ORIGINAL ARTICLE

Estimation of appendicular muscle mass and fat mass by near infrared spectroscopy in older persons

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Aim: Near infrared spectroscopy has been reported to have a high reliability and accuracy in assessing the percentage of body fat. However, whether muscle mass can be accurately estimated using this method has not been established. This study examined whether a near infrared spectroscopy method could estimate appendicular muscle mass and fat mass, with dual-energy X-ray absorptiometry as the standard method for comparison.

Methods: A total of 20 orthopedic inpatients (mean age 73.2 ± 6.8 years) were recruited for this study. Their body composition was assessed using near infrared spectroscopy and dual-energy X-ray absorptiometry. Appendicular muscle mass and fat mass were estimated from height, weight and optical densities.

Results: The optical densities for the upper arm (biceps, triceps) and forearm (flexor carpi radialis) were significantly correlated with appendicular muscle mass ($r = 0.534$ to 0.623) or fat mass ($r = -0.483$ to -0.827). Estimated appendicular muscle mass and fat mass explained 89% and 80% of the variance in the dual-energy X-ray absorptiometry-derived muscle mass and fat mass estimates using height, weight and optical density values of the proximal flexor carpi radialis.

Conclusions: Near infrared spectroscopy is a useful method to assess not only fat mass, but also muscle mass in older adults. **Geriatr Gerontol Int 2012; ••: ••–••.**

Keywords: aged, body composition, body fat, sarcopenia, skeletal muscles.

Introduction

Age-related loss of muscle mass (so-called sarcopenia) can lead to functional decline in older persons.[1-5] Two published Health, Aging and Body Composition reports

showed that sarcopenia, as determined by computed tomography (CT) in the mid-thigh, was a weak to modest predictor of loss of physical function over the following 2 to 3 years.[6,7] Furthermore, one study reported that older sarcopenic patients were twice as likely to contract infection during a hospital stay compared with older patients with a normal muscle mass.[8] This suggested that sarcopenic individuals might have decreased immunity, which might provide a mechanistic link between sarcopenia and mortality risk. In addition, reduced arm muscle area was reported to be an independent predictor of long-term mortality in community-dwelling older adults.[9] According to the

New Mexico Elder Health Survey, the prevalence of sarcopenia increased from 13 to 24% in persons aged under 70 years to >50% in persons aged over 80 years.[1] To achieve successful aging, it is important to preserve muscle mass to maintain function.

Recently, some researchers reported that sarcopenic patients who were obese were at particularly high risk of functional impairment and physical disability.[10-13] The condition was termed sarcopenic obesity, and it was suggested that approximately 15% of those with sarcopenia were also obese.[10] This suggests that it is necessary to assess not only muscle mass, but also fat mass accurately in the elderly.

There are various methods for measurement of body composition. Total body and regional skeletal muscle mass can now be accurately quantified using imaging methods, including CT and magnetic resonance imaging (MRI).[14] However, CT and MRI are costly methods and access to the equipment can be limited. Dual-energy X-ray absorptiometry (DXA) has been widely used in clinical practice, not only for osteoporosis screening and diagnosis, but also for assessment of body composition, such as skeletal muscle mass and fat mass. DXA is less expensive and less invasive compared with MRI and CT. Previous studies have shown good correlations between DXA-derived lean soft tissue mass and skeletal muscle mass in the lower limb region when CT and MRI were used as the standards for comparison.[15,16] However, DXA methods take more time, although whole-body scanning by this method exposes the patient to minimal radiation.

Bioelectrical impedance analysis (BIA) is a non-invasive, portable, quick and inexpensive method for measuring body composition.[17] Previous studies have shown that there is a strong correlation between BIA resistance and skeletal muscle measurements in the arms[18] and legs.[19] In addition, one report suggested that BIA could provide rapid and accurate estimates of whole body skeletal muscle mass in adults.[20] There are some disadvantages with the BIA method. First, fat tissue also holds water, although the proportion is small.[21] Second, the volume of muscle derived by BIA might overestimate the actual volume. Third, there are a large proportion of older adults who have a changed distribution of body water, such as edema. One report showed that the expansion of extracellular water relative to intracellular water and to regional lean volume masks actual muscle cell atrophy during aging.[22] This suggested that it might be difficult to accurately assess body composition in older adults.

Another development that might have potential for use in older adults is near infrared spectroscopy (NIRS). NIRS is also a non-invasive, simple and rapid method of assessing the percentage of body fat. There are some reports that the NIRS method has a high reliability and accuracy in determination of the percentage of body

fat.[23-25] In contrast, it has not been established whether muscle mass can be estimated accurately by NIRS.

The present study investigated whether a NIRS method could provide an accurate estimate of appendicular muscle mass (AMM) and appendicular fat mass (AFM) using DXA as the standard method for comparison.

Methods

Participants

A total of 20 orthopedic patients who were admitted to the National Hospital for Geriatric Medicine and who were aged 60 years or older were recruited for the present study. Patients with dementia or who had major laterality of muscle mass in the arms and legs, or who had surgery just before the study were excluded. All participants had their height (to the nearest 0.1 cm) and weight (to the nearest 0.1 kg) measured after admission. The details of the study were explained in advance and written consent was obtained from each participant. In addition, the present study was approved by the ethics committee of the National Center for Geriatrics and Gerontology.

Measurement of body composition

Whole and regional body composition was measured using DXA (Lunar DPX, Madison, WI, USA). This system provided the mass of lean soft tissue, fat and bone mineral for both the whole body and specific regions. Appendages were isolated from the trunk and head by using a DXA regional computer-generated default line. AMM or AFM was derived as the sum of the fat-free soft tissues or fat tissue of the arms and legs. A previous study reported that total body skeletal muscle mass can be accurately predicted from DXA-estimated appendicular lean soft tissue mass.[26,27]

NIRS

The NIRS measurements were carried out with the Fitness Analyzer BFT-3000 (Kett Electrical Laboratory, Tokyo, Japan, Fig. 1), the Japanese version of the Futrex 5000 (Futrex, Gaithersburg, MD, USA; 1988), which has potential for estimating body composition.[22,28] This device uses optical densities (OD) at two wavelengths (OD1 = 937 nm, OD2 = 947 nm) measured at each site. The NIRS instrument was tested immediately before taking measurements on each patients by using an optical standard, which was provided with the instrument and situated in a flexible light shield, to ensure that its performance was consistent throughout the study.

OD values were obtained at six sites: distal biceps (5 cm from the olecranon), distal triceps (5 cm from the

Figure 1 The near infrared spectroscopy instrument (Fitness Analyzer BFT-3000).

Figure 2 Method of measurement.

olecranon), proximal flexor carpi radialis (5 cm from the olecranon), distal quadriceps (5 cm from the upper edge of patella), proximal tibialis anterior (5 cm from the caput fibulae) and proximal calf (5 cm from the caput fibulae). The reliability was confirmed by test–retest. The test–retest reproducibility was excellent (intraclass correlation coefficient = 0.95–0.97, $P < 0.01$). Patients were required to maintain a seated position, with their arms relaxed at their sides (Fig. 2). NIRS measurements were carried out by a single trained physical therapist, and completed within a few minutes.

Statistical analysis

Pearson's correlation coefficient was used to determine the relationship between AMM or AFM and each OD value. Equations for estimation of AMM and AFM were developed with the use of multiple linear regression analysis. Potential explanatory variables included OD value, height and weight. DXA-measured AMM and AFM were set as the objective variable. The coefficient of determination (R^2) values were used to quantify the accuracy of model fit. The mean difference between DXA-measured AMM (AFM) and estimated AMM (AFM) was tested using the paired Student's t-test. Statistical analyses were carried out using PASW Statistics 18 for Windows (SPSS, Chicago, IL, USA) and the significance level was less than 5%.

