TABLE 1. Baseline Characteristics of Study Participants According to ZBI Score Quartile of Caregivers

	Quartile Group of Caregiver ZBI Score					
	1st, Score: 0–15, n = 284	2nd, Score: 16–26, n = 253	3rd, Score: 27–39, n = 269	4th, Score: 40–84, n = 261	*F*	p
Care recipients (n = 1067)						
Men/women, N (% of men)	89/195 (31.3)	88/165 (34.8)	100/169 (37.2)	110/151 (42.1)		0.065
Age, M (SD), year[a]	81.0 (7.1)	81.1 (7.7)	81.2 (7.8)	80.8 (8.5)	0.10	0.962
Basic ADL (rage: 0–20), *M* (SD)[a]	14.2 (6.1)	12.5 (6.2)	11.0 (6.5)	10.4 (6.3)	20.07	<0.001
Charlson comorbidity index, *M* (SD)[a]	1.8 (1.5)	2.2 (1.5)	2.3 (1.5)	2.4 (1.7)	7.06	<0.001
GDS-15 (range: 0–15), *M* (SD)[a,b]	5.4 (3.4)	6.2 (3.2)	6.6 (3.4)	8.1 (3.7)	21.19	<0.001
No. of service uses (range: 0–7), *M* (SD)[a]	2.0 (1.1)	2.2 (1.2)	2.4 (1.3)	2.5 (1.3)	8.97	<0.001
Presence of chronic disease, no. (%)						
Ischemic heart disease	36 (12.7)	31 (12.3)	36 (13.4)	29 (11.1)		0.882
Congestive heart failure	17 (6.0)	20 (7.9)	22 (8.2)	27 (10.3)		0.321
Cerebrovascular disease	93 (32.7)	110 (43.5)	120 (44.6)	111 (42.5)		0.016
COPD	11 (3.9)	11 (4.3)	14 (5.2)	18 (6.9)		0.400
Dementia	66 (23.2)	92 (36.4)	135 (50.2)	135 (51.7)		<0.001
Cancer	29 (10.2)	22 (8.7)	14 (5.2)	28 (10.7)		0.098
Presence of behavioral problems, no. (%)	8 (2.8)	11 (4.3)	21 (7.8)	26 (10.0)		0.002
Caregiver variables (n = 1067)						
Men/women, no. (% of men)	71/213 (25.0)	69/184 (27.3)	56/213 (20.3)	60/201 (23.0)		0.350
Age, *M* (SD), year[a]	64.1 (13.0)	65.4 (12.2)	63.5 (12.6)	65.8 (11.3)	2.05	0.106
GDS-15 (range: 0–15), *M* (SD)[a,c]	3.4 (3.0)	4.7 (3.2)	5.8 (3.5)	8.5 (3.4)	107.98	<0.001
Relationship to care recipient, no. (%)						
Spouse	115 (40.5)	119 (47.0)	106 (39.4)	128 (49.0)		0.061
Nonspouse	169 (59.5)	134 (53.0)	163 (60.6)	133 (51.0)		
Health status, no. (%)						
Good to excellent	150 (52.8)	101 (39.9)	98 (36.4)	80 (26.2)		<0.001
Fair	103 (36.3)	127 (50.2)	148 (55.0)	169 (55.4)		
Poor	31 (10.9)	25 (9.9)	23 (8.6)	56 (18.4)		
ZBI score (range: 0–88), *M* (SD)[a]	9.4 (4.7)	21.0 (3.1)	32.6 (4.0)	52.5 (9.8)	2553.05	<0.001

Notes: *M*: mean; SD: standard deviation; COPD: chronic obstructive pulmonary diseasee.
[a]Analysis of variance for multiple comparisons was used to determine differences among the quartiles of the ZBI score for continuous variables ($df = 3,1063$ except for recipient GDS-15 [$df = 3, 804$] and caregiver GDS-15 [$df = 3,962$]), and the Pearson χ^2 test was used to test categorical variables ($df = 3$).
[b]n = 808.
[c]n = 966.

TABLE 2. Adverse Events During 3-year Period According to the Quartile Group of ZBI Score

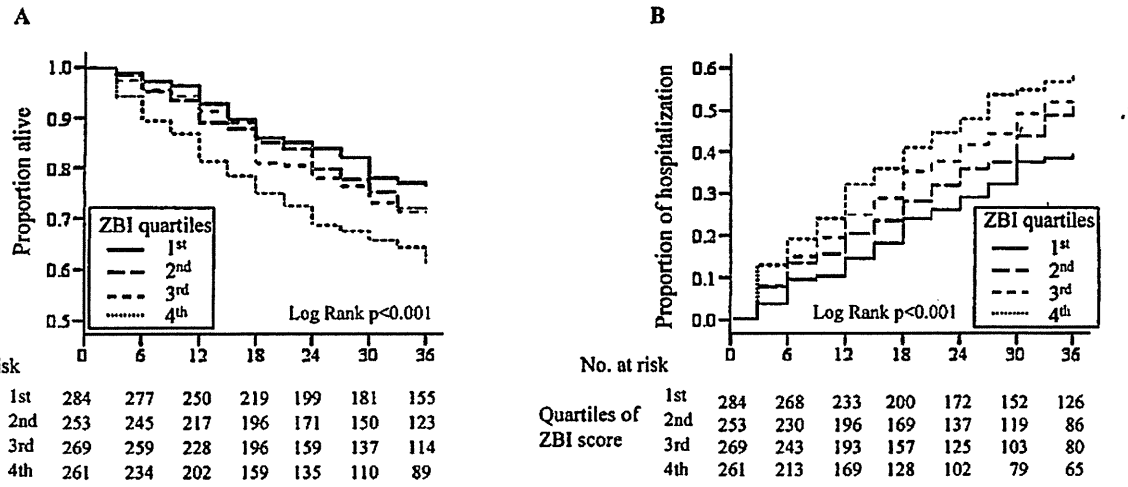
	Quartile Group of ZBI Score					
	1st, n = 284	2nd, n = 253	3rd, n = 269	4th, n = 261	Total, n = 1,067	p[a]
Adverse outcomes, no. (% of each quartile)						
All-cause death	58 (20.4)	63 (24.9)	64 (23.8)	83 (31.8)	268 (25.1)	0.020
Hospitalization	98 (34.5)	111 (43.9)	119 (44.2)	127 (48.7)	455 (42.6)	0.007

[a]Pearson χ^2 test. Degree of freedom is equal to 3.

test, $\chi^2 = 1.52$, $df = 1$, p = 0.218, and HR: 1.02; 95% CI: 0.98–1.05, Wald $\chi^2 = 1.072$, $df = 1$, p = 0,301, respectively; hospitalization: HR: 1.02; 95% CI: 0.99–1.05; Wald χ^2 test, $\chi^2 = 1.54$, $df = 1$, p = 0.215, and HR: 1.01; 95% CI: 0.99–1.04; Wald $\chi^2 = 0.81$, $df = 1$, p = 0.369, respectively).

As shown in Table 3, multivariate adjustment for confounders, including recipient gender and age, bADL score, number of community-based services used, the Charlson comorbidity index, caregiver gender and age, presence or absence of behavioral problems (only for all-cause mortality analysis), and the subjective health status of the caregiver (only for hospitalization analysis), showed that the highest quartile of caregivers' ZBI scores (compared with the lowest quartile) was associated with a 1.54-fold risk

FIGURE 1. Kaplan–Meier Plot for Probability of Event-Free Survival (A) and Probability of Hospital Admission (B) According to Increasing Quintiles of the Zarit Burden Interview Score



No. at risk

Quartiles of ZBI score								
	1st	284	277	250	219	199	181	155
	2nd	253	245	217	196	171	150	123
	3rd	269	259	228	196	159	137	114
	4th	261	234	202	159	135	110	89

No. at risk

Quartiles of ZBI score								
	1st	284	268	233	200	172	152	126
	2nd	253	230	196	169	137	119	86
	3rd	269	243	193	157	125	103	80
	4th	261	213	169	128	102	79	65

Log-rank χ^2 test, mortality (A): $\chi^2 = 17.29$, $df = 3$, $p < 0.001$; hospitalization (B): $\chi^2 = 23.61$, $df = 3$, $p < 0.001$.

of all-cause death and a 1.51-fold risk of recipient hospitalization. When the analyses were conducted using the ZBI score as a continuous variable, the recipients who had caregivers with higher ZBI scores were associated with higher risk of mortality and hospitalization (HR: 1.01, 95% CI: 1.00–1.02, Wald χ^2 test, $\chi^2 = 6.92$, $df = 1$, $p = 0.009$, and HR: 1.01, 95% CI: 1.00–1.01, Wald $\chi^2 = 8.86$, $df = 1$, $p = 0.003$, respectively). The HRs of the top quartile were similar when the comorbidity index score was replaced with the presence or absence of chronic diseases that were identified as risk factors by univariate analysis in each event (Table 3).

Based on ANCOVA adjusted for recipient gender, age, bADL score, the Charlson comorbidity index, the presence or absence of dementia and behavior problems, caregiver gender, and caregiver age, no differences in the adjusted average ZBI scores were detected between users and nonusers of these services (ANCOVA, adjusted mean ZBI score (standard deviation): day-care service, nonuse, 27.9 (16.4) versus use, 29.3 (16.5), F value = 1.92, df = 1,1057, $p = 0.166$; home-help service: nonuse, 29.0 (16.4) versus use, 27.9 (16.4), F value = 1.06, $df = 1$, 1057, $p = 0.304$) except for the nursing-home respite service, for which users showed higher ZBI scores than nonusers (nonuse, 28.1 [16.4], versus use, 32.1 [16.8], F value = 5.26, $df = 1$, 1057, $p = 0.022$).

