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ORIGINAL ARTICLE

Bone mineral density in post-menopausal female subjects is associated with serum antioxidant carotenoids

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Abstract
Summary High intake of fruit and vegetables may reduce the risk of osteoporosis. Carotenoids exist in abundance in these foods. This study showed the association of bone mineral density with serum carotenoids. The findings suggest that β-cryptoxanthin and β-carotene might provide benefits to bone health in post-menopausal female subjects.
Introduction Antioxidant carotenoids are abundant in fruit and vegetables. Recent epidemiological studies show that high intakes of fruit and vegetables may reduce the risk of osteoporosis, but little is known about the association of bone mineral density (BMD) with serum carotenoids.
Methods A total of 699 subjects (222 males and 477 females) who had received health examinations in the town of Mikkabi, Shizuoka Prefecture, Japan, participated in the study. Radial BMD was measured using dual-energy X-ray absorptiometry (DXA). The associations of serum carotenoid concentrations with the radial BMD were evaluated cross-sectionally.
Results In male and pre-menopausal female subjects, the six serum carotenoids were not associated with the radial BMD. On the other hand, in post-menopausal female subjects, serum β-cryptoxanthin and β-carotene were weakly but positively correlated with the radial BMD. After adjustment for confounders, the odds ratio (OR) for the lowest quartile of BMD in the high groups (Q2–Q4) of serum β-cryptoxanthin against the lowest quartile (Q1) was 0.45 (95% confidence interval: 0.22–0.95) in post-menopausal female subjects. However, this association was not significant after further adjusting for intakes of minerals and vitamins.
Conclusions Antioxidant carotenoids, especially β-cryptoxanthin, significantly but partly associate with the radial BMD in post-menopausal female subjects.

Keywords Bone mineral density · Carotenoids · Fruit and vegetables · Post-menopausal female

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Introduction

Osteoporosis and related fractures are a major public health problem [1]. Osteoporosis is a chronic disease characterized by low bone density and microarchitectural disruption, leading to bone fragility and an increased susceptibility to fractures [2]. Nutrition is an important modifiable factor in the development and maintenance of bone health, and numerous studies on nutrition and bone health have been conducted [3, 4]. With regard to nutritional approaches to bone metabolism, calcium and vitamin D have been identified as important nutritional factors to maintain normal bone metabolism. Other nutrients, such as potassium, magnesium, zinc, copper, iron, vitamin C, and vitamin K, may also have beneficial effects. Furthermore, recent epidemiological studies have shown an association between fruit and vegetable intake with the bone mineral density (BMD) in both young and elderly subjects [5–10]. Fruit and vegetables are rich sources of nutrients for bone metabolism, such as potassium, magnesium, vitamin C and K, folate, and other constituents

that may influence bone health, such as flavonoids. Therefore, the intake of these types of food might affect bone health.

Antioxidant carotenoids exist in abundance in fruit and vegetables, and its serum levels elevate with increasing fruit and vegetable intake [11–14]. Recent studies show that carotenoids have been known to contribute to the body's defense against reactive oxygen species [15, 16]. Oxidative stress is thought to play an important role in the development of several chronic diseases [17]. Therefore, antioxidant carotenoids may have a beneficial effect against oxidative stress-related chronic diseases. On the other hand, recent epidemiological studies have shown the relationship between oxidative stress and BMD or osteoporosis [18–21]. Although smoking is recognized as a major risk factor for osteoporotic fracture [22], Melhus et al. found that an insufficient dietary intake of antioxidant vitamins increased the risk of hip fracture in current smokers [18]. Furthermore, Maggio et al. reported a marked decrease in plasma antioxidants in aged osteoporotic women [20]. In this report, Maggio et al. demonstrated that plasma vitamins A, C, and E, and antioxidant enzyme activities in osteoporotic women were significantly lower than those in control subjects. Furthermore, very recently, they found that plasma levels of carotenoids and retinol in involutional osteoporotic women were significantly low compared with those in control subjects [23]. These results suggest that antioxidant carotenoids are beneficial micronutrients for the maintenance of normal bone metabolism. However, a nutritional epidemiological study about the association of BMD with serum antioxidant carotenoids has not been thoroughly studied.

The objective of this study was to investigate whether the BMD is higher in the presence of a high serum carotenoid concentration. The associations of six serum carotenoid concentrations, i.e., lutein, lycopene, α-carotene, β-carotene, β-cryptoxanthin, and zeaxanthin, with the radial BMD were evaluated cross-sectionally.

Subjects and methods

Subjects

In this survey, study subjects were recruited from participants in an annual health check-up program conducted by the local government of the town of Mikkabi, Shizuoka Prefecture, Japan in April 2005. Mikkabi is located in western Shizuoka, and about 40% of its residents work in agriculture. Fruit trees are the key industry in Mikkabi, which is an important producer of mandarin orange in Japan. A total of 1,891 males and females were subjects for the annual health check-up program. As results, 1,369 males and females (72.4% of total subjects), ranging in age from 30 to 70 years, had received the health check-up program. Participants were recruited for this study, and informed consent was obtained from 699 subjects (222 males and 477 females). The response rate was 51.1%. This study was approved by the ethics committee of the National Institute of Fruit Tree Science and the Hamamatsu University School of Medicine.

Serum carotenoid analysis

Blood samples were obtained in the morning after overnight fasting. Serum was separated from blood cells by centrifugation and stored at −80°C until analysis of the serum carotenoid concentrations. The concentrations of six serum carotenoids, lutein, lycopene, α-carotene, β-carotene, β-cryptoxanthin, and zeaxanthin, were analyzed by reverse-phase high-performance liquid chromatography (HPLC) using β-apo-8′-carotenal as an internal standard at the Laboratory of Public Health and Environmental Chemistry, Kyoto Biseibutsu Kenkyusho (Kyoto, Japan), as described previously [24].

Bone mineral density measurement

The radial BMD was measured using dual-energy X-ray absorptiometry (DXA) of each participant's non-dominant forearm with an osteometer (model DCS-600EX-III, ALOKA Co., LTD., Tokyo, Japan). This osteometer automatically measured the forearm length from the styloid process on the ulna, and DXA scan was automatically placed on the radial centered 1/3 of the forearm length. Calibration of the machine was performed daily, and quality assurance was performed by measuring the manufacturer's phantom. The CV of the radial BMD measurement was within 0.5%. In this study, the measurement of the radial BMD of each participant was performed by well-trained clinical technologist of the Seirei Preventive Health Care Center (Shizuoka, Japan).

Self-administered questionnaire

A self-administered questionnaire was used to collect information about a subject's history of osteoporosis, hormone use, and lifestyle, including tobacco use (current smoker, ex-smoker, or non-smoker), exercise (1+ times per week), regular alcohol intake (1+ times per 15 week), dietary supplement use (non-user, occasional-user, current-user), and dietary habits. Diet was assessed with a modified validated simple food-frequency questionnaire (FFQ) developed especially for the Japanese [25, 26]. In this FFQ, Wakai et al. selected a total of 97 foods and dishes through a two-step procedure: first by ranking food items according to the contribution to the population intake of energy and nutrients, and second by stepwise multiple regression analysis of individual food items as the independent variables and of total nutrient intake

as the dependent variable. For simplicity, questions on portion sizes were not included except for a few selected food items; resulting in short time to complete the questionnaire. They validated this FFQ for food groups by referring to four 4-day dietary records (DRs), and correlation coefficients between FFQ and DRs were larger than 0.4 for most food groups. Information about alcohol consumption and the daily intake of 18 nutrients was estimated from the monthly food intake frequencies with either standard portion size (for most types of food) or subject-specified usual portion size (for rice, bread, and alcoholic and non-alcoholic beverages) using FFQ analysis software package for windows (Food Frequency Questionnaire System, System Supply Co., LTD., Kanagawa, Japan). This FFQ analysis software computes individual's foods and nutrients intake from FFQ data based on "Standard tables of food composition in Japan" [27, 28]. The intake of total energy, calcium, potassium, magnesium, and vitamins C, D, and E of each subjects was used in this report.

Statistical analysis

For this study, the following subjects were excluded from the data analysis: (1) those who reported a history of osteoporosis in the self-administered questionnaire (n=8); (2) those for whom the self-administered questionnaire data-were as incomplete (n=14); and (3) those for whom blood samples for serum-carotenoid analysis were not collected (n=1). As a result, a total of 222 male and 454 female subjects were included in further data analysis.

All subjects were categorized into three groups stratified by sex and menopausal status. Serum carotenoid concentrations and intake of vitamins C, D, and E were skewed toward the higher concentrations. These values were $\log_e$ (natural)-transformed to improve the normality of their distribution. Analysis of covariance adjusted for age followed by Bonferroni multiple comparisons was used to test between-group differences. All variables were presented as an original scale. The data are expressed as means (standard deviation), geometric mean (95% confidence interval), range, or percent.

The standard regression coefficients of serum carotenoid concentration with the radial BMD were calculated after adjusting for confounding factors by multiple linear regression analysis. In male and pre-menopausal female models, age, weight, height, current tobacco use, regular alcohol intake, exercise habits, use of dietary supplements, and intake of total energy, calcium, magnesium, potassium, and vitamins C, D, and E were adjusted. In the post-menopausal female model, we adjusted further for years since menopause.

The multivariate adjusted mean of the radial BMD by the quartiles of the serum carotenoid concentration was calculated after adjusting for confounding factors. Differences in the multivariate adjusted mean of the radial BMD among each quartile of serum carotenoid concentration were tested by Bonferroni multiple comparison. The serum carotenoid concentrations were assigned to four categories, using the mean of the serum carotenoid concentrations in each quartile, and the associations among the radial BMD across four categories were assessed with a test for linear trends using linear regression.

Low radial BMD was defined as the lowest quartile of the value among study participants; i.e., equal to or less than 0.725 g/cm^2 in male subjects, equal to or less than 0.646 g/cm^2 in pre-menopausal female subjects, and equal to or less than 0.501 g/cm^2 in post-menopausal female subjects. To assess the relationship between the serum carotenoid concentrations with low radial BMD, logistic regression analyses were performed after adjusting for age, weight and height. Multivariable adjustment was further conducted to control for potential confounders. We did not adjust each carotenoid concentration in the multivariate models because Pearson's correlation analyses of serum carotenoid concentrations revealed significant positive correlations among all combinations of the six carotenoids.

