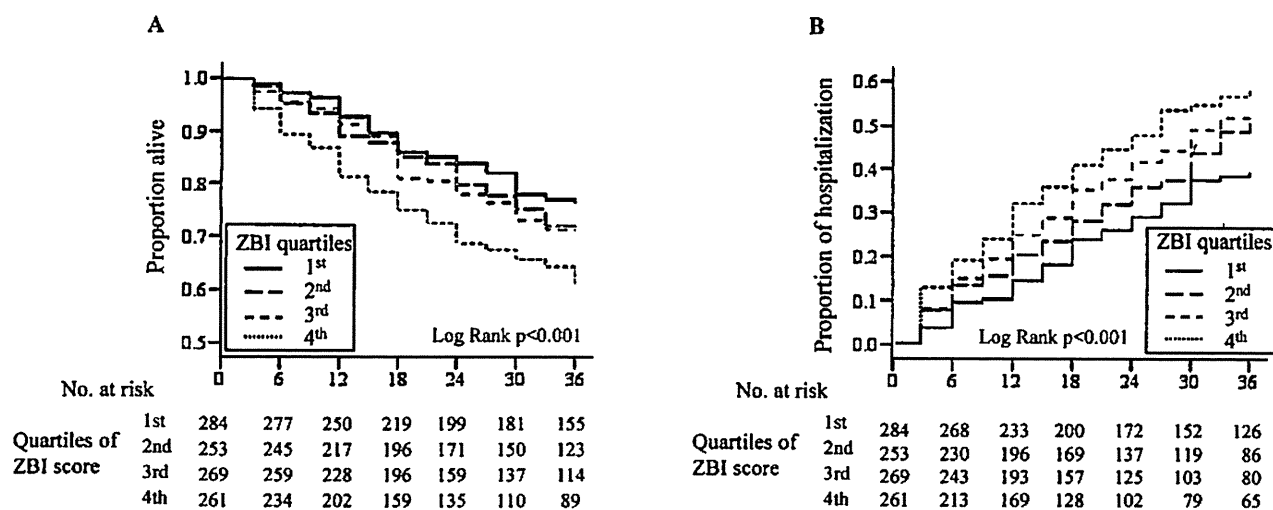


**FIGURE 1.** Kaplan–Meier Plot for Probability of Event-Free Survival (A) and Probability of Hospital Admission (B) According to Increasing Quintiles of the Zarit Burden Interview Score



Log-rank  $\chi^2$  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  $\chi^2$  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 the 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  $\chi^2$  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 <sup>a</sup>			Model 2 <sup>b</sup>			Model 1 <sup>c</sup>			Model 2 <sup>d</sup>		
	Wald $\chi^2$	p	HR (95% CI)	Wald $\chi^2$	p	HR (95% CI)	Wald $\chi^2$	p	HR (95% CI)	Wald $\chi^2$	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 <sup>e</sup>	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 <sup>e</sup>	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 <sup>e</sup>	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 <sup>e</sup>	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 <sup>e</sup>	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.

<sup>a</sup>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.

<sup>b</sup>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.

<sup>c</sup>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.

<sup>d</sup>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.

<sup>e</sup>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			3rd			4th		
		Wald $\chi^2$	p	HR (95% CI)	Wald $\chi^2$	p	HR (95% CI)	Wald $\chi^2$	p	HR (95% CI)
<b>All death<sup>a</sup></b>										
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)
<b>Hospitalization<sup>b</sup></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.

<sup>a</sup>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.

<sup>b</sup>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.<sup>2,20</sup> 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.<sup>21,22</sup> In fact, it has been demon-

strated that a lack of needed care for disabled older individuals or a decreased quality of family caregiving results in poor outcomes for care recipients.<sup>23-25</sup> 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.<sup>26-28</sup> 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

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investigation had multiple medical problems and functional limitations and were probably at higher mortality and hospitalization risk than those in these prior studies.<sup>26-28</sup>

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.<sup>29,30</sup> A possible mechanism of this association is that spousal illness or death may deprive a partner of social, emotional, or other practical support.<sup>29,30</sup> 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.<sup>31</sup> 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.<sup>32-36</sup> 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.

The authors thank all the patients, caregivers, and the many nurses participating in the study, and the Nagoya City Health Care Service Foundation for Older People for their vigorous cooperation.

