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

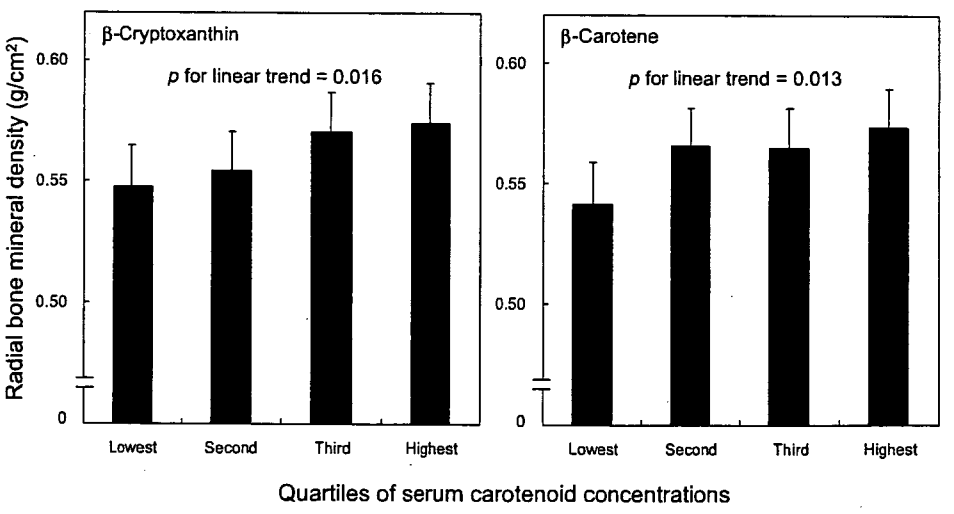

Fig. 1 Multivariate-adjusted means of bone mineral density by quartiles of serum β-carotene and β-cryptoxanthin concentrations in post-menopausal female subjects. Multivariate-adjusted means of bone mineral density were calculated after adjusting for age, weight, height, years since menopause, current 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 antioxidant 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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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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Key words: polymorphism, genetics, osteoporosis, bone density, *MAOA*, *SH2B1*

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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第3章 老化の概念と学説 (新野直明)

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第3章 老化の概念と学説

1．老化の生物学説

（１）老化の概念

老化の概念には，広義のものと狭義のものの２つがある。広義の老化は，生まれてから死ぬまでの生涯の全変化をさし，加齢（aging）と呼ばれるものである。それに対し，狭義の老化は，成熟期以降の退行期の変化を示すもので，いわゆる老化，老衰（senescence, senility）である。このため，老化を一言で定義するのは難しいが，老年学，老年医学の分野では，狭義の老化を意識したもの，たとえば「老化とは，加齢に伴い誰にでも起こる諸機能の低下，減退であり，死の確率が増す過程（現象）」という考え方が一般的のようである。本章でも，原則的にこの考え方で老化を捉えることとする。

（２）生理的老化

老化は，疾病，事故，栄養，生活環境など様々な要因の影響を受けると考えられるが，これらの外因により規定されない老化，すなわち，大きな病気や事故がなく，寿命をまっとうした場合の老化を生理的老化（正常老化）という。

生理的老化の特徴として，ストレーラー（Strehler）は次の４つをあげている[1)]。

1）普遍性：全ての生命体に認められる現象であり，（同一の種に属する個体は）

ほぼ同様の経過を示す。

2）内在性：個体に内在するものである（ある程度，遺伝的に規定されている）。

3）進行性：進行性であり，後戻りしない。

4）有害性：個体の機能を低下させるものである。

以上の4条件を満たす変化が（生理的）老化であり，最終的に機能の低下から死につながる。また，病気などによる機能の低下や寿命の短縮は，すべての人に起こるわけではないので，生理的老化ではなく病的老化と呼ばれる（ただし，高齢になると，多数の人が似たような症状，訴えを呈することもあり，生理的老化と病的老化を簡単に分けることができない場合もある）。

人間の生理的老化について，皺が増える，髪が白くなる，背が曲がるなど，経験的な事実としては多くのことが語られているが，系統的，科学的には解明されていないことも多い。そのため，個人，あるいは，集団を長年，追跡的に調査し，形態や機能の生理的変化を明らかにしようとする，長期縦断研究が実施され，生理的老化に関する結果が蓄積されている。わが国でも，東京都老人総合研究所による「中年からの老化予防総合的長期追跡研究（TMIG－LISA）」[2]，国立長寿医療センター研究所疫学研究部による「老化に関する長期縦断疫学調査（NILS-LSA）」[3]などの大規模な研究が行われている。