The detection limit for the serum lycopene concentration for the method used in the study was 0.04 μg/mL (0.075 μmol/L), and the values below the limit of detection of the assay were marked as 0.03 μg/mL (0.056 μmol/L) in the analysis. All statistical analyses were performed using statistical software package for Windows (SPSS ver. 12.0J, SPSS Inc., Chicago, IL, USA) on personal computers.

Results

Clinical, biochemical and nutrient intake profiles of study subjects

Table 1 shows the characteristics of the study subjects stratified by sex and menopausal status. The radial BMD in pre- and post-menopausal female subjects was significantly lower than that in male subjects. In post-menopausal female subjects, the radial BMD was particularly low. Although the total energy intake including ethanol was significantly lower in pre- and post-female subjects than in male subjects, the total energy intake excluding ethanol did not differ among the three groups. The intake of calcium, potassium, and vitamins C, D, and E in post-menopausal female subjects were significantly higher than that in male subjects. The serum carotenoid concentrations in post-menopausal female subjects were significantly higher than those in male subjects. In comparison with those of pre-menopausal female subjects, the serum carotenoid concentrations of post-menopausal female subjects were not statistically but slightly higher, except for lycopene.

Table 1 Characteristics of the study subject stratified by sex or menopausal status[a]

	Male		Female					
			Pre-menopausal			Post-menopausal		
n	222		161			293		
Age (y)	56.1	(9.2)	44.1	(5.3)		60.2	(6.2)	
Body weight (kg)	165.8	(6.2)	156.0	(5.0)	g	152.0	(5.5)	g
Body height (cm)	65.5	(9.8)	54.6	(8.9)	g	51.9	(7.6)	g
Body mass index (kg/m^2)	23.8	(2.9)	22.4	(3.7)	g	22.5	(3.0)	g
Total energy intake (MJ/day)								
Including ethanol	8.90	(2.04)	8.03	(1.83)	g	8.20	(2.01)	g
Excluding ethanol	8.31	(1.95)	7.96	(1.84)		8.15	(2.00)	
Calcium intake (mg/day)	517	(230)	566	(190)	f	651	(256)	g
Potassium intake (mg/day)	2448	(817)	2474	(799)		2910	(967)	g
Magnesium intake (mg/day)	265	(72)	242	(71)		281	(81)	
Vitamin C intake (mg/day)[b]	120	(112–129)	110	(102–119)		170	(161–179)	g
Vitamin D intake (μg/day)[b]	201	(184–219)	195	(177–215)		256	(238–276)	f
Vitamin E intake (mg/day)[b]	7.3	(7.0–7.6)	7.6	(7.2–8.0)		8.1	(7.8–8.4)	f
Bone mineral density (g/cm^2)	0.771	(0.067)	0.677	(0.055)	g	0.561	(0.084)	d, g
Range		0.593–0.934		0.412–0.817			0.366–0.820	
Serum carotenoid concentrations (μmol/L)[b]								
Lutein	0.44	(0.42–0.46)	0.46	(0.44–0.48)	f	0.54	(0.51–0.56)	g
Lycopene	0.30	(0.28–0.32)	0.46	(0.43–0.49)	f	0.37	(0.35–0.39)	g
α-Carotene	0.12	(0.12–0.14)	0.19	(0.17–0.20)	g	0.21	(0.20–0.23)	g
β-Carotene	0.54	(0.50–0.59)	0.84	(0.77–0.91)	g	1.12	(1.06–1.18)	g
β-Cryptoxanthin	1.11	(0.98–1.25)	0.89	(0.79–1.01)		1.75	(1.61–1.90)	g
Zeaxanthin	0.19	(0.18–0.19)	0.19	(0.18–0.20)		0.20	(0.20–0.21)	e
Current tobacco use (%)	28.8		3.7			1.7		
Exercise habits (%)[c]	21.2		14.9			21.5		
Regular alcohol intake (%)[c]	59.9		19.9			11.0		
Current supplement use (%)	2.7		9.9			9.6		

[a] Data are mean (standard deviation), geometric mean (95% confidence interval), range, or percent
[b] These variables were represented as original scale after analysis by log (natural) transformed values
[c] > = 1 times/wk
[d] Significantly different vs. pre-menopausal female by analysis of covariance adjusted for age followed by Bonferroni multiple comparison test: [d] $P<0.001$
[e, f, g] Significantly different vs. male by analysis of covariance adjusted for age followed by Bonferroni multiple comparison test: [e] $P<0.05$, [f] $P<0.01$, [g] $P<0.001$

Multiple-linear regression analysis of the association of serum carotenoid with the radial BMD

In multiple linear regression analysis, after adjustment for age, weight, height, current tobacco use, regular alcohol intake, exercise habits, use of dietary supplements, and total energy intake, the radial BMD in male and pre-menopausal female subjects were not correlated with serum carotenoid concentrations (Table 2). On the other hand, in post-menopausal female subjects, the radial BMD was weakly but significantly correlated with serum β-carotene and β-cryptoxanthin concentrations after adjusting for age, years since menopause, weight, height, current tobacco use, regular alcohol intake, exercise habits, use of dietary supplements, and total energy intake (Table 2). After further adjusting for intake of calcium, magnesium, potassium and vitamins C, D, and E, a significant correlation was observed in β-cryptoxanthin. No other statistically significant correlations were observed.

Multivariate adjusted means of the radial BMD associated with the quartiles of each serum carotenoid concentrations

In post-menopausal female subjects, although no significant differences among each quartile were observed in all six serum carotenoids, the multivariate adjusted means of the radial BMD showed significant increasing trends under linearity with the quartiles of serum β-cryptoxanthin and β-carotene (Fig. 1). The results show the multivariate adjusted means of the radial BMD by quartiles of serum β-carotene

Table 2 Multiple linear regression analysis for the association between bone mineral density with serum carotenoid concentrations stratified by sex or menopausal status[a]

Serum carotenoids	Model 1		Model 2		Model 3	
	β	p-value	β	p-value	β	p-value
Male						
Lutein	−0.029	0.659	−0.024	0.712	0.039	0.563
Lycopene	−0.094	0.171	−0.115	0.102	−0.033	0.657
α-Carotene	−0.066	0.308	−0.102	0.143	−0.051	0.488
β-Carotene	−0.020	0.761	−0.052	0.481	−0.034	0.659
β-Cryptoxanthin	0.070	0.292	0.052	0.465	0.049	0.514
Zeaxanthin	−0.026	0.689	−0.023	0.721	0.020	0.762
Pre-menopausal female						
Lutein	0.016	0.832	0.001	0.992	−0.004	0.961
Lycopene	−0.146	0.049	−0.127	0.091	−0.114	0.133
α-Carotene	0.039	0.603	0.038	0.619	0.045	0.558
β-Carotene	−0.013	0.862	−0.001	0.987	0.011	0.896
β-Cryptoxanthin	−0.080	0.322	−0.068	0.405	−0.043	0.645
Zeaxanthin	0.001	0.986	−0.012	0.872	−0.007	0.930
Post-menopausal female						
Lutein	−0.067	0.184	−0.064	0.209	−0.081	0.118
Lycopene	0.018	0.725	0.007	0.891	0.003	0.952
α-Carotene	0.032	0.523	0.028	0.582	0.022	0.677
β-Carotene	0.101	0.046	0.102	0.047	0.103	0.060
β-Cryptoxanthin	0.097	0.060	0.105	0.047	0.116	0.047
Zeaxanthin	−0.044	0.374	−0.051	0.307	−0.071	0.166

[a] Standard regression coefficients of the radial BMD with serum carotenoid concentrations were calculated by multiple linear regression analysis after adjusting for confounding factors. Model 1: Age, weight, and height were adjusted.
Model 2: Age, weight, height, current tobacco use, regular alcohol intake, exercise habits, supplement use, and total energy intake were adjusted in male and pre-menopausal female models. Years since menopause was further adjusted in post-menopausal female model. Model 3: Age, weight, height, current tobacco use, regular alcohol intake, exercise habits, supplement use, and intake of total energy, calcium, magnesium, potassium, and vitamins D, C, and E were adjusted in male and pre-menopausal female models. Years since menopause was further adjusted in postmenopausal female model.

and β-cryptoxanthin concentrations in post-menopausal female subjects after adjusting for age, weight, height, years since menopause, current tobacco use, regular alcohol intake, exercise habits, supplement use, and intake of total energy. Similar associations were also observed after further adjusting for intake of calcium, magnesium, potassium, and vitamins D, C, and E (*P* for linear tend: 0.022 for β-carotene, 0.018 for β-cryptoxanthin). On the other hand, in

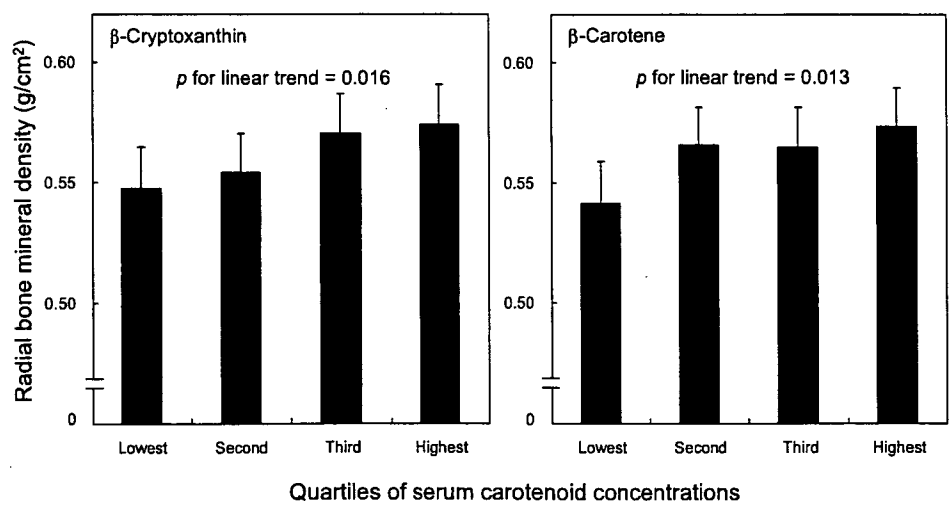

Fig. 1 Multivariate-adjusted means of bone mineral density by quartiles of serum β-carotene and β-cryptoxanthin concentrations in post-menopausal female subjects. Multivariate-adjusted means of bone mineral density were calculated after adjusting for age, weight, height, years since menopause, current tobacco use, regular alcohol intake, exercise habits, supplement use, and total energy intake. *P*-values over the quartiles of serum carotenoids were assessed with a test for linear trends using linear regression

male and pre-menopausal female subjects, no significant associations were observed in all six serum carotenoids (data not shown).