In Table 4, using the multivariate Cox proportional hazards model, we examined the association between higher versus lowest quartile of the ZBI score and care recipient all-cause mortality and hospitalization within subgroups of various community-based respite service use status. Overall, within nonusers of these respite care services, the highest quartile of caregiver burden was associated with all-cause mortality and hospitalization. No apparent association was observed within users of these services except for users of the day-care service, who showed a statistically significant association between the highest quartile and the risk of hospitalization (HR: 1.56, 95% CI: 1.03–2.36, Wald χ^2 test, $\chi^2 = 4.50$, $df = 1$, $p = 0.034$).

DISCUSSION

In the present study, we observed that the recipients with caregivers with a baseline ZBI score in the highest quartile were 1.54 and 1.51 times more likely to show increased risk of all-cause mortality and hospitalization during a 3-year follow-up period, respectively, in comparison with those with caregivers in the lowest. These relationships existed independently of various other risk factors for mortality and hospitalization, including gender, age, number of community-based services used, ADL status, and

TABLE 3. Multivariate Cox Proportional Hazards Models and Association Between Baseline Characteristics and Risk of Mortality and Hospitalization During 3-year Follow-up

	All Death						Hospitalization					
	Model 1[a]			Model 2[b]			Model 1[c]			Model 2[d]		
	Wald χ^2	p	HR (95% CI)	Wald χ^2	p	HR (95% CI)	Wald χ^2	p	HR (95% CI)	Wald χ^2	p	HR (95% CI)
Care recipient variables												
Men (versus women)	22.41	<0.001	1.98 (1.49–2.63)	24.25	<0.001	2.06 (1.54–2.75)	3.39	0.065	1.23 (0.99–1.54)	3.05	0.081	1.22 (0.98–1.52)
Age[e]	66.21	<0.001	1.07 (1.05–1.09)	45.71	<0.001	1.06 (1.04–1.08)	6.08	0.014	1.02 (1.00–1.03)	3.24	0.072	1.01 (1.00–1.03)
Basic ADL score[e]	26.01	<0.001	0.94 (0.92–0.96)	32.04	<0.001	0.93 (0.91–0.96)	7.89	0.005	0.97 (0.96–0.99)	12.95	<0.001	0.97 (0.95–0.99)
No. of service uses[e]	0.84	0.360	0.94 (0.83–1.05)	0.90	0.343	0.93 (0.83–1.05)	2.28	0.131	1.07 (0.97–1.17)	1.75	0.186	1.06 (0.97–1.16)
Charlson comorbidity index[e]	8.84	0.003	1.12 (1.04–1.22)			—	2.13	0.145	1.05 (0.98–1.12)		—	
Chronic disease (versus absence)												
Congestive heart failure		—		5.16	0.023	1.54 (1.06–2.23)		—			—	
COPD		—				—		—		3.090	0.079	1.40 (0.96–2.03)
Dementia		—		4.298	<0.001	1.33 (1.02–1.74)		—			—	
Cancer		—		14.64	<0.001	1.99 (1.40–2.82)		—		18.10	<0.001	1.84 (1.39–2.44)
Behavioral problems (versus absence)	0.20	0.657	1.11 (0.72 to 1.70)	0.23	0.629	1.12 (0.72–1.74)		—			—	
Caregiver's variables												
Men (versus women)	0.03	0.863	1.02 (0.74–1.42)	0.01	0.984	0.99 (0.71–1.38)	0.01	0.942	0.99 (0.77–1.27)	0.13	0.714	0.95 (0.74–1.22)
Age[e]	0.36	0.551	1.00 (0.99–1.02)	0.47	0.492	1.00 (0.99–1.02)	0.07	0.788	1.00 (0.99–1.01)	0.04	0.847	1.00 (0.99–1.01)
Subjective caregiver health status (versus good to excellent)												
Fair		—				—	2.95	0.086	1.21 (0.97–1.50)	2.52	0.113	1.19 (0.96–1.48)
Poor		—				—	2.99	0.084	1.32 (0.96–1.82)	2.69	0.101	1.31 (0.95–1.80)
ZBI score (versus 1st quartile)												
2nd	0.13	0.717	1.07 (0.75–1.53)	0.23	0.629	1.09 (0.76–1.57)	2.06	0.151	1.22 (0.93–1.61)	2.51	0.113	1.25 (0.95–1.64)
3rd	0.01	0.942	1.01 (0.71–1.46)	0.06	0.809	1.05 (0.73–1.51)	2.93	0.087	1.27 (0.97–1.68)	3.95	0.047	1.32 (1.00–1.75)
4th	5.80	0.016	1.54 (1.09–2.17)	4.44	0.035	1.45 (1.03–2.05)	8.62	0.003	1.51 (1.15–1.98)	8.29	0.004	1.50 (1.14–1.97)

Notes: Degree of freedom is equal to 1. COPD: chronic obstructive pulmonary disease.

[a]Model 1 included gender, age, bADL score, number of community-based services used, regular medical checkups, Charlson comorbidity index, behavioral problems, caregiver's age and gender, the Zarit categories that are associated with mortality in univariate analysis.

[b]Model 2 for analysis of all-death, which included variables used in Model 1 plus presence or absence of heart failure, dementia, and cancer, which are associated with all-death in univariate analysis, instead of Charlson comorbidity index.

[c]Model 1 included gender, age, bADL score, number of community-based services used, regular medical checkups, Charlson comorbidity index, caregiver's age and gender, subjective caregiver health status, and the Zarit categories that are associated with mortality in univariate analysis.

[d]Model 2 for analysis of hospitalization, which included variables used in Model 1 plus presence or absence of cancer and COPD, which are associated with all-death in univariate analysis, instead of Charlson comorbidity index.

[e]Continuous variables.

TABLE 4. Subgroup Cox Hazard Analysis According to Quartiles of the ZBI Score

Use or Nonuse	No. of Case Total	Quartile Group of ZBI Score (1st: Reference) 2nd Wald χ^2	p	HR (95% CI)	3rd Wald χ^2	p	HR (95% CI)	4th Wald χ^2	p	HR (95% CI)
All death[a]										
Day-care service										
Nonuse	165/573	1.03	0.310	1.26 (0.81-1.96)	0.07	0.791	0.95 (0.58-1.54)	5.06	0.024	1.66 (1.07-2.59)
Use	103/494	0.31	0.579	0.84 (0.45-1.58)	0.15	0.695	1.12 (0.64-1.97)	0.75	0.387	1.29 (0.72-2.31)
Home-help service										
Nonuse	155/618	0.76	0.382	1.25 (0.76-2.05)	0.16	0.688	1.11 (0.67-1.85)	12.7	<0.001	2.32 (1.46-3.69)
Use	113/449	0.10	0.757	0.92 (0.54-1.57)	0.23	0.633	0.88 (0.52-1.49)	0.63	0.426	0.80 (0.46-1.39)
Nursing-home respite stay service										
Nonuse	237/959	0.34	0.558	1.12 (0.76-1.65)	0.09	0.768	1.06 (0.72-1.56)	9.75	0.002	1.79 (1.24-2.58)
Use	31/108	0.42	0.517	0.70 (0.24-1.79)	0.77	0.381	0.63 (0.23-1.76)	1.86	0.173	0.46 (0.15-1.41)
Hospitalization[b]										
Day-care service										
Nonuse	250/573	1.72	0.189	1.27 (0.89-1.82)	3.46	0.063	1.43 (0.98-2.10)	5.07	0.024	1.54 (1.06-2.24)
Use	205/494	0.86	0.353	1.23 (0.80-1.88)	0.93	0.334	1.22 (0.81-1.84)	4.50	0.034	1.56 (1.03-2.36)
Home-help service										
Nonuse	260/618	3.56	0.059	1.45 (0.99-2.14)	4.88	0.027	1.54 (1.05-2.25)	18.00	<0.001	2.25 (1.55-3.27)
Use	195/449	0.01	0.945	1.01 (0.68-1.51)	0.01	0.930	1.02 (0.67-1.54)	0.40	0.528	0.87 (0.57-1.33)
Nursing-home respite stay service										
Nonuse	400/959	2.30	0.130	1.25 (0.94-1.67)	2.09	0.148	1.24 (0.93-1.67)	9.32	0.002	1.57 (1.18-2.10)
Use	55/108	0.21	0.646	1.26 (0.47-3.34)	0.65	0.419	1.43 (0.60-3.41)	0.02	0.891	1.06 (0.44-2.56)

Notes: Multivariate Cox proportional hazard models. Degree of freedom for all variables is equal to 1.
[a]Model included gender, age, basic activities of daily living (bADL) score, number of community-based services used, Charlson comorbidity index, behavioral problems, caregiver's age and gender, and the Zarit categories.
[b]Model included gender, age, bADL score, number of community-based services used, Charlson comorbidity index, caregiver's age and gender, subjective caregiver health status, and the Zarit categories.

comorbidity. To our knowledge, this is the first report addressing the relationships between caregiver burden and mortality or hospitalization for dependent older care recipients living in the community.