（3）老化の測定

老化について研究し，その進行を少しでも遅らせる対応策，予防法などを考えるためには，老化の程度を正確に測定・評価することが重要になる。普段の生活では，暦年齢で老化度を評価することが少なくない。しかし，老化に伴う心身機能の変化は，基本的には低下の方向を示すものの，個人差が非常に大きい。また，栄養，生活習慣，環境など多くの外的要因が影響を与える。そのため，単純な暦年齢のみで，正確かつ客観的に老化を測定することは難しい。下方は，望ましい老化指標の条件として，次の4つをあげ，老化を総合的に評価するには多くの生体機能を含む数多くの指標が必要と述べている[4]。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。②測定に苦

痛や障害を伴わない。③正常人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。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。

近年，しばしば使われる老化の指標に生物学的年齢がある。これは，生体のもつ種々の機能，たとえば，生理機能，知的機能，運動機能などを基準として推定される年齢である。暦（生年月日）という外的基準による暦年齢とは異なり，臓器や組織の機能など，個体に特有な内的基準による年齢と考えることができる。この生物学的年齢は，1つの指標（機能）から判定することもできるが，重回帰モデルなどの多変量の統計モデルを用いて，複数の指標を同時に考慮しながら推定する方法も検討，利用されている。

（4）老化学説

個体，器官，組織，細胞などの老化が起こる原因，あるいは老化の機序については多くの学説がある。武田は，老化学説として，消耗説（wear and tear theory），ストレス説（stress theory），生活代謝率説（living rate theory），プログラム説（program theory），エラー破綻説（error catastrophe theory），体細胞突然変異説（somatic mutation theory），自己免疫説（autoimmune theory），代謝産物原因説（waste product theory），生物時計説（biological clock theory），内分泌説（endocrine theory），遊離基説（free radical theory），架橋結合説（cross-linking theory）の12種類をあげている[5]。

また，三木は，老化学説をプログラム説とエラー蓄積説の2つに大別し，以下のように説明している[6]。

1）プログラム説：老化が遺伝子レベル（遺伝因子）により制御されているという考え方。動物は種により固有の最大寿命を有すること[注1]，ヒトの細胞に寿命があること[注2]，遺伝的早老症（progeroid syndrome）[注3]があること，老化遺伝子[注4]，テロメア[注5]，アポトーシス[注6]の研究など遺伝が老化を制御することを示す研究事実があること，などがこの説を支持する。

2）エラー蓄積説：数々の障害や老化物質の蓄積（遺伝外因子）がDNAやタン

パク質に発生することで老化が起こるという考え方。以下の説がある。①磨耗説：放射線や化学物質によりDNAに損傷が起き，その蓄積が寿命を決定する。②活性酸素説：フリーラジカル（遊離基）が原因[注7)]。③架橋結合説：加齢に伴いタンパク質分子間に科学的な結合（架橋）ができ，細胞機能が障害される。④誤り説：DNAの複製や損傷修復時に塩基配列を誤って写し，その結果，異常タンパク質集積，細胞機能障害が出現する。⑤老廃物蓄積説：加齢に伴う変異酵素や変異タンパク質（リポフスチン，アミロイドなど）の出現・蓄積による。⑥自己免疫説：免疫機構の破綻などによる。

以上のように老化学説は多種存在するが，どれか1つの説で老化の全体を説明するのは困難であり，複数の説（あるいはすべての説）を取り入れる必要があるだろう。また，老化に，遺伝要因と非遺伝要因（環境要因）の両者が関与することは確実と考えられる。遺伝要因については，現状では，まだ介入困難な部分が多い。しかし，環境要因に対しては，生活習慣の改善，疾病の予防など介入が可能であり，結果として，老化の遅延や寿命の延長などが期待できるだろう。

■注：

注1）寿命の限界値（限界寿命）は動物の種によりほぼ決まっているといわれる。限界寿命にはある程度法則性があり，体重，脳重量などを用いた推定法が考案されている。代表的なものにザッハー（Sacher）の式がある。

注2）ヒトの体細胞を培養しても無限には分裂できず，50～70回分裂すれば限界で，もはや分裂できない。いわゆる細胞寿命と考えられる。

注3）遺伝的早期老化症候群の略。老化現象の促進（小児期，若年期など早期に老化現象が出現，進行）と短命を呈する。ウェルナー症候群，ハッチンソン・ギルフォード症候群（プロジェリア），コケイン症候群の3疾患が含まれる。40歳～64歳のヒトが介護保険の認定を受ける際に必要な特定疾患の1つである。

注4）老化現象の発現，進行，あるいは，抑制に関わる「老化関連遺伝子」，高齢者に多い高血圧，虚血性心疾患などの疾病と関係する「老年病関連遺伝子」，長生きと関係

する「長寿遺伝子」などが，老化遺伝子として研究されている。アルツハイマー病に対するアポリポタンパクE遺伝子のように，すでにその関係が確立されたものもある。

注5）テロメアは，染色体の両末端にある保護構造であり，細胞分裂によりDNA複製が行われる度に短縮し，一定の長さ以下になると細胞は分裂を停止してしまう。そのためテロメアは「細胞内時計」，「分裂時計」などといわれ，細胞寿命を規定するものとして注目されている。