Results

The characteristics of the patients are shown in Table 1. Mean age was 73.2 ± 6.8 years (range 62–84 years) and 70% were female. The subjects were diagnosed with the following: spinal canal stenosis ($n = 11$), disc herniation ($n = 1$), spinal tumor ($n = 2$), knee osteoarthritis ($n = 2$), compression fracture ($n = 1$), femoral neck fracture ($n = 1$) and others ($n = 2$).

The correlation coefficients between AMM or AFM and each OD value are listed in Table 2. AMM was significantly correlated with OD values at the distal triceps (OD1: $r = 0.623$; OD2: $r = 0.534$). AFM was significantly correlated with OD values at the distal biceps (OD1 $r = -0.570$; OD2 $r = -0.551$), distal triceps (OD1 $r = -0.483$; OD2 $r = -0.494$) and proximal flexor carpi radialis (OD1 $r = -0.827$; OD2 $r = -0.821$). In the correlation analysis between muscle mass or fat mass and the OD value, correlation coefficients were mostly higher with OD1 than with OD2. Thus, OD1 was used as the representative value of NIRS data for the estimation equation.

The results from linear regression analyses for the multivariate models are presented in Table 3. The multiple regression equations incorporated height, weight and OD1. Using anthropometric data (height and weight) as the explanatory variables, the R^2 value of AMM and AFM were 0.81 (standard error of the estimate [SEE] = 1.67 kg) and 0.50 (SEE = 1.77 kg), respectively (model 1). When OD1 was added to the explanatory variables, the R^2 values of AMM and AFM ranged from 0.85 to 0.89, and 0.58 to 0.80, respectively (models 2–5). The highest R^2 values of AMM and AFM were 0.89 (SEE = 1.33 kg) and 0.80 (SEE = 1.16 kg), respectively, when OD1 at the proximal flexor carpi radialis was added to the explanatory variables. For separate estimation equations (upper and lower limb), the accuracy of model fit was slightly less (muscle mass $R^2 = 0.82$–0.87, fat mass $R^2 = 0.53$–0.55). There were no significant differences between DXA-measured AMM and estimated AMM (mean difference 0.01, 95% confidence interval –0.56 to 0.58), or between DXA-measured AFM and estimated AFM (mean difference –0.25, 95% confidence interval –0.75 to 0.25).

Table 1 Physical characteristics of the study participants

Variables	All subjects (n = 20)	Men (n = 6)	Women (n = 14)
Age (years)	73.2 ± 6.8	67.8 ± 8.1	75.5 ± 4.9
Height (cm)	153.2 ± 9.5	166.1 ± 3.2	147.8 ± 4.3
Weight (kg)	53.9 ± 10.3	64.3 ± 6.8	49.4 ± 8.2
BMI (kg/m^2)	22.8 ± 2.9	23.3 ± 2.1	22.6 ± 3.2
AMM (kg)	15.7 ± 3.7	20.5 ± 1.2	13.6 ± 2.0
AFM (kg)	4.8 ± 2.4	4.1 ± 2.2	5.1 ± 2.4
Diagnosis n (%)			
Spinal canal stenosis	11 (55%)		
Disc herniation	1 (5%)		
Spinal tumor	2 (10%)		
Knee osteoarthritis	2 (10%)		
Compression fracture	1 (5%)		
Femoral neck fracture	1 (5%)		
Others	2 (10%)		

Values are mean ± standard deviation or n (%).
AFM, dual-energy X-ray absorptiometry-derived appendicular fat mass; AMM, dual-energy X-ray absorptiometry-derived appendicular muscle mass; BMI, body mass index.

Table 2 Correlation coefficients between limb muscle mass or fat mass and each optical densities value

	Biceps OD1	OD2	Triceps OD1	OD2	Flexor carpi radialis OD1	OD2
Upper limb muscle mass						
Four limbs	0.369	0.350	0.623**	0.534*	0.343	0.324
Upper limb	0.292	0.286	0.572**	0.462*	0.279	0.267
Upper limb fat mass						
Four limbs	−0.570**	−0.551*	−0.483*	−0.494*	−0.827**	−0.821**
Upper limb	−0.423	−0.394	−0.403	−0.411	−0.723**	−0.705**

	Quadriceps OD1	OD2	Tibialis anterior OD1	OD2	Calf OD1	OD2
Lower limb muscle mass						
Four limbs	0.332	0.190	0.139	0.118	0.297	0.327
Lower limb	0.383	0.248	0.138	0.125	0.346	0.373
Lower limb fat mass						
Four limbs	−0.348	−0.220	−0.421	−0.388	−0.426	−0.443
Lower limb	−0.333	−0.218	−0.434	−0.401	−0.458*	−0.472*

*$P < 0.05$; **$P < 0.01$. Optical density (OD)1 = 937 nm, OD2 = 947 nm.

Discussion

Recently, Sanada et al. reported prediction models for skeletal muscle index using body mass index (BMI) in Japanese adults.[29] The results showed that the R^2 values for the skeletal muscle index were 0.56 in men and 0.45 in women. Similarly, Gallagher et al. reported that height and weight accounted for 64% and 67% of the total variance of the appendicular skeletal muscle mass in African-American and Caucasian women, respec-
tively, and 63% and 39% of the total variance in African-American and Caucasian men, respectively.[30] These results showed the difficulty in estimating the AMM accurately using only anthropometric measurements, and the need for an objective method for accurate measurement of body composition.

To address this problem, we investigated whether AMM and AFM could be estimated by a combination of height, weight and NIRS data (OD values). The present results showed that OD1 of the proximal flexor carpi

Table 3 Regression equation for estimating appendicular muscle mass and fat mass

Model	Equation	R^2	SEE
Appendicular muscle mass			
1	$y = 0.23 \times$ (height) $+ 0.13 \times$ (weight) $- 26.35$	0.81	1.67
2	$y = 0.17 \times$ (height) $+ 0.17 \times$ (weight) $+ 8.45 \times$ [OD1 [biceps]) $- 28.97$	0.89	1.34
3	$y = 0.13 \times$ (height) $+ 0.18 \times$ (weight) $+ 10.49 \times$ (OD1 [triceps]) $- 23.19$	0.85	1.55
4	$y = 0.10 \times$ (height) $+ 0.24 \times$ (weight) $+ 7.82 \times$ (OD1 [flexor carpi radialis]) $- 21.42$	0.89	1.33
5	$y = 0.20 \times$ (height) $+ 0.15 \times$ (weight) $+ 6.12 \times$ (OD1 [calf]) $- 29.44$	0.85	1.57
Appendicular fat mass			
1	$y = -0.22 \times$ (height) $+ 0.25 \times$ (weight) $+ 25.39$	0.50	1.77
2	$y = -0.17 \times$ (height) $+ 0.21 \times$ (weight) $- 7.89 \times$ (OD1 [biceps]) $+ 27.84$	0.65	1.52
3	$y = -0.10 \times$ (height) $+ 0.20 \times$ (weight) $- 12.11 \times$ (OD1 [triceps]) $+ 21.73$	0.61	1.60
4	$y = -0.06 \times$ (height) $+ 0.12 \times$ (weight) $- 10.01 \times$ (OD1 [flexor carpi radialis]) $+ 19.08$	0.80	1.16
5	$y = -0.19 \times$ (height) $+ 0.23 \times$ (weight) $- 6.55 \times$ (OD1 [calf]) $+ 28.70$	0.58	1.66

R^2, coefficient of determination; SEE, standard error of the estimate.

radialis, in association with anthropometric data, can provide accurate estimates of both AMM and AFM in older adults, although the NIRS data alone did not reflect muscle mass except at the distal triceps. Furthermore, compared with the estimation equation that included only anthropometric data, the estimation equation that included both anthropometric and NIRS data had a higher coefficient of determination.

