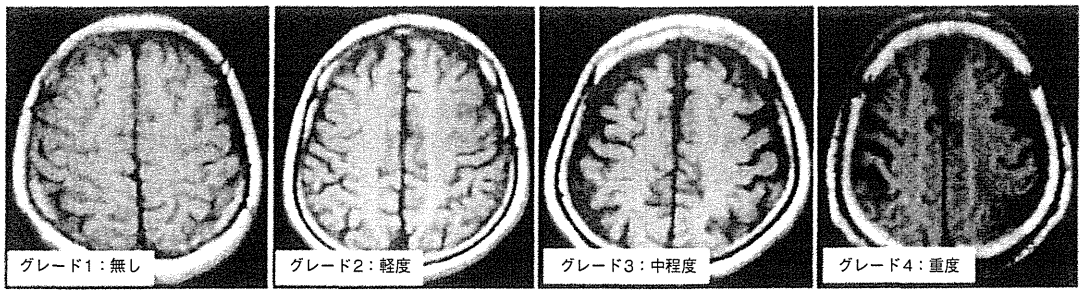

図1　脳萎縮の分類

1．4　歩行量調査

本研究では身体活動量指標として，歩行量を用いた．歩行量調査は，ベースラインに当たる第2次調査時において実施した．歩行量は，加速度計（Lifecorder, スズケン）を対象者の腰部に装着してもらうことで得た．調査期間は，旅行などの特別なイベントの無い7日間とし，入浴時及び就寝時は除外した．得られた7日間の歩行量のうち，最大値と最小値を除外した計5日間分のデータより，一日当たりの平均歩数を算出した．

1．5　対象者の特性に関する検査及び調査

第2次調査時における身長及び体重より，BMIを求めた．体脂肪率は，二重エネルギーX線吸収法による全身スキャンによって算出した（QDR-4500A, Hologic）．一日当たりのアルコール摂取量，現在の喫煙の状況，教育年数は自記式の調査票により得た．脳卒中，虚血性心疾患，糖尿病，高血圧症，脂質異常症の既往歴について，対象者に自記式の調査票への回答を求めるとともに，医師の問診による確認を行った．またNILS-LSAでは，対象者の過去2週間において使用したすべての処方薬及び市販薬について調査を行っている[22]．本研究では，血糖降下薬，降圧薬，脂質降下薬の使用者はそれぞれ，糖尿病，高血圧症，脂質異常症既往歴保有者に分類した．

1．6　統計解析

各変数のデータは，平均値 ± 標準偏差，または標準誤差で示すとともに，t検定または χ^2 検定を用い，群間における差及び分布状況についての比較を行った．対象者の年代と脳萎縮の進行の関係について，Cochran-Mantel-Haenszel検定を用いて年代上昇による増減傾向を検定した．

ベースラインから8年間の脳萎縮の進行状況と一日平均歩数の関連について，多重ロジスティック回帰分析を用いて検討した．多重ロジスティック回帰分析は，目的変数に萎縮進行の有無を，説明変数として一日平均歩数を投入し，年齢[25]，BMI[11]，教育年数[11]，脳卒中，虚血性心疾患，糖尿病，高血圧症，脂質異常症既往歴の有無[2,7]，現在の喫煙状況[6]，アルコール摂取量で調整した[24]．一日平均歩数は，連続変数，5分位としたカテゴリー変数としてそれぞれ投入し，脳萎縮進行のオッズ比を求めた．解析はStatistical Analysis System ver. 9.3（SAS Institute Inc）を用いて行い，有意水準は5%未満とした．

2．結　果

2．1　対象者の特性

表1に，ベースライン時における対象者の特性について男女別に示した．平均追跡期間は男女共に8.2± 0.3年であった．年齢，BMI，一日平均歩数は男女間に差を認めなかった．身長及び体重，アルコール摂取量，教育年数は，女性と比較して男性で高値を示した（各$p<0.0001$）．体脂肪率は，男性と比較して女性で高値を示した（$p<0.0001$）．脳卒中，虚血性心疾患，高血圧症の

