# Impact of Caregiver Burden on Adverse Health Outcomes in Community-Dwelling Dependent Older Care Recipients

*Masafumi Kuzuya, M.D., Ph.D., Hiromi Enoki, Ph.D., Jun Hasegawa, M.D., Sachiko Izawa, Ph.D., Yoshihisa Hirakawa, M.D., Ph.D., Hiroshi Shimokata, M.D., Ph.D., Akihisa Iguchi, M.D., Ph.D.*

**Objective:** *To determine whether caregiver burden is associated with subsequent all-cause mortality or hospitalization among dependent community-dwelling older care recipients.* **Methods:** *A prospective cohort study of 1,067 pairs of community-dwelling 65-year-old or older care recipients and their informal caregivers was conducted. The 1,067 pairs completed the baseline assessment including caregiver burden assessed by the Zarit Burden Interview and a 3-year follow-up for all-cause mortality and hospitalization.* **Results:** *During the 3-year follow-up, 268 recipients died and 455 were admitted to hospitals. The multivariate Cox proportional hazards model revealed 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 risks of all-cause mortality and hospitalization, respectively, in comparison with those with caregivers in the lowest quartile after adjustment for potential confounders. The highest quartile of caregiver burden was associated with all-cause mortality and hospitalization within nonusers of respite services including day-care services, home-help services, and nursing-home respite stay services. No apparent association was observed within the users of these services except for day-care services, for which users showed a statistically significant association between the highest quartile and the risk of hospitalization.* **Conclusions:** *Heavy caregiver burden is associated with mortality and hospitalization among community-dwelling dependent older adults, even after adjusting for potential confounders. The reduction of caregiver burden and improvement of caregiver well-being may not only prevent the deterioration of caregiver health but also reduce adverse health outcomes for care recipients.* (Am J Geriatr Psychiatry 2011; 19:382–391)

**Key Words:** Caregiver burden, mortlity, hospitalization, adverse health outcomes of care recipient

Received August 16, 2009; revised April 24, 2010; accepted April 26, 2010. From the Department of Geriatrics, Nagoya University Graduate School of Medicine, Nagoya (MK, HE, JH, SI, YH); Department of Epidemiology, National Institute for Longevity Sciences, Aichi Prefecture (HS); and Faculty of Medical Welfare Department of Community Care Philanthropy, Aichi Shukutoku University, Aichi (AI), Japan. Send correspondence and reprint requests to Masafumi Kuzuya, M.D., Ph.D., Department of Geriatrics, Nagoya University Graduate School of Medicine, 65 Tusruma-cho, Showa-ku, Nagoya 466-8550, Japan. e-mail: kuzuya@med.nagoya-u.ac.jp.



DOI: 10.1097/JGP.0b013e3181e9b98d

The current trend toward a community-based healthcare system means that when older people require care, much of it is provided at home. Thus, family members are providing care for ill or disabled older relatives. Family caregiving has been intensively studied in the past decade, particularly the impact on caregivers of providing home care to a family member. Caregiver burden has been defined as a negative reaction to the impact of providing care on the caregiver's social, occupational, and personal roles.[1–3] It is well documented that informal care for the disabled elderly places heavy burdens on family caregivers.[1–3] Previous studies demonstrated that caregiver burden is associated with the substantial care needs of seriously ill patients, which are in turn associated with the presence of dementia, behavioral problems, poorer physical functioning, and factors that are not readily modifiable.[4–7] Caregiver burden can lead to a chronic stress response that can worsen caregiver health, contribute to psychiatric morbidity in the form of increased depression,[8] contribute to the risk of health problems such as wound healing impairment, elevated blood pressure, and coronary heart disease risk and immune function impairment,[9–11] and is an independent risk factor for mortality.[12]

Thus, most of the previous studies on caregiver burden have focused on examining its cause(s) and extensively examining caregiver health. However, conversely, much less attention has been paid to the impact of caregiver burden or distress on the health of the partner, the care recipient. In fact, it remains uncertain whether caregiver burden or distress has any influence on the health-related outcomes of care recipients, although the association of caregiver burden with long-term care placement has been well demonstrated.[13,14] In this study, we investigated whether caregiver burden is associated with adverse health outcomes of the care recipients, including all-cause mortality and hospitalization for acute illness, during a 3-year study period. In addition, we examined the effect of community-based respite care services, including day-care, home-help, and nursing-home respite stay services on the adverse outcomes of care recipients.