This study was supported by a Grant-in Aid for the Comprehensive Research on Aging and Health from the Ministry of Health, Labor, and Welfare of Japan and a grant from Mitsui Sumitomo Insurance Welfare Foundation.

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# 老年内科

## Geriatrics 標榜をめざして

下方浩史 国立長寿医療研究センター  
予防開発部長

## 2. 高齢者の疾病 —疫学，臨床的特徴



### はじめに

日本では平均寿命の延長が続き、高齢者、特に75歳以上の後期高齢者あるいは85歳以上、90歳以上の超高齢者の人口が急速に増加しつつある。医療機関を受診する患者の多くが高齢者となり、高齢者疾患の特性を理解した上での診断・治療が日常の医療を行う際の重要な課題となっている。老年内科の診療には、高齢者に多い疾患や症状、高齢者疾患の臨床的特徴や、検査値を判定する際の留意点などの理解が必要である。



### 高齢者と超高齢者の健康状況・疾病

国民生活基礎調査では、3年ごとに世帯員の健康状況や介護状況の調査を行っている。平成19年度の国民生活基礎調査では、要介護者のうち、94.7%が65歳以上の高齢者であった。特に80歳代で要介護になっている者が43.0%と半数近くを占めていた<sup>1)</sup>。自分自

身の健康状態を良いと思っている人は65歳以上で20.0%、85歳以上では13.7%にすぎない。日常生活への影響がある者は65歳以上で22.6%、85歳以上で39.5%に及ぶ。影響のある日常生活活動としては、外出が高頻度になっていた。

65歳以上の63.8%、85歳以上の61.8%が病気のために医療機関に通院していた。65歳以上の高齢通院患者の罹患疾病は男女ともに第1位は高血圧症、第2位は眼疾患であった(表1)。高血圧症は男性で通院者の24.0%、女性で25.3%を占めている。

自覚症状については、65歳以上では1位は「腰痛」であり、これは85歳以上でも同じである。「手足の関節が痛む」、「手足の動きが悪い」などの整形外科的な症状が多い。次いで「咳や痰」、「頻尿」が多くなっている。85歳以上では「もの忘れ」、「聞こえにくい」が多くなっているのが特徴である。

一人あたりの症状数は65歳以上で平均5.2個、85歳以上で5.8個となっており、一人が同時に多くの症状を持っている。高齢者の健

表1 65歳以上の高齢者の通院者率上位5傷病（複数回答）

性別	第1位	第2位	第3位	第4位	第5位
男性	高血圧症 24.0%	眼の病気 10.7%	糖尿病 10.7%	腰痛症 9.5%	前立腺肥大症 8.2%
女性	高血圧症 25.3%	眼の病気 14.6%	腰痛症 13.2%	脂質異常症 9.4%	骨粗鬆症 8.1%

（文献<sup>1)</sup>より）

康状況は加齢とともに悪くなり、85歳以上の男性の38.4%、女性の41.7%が自覚症状、通院、健康状態に起因する生活への影響のすべてがあると回答している（図1）。

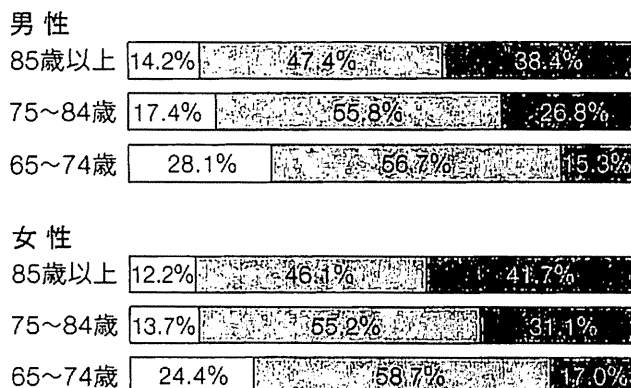
### 高齢者と超高齢者の死因

人口動態統計による平成21年度の年齢階級別死因は、55～84歳までが悪性新生物、心疾患、脳血管疾患の順であり<sup>2)</sup>、これよりも若い世代では自殺、不慮の事故が上位にあるのが特徴である。中年者でも高齢者でも基本的には死因に大きな違いはない。しかし、90歳以上の超高齢者では悪性新生物による死亡の割合が低下し、心疾患による死亡の割合が増加する。肺炎による死亡も超高齢者では増加している。また死因としての「老衰」が第5位までに登場しているのも超高齢者の特徴である（表2）。