In the present study, the NIRS data were obtained at six sites to determine the best location for estimating AMM and AFM. As a result, OD values measured at the distal triceps and proximal flexor carpi radialis showed a good correlation coefficient with limb muscle mass and fat mass, respectively. Yasukawa et al. reported that the NIRS data (OD values) measured by BFT-2000 (old model of BFT-3000) had higher correlations with percentage fat at the thinner adipose sites than thicker adipose sites,[31] and similar results were observed by Futrex 5000 in another report.[25] Inconsistent strengths of the association of OD values with total body fat at the various sites might simply be a result of differences in the depth of penetration of the infrared radiation. These results suggested that it might be preferable to carry out measurements at sites where there is little subcutaneous fat, such as the flexor carpi radialis.

There are several reports of NIRS being a valid method to assess the percentage of fat or fat mass. For example, Sawai et al. reported that the correlation coefficient between percentage body fat as predicted by the NIRS method and as predicted by the hydrostatic weighing technique was 0.88 ($P < 0.001$, SEE = 3.2).[24] Fuller et al. also suggested that NIRS methods using Futrex 5000 have the potential to replace skinfold thickness (SFT) for estimation of body composition.[25] The BFT-3000 used in the present study was developed for Japanese patients, and the principle of measurement was the same as for Futrex 5000. Our findings that

NIRS data could accurately reflect fat mass are consistent with a previous study.[25] These results suggest that NIRS is a valid method for the estimation of AFM.

Other reports (by Futrex 5000) showed that NIRS might have little or no advantage over SFT in determining body composition.[32,33] One of the reasons for this controversy is that the degree of obesity differs in each patient. Elia et al. concluded that NIRS might underestimate body fat in very obese patients.[32] In the present study, the mean BMI of the patients was 23.3 ± 2.1 kg/m^2 in men and 22.6 ± 3.2 kg/m^2 in women, and there was no patient whose BMI was over 30 kg/m^2. Previous studies of older Japanese patients also reported a BMI ranging from 19.9 to 23.3 kg/m^2.[21,22] These results imply that NIRS data might be less affected by subcutaneous fat in older Japanese patients, and that NIRS is a valid method to assess their percentage fat and fat mass.

In contrast, NIRS data were not correlated significantly with whole and regional muscle mass except in the distal triceps. It is possible that quantitative assessment of skeletal muscle mass might be difficult using only NIRS data, because near infrared light might not reach the deeper muscle layer. However, when bodyweight is divided into fat mass and fat-free mass, skeletal muscle constitutes the largest fraction of appendicular fat-free mass. Previous investigators also proposed several models for predicting skeletal muscle mass with DXA. Lean body mass consists mostly of skeletal muscle. If we obtain an accurate bodyweight and the fat mass, the lean body weight (i.e. skeletal muscle mass) can be calculated automatically. The results in the present study suggest that AMM might be estimated indirectly by using NIRS data and bodyweight.

The present study is limited by the small sample size and orthopedic patients who were mostly women. The estimation equations of AMM and AFM developed in

the present study might have high specificity. In addition, we did not confirm the validity of these estimation equations. Thus, further studies are required to check the validity of these equations in other older adults (cross-validity) and longitudinally monitored populations (predictive validity) in the future. Furthermore, these equations will be developed for each sex using larger samples. Finally, to our knowledge, it is unclear whether the OD value (wavelength 937–947 nm) is influenced by blood flow and oxygen saturation. In the previous study, investigators did not mention this point. However, all patients were maintained in a resting position before and during the measurement in the present study. We think that the influence of blood flow and oxygen saturation is not likely to be marked, but this should be considered in a future study.

In conclusion, NIRS data can provide reliable and accurate estimates of AMM and AFM in older adults with the use of anthropometric data (height and weight). The estimation equations of AMM and AFM suggest the possibility that NIRS is a convenient method to assess body composition and to screen sarcopenic (or sarcopenic-obesity) patients. For further adjustment of this equation, it might be expected that sarcopenia or sarcopenic-obesity patients can be screened easily.

Acknowledgments

Disclosure statement

The authors have no financial disclosures or other conflicts of interest to report.

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Brain Atrophy and Trunk Stability During Dual-Task Walking Among Older Adults

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Background. Dual-task walking is believed to be more cognitively demanding than normal walking and alters trunk movement among older adults. However, the possible association between brain atrophy and spatiotemporal gait parameters, particularly during dual-task walking, is poorly understood. In this study, we examined the relationship between dual-task walking and brain atrophy.

Methods. One hundred ten elderly adults (aged 65–94 years, women $n = 55$) underwent magnetic resonance imaging scanning and gait experiments under normal and dual-task walking conditions. Linear accelerations of the trunk were measured in vertical, anteroposterior, and mediolateral directions using a triaxial accelerometer attached to the lower trunk. Gait speed, stride length, and cadence were recorded. The harmonic ratio, a measure of trunk stability, was computed separately in each direction to evaluate the smoothness of trunk movement during walking. Brain atrophy was quantitatively assessed using magnetic resonance image data.

Results. Gait speed, stride length, cadence, and harmonic ratio in all directions were lower in dual-task walking than in normal walking ($p < .05$). The dual-task–related changes in harmonic ratio were independently correlated with brain atrophy adjusted for subject characteristics only in the vertical direction ($p < .05$).

Conclusions. Our findings support the hypothesis that dual-task walking is more cognitively demanding than normal walking. Decreased trunk stability during dual-task walking is associated with brain atrophy. Additional studies are necessary to elucidate the effects of regional brain atrophy on the control of walking.

Key Words: Brain atrophy—Gait analysis—Dual-task walking—Acceleration.

SUCCESSFUL locomotion is thought to require stability during gait. During normal walking, control of trunk movement is prioritized and contributes to head stability to maintain gait stability (1). Age-related gait changes among older adults induce trunk instability, which is reflected in reduced smoothness of trunk motion (2,3), and is more pronounced during more challenging walking tasks than during normal walking (4). Walking is a motor task that requires consecutive movement and adaptability to a changing environment. Successful locomotion not only requires input from the neuromuscular system but also from high-order cognitive systems such as executive function.

The performance of executive function has been associated with gait performance, and this relationship is stronger during more challenging walking tasks such as dual-task walking (5,6,7). To investigate the cognitive demands of

walking, dual-task walking has been researched, for example, walking while performing a cognitive task or walking while talking. Dual-task walking markedly increased the variability of lower limb gait variables in older adults with cognitive impairment (8,9) and even in healthy older adults (10,11,12). Additionally, dual-task walking affected trunk movement in healthy older adults (7,13,14,15). Cognitive demands during dual-task walking affect spatiotemporal gait parameters. Dual-task–related changes (DTC) in gait variables correlate with both mobility and cognitive function in healthy older adults with normal gait performance (5). Moreover, dual-task training involving mobility tasks improved not only mobility function but also cognitive function (16, 17). Thus, dual-task walking may require and activate more multidomain neural resources in the brain than normal walking.

Emerging evidence suggests that age-related changes in the brain are linked to mobility deficits. Examples of these age-related changes include structural changes and changes to the biochemistry in the brain (18). Changes in the white matter (19,20,21) or the volume of gray matter (21,22,23), that is, macrostructural changes seen on magnetic resonance images (MRI), are also associated with changes in gait parameters. MRI-based measures of atrophy are a neurodegeneration marker, and they correlate with cognitive deficits and disease progress (24). However, a consensus has not been reached on which specific gait parameters are related to brain atrophy. Furthermore, it is still unclear if DTC in gait variables, including trunk movement, are related to MRI-based markers.

The purpose of this study was to investigate the relationships between brain atrophy and spatiotemporal gait parameters during normal and dual-task walking in older adults. We hypothesized that DTC in spatiotemporal gait parameters in older adults are related to brain atrophy described by MRI-based markers. To acquire quantitative gait variables including variables describing trunk movement and for a variety of conditions, we used a triaxial accelerometer that minimizes restrictions of walking movements (25). Brain atrophy was quantitatively and automatically calculated using a voxel-based analysis system from MRI (26,27).