## METHODS

### Study Setting and Cohort Participants

In this study, we employed baseline data on the care recipient and caregiver pairs in the Nagoya Longitudinal Study for Frail Elderly (NLS-FE) and data on the mortality and hospitalization of the care recipients during the 3-year follow-up period. Japan introduced a universal-coverage long-term care insurance (LTCI) program in 2000. Under the LTCI program, each applicant's care levels are determined according to eligibility criteria. Eligibility status is classified into six levels ("needs support" and care levels 1–5) by the estimation of care needs based on an assessment of the current physical and mental status of the patient and their use of medical procedures.[15] The NLS-FE was designed to compare the outcomes of different uses of community-based care services provided by the LTCI program.[16,17] The study sample consisted of 1,875 community-dwelling elderly (632 men and 1,243 women, age 65 years or older) with some degree of physical or mental disability. They were eligible for the LTCI program, lived in Nagoya City, Japan, and received various kinds of community-based services from the Nagoya City Health Care Service Foundation for Older People, which has 17 visiting nursing stations associated with care-managing centers. These 1,875 NLS-FE participants and 1,502 caregivers (373 of the 1,875 participants lacked a primary caregiver), who were enrolled between December 1, 2003, and January 31, 2004, were scheduled to undergo comprehensive in-home assessments by trained nurses at the baseline and at 6, 12, and 24 months. At 3-month intervals, data were collected about any important events in the lives of the participants, including mortality and admission to the hospital for acute illness during the 3-year follow-up. Written informed consent for participation was obtained from the participants, care recipients, and caregivers, or, for those with substantial cognitive impairment, from a surrogate (usually the closest relative or legal guardian), according to procedures approved by the Institutional Review Board of Nagoya University Graduate School of Medicine.

### Data Collection

The data were collected at the clients' homes through structured interviews with care recipients or surrogates and caregivers and from care-managing center records taken by trained nurses. The data included each participant's demographic characteristics, general socioeconomic status, living arrangements, subjective economic status, use of medical services, and the utilization of a total of seven community-based services available under LTCI programs, including the day-care service, visiting nurse service, home-help service, visiting bathing service, visiting rehabilitation, assistive device leasing, and nursing-home respite stay (overnight respite, temporary stays at nursing facilities). The data also included depressive symptoms as assessed by the 15-item Geriatric Depression Scale (GDS-15) (range: 0–15, with higher values indicating more depressive symptoms)[18] and a rating for eight basic activities of daily living (bADL) using summary scores ranging from 0 (total disability) to 20 (no disability). The information on the following physician-diagnosed chronic conditions was obtained from care-managing center records: ischemic heart disease, congestive heart failure, cerebrovascular disease, diabetes mellitus, dementia, chronic obstructive pulmonary disease, cancer, hypertension, and other diseases comprising the Charlson Comorbidity Index,[19] which represents a sum of weighted indexes that takes into account the number and seriousness of preexisting comorbid conditions (range: 0–19, with a higher value indicating higher comorbidity).

Data were also obtained from caregivers concerning their own personal demographic characteristics including caregiver relationship to care recipient (spouse or not), and the presence of behavioral disturbance of the care recipient according to the primary assessment dataset of the public LTCI, including wandering, hallucinations, physically aggressive behaviors, verbal aggression, delusions, altered sleep-wake cycles, sexually disinhibited behaviors, aberrant behaviors, abnormal eating behaviors, and resistance to care. Depressive symptoms were assessed by the GDS-15, and the caregiver's subjective burden was assessed by the Japanese version of the Zarit Burden Interview (ZBI), which is a 22-item self-report inventory that examines the burden associated with functional behavioral impairments in the home care situation (range: 0–88, with higher values indicating a greater burden). The primary caregivers were also asked to rate their current overall health in three categories of subjective health status (poor, fair, and good to excellent).

### Subjects for the Analysis

Among the original 1,502 pairs at baseline, 276 caregivers could not complete or refused to assess the ZBI, and the data on comorbidity condition or sociodemographic characteristics were lacking for 159 participants. The study sample, therefore, consisted of 1,067 community-dwelling disabled elderly (387 men, 680 women, age range: 65–104 years) and paired caregivers (256 men, 811 women, age range: 31–90 years). There were no statistical differences in mortality and hospitalization rates during the follow-up period between participants with and without caregiver ZBI measurements among the 1,502 participants. Of these 1,067 pairs, 259 care recipients could not complete the GDS-15 because of severe cognitive impairment or communication impairment, and 101 caregivers because of refusal to do the assessment.