### 高齢者疾患の臨床的特徴

高齢者疾患には認知症や骨粗鬆症、加齢性難聴、白内障など高齢者特有の疾患もあるが、腰痛症や関節症などの整形外科的な疾患、高血圧症や糖尿病、脂質異常症、癌、動脈硬化性疾患など一般成人にも見られる疾患も多い。

しかも高齢者では、これらの疾患を同時に持っており、また症状が出にくく診断が難しかったり、治療への反応が悪く慢性化しやすかったりする。特に疾患がなくても、筋肉量



- 自覚症状・通院・生活への影響ともなし
- 自覚症状・通院・生活への影響いずれかあり
- 自覚症状・通院・生活への影響ともあり

図1 性別・年齢別に見た高齢者の健康状態の構成割合（入院中、回答不詳の者を除く）

（文献<sup>1)</sup>より）

が減少するサルコペニアと呼ばれる状態や、日常生活活動に支障の生じている脆弱高齢者、要介護高齢者も多い<sup>3)</sup>。

高齢者疾患を診る場合には、栄養問題を考慮することがきわめて重要である<sup>4)</sup>。老化に伴う生理学的変化として、消化吸収機能が低下し、エネルギー消費量が減って、食欲が低下する。感覚機能、特に食欲に密接に関わる味覚、臭覚、視覚などの機能の低下がいわゆる食欲不振を増強させる。高齢者に多い心疾患に使われるジギタリス薬などには食欲を減退させる副作用が往々にして見られる。

また亜鉛欠乏は味覚障害を起こし、食欲低下の原因となる。亜鉛欠乏症は若年者に多いが、最近では高齢者にも多く見られる。亜鉛





大きくなっていることにも注意しなければならない。高齢者では肥満よりもやせの重要性を認識すべきである。高度の肥満に伴う高血圧症や糖尿病などが無い限り、高齢者に食事制限を勧めるべきではない。

## 高年齢者における検査値の見方

高齢者における検査値の判定には注意が必要である<sup>5)</sup>。検査値に異常を来す要因としては、検査手技などの技術的要因、体質性黄疸など遺伝的要因による個人差、さらに同じ個人でも、検査の時刻や季節、体位、運動や食事の影響などによる個人内変動がある。技術的要因としては、高齢者では静脈が脆く、しばしば採血が困難であり、採血時の溶血が問題となる場合が多い。溶血により血清カリウムやLDHが高値になることがある。

同一個人でも測定時の条件で異常値になることがある。こうした個人内変動は検体採取時の時刻や体位、生活習慣による影響、薬物の影響などによることが多い。時間的要因には日内変動、日差、季節差が挙げられる。日内変動を示す検査値としては血糖値、血清鉄、中性脂肪、脂肪酸、ビリルビン、遊離脂肪酸、副腎皮質ホルモンなどがある。これらの検査では採血の時刻に留意しなければならない。

筋組織からの逸脱酵素であるCK、AST、LDHの上昇が運動後に見られる。その上昇は運動翌朝に最大となる。これらの検査値は登山などの負荷の大きい運動でなくても、高齢者ではレクリエーション程度の軽度の運動で上昇が見られることもある。逆に、CKは寝たきり状態では低値になる。

安静に横になっている場合と、起立し活動している場合では循環血漿量が異なる。立位で活動をしている場合には血液の濃縮が起

り、血清蛋白質の濃度が上昇する。血清総蛋白量や血清アルブミンのみならず血清蛋白に結合して血清中に存在するカルシウムやビリルビン、コレステロールなどの測定値も立位活動時に上昇し、安静仰臥時では低下する。この低下は立位を30分ほど取ることで回復するが、同じ患者でも外来と入院で、また寝たきりの患者と自由に歩き回っている患者で、検査値の判定について考慮する必要がある。

## おわりに

高齢者は多彩な自覚症状、多数の疾患を同時に有していることが多い。個々の疾患や症状については、それぞれの分野の専門医が対応しては、検査や治療が増えて適切な対応ができない。老年内科の標榜を目指すためには、臓器別、分野別の縦割り医療ではなく、内科全般にわたる幅広い知識を持ち、高齢者の特性を理解することが必要である。さらに介護保険制度や高齢者医療、在宅医療など、家庭や社会的な環境についての理解と知識が重要であろう。高齢者、特に後期高齢者、超高齢者が今後急速に増えていく日本の社会で、老年内科医の果たすべき役割は大きい。老年内科医の今後の活躍に期待したい。