表１　対象者の特性

	男性 (n = 381)	女性 (n = 393)	p value
追跡期間（年）	8.2 ± 0.3	8.2 ± 0.3	0.5777
年齢	60.4 ± 7.3	60.8 ± 7.6	0.5421
身長（cm）	164.7 ± 5.4	152.2 ± 5.2	< 0.0001
体重（kg）	62.5 ± 7.1	52.7 ± 7.0	< 0.0001
BMI（kg/m2）	23.0 ± 2.4	22.7 ± 2.9	0.1279
体脂肪率（%）	21.0 ± 4.0	31.3 ± 4.9	< 0.0001
アルコール摂取量（g/day）	16.6 ± 20.9	2.7 ± 6.1	< 0.0001
教育年数（年）	12.3 ± 2.7	11.4 ± 2.3	< 0.0001
一日平均歩数（/day）	7993.2 ± 2588.0	7925.6 ± 2297.1	0.7011
脳卒中既往歴（n）	14(3.7%)	7 (1.8%)	0.105
虚血性心疾患既往歴（n）	13(3.5%)	19 (4.8%)	0.3203
高血圧症既往歴（n）	40(10.5%)	40 (10.2%)	0.8836
脂質異常症既往歴（n）	61(16.0%)	94 (23.9%)	0.006
糖尿病既往歴（n）	32(8.4%)	16 (4.1%)	0.0126
喫煙者（n）	102(26.8%)	27 (6.9%)	< 0.0001

平均値 ± 標準偏差　p 値は t 検定，χ^2 検定による

既往歴保有者の割合は，男女間で差を認めなかった．脂質異常症の既往歴保有者の割合は，男性と比較して女性で高かった（p=0.0060）．糖尿病既往歴保有者，喫煙者割合は，女性と比較して男性で高かった（糖尿病 p=0.0126; 喫煙者 p<0.0001）．

２．２　年代別にみた脳萎縮進行の頻度

表２に，ベースラインから８年間の前頭葉及び側頭葉における萎縮の進行状況について，性，年代別に示した．男性対象者 381 名中 55 名（14.4%），女性対象者 393 名中 35 名（8.9%）に萎縮の進行が認められ，その割合は女性と比較して男性で高かった（χ^2 検定，p = 0.0213）．また，男女とも年代上昇で前頭葉萎縮進行者の割合は増加した（p trend < 0.0001）．側頭葉では，男性対象者 381 名中 100 名（26.3%），女性対象者 393 名中 78 名（19.8%）に萎縮の進行が認められ，その割合は女性と比較して男性で高かった（χ^2 検定，p = 0.0344）．また，男女とも年代上昇で側頭葉萎縮進行者の割合は増加した（p trend < 0.0001）．

２．３　脳萎縮の進行状況と一日平均歩数

図２に，一日平均歩数について，前頭葉及び側頭葉の萎縮進行群別に示した．男性では，前頭葉の萎縮進行なし群と比較して，萎縮進行あり群では一日平均歩数が低値を示した（p = 0.0131）．一方側頭葉では，群間に差を認めなかった．また女性では前頭葉及び側頭葉ともに，一日平均歩数は群間に差を認めなかった．

表２　脳萎縮進行者の年代別分布

		前頭葉萎縮		trend p value	側頭葉萎縮		trend p value
		進行なし	進行あり		進行なし	進行あり	
男性（n）	50-59 歳	176 (95.1%)	9 (4.9%)		156 (84.3%)	29 (15.7%)	
	60-69 歳	112 (79.4%)	29 (20.6%)	< 0.0001	87 (61.7%)	54 (38.3%)	< 0.0001
	70-79 歳	38 (69.1%)	17 (30.9%)		38 (69.1%)	17 (30.9%)	
	計	326 (85.6%)	55 (14.4%)		281 (73.8%)	100 (26.3%)	
女性（n）	50-59 歳	191 (96.0%)	8 (4.0%)		188 (94.5%)	11 (5.5%)	
	60-69 歳	117 (90.0%)	13 (10.0%)	< 0.0001	92 (70.8%)	38 (29.2%)	< 0.0001
	70-79 歳	50 (78.1%)	14 (21.9%)		35 (54.7%)	29 (45.3%)	
	計	358 (91.1%)	35 (8.9%)		315 (80.2%)	78 (19.8%)	