### Statistical Analysis

The ZBI score was categorized into quartiles (quartile 1: score, 0–15, N = 284; quartile 2: 16–26, N = 253; quartile 3: 27–39, N = 269; quartile 4: 40–84, N = 261). Baseline characteristics of the study participants, including both care recipients and caregivers, were examined using the Jonckheere–Terpstra test or the General Linear Models for trends across the quartiles of the ZBI score. Analysis of variance for multiple comparisons was used to determine differences among the quartiles of the ZBI score for continuous variables, and the Pearson $\chi^2$ test was used to test categorical variables. The end point of this study was defined as the time to all-cause death or hospitalization because of acute illness, whichever occurred first, during follow-up. Cox proportional hazard models and the Kaplan–Meier method (differences between strata of the ZBI score levels determined using log-rank tests) were used to assess the association of quartiles of the ZBI score with those adverse outcomes after enrollment during a 3-year period (3-month intervals). To create an ideal model for a multivariate Cox proportional hazards model, we first evaluated the association between

each covariate and all-cause death or hospitalization, using the univariate Cox proportional hazards model. Covariates included, for the recipient, sociodemographic characteristics, the presence or absence of regular medical checkups, the number of community-based services, economic status, bADL score, the Charlson comorbidity index, and the presence or absence of selected major comorbidities and behavioral problems. Covariates also included, for the caregiver, sociodemographic characteristics, subjective health status, and categorized ZBI score. In the multivariate analysis, the covariates included were variables associated with each event with $p < 0.05$ in univariate analysis. In models considering the quartiles of the caregiver ZBI score, we compared hazard ratios (HRs) with a corresponding 95% confidence interval (CI) in the second, third, and fourth quartiles with those in the first quartile (referent).

Additional analyses stratified by the use or nonuse of community-based respite care services including day-care, home-help, and nursing-home respite stay services were also performed using a consistent set of covariates to examine the data for possible interactions of these variables with the adverse health outcomes of care recipients. Student's *t*-test and analysis of covariance (ANCOVA) were used to compare the caregiver ZBI score according to the service use and nonuse groups. Covariates of ANCOVA included recipient gender, age, bADL score, the Charlson comorbidity index, the presence or absence of dementia and behavior problems, caregiver gender, and caregiver age.

The data were analyzed using the SAS, Release 9.13. Probability value of <0.05 was considered significant.

## RESULTS

The baseline distribution of the sociodemographic characteristics of the care recipients and caregivers according to the quartiles of the ZBI score is shown in Table 1. We used analysis of variance or Pearson $\chi^2$ test to evaluate differences among the quartiles of the ZBI score. The bADL score decreased, and the number of community-based services used, the Charlson comorbidity index, and recipient GDS-15 score increased as the level of the ZBI quartile increased. The care recipients whose caregivers' ZBI scores were in higher quartiles were more likely to show a higher prevalence of dementia ($\chi^2$ test: $\chi^2 = 61.09$, degrees of freedom [*df*] = 3, $p < 0.001$; Jonckheere-Terpstra test: z statistics, Z value = 7.51, N = 1,067, $p < 0.001$), behavioral problems ($\chi^2 = 14.75$, $df = 3$, $p = 0.002$; Jonckheere-Terpstra test, Z value = 8.58, N = 1,067, $p < 0.001$) and a history of cerebrovascular disease ($\chi^2 = 10.31$, $df = 3$, $p = 0.016$; Jonckheere-Terpstra test, Z value = 2.37, N = 1,067, $p = 0.018$). The caregiver's GDS-15 score increased (General Linear Model, *F* value = 313.48, $df = 1,964$, $p < 0.001$), and the prevalence of good to excellent subjective health status of the caregiver decreased with increasing quartiles of the ZBI score (Jonckheere-Terpstra test, Z value = 5.37, N = 1,067, $p < 0.001$). There were no differences in the rate of regular medical checkups ($\chi^2$ test, $\chi^2 = 5.66$, $df = 3$, $p = 0.130$), living arrangements (living alone or with one person versus living with two or more, $\chi^2 = 1.46$, $df = 3$, $p = 0.692$), and three categories of economic status ($\chi^2 = 6.70$, $df = 3$, $p = 0.349$) among the quartiles of the ZBI score.