In addition, subgroup analysis revealed that the association between a high caregiver burden and adverse health outcomes of care recipients was mainly observed in nonusers of community-based respite services, including day-care, home help, or nursing-home respite stay services. No association was found between high caregiver burden and adverse health outcomes of care recipients among users of these services except for users of day-care services with a hospitalization risk.

There are a number of possible mechanisms for these associations. Previous research has found caregiver burden to be a factor in determining the quality of care given and, specifically, a negative indicator of the willingness of caregivers to continue in the caregiving role.[2,20] The caregiver burden may lead to a lower quality of care, leading over time to abuse or neglect and, ultimately, to negative health outcomes for the care recipient.[21,22] In fact, it has been demonstrated that a lack of needed care for disabled older individuals or a decreased quality of family caregiving results in poor outcomes for care recipients.[23–25] Thus, caregiver burden and emotional distress can be a detriment to the health and well-being of care recipients through inadequate provision of care.

In this study, we demonstrated that the GDS-15 score of the caregiver as well as the care recipient increased as the level of the ZBI quartile increased. These results may indicate that caregiver burden is associated with depressive symptoms in the care recipients and that there may be interrelationships between the emotional distress of the caregiver and depressive symptoms in care recipients. A number of reports have suggested that depressive symptoms have been shown to be an important risk factor for mortality and to increase the risk of physical disability through poorer adherence to healthy life styles.[26–28] However, the GDS-15 scores of care recipients were not associated with all-cause mortality and hospitalization in this cohort. There are several possible reasons for this difference between our cohort and others. The subjects of the current

investigation had multiple medical problems and functional limitations and were probably at higher mortality and hospitalization risk than those in these prior studies.[26–28]

It has been demonstrated that the death or serious illness of a spouse increases the risk of death or affects the health of a partner.[29,30] A possible mechanism of this association is that spousal illness or death may deprive a partner of social, emotional, or other practical support.[29,30] In the present study, subjective poor health status of the caregiver was associated with risk of hospitalization for the care recipient but not with mortality in this cohort. The highest level of caregiver burden was associated with hospitalization for dependent elderly care recipients, even after adjustment for the subjective health status of the caregivers, making it unlikely that our findings were confounded by the poor health status of the caregiver, at least at baseline.

Community-based long-term care services are believed to relieve stress on family caregivers and enable older people with disabilities to remain at home for a longer period of time.[31] However, the usefulness of these services for reducing caregiver burden is still controversial. Some studies have demonstrated a positive effect of respite service on caregiver burden, but others have shown no effect or a negative relationship between respite service use and caregiver burden.[32–36] In our cohort, no difference in the caregiver ZBI score was observed between users and nonusers of day-care services and home-help services, and a rather higher average ZBI score in users of nursing-home respite stay services compared with nonusers was observed, although cross-sectional determination of respite care service use and the ZBI measurement do not allow evaluation of the causal and consequent relationships between service use and caregiver burden. However, the present study found that the adverse outcome for care recipients with caregivers with the highest burden is more evident in nonusers of respite services than in service users. It is possible that the use of these long-term care services decreased the adverse health outcomes of care recipients through other factors beyond caregiver burden.

Our study has several strengths, including the relatively large number of paired participants and outcome events, a prospective design, and a well-defined population. Our analyses took into account potential confounders including age, gender, bADL, comorbidity, and subjective health status of the caregiver. We also adjusted for the number of community-based services used and conducted an analysis stratified by the use or nonuse of community-based respite care services.

This study has potential limitations. Subjects with acute illness at enrollment were excluded from participating in the NLS-FE, and the present study used statistical control of potential confounding variables to rule out third factors that might produce an association between caregiver burden and care recipient adverse health outcomes during the follow-up period. However, because of the observational design of the present study, differences in unmeasured factors, including social circumstances, caregiver's competence in caring for a disabled recipient, the health condition of the caregivers during the study period not at baseline, and the length of caregiving may in part account for the findings. We used only the presence or absence of selected major comorbidities and behavioral problems as covariates in the analyses. The lack of assessment of the severity of the recipient's medical illness or significant behavioral problems, both of which would require more time for care providing, may have influenced the results in the present study. The present findings may not be generalizable to other populations, given that health practices, a variety of social and economic factors, ethnic attitudes about caring for very old people, and the cost of healthcare may have influenced these results. It should be noted that multiple analyses in the present study increased the chances of making high likelihood of Type I errors.

We demonstrated high caregiver burden as an important risk of the adverse health outcomes of care recipients, including all-cause mortality and hospitalization. This risk of care recipient adverse health outcomes associated with a heavy caregiver burden was attenuated in community-based respite care service users. In the community setting, interventions directed toward the reduction of caregiver burden and improving caregiver well-being may not only delay long-term care placement and prevent the deterioration of caregiver health but also reduce care recipient adverse health outcomes. A community-based service may thus yield benefits for care recipients and may favorably affect the complex and interrelated variables of the caregiver and the recipient. These efforts may facilitate the continuation of home care of the disabled elderly.

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ORIGINAL ARTICLE

Dietary patterns of antioxidant vitamin and carotenoid intake associated with bone mineral density: findings from post-menopausal Japanese female subjects

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Abstract
Summary Recent studies show that antioxidants may reduce the risk of osteoporosis. This study showed the associations of bone mineral density with dietary patterns of antioxidant vitamins and carotenoids. The findings suggest the combination of vitamin C and β-cryptoxanthin intakes might provide benefit to bone health in post-menopausal Japanese female subjects.
Introduction Recent epidemiological studies show antioxidants may reduce the risk of osteoporosis, but little is known about the dietary patterns of antioxidant vitamin and carotenoid intakes and their relation with bone mineral density (BMD).
Methods A total of 293 post-menopausal female subjects 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. Dietary intakes of antioxidant vitamins and carotenoids were assessed by using a validated food-frequency questionnaire. Dietary patterns were identified on a selected set of antioxidants through principal component factor analysis.
Results Three dietary patterns were identified. The "retinol" pattern, characterized by notably high intakes of preformed retinol, zeaxanthin, and vitamin E, was positively associated with the risk for low BMD. In contrast, the "β-cryptoxanthin" pattern, characterized by notably high intakes of β-cryptoxanthin and vitamin C, was negatively associated with low BMD. The odds ratios for low BMD in the highest tertiles of dietary intakes of preformed retinol, vitamin C, and β-cryptoxanthin against the lowest tertiles were 3.22 [95% confidence interval (CI), 1.38–7.51], 0.25 (CI, 0.10–0.66), and 0.40 (CI, 0.17–0.92), respectively, after adjustments for confounders. However, negative associations of vitamin C and β-cryptoxanthin with low BMD were not significant after further adjustment for intake of β-cryptoxanthin or vitamin C, respectively. Higher intakes of both vitamin C and β-cryptoxanthin were significantly associated with low BMD ($P<0.05$).
Conclusions The combination of vitamin C and β-cryptoxanthin may be associated with radial BMD in post-menopausal Japanese female subjects.

Keywords Bone mineral density · Carotenoid · Dietary pattern · Preformed retinol · Vitamin

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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 mineral 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]. Recent epidemiological studies have shown an association between fruit and vegetable intake and bone mineral density (BMD) in both young and elderly subjects [5–10].

Fruits and vegetables are rich sources of antioxidant vitamins and carotenoids, which have been shown to contribute to the body's defense against reactive oxygen species [11, 12].

Recent animal experiments and in vitro studies have shown that reactive oxygen species and free radicals are involved in osteoclastogenesis, in apoptosis of osteoblasts and osteocytes and therefore also in bone resorption [13–15]. Furthermore, recent epidemiological studies have shown a relationship between oxidative stress and BMD or osteoporosis [16–18]. These previous findings in epidemiological and experimental studies suggest that antioxidant micronutrients may provide benefits to bone metabolism against oxidative stress. In fact, recent epidemiological studies have reported inverse associations of antioxidant vitamin and carotenoid intake and/or serum level with low BMD, risk of fracture, and/or osteoporosis [19–24]. Very recently, we found that serum concentrations of carotenoids such as β-cryptoxanthin and β-carotene were weakly but positively associated with radial BMD in post-menopausal female subjects [25]. Therefore, antioxidant vitamins and carotenoids may be beneficial to the maintenance of bone health.

With regard to antioxidant vitamins and carotenoids, most studies have focused on a single antioxidant and examined the relationship between antioxidant intake and/or serum level and the status of bone health. However, these common approaches may not adequately account for the complicated interactions of these antioxidants because people consume diets consisting of a variety of foods with complex combinations of antioxidants rather than single antioxidant. Furthermore, it is unclear whether the beneficial effects of these antioxidants on bone health are synergistic or additive. To answer such questions, the identification of dietary patterns using factor analysis has been widely used to elucidate the relationship between diet and disease. This type of statistical analysis allows the development of appropriate recommendations for overall dietary habits.

The objective of this study was to identify the dietary patterns of antioxidant vitamin and carotenoid intake associated with radial BMD in post-menopausal Japanese female subjects and to investigate the association of interactions of these antioxidants with bone health. The dietary patterns of antioxidant vitamin and carotenoid intake with 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. In total, 1,369 males and females (72.4% of total subjects), ranging in age from 30 to 70 years, had received the health check-up through the 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 committees of the National Institute of Fruit Tree Science and the Hamamatsu University School of Medicine. For the present study, we used the data of post-menopausal female subjects because, in our previous study, we had found inverse associations of serum antioxidant carotenoids with risk for low BMD in post-menopausal female subjects [25].