trend p 値は Cochran–Mantel–Haenszel 検定による

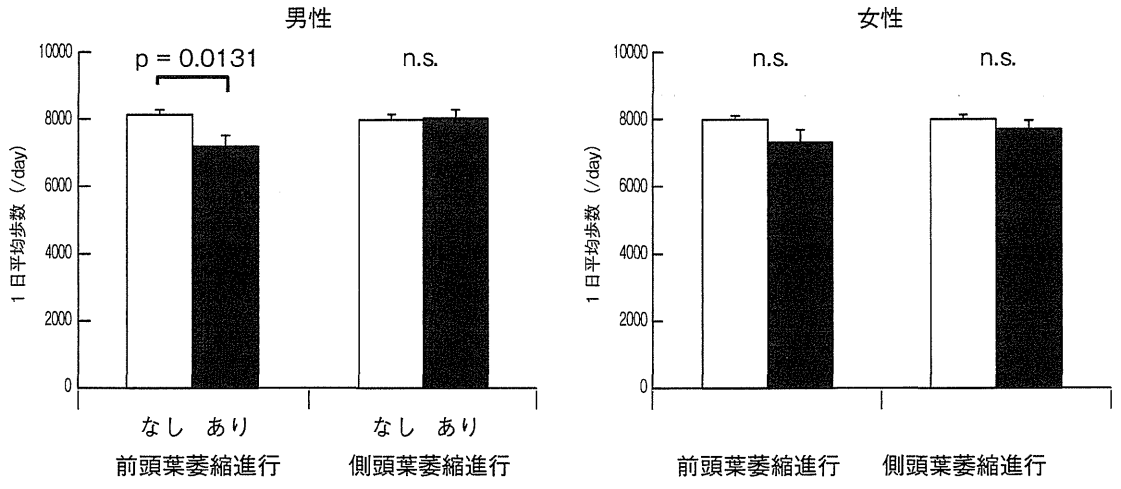

図2　一日平均歩数の比較
平均値±標準誤差　p値はt検定による

2．4　脳萎縮進行のリスクと関連する一日平均歩数

表3及び表4に，前頭葉及び側頭葉における萎縮の進行と，一日平均歩数の関連性について検討したロジスティック回帰分析の結果を示す．男性において，8年後の前頭葉萎縮の進行と一日平均歩数との間に関連性を認めた．一日平均歩数を連続変数（マイナス3,000歩ごと）とした際の前頭葉萎縮進行のオッズ比は，1.480（95%信頼区間，1.007－2.175; p=0.0460）と有意な関連を示した．また一日平均歩数を5分位とし，第5分位を基準した際の各分位の前頭葉萎縮進行のオッズ比を求めたところ，第1分位におけるオッズ比は3.651（95%信頼区間，1.304－10.219; p=0.0072）と有意な関連を示した．一方，側頭葉萎縮進行と一日平均歩数との間に関連を認めなかった．女性では，前頭葉及び側頭葉のいずれも，萎縮の進行と一日平均歩数との間に関連を認め

表3　男性対象者における脳萎縮進行のオッズ比

		n	前頭葉萎縮進行		側頭葉萎縮進行	
			オッズ比（95%信頼区間）	p value	オッズ比（95%信頼区間）	p value
1日平均歩行量	-3000歩ごと	381	1.480（1.007－2.175）	0.046	0.979（0.742－1.290）	0.8787
	Q1: 5736.0歩未満	76	3.651（1.304－10.219）	0.0072	0.938（0.435－2.024）	0.6269
	Q2: 5736.0－6955.0歩未満	76	1.261（0.383－3.863）	0.3108	1.100（0.519－2.330）	0.8715
	Q3: 6955.0－8261.4歩未満	76	1.487（0.471－4.689）	0.6501	1.142（0.538－2.425）	0.7501
	Q4: 8261.4－10407.4歩未満	76	2.403（0.819－7.052）	0.2874	1.123（0.528－2.389）	0.8039
	Q5: 10407.4歩以上	77	1.00（基準）		1.00（基準）	