During the 3-year period, 268 care recipients died and 455 were admitted to hospitals (Table 2). The participants whose caregivers' ZBI scores were in the higher quartiles were more likely to die and be hospitalized during the follow-up period than those whose caregivers' scores were in the lower quartile categories ($\chi^2$ test, $\chi^2 = 9.78$, $df = 3$, $p = 0.020$; $\chi^2 = 11.09$, $df = 3$, $p = 0.007$, respectively).

Kaplan–Meier curves of survival and the cumulative incidence of hospitalization during the 3-year period among care recipients according to the quartile of the caregivers' ZBI scores demonstrated that all-cause mortality and hospitalization increased with higher quartiles of caregiver ZBI at baseline (log-rank $\chi^2$ test, mortality: $\chi^2 = 17.29$, $df = 3$, $p < 0.001$; hospitalization: $\chi^2 = 23.61$, $df = 3$, $p < 0.001$; Fig. 1).

The univariate Cox proportional hazards model revealed that the recipients whose caregivers' ZBI scores were in the highest quartile were 1.93 times and 1.86 times more likely to suffer all-cause mortality and hospitalization, respectively, during the 3-year period than those in the lowest quartile (95% CI: 1.38–2.71, Wald $\chi^2$ test, $\chi^2 = 14.80$, $df = 1$, $p < 0.001$; 95% CI: 1.43–2.42, Wald $\chi^2 = 21.16$, $df = 1$, $p < 0.001$). The GDS-15 score of the care recipients and caregivers was not associated with mortality and hospitalization in univariate analysis (mortality: HR: 1.03; 95% CI: 0.98–1.07, Wald $\chi^2$

**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 $\chi^2$ 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**

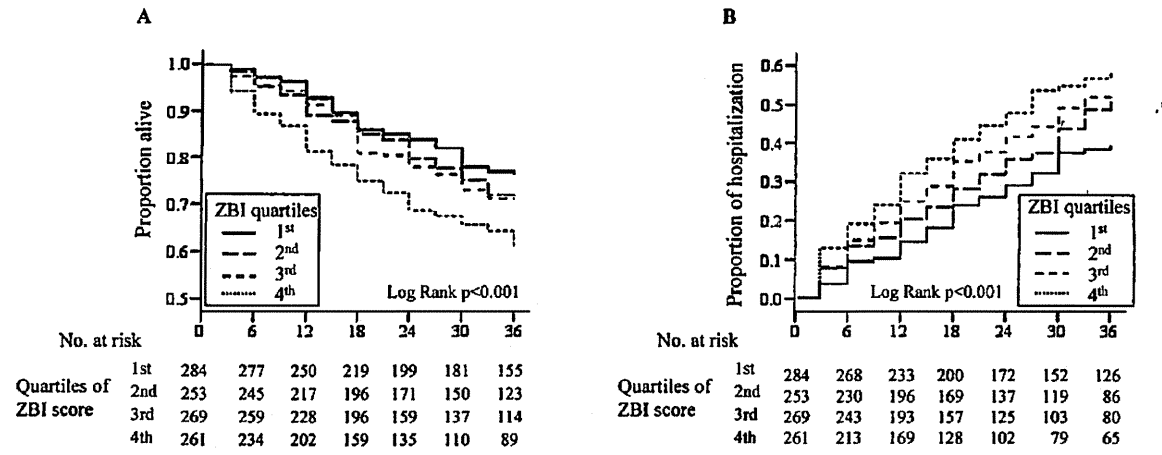
| | Quartile Group of ZBI Score | | | | | |
|---|---|---|---|---|---|---|
| | 1st, n = 284 | 2nd, n = 253 | 3rd, n = 269 | 4th, n = 261 | Total, n = 1,067 | p[a] |
| Adverse outcomes, no. (% of each quartile) | | | | | | |
| All-cause death | 58 (20.4) | 63 (24.9) | 64 (23.8) | 83 (31.8) | 268 (25.1)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

[a]Pearson $\chi^2$ 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 $\chi^2$ 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**




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 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[a] | | | Model 2[b] | | | Model 1[c] | | | Model 2[d] | | |
| | 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[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 $\chi^2$ | p | HR (95% CI) | 3rd Wald $\chi^2$ | p | HR (95% CI) | 4th Wald $\chi^2$ | 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.