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Japan Medical Journal

No.4544  
2011年  
(平成23年)  
5月28日

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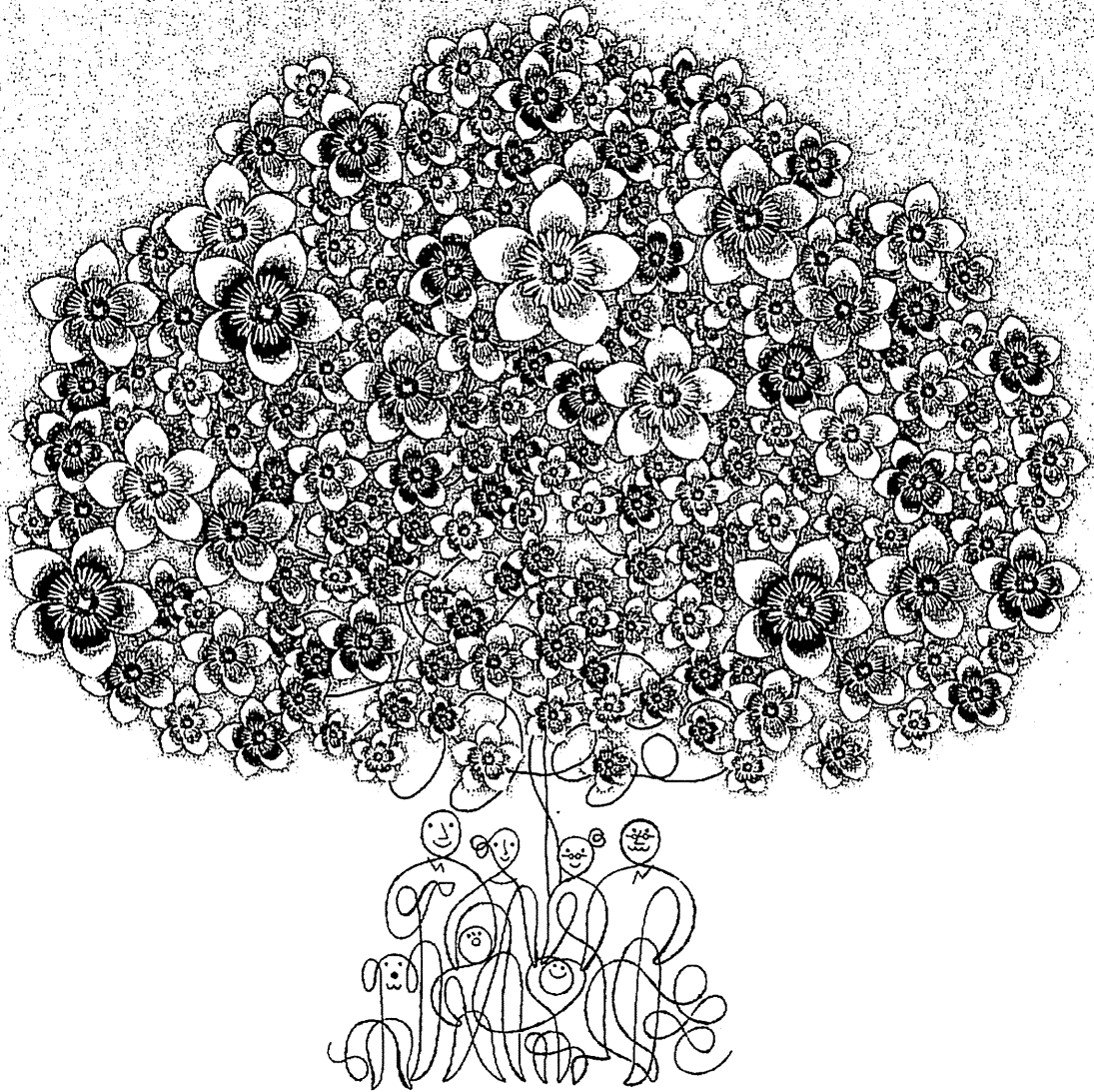
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## Dietary patterns of antioxidant vitamin and carotenoid intake associated with bone mineral density: findings from post-menopausal Japanese female subjects