METHODS

Participants

One hundred thirty-five people were recruited from our volunteer database, which included older adults aged 65 and older. The inclusion criteria required that participants were living independently in the community and had adequate speech, hearing, and visual acuity to participate in the examinations. Exclusion criteria included having a history of major psychiatric illness, serious neurological or musculoskeletal diagnoses, or depression [Geriatric Depression Scale score $\geq$ 10 (28)]. Each participant underwent gait experiments and assessments including a face-to-face interview with a clinical nurse, a cognitive assessment by a speech therapist, physical performance tests, and MRI scanning. One hundred ten people met the criteria and participated in this study. The following data were recorded: age, sex, body mass index, and educational history. To assess functional capacity, we used the Tokyo Metropolitan Institute of Gerontology Index of Competence (29) questionnaire (0–13 points). This questionnaire consists of three subscales and each item has 1 point: instrumental self-maintenance (five items), intellectual activity (four items), and social role (four items). General physical function was examined using grip strength and the timed up and go test (30). Grip strength was measured twice while standing, and the higher value was used. The timed up and go test is a mobility test, and participants were asked to walk 3 m, then turn around and walk 3 m, all at their self-selected normal speed in a well-lit environment.

Neuropsychological function was evaluated using the Mini-Mental State Examination (31). The ethics committee of the National Center for Geriatrics and Gerontology approved this study. All participants provided written, informed consent.

Gait Analysis

Participants were checked to make sure they were wearing shoes of an appropriate size before each experiment. Then, subjects were instructed to walk on an 11-m smooth, horizontal walkway, with a 2-m space at both ends of the walkway for acceleration and deceleration. Two gait experiments were performed in order: (a) normal walking at the participant's preferred speed and (b) dual-task walking: walking while counting backward in double digits with a randomly chosen starting number between 50 and 99. The mid 5-m walking time was measured, and gait speed was expressed in meters per second. A triaxial accelerometer (MVP-RF8, acceleration range: ± 60 m/sec^2, size: 45 mm width, 45 mm depth, 18.5 mm height, weight: 60 g, sampling rate: 200 Hz; MicroStone, Nagano, Japan) was attached to the L3 spinous process using a Velcro™ belt. The accuracy of data acquisition had been confirmed in a previous study using the same type of sensor (32). Before measurements, the accelerometer was calibrated statically against gravity. After analogue to digital transformation (10-bit resolution), signals were immediately transferred to a laptop PC (Let's Note CF-W5, Panasonic, Osaka, Japan) via a Bluetooth Personal Area Network. The working range of the accelerometer to the PC was approximately 50 m. Signal processing was performed using commercially available software (MATLAB, Release 2008b, The MathWorks Japan, Tokyo, Japan). The person who processed the acceleration data was blinded to any other results. Before analysis, all acceleration data were low-pass filtered (dual pass zero lag Butterworth filtered) with a cutoff frequency of 20 Hz. Stride time was determined by a validated method reported as the interval from an initial contact event to the next ipsilateral event (33). The mean stride time was calculated from five consecutive stride times. The average stride length was determined by multiplying gait speed by mean stride duration. The harmonic ratio (HR) was used to evaluate the smoothness and stability of trunk movement during gait (3,4,34). Higher HR values indicate greater stability during walking. HR was computed using a digital Fourier transform separately in each direction (vertical: VT direction, mediolateral direction, and anteroposterior direction). The procedure for calculating HR has been reported elsewhere (3,4,34).

Brain MRI

MRI was performed on a 1.5-T system (Magnetom Vision, Siemens, Germany). Three-dimensional volumetric acquisition of a T1-weighted gradient echo sequence was 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). The voxel-based specific regional analysis system in this study has been validated (26,27). This system was reformatted to produce gapless 2-mm thin-slice transaxial images, and the first anatomical standardization used affine transformation. The normalized MRI images were then segmented into gray matter, white matter, cerebrospinal fluid, and other components using a modified version of the clustering algorithm, the maximum likelihood "mixture model" algorithm. The segmentation procedure involved a calculation for each voxel using a Bayesian probability of belonging to each tissue class based on a priori MRI information with a nonuniformity correction. The segmented gray matter images were then subjected to an affine and nonlinear anatomical standardization using an a priori gray matter template. The anatomically standardized gray matter images were smoothed with an isotropic Gaussian kernel 12-mm full-width at half-maximum to exploit the partial volume effects, and a spectrum of gray matter intensities was created. We compared the gray matter image of each patient with the mean and standard deviation 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:

$$Z \text{ score} = ([\text{control mean}] - [\text{individual value}]) / \text{control } SD$$

The region of brain atrophy was defined as voxels with a Z score greater than 2. The brain atrophy index was defined as the proportion of the number of voxels defined atrophic relative to the total number of voxels of the entire brain.

Statistical Analysis

All analyses were performed using commercially available software (JMP8.0J for Windows, SAS Institute Japan, Tokyo, Japan). The data were normally distributed for all spatiotemporal gait parameters under both normal walking and dual-task walking conditions. Gait parameters were compared between normal walking and dual-task walking using a repeated measures analysis of variance. To assess the association between DTC in gait parameters and brain atrophy, we first confirmed the interaction of the factors brain atrophy (continuous measure) and walking condition (normal walking vs dual-task walking) for each gait parameters using a repeated multivariate analysis of covariance adjusted for covariates (covariates: age, sex, and Mini-Mental State Examination score). Covariates for the interaction were then confirmed using an analysis of variance comparing tertiles of brain atrophy. A linear regression model adjusted for gait speed was used to detect a significant association between brain atrophy and DTC in those gait parameters with a significant interaction between brain atrophy and walking condition. Independent variables included subject characteristics and DTC in gait parameters between walking conditions and were presented as percentage

of changes (|dual-task walking − normal walking|/normal walking × 100). Statistical significance was set a priori at $p < .05$.

RESULTS

The 110 subjects (50% women) were aged between 65 and 94 years with a mean body mass index of 23.1 kg/m². The demographic data, general physical performance, functional capacity, and brain atrophy for all subjects are summarized in Table 1. The spatiotemporal gait parameters under normal walking and dual-task walking conditions and a comparison between conditions are presented in Table 2. Gait speed was significantly lower for the dual-task walking compared with the normal walking condition even when adjusted for sex ($p = .029$). Stride length and cadence were lower for dual-task walking condition compared with the normal walking condition even when adjusted for sex and gait speed (stride length: $p < .001$, cadence: $p < .001$). The HR of trunk movement in all directions was significantly lower for the dual-task walking condition compared with the normal walking condition even when adjusted for sex and gait speed (VT direction: $p < .001$, mediolateral direction: $p = .002$, anteroposterior direction: $p < .001$). The repeated multivariate analysis of covariance revealed a significant interaction between walking condition (normal walking vs dual-task walking) and brain atrophy only for HR in VT direction (walking condition × brain atrophy: $F = 4.334$, $p = .040$). Linear regression analysis revealed that brain atrophy is independently related to DTC in HR in VT direction ($\beta = .231$, $p = .024$; Table 3).

DISCUSSION

This study revealed that decreased trunk stability during dual-task walking is significantly associated with brain atrophy in older adults. This association was independent of other variables in a regression model. In addition, dual-task walking resulted in a change of spatiotemporal gait parameters compared with normal walking, even when adjusted for sex and gait speed. The deterioration in HR during dual-task walking

Table 1. Subject Characteristics and Percentage of Brain Atrophy

Characteristics	$M \pm SD$
Age (y)	75.4 ± 7.1
Sex, women subjects (%)	55 (50)
Body mass index (kg/m²)	23.1 ± 3.3
Educational history (y)	10.7 ± 2.6
Mini-Mental State Examination (total score)	26.4 ± 2.5
Grip strength (kg)	23.5 ± 7.5
Timed up and go test (seconds)	9.2 ± 2.3
Geriatric Depression Scale (total score)	3.7 ± 3.0
Tokyo Metropolitan Institute of Gerontology Index of Competence (total score)	12.2 ± 1.1
Brain atrophy (%)	7.6 ± 4.2

Notes: Values are mean ± *SD* and numbers (proportion) for sex. Brain atrophy was calculated using a specific voxel-based regional analysis system for MRI data.