Bone mineral density measurement

The radial BMD was measured using dual-energy X-ray absorptiometry (DXA) of each participant's nondominant 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 technologists 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, medications and/or hormone use, and lifestyle, including tobacco use (current smoker, ex-smoker, or non-smoker), exercise (1+ times per week), regular alcohol intake (1+ time per week), dietary supplement use (non-user, occasional-user, and current-user), and dietary habits. Diet

was assessed with a modified validated simple food-frequency questionnaire (FFQ) developed especially for the Japanese [26, 27]. 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 an individual's food and nutrient intake form FFQ data based on "Standard tables of food composition in Japan" [28, 29].

The dietary carotenoid intakes of each individual were computed to obtain the amount of six carotenoids, lycopene, α-carotene, β-carotene, lutein, β-cryptoxanthin, and zeaxanthin using a published database of the carotenoid composition of fruit and vegetables [30, 31]. In our survey, we calculated an individual's carotenoid intake from important sources of carotenoids. In this data analysis, the dietary carotenoid intakes were calculated from the FFQ data of individual food items not dishes [32].

The dietary intakes of total energy, calcium, potassium, magnesium, vitamins C, D, and E, preformed retinol, lycopene, α-carotene, β-carotene, lutein, β-cryptoxanthin, and zeaxanthin of each subject were used in this report.

Statistical analyses

For this study, the following subjects were excluded from the data analyses: (1) those who reported a history of osteoporosis or taking medications for bone metabolism in the self-administered questionnaire (n=14); (2) those for whom the self-administered questionnaire data were incomplete (n=1); and (3) those for whom blood samples for serum-carotenoid analysis were not collected (n=1). As a result, a total of 293 post-menopausal female subjects were included in further data analysis.

Intakes of preformed retinol, vitamins C, D, and E, and six carotenoids were skewed toward the higher concentrations. These values were loge (natural)-transformed to improve the normality of their distribution. All variables were presented as an original scale. The data are expressed as means (standard deviation), geometric mean (95% confidence interval), range, or percent.

A principal component analysis was used to derive the dietary patterns on the basis of the intakes of nine antioxidant vitamins and carotenoids obtained from the FFQ. To identify the number of factors to be retained, we used the criterion of eigenvalues>1.0, the most widely used criterion in factor analysis. Finally, we decided to retain three factors for further analysis. We applied a varimax rotation to the factor-loading matrix to achieve a simpler structure with greater interpretability. After the varimax rotation, the factor scores for each subject were saved from the principal component analysis. The factor-loading matrix represents correlation coefficients between individual antioxidants and dietary patterns. The percentage of variance explained by each factor was calculated by dividing the sum of the squares of the respective factor loadings by the number of variables.

Participants were divided into three categories according to tertiles of factor scores. Low radial BMD was defined as the lowest quartile of the value among study participants, i.e., equal to or less than 0.501 g/cm^2 in post-menopausal female subjects. To assess the relationship between dietary patterns and low radial BMD, logistic regression analyses were performed using three models. In model 1, we adjusted for age, weight, and height. Model 2: Years since menopause, current tobacco use, regular alcohol intake, exercise habits, supplement use, and total energy intake were further adjusted. Model 3: Intakes of calcium, magnesium, potassium, and vitamin D were further adjusted. The goodness-of-fit for logistic regression model was evaluated by Hosmer–Lemeshow Goodness-of-Fit test, and then, we calculated odds ratios.

For dietary intake of each antioxidant vitamin and carotenoid, participants were further divided into three categories according to tertiles of antioxidant vitamin and carotenoid intake, and logistic regression analyses were performed to assess the relationship between antioxidant vitamin and carotenoid intake with low radial BMD.

All statistical analyses were performed using a statistical software package for Windows (SPSS ver. 17.0, SPSS Inc., Chicago, IL, USA) on a personal computer.

Results

Clinical, biochemical, and nutrient intake profiles of study subjects

Table 1 shows the characteristics of the study subjects. The mean radial BMD in post-menopausal Japanese female

Table 1 Characteristics of the study subject

	Post-menopausal female	
Number study subjects	293	
Age (years)	60.2	(6.2)
Body height (cm)	152.0	(5.5)
Body weight (kg)	51.9	(7.6)
Body mass index (kg/m2)	22.5	(3.0)
Bone mineral density (g/cm2)	0.561	(0.084)
Range		0.366–0.820
Intake		
Total energy including ethanol (MJ/day)	8.20	(2.01)
Total energy excluding ethanol (MJ/day)	8.15	(2.00)
Calcium (mg/day)	651	(256)
Potassium (mg/day)	2910	(967)
Magnesium (mg/day)	281	(81)
Retinol (μg/day)[a, b]	281	(259–305)
Vitamin C (mg/day)[b]	170	(161–179)
Vitamin D (μg/day)[b]	6.4	(5.9–6.9)
Vitamin E (mg/day)[b]	8.1	(7.8–8.4)
Lycopene (mg/day)[b]	0.15	(0.13–0.17)
α-Carotene (mg/day)[b]	0.25	(0.23–0.27)
β-Carotene (mg/day)[b]	1.86	(1.74–1.99)
Lutein (mg/day)[b]	2.06	(1.92–2.20)
β-Cryptoxanthin (mg/day)[b]	0.62	(0.52–0.73)
Zeaxanthin (mg/day)[b]	0.67	(0.61–0.73)
Current tobacco use (%)	1.7	
Exercise habits (%)[c]	21.5	
Regular alcohol intake (%)[c]	11.0	
Current supplement use (%)	9.6	

Data are mean (standard deviation), geometric mean (95% confidence interval)

[a] Preformed retinol

[b] These variables were represented as original scale after analysis by log

[c] ≥1 time per week

subjects was 0.561 g/cm^2. The percent of subjects with osteoporosis whose radial BMD was less than 70% of that of the young adult mean was 9.2% [33]. The mean daily intakes of calcium, potassium, magnesium, preformed retinol, and vitamins C, D, and E were at least comparable to the recommended dietary allowance. Of the six carotenoids analyzed, that with the highest intake was lutein; the second was β-carotene, and the lowest was lycopene. In our survey, 9.6% of study subjects used supplements, but most used multivitamin supplements. The rate of supplement users among study subjects for vitamin C and D, β-carotene, and calcium were 3.1%, 0.3%, 0.7%, and 5.5%, respectively. Therefore, we think that specific quantitative intakes of vitamin, carotenoid, and mineral from supplement were negligible compared with those from foods.

Principal component analysis of dietary patterns of antioxidant vitamin and carotenoid intake

The factor-loading matrices for the three retained factors are shown in Table 2. The high positive loadings indicate strong associations between given antioxidants and dietary patterns. Factor 1 had heavy loadings on β-carotene, α-carotene, lutein, lycopene, and vitamins E and C. This pattern was especially heavily loaded on carotenoids and was labeled the "Carotene" pattern. Factor 2 had heavy loadings on preformed retinol, zeaxanthin, vitamin E, lutein, vitamin C, and β-carotene. This pattern, heavily loaded on preformed retinol, zeaxanthin, and vitamin E, was labeled the "Retinol" pattern. Factor 3 had heavy loadings on β-cryptoxanthin, vitamin C, β-carotene, lutein, and vitamin E. This pattern, heavily loaded on β-cryptoxanthin and vitamin C, was labeled the "β-cryptoxanthin" pattern. Overall, the three dietary patterns accounted for 73.1% of the variance in antioxidant vitamin and carotenoid intake.

Odds ratio of low radial BMD in the highest group of factor scores of each dietary pattern

The odds ratios of low radial BMD associated with the tertiles of factor scores of each of the three dietary patterns after adjustments for confounding factors are shown in Table 3. The odds ratios for the risk of low radial BMD in the highest tertile of factor scores against the lowest tertile used for the reference group were calculated. In the

Table 2 Factor-loading matrix for the three dietary patterns of antioxidant vitamins and carotenoid intakes identified among 293 post-menopausal Japanese female subjects

	Factor 1: carotene	Factor 2: retinol	Factor 3: β-cryptoxanthin
Retinol[a]		0.825	
Vitamin C	0.435	0.285	0.773
Vitamin E	0.464	0.711	0.258
Lycopene	0.633		
α-Carotene	0.788		
β-Carotene	0.852	0.257	0.369
Lutein	0.740	0.447	0.270
β-Cryptoxanthin			0.920
Zeaxanthin		0.712	
Percentage of variance (%)	30.3	22.8	20.1

Data for 293 subjects from the self-administered food-frequency questionnaire. Absolute values <0.25 were excluded from the table for simplicity

[a] Preformed retinol

Table 3 The odds ratios (and 95% confidence intervals) of tertiles of three dietary patterns on low bone mineral density in post-menopausal Japanese female subjects

Dietary patterns	Factor score	Number	Model 1			Model 2			Model 3		
			OR	95% CI	*P* for trend	OR	95% CI	*P* for trend	OR	95% CI	*P* for trend
Factor 1: carotene	Lowest (Q1)	97	1.00			1.00			1.00		
	Middle (Q2)	98	0.83	(0.40–1.72)		0.94	(0.44–2.00)		1.14	(0.51–2.54)	
	Highest (Q3)	98	1.31	(0.65–2.64)	0.370	1.38	(0.66–2.89)	0.340	2.30	(0.93–5.70)	0.064
Factor 2: retinol	Lowest (Q1)	97	1.00			1.00			1.00		
	Middle (Q2)	98	1.16	(0.55–2.45)		1.35	(0.61–2.98)		1.08	(0.47–2.47)	
	Highest (Q3)	98	2.02	(0.99–4.09)	0.041	3.09	(1.28–7.47)	0.009	2.31	(0.90–5.89)	0.059
Factor 3: β-cryptoxanthin	Lowest (Q1)	97	1.00			1.00			1.00		
	Middle (Q2)	98	0.55	(0.26–1.16)		0.54	(0.25–1.18)		0.53	(0.24–1.17)	
	Highest (Q3)	98	0.26	(0.11–0.59)	0.001	0.22	(0.09–0.54)	0.001	0.30	(0.11–0.77)	0.017