Odds ratio of low radial BMD in the high group of serum carotenoid concentrations

The odds ratios of low radial BMD associated with the quartiles of six serum carotenoid concentrations after adjusting for confounding factors are shown in Table 3. In the data analyses, the second (Q2), third (Q3), and highest (Q4) quartiles of serum carotenoid concentrations were combined as a high group (Q2–Q4). The odds ratios of low radial BMD in the high groups (Q2–Q4) against the lowest quartile (Q1) used for the reference group were calculated. After adjusting for age, weight, and height, significantly lower odds ratio for low radial BMD in post-menopausal female subjects was observed in the group with high serum β-cryptoxanthin. Multivariate adjustment was further conducted to control for potential confounders. After adjusting for age, years since menopause, weight, height, current tobacco use, regular alcohol intake, exercise habits, use of dietary supplements, and total energy intake in post-menopausal female subjects, significantly lower odds ratio for low radial BMD was observed in the group with high serum β-cryptoxanthin compared to the respective lowest quartile used for reference. However, after further adjusting for intake of calcium, magnesium, potassium, and vitamins C, D, and E, this significantly lower odds ratio was not observed in the group with high serum β-cryptoxanthin. In male and pre-menopausal female subjects, no statistically significant associations were observed (data not shown).

In this study population, although four post-menopausal female subjects were currently using female hormones, such as estrogen, the associations of the radial BMD with serum carotenoid concentrations did not change after excluding these four subjects.

Discussion

The objective of this study was to investigate whether the BMD is higher in the presence of a high serum carotenoid concentration. The results indicated that the radial BMD in post-menopausal female subjects was weakly but positively associated with serum β-cryptoxanthin and β-carotene. This investigation is the first-reported cross-sectional study to examine the association of serum carotenoid concentration with BMD. Numerous minerals and vitamins are contained in fruit and vegetables, and several epidemiologic reports have shown an association between dietary vitamin C intake and BMD in post-menopausal women [29–31]. Furthermore, it has been reported that lower dietary intake of vitamin C and E may substantially increase the risk of hip fracture in smokers [18]. These previous reports suggest that antioxidant micronutrients have beneficial effects on bone formation or bone loss. However, the association of BMD with serum antioxidant carot-

Table 3 The dds ratios (and 95% confidence intervals) of high group (upper three quartiles) compared with lowest quartile of serum carotenoid concentrations on low bone mineral density in post-menopausal female subjects[a]

Serum carotenoids		*n*	Mean and range of serum carotenoid (mmol/L)		Model 1		Model 2		Model 3	
					OR	95% CI	OR	95% CI	OR	95% CI
Lutein	Lowest (Q1)	71	0.34	(0.21–0.42)	1.00		1.00		1.00	
	High (Q2–Q4)	222	0.62	(0.44–2.11)	0.64	(0.32–1.28)	0.59	(0.29–1.22)	0.69	(0.32–1.49)
Lycopene	Lowest (Q1)	76	0.20	(0.07–0.30)	1.00		1.00		1.00	
	High (Q2–Q4)	217	0.46	(0.32–1.10)	1.39	(0.72–2.70)	1.53	(0.77–3.03)	1.60	(0.78–3.29)
α-Carotene	Lowest (Q1)	81	0.12	(0.06–0.15)	1.00		1.00		1.00	
	High (Q2–Q4)	212	0.27	(0.17–2.74)	1.15	(0.59–2.25)	1.19	(0.60–2.36)	1.39	(0.65–2.95)
β-Carotene	Lowest (Q1)	70	0.60	(0.32–0.82)	1.00		1.00		1.00	
	High (Q2–Q4)	223	1.36	(0.84–3.37)	0.59	(0.30–1.17)	0.51	(0.25–1.04)	0.61	(0.28–1.31)
β-Cryptoxanthin	Lowest (Q1)	73	0.67	(0.22–1.07)	1.00		1.00		1.00	
	High (Q2–Q4)	220	2.41	(1.10–10.53)	0.48	(0.24–0.96)	0.45	(0.22–0.95)	0.49	(0.22–1.09)
Zeaxanthin	Lowest (Q1)	63	0.14	(0.09–0.16)	1.00		1.00		1.00	
	High (Q2–Q4)	230	0.23	(0.18–0.46)	1.07	(0.53–2.18)	1.02	(0.49–2.11)	1.32	(0.61–2.85)

[a] Odds ratios (and 95% confidence intervals) of high group (upper three quartiles) compared with lowest quartile of serum carotenoid concentrations on low bone mineral density were calculated by logistic regression analysis after adjusting for confounding factors. Model 1: Age, weight, and height were adjusted.

Model 2: Age, weight, height, years since menopause, current tobacco use, regular alcohol intake, exercise habits, supplement use, and total energy intake were adjusted. Model 3: Age, weight, height, years since menopause, current tobacco use, regular alcohol intake, exercise habits, supplement use, and intake of total energy, calcium, magnesium, potassium, and vitamins D, C, and E were adjusted.

enoids has not been thoroughly studied. Our findings further support the hypothesis that high intake of fruit and vegetables rich in antioxidant carotenoids, especially β-cryptoxanthin and β-carotene might provide benefits to bone health in post-menopausal women.

Numerous case-control or prospective cohort studies have shown the relationship of fruit and vegetable intake with chronic diseases [32]. However, the components and mechanisms of fruit and vegetables against chronic diseases are well-unknown. Recent studies have been focused on anti-oxidant micronutrients in fruit and vegetables, especially phytochemicals, such as carotenoids. Carotenoids exist in abundance in fruit and vegetables, and it is well known that the serum carotenoid levels are well correlated with the intake of fruit and vegetables [11–14]. Antioxidant carotenoids have been known to contribute to the body's defense against reactive oxygen species [15, 16]. Therefore, carotenoids may play an important role in the prevention of oxidative stress-related chronic diseases.

The relationship between oxidative stress and BMD or osteoporosis has recently been reported [18–21]. From the finding of osteopetrosis in mice lacking NF-κB1 and NF-κB2, Iotsova et al. reported that NF-κB proteins are important for osteoclastogenesis [33]. NF-κB is activated by exposure to cells to oxidative stress [34]. Therefore, it seems that reactive oxygen species enhance osteoclastogenesis and bone resorption. In fact, some studies have implicated reactive oxygen species in bone regulation. Garrett et al. reported that bone resorption stimulated by parathyroid hormone and interleukin-1 was inhibited by removal of superoxide anions [35]. In contrast, Bax et al. reported that osteoclastic bone resorption was enhanced by the addition of hydrogen peroxide [36]. Furthermore, in epidemiological studies, it was reported that oxidative stress levels were negatively associated with BMD and that antioxidant levels were lower in osteoporotic patients [19, 20, 23]. These previous findings in epidemiological and experimental studies suggest that antioxidant micronutrients may provide benefits to bone metabolism against oxidative stress. Therefore, antioxidant carotenoids are useful to keep bone health.

In our study, serum β-cryptoxanthin in post-menopausal female subjects was significantly correlated with the radial BMD. This association was also observed after further adjusting for intake of vitamins C, D, and E and minerals associated with BMD. In contrast, in logistic regression analysis, significantly lower odds ratio of low radial BMD was observed in the group with high serum β-cryptoxanthin, after adjusting for age, years since menopause, weight, height, current tobacco use, regular alcohol intake, exercise habits, use of dietary supplements, and total energy intake. However, after further adjusting for intake of vitamins C, D, and E and minerals, obvious change of odds ratio were not observed, but this association was not significant. From these results, we concluded that β-cryptoxanthin might positively but partly associate with the BMD. Also, in this study, sample size in post-menopausal female group (n=293) was not so large, and it might be difficult to reach statistical significance. A larger scale would increase the significance of the results.

In our data analysis, we also examined the associations of fruit and vegetables intake and serum carotenoids with the radial BMD. In post-menopausal female subjects, although fruit and vegetable intake was significantly correlated with serum concentrations of lycopene, α-carotene, β-carotene, and β-cryptoxanthin, remarkable correlation was observed among fruit intake with serum β-cryptoxanthin concentration ($r=0.417$, $P<0.001$). In addition, significantly lower odds ratio of low radial BMD was also observed in the group with high intake of fruit after adjusting for confounders (OR: 0.44, 95% CI: 0.20–0.97). The average amount of fruit intake in the group with high serum β-cryptoxanthin was about 254 grams per day and was approximately equal to that in the group with high intake of fruit. In our study population, intake of fruit, especially mandarin orange, might provide benefits to keep bone health, because the subjects in this survey were residents of an area in which the mandarin is considerably more popular than in the rest of Japan. Beta-cryptoxanthin is a 15 carotenoid pigment that is particularly abundant in the Japanese mandarin orange [37, 38]. Therefore, the serum concentrations of β-cryptoxanthin in this study population were widely distributed. In addition to the mandarin orange, tangerine and papaya also contain an abundance of β-cryptoxanthin, and these fruits might also provide benefits to bone health. On the other hand, mandarin orange also includes numerous bioactive flavonoids, such as hesperidin, which have recently been reported to influence bone metabolism [39, 40]. It is also conceivable that these active components might be associated with the radial BMD. To make clear whether other components, such as hesperidin in mandarin orange, are associated with bone health, further studies are required.

Our observation is consistent with experimental results previously reported. Very recently, Yamaguchi et al. reported the beneficial effects of β-cryptoxanthin on bone metabolism in in vitro and in vivo studies [41–43]. They found that β-cryptoxanthin enhanced the calcium content and alkaline phosphatase activity in the femoral-diaphyseal and femoral-metaphyseal tissues of young rats at physiological low concentrations in vitro, while lycopene and lutein had no effects at the same dose [41]. Furthermore, they found a stimulatory effect on bone formation and an inhibitory effect on bone resorption in a tissue culture [42]. In an in vivo study, they found that the oral administration of β-cryptoxanthin caused a significant increase in the calcium content and alkaline phosphatase activity in the femoral-diaphyseal and femoral-metaphyseal tissues [43]. These previous results support our findings showing that β-cryptoxanthin may have a direct

stimulatory effect on bone formation and an inhibitory effect on bone resorption. The development of osteoporosis may be reduced by the dietary intake of β-cryptoxanthin.