年齢，BMI，教育年数，脳卒中，虚血性心疾患，糖尿病，高血圧症，脂質異常症既往歴の有無，喫煙状況，アルコール摂取量で調整

表4　女性対象者における脳萎縮進行のオッズ比

		n	前頭葉萎縮進行		側頭葉萎縮進行	
			オッズ比（95%信頼区間）	p value	オッズ比（95%信頼区間）	p value
1日平均歩行量	-3000歩ごと	393	1.298（0.766－2.197）	0.3323	0.961（0.656－1.407）	0.8361
	Q1: 5825.2歩未満	78	1.559（0.420－5.791）	0.78	0.879（0.355－2.178）	0.8452
	Q2: 5825.2－7090.0歩未満	79	2.269（0.627－8.209）	0.1784	0.789（0.311－2.005）	0.5798
	Q3: 7090.0－8374.0歩未満	78	0.826（0.181－3.769）	0.2578	0.825（0.317－2.147）	0.7003
	Q4: 8374.0－9910.4歩未満	79	1.887（0.505－7.053）	0.426	1.206（0.489－2.974）	0.3522
	Q5: 9910.4歩以上	79	1.00（基準）		1.00（基準）	

年齢，BMI，教育年数，脳卒中，虚血性心疾患，糖尿病，高血圧症，脂質異常症既往歴の有無，喫煙状況，アルコール摂取量で調整

なかった．

3．考　察

地域から無作為に抽出された中高年者を対象とし，加齢による脳萎縮進行と関連を示す一日平均歩数について縦断解析を行った結果，男性では前頭葉萎縮進行と一日平均歩数との間に関連を認めた（表4）．一日平均歩数を連続変数とした際の前頭葉萎縮進行のリスクは，歩数が3,000歩ずつ減少するごとに約1.5倍ずつの上昇を示し，日本人の中高年男性では日常の歩行量を高く保つことで，加齢による前頭葉萎縮の進行を抑制する可能性が示唆された．さらに一日平均歩数を5分位とし，歩行量が最も多い群（10,407.4歩以上）を基準として，各分位における前頭葉萎縮進行のリスクを検討したところ，歩行量が最も少ない群（5,736.0歩未満）では前頭葉萎縮進行のリスクが約3.7倍高いことが示された．このことから，男性では前頭葉萎縮の進行を予防する日常の歩行量の最少閾値が，約5,800歩付近に存在している可能性が考えられた．一般に歩行量は加齢に伴い減少することが知られている．日本人男性の一日平均歩数は，50歳代が7,772歩，60歳代が6,949歩，70歳以上が4,707歩と報告されており[17]，70歳以降の男性では前頭葉萎縮の進行リスクが他の年代と比較して特に高いことが推察される．従って日本人の中高年男性では，一日の歩数を概ね5,800歩以上に保つこと，また特に70歳以降において歩行量を増やすことが，加齢による前頭葉の萎縮進行の予防において重要である可能性が示唆された．

対照的に，女性では加齢による前頭葉萎縮進行と日常の歩行量の間に関連を認めなかった（表4）．一般的に，男性は女性と比較して脳萎縮の頻度は高い[25]．そして実際に本研究においても，男性では前頭葉萎縮進行の頻度が女性と比較して高く（表2），身体活動の効果が男性でより明確化したことが考えられる．また，テストステロンやエストロゲンなどの性ホルモンについても，脳量に影響を及ぼす因子であることが報告されており[8, 15]，身体活動に対する脳の可塑性には性差が存在する可能性も考えられる．

本研究はヒトを対象とし，脳萎縮の進行についてMRI画像を基に評価した非侵襲的研究であることから，身体活動が前頭葉萎縮進行を抑制したメカニズムを明らかにすることはできない．マウスの脳ではアミロイドβの蓄積量と活動量との間に関連が認められることが報告されており[14]，身体活動が高いことでアミロイドβの蓄積が抑制された可能性が考えられる．また，身体活動により神経細胞の増殖や生存に不可欠とされる成長因子の発現量が変動した可能性もある[26]．