注6）不要な細胞や危険な細胞が，自発的に死亡していく現象。「プログラムされた細胞死」，「細胞の自然死」，「自発的な細胞死」などといわれる。生物の発生過程においては，決まった時点と部位で細胞死が起こり形態が変化する。感染細胞など生命に危険な細胞は，自らプログラムを起動して自滅するように死んでいく。これらの現象は，アポトーシスの例である。

注7）フリーラジカルは，生体が酸素を消費すると産生され，他の物質に作用して過酸化物を生じ，この蓄積が老化につながると考えられる。

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2．自転車運動

本項では，サイクリングエルゴメトリを用いた全身持久性体力の簡易評価法として，①心拍数を利用した方法，②自覚的運動強度を利用した方法を紹介する．

1）心拍数を利用した方法

心拍数を利用した先駆的な方法としては，Sjostrand（1947）と Wahlund（1948）が提唱した PWC（physical work capacity）テストと Åstrand and Ryhming[1]のノモグラムを利用する方法（以下，Åstrand and Ryhming 法とする）があげられる．その後，Åstrand and Ryhming 法は Siconolfi ら[2]の年齢補正によって推定精度が向上した．一方，日本において，宮下ら[3]は PWC テストに一部修正を加え PWC 75% HRmax テストを提案することで適用範囲を若年層から中高年者にまで広げた．また，田中ら（1989）は，運動中心の心拍数変動フィードバック管理により運動負荷を個人ごとに制御するシステム開発し，同システムによって評価される aerobic score（AS）は $\dot{V}O_2$ max および $\dot{V}O_{2LT}$ と強い相関関係にあったと報告している．本項では，以上で述べた方法のうち Åstrand and Ryhming 法，Siconolfi らの方法，PWC 75% HRmax テストの 3 つの方法について概説した．

①Åstrand and Ryhming 法

Åstrand and Ryhming 法において利用することのできる運動様式は，ステップ運動，トレッドミル走およびサイクリングの 3 種類であるが，ここでは，サイクリングによるテスト方法について述べてみる．この方法は，最大下運動中の心拍数および物理的仕事量（酸素摂取量）から独自のノモグラム（図 1－5）を利用するものである．心拍数が男性で 128 ～ 154 拍/min，女性で 138 ～ 164 拍/min となる物理的仕事量を設定し，6 分間のサイクリングテストをおこなわせる中で最後の 1 分間の心拍数を利用する．最後の 1 分間の心拍数とその時点の物理的仕事量をノモグラム上の各軸にプロットし，2 点を結んだ直線が $\dot{V}O_2$max 軸と交わる点が推定された $\dot{V}O_2$ max（l/min）である．この方法の開発に用いられた対象者は運動習慣のある若年男性であったため，中年者への適用は注意を要する．