In our study, significant associations of serum carotenoid concentrations with the radial BMD were seen in only post-female subjects. We have no clear explanation for this reason.

As one possible explanation, in our study, almost all male and pre-menopausal female subjects have normal BMD. In contrast, the radial BMD in post-menopausal female subjects was significantly low compared with those of male and pre-menopausal female subjects. Therefore, the association of serum carotenoid with the radial BMD might be detectable in post-menopausal female subjects. Alternatively, antioxidant carotenoids might affect against not normal bone metabolism in male or pre-menopausal female subjects, but marked bone loss in female subjects after menopause.

This study had some limitations. First, we could not evaluate the association of blood levels of vitamins C and E with the radial BMD. Some studies have shown an association of antioxidant vitamins with the risk of hip fracture in current smokers and aged osteoporotic women [18, 20]. It would be necessary to measure blood levels of vitamins C and E in order to examine the associations of these antioxidant vitamin concentrations with the radial BMD. Second, the data obtained here consisted of cross-sectional analyses. Therefore, only limited inferences can be made regarding temporality and causation. Third, in this report, we evaluated the radial BMD at 1/3 of the forearm length measured from the styloid process on the ulna. Therefore, an analysis of the association of serum carotenoids with BMD in cancellous bone, such as the femoral neck or lumbar spine, will be required. Last, in our study, sample size in post-menopausal female subjects was not particularly large; thus, it had less statistical power. Further study on a large scale will be required.

In conclusion, serum concentrations of β-cryptoxanthin and β-carotene were weakly but positively associated with the radial BMD in post-menopausal female subjects. These associations suggest that high intake of fruit and vegetables rich in β-cryptoxanthin and β-carotene might provide benefits to bone health in post-menopausal women. To determine whether antioxidant carotenoids are beneficial micronutrients on bone health, further cohort or intervention studies will be required.

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References

1. Ministry of Health, Labor and Welfare. Comprehensive survey of living conditions of the people on Health and Welfare. Section3 2004. Internet: http://www.mhlw.go.jp/toukei/saikin/hw/k-tyosa/k-tyosa04/4-2.html (accessed 16 March 2007)
2. Christodoulou C, Cooper C (2003) What is osteoporosis? Postgrad Med J 79:133–138
3. Gennari C (2001) Calcium and vitamin D nutrition and bone disease of the elderly. Public Health Nutr 4:547–559
4. Prentice A (2004) Diet, nutrition and the prevention of osteoporosis. Public Health Nutr 7:227–243
5. Macdonald HM, New SA, Golden MH et al (2004) Nutritional associations with bone loss during the menopausal transition: evidence of a beneficial effect of calcium, alcohol, and fruit and vegetable nutrients and of a detrimental effect of fatty acids. Am J Clin Nutr 79:155–165
6. New SA, Bolton-Smith C, Grubb DA et al (1997) Nutritional influences on bone mineral density: a cross-sectional study in premenopausal women. Am J Clin Nutr 65:1831–1839
7. Prynne CJ, Mishra GD, O'Connell MA et al (2006) Fruit and vegetable intakes and bone mineral status: a cross sectional study in 5 age and sex cohorts. Am J Clin Nutr 83:1420–1428
8. Okubo H, Sasaki S, Horiguchi H et al (2006) Dietary patterns associated with bone mineral density in premenopausal Japanese farmwomen. Am J Clin Nutr 83:1185–1192
9. McGartland CP, Robson PJ, Murray LJ et al (2004) Fruit and vegetable consumption and bone mineral density: the Northern Ireland Young Hearts Project. Am J Clin Nutr 80:1019–1023
10. Tucker KL, Chen H, Hannan MT et al (2002) Bone mineral density and dietary patterns in older adults: the Framingham Osteoporosis Study. Am J Clin Nutr 76:245–252
11. Andersen LF, Veierod MB, Johansson L et al (2005) Evaluation of three dietary assessment methods and serum biomarkers as measures of fruit and vegetable intake, using the method of triads. Br J Nutr 93:519–527
12. Coyne T, Ibiebele TI, McNaughton S et al (2005) Evaluation of brief dietary questions to estimate vegetable and fruit consumption - using serum carotenoids and red-cell folate. Public Health Nutr 8:298–308
13. Weinstein SJ, Vogt TM, Gerrior SA (2004) Healthy Eating Index scores are associated with blood nutrient concentrations in the third National Health and Nutrition Examination Survey. J Am Diet Assoc 104:576–584
14. van Kappel AL, Steghens JP, Zeleniuch-Jacquotte A et al (2001) Serum carotenoids as biomarkers of fruit and vegetable consumption in the New York Women's Health Study. Public Health Nutr 4:829–835
15. Gutteridge JM (1994) Biological origin of free radicals, and mechanisms of antioxidant protection. Chem Biol Interact 91:133–140
16. Rock CL, Jacob RA, Bowen PE (1996) Update on the biological characteristics of the antioxidant micronutrients: vitamin C, vitamin E, and the carotenoids. J Am Diet Assoc 96:693–702
17. Valko M, Leibfritz D, Moncol J et al (2007) Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 39:44–84
18. Melhus H, Michaelsson K, Holmberg L et al (1999) Smoking, antioxidant vitamins, and the risk of hip fracture. J Bone Miner Res 14:129–135
19. Basu S, Michaelsson K, Olofsson H et al (2001) Association between oxidative stress and bone mineral density. Biochem Biophys Res Commun 288:275–279
20. Maggio D, Barabani M, Pierandrei M et al (2003) Marked decrease in plasma antioxidants in aged osteoporotic women:

results of a cross-sectional study. J Clin Endocrinol Metab 88:1523–1527
21. Yalin S, Bagis S, Polat G et al (2005) Is there a role of free oxygen radicals in primary male osteoporosis? Clin Exp Rheumatol 23:689–692
22. Law MR, Hackshaw AK (1997) A meta-analysis of cigarette smoking, bone mineral density and risk of hip fracture: recognition of a major effect. BMJ 315:841–846
23. Maggio D, Polidori MC, Barabani M et al (2006) Low levels of carotenoids and retinol in involutional osteoporosis. Bone 38:244–248
24. Sugiura M, Nakamura M, Ikoma Y et al (2005) High serum carotenoids are inversely associated with serum gamma-glutamyl-transferase in alcohol drinkers within normal liver function. J Epidemiol 15:180–186
25. Wakai K, Egami I, Kato K et al (1999) A simple food frequency questionnaire for Japanese diet-Part I. Development of the questionnaire, and reproducibility and validity for food groups. J Epidemiol 9:216–226
26. Egami I, Wakai K, Kato K et al (1999) A simple food frequency questionnaire for Japanese diet-Part II. Reproducibility and validity for nutrient intakes. J Epidemiol 9:227–234
27. Science and Technology Agency (1983) Standard tables of food composition in Japan, 4th edn. Printing Bureau, Ministry of Finance, Tokyo, Japan. (in Japanese)
28. Science and Technology Agency (1997) Standard tables of food composition in Japan, 5th edn. (for new foods). Printing Bureau, Ministry of Finance, Tokyo, Japan (in Japanese)
29. Hall SL, Greendale GA (1998) The relation of dietary vitamin C intake to bone mineral density: results from the PEPI study. Calcif Tissue Int 63:183–189
30. Leveille SG, LaCroix AZ, Koepsell TD et al (1997) Dietary vitamin C and bone mineral density in postmenopausal women in Washington State, USA. J Epidemiol Community Health 51:479–485
31. Wang MC, Luz Villa M, Marcus R et al (1997) Associations of vitamin C, calcium and protein with bone mass in postmenopausal Mexican American women. Osteoporos Int 7:533–538
32. World Health Organization (2003) Diet, nutrition and the prevention of chronic diseases. World Health Organ Tech Rep Ser 916:i–viii, 1–149, 2003
33. Iotsova V, Caamano J, Loy J et al (1997) Osteopetrosis in mice lacking NF-kappaB1 and NF-kappaB2. Nat Med 3:1285–1289
34. Baeuerle PA, Rupec RA, Pahl HL (1996) Reactive oxygen intermediates as second messengers of a general pathogen response. Pathol Biol (Paris) 44:29–35
35. Garrett IR, Boyce BF, Oreffo RO et al (1990) Oxygen-derived free radicals stimulate osteoclastic bone resorption in rodent bone in vitro and in vivo. J Clin Invest 85:632–639
36. Bax BE, Alam AS, Banerji B et al (1992) Stimulation of osteoclastic bone resorption by hydrogen peroxide. Biochem Biophys Res Commun 183:1153–1158
37. Goodner KL, Rouseff RL, Hofsommer HJ (2001) Orange, mandarin, and hybrid classification using multivariate statistics based on carotenoid profiles. J Agric Food Chem 49:1146–1150
38. Holden JM, Eldridge AL, Beecher GR et al (1999) Carotenoid content of U. S. foods: an update of the database. J Food Comp Anal 12:169–196
39. Hosseinimehr SJ, Nemati A (2006) Radioprotective effects of hesperidin against gamma irradiation in mouse bone marrow cells. Br J Radiol 79:415–418
40. Chiba H, Uehara M, Wu J et al (2003) Hesperidin, a citrus flavonoid, inhibits bone loss and decreases serum and hepatic lipids in ovariectomized mice. J Nutr 133:1892–1897
41. Yamaguchi M, Uchiyama S (2003) Effect of carotenoid on calcium content and alkaline phosphatase activity in rat femoral tissues in vitro: the unique anabolic effect of beta-cryptoxanthin. Biol Pharm Bull 26:1188–1191
42. Yamaguchi M, Uchiyama S (2004) Beta-Cryptoxanthin stimulates bone formation and inhibits bone resorption in tissue culture in vitro. Mol Cell Biochem 258:137–144
43. Uchiyama S, Yamaguchi M (2004) Oral administration of beta-cryptoxanthin induces anabolic effects on bone components in the femoral tissues of rats in vivo. Biol Pharm Bull 27:232–235

Association of genetic variants of *MAOA* and *SH2B1* with bone mineral density in community-dwelling Japanese women