Model 1: Age, weight and height were adjusted. Model 2: Years since menopause, current tobacco use, regular alcohol intake, exercise habits, supplement use, and total energy intake were further adjusted. Model 3: Intakes of calcium, magnesium, potassium, and vitamins D were further adjusted

"Carotene" pattern, there was no significant association between the factor score and low radial BMD. In the "Retinol" pattern, a significantly higher odds ratio was observed in the highest tertile of factor score after adjustments for age, years since menopause, weight, height, current tobacco use, regular alcohol intake, exercise habits, use of dietary supplements, and total energy. However, this significant association was not observed after further adjustments for intake of calcium, magnesium, potassium, and vitamin D. On the other hand, in the "β-cryptoxanthin" pattern, a significantly lower odds ratio was observed in the highest tertile of factor scores after multivariate adjustment.

Odds ratios of low radial BMD in the highest group of antioxidant vitamin and carotenoid intake

The odds ratios for the risk of low radial BMD associated with the tertiles of daily intakes of each antioxidant vitamin and carotenoid after adjustments for confounding factors are shown in Table 4. A significantly higher odds ratio was observed in the highest tertile of preformed retinol intake after adjustments for age, weight, and height. This significant association was also observed after multivariate adjustments. Similarly, a significantly higher odds ratio was observed in the highest tertile of zeaxanthin intake after adjustments for age, years since menopause, weight, height, current tobacco use, regular alcohol intake, exercise habits, use of dietary supplements, and total energy, but this significant association was not observed after further adjustments for intakes of calcium, magnesium, potassium, and vitamin D. In contrast, a significantly lower odds ratio was observed in the highest tertile of vitamin C intake after multivariate adjustments. Also, a significantly lower odds ratio was observed in the highest tertile of β-cryptoxanthin intake after adjustments for age, years since menopause, weight, height, current tobacco use, regular alcohol intake, exercise habits, use of dietary supplements, and total energy, but this significant association was not observed after further adjustments for intakes of calcium, magnesium, potassium, and vitamin D.

Next, study subjects were divided into two groups by median values of vitamin C and/or β-cryptoxanthin intake. And then, all subjects were ranked into four groups as follows: group 1: lower intake of vitamin C (47–169 mg/day) with lower intake of β-cryptoxanthin (0–0.96 mg/day); group 2: lower intake of vitamin C (47–169 mg/day) with higher intake of β-cryptoxanthin (0.97–7.91 mg/day); group 3, higher intake of vitamin C (170–625 mg/day) with lower intake of β-cryptoxanthin (0–0.96 mg/day); group 4, higher intake of vitamin C (170–625 mg/day) with higher intake of β-cryptoxanthin (0.97–7.91 mg/day). In both groups of higher intake of vitamin C with lower intake of β-cryptoxanthin and/or lower intake of vitamin C with higher intake of β-cryptoxanthin, significantly lower odds ratios were not observed against the lower intake group of both of them used for the reference group. In contrast, a significantly lower odds ratio was observed in the higher intake group of both of them after adjustments for age, years since menopause, weight, height, current tobacco use, regular alcohol intake, exercise habits, use of dietary supplements, and total energy (Table 5). However, this significant lower odds ratio became insignificant after further adjustments for intakes of calcium, magnesium, potassium, and vitamin D (data not shown).

Table 4 The odds ratios (and 95% confidence intervals) of tertiles of antioxidant intakes on low bone mineral density in post-menopausal Japanese female subjects

Dietary intake		Number	Range (mg/d) or (μg/d)	Model 1			Model 2			Model 3		
				OR	95% CI	*P* for trend	OR	95% CI	*P* for trend	OR	95% CI	*P* for trend
Retinol[a]	Lowest (Q1)	97	(29–213)	1.00			1.00			1.00		
	Middle (Q2)	98	(218–383)	1.30	(0.61–2.75)		1.65	(0.74–3.69)		1.28	(0.56–2.94)	
	Highest (Q3)	98	(386–3531)	2.37	(1.16–4.85)	0.014	3.22	(1.38–7.51)	0.007	2.52	(1.03–6.14)	0.031
Vitamin C	Lowest (Q1)	96	(47–139)	1.00			1.00			1.00		
	Middle (Q2)	99	(140–214)	1.15	(0.55–2.40)		1.02	(0.47–2.22)		1.03	(0.45–2.36)	
	Highest (Q3)	98	(215–625)	0.35	(0.15–0.80)	0.004	0.25	(0.10–0.66)	0.001	0.25	(0.07–0.82)	0.010
Vitamin E	Lowest (Q1)	101	(3.2–7.2)	1.00			1.00			1.00		
	Middle (Q2)	97	(7.3–9.1)	0.61	(0.29–1.27)		0.56	(0.25–1.25)		0.49	(0.21–1.14)	
	Highest (Q3)	95	(9.2–30.9)	0.61	(0.29–1.27)	0.244	0.45	(0.16–1.31)	0.176	0.43	(0.14–1.36)	0.193
Lycopene	Lowest (Q1)	121	(0.00–0.06)	1.00			1.00			1.00		
	Middle (Q2)	76	(0.15–0.15)	1.02	(0.48–2.15)		1.10	(0.51–2.35)		1.06	(0.48–2.34)	
	Highest (Q3)	96	(0.36–1.78)	1.55	(0.79–3.04)	0.177	1.72	(0.85–3.47)	0.117	1.60	(0.75–3.38)	0.201
α-Carotene	Lowest (Q1)	95	(0.03–0.23)	1.00			1.00			1.00		
	Middle (Q2)	97	(0.24–0.37)	0.73	(0.36–1.48)		0.79	(0.38–1.66)		0.90	(0.42–1.95)	
	Highest (Q3)	101	(0.38–1.27)	0.77	(0.38–1.57)	0.522	0.78	(0.36–1.67)	0.551	1.05	(0.45–2.45)	0.882
β-Carotene	Lowest (Q1)	97	(0.34–1.52)	1.00			1.00			1.00		
	Middle (Q2)	99	(1.53–2.36)	0.61	(0.29–1.27)		0.63	(0.29–1.35)		0.74	(0.32–1.70)	
	Highest (Q3)	97	(2.37–8.19)	0.75	(0.37–1.53)	0.586	0.69	(0.31–1.55)	0.487	0.93	(0.33–2.62)	0.981
Lutein	Lowest (Q1)	98	(0.49–1.68)	1.00			1.00			1.00		
	Middle (Q2)	97	(1.70–2.58)	1.56	(0.74–3.28)		1.84	(0.83–4.06)		2.10	(0.89–4.93)	
	Highest (Q3)	98	(2.59–10.01)	1.25	(0.59–2.62)	0.762	1.39	(0.60–3.23)	0.698	1.94	(0.69–5.48)	0.339
β-Cryptoxanthin	Lowest (Q1)	98	(0.00–0.30)	1.00			1.00			1.00		
	Middle (Q2)	101	(0.31–1.21)	0.52	(0.25–1.10)		0.47	(0.22–1.01)		0.49	(0.22–1.10)	
	Highest (Q3)	94	(1.22–7.91)	0.46	(0.21–1.00)	0.099	0.40	(0.17–0.92)	0.068	0.53	(0.22–1.28)	0.295
Zeaxanthin	Lowest (Q1)	95	(0.08–0.46)	1.00			1.00			1.00		
	Middle (Q2)	100	(0.47–0.96)	1.73	(0.82–3.65)		1.95	(0.89–4.27)		1.71	(0.75–3.87)	
	Highest (Q3)	98	(0.97–6.09)	1.96	(0.93–4.13)	0.104	2.65	(1.11–6.31)	0.038	2.51	(0.99–6.33)	0.061

Model 1: Age, weight and height were adjusted. Model 2: Years since menopause, current tobacco use, regular alcohol intake, exercise habits, supplement use, and total energy intake were further adjusted. Model 3: Intakes of calcium, magnesium, potassium, and vitamins D were further adjusted

[a] Preformed retinol

Table 5 The odds ratios (and 95% confidence intervals) of four groups stratified by dietary intakes of vitamin C and β-cryptoxanthin on low bone mineral density in post-menopausal Japanese female subjects

		β-Cryptoxanthin intake					
		Low intake (0–0.96mg/d)			High intake (0.97–7.91mg/d)		
		Number	OR	95%CI	Number	OR	95%CI
Vitamin C intake	Low intake (47–169 mg/d)	113	1.00	(Reference)	34	0.73	(0.27–1.99)
	High intake (170–625 mg/d)	36	0.52	(0.18–1.52)	110	0.42	(0.19–0.93)

Age, weight, height, years since menopause, current tobacco use, regular alcohol intake, exercise habits, supplement use, and total energy intake were adjusted

Discussion

The objective of this study was to investigate the associations of dietary patterns of antioxidant vitamin and carotenoid intake with radial BMD in post-menopausal Japanese female subjects. The results indicate that radial BMD was significantly associated with a dietary pattern heavily loaded on β-cryptoxanthin and vitamin C. Furthermore, we found that a high intake of vitamin C with β-cryptoxanthin was inversely associated with a low radial BMD. This investigation is the first reported cross-sectional study to examine the association of dietary patterns of antioxidant vitamin and carotenoid intake with BMD. Numerous antioxidant vitamins and carotenoids are contained in fruits and vegetables, and several recent epidemiological reports have shown inverse associations of antioxidant vitamin and carotenoid intake or serum level with low BMD, risk of fracture, and/or risk of osteoporosis [16–18]. However, the association of BMD with dietary patterns of antioxidant vitamin and carotenoid intake has not been thoroughly studied. Our findings further support the hypothesis that high intakes of fruits and vegetables rich in antioxidant vitamins and carotenoids, especially vitamin C and β-cryptoxanthin, may be beneficial to bone health in post-menopausal women.