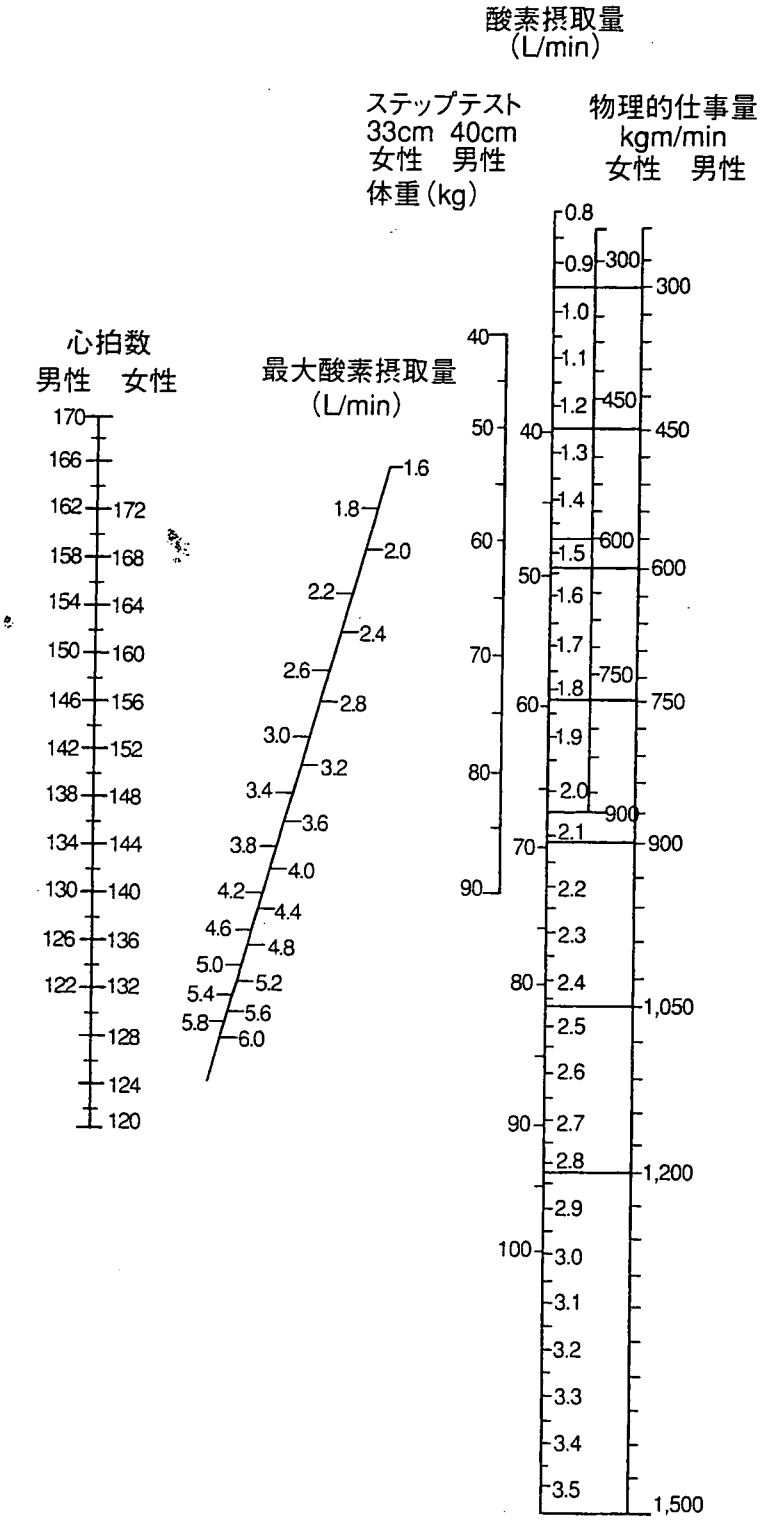

図1－5　Åstrand and Ryhming のノモグラム[4]

② Siconolfi らの方法

Åstrand and Ryhming 法の対象者の年齢に関する問題点を改善するために，Siconolfi らは，推定される $\dot{V}O_2max$ の値をより実測値に近づけるため

に年齢修正を加えた以下の補正式を考案した.

男性用　$\dot{V}O_2max$（1/min）＝0.348×推定値－0.035×年齢＋3.011

女性用　$\dot{V}O_2max$（1/min）＝0.302×推定値－0.019×年齢＋1.593

この補正式により算出された値と実測値との相関係数は男性 r＝0.86, 女性 r＝0.97 であった. 推定標準誤差（SEE）は男性 0.359 l/min, 女性 0.199 l/min であり, 精度の高い推定が可能と述べられている. 竹島ら(1992)は, 両者の妥当性を比較検討した結果, Siconolfi らの方法が Åstrand and Ryhming 法よりも優れると報告している.

③ PWC 75% HRmax テスト

この方法では, 対象者は自転車エルゴメータ上にて数分間の安静を保った後, 3分間ずつの漸増法によるサイクリングテストを9分間おこなう. 物理的仕事量は 25 watts より開始し, 運動終了時の心拍数が, 性・年齢別推定最高心拍数の 70 % を超えない程度となるように, 第2, 第3の物理的仕事量を設定する. 運動終了後, 各物理的仕事量とそのステージにおける最後の1分間の心拍数との関係を, 最小2乗法を用いて1次回帰し, 推定最高心拍数の 75 % に相当する心拍数となるときの物理的仕事量を外挿して求める. この値を PWC 75% HRmax とする.

宮下らはこのような方法で男性 151 名, 女性 275 名を測定し, それに基づく全身持久性体力評価表（表1－3）を作成した. この評価表の妥当性は, 後に異なる集団（362 名）への適用によって確認されている.

2）自覚的運動強度を利用した方法

RPE は, Borg（1973）の提唱した「運動刺激に対して受容された身体全体または各部位における感覚の統合的心理反応を7種類の簡易な言語記述子〔“楽である（light)”や“きつい（hard)”など〕にて15段階に尺度化された表」のことを指している. Robertson ら[5]は, RPE_{15} に相当する物理的仕事量（PWC_{R15}）を指標とした全身持久性体力の相対的評価法を報告している. 日本においては大藏ら（1998）が最大下多段階漸増負荷サイクリングテスト中に記録される RPE を利用することで $\dot{V}O_2max$ や $\dot{V}O_{2AT}$ を推定する方法を提案している. ここでは, これら2つの方法について概説す