運動が脳量に与える効果は，前頭葉に限らず，側頭葉や頭頂葉，海馬など多くの脳領域に及ぶことが報告されている[3, 5, 11]．興味深いことに，本研究では前頭葉と側頭葉を脳萎縮進行の評価の対象としたが，高い身体活動量との関連は前頭葉に限られている．脳における神経新生を促す要因として，脳血流量の増加が指摘されている[19]．そして身体運動は脳血流量を変動させるが，その変動様式は運動の種類や強度により異なるとされる[12, 18]．本研究は加速度計を用いて得た歩数を身体活動レベルの指標としており，身体活動の種類や強度などは考慮されていない．今後は，身体活動の種類や強度などの影響を考慮した上で，さらなる検討を行う必要があると思われる．

4．まとめ

本研究は地域から無作為に抽出された50歳から79歳までの男女774名を対象に，日常歩行量と加齢による脳萎縮進行の関連について，縦断的に検討した．その結果，男性において日常の

歩行量を高く保つことが，前頭葉萎縮進行を抑制することが示された．また5,800歩が，前頭葉萎縮の進行を抑制する一日当たりの歩行量閾値として示され，認知機能低下の予防に繋がる身体活動量の目標値の一つとなる可能性が示唆された．

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文　献

1) 青山友子，浅香明子，金子香織，石島寿道，河野寛，坂本静男，田畑泉，樋口満．中高年男性における心肺体力と身体活動の量およびその強度との関係．体力科学．59: 191-198(2010)
2) Bruehl H., Wolf O.T., Sweat V., Tirsi A., Richardson S., Convit A., Modifiers of cognitive function and brain structure in middle-aged and elderly individuals with type 2 diabetes mellitus. *Brain Res.*, 1280: 1896-194(2009)
3) Bugg J.M., Head D., Exercise moderates age-related atrophy of the medial temporal lobe. *Neurobiol Aging.*, 32: 506-514(2011)
4) Cao Z.B., Miyatake N., Higuchi M., et al., Prediction of VO2max with daily step counts for Japanese adult women. *Eur. J. Appl. Physiol.*, 105: 289-296(2009)
5) Colcombe S.J., Erickson K.I., Scalf P.E., et al., Aerobic exercise training increases brain volume in aging humans. *J. Gerontol. A Biol. Sci. Med. Sci.*, 61: 1166-1170(2006)
6) Debette S., Seshadri S., Beiser A., et al., Midlife vascular risk factor exposure accelerates structural brain aging and cognitive decline. *Neurology.*, 77: 461-468(2011)
7) Enzinger C., Fazekas F., Matthews P.M., et al., Risk factors for progression of brain atrophy in aging: six-year follow-up of normal subjects. *Neurology.*, 64: 1704-1711(2005)
8) Erickson K.I., Colcombe S.J., Raz N, et al., Selective sparing of brain tissue in postmenopausal women receiving hormone replacement therapy. *Neurobiol Aging.*, 26: 1205-1213(2005)
9) Erickson K.I., Kramer A.F., Aerobic exercise effects on cognitive and neural plasticity in older adults. *Br. J. Sports Med.*, 43: 22-24(2009)
10) Erickson K.I., Voss M.W., Prakash R.S., et al., Exercise training increases size of hippocampus and improves memory. *Proc. Natl. Acad. Sci. USA.*, 108: 3017-3022(2011)
11) Ho A.J., Raji C.A., Becker J.T., et al., The effects of physical activity, education, and body mass index on the aging brain. *Hum. Brain Mapp.*, 32: 1371-1382(2011)
12) Ide K., Horn A., Secher N.H., Cerebral metabolic response to submaximal exercise. *J. Appl. Physiol.*, 87: 1604-1608(1999)
13) Killiany R.J., Gomez-Isla T., Moss M., et al., Use of structural magnetic resonance imaging to predict who will get Alzheimer's disease. *Ann. Neurol.*, 47: 430-439(2000)
14) Lazarov O., Robinson J., Tang Y.P., et al., Environmental enrichment reduces Abeta levels and amyloid deposition in transgenic mice. *Cell.*, 120: 701-713(2005)
15) Lessov-Schlaggar C.N., Reed T., Swan G.E., et al., Association of sex steroid hormones with brain morphology and cognition in healthy elderly men. *Neurology.*, 22: 1591-1596(2005)
16) Manolio T.A., Kronmal R.A., Burke G.L., et al., Magnetic resonance abnormalities and cardiovascular disease in older adults. The Cardiovascular Health Study. *Stroke.*, 25: 318-327(1994)
17) 厚生労働省．平成21年度国民健康・栄養調査結果．(2010)
18) Nielsen H.B., Boushel R., Madsen P., Secher N.H., Cerebral desaturation during exercise reversed by O2 supplementation. *Am. J. Physiol.*, 277: H1045-1052(1999)
19) Pereira A.C., Huddleston D.E., Brickman A.M., et al., An in vivo correlate of exercise-induced neurogenesis in the adult dentate gyrus. *Proc. Natl. Acad. Sci. USA.*, 104: 5638-5643(2007)
20) Rusinek H., De Santi S., Frid D., et al., Regional brain atrophy rate predicts future cognitive decline:

6-year longitudinal MR imaging study of normal aging. *Radiology*., 229: 691-696 (2003)

21) Sattler C., Erickson K.I., Toro P., et al., Physical fitness as a protective factor for cognitive impairment in a prospective population-based study in Germany. *J. Alzheimers Dis*., 26: 709-718 (2011)

22) Shimokata H., Ando F., Niino N., A new comprehensive study on aging the National Institute for Longevity Sciences, Longitudinal Study of Aging (NILS-LSA). *J. Epidemiol*., 10: S1-9 (2000)

23) Shimokata H., Ando F., Fukukawa Y., et al., Klotho gene promoter polymorphism and cognitive impairment. *Geriatrics & Gerontology International*., 6: 136-141 (2006)

24) Taki Y., Kinomura S., Sato K., et al., Both global gray matter volume and regional gray matter volume negatively correlate with lifetime alcohol intake in non-alcohol-dependent Japanese men: a volumetric analysis and a voxel-based morphometry. *Alcohol Clin. Exp. Res*., 30: 1045-1050 (2006)

25) Taki Y., Kinomura S., Sato K., et al., A longitudinal study of gray matter volume decline with age and modifying factors. *Neurobiol. Aging*., 32: 907-915 (2011)

26) Thoenen H., Neurotrophins and neuronal plasticity. *Science*., 270: 593-598 (1995)

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Does high educational level protect against intellectual decline in older adults?: A 10-year longitudinal study[1]

YUKIKO NISHITA*, CHIKAKO TANGE, and MAKIKO TOMIDA *National Center for Geriatrics and Gerontology*

FUJIKO ANDO *Aichi Shukutoku University*

HIROSHI SHIMOKATA *Nagoya University of Arts and Sciences*

Abstract: This study examined the relation between educational level and intellectual change in Japanese older adults. Participants (age = 65–79 years, n = 593) comprised the first-wave participants of the National Institute for Longevity Sciences-Longitudinal Study of Aging (NILS-LSA). They were followed for 10 years and were tested six times. Educational levels were divided into two groups (low-educated or high-educated), and intellectual changes for the 10 years were assessed using the Japanese Wechsler Adult Intelligence Scale-Revised Short Forms (JWAIS-R-SF); subtests included Information, Similarities, Picture Completion, and Digit Symbol. General linear mixed-model analyses revealed that education had not affected 10-year changes of the Information, Similarities, and Picture Completion subtest scores. In contrast, education was significantly associated with a change in the Digit Symbol subtest score; individuals with higher levels of education showed greater decline than those with less education, although they had higher ability at every time point. These findings suggest that higher education does not protect against intellectual decline in late life, although it is associated with long-term individual differences in intelligence.

Key words: intelligence, education, older adults, longitudinal study.