Table 2. Paired Comparison of Spatiotemporal Gait Parameters for Normal Walking and Dual-Task Walking

Variables	Normal Walking ($M \pm SD$)	Dual-Task Walking ($M \pm SD$)	Mean Difference (95% CI)	p Value	Adjusted p Value*
Gait speed (m/s)	1.10 ± 0.26	1.04 ± 0.31	−0.05 (−0.10, −0.01)	.022	.029†
Stride length (m)	1.13 ± 0.21	1.19 ± 0.41	0.06 (−0.01, 0.13)	.103	<.001
Cadence (steps/min)	115.8 ± 12.3	107.6 ± 17.8	−8.0 (−12.21, −3.80)	<.001	< .001
Harmonic ratio					
Vertical	2.84 ± 0.86	2.44 ± 0.81	−0.38 (−0.64, −0.12)	.005	<.001
Mediolateral	2.12 ± 0.65	1.95 ± 0.53	−0.19 (−0.36, −0.01)	.036	.002
Anteroposterior	3.13 ± 1.04	2.61 ± 0.83	−0.53 (−0.79, −0.25)	<.001	<.001

Notes: CI = confidence interval.
*Adjusted for sex and gait speed.
†Adjusted only for sex.

was observed in all three directions. However, the association between brain atrophy and DTC in HR was only present in VT direction.

Both the motor system and the cognitive system act reciprocally to ensure successful locomotion. To investigate this interaction, many experiments have been conducted using the dual-task method (10,11,12). DTC in gait parameters among older adults as a result of cognitive motor interference reflect an adaptation to a more challenging conditions and the fact that locomotion requires high-order cognitive processing such as executive function (5,6,7). Dual tasking generally affects spatiotemporal gait parameters including lower extremity (10,12) and trunk movement (7,13,14,15). Our results were consistent with reported dual-task changes for spatiotemporal gait measures, although the magnitude of changes varied among gait variables, type of tasks, or task difficulty (10,12). Dual tasking decreases HR as indicated by decreased smoothness of trunk movement and increased trunk instability in all directions. Furthermore, decreased HR may be caused by an adaptation because similar changes in HR have been reported for walking with additional challenges (eg, walking on an irregular surface) (4). The DTC in spatiotemporal gait parameters observed in our study suggest that dual tasking influences the control of both lower extremity and trunk movement.

Table 3. A Linear Regression Model for Brain Atrophy

Variables	Brain Atrophy	
	β (SE)	p Value
Age	.352 (.004)	<.001
Gender	.462 (.034)	<.001
Body mass index	.240 (.007)	.010
Educational history	−.028 (.010)	.779
Mini-Mental State Examination score	−.143 (.011)	.164
Grip strength	−.082 (.005)	.540
Tokyo Metropolitan Institute of Gerontology Index of Competence	.072 (.023)	.469
Geriatric Depression Scale	.249 (.008)	.016
Dual-task–related changes of HR in VT direction	.231 (.062)	.024
R^2		.362

Notes: HR = harmonic ratio; VT = vertical. A linear regression model was used to examine the association between dual-task–related changes of the gait parameter and brain atrophy, adjusted for gait speed.

MRI-based measures of brain atrophy are valid parameters because macrostructural brain abnormalities inevitably lead to neurodegeneration, neuropsychological deficits, tangle deposition, and microstructural loss (24). The macrostructural brain abnormalities associated with gait are hyperintensities of the white matter (19,20,21) and atrophy of the gray matter (21,22,23). The brain volume in the sensorimotor and frontoparietal regions including the prefrontal lobes is associated with step time and double support time during normal gait (22), and the differences between intracranial and brain volume were independently related to slower gait speed in women after adjusting for covariates (21). While one study reported that hippocampal volume is related to gait speed (23), results of another study suggest that gait performance among older adults is not necessarily related to atrophy in the memory domain including the hippocampus (22). The latter study also reported a weak association between gait measures and brain volume in the cerebellum or basal ganglia structures—regions that play key roles in the control of balance. A consensus has not been reached on the relationship between quantitative MRI-based measures of brain atrophy and gait variables. The results of our study indicate that DTC in trunk movement is significantly related to brain atrophy measured using the voxel-by-voxel method, which has been validated in other studies (26,27). Rosano and colleagues (22) suggested that gait variables under several conditions, including difficult conditions, should be investigated to clarify the task-specific network in the brain. Our initial results indicate that DTC in trunk movement might be associated with brain atrophy.

The control of trunk movement contributes to successful locomotion and is under continuous active neural control (1). The neural network may prioritize trunk stability to increase head stability during walking (35). Additionally, dual-task walking requires successful allocation of attention to both walking and the other task, which relies on executive function. In fact, dual-task decrements of gait measures are related to cognitive performance such as executive function (5,6,7), and both mobility and cognitive function are enhanced by dual-task intervention training as shown by results of randomized clinical trials (16,17). Because dual-task walking requires the

simultaneous control of walking and an additional task, the demand on neural resources for postural adjustments during walking may be greater for dual-task walking compared with normal walking. The analysis of HR during dual-task walking revealed an association between DTC in HR and brain atrophy; however, there was no relationship between DTC in other gait variables and brain atrophy. These results suggest that HR during dual-task walking may be a biomechanical marker for identifying a decline in brain volume.

Although dual-task walking decreased HR for trunk movement in all directions, an association between brain atrophy and DTC in HR was only observed in VT direction. These observations agree with results of other studies that HR data for lower trunk acceleration may represent different phenomena depended on the direction (2,36). Menz and colleagues (2) reported that directional specificity in HR in older adults was greater while walking under more challenging conditions. Results of their study suggested that the HR value of the lower trunk in VT direction had the ability to detect instability under challenging conditions. In another study, Brach and colleagues (3) suggested that HR in anteroposterior direction represents age-related changes that are not even affected by gait speed. The directional specificity of HR was not fully clarified, and further evidence for this specificity is required. Nevertheless, the results of our study indicate that brain atrophy is more likely to be related to trunk instability in the VT direction than in the anteroposterior and mediolateral directions induced by dual-task walking.

One limitation of this study is the relatively small sample size. Additionally, some physical dimensions, such as fitness level (37) and static postural instability (38), may have acted as confounding factors but were not included in this study. Furthermore, the effects of executive function and attention as confounding factors could influence dual-task gait performance (6,12) and should be considered to generalize these results. Moreover, the type and/or difficulty of dual-task walking in this study could have affected the results. Hence, dual-task walking using other types of cognitive tasks (eg, verbal fluency) should further be investigated. Finally, in this study, we measured atrophy of the entire brain. It is likely that regional atrophy assessed by MRI and other macrostructural measures (eg, white matter lesions) will provide a better insight into the mechanistic relationship between brain atrophy and gait function.

CONCLUSION

Brain atrophy correlated with a decline in the control of trunk movement during dual-task walking. This result indicates that dual-task walking induces trunk instability because additional cognitive resources are required compared with that during normal walking. Further studies are needed to clarify the effects of regional structural brain loss on the control of trunk movement and limb control during walking.

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A Significant Relationship between Plasma Vitamin C Concentration and Physical Performance among Japanese Elderly Women

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Background. Maintenance of physical performance could improve the quality of life in old age. Recent studies suggested a beneficial relationship between antioxidant vitamin (eg, vitamin C) intake and physical performance in elderly people. The purpose of this study was to examine the relationship between plasma vitamin C concentration and physical performance among Japanese community-dwelling elderly women.