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Abstract. Although bone mineral density (BMD) is a complex trait that is influenced by both genetic and environmental factors, heritability studies in twins and families have shown that genetic factors account for 60-85% of its variance. We examined the relation of the variable number of tandem repeats (VNTR) polymorphism of the monoamine oxidase A gene (*MAOA*) and the A→G (Thr484Ala) polymorphism of the SH2B adaptor protein 1 gene (*SH2B1*) to BMD in community-dwelling Japanese women and men. The 2235 subjects (1107 women, 1128 men) were aged 40-79 years and were randomly recruited for a population-based prospective cohort study of aging and age-related diseases in Japan. BMD at the distal and proximal radius was measured by peripheral quantitative computed tomography, and the BMD of the total body, lumbar spine (L2-L4), right femoral neck and right trochanter was measured by dual-energy X-ray absorptiometry. The genotypes of the VNTR polymorphism of *MAOA* were determined by DNA fragment analysis, and those of the A→G (Thr484Ala) polymorphism of *SH2B1* by melting curve analysis. The VNTR polymorphism of *MAOA* was associated with the BMD of the distal radius, total body, lumbar spine and trochanter in all women, and with the BMD of the total body and trochanter in postmenopausal ones, with the *L* (four repeats) and *S* (two or three repeats) alleles reflecting increased and decreased BMD, respectively. The A→G (Thr484Ala) polymorphism of *SH2B1* was associated with the BMD of the lumbar spine in all women, with the BMD of the proximal radius in premenopausal women and with the BMD of the lumbar spine, femoral neck and trochanter in postmenopausal women, with the variant *G* allele being related to increased BMD. These results suggest that *MAOA* and *SH2B1* are determinative loci for bone mass in Japanese women, especially in postmenopausal ones.

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Introduction

Osteoporosis, a major health problem of the elderly, is characterized by a reduction in bone mineral density (BMD) and a deterioration in the microarchitecture of the bone, both resulting in a predisposition to fractures (1). Although reproductive, nutritional and lifestyle factors influence BMD, family and twin studies have suggested that it is largely (60-85%) heritable and controlled by multiple genes (2-4). Personalized prevention of osteoporosis and osteoporotic fractures is an important public health goal and can be approached by identifying disease susceptibility genes. Although genetic linkage analyses (5-7) and candidate gene association studies (7-10) have implicated various loci and genes in the predisposition to osteoporosis or fractures, the genes that confer susceptibility to this condition have yet to be definitively identified. In addition, because of ethnic differences in gene polymorphisms as well as in lifestyle and other environmental factors, it is important to examine polymorphisms in relation to BMD in individual ethnic groups.

We have been attempting to identify, with a candidate gene approach, the genetic variants associated with BMD in Japanese women or men recruited for a population-based prospective cohort study. In the present study, we selected the monoamine oxidase A gene (*MAOA*) and SH2B adaptor protein 1 gene (*SH2B1*) as ones that might contribute to bone remodeling (Table I), and examined the relation between the polymorphisms of these genes and BMD, even though there is no apparent biological link between them. Our aim was to identify a single polymorphism significantly associated with BMD in each gene. Of the polymorphisms previously identified, we selected those that might be expected to affect gene function. We then examined the relation between these polymorphisms and BMD in community-dwelling Japanese women and men.

Materials and methods

Study population. The National Institute for Longevity Sciences, Longitudinal Study of Aging, is a population-based prospective cohort study of aging and age-related diseases, the details of which have been described previously (11-15). Individuals with disorders known to cause abnormalities of bone metabolism, including diabetes mellitus, chronic renal failure, rheumatoid arthritis, as well as thyroid, parathyroid,

Table I. The two gene polymorphisms examined in the study.

Locus	Gene	Symbol	Polymorphism	NCBI database
Xp11.3	Monoamine oxidase A	*MAOA*	VNTR [(ACCGGCACCGGCACCAGTACCCGCACCAGT)$_n$]	M89636 (nt 208-327)
16p11.2	SH2B adaptor protein 1	*SH2B1*	A→G (Thr484Ala)	rs7498665

adrenal and other endocrine diseases, or those who had taken drugs that affect bone metabolism such as estrogen, glucocorticoids, bisphosphonates and vitamin D, were excluded from the present study. We thus examined the relation between gene polymorphisms and BMD in 2235 individuals (1107 women, 1128 men). Individuals whose genotypes were not successfully determined were also excluded from the analysis. In addition, to uncover potential differences between women according to menopausal status, we conducted all determinations for associations in premenopausal and postmenopausal women separately. Menopausal status was evaluated by a detailed questionnaire, with menopause defined as complete cessation of menstruation. Because of their small number (n=17), perimenopausal women were excluded from the study. The study protocol complied with the Declaration of Helsinki and was approved by the Committee on Ethics of Human Research of the National Institute for Longevity Sciences. Written and informed consent was obtained from each subject.

Measurement of BMD. BMD at the radius was measured by peripheral quantitative computed tomography (pQCT) with a Desiscan 1000 instrument (Scanco Medical, Bassersdorf, Switzerland) and was expressed as D50 (BMD for the inner 50% of the cross-sectional area of the distal radius, comprising mostly cancellous bone), D100 (BMD for the entire cross-sectional area of the distal radius, including both cancellous and cortical bone) and P100 (BMD for the entire cross-sectional area of the proximal radius, consisting mostly of cortical bone). The BMD of the total body, lumbar spine (L2-L4), right femoral neck and right trochanter was measured by dual-energy X-ray absorptiometry (DXA) with a QDR 4500 instrument (Hologic, Bedford, MA, USA). The coefficients of variation of the pQCT instrument for BMD values were 0.7% (D50), 1.0% (D100) and 0.6% (P100), and those of the DXA instrument 0.9% (total body), 0.9% (L2-L4), 1.3% (femoral neck) and 1.0% (trochanter).

Determination of genotype. Genotypes for the variable number of tandem repeats (VNTR) polymorphism in the promoter region of *MAOA* were determined by DNA fragment analysis. The polymorphic region of *MAOA* was amplified by polymerase chain reaction (PCR) with a sense primer (5'-CCCA GGCTGCTCCAGAAAC-3') labeled at the 5' end with 6-carboxyfluorescein and with an antisense primer (5'-GGA CCTGGGCAGTTGTGC-3'). The reaction mixture (25 μl) contained 20 ng of DNA, 5 pmol of each primer, 0.2 mmol/l of each deoxynucleoside triphosphate, 2.5 mmol/l $MgSO_4$ and 1.25 U of rTaq DNA polymerase (Toyobo, Osaka, Japan) in polymerase buffer. The amplification protocol comprised initial denaturation at 95°C for 5 min, 35 cycles of denaturation at 95°C for 30 sec, annealing at 65°C for 30 sec and extension at 72°C for 30 sec, and a final extension at 72°C for 2 min. The fragment size of VNTR was determined with a PRISM 3100 DNA sequencer and with GeneScan and Genotyper software (Applied Biosystems, Foster City, CA, USA).

Genotypes for the A→G (Thr484Ala) polymorphism of *SH2B1* were determined by melting curve analysis (intercalater-mediated fluorescence resonance energy transfer probe method). The polymorphic region of *SH2B1* was amplified by PCR in a reaction mixture (25 μl) containing 20 ng of DNA, 5 pmol each of sense (5'-TGGAACTGC TTCCCCCAGAGTTG-3') and antisense (5'-TACCTG TGGCTGTTTCCGGAGTGTC-3') primers, 0.2 mmol/l of each deoxynucleoside triphosphate, 2 mmol/l $MgCl_2$ and 1.25 U of rTaq DNA polymerase in polymerase buffer. The amplification protocol comprised initial denaturation at 95°C for 5 min, 40 cycles of denaturation at 95°C for 30 sec, annealing at 65°C for 30 sec and extension at 72°C for 30 sec, and a final extension at 72°C for 2 min. A solution (2 μl) containing 10 pmol of probe (5'-GAACTGTCCCTG CTGGGG-3') labeled at the 5' end with Texas red and 1/400 diluted SYBR Green I was added to the PCR products, which were then transferred to a PRISM 7700 instrument (Applied Biosystems) for measurement of melting temperature. The program for analytic melting comprised incubation at 95°C for 30 sec, 40°C for 1 min, and temperatures increasing to 80°C over 10 min. The fluorescence signals were detected at excitation and emission wavelengths of 485 and 612 nm, respectively.

Statistical analysis. Data were presented as means ± SE or ± SD, as indicated. Statistical analysis was performed with SAS software (SAS Institute, Cary, NC, USA). Data from three genotype groups were compared by one-way analysis of variance and the Tukey-Kramer post hoc test, and between two groups (dominant or recessive model) by the unpaired Student's t-test. The BMD values of genotypes for each polymorphism were compared with adjustment for age, height and body weight by the least squares method in a general linear model. The relation of the number of repeats in the VNTR polymorphism of *MAOA* to BMD was analyzed by multiple regression analysis with adjustment for age, height and body weight. Allele frequencies were estimated by the gene-counting method, and the χ^2 test was used to identify a significant departure from Hardy-Weinberg equilibrium. A P-value of <0.05 was considered statistically significant.

Results

Relation between the VNTR polymorphism of MAOA and BMD. The number of repeats in the VNTR polymorphism of

Table II. BMD and other characteristics of all women (n=1099) according to *MAOA* genotype.[a]

Characteristic	*SS*	*SL*	*LL*	*SS + SL*	*SL + LL*
Number (%)	420 (38.2)	499 (45.4)	180 (16.4)	919 (83.6)	679 (61.8)
Age (years)	59.4±0.5	58.9±0.5	59.6±0.8	59.1±0.4	59.1±0.4
Height (cm)	150.8±0.3	151.5±0.3	152.1±0.5[b]	151.1±0.2	151.6±0.2[c]
Body weight (kg)	52.2±0.4	52.9±0.4	52.7±0.6	52.6±0.3	52.9±0.3
BMD measured with pQCT (mg/cm^3)					
D50	181.2±3.0	187.7±2.8	191.1±4.7	184.7±2.0	188.5±2.4
D100	478.9±4.4	490.8±4.0	493.8±6.7	485.4±3.0	491.6±3.4[d]
P100	1157.0±7.1	1154.8±6.5	1148.1±10.9	1155.8±4.8	1153.1±5.6
BMD measured with DXA (g/cm^2)					
Total body	0.953±0.004	0.975±0.004[e]	0.971±0.006	0.965±0.003	0.974±0.003[f]
L2-L4	0.856±0.006	0.875±0.006	0.865±0.009	0.866±0.004	0.873±0.005[g]
Femoral neck	0.672±0.004	0.681±0.004	0.684±0.006	0.677±0.003	0.682±0.003
Trochanter	0.564±0.004	0.574±0.004	0.582±0.006[h]	0.569±0.003	0.576±0.003[i]

[a]BMD is adjusted for age, height and body weight. Data are the means ± SE. [b]P=0.0434, [c]P=0.0270, [d]P=0.0235, [e]P=0.004, [f]P=0.0001, [g]P=0.0343, [h]P=0.0385, [i]P=0.0166 versus *SS*.