Many studies have suggested that early-life educational level is associated with better intellectual abilities in late life (e.g., Kaufman & Lichtenberger, 2006; Schaie, 2005; Wechsler, 1981). However, recent articles based on longitudinal data have shown conflicting results with respect to the relation between educational level and intellectual changes in old age. Some longitudinal studies have reported that educational attainment moderates intellectual decline in samples of older adults (e.g., Alvarado, Zunzunequi, Del Ser, & Beland, 2002; Arbuckle, Maag, Pushkar, & Chaikelson, 1998; Evans, Beckett, Albert, Hebert, Scherr, Funkenstein, & Taylor, 1993; Farmer, Kittner, Rae, Bartko, & Regier, 1995; Koster, Penninx, Bosma, Kempen, Newman, Rubin, Satterfield, Atkinson, Ayonayon, Rosano, Yaffe, Harris, Rooks, Van Eijk, & Kritchevsky, 2005; Lee, Kawachi, Berkman, & Grodstein, 2003; Lyketsos, Chen, & Anthony, 1999). However, others disagree with these findings, suggesting

*Correspondence concerning this article should be sent to: Yukiko Nishita, Section of NILS-LSA, Center for Gerontology and Social Science, National Center for Geriatrics and Gerontology, Gengo, Morioka-cho, Obu 477-8511, Japan. (E-mail: nishita@ncgg.go.jp)

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that higher education does not protect against intellectual decline (e.g., Seeman, Huang, Bretsky, Crimmins, Launer, & Guralnik, 2005; Tucker-Drob, Johnson, & Jones, 2009; Van Dijk, Van Gerven, Van Boxtel, Van der Elst, & Jolles, 2008; Wilson, Hebert, Scherr, Barnes, Mendes de Leon, & Evans, 2009; Zahodne, Glymour, Sparks, Bontempo, Dixon, Macdonald, & Manly, 2011), or that relations between education and intellectual change appear to differ by intellectual domain (e.g., Alley, Suthers, & Crimmins, 2007; Anstey & Christensen, 2000; Anstey, Hofer, & Luszcz, 2003).

From the perspective of the *cognitive reserve* hypothesis, it is noteworthy that previous longitudinal studies have reported the mixed results described above. The hypothesis of cognitive reserve asserts that older individuals with greater experiential resources exhibit better cognitive functioning and are able to tolerate brain pathology before displaying clinical symptoms (Scarmeas & Stern, 2004; Stern, 2002). Stern (2002) postulated that high cognitive reserve may allow individuals to cope more successfully with age-related brain changes, and that one of the most well-established proxy measures of cognitive reserve capacity in the elderly was educational attainment, which is thought to reflect more effective use of brain networks or cognitive paradigms.

Two competing cognitive reserve models could offer insight into the effect of education on the rate of cognitive change (Stern, 2002; Van Dijk et al., 2008). First, if high education was found to slow the rate of cognitive decline, this finding would support an *active* cognitive reserve hypothesis. In this case, individuals with higher education would be hypothesized to process tasks more efficiently. Further, because they make more efficient use of brain networks, the same amount of organic cognitive damage would result in a smaller decline in cognitive function relative to those with less education. Second and alternately, if educational level does not relate to the rate of cognitive change, this would support a *passive* cognitive reserve hypothesis. If aging individuals begin to lose cognitive function from a common cause, such as normal aging brains, people with higher education would change at a rate similar to the total population, but would continue to perform at a higher level at any age because of greater baseline brain reserve. These theories of *active* and *passive* cognitive reserve processes are often evaluated with respect to the implications for *moderation* versus *stability* (Salthouse, 2003; Tucker-Drob et al., 2009) or *differential-preservation* versus *preserved-differentiation* (Bielak, Anstey, Christensen, & Windsor, 2012; Salthouse, 2006).

Inconsistencies in previous longitudinal studies may be due to some methodological differences among the studies. For example, studies differed in the number of consecutive assessments, or the measures of intellectual abilities used.