Methods. This is a cross-sectional study involving elderly females residing in an urban area in Tokyo, Japan, in October 2006. We examined anthropometric measurements, physical performance, lifestyles, and plasma vitamin C concentration of participants.

Results. A total of 655 subjects who did not take supplements were analyzed. The mean age ($\pm$standard deviation) of participants was 75.7 ± 4.1 years in this study. The geometric mean (geometric standard deviation) of plasma vitamin C concentration was 8.9 (1.5) μg/mL. The plasma vitamin C concentration was positively correlated with handgrip strength, length of time standing on one leg with eyes open and walking speed, and inversely correlated with body mass index. After adjusting for the confounding factors, the quartile plasma vitamin C level was significantly correlated with the subject's handgrip strength (p for trend = .0004) and ability to stand on one leg with eyes open (p for trend = .049).

Conclusions. In community-dwelling elderly women, the concentration of plasma vitamin C related well to their muscle strength and physical performance.

Key Words: Plasma vitamin C—Physical performance—Elderly women—Japanese.

PHYSICAL performance and physical ability are the most important indicators of health status in elderly people and are also closely related to the quality of life. Declines in physical performance and physical activity, whether from specific disease, fall, fracture, poor nutrition, or aging itself, are associated with future disability, morbidity, and death (1,2).

In recent years, many studies have examined the roles of diet, protein, and vitamins in physical performance and physical activity(3–5). Several studies have associated low serum albumin concentration with deteriorated muscle strength and function (6,7). Some other studies have examined the relationship between serum vitamin D level and physical performance such as muscle mass, muscle strength, handgrip, walking speed, and functional capacity (8,9). Cesari et al. (3) examined the relationship between antioxidant vitamin intake (vitamin C, vitamin E, β-carotene, and retinol) and physical performance in elderly people and showed significant positive correlations between most antioxidants, especially vitamin C, and higher skeletal muscular strength in this group of people.

There are a number of mechanistic hypotheses about the potential beneficial effects of antioxidant vitamins(10–12). Vitamin C, vitamin E, β-carotene, and retinol are important antioxidants that are not synthesized by humans and, therefore, are mainly supplied via dietary intake. Vitamin C

(ascorbic acid) is a water-soluble antioxidant present in the cytosol and extracellular fluid and can directly react with free radicals such as superoxide ($O_2\cdot^-$) and hydroxyl radicals ($\cdot OH$) (13,14). Each one of these oxygen-derived intermediates is considered highly reactive because of their unstable electron configurations, which could attract electrons from other molecules, resulting in another free radical that is capable of reacting with yet another molecule. This chain reaction is thought to contribute to lipid peroxidation, DNA damage, and protein degradation during oxidative stress. Oxidative damage is thought to play an important role in the age-related decline of functional activity in human skeletal muscle (15). Concentration of plasma vitamin C, which has potent antioxidant activity, is known to increase after exercise (4).

An increase in the amount of blood vitamin C content has been used as an indicator of increased oxidative reaction (11). Previous studies have examined the effects of vitamin C supplementation on physical performance and exercise (4,11). Although findings from some of the previous studies do not support any beneficial effect of increased antioxidant intake on physical performance, other studies have shown improved recovery from exercise with antioxidant intake and have also shown a preventive role of antioxidant supplementation against oxidative damage. These studies were carried out on athletes after heavy exercise. So far, however, there has been no study examining the relationship between physical performance and blood levels of vitamin C, which may be a more direct marker of the antioxidative ability of the human body.

The present study, to the best of our knowledge, is the first report that examines the relationship between plasma vitamin C concentration and physical performance in Japanese community-dwelling elderly women.

SUBJECTS AND METHODS

Study Subjects

The present cross-sectional study was carried out as part of a project involving mass health examination of community-dwelling people ("Otasha-kenshin" in Japanese) aged 70 years and older living in Itabashi-ku, Tokyo. "Otasha-kenshin," which literally means "health examination for successful aging," is a comprehensive health examination program for community-dwelling older adults aimed at preventing geriatric syndromes including falls and fractures, incontinence, mild cognitive impairment, depression, and undernutrition (16).

The eligible subjects were all female residents, aged between 70 and 84 years, living in the Itabashi area, an urban part of Itabashi-ku, Tokyo, Japan in October 2006. The population of women belonging to this age range and residing in the Itabashi area was 5937, and they were recruited by invitation through postal mail. Of them, 1,112 women applied for admission and 957 women ultimately participated in this study. The participants who were taking vitamin C

supplements ($n = 238$) were excluded from the primary analyses for examination of the relationship between plasma vitamin C and physical performance because intake of supplements could strongly influence the plasma vitamin C level. Thus, data from 655 subjects were ultimately used for the primary analysis. However, data from the 238 supplement users were also used for subanalysis to determine whether any relationship exists between vitamin C supplementation and physical performance.

All participants were examined at the Tokyo Metropolitan Institute of Gerontology's hall. Physical performance, blood examinations, lifestyle assessments, and anthropometric measurements were performed as described below (9).

The present study was approved by the ethics review committee of the Tokyo Metropolitan Institute of Gerontology. All subjects gave written informed consent.

Anthropometric Measurements

Height and weight of each participant were measured, and body mass index was defined as weight/height^2 (kg/m^2). Body composition measurements (percent body fat) were obtained by segmental bioelectrical impedance using eight tactile electrodes according to the manufacturer's instructions (In Body 3.0; Biospace, Seoul, Korea). Measurements for the triceps surae muscles were taken between the knee and the ankle, at the level of maximum circumference of the medial and anterior calf of the left leg of each participant at sitting position.

Physical Performance

Physical performance was assessed by muscle strength (handgrip strength), balance capability, and usual and maximal walking speeds, without prior practice before the actual measurements. These assessments are routinely conducted for the elderly community as described previously (9). Handgrip strength (kg) was measured once for the dominant hand with the subjects in a standing position using a Smedley's Hand Dynamometer (Yagami, Tokyo, Japan). Grip devices were calibrated with known weights. Subjects held the dynamometer at thigh level and were encouraged to exert the strongest possible force. Balance capability was measured in terms of the length of time standing on one leg, that is, we asked the subjects to look straight ahead at a dot 1 m in front of them and to stand on the preferred leg with their eyes open and hands down alongside the trunk. The time until balance was lost (or maximum 60 seconds) was recorded. We used the better of two trials in the analysis. To determine the walking speed, participants were asked to walk on a flat surface at their "usual and maximum walking speeds." Two marks were used to delineate the start and end of a 5-m path. The start mark was preceded by a 3-m approach to ensure that the participants achieved their pace of usual or maximum before entering the test path. The participants were also instructed to continue walking past the end of the 5-m path for a further 3 m to ensure that their walking pace was maintained

throughout the test path. The time taken to complete the 5-m walk was measured by an investigator and used for analysis. Walking test at maximum speed was repeated twice, and the faster speed was recorded for the test.

All physical performance tests were performed between 9 AM and 4 PM during the day. We have no data on the reproducibility of the measurements. To reduce interexaminer variation, each test was conducted by the same staff member specifically trained for this study.

Blood Examinations

Blood samples (nonfasting) were collected from the subjects between 9 am and 4 pm during the day. There was no difference in mean plasma vitamin C concentration with regard to the time of collection (data not shown). Venous blood samples were drawn into Ethylene diamine tetraacetic acid tubes. Plasma was then obtained by centrifugation at 3,000 rpm for 15 min at 4°C and subsequently used for biochemical assays. Plasma was treated with Ethylene diamine tetraacetic acid to prevent the spontaneous vitamin C degradation. Next, 100 μl of the plasma was dispensed into storage tubes, to which 450 μl of 3% metaphosphoric acid solution was added, and the mixture was stored at −80°C until further use. Vitamin C concentration was determined by an High performance liquid chromatography-electrochemical detection–based method (17). The analysis was carried out centrally in our laboratory. Serum albumin concentration was measured by the Bromocresol Green method (Special Reference Laboratories Inc., Tokyo, Japan). The coefficient of variation for serum albumin found using this method was less than 1% (9).