On the other hand, some epidemiological studies have reported that excessive intake of retinol may have adverse effects on BMD [34–36]. In our study, a positive association between the factor score of the "Retinol" pattern and low radial BMD was observed after adjustments for age, years since menopause, weight, height, current tobacco use, regular alcohol intake, exercise habits, use of dietary supplements, and total energy. For dietary antioxidants, a significantly higher odds ratio was observed in the highest tertile of preformed retinol intake against the lowest tertile used for the reference group after multivariate adjustments. The recommended daily intake of retinol activity equivalents is 600 μgRE/day for Japanese women, with a tolerable upper intake of 3,000 μgRE/day [37]. In the highest tertile of preformed retinol intake, all of them consumed more than the recommended dietary allowance for Japanese adult females (600 μgRE/day) although most subjects consumed less than 3,000 μgRE/day. The effect of the dietary amount of preformed retinol on bone metabolism in Japanese female subjects has not been studied in detail, but a high intake of preformed retinol may be associated with the risk for low radial BMD. Further study is required.

In our data analyses, we identified three dietary patterns of antioxidant vitamin and carotenoid intake from the principal component analysis. Although all dietary patterns were heavily loaded on vitamin C intake, the highest positive loading between vitamin C, and dietary pattern was observed in the "β-cryptoxanthin" pattern. On the other hand, an association between β-cryptoxanthin and dietary pattern was observed in only the "β-cryptoxanthin" pattern, which had an extremely high factor loading. Vitamin C and β-cryptoxanthin are especially concentrated in citrus fruits such as Japanese mandarin orange. Therefore, a high intake of citrus fruit may be inversely associated with low BMD. In fact, in our previous study, we found that fruit intake was inversely associated with low radial BMD [25].

In animals, an experimentally induced deficiency of vitamin C led to impairments in bone mass, cartilage, and connective tissues [38, 39]. The protein in the bone matrix is over 90% collagen [40]. Vitamin C is an essential cofactor for the formation of collagen and the synthesis of hydroxyproline and hydroxylysine [41]. Therefore, vitamin C is an important micronutrient for the maintenance of bone health. Furthermore, it is well known that vitamin C reduces oxidative stress by scavenging singlet oxygen and peroxyl radicals. The relationship between oxidative stress and BMD or osteoporosis has recently been reported [16–18]. 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 [42]. NF-κB is activated by the exposure of cells to oxidative stress [43]. Therefore, it seems that reactive oxygen species enhance osteoclastogenesis and bone resorption. In fact, some studies have implicated reactive oxygen species in bone regulation [44, 45]. 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 [19–24]. These previous findings in epidemiological and experimental studies suggest that antioxidant micronutrients may provide benefits to bone metabolism against oxidative stress. Therefore, it seems that vitamin C is an important micronutrient for the maintenance of bone health through its biological action on cofactors for collagen formation, the synthesis of hydroxyproline and hydroxylysine, and antioxidant activity.

Carotenoids, as antioxidants, may also play an important role in the prevention of oxidative stress-related osteoclastogenesis and bone resorption. Very recently, Yamaguchi et al. reported the beneficial effects of β-cryptoxanthin on bone metabolism [46–48]. Through in vitro and in vivo studies, they found that β-cryptoxanthin stimulated bone formation and inhibited bone resorption. Their results support the idea that β-cryptoxanthin may have a direct stimulatory effect on bone formation and an inhibitory effect on bone resorption. Recent epidemiological studies have shown an association of serum β-cryptoxanthin with bone health. Yang et al. examined serum-carotenoid concentrations in postmenopausal American female subjects and found that the serum concentrations of β-cryptoxanthin and lycopene were significantly lower in osteoporotic subjects than in non-osteoporotic subjects [22]. Furthermore, we found that serum β-cryptoxanthin was significantly but partially associated with radial BMD [25]. The results of these experimental and epidemiological studies strongly support the hypothesis that the development of osteoporosis may be reduced by β-cryptoxanthin intake.

In our data analysis, significantly lower odds ratios in the highest tertiles of vitamin C and β-cryptoxanthin intakes were observed, but these significant associations were not observed after adjusting for β-cryptoxanthin and/or vitamin C intakes, respectively (OR, 0.36; CI, 0.12–1.11 for vitamin C and OR, 0.70; CI, 0.27–1.90 for β-cryptoxanthin). These results indicate that a combined intake of vitamin C and β-cryptoxanthin may be associated with radial BMD. Next, we examined the association of low radial BMD with the combined intake of vitamin C and β-cryptoxanthin. A significantly lower odds ratio was observed in the high-intake group for both of vitamin C and β-cryptoxanthin than in the low-intake group for both nutrients after adjustments for age, years since menopause, weight, height, current tobacco use, regular alcohol intake, exercise habits, use of dietary supplements, and total energy. However, this significantly lower odds ratio became insignificant after further adjustments for intakes of calcium, magnesium, potassium, and vitamin D (data not shown). For this reason, we think that these micronutrients might be more relevant factors for BMD rather than vitamin C and β-cryptoxanthin, or there is no denying the possibility of multicollinearity among these nutrients because these micronutrients were also rich in fruit and vegetables. From these results, we concluded that the intakes of vitamin C and β-cryptoxanthin may be significantly but partially associated with radial BMD, and these associations may be caused by a combination of vitamin C and β-cryptoxanthin. To our knowledge, there has been no experimental or epidemiological study of the combined effect of vitamin C and carotenoid on bone metabolism. It is conceivable that, rather than vitamin C alone, vitamin C intake combined with the intakes of other antioxidants such as carotenoids may yield an important dietary pattern conducive to the maintenance of bone health. Further studies on the complicated interactions of antioxidants on bone metabolism are required.

This study had some limitations. First, the data obtained here cross-sectional; therefore, only limited inferences can be made regarding temporality and causation. Furthermore, the sample size was limited, and thus further large-scale studies are required. Second, in our survey, portion size questions were not included for most items. Absolute nutrient intake could not be estimated from FFQ without portion size questions. Third, we evaluated 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, is required. Lastly, we could not evaluate the dietary patterns of other antioxidants such as flavonoids. Some studies have shown a beneficial effect of bioactive flavonoids on bone metabolism [49, 50].

In conclusion, dietary patterns heavily loaded on β-cryptoxanthin, and vitamin C are associated with radial BMD in post-menopausal Japanese female subjects. A high intake of vitamin C with β-cryptoxanthin is inversely associated with low radial BMD and may be beneficial to bone health. To determine whether antioxidant vitamins and carotenoids are beneficial to bone health, further cohort or intervention studies are required.

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Conflicts of interest None.

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Vitamin D Deficiency in Elderly Women in Nursing Homes: Investigation with Consideration of Decreased Activation Function from the Kidneys

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OBJECTIVES: To determine the approximate percentage of women in nursing homes who have vitamin D deficiency and to investigate whether, in assessing vitamin D status in elderly women, there are problems with measuring only 25 hydroxy-vitamin D_3 ($25(OH)D_3$) and whether decreased vitamin D activation as a result of poor renal function needs to be considered.

DESIGN: Cross-sectional study.

SETTING: Forty-eight nursing homes in Japan.

PARTICIPANTS: Four hundred three women with a mean age of 86.5 living in nursing homes who had participated in a clinical trial for hip protectors and were not bedridden.

MEASUREMENTS: At the start of the trial, in addition to general biochemical data, $25(OH)D_3$, 1,25-dihydroxy-vitamin D_3 ($1,25(OH)_2D_3$), intact parathyroid hormone (intact PTH), calcium (Ca), phosphorus (P), bone alkaline phosphate (BAP), cross-linked *N*-telopeptide of type I collagen (NTx), and osteocalcin were measured in participants' blood, and statistical analysis was performed.

RESULTS: $25(OH)D_3$, which is thought to reflect vitamin D status in the body, was surveyed and found to have a mean value of 16.7 ng/mL. $25(OH)D_3$ was less than 16 ng/mL in 49.1% of all participants. Creatinine clearance (CCr) was less than 30 mL/min in 20.1% of participants. Participants with serum $25(OH)D_3$ less than 16 ng/mL and CCr less than 30 mL/min had significantly higher levels of intact PTH and serum NTx. Participants with a CCr less than 30 mL/min had significantly lower levels of $1,25(OH)_2D_3$.