M. Sugiura · M. Nakamura · K. Ogawa · Y. Ikoma ·  
F. Ando · H. Shimokata · M. Yano

Received: 8 December 2009 / Accepted: 1 March 2010 / Published online: 18 May 2010  
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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  $\beta$ -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 “ $\beta$ -cryptoxanthin” pattern, characterized by notably high intakes of  $\beta$ -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  $\beta$ -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  $\beta$ -cryptoxanthin with low BMD were not significant after further adjustment for intake of  $\beta$ -cryptoxanthin or vitamin C, respectively. Higher intakes of both vitamin C and  $\beta$ -cryptoxanthin were significantly associated with low BMD ( $P < 0.05$ ).

**Conclusions** The combination of vitamin C and  $\beta$ -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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M. Sugiura (✉) · K. Ogawa · Y. Ikoma · M. Yano  
Research team for health benefit of fruit,  
National Institute of Fruit Tree Science,  
485-6 Shimizu-Okitsu-nakachou,  
Shizuoka-shi, Shizuoka 424-0292, Japan  
e-mail: msugiura@affrc.go.jp

M. Nakamura  
Department of Community Health and Preventive Medicine,  
Hamamatsu University School of Medicine,  
1-20-1 Handayama,  
Hamamatsu-shi, Shizuoka 431-3192, Japan

F. Ando  
Department of Community Care Philanthropy,  
Aichi Shukutoku University,  
23 Sakuragaoka,  
Nagoya-shi, Aichi 464-8671, Japan

H. Shimokata  
Department of Epidemiology,  
National Institute for Longevity Sciences,  
36-3 Moriokachou,  
Obu-shi, Aichi 474-8522, Japan

### 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  $\beta$ -cryptoxanthin and  $\beta$ -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 from 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,  $\alpha$ -carotene,  $\beta$ -carotene, lutein,  $\beta$ -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,  $\alpha$ -carotene,  $\beta$ -carotene, lutein,  $\beta$ -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  $0.501 \text{ g/cm}^2$  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/m <sup>2</sup> )	22.5	(3.0)
Bone mineral density (g/cm <sup>2</sup> )	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 ( $\mu$ g/day) <sup>a, b</sup>	281	(259–305)
Vitamin C (mg/day) <sup>b</sup>	170	(161–179)
Vitamin D ( $\mu$ g/day) <sup>b</sup>	6.4	(5.9–6.9)
Vitamin E (mg/day) <sup>b</sup>	8.1	(7.8–8.4)
Lycopene (mg/day) <sup>b</sup>	0.15	(0.13–0.17)
$\alpha$ -Carotene (mg/day) <sup>b</sup>	0.25	(0.23–0.27)
$\beta$ -Carotene (mg/day) <sup>b</sup>	1.86	(1.74–1.99)
Lutein (mg/day) <sup>b</sup>	2.06	(1.92–2.20)
$\beta$ -Cryptoxanthin (mg/day) <sup>b</sup>	0.62	(0.52–0.73)
Zeaxanthin (mg/day) <sup>b</sup>	0.67	(0.61–0.73)
Current tobacco use (%)	1.7	
Exercise habits (%) <sup>c</sup>	21.5	
Regular alcohol intake (%) <sup>c</sup>	11.0	
Current supplement use (%)	9.6	

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

<sup>a</sup> Preformed retinol

<sup>b</sup> These variables were represented as original scale after analysis by log

<sup>c</sup>  $\geq 1$  time per week

subjects was 0.561 g/cm<sup>2</sup>. 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  $\beta$ -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,  $\beta$ -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  $\beta$ -carotene,  $\alpha$ -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  $\beta$ -carotene. This pattern, heavily loaded on preformed retinol, zeaxanthin, and vitamin E, was labeled the “Retinol” pattern. Factor 3 had heavy loadings on  $\beta$ -cryptoxanthin, vitamin C,  $\beta$ -carotene, lutein, and vitamin E. This pattern, heavily loaded on  $\beta$ -cryptoxanthin and vitamin C, was labeled the “ $\beta$ -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: $\beta$ - cryptoxanthin
Retinol <sup>a</sup>		0.825	
Vitamin C	0.435	0.285	0.773
Vitamin E	0.464	0.711	0.258
Lycopene	0.633		
$\alpha$ -Carotene	0.788		
$\beta$ -Carotene	0.852	0.257	0.369
Lutein	0.740	0.447	0.270
$\beta$ -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