Table III. BMD and other characteristics of postmenopausal women (n=807) according to *MAOA* genotype.[a]

Characteristic	*SS*	*SL*	*LL*	*SS + SL*	*SL + LL*
Number (%)	319 (39.5)	347 (43.0)	141 (17.5)	666 (82.5)	488 (60.5)
Age (years)	63.4±0.5	64.4±0.5	63.7±0.7	63.9±0.3	64.2±0.4
Height (cm)	149.7±0.3	150.2±0.3	151.1±0.5	150.0±0.2[b]	150.4±0.3
Body weight (kg)	51.5±0.5	52.2±0.4	52.5±0.7	51.9±0.3	52.3±0.4
BMD measured with pQCT (mg/cm^3)					
D50	159.7±3.6	164.8±3.4	172.5±5.4	162.3±2.5	167.0±2.9
D100	436.9±5.2	446.6±4.9	453.1±7.7	441.9±3.6	448.5±4.2
P100	1084.6±8.5	1079.9±8.2	1071.7±12.7	1082.1±5.9	1077.5±6.9
BMD measured with DXA (g/cm^2)					
Total body	0.907±0.005	0.926±0.005[c]	0.931±0.007[d]	0.917±0.003	0.927±0.004[e]
L2-L4	0.799±0.007	0.818±0.007	0.814±0.011	0.809±0.005	0.817±0.006
Femoral neck	0.640±0.005	0.645±0.005	0.655±0.007	0.642±0.003	0.648±0.004
Trochanter	0.534±0.005	0.541±0.004	0.553±0.007[f]	0.537±0.003[g]	0.544±0.004

[a]BMD is adjusted for age, height and body weight. Data are the means ± SE. [b]P=0.0444, [g]P=0.0337 versus *LL*; [c]P=0.0103, [d]P=0.0169, [e]P=0.0009, [f]P=0.0454 versus *SS*.

MAOA was two, three or four for all women (mean ± SD, 3.4±0.5; n=2198 alleles) and men (3.4±0.5; n=1096 alleles). The number of repeats in this polymorphism was related to the BMD in terms of the distal radius (D100, P=0.0102), total body (P=0.0103) or trochanter (P=0.0210) in all women, whereas no relation was detected in men. At each of these sites, the BMD was greater in women with four repeats than in those with three repeats. Given that the mean number of repeats was 3.4 for men and women, we designated alleles containing two or three repeats as short (*S*) and those containing four repeats as long (*L*).

Age and body weight did not differ among all women (Table II), premenopausal women (data not shown), and postmenopausal women (Table III) with the *SS*, *SL* and *LL* genotypes of *MAOA*. Height was greater in individuals with the *LL* genotype and in the combined group of *SL* and *LL* genotypes than in individuals with the *SS* genotype for all women, and greater in individuals with the *LL* genotype than in the combined group of *SS* and *SL* genotypes for postmenopausal women. Height did not differ among the premenopausal women with *MAOA* genotypes. In all women, the BMD of D100 and the lumbar spine was greater in the combined

Table IV. BMD and other characteristics of all women (n=1107) according to *SH2B1* genotype.[a]

Characteristic	*AA*	*AG*	*GG*	*AA + AG*	*AG + GG*
Number (%)	820 (74.1)	272 (24.6)	15 (1.4)	1092 (98.6)	287 (25.9)
Age (years)	59.5±0.4	58.7±0.7	56.8±2.8	59.3±0.3	58.6±0.6
Height (cm)	151.2±0.2	151.5±0.4	153.3±1.6	151.3±0.2	151.6±0.4
Body weight (kg)	52.7±0.3	52.3±0.5	55.6±2.1	52.6±0.2	52.5±0.5
BMD measured with pQCT (mg/cm^3)					
D50	184.7±2.2	186.1±3.7	192.3±15.6	185.1±1.9	186.5±3.6
D100	485.1±3.1	486.8±5.4	507.6±22.6	485.6±2.7	487.9±5.2
P100	1151.5±5.1	1157.8±8.6	1194.4±36.4	1153.1±4.4	1159.8±8.4
BMD measured with DXA (g/cm^2)					
Total body	0.964±0.003	0.965±0.005	0.998±0.022	0.965±0.003	0.967±0.005
L2-L4	0.867±0.004	0.857±0.008[b]	0.938±0.033	0.864±0.004[c]	0.861±0.007
Femoral neck	0.676±0.003	0.680±0.005	0.711±0.022	0.677±0.003	0.682±0.005
Trochanter	0.569±0.003	0.573±0.005	0.601±0.021	0.570±0.002	0.575±0.005

[a]BMD is adjusted for age, height and body weight. Data are the means ± SE. [b]P=0.0432, [c]P=0.0260 versus *GG*.

group of *SL* and *LL* genotypes than in individuals with the *SS* genotype (Table II). BMD for the total body was greater in individuals with the *SL* genotype and in the combined group of *SL* and *LL* genotypes than in individuals with the *SS* genotype. BMD for the trochanter was greater in individuals with the *LL* genotype or the combined group of *SL* and *LL* genotypes than in individuals with the *SS* genotype. The differences in the BMD of D100, total body and lumbar spine between the combined group of *SL* and *LL* genotypes and individuals with the *SS* genotype (expressed as a percentage of the larger value) were 2.6, 2.2, and 1.9%, respectively, and the difference in the BMD of the trochanter between individuals with the *LL* genotype and those with the *SS* genotype was 3.1%. In postmenopausal women, the BMD of the total body was greater in individuals with the *LL* genotype, *SL* genotype and the combined group of *SL* and *LL* genotypes than in individuals with the *SS* genotype (Table III). The BMD of the trochanter was greater in individuals with the *LL* genotype than in those with the *SS* genotype or in the combined group of *SS* and *SL* genotypes. The differences in the BMD of the total body and trochanter between individuals with the *LL* and *SS* genotype were 2.6 and 3.4%, respectively. In premenopausal women or men, the BMD did not differ among the *MAOA* genotypes (data not shown).

Relation of the A→G (Thr484Ala) polymorphism of SH2B1 to BMD. The distribution of A→G genotypes of *SH2B1* was in Hardy-Weinberg equilibrium. Age, height and body weight did not differ among genotype groups for all women (Table IV), premenopausal women (data not shown) or postmenopausal women (Table V). In all the women, the BMD of the lumbar spine was greater in individuals with the *GG* genotype than in those with the *AG* genotype or the combined group of *AA* and *AG* genotypes. The difference in the BMD of the lumbar spine between individuals with the *GG* genotype and the combined group of *AA* and *AG* genotypes was 7.9%. In premenopausal women, the BMD of P100 in individuals with the *AG* genotype and in the combined group of *AG* and *GG* genotypes was greater than in individuals with the *AA* genotype (data not shown). In postmenopausal women, the BMD of the lumbar spine was greater in individuals with the *GG* genotype than in the combined group of *AA* and *AG* genotypes (Table V). The BMD of the femoral neck was greater in the combined group of *AG* and *GG* genotypes than in individuals with the *AA* genotype. The BMD of the trochanter was greater in individuals with the *GG* genotype than in the combined group of *AA* and *AG* genotypes, and greater in the combined group of *AG* and *GG* genotypes than in individuals with the *AA* genotype. The differences in the BMD of the lumbar spine and trochanter between individuals with the *GG* genotype and the combined group of *AA* and *AG* genotypes was 9.2 and 8.5%, respectively, and the difference in the BMD of the femoral neck between the combined group of *AG* and *GG* genotypes and individuals with the AA genotype was 2.4%. For men, the distribution of *SH2B1* genotypes was in Hardy-Weinberg equilibrium; there was no difference in BMD among *SH2B1* genotypes (data not shown).

Discussion

We examined the relation of the VNTR polymorphism of *MAOA* and the A→G (Thr484Ala) polymorphism of *SH2B1* to BMD at various sites in community-dwelling Japanese women and men. Our results showed that the polymorphisms of *MAOA* and *SH2B1* were associated with BMD in women, especially in postmenopausal individuals, suggesting that *MAOA* and *SH2B1* are determinative loci for bone mass in Japanese women.

MAOA is an important catabolic enzyme that regulates levels of monoamine neurotransmitters, including serotonin, dopamine and noradrenaline, in the central nervous system. The VNTR polymorphism in the promoter region of *MAOA* consists of a 30-bp repeated sequence that is present in 3, 3.5, 4 or 5 copies (16), and has been shown to affect the tran-

Table V. BMD and other characteristics of postmenopausal women (n=814) according to *SH2B1* genotype.[a]

Characteristic	*AA*	*AG*	*GG*	*AA + AG*	*AG + GG*
Number (%)	605 (74.3)	198 (24.3)	11 (1.4)	803 (98.6)	209 (25.7)
Age (years)	64.0±0.3	63.6±0.6	60.5±2.6	63.9±0.3	63.4±0.6
Height (cm)	150.1±0.2	150.4±0.4	152.0±1.8	150.2±0.2	150.5±0.4
Body weight (kg)	52.1±0.3	51.7±0.6	54.5±2.4	52.0±0.3	51.8±0.6
BMD measured with pQCT (mg/cm^3)					
D50	162.4±2.6	166.9±4.5	179.7±18.8	163.5±2.3	167.6±4.4
D100	441.6±3.7	446.3±6.4	481.8±26.9	442.8±3.2	448.2±6.3
P100	1079.0±6.2	1078.9±10.7	1057.1±44.6	1079.0±5.3	1083.1±10.4
BMD measured with DXA (g/cm^2)					
Total body	0.916±0.004	0.925±0.006	0.963±0.026	0.918±0.003	0.927±0.006
L2-L4	0.808±0.005	0.810±0.009	0.890±0.038	0.808±0.004[b]	0.814±0.009
Femoral neck	0.640±0.003	0.655±0.006	0.683±0.025	0.644±0.003	0.656±0.006[c]
Trochanter	0.537±0.003	0.547±0.006	0.589±0.024	0.539±0.003[d]	0.550±0.006[e]

[a]BMD is adjusted for age, height and body weight. Data are the means ± SE. [b]P=0.0335, [d]P=0.0418 versus *GG*; [c]P=0.0182, [e]P=0.0471 versus *AA*.

scriptional activity of the gene *in vitro* (16-18). Transcription of VNTR alleles with 3.5 or 4 repeats is more efficient than in the allele with 3 repeats in various cell lines and human male skin fibroblasts (16-18). This polymorphism was also shown to affect the expression and activity of MAOA in the brain of individuals with Alzheimer's disease (19). In addition, it has been associated with various pathological behavioral traits, such as mood disorders, autism, aggression and impulsivity (20-23). We have now shown that the VNTR polymorphism of *MAOA* was associated with BMD in postmenopausal women, with the *L* and *S* alleles reflecting increased and decreased BMD, respectively. As far as we are aware, this is the first demonstration of the association of this polymorphism of *MAOA* with BMD, although the underlying molecular mechanism remains to be elucidated. This association may be attributable, however, to the effects of the polymorphism on the neuroendocrine systems, given that neuroendocrine disorders can result in a decrease in the concentration of growth hormone and sex steroids and thus accelerate the development of osteoporosis (24).