In terms of the number of assessments, some studies (e.g., Alvarado et al., 2002; Arbuckle et al., 1998; Evans et al., 1993; Farmer et al., 1995; Koster et al., 2005; Lee et al., 2003; Lyketsos et al., 1999) examined intellectual change by calculating a difference between only two test occasions and then used traditional regression analysis or repeated measures analysis of variance techniques. However, ideally, to estimate a true change, intellectual ability should be assessed at multiple time points rather than using a simple difference in two test administrations (Alley et al., 2007; Wilson et al., 2009). The use of three or more assessments of longitudinal intellectual aging can reduce measurement error (Winkens, Schouten, Van Breukelen, & Berger, 2006) as well as avoid the regression toward the mean phenomenon (Dufouil, Fuhrer, Dartigues, & Alpérovitch, 1996; Zahodne et al., 2011). Moreover, the use of three or more assessments makes possible the use of more sophisticated analytic techniques, such as multilevel modeling or general linear mixed modeling (Laird & Ware, 1982; Morrell, Brant, & Ferrucci, 2009; Verbeke & Molenberghs, 1997).

A second methodological difference concerns which domain of intellectual ability was being measured; it remains possible that education may have different effects on the changes in different intellectual domains. For example,

in their review of the literature, Anstey and Christensen (2000) found that education appears to be more predictive for crystallized ability, but less predictive for fluid ability or processing speed. Similarly, Wilson et al. (2009) pointed out that their results were based on overall global cognition, so they could not establish whether education was related to decline in some intellectual domains but not others. Additionally, some studies (e.g., Evans et al., 1993; Farmer et al., 1995; Lee et al., 2003) have used mental status measures that assess the most basic level of cognitive abilities (e.g., the Mini Mental State Examination; Folstein, Folstein, McHugh, Practical, & Patients, 1975). Such basic level measures may be insensitive to change among well-educated older adults due to ceiling effects that prevent detection of changes within the upper levels of functioning, resulting in spurious relations between initial performance and change (Tucker-Drob et al., 2009). Thus, multiple and more sensitive assessments that reflect greater variability in intellectual functions might better address educational differences in future research.

The present study

The purpose of the present study was to determine whether educational level is associated with the rate of intellectual change in community-dwelling older Japanese. The important characteristics of this study included the following: (a) the participants were followed for 10 years, tested six times, and general linear mixed models were used to analyze the data; and (b) to measure intelligence in late life, we used neuropsychological tests to cover the multiple intellectual abilities of the adults: the Japanese Wechsler Adult Intelligence Scales-Revised Short Forms (JWAIS-R-SF; Kobayashi, Fujita, Maekawa, & Dairoku, 1993). The JWAIS-R-SF includes four standardized subtests (Information, Similarities, Picture Completion, and Digit Symbol). To our knowledge, this may be the first study that approaches the effect of educational levels on intellectual changes for Japanese older adults.

Methods

Participants

The data for the present study were collected as a part of the National Institute for Longevity Sciences-Longitudinal Study of Aging (NILS-LSA; Shimokata, Ando, & Niino, 2000). The NILS-LSA is a population-based prospective cohort study of aging and age-related diseases. The participants were sex- and age-stratified random samples of Japanese community-dwelling adults aged from 40 to 79 years at baseline (Wave1: 1997–2000). This baseline sample consisted of 2267 participants who were followed up every 2 years (Wave2: 2000–2002, Wave3: 2002–2004, Wave4: 2004–2006, Wave5: 2006–2008, Wave6: 2008–2010). Informed consent was obtained from each participant at the beginning of the study.

We selected an initial sample of individuals who were aged 65 years or older at baseline ($n = 816$). We excluded individuals who: (a) provided data only at baseline ($n = 210$) because longitudinal analyses required a minimum of two valid scores per individual, (b) had a history of dementia at baseline ($n = 1$), or (c) had missing data on all dependent variables at baseline or on the independent variables ($n = 12$). Based on these criteria, the data from 593 individuals were included at baseline. Mean age at baseline was 70.96 years ($SD = 3.90$ years, age range = 65–79 years), with 46.54% of the sample being women.

Measures

Intelligence. The Wechsler Adult Intelligence Scale (WAIS) is one of the most popular tools for assessing intelligence (Wechsler, 1944). In this study, intelligence was assessed using the JWAIS-R-SF (Kobayashi et al., 1993). The trained testers (clinical psychologists or psychology graduate students) administered the test to each participant one on one. The JWAIS-R-SF consists of the following four subtests: Information, Similarities, Picture Completion, and Digit Symbol.