CONCLUSION: Frail elderly adults living in nursing homes with poor renal function had lower $1,25(OH)_2D_3$ and higher intact PTH levels and were thus thought to have poorer vitamin D activating capacity. Supplementation with cholecalciferol may be insufficient in people who have poor renal function. **J Am Geriatr Soc 60:251–255, 2012.**

Key words: 25-hydroxy-vitamin D_3; 1,25-dihydroxy-vitamin D_3; nursing homes

The importance of vitamin D for bones has been indicated in previous studies.[1,2] Frail elderly adults with limited ability to perform activities of daily living (ADL) who enter a nursing home are at high risk for low vitamin D as a result of poor nutrition and lack of sunlight. Vitamin D deficiency is an important risk factor for osteoporosis and fractures from falls in elderly adults.[3–5] When assessing serum 25 hydroxy-vitamin D_3 ($25(OH)D_3$) levels to define vitamin D deficiency, many reports have adopted a cutoff of 20 ng/mL.[6–8] It has also been reported that individuals with hip fracture or those with a history of falls have low $25(OH)D_3$ levels.[9,10] Secondary hyperparathyroidism from poor renal function in elderly adults must also not be overlooked.[11] The group that is probably at the highest risk of falls and fractures is elderly women living in nursing homes who are not completely bedridden but have a mobility level of at least being able to move about in a wheelchair with assistance. The participants in this study were such a group of people, who had previously participated in a fracture prevention trial using hip protectors.[12] Vitamin D levels, renal function, and the relationship between the two were investigated in these women, and the approximate percentage of these nursing home residents who needed supplemental vitamin D was considered.

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METHODS

Participants were 403 women aged 70 and older (range: 70–103) who lived in 48 nursing homes from whom consent was obtained for participation in a fracture prevention trial using hip protectors.[12] They had a mobility level of at least being able to move about in a wheelchair with assistance. A history of bilateral hip fracture was a condition for exclusion. Written informed consent was obtained from all participants. The Ethics Committee of the National Center for Geriatrics and Gerontology approved the study. Blood was collected from participants as the 48 nursing homes in the southern part of central Japan were visited in turn between January 2005 and May 2008. At the start of the trial, in addition to general biochemical data, 25(OH)D_3, 1,25-dihydroxy-vitamin D_3 (1,25 $(OH)_2D_3$), intact parathyroid hormone (PTH), calcium (Ca), phosphorus (P), bone alkaline phosphate (BAP), cross-linked *N*-telopeptide of type I collagen (NTx), and osteocalcin were measured using participants' blood, and statistical analysis was performed. 25(OH)D_3 was measured using the radioimmunoassay double antibody method. Frail elderly adults have little muscle, and even if creatinine (Cr) is in the normal range, it cannot be concluded that renal function is normal. For a simpler assessment of renal function, we estimated Cr clearance (CCr) with adjustments for age and body weight using the widely adopted Cockcroft-Gault formula.[13]

Statistical Analyses

SPSS (version 17.0, SPSS, Inc., Chicago, IL) was used in the statistical analysis. Adjustment was made for age as a control variable in partial correlation. Two-tailed significance probability <.05 was taken to be significant. The Student *t*-test was used to test for differences between the mean values of the two groups, with $P < .05$ taken to indicate significance. The Bonferroni test was used to compare the mean values in the groups, using a general linear model adjusted for age. $P < .05$ was taken to indicate a significant difference.

RESULTS

Participants were aged 70 were to 103 (mean 86.5). Mean 25(OH)D_3 level, which is an indicator of vitamin D level, was low (16.7 ng/mL). The mean values for the following tests were: 1,25$(OH)_2D_3$, 44.4 ± 17.5 pg/mL; intact PTH, 57.4 ± 38.7 pg/mL; BAP, 32.4 ± 13.2 U/L; osteocalcin, 7.8 ± 3.8 ng/mL; and NTx, 17.6 ± 9.7 nmol bone collagen equivalent/L. The percentile distribution in the 25(OH)D_3 distribution is shown in Figure 1. When 25(OH)D_3 concentration of less than 20 ng/mL was taken to indicate vitamin D deficiency, 78.1% of participants were found to be vitamin D deficient.

To further investigate 25(OH)D_3, the partial correlation was first examined adjusted for age. There were significant positive correlations between 25(OH)D_3 and 1,25$(OH)_2D_3$ (correlation coefficient (r) = 0.149, $P = .003$), albumin ($r = 0.185$, $P < .001$), total cholesterol ($r = 0.165$, $P = .001$), blood urea nitrogen ($r = 0.116$, $P = .02$), Ca ($r = 0.153$, $P = .002$), and P ($r = 0.100$, $P = .04$). Significant negative correlations were shown with serum NTx ($r = -0.153$, $P = .002$) and intact PTH ($r = -0.178$, $P < .001$). It was then decided to further investigate intact PTH, which had shown a high correlation. Mean intact PTH levels in the group with a serum 25 $(OH)D_3$ concentration less than 12.0 ng/mL, 12.0 to 15.9 ng/mL, and 16.0 ng/mL or higher were 72.3 pg/mL, 60.4 pg/mL, and 51.1 pg/mL, respectively. Mean intact PTH level was significantly higher in participants with a serum 25(OH)D_3 concentration less than 12.0 ng/mL ($P < .001$) and 12.0 to 15.9 ng/mL ($P = .02$) than in those with a concentration of 16.0 ng/mL or higher. Participants younger than 85 were then compared with those aged 85 and older to determine whether the various data differed depending on age (Table 1). Significant differences were seen in 25(OH)D_3, 1,25$(OH)_2D_3$, and intact PTH. Because 1,25$(OH)_2D_3$, a form of activated vitamin D, also decreases with age, it was decided to investigate 1,25 $(OH)_2D_3$. First, in the age-adjusted partial correlation, 1,25$(OH)_2D_3$ showed the strongest negative correlation with Cr ($r = -0.323$, $P < .001$). This finding suggests that renal function strongly affects 1,25$(OH)_2D_3$. The relationship between 1,25$(OH)_2D_3$ concentration and estimated CCr is shown in Table 2. 1,25$(OH)_2D_3$ concentration was significantly lower in participants with CCr less than 30 mL/min. Similarly, intact PTH concentration was significantly higher in participants with CCr less than 30 mL/min, in whom 1,25$(OH)_2D_3$ concentration was significantly lower (Table 2). A tendency was seen for 25(OH) D_3 levels to be higher with lower CCr, and a significant difference was seen between groups with CCr of less than 30 and 45 mL/min or greater ($P < .05$, general linear model Bonferroni test). To improve understanding of how participants were distributed according to 25(OH)D_3 concentration and CCr value, they were divided into four groups with 25(OH)D_3 concentrations of less than 16 and 16 ng/mL and greater and CCr of less than 30 and 30 mL/min and greater. Concentrations of 1,25$(OH)_2D_3$, intact PTH, and serum NTx of the groups were then compared (Table 3). Of 198 participants with 25(OH)D_3 concentrations of less than 16 ng/mL, 36 (18.4%) had poor renal function (CCr < 30 mL/min), and of 205 participants with

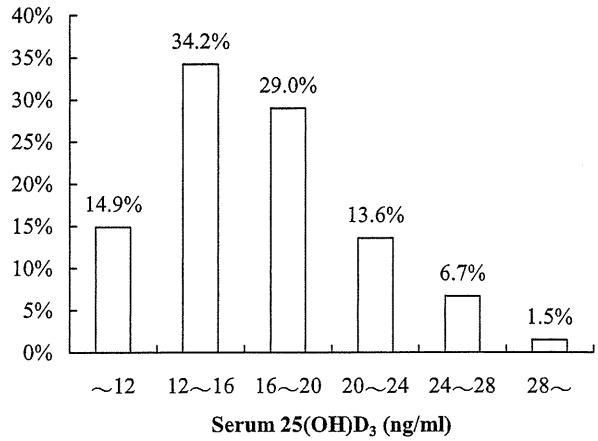

Figure 1. Percentile distribution of serum 25 hydroxy-vitamin D_3 (25(OH)D_3) concentrations. 25(OH)D_3 level was < 20 ng/mL in 78.1% and < 16 ng/mL in approximately half.

Table 1. Comparison of Mean Data Values According to Age

Characteristic	Normal Range	Mean ± Standard Deviation <85 (n = 139)	Mean ± Standard Deviation ≥85 (n = 264)	*P*-Value
Age	—	79.1 ± 3.8	90.4 ± 3.7	<.001
Height, cm	—	145.2 ± 7.5	142.8 ± 7.2	.003
Weight, kg	—	44.1 ± 8.3	41.6 ± 7.5	.003
Body mass index, kg/m^2	—	20.7 ± 4.4	20.0 ± 3.3	.28
25 hydroxy-vitamin D_3, ng/mL	—	17.5 ± 4.9	16.3 ± 4.7	.01
1,25-dihydroxy-vitamin D_3, pg/mL	20–60	47.5 ± 18.1	42.7 ± 16.9	.008
Intact parathyroid hormone, pg/mL	10–65	51.6 ± 27.4	60.4 ± 43.2	.03
Albumin, g/dL	3.9–4.9	3.9 ± 0.3	3.9 ± 0.4	.01
Total protein, g/dL	6.5–8.2	6.9 ± 0.5	6.9 ± 0.5	.26
Total cholesterol, mg/dL	120–220	207.6 ± 38.0	195.9 ± 36.3	.003
Blood urea nitrogen, mg/dL	8–20	17.8 ± 6.5	18.7 ± 7.7	.25
Creatinine, mg/dL	0.5–0.8	0.66 ± 0.3	0.72 ± 0.4	.13
Creatinine clearance (Cockcroft-Gault formula), mL/min	—	55.2 ± 18.6	38.9 ± 12.7	<.001
Glomerular filtration rate (modified diet in renal disease formula), mL/min	—	73.9 ± 25.0	65.4 ± 22.1	.001
Calcium, mg/dL	8.7–10.1	8.8 ± 0.4	8.8 ± 0.5	.25
Phosphorus, mg/dL	2.5–4.5	3.6 ± 0.4	3.6 ± 0.5	.21
Aspartate aminotransferase, U/L	10–40	19.2 ± 6.2	19.7 ± 6.2	.39
Alanine aminotransferase, U/L	5–45	13.2 ± 7.5	11.5 ± 6.0	.02