SH2B1 is a widely-expressed cytoplasmic protein that simultaneously binds, via its Src homology 2 (SH2) domain, to both Janus kinase 2 (JAK2) and insulin receptor substrate 2 (IRS2), thereby promoting the leptin-induced activation of the phosphoinositide 3-kinase signaling pathway in cultured cells (25,26). SH2B1-deficient mice develop insulin resistance and type 2 diabetes mellitus (27) as well as severe leptin resistance, hyperphagia and obesity (28). SH2B1 is thus a key cytoplasmic signaling molecule that acts as a positive regulator of leptin and insulin signal transduction in mice. The A→G (Thr484Ala) polymorphism of *SH2B1* (rs7498665) is a tag single nucleotide polymorphism (SNP) that represents five common SNPs in complete linkage disequilibrium within a 16-kb region encompassing *SH2B1* (29). This polymorphism was associated with the serum concentration of leptin, total body fat, waist circumference and body weight in Caucasian female twins, although it is predicted to not affect protein structure or function and is likely in linkage disequilibrium with an as yet unidentified functional variant of *SH2B1* (29). We have now shown that the A→G (Thr484Ala) polymorphism of *SH2B1* is associated with BMD in women, especially in postmenopausal women, with the variant *G* allele being related to increased BMD. Given that leptin plays an important role in bone remodeling (30-32), the association of this polymorphism of *SH2B1* with BMD may be attributable to effects on leptin signaling.

Given the multiple comparisons of genotypes with BMD at various sites in the present study, it is not possible to exclude potential type I errors (false positives). It is also possible that the polymorphisms associated with BMD in our study are in linkage disequilibrium with other polymorphisms in the same gene or with polymorphisms of nearby genes that are actually the determinants of BMD. Furthermore, the relevance of the polymorphisms to gene transcription or to protein structure or function and their effects on bone remodeling were not determined in the present study.

In conclusion, our present results suggest that *MAOA* and *SH2B1* are determinative loci for BMD in Japanese women. Determination of genotypes for these polymorphisms may prove informative for the assessment of the genetic risk of reduced BMD. Given that multiple variants, each having a small effect, will likely be found to be responsible for a large fraction of the genetic component of osteoporosis, identification of additional osteoporosis susceptibility genes will allow for a more accurate assessment of the genetic component of this condition.

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References

1. Kanis JA, Melton LJ III, Christiansen C, Johnston CC and Khaltaev N: The diagnosis of osteoporosis. J Bone Miner Res 9: 1137-1141, 1994.
2. Pocock NA, Eisman JA, Hopper JL, Yeates MG, Sambrook PN and Eberl S: Genetic determinations of bone mass in adults: a twin study. J Clin Invest 80: 706-710, 1987.
3. Gueguen R, Jouanny P, Guillemin F, Kuntz C, Pourel J and Siest G: Segregation analysis and variance components analysis of bone mineral density in healthy families. J Bone Miner Res 10: 2017-2022, 1995.
4. Ralston SH: Genetic determinants of susceptibility to osteoporosis. Curr Opin Pharmacol 3: 286-290, 2003.
5. Johnson ML, Gong G, Kimberling W, Recker SM, Kimmel DB and Recker RB: Linkage of a gene causing high bone mass to human chromosome 11 (11q12-13). Am J Hum Genet 60: 1326-1332, 1997.
6. Hsu YH, Xu X, Terwedow HA, *et al*: Large-scale genome-wide linkage analysis for loci linked to BMD at different skeletal sites in extreme selected sibships. J Bone Miner Res 22: 184-194, 2007.
7. Morrison NA, Qi JC, Tokita A, *et al*: Prediction of bone density from vitamin D receptor alleles. Nature 367: 284-287, 1994.
8. Uitterlinden AG, Burger H, Huang Q, *et al*: Relation of alleles of the collagen type Iα1 gene to bone density and the risk of osteoporotic fractures in postmenopausal women. N Engl J Med 338: 1016-1021, 1998.
9. Yamada Y, Ando F, Niino N and Shimokata H: Transforming growth factor-ß1 gene polymorphism and bone mineral density. JAMA 285: 167-168, 2001.
10. Xiong DH, Shen H, Zhao LJ, *et al*: Robust and comprehensive analysis of 20 osteoporosis candidate genes by very high-density single-nucleotide polymorphism screen among 405 white nuclear families identified significant association and gene-gene interaction. J Bone Miner Res 21: 1678-1695, 2006.
11. Shimokata H, Ando F and Niino N: A new comprehensive study on aging - the National Institute for Longevity Sciences, Longitudinal Study of Aging (NILS-LSA). J Epidemiol 10: S1-S9, 2000.
12. Yamada Y, Ando F, Niino N and Shimokata H: Association of polymorphisms of interleukin-6, osteocalcin, and vitamin D receptor genes, alone or in combination, with bone mineral density in community-dwelling Japanese women and men. J Clin Endocrinol Metab 88: 3372-3378, 2003.
13. Yamada Y, Ando F, Niino N and Shimokata H: Association of polymorphisms of androgen receptor and klotho genes with bone mineral density in Japanese women. J Mol Med 83: 50-57, 2005.
14. Yamada Y, Ando F and Shimikata H: Association of polymorphisms in forkhead box C2 and perilipin genes with bone mineral density in community-dwelling Japanese individuals. Int J Mol Med 18: 119-127, 2006.
15. Yamada Y, Ando F and Shimokata H: Association of candidate gene polymorphisms with bone mineral density in community-dwelling Japanese women and men. Int J Mol Med 19: 791-801, 2007.
16. Sabol SZ, Hu S and Hamer D: A functional polymorphism in the monoamine oxidase A gene promoter. Hum Genet 103: 273-279, 1998.
17. Deckert J, Catalano M, Syagailo YV, *et al*: Excess of high activity monoamine oxidase A gene promoter alleles in female patients with panic disorder. Hum Mol Genet 8: 621-624, 1999.
18. Denney RM, Koch H and Craig IW: Association between monoamine oxidase A activity in human male skin fibroblasts and genotype of the MAOA promoter-associated variable number tandem repeat. Hum Genet 105: 542-551, 1999.
19. Wu YH, Fischer DF and Swaab DF: A promoter polymorphism in the monoamine oxidase A gene is associated with the pineal MAOA activity in Alzheimer's disease patients. Brain Res 1167: 13-19, 2007.
20. Manuck SB, Flory JD, Ferrell RE, Mann JJ and Muldoon MF: A regulatory polymorphism of the monoamine oxidase-A gene may be associated with variability in aggression, impulsivity, and central nervous system serotonergic responsivity. Psychiatry Res 95: 9-23, 2000.
21. Cohen IL, Liu X, Schutz C, *et al*: Association of autism severity with a monoamine oxidase A functional polymorphism. Clin Genet 64: 190-197, 2003.
22. Yu YW, Tsai SJ, Hong CJ, Chen TJ, Chen MC and Yang CW: Association study of a monoamine oxidase A gene promoter polymorphism with major depressive disorder and anti-depressant response. Neuropsychopharmacology 30: 1719-1723, 2005.
23. Balciuniene J and Jazin E: Human monoamine oxidase: from genetic variation to complex human phenotypes. Gene Funct Dis 1: 26-37, 2001.
24. Rehman HU and Masson EA: Neuroendocrinology of ageing. Age Ageing 30: 279-287, 2001.
25. Rui L and Carter-Su C: Identification of SH2-beta as a potent cytoplasmic activator of the tyrosine kinase Janus kinase 2. Proc Natl Acad Sci USA 96: 7172-7177, 1999.
26. Duan C, Li M and Rui L: SH2-B promotes insulin receptor substrate (IRS)1- and IRS2-mediated activation of the phosphatidylinositol 3-kinase pathway in response to leptin. J Biol Chem 279: 43684-43691, 2004.
27. Duan C, Yang H, White MF and Rui L: Disruption of the SH2-B gene causes age-dependent insulin resistance and glucose intolerance. Mol Cell Biol 24: 7435-7443, 2004.
28. Ren D, Li M, Duan C and Rui L: Identification of SH2-B as a key regulator of leptin sensitivity, energy balance, and body weight in mice. Cell Metab 2: 95-104, 2005.
29. Jamshidi Y, Snieder H, Ge D, Spector TD and O'Dell SD: The SH2B gene is associated with serum leptin and body fat in normal female twins. Obesity 15: 5-9, 2007.
30. Ducy P, Amling M, Takeda S, *et al*: Leptin inhibits bone formation through a hypothalamic relay: a central control of bone mass. Cell 100: 197-207, 2000.
31. Takeda S, Elefteriou F, Levasseur R, *et al*: Leptin regulates bone formation via the sympathetic nervous system. Cell 111: 305-317, 2002.
32. Elefteriou F, Ahn JD, Takeda S, *et al*: Leptin regulation of bone resorption by the sympathetic nervous system and CART. Nature 434: 514-520, 2005.