*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.*

## References

1. George LK, Gwyther LP: Caregiver well-being: a multidimensional examination of family caregivers of demented adults. Gerontologist 1986; 26:253–259
2. Zarit SH, Todd PA, Zarit JM: Subjective burden of husbands and wives as caregivers. Gerontologist 1986; 26:260–266
3. Schulz R, Visintainer P, Williamson GM: Psychiatric and physical morbidity effects of caregiving. J Gerontol 1990; 45:181–191
4. Torti FM Jr, Gwyther LP, Reed SD, et al: A multinational review of recent trends and reports in dementia caregiver burden. Alzheimer Dis Assoc Disord 2004; 18:99–109
5. Covinsky KE, Goldman L, Cook EF, et al: The impact of serious illness on patients' families. SUPPORT Investigators. Study to Understand Prognoses and Preferences for Outcomes and Risks of Treatment. JAMA 1994; 272:1839–1844
6. Scholte op Reimer WJ, de Haan RJ, et al: The burden of caregiving in partners of long-term stroke survivors. Stroke 1998; 29: 1605–1611
7. Vitaliano PP, Katon W, Unützer J: Making the case for caregiver research in geriatric psychiatry. Am J Geriatr Psychiatry 2005; 13:834–843
8. Gallicchio L, Siddiqi N, Langenberg P, et al: Gender differences in burden and depression among informal caregivers of demented elders in the community. Int J Geriatr Psychiatry 2002; 17:154–163
9. Kiecolt-Glaser JK, Marucha PT, Malarkey WB, et al: Slowing of wound healing by psychological stress. Lancet 1995; 346:1194–1196
10. Kiecolt-Glaser JK, Glaser R, Gravenstein S, et al: Chronic stress alters the immune response to influenza virus vaccine in older adults. Proc Natl Acad Sci USA 1996; 93:3043–3047
11. von Känel R, Mausbach BT, Patterson TL, et al: Increased Framingham Coronary Heart Disease Risk Score in dementia caregivers relative to non-caregiving controls. Gerontology 2008; 54:131–137
12. Schulz R, Beach SR: Caregiving as a risk factor for mortality: the Caregiver Health Effects Study. JAMA 1999; 282:2215–2219
13. Yaffe K, Fox P, Newcomer R, et al: Patient and caregiver characteristics and nursing home placement in patients with dementia. JAMA 2002; 287:2090–2097
14. Gaugler JE, Kane RL, Kane RA, et al: Caregiving and institutionalization of cognitively impaired older people: utilizing dynamic predictors of change. Gerontologist 2003; 43:219–229
15. Tsutsui T, Muramatsu N: Care-needs certification in the long-term care insurance system of Japan. J Am Geriatr Soc 2005; 53:522–527
16. Kuzuya M, Masuda Y, Hirakawa Y, et al: Day care service use is associated with lower mortality in community-dwelling frail older people. J Am Geriatr Soc 2006; 54:1364–1371
17. Kuzuya M, Masuda Y, Hirakawa Y, et al: Underuse of medications for chronic diseases in the oldest of community-dwelling older frail Japanese. J Am Geriatr Soc 2006; 54:598–605
18. Yesavage JA: Geriatric Depression Scale. Psychopharmacol Bull 1988; 24:709–711
19. Charlson ME, Pompei P, Ales KL, et al: A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis 1987; 40:373–383
20. Pearlin LI, Mullan JT, Semple SJ, et al: Caregiving and the stress process: an overview of concepts and their measures. Gerontologist 1990; 30:583–594
21. Lachs MS, Pillemer K. Abuse and neglect of elderly persons. N Engl J Med 1995; 332:437–443
22. Beach SR, Schulz R, Williamson GM, et al: Risk factors for potentially harmful informal caregiver behavior. J Am Geriatr Soc 2005; 53:255–261
23. Blazer DG, Sachs-Ericsson N, Hybels CF: Perception of unmet basic needs as a predictor of mortality among community-dwelling older adults. Am J Public Health 2005; 95:299–304
24. Sands LP, Wang Y, McCabe GP, et al: Rates of acute care admissions for frail older people living with met versus unmet activity of daily living needs. J Am Geriatr Soc 2006; 54: 339–344
25. Lachs MS, Williams CS, O'Brien S, et al: The mortality of elder mistreatment. JAMA 1998; 280:428–432
26. Schulz R, Drayer RA, Rollman BL: Depression as a risk factor for non-suicide mortality in the elderly. Biol Psychiatry 2002; 52:205–225
27. Cronin-Stubbs D, de Leon CF, Beckett LA, et al: Six-year effect of depressive symptoms on the course of physical disability in community-living older adults. Arch Intern Med 2000; 160:3074–3080
28. Williams LS, Ghose SS, Swindle RW: Depression and other mental health diagnoses increase mortality risk after ischemic stroke. Am J Psychiatry 2004; 161:1090–1095
29. Martikainen P, Valkonen T: Mortality after the death of a spouse: rates and causes of death in a large Finnish cohort. Am J Public Health 1996; 86:1087–1093
30. Christakis NA, Allison PD: Mortality after the hospitalization of a spouse. N Engl J Med 2006; 354:719–730
31. Zarit SH, Gaugler JE, Jarrott SE: Useful services for families: research findings and directions. Int J Geriatr Psychiatry 1999; 14:165–177
32. Sörensen S, Pinquart M, Duberstein P: How effective are interventions with caregivers? An updated meta-analysis. Gerontologist 2002; 42:356–372
33. Shaw C, McNamara R, Abrams K, et al: Systematic review of respite care in the frail elderly. Health Technol Assess 2009; 13: 1–224
34. Cox C: Findings from a statewide program of respite care: a comparison of service users, stoppers, and nonusers. Gerontologist 1997; 37:511–517
35. Baumgarten M, Lebel P, Laprise H, et al: Adult day care for the frail elderly: outcomes, satisfaction, and cost. J Aging Health 2002; 14:237–259
36. Grant I, McKibbin CL, Taylor MJ, et al: In-home respite intervention reduces plasma epinephrine in stressed Alzheimer caregivers. Am J Geriatr Psychiatry 2003; 11:62–72