Table 2. Comparison of 1,25-Dihydroxy-Vitamin D_3 ($1,25(OH)_2D_3$), Intact Parathyroid Hormone (PTH), and 25 Hydroxy-Vitamin D_3 ($25(OH)D_3$) Concentrations According to Creatinine Clearance (CCr)

CCr, mL/min	Mean (Standard Error) $1,25(OH)_2D_3$, pg/mL	Intact PTH, pg/mL	25 Hydroxy-Vitamin D_3, ng/mL
<30.0 (n = 82)	33.0 (1.9)*	80.1 (4.3)*	17.9 (5.2)
30.0–44.9 (n = 160)	45.8 (1.3)	52.7 (3.0)	17.0 (4.9)
≥45 (n = 161)	48.8 (1.4)	50.5 (3.2)	15.9 (4.4)

* $P < .05$, general linear model Bonferroni test.

Table 3. Comparison of 1,25-Dihydroxy-Vitamin D_3 ($1,25(OH)_2D_3$), Intact Parathyroid Hormone (PTH), and Cross-Linked *N*-Telopeptide of Type I Collagen (NTx) Concentrations According to Creatinine Clearance (CCr) and 25 Hydroxy-Vitamin D_3 ($25(OH)D_3$) Concentration

CCr, mL/min	Mean (Standard Error) $25(OH)D_3$, ng/mL <16	$25(OH)D_3$, ng/mL ≥16
<30		
$1,25(OH)_2D_3$, pg/mL	29.0 (2.7)*	36.3 (2.5)*
Intact PTH, pg/mL	104.8 (6.1)*	60.7 (5.4)
NTx, nmolBCE/L	28.3 (1.6)*	18.9 (1.4)
≥30		
$1,25(OH)_2D_3$, pg/mL	45.2 (1.2)	49.3 (1.3)
Intact PTH, pg/mL	55.1 (2.8)	48.1 (2.9)
NTx, nmolBCE/L	17.1 (0.7)	15.3 (0.7)

$1,25(OH)_2D_3$ levels were significantly lower in participants with CCr lower than 30 mL/min than those with CCr of 30 mL/min and higher. Mean intact PTH and NTx concentrations in participants with CCr lower than 30 mL/min and $25(OH)D_3$ of less than 16 ng/mL were significantly higher than in the other participants.

* $P < .05$, general linear Bonferroni test.

$25(OH)D_3$ concentrations of 16 ng/mL and higher, 45 (22.0%) had poor renal function. These percentages were approximately the same, but concentrations of intact PTH and NTx were significantly higher in the group with $25(OH)D_3$ of less than 16 ng/mL and CCr of less than 30 mL/min. In addition, in the group with CCr of less than 30 mL/min, $1,25(OH)_2D_3$ concentration was significantly lower than in the group with CCr of 30 mL/min and higher, regardless of $25(OH)D_3$ concentration.

DISCUSSION

Table 4 summarizes the reports on $25(OH)D_3$ concentration in elderly cohorts.[14–20] A comparison of reports in which participants were living in institutions and reports in which participants were living independently revealed lower levels of $25(OH)D_3$ in residents of institutions, who are thought to have greater difficulty with activities of daily living. Experts have proposed that $25(HO)D_3$ concentrations of 20 to 32 ng/mL, or roughly 30 ng/mL, are the minimum necessary concentration to prevent fractures.[21] A recent meta-analysis also reported that concentrations of 75 to 100 nmol/L balanced the benefits and risks of the health of elderly people.[22] Many studies take PTH to be an indicator of the cutoff value for $25(OH)D_3$ concentration.[6–8] When PTH is taken as an indicator, a $25(OH)D_3$ concentration of 20 ng/mL is taken as the cutoff

Table 4. Past Reports of 25 Hydroxy-Vitamin D_3 (25 $(OH)D_3$) Levels in Elderly Cohorts

Study Participants	n	Age, Mean	25(OH)D_3, ng/mL, Mean	References
Nursing home (Japan)	133	84.6	11.9	14
Nursing home or housebound (United States)	116	81	12.6	15
Nursing home (this study, Japan)	425	86.4	16.8	—
Nursing home (United States)	35	74	17.4	16
Independent women (Canada)	186	73	15.6	17
Independent women (France)	440	80	17.0	18
Community-dwelling elderly women (Japan)	2,007	75.4	24.2	19
Independent women (United States)	500	71	29.6	20

in many reports.[6–8] In the participants in this study, 78.1% had 25(OH)D_3 levels less than 20 ng/mL. Another study reported that 25(OH)D_3 of 20 ng/mL and greater is needed when intact PTH is taken as the indicator and that 28 ng/mL and greater is needed when bone density in the femoral neck is taken as the indicator.[6] From the present results, the cutoff value for 25(OH)D_3 as an indicator of intact PTH was thought to be 16 ng/mL; 49.1% of participants had 25(OH)D_3 of less than 16 ng/mL (Figure 1). In general, people with poor renal function have lower levels of 1,25$(OH)_2D_3$, an activated form of vitamin D, as a result of poor vitamin D activating capacity. Moreover, secondary hyperparathyroidism from poor renal function is not unusual in elderly people.[11] In the present results as well, there was a strong negative correlation between 1,25 $(OH)_2D_3$ and CCr ($r = -0.323$, $P < .001$), which suggests that renal function strongly affects 1,25$(OH)_2D_3$. As shown in Table 2, intact PTH levels were significantly higher and 1,25$(OH)_2D_3$ significantly lower with a CCr of less than 30 mL/min. From this it can be conjectured that vitamin D activation in the kidneys may decrease in cases of secondary hyperparathyroidism from poor renal function. In addition, as shown in Table 3, the percentage of people with poor renal function (CCr < 30 mL/min) was nearly the same in participants with 25(OH)D_3 levels greater and less than 16 ng/mL. Women with such vitamin D activating capacity made up 20.1% of all participants, although according to guidelines published in the United States in 2003[23] for bone metabolism disorders in individuals with chronic kidney disease, if PTH is measured and found to be high in people undergoing dialysis and those with chronic renal failure with less than 60% renal function, it is recommended that serum 25(OH)D_3 be measured and vitamin D_2 be administered if it is less than 30 ng/mL. Considering these guidelines, a greater number of people would probably be judged to have poor renal function, although there are limitations to this investigation. All CCr values were derived through calculation, not from actual measurements of CCr or glomerular filtration rate (GFR). Cystatin C was not measured either. The Cockcroft-Gault formula was first used to calculate CCr, but the Modification of Diet in Renal Disease (MDRD) formula[24] was also used to investigate CCr. The correlation between CCr calculated using the Cockcroft-Gault formula and GFR calculated using the MDRD formula was high ($r = 0.769$, $P < .001$). Moreover, in the group with GFR of less than 50 mL/min (n = 84, 20.8%), a significant difference, similar to that in the results obtained with the Cockcroft-Gaults formula, was seen. Thus, although CCr obtained from calculations is not ideal, it seems to be reliable. In addition, intact PTH level may be a useful indicator in establishing a cutoff value for 25(OH) D_3 in frail elderly adults such as the present participants. Moreover, because plainly higher intact PTH levels were shown in participants with poor vitamin D activation in the kidneys, intact PTH may have an important role in considering vitamin D supplementation in frail elderly adults. Many experts recommend vitamin D supplementation with cholecalciferol when 25(OH)D_3 level drops below 30 to 32 ng/mL. A recent Institute of Medicine report[25] recommends supplementation when 25(OH)D_3 is less than 20 ng/mL, but it does not specifically address frail elderly adults. Vitamin D is not activated efficiently even with cholecalciferol supplementation in frail elderly adults, such as the present participants, who seem to have poor activation of vitamin D. Theoretically, therefore, it would seem that supplementation with a form of activated vitamin D such as paricalcitol or alfacalcidol may be beneficial in the case of frail elderly adults with poor renal function.

CONCLUSION

In this study, 25(OH)D_3 levels were found to be low in women living in nursing homes who were at least able to move about in a wheelchair with assistance. Approximately 50% to 80% of participants were thought to be vitamin D deficient, although this depends somewhat on the cutoff value used for 25(OH)D_3. In addition, approximately 20% of all participants were thought to have decreased vitamin D activating capacity in the kidneys. Such poor vitamin D activation capacity in the kidneys was present in a similar 20% of people whose 25(OH)D_3 level was above the cutoff level (16 ng/mL). An unexpectedly large number of women in nursing homes thus had poor vitamin D activation secondary to poor renal function. For vitamin D supplementation, therefore, it may be necessary to make a comprehensive judgment with measurements of intact PTH and CCr or GFR and 1,25 $(OH)_2D_3$ rather than cholecalciferol supplementation based simply on 25$(OH)_3$ level.

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