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第17巻 老人の医療

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4) Wechsler D: The measurement and appraisal of adult intelligence. 4th ed. Baltimore: Williams and Wilkins; 1958.
5) Wechsler D: WAIS-R manual. New York: Psychological Corporation; 1987.
6) Schaie KW: Longitudinal studies of adult psychological development. New York: Guilford; 1983.
7) Botwinick J: Intellectual abilities. In: Birren JE, et al, editors. Handbook of the psychology of aging. New York: Van Nostrand Reinhold; 1977. p.580-605.
8) Horn JL, et al: Age differences in fluid and crystallized intelligence. Acta Psychol 1967; 26: 107-129.
9) Thornton WJ, et al: Age differences in everyday problem-solving and decision-making effectiveness: a meta-analytic review. Psychol Aging 2005; 20(1):85-99.
10) Taub GE, et al: Relative roles of cognitive ability and practical intelligence in the prediction of success. Psychol Rep 2001; 88(3 Pt 1):931-942.

❻個人差

ひとりひとりの人間は，それぞれに異なっている．この世の中に1人として自分とまったく同じ人はいない．これが個性である．たとえ，まったく同じ遺伝子をもつ一卵性双生児でも，そのそれぞれが異なった個性をもっており，異なった人生を歩んでいく．

医療の場においても，こうした個人差を絶えず考慮に入れていかねばならない．同じ疾患でも，臨床的にとらえられる症状は，個人個人で大きく違う．さらに，同じ疾患に対して同じ治療を行っても，その効果はまた個人によって大きく異なる．特に老年者では，こうした個人差が大きい．暦のうえでは同じ年齢でも，元気で社会的に活躍している人，寝たきりで身動きすらできない人など，多様である．

ここでは，老化に伴う個人差について，その規定要因やとらえ方などを述べ，さらに，こうした個人差に対応した医療のあり方についても述べていく．

老年者の身体的特徴と個人差

老化による身体的な変化

老化によって，身体にはいろいろな変化が生じる．骨塩量★1が低下し，骨がもろく折れやすくなる．関節が変形し，炎症を起こしやすくなる．筋力が低下し，これらの結果，身体の運動機能が低下する．平衡覚が低下して，歩行が不安定になり，転倒しやすくなる．

また種々の生理機能の低下，特に循環器機能や肺機能の低下によ

★1 骨塩量
骨塩はリン酸とカルシウムから成り，コラーゲン分子から成る有機骨基質と結合して骨を形成している．骨塩量はX線の透化度などで測定され，これから骨の量が推定される．

って持久力が低下する．免疫機能などの生体防御の機能がうまく働かず，感染症にかかりやすくなり，軽い病気でも重篤化するようになる．老年者では一見健康であっても慢性の潜在性の疾患をもっていることも多い．

老化とともに感覚器機能が低下することも特徴である．視力では，矯正遠距離視力は高齢まで保たれるが，近距離視力，動体視力，暗視力，視野，立体視機能，青黄色の色覚の低下が進む．聴力では高音域の低下が顕著である．皮膚知覚は低下したり，逆に過敏になったりすることもある．

エネルギーの消費量・摂取量の低下

老年者では若年者に比べて食欲が低下することが多い．老年者では身体活動によるエネルギー消費が少なくなる．骨格筋が萎縮し，体脂肪が増加する．骨格筋は多くのエネルギーを消費するが，脂肪組織ではエネルギーはほとんど消費されず，体脂肪率の上昇とともに全身の基礎代謝率は低下する．エネルギー要求量が低くなり，その結果，食欲が低下することになる．感覚機能，特に食欲に密接にかかわる味覚，嗅覚，視覚などの機能の低下が，いっそう食欲不振を増強させる．

老年者における最大の特徴は，こうした身体的な変化の個人差が大きいことである．いろいろな検査データの分布幅は，小児期→青年期→中年期→初老期と，加齢とともに増大していき，老年者では一般成人よりも大きな分布幅を示すようになる．

個人差をもたらす要因

個人差には多くの要因がある．個人差の要因には先天的・遺伝的要因，環境・生活要因，年齢的要因が含まれる（図18）．

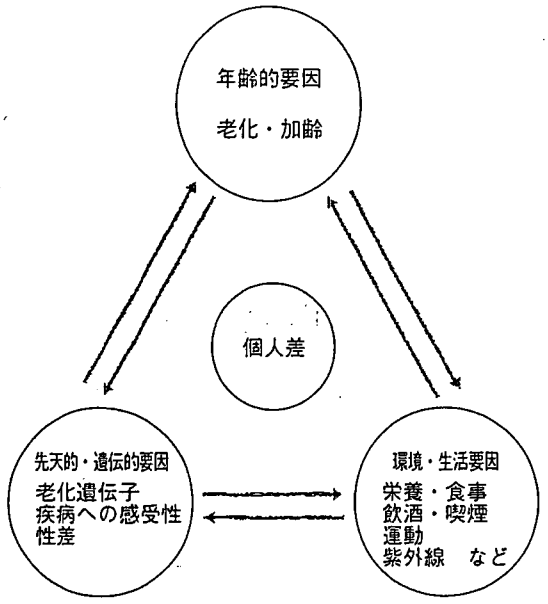
先天的・遺伝的要因

遺伝子：老化そのものの進行が，遺伝子によってある程度制御されていることは確かである．老化が異常に早く進む病態として早老症☆2（プロゲリア）が知られており，これらは遺伝子の異常であることが確かめられている．糖尿病や高血圧症などの慢性疾患は，老化を促進させることが知られている．こうした疾患への感受性もまた，遺伝的因子によって左右されている．

性差：先天的な要因として性差がある．男女で多くの生理機能に差があり，また老化の進行の仕方にも差がある．閉経前には血清脂質，耐糖能など生理機能に性差がみられる場合が多いが，閉経後は年齢とともに性差は小さくなっていく．これは生理機能の性差が性ホルモンに由来する部分が大きいからである．特に女性ホルモンのエストロゲンにはHDLコレステロールを増加させ，LDLコレステロール

☆2 早老症
実際の年齢よりも早期に老化もしくは老化に似た身体の変化をきたす症候群．遺伝子の異常によって起こるとされている．

図18　個人差をきたす要因



を低下させるなど，動脈硬化の進行を防ぐ作用が大きい．動脈硬化は「人は血管とともに老いる」と言われるように老化によって進行し，冠動脈疾患や脳卒中を起こす原因となる．喫煙率の性差など社会的な要因による影響もあるが，たとえば冠動脈疾患の発症年齢は女性は男性よりも10歳以上高くなっている．一方，骨粗鬆症やアルツハイマー病などの老年病は女性に多い．老化の過程と老化に伴って生じるさまざまな疾患にはこうした性差があり，それを考慮していく必要がある．

環境・生活要因

一般に，環境・生活要因に曝露される期間が長くなるほど，その影響は大きくなる．したがって，高齢になるほど環境・生活などの後天的要因は，遺伝などの先天的要因よりも，個人差を引き起こす原因として重要となってくる．子どものころには区別がつかなかった一卵性双生児でも，高齢に至れば容易に区別がつくようになる．

栄養・食事：個人差を生じるような生活要因のうち，最も重要なのは栄養である．食物は個人の嗜好により選択され摂取される．文化や風土によって大きく異なる多彩な内容をもつものである．だれもが生涯にわたって毎日，何度も曝露されて影響を受ける環境要因であって，身体に及ぼす影響が大である．摂取エネルギー量の過剰や不足は体格に変化を与える．脂質の摂りすぎは癌や動脈硬化を促進させる．老化そのものの進行にも栄養は大きな影響を与え，個人差を引き起こす．

喫煙・飲酒：喫煙・飲酒といった嗜好も，老化の進行には大きな影響を及ぼす．喫煙は，癌や循環器疾患の危険因子として重要である．

一方でエネルギー消費を促進させ，体重を減少させる．精神機能にも影響を与えている可能性もある．さらに，身体の老化を促進させるという指摘もある．飲酒は少量では循環器疾患を予防し，また老化の進行そのものを遅らせる．しかし，大量に摂取すれば肝機能障害や，膵炎，精神障害などの原因にもなってしまう．

運動・紫外線：運動習慣の違いによる骨や身体機能への影響，紫外線の影響による皮膚の老化の促進なども，老化に伴う個人差の要因となる．

個人差には，このようにさまざまな要因がかかわっており，またそれらの要因がそれぞれ互いに影響を及ぼし合って，さらに個人差を生み出している．

老化度

老化の指標

老化の進行の個人差を明らかにするには，個人個人の老化の進み方を客観的に示すための指標が必要である．多くの生理機能は加齢とともに変動し，高齢に至ればその機能は低下することが多いが，逆に加齢によって大きく変動する検査項目を用いて，老化の進行の程度を判定しようとする試みもなされている．老化の指標として，表24に示すような条件を備えていることが望ましい．

骨年齢：図19に示すように，骨塩量（骨密度）は加齢とともに低下していく．40歳の人の骨塩量測定値が60歳の人の平均的な骨塩量の値に等しければ，その人の骨年齢は60歳であるというような表現ができ，わかりやすい．

人間の老化において，生理機能の加齢変化は，認知機能，運動機能，外見上の変化などの加齢変化と必ずしも同じ速度で進むわけではない．また生理機能のなかでも，感覚器機能，肝機能，腎機能，循環器機能，免疫機能，呼吸器機能，消化管機能，内分泌機能など，臓器ごとに加齢変化は異なることが多い．このため，ヒトの老化の進行を総合的に評価するためには多くの機能面からみた数多くの老化の指標が必要となる．

生物学的年齢：しかし，老化の指標を多く採用すればするほど，その解釈は複雑なものとなり，老化の進行状況を正確に把握することはかえって難しくなってしまう．そこで，数多くの老化の指標から，老化の進行の程度を表す総合的な指標をつくって，それを用いて老化の判定を行うという方法が考えられる．その方法の1つが生物学的年齢である．これは暦のうえの実際の年齢ではなくて，生体機能の老化の程度から推定された年齢である．

図20に示すように，平均的な人は暦年齢と生物学的年齢はほぼ一

表24　老化の指標

1. 測定方法が確立しており，測定法が簡便で，費用が安く，誤差が少ないこと．
2. 測定に苦痛や障害を伴わないこと．
3. 健常者では加齢とともに有意に変化し，理想的にはその変動が直線的であり，変動に性差が少ないこと．
4. 他の老化指標との相関が低く，機能の変化を代表する独立した指標であること．