Lifestyle Assessment

Information regarding the participants' general health (such as medical history, smoking habits, alcohol drinking habits, regular exercise habits, vegetable intake, fruit intake and use of vitamin C supplement) was collected by interview, and history of medical conditions including hypertension, stroke, heart attack, diabetes mellitus, and hyperlipidemia was self-reported.

Alcohol drinking habits of the subjects were classified as nondrinker, current drinker, or ex-drinker. Smoking habits of the subjects were classified using three categories: never smokers, current smokers, and ex-smokers. The frequency of vegetable and fruit intake was asked using four categories: almost every day, once every two days, once or twice per week, and almost never. Subsequently, for analysis, the categories were summarized as almost every day and others.

Statistical Analysis

Data were summarized as mean and standard deviation or percentage values. The data of plasma vitamin C concentration was logarithmically transformed to approximate a normal distribution and was summarized as the geometric mean and geometric standard deviation.

Table 1. Characteristics of Study Subjects ($N = 655$)

Characteristic	Mean (SD)
Age (y)	75.7 (4.1)
Height (cm)	149.1 (5.7)
Weight (kg)	51.0 (8.3)
Body mass index (kg/m²)	22.9 (3.4)
Triceps surae muscle (cm)	33.1 (2.8)
Plasma vitamin C (μg/ml)*	8.9 (1.5)
Serum albumin (mg/dL)	4.3 (0.2)
Body composition	
Percent body fat (%)	32.2 (7.0)
Physical performance tests	
Handgrip strength (kg)	18.7 (4.4)
One leg standing with eyes open (s)	35.2 (23.5)
Usual walking speed (m/s)	1.2 (0.3)
Maximal walking speed (m/s)	1.8 (0.4)
	%
Medical history	
Hypertension	50.7
Stroke	6.6
Heart attack	21.2
Diabetes mellitus	9.0
Hyperlipidemia	34.7
Alcohol drinking habit	
Current	25.3
Former	5.0
Never	69.6
Smoking habit	
Current	3.7
Former	5.7
Never	90.7
Regular exercise habit	
Yes	69.2
No	30.8
Vegetable intake	
Everyday	84.2
Others†	15.8
Fruit intake	
Everyday	81.8
Others†	18.2

Notes: Data of vitamin C supplement users were excluded.
* The geometric mean and geometric SD.
† Including participants taking vegetables/fruits not everyday or almost never.

The age-adjusted Pearson's correlation coefficient between the plasma vitamin C concentration and other factors were calculated. The least square means and SEs adjusted for potential confounders were calculated and compared between categories by analysis of covariance. To examine the relationship between plasma vitamin C concentration and physical performance, statistical adjustment was done by analysis of covariance for variables (except for other physical performance variables) that were correlated to plasma vitamin C concentration with $p < .20$. The same analyses were repeated for the 238 users of vitamin C supplement. All statistical analyses were performed using the SAS (version 9.0; SAS Institute Inc., NC).

RESULTS

Table 1 summarizes the basic characteristics of the subjects. As shown, the mean age (±standard deviation) of the

Table 2. Correlation between Plasma Vitamin C Concentration and Selected Factors ($N = 655$)

Factor	Correlation*	
	r	p
Age	−0.004	.91
Height	0.04	.27
Weight	−0.05	.19
Body mass index	−0.08	.054
Triceps surae muscle	0.001	.98
Serum albumin	−0.04	.33
Percent body fat	−0.12	.002
Handgrip strength	0.16	<.001
One leg standing with eyes open	0.15	<.001
Usual walking speed	0.14	<.001
Maximal walking speed	0.09	.036

Notes: Number of subjects is slightly different for the selected factors because of missing values.

*Age-adjusted Pearson's correlation coefficient between logarithm of vitamin C concentration and each factor.

subjects was 75.7 ± 4.1 years. The geometric mean (geometric standard deviation) of plasma vitamin C concentration was 8.9 (1.5) μg/mL. The prevalence of women eating vegetables everyday was 84.2% and those eating fruits everyday was 81.8%.

The age-adjusted geometric mean of plasma vitamin C concentration was significantly lower in subjects who had a medical history of hypertension (8.53 vs 9.22, $p = .0015$) and diabetes mellitus (7.59 vs 9.00, $p = .002$) as compared with those who did not. A history of stroke, heart attack, or hyperlipidemia was not associated with plasma vitamin C concentration. Subjects who took fruits every day had a significantly higher concentration of vitamin C than those who did not (9.14 vs 7.78, $p < .0001$). Vegetable intake, alcohol drinking habit and smoking habit were not related to plasma vitamin C concentration (not shown in table).

Table 2 shows the age-adjusted correlations between the plasma vitamin C concentration and selected factors. As

shown, the plasma vitamin C concentration was positively but modestly correlated with handgrip strength, length of time standing on one leg with eyes open, as well as usual walking speed and maximal walking speed, and modestly inversely correlated with body mass index and percent body fat of the subjects.

Table 3 shows the relationship between plasma vitamin C concentration and each physical performance after adjusting for confounding factors. Results obtained after the adjustment for potential confounders confirmed that the plasma vitamin C concentration was correlated with the handgrip strength independently from the other factors (eg, p for trend = .0004 after adjusting for age, body mass index, percent body fat, hypertension, diabetes mellitus, and fruit intake; Table 3). There was also a significant relationship between the plasma vitamin C level and the subject's length of time standing on one leg with eyes open after adjustments for age, body mass index, percent body fat, hypertension, diabetes mellitus, and fruit intake (Table 3; p for trend = .049). We did not observe any significant association between the plasma vitamin C level and the usual or the maximal walking speed of the subjects.

A subanalysis using data from the 238 vitamin C supplement users showed almost null relationship between handgrip strength and plasma vitamin C concentration (data not shown).

DISCUSSION

A previous study has shown an association between higher daily dietary intake of vitamin C and skeletal muscle strength in elderly people (3). Results described in the present study indicated that plasma vitamin C concentration was positively related with muscle and physical performance in community-dwelling elderly women. To the best of our knowledge, this is the first study showing a significant

Table 3. Relationship between Plasma Vitamin C Concentration and Physical Performance Adjusted for Potential Confounder

Physical performance	Quartile of plasma vitamin C level				
	Q1	Q2	Q3	Q4	
	Mean ± *SE*	Mean ± *SE*	Mean ± *SE*	Mean ± *SE*	p for trend
Handgrip strength (kg), N	154	159	154	152	
Age adjusted	17.70 ± 0.34	18.75 ± 0.33	18.75 ± 0.34	19.60 ± 0.34	.0001
Multivariate adjusted*	17.83 ± 0.34	18.83 ± 0.32	18.89 ± 0.33	19.60 ± 0.33	.0004
One leg standing with eyes open† (s), N	162	163	164	161	
Age adjusted	31.44 ± 1.71	33.98 ± 1.70	37.70 ± 1.70	37.83 ± 1.71	.003
Multivariate adjusted*	33.39 ± 1.74	34.08 ± 1.67	37.63 ± 1.67	37.50 ± 1.70	.049
Usual walking speed (m/s), N	146	154	145	147	
Age adjusted	1.13 ± 0.02	1.19 ± 0.02	1.23 ± 0.02	1.21 ± 0.02	.008
Multivariate adjusted*	1.18 ± 0.02	1.19 ± 0.02	1.22 ± 0.02	1.21 ± 0.02	.23
Maximal walking speed (m/s), N	146	154	154	147	
Age adjusted	1.70 ± 0.03	1.76 ± 0.03	1.82 ± 0.03	1.76 ± 0.03	.15
Multivariate adjusted*	1.76 ± 0.03	1.77 ± 0.03	1.80 ± 0.03	1.75 ± 0.03	.94

Notes: Values are least squares mean and *SE* adjusted for the factors by analysis of covariance. Q1–Q4: first to fourth quartile groups of plasma vitamin C concentration, respectively.