Osteoporos Int (2011) 22:143–152
DOI 10.1007/s00198-010-1239-9

ORIGINAL ARTICLE

# 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


**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

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 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.

**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.

## References

1. Ministry of Health, Labor and Welfare. Comprehensive survey of living conditions of the people on Health and Welfare. Section3 2004. Available at: http://www.mhlw.go.jp/toukei/saikin/hw/k-tyosa/k-tyosa04/4-2.html (accessed 1 December 2009)

2. Christodoulou C, Cooper C (2003) What is osteoporosis? Postgrad Med J 79:133–138
3. Gennari C (2001) Calcium and vitamin D nutrition and bone disease of the elderly. Public Health Nutr 4:547–559
4. Prentice A (2004) Diet, nutrition and the prevention of osteoporosis. Public Health Nutr 7:227–243
5. Macdonald HM, New SA, Golden MH, Campbell MK, Reid DM (2004) Nutritional associations with bone loss during the menopausal transition: evidence of a beneficial effect of calcium, alcohol, and fruit and vegetable nutrients and of a detrimental effect of fatty acids. Am J Clin Nutr 79:155–165
6. New SA, Bolton-Smith C, Grubb DA, Reid DM (1997) Nutritional influences on bone mineral density: a cross-sectional study in premenopausal women. Am J Clin Nutr 65:1831–1839
7. Prynne CJ, Mishra GD, O'Connell MA, Muniz G, Laskey MA, Yan L, Prentice A, Ginty F (2006) Fruit and vegetable intakes and bone mineral status: a cross-sectional study in 5 age and sex cohorts. Am J Clin Nutr 83:1420–1428
8. Okubo H, Sasaki S, Horiguchi H, Oguma E, Miyamoto K, Hosoi Y, Kim MK, Kayama F (2006) Dietary patterns associated with bone mineral density in premenopausal Japanese farmwomen. Am J Clin Nutr 83:1185–1192
9. McGartland CP, Robson PJ, Murray LJ, Cran GW, Savage MJ, Watkins DC, Rooney MM, Boreham CA (2004) Fruit and vegetable consumption and bone mineral density: the Northern Ireland Young Hearts Project. Am J Clin Nutr 80:1019–1023
10. Tucker KL, Chen H, Hannan MT, Cupples LA, Wilson PW, Felson D, Kiel DP (2002) Bone mineral density and dietary patterns in older adults: the Framingham Osteoporosis Study. Am J Clin Nutr 76:245–252
11. Gutteridge JM (1994) Biological origin of free radicals, and mechanisms of antioxidant protection. Chem Biol Interact 91:133–140
12. Rock CL, Jacob RA, Bowen PE (1996) Update on the biological characteristics of the antioxidant micronutrients: vitamin C, vitamin E, and the carotenoids. J Am Diet Assoc 96:693–702
13. Almeida M, Han L, Martin-Millan M, O'Brien CA, Manolagas SC (2007) Oxidative stress antagonizes Wnt signaling in osteoblast precursors by diverting beta-catenin from T cell factor- to forkhead box *O*-mediated transcription. J Biol Chem 282:27298–27305
14. Jilka RL, Weinstein RS, Parfitt AM, Manolagas SC (2007) Quantifying osteoblast and osteocyte apoptosis: challenges and rewards. J Bone Miner Res 22:1492–1501
15. Garrett IR, Boyce BF, Oreffo RO, Bonewald L, Poser J, Mundy GR (1990) Oxygen-derived free radicals stimulate osteoclastic bone resorption in rodent bone in vitro and in vivo. J Clin Invest 85:632–639
16. Basu S, Michaelsson K, Olofsson H, Johansson S, Melhus H (2001) Association between oxidative stress and bone mineral density. Biochem Biophys Res Commun 288:275–279
17. Yalin S, Bagis S, Polat G (2005) Is there a role of free oxygen radicals in primary male osteoporosis? Clin Exp Rheumatol 23:689–692
18. Law MR, Hackshaw AK (1997) A meta-analysis of cigarette smoking, bone mineral density and risk of hip fracture: recognition of a major effect. BMJ 315:841–846
19. Melhus H, Michaelsson K, Holmberg L, Wolk A, Ljunghall S (1999) Smoking, antioxidant vitamins, and the risk of hip fracture. J Bone Miner Res 14:129–135