<sup>a</sup> 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	<i>P</i> for trend	OR	95% CI	<i>P</i> for trend	OR	95% CI	<i>P</i> 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: $\beta$ -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 “ $\beta$ -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  $\beta$ -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  $\beta$ -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  $\beta$ -cryptoxanthin (0–0.96 mg/day); group 2: lower intake of vitamin C (47–169 mg/day) with higher intake of  $\beta$ -cryptoxanthin (0.97–7.91 mg/day); group 3, higher intake of vitamin C (170–625 mg/day) with lower intake of  $\beta$ -cryptoxanthin (0–0.96 mg/day); group 4, higher intake of vitamin C (170–625 mg/day) with higher intake of  $\beta$ -cryptoxanthin (0.97–7.91 mg/day). In both groups of higher intake of vitamin C with lower intake of  $\beta$ -cryptoxanthin and/or lower intake of vitamin C with higher intake of  $\beta$ -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 ( $\mu\text{g}/\text{d}$ )	Model 1			Model 2			Model 3		
				OR	95% CI	<i>P</i> for trend	OR	95% CI	<i>P</i> for trend	OR	95% CI	<i>P</i> for trend
Retinol <sup>a</sup>	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
$\alpha$ -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
$\beta$ -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
$\beta$ -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

<sup>a</sup> Prefomed retinol

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

		$\beta$ -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  $\beta$ -cryptoxanthin and vitamin C. Furthermore, we found that a high intake of vitamin C with  $\beta$ -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  $\beta$ -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  $\mu$ gRE/day for Japanese women, with a tolerable upper intake of 3,000  $\mu$ gRE/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  $\mu$ gRE/day) although most subjects consumed less than 3,000  $\mu$ gRE/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 “ $\beta$ -cryptoxanthin” pattern. On the other hand, an association between  $\beta$ -cryptoxanthin and dietary pattern was observed in only the “ $\beta$ -cryptoxanthin” pattern, which had an extremely high factor loading. Vitamin C and  $\beta$ -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 peroxy 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- $\kappa$ B1 and NF- $\kappa$ B2, Iotsova et al. reported that NF- $\kappa$ B proteins are important for osteoclastogenesis [42]. NF- $\kappa$ B 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  $\beta$ -cryptoxanthin on bone metabolism [46–48]. Through *in vitro* and *in vivo* studies, they found that  $\beta$ -cryptoxanthin stimulated bone formation and inhibited bone resorption. Their results support the idea that  $\beta$ -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  $\beta$ -cryptoxanthin with bone health. Yang et al. examined serum-carotenoid concentrations in postmenopausal American female subjects and found that the serum concentrations of  $\beta$ -cryptoxanthin and lycopene were significantly lower in osteoporotic subjects than in non-osteoporotic subjects [22]. Furthermore, we found that serum  $\beta$ -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  $\beta$ -cryptoxanthin intake.

In our data analysis, significantly lower odds ratios in the highest tertiles of vitamin C and  $\beta$ -cryptoxanthin intakes were observed, but these significant associations were not observed after adjusting for  $\beta$ -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  $\beta$ -cryptoxanthin). These results indicate that a combined intake of vitamin C and  $\beta$ -cryptoxanthin may be associated with radial BMD. Next, we examined the association of low radial BMD with the combined intake of vitamin C and  $\beta$ -cryptoxanthin. A significantly lower odds ratio was observed in the high-intake group for both of vitamin C and  $\beta$ -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  $\beta$ -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  $\beta$ -cryptoxanthin may be significantly but partially associated with radial BMD, and these associations may be caused by a combination of vitamin C and  $\beta$ -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  $\beta$ -cryptoxanthin, and vitamin C are associated with radial BMD in postmenopausal Japanese female subjects. A high intake of vitamin C with  $\beta$ -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.

**Acknowledgment** This work was supported by a grant from the Ministry of Agriculture, Forestry, and Fisheries (MAFF) for a food research project titled “Integrated Research on Safety and Physiological Function of Food” and a grant from the Council for Advancement of Fruit Tree Science. We are grateful to the participants in our survey and to the staff of the health examination program for residents of the town of Mikkabi, Shizuoka, Japan. We are also grateful to the staff of the Seirei Preventive Health Care Center (Shizuoka, Japan).

**Conflicts of interest** None.

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