*Adjusted for age, body mass index, percent body fat, hypertension, diabetes mellitus and **fruit intake**.

†Length of time standing on one leg with eyes open.

correlation between plasma vitamin C concentration and handgrip strength and ability to stand on one leg with eyes open. We, however, were unable to find any relationship between skeletal muscle mass and plasma vitamin C concentration. Handgrip strength has been found to correlate well with the strength of other muscle groups and is thus a good indicator of overall strength (18). Consistent with this idea, handgrip strength was found to be a strong and consistent predictor of all-cause mortality and morbidity of Activities of Daily Living in middle-aged people (19). The handgrip test is considered an easy and inexpensive screening tool to identify elderly people at risk of disability. Handgrip strength, an indicator of overall muscle strength, is thought to predict mortality through mechanisms other than underlying disease that could cause muscle impairment (18,19). The one leg standing test is one of the balance tests (20). The test is a clinical tool to assess postural steadiness in a static position by quantitative measurement. Many studies have shown that the decreased one leg standing time is associated with declines in Activities of Daily Living and increases in other morbidities including osteoporosis and fall (20).

Our findings suggest that vitamin C may play an important role in maintaining physical performance and thereby may help to improve healthy life expectancy in the elderly. However, the usual and maximal walking speeds did not relate to plasma vitamin C concentration. Walking speed test may be an efficient tool in screening older persons with higher risk of mortality and may easily identify high-risk groups in the community (21). Walking is a rhythmic, dynamic, and aerobic activity of the large skeletal muscles that confers multifarious benefits with minimal adverse effects. Muscles of the legs, limbs, and lower trunk are strengthened, and the flexibility of their joints are preserved (22). One of the reasons why walking speed was not related to vitamin C concentration may be because walking requires coordinated movements of arms, legs, and many parts of the body rather than a simple muscle and balance function. Previous reports showed that walking balance function did not correlate with standing balance function (23). Although we did not find any clear association between walking and plasma vitamin C concentration in this study, vitamin C may still have effects on relatively simple strength and balance functions.

One of the possible explanations for the observed relationship between vitamin C and physical performance, especially handgrip strength and the ability to stand on one leg with eyes open, may be the potential protective effects of the antioxidant vitamins against muscle damage (4,11). Vitamin C is a six-carbon lactone that is synthesized from glucose in the liver of most mammalian species, but not in humans (12). Vitamin C is an antioxidant because, by donating its electrons, it prevents other compounds from being oxidized (12). Thus, vitamin C readily scavenges reactive oxygen and nitrogen species, thereby effectively protects other substrates from oxidative damage (10,24). Although

habitual exercise reduces systemic inflammation and oxidative stress as the production of endogenous antioxidants are enhanced, acute exercise increases the generation of oxygen-free radicals and lipid peroxidation (4,25). Strenuous physical performance can increase oxygen consumption by 10- to 15-folds over the resting state to meet the energy demands and results in muscle injury (26). Prolonged submaximal exercise was shown to increase the amount of both whole-body and skeletal muscle lipid peroxidation by-products; in the case of the former, the increase was indicated by greater exhalation of pentane but not of ethane (4,27,28). Supplementation with vitamin C was shown to decrease the exercise-induced increase in the rate of lipid peroxidation (27,28). Several studies suggested that oxidative damage may play a crucial role in the decline of functional activity in human skeletal muscle with normal aging (15). Consistent with this idea, several studies showed significantly lower plasma vitamin C level in the elderly population than in the younger adult population (29–31). Because the plasma vitamin C levels in these apparently healthy elderly persons rose markedly after an oral dose of vitamin C, their initially low plasma levels can be attributed to the low intake rather than to an age-related physiological defect.

In fact, the relationship between handgrip strength and plasma vitamin C concentration was significantly different between supplement users and nonusers, that is, an almost null relationship in the former and a positive relationship in the latter (data not shown). This finding suggested that vitamin C supplementation did not have any beneficial effect on the physical performance and muscle strength despite the increased plasma level of vitamin C. A number of studies reported that vitamin C supplement users had significantly higher blood vitamin C concentration than non-users (29, 32, 33). Several studies have examined the effects of exercise on changes in the serum vitamin C concentration (34–36). Some other experimental studies have shown that vitamin C supplementation can reduce symptoms or indicators of exercise-induced oxidative stress (37–40). However, the results regarding vitamin C supplementation are equivocal, and most well-controlled intervention studies report no beneficial effect of vitamin C supplementation on either endurance or strength performance (41,42). Likewise, vitamin C restriction studies showed that a marginal vitamin C deficiency did not affect the physical performance (43). Although evidence from a number of studies show that vitamin C is a powerful antioxidant in biological systems in vitro, its antioxidant role in humans has not been supported by currently available clinical studies.

Vitamin C is especially plentiful in fresh fruits and vegetables. Plasma vitamin C concentration may be merely a marker for intake of other nutrients that are abundant in fruits and vegetables. However, the statistical adjustment for fruit intake did not attenuate the relationship between plasma vitamin C and physical performance (Table 3), suggesting that vitamin C did have some beneficial effects

independently of other nutrients. A number of biochemical, clinical, and observational epidemiologic studies have indicated that diets rich in fruits, vegetables, and vitamin C may be of benefit for the prevention of chronic diseases such as cardiovascular disease and cancer (44,45). Several cohort studies have examined associations between plasma vitamin C concentration and mortality from stroke or coronary heart disease (30,46,47). The effects of vitamin C supplementation are, however, still unclear. A pooled study suggested reduced incidences of coronary heart disease events with higher intake of vitamin C supplement (48), while another study showed that a high intake of vitamin C supplement is associated with an increased risk of mortality due to cardiovascular diseases in postmenopausal women with diabetes (49). A randomized placebo controlled 5-year trial, however, did not show any significant reduction in the mortality from, or incidence of, any type of vascular disease or cancer (50). These studies, in fact, have failed to demonstrate any benefit from such supplementation.

There are a number of potential weaknesses in our study that should be mentioned here. The subjects used in this study were not selected randomly from the study population, and they may be relatively healthy elderly women who were able to come to the health examination hall from their homes. A previous study assessed the correlation of antioxidants with physical performance and muscular strength (3) and demonstrated that a higher daily intake of vitamin C and carotene associated with skeletal muscle strength. However, we have no data regarding the presence of other dietary antioxidants in blood such as vitamin E, retinol, and carotene. In our questionnaire, participants were asked to respond "Yes" or "No" to whether they took supplements, and not about the frequency and quantity of intake of the supplements. Thus, we were unable to examine the reason why plasma vitamin C was not related to the handgrip strength in the supplement users by considering the dose of vitamin C they took.

This study was a cross-sectional study and, therefore, does not provide cause/effect relationships, although we demonstrated a significant correlation between physical performance and concentration of plasma vitamin C. Therefore, longitudinal follow-up studies and controlled clinical trials are necessary to confirm the role of plasma vitamin C and physical performance of the elderly women. These limitations should be considered in future studies.

In conclusion, we found a strong correlation of a higher plasma vitamin C concentration with handgrip strength and one leg standing time in community-dwelling elderly women. Although the elderly are prone to vitamin C deficiency, and they appear to have a higher dietary requirement for vitamin C, the beneficial effects of vitamin C supplementation to maintain physical performance in elderly people are equivocal and thus, need further in-depth studies.

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