20. Maggio D, Barabani M, Pierandrei M (2003) Marked decrease in plasma antioxidants in aged osteoporotic women: results of a cross-sectional study. J Clin Endocrinol Metab 88:1523–1527
21. Maggio D, Polidori MC, Barabani M, Tufi A, Ruggiero C, Cecchetti R, Aisa MC, Stahl W, Cherubini A (2006) Low levels of carotenoids and retinol in involutional osteoporosis. Bone 38:244–248
22. Yang Z, Zhang Z, Penniston KL, Binkley N, Tanumihardjo SA (2008) Serum carotenoid concentrations in postmenopausal women from the United States with and without osteoporosis. Int J Vitam Nutr Res 78:105–111
23. Sahni S, Hannan MT, Blumberg J, Cupples LA, Kiel DP, Tucker KL (2009) Inverse association of carotenoid intakes with 4-y change in bone mineral density in elderly men and women: the Framingham Osteoporosis Study. Am J Clin Nutr 89:416–424
24. Sahni S, Hannan MT, Gagnon D, Blumberg J, Cupples LA, Kiel DP, Tucker KL (2009) Protective effect of total and supplemental vitamin C intake on the risk of hip fracture—a 17-year follow-up from the Framingham Osteoporosis Study. Osteoporos Int 20:1853–1861
25. Sugiura M, Nakamura M, Ogawa K, Ikoma Y, Ando F, Yano M (2008) Bone mineral density in post-menopausal female subjects is associated with serum antioxidant carotenoids. Osteoporos Int 19:211–219
26. Wakai K, Egami I, Kato K, Lin Y, Kawamura T, Tamakoshi A, Aoki R, Kojima M, Nakayama T, Wada M, Ohno Y (1999) A simple food frequency questionnaire for Japanese diet—Part I. Development of the questionnaire, and reproducibility and validity for food groups. J Epidemiol 9:216–226
27. Egami I, Wakai K, Kato K, Lin Y, Kawamura T, Tamakoshi A, Aoki R, Kojima M, Nakayama T, Wada M, Ohno Y (1999) A simple food frequency questionnaire for Japanese diet—Part II. Reproducibility and validity for nutrient intakes. J Epidemiol 9:227–234
28. Science and Technology Agency (1983) Standard tables of food composition in Japan, 4th edn. Printing Bureau, Ministry of Finance, Tokyo, in Japanese
29. Science and Technology Agency (1997) Standard tables of food composition in Japan. (for new foods], 5th edn. Printing Bureau, Ministry of Finance, Tokyo, in Japanese
30. Yano M, Kato M, Ikoma Y, Kawasaki A, Fukazawa Y, Sugiura M, Matsumoto H, Ohara Y, Nagao A, Ogawa K (2005) Quantitation of carotenoids in raw and processed fruits in Japan. Food Sci Technol Res 11:13–18
31. Aizawa K, Inakuma T (2007) Quantitation of carotenoids in commonly consumed vegetables in Japan. Food Sci Technol Res 13:247–252
32. Sugiura M, Nakamura M, Ogawa K, Ikoma Y, Matsumoto H, Ando F, Shimokata H, Yano M (2009) Synergistic interaction of cigarette smoking and alcohol drinking with serum carotenoid concentrations: findings from a middle-aged Japanese population. Br J Nutr 102:1211–1219
33. Orimo H, Hayashi Y, Fukunaga M et al (2001) Diagnostic criteria for primary osteoporosis. J Bone Miner Metab 19:331–337
34. Feskanich D, Singh V, Willett WC, Colditz GA (2002) Vitamin A intake and hip fractures among postmenopausal women. JAMA 287:47–54
35. Melhus H, Michaëlsson K, Kindmark A, Bergström R, Holmberg L, Mallmin H, Wolk A, Ljunghall S (1998) Excessive dietary intake of vitamin A is associated with reduced bone mineral density and increased risk for hip fracture. Ann Intern Med 129:770–778
36. Promislow JH, Goodman-Gruen D, Slymen DJ, Barrett-Connor E (2002) Retinol intake and bone mineral density in the elderly: the Rancho Bernardo Study. J Bone Miner Res 17:1349–1358
37. Ministry of Health, Labor and Welfare. Recommended dietary allowance for Japanese: dietary reference intakes. Section 2 2009. Available at: http://www.mhlw.go.jp/shingi/2009/05/dl/s0529-4i.pdf (accessed 1 December 2009)

38. Poal-Manresa J, Little K, Trueta J (1970) Some observations on the effects of vitamin C deficiency on bone. Br J Exp Pathol 51:372–378
39. Kipp DE, McElvain M, Kimmel DB, Akhter MP, Robinson RG, Lukert BP (1996) Scurvy results in decreased collagen synthesis and bone density in the guinea pig animal model. Bone 18:281–288
40. Termine JD (1990) Cellular activity, matrix proteins, and aging bone. Exp Gerontol 25:217–221
41. Peterkofsky B (1991) Ascorbate requirement for hydroxylation and secretion of procollagen: relationship to inhibition of collagen synthesis in scurvy. Am J Clin Nutr 54:1135S–1140S
42. Iotsova V, Caamano J, Loy J, Yang Y, Lewin A, Bravo R (1997) Osteopetrosis in mice lacking NF-kappaB1 and NF-kappaB2. Nat Med 3:1285–1289
43. Baeuerle PA, Rupec RA, Pahl HL (1996) Reactive oxygen intermediates as second messengers of a general pathogen response. Pathol Biol (Paris) 44:29–35
44. Garrett IR, Boyce BF, Oreffo RO, Bonewald L, Poser J, Mundy GR (1996) Oxygen-derived free radicals stimulate osteoclastic bone resorption in rodent bone in vitro and in vivo. J Clin Invest 85:632–639
45. Bax BE, Alam AS, Banerji B, Bax CM, Bevis PJ, Stevens CR, Moonga BS, Blake DR, Zaidi M (1992) Stimulation of osteoclastic bone resorption by hydrogen peroxide. Biochem Biophys Res Commun 183:1153–1158
46. Yamaguchi M, Uchiyama S (2003) Effect of carotenoid on calcium content and alkaline phosphatase activity in rat femoral tissues in vitro: the unique anabolic effect of beta-cryptoxanthin. Biol Pharm Bull 26:1188–1191
47. Yamaguchi M, Uchiyama S (2004) Beta-cryptoxanthin stimulates bone formation and inhibits bone resorption in tissue culture in vitro. Mol Cell Biochem 258:137–144
48. Uchiyama S, Yamaguchi M (2004) Oral administration of beta-cryptoxanthin induces anabolic effects on bone components in the femoral tissues of rats in vivo. Biol Pharm Bull 27:232–235
49. Hosseinimehr SJ, Nemati A (2006) Radioprotective effects of hesperidin against gamma irradiation in mouse bone marrow cells. Br J Radiol 79:415–418
50. Chiba H, Uehara M, Wu J, Wang X, Masuyama R, Suzuki K, Kanazawa K, Ishimi Y (2003) Hesperidin, a citrus flavonoid, inhibits bone loss and decreases serum and hepatic lipids in ovariectomized mice. J Nutr 133:1892–1897