High Vitamin C Intake with High Serum β-Cryptoxanthin Associated with Lower Risk for Osteoporosis in Post-Menopausal Japanese Female Subjects: Mikkabi Cohort Study

Minoru SUGIURA1, Mieko NAKAMURA2, Kazunori OGAWA1, Yoshinori IKOMA1 and Masamichi YANO1

1 Citrus Research Division, NARO Institute of Fruit Tree Science, National Agriculture and Food Research Organization (NARO), 485–6 Okitsu-nakachou, Shimizu, Shizuoka, Shizuoka 424–0292, Japan
2 Department of Community Health and Preventive Medicine, Hamamatsu University School of Medicine, 1–20–1 Handayama, Hamamatsu, Shizuoka 431–3192, Japan

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Summary Recent epidemiological studies show that antioxidant vitamins and carotenoids might be beneficial to the maintenance of bone health. Recently, we found that serum carotenoids were inversely associated with the risk of developing osteoporosis in post-menopausal Japanese female subjects. However, little is known about the vitamin alone and/or the combination of the vitamin and carotenoid with the risk of osteoporosis. The objective of this study was to investigate longitudinally whether antioxidant vitamins and their combination with carotenoids are associated with the risk of developing osteoporosis. We conducted a follow-up study on 187 post-menopausal female subjects from the Mikkabi prospective cohort study. Those who participated in previous bone mineral density (BMD) surveys and completed four years of follow-up were examined longitudinally. During a four-year follow-up, fifteen of the post-menopausal female subjects developed new-onset osteoporosis. After adjustment for confounders, the odds ratios (OR) for osteoporosis in the highest tertiles of vitamins C and E and retinol intakes against the lowest tertiles were 0.15 (95% confidence interval (CI): 0.02–0.99), 0.50 (CI: 0.08–3.23), and 1.49 (CI: 0.36–6.22), respectively. Furthermore, a significantly lower odds ratio was observed in the higher vitamin C intake group (169–625 mg/d) with higher serum β-cryptoxanthin (1.88–10.53 μM) against the lower vitamin C intake group (47–168 mg/d) with lower serum β-cryptoxanthin (0.24–1.84 μM) used for the reference group (p<0.05). The combination of β-cryptoxanthin and vitamin C is inversely associated with the risk of developing osteoporosis in post-menopausal Japanese female subjects.

Key Words bone mineral density, osteoporosis, carotenoid, vitamin, post-menopausal female

Bone loss with aging induces osteoporosis. Osteoporosis is a chronic disease characterized by low bone density and microarchitectural disruption, leading to bone fragility and increased susceptibility to fractures (1). Therefore, osteoporosis and its related fractures are a major public health problem, not only in Japan, but worldwide (2). Nutritional factors may have the effect of preventing the development of osteoporosis and its related fractures with increasing age. Calcium and vitamin D are well-known, important nutritional factors in maintaining normal bone metabolism (3). In addition, other nutrients, such as potassium, magnesium, zinc, copper, iron, vitamin C and vitamin K, may also have beneficial effects on bone metabolism (4). Fruits and vegetables are rich in these micronutrients; therefore, the intake of these types of foods might affect bone health. In fact, many recent epidemiological studies have shown an association between fruit and vegetable intakes and bone mineral density (BMD) in both young and elderly subjects (5–10).

Antioxidant vitamins and carotenoids are widely included in fruit and vegetables, and these micronutrients have been shown to contribute to the body’s defense against reactive oxygen species (11, 12). Recently, many experimental studies have shown that a reduction in the formation of reactive oxygen species and free radicals might reduce the rate of bone loss because oxidative stress is involved in osteoclastogenesis, in apoptosis of osteoblasts and osteocytes, and therefore in bone resorption (13–15). Furthermore, recent epidemiologic studies have also shown a relationship between oxidative stress and BMD or osteoporosis (16–18). From these previous findings in epidemiological and experimental studies, we assume that antioxidant vitamins and carotenoids may provide benefits to bone metabolism against oxidative stress. Actually, many recent epidemiologic reports have shown inverse associations of antioxidant micronutrient intake or serum level with low BMD, risk of fracture, and/or risk of osteoporosis (19–26).

Previously, we found that the serum concentrations of β-cryptoxanthin and β-carotene were weakly but positively associated with the radial BMD in post-meno-
paulal Japanese female subjects (27). These associations were also observed in a prospective cohort study: that is, high serum β-cryptoxanthin was inversely associated with the risk of developing osteoporosis (28). On the other hand, we also previously found that a high intake of both of vitamin C and β-cryptoxanthin was inversely associated with the risk for low BMD cross-sectionally (29). However, a thorough longitudinal cohort study about the association of the combination of antioxidant vitamins and carotenoids with the incidence of osteoporosis has not been conducted.

The objective of this study was to investigate longitudinally whether antioxidant vitamins and their combination with carotenoids are associated with the risk of developing osteoporosis in post-menopausal Japanese female subjects.

MATERIALS AND METHODS

Ethics statement. This study was carried out in accordance with the Declaration of Helsinki and approved by the ethics committee of the National Institute of Fruit Tree Science. We obtained written, informed consent from all participants involved in our study.

Study design. This was a prospective survey involving participants in the Mikkabi cohort study conducted in the town of Mikkabi, Shizuoka Prefecture, Japan. In a baseline survey, study subjects were recruited from participants in an annual health check-up program conducted by the local government of Mikkabi in April 2005 (27). The study design has been described previously (28). In this study, longitudinal cohort analyses about the associations of vitamin intakes and/or the combination of these vitamin intake with the serum levels of carotenoids and the risk of developing osteoporosis were conducted using 187 post-menopausal female subjects whose T-score (which shows how a subject’s BMD compares with that of the young adult mean) at the baseline survey exceeded 70% (28). In our study, a person whose T-score was less than 70% according to the guidelines on the management of osteoporosis by the Japan Osteoporosis Society was considered to have osteoporosis (30). A person whose T-score exceeded 70% but was less than 80% was defined as having osteopenia.

The concentrations of six serum carotenoids at the baseline survey, lutein, lycopene, α-carotene, β-carotene, β-cryptoxanthin, and zeaxanthin, were analyzed by reverse-phase high-performance liquid chromatography using β-apo-8’-carotenal as an internal standard at the Laboratory of Public Health and Environmental Chemistry, Kyoto Biseibutsu Kenkyusho (Kyoto, Japan), as described previously (31). Preceding the study, intra-observer reproducibility of the measurement was evaluated. The range of coefficients of variation of measurements made five times for each of five subjects were 1.9–7.6% (median, 3.0%) for α-carotene, 1.1–7.0% (1.2%) for β-carotene, 0.9–2.7% (1.5%) for β-cryptoxanthin, 1.6–3.9% (3.0%) for lutein, 3.4–10.5% (6.7%) for lycopene and 1.7–10.6% (3.0%) for zeaxanthin. In this study, serum carotenoid concentration was measured by a single technical expert. Quality control of the measurements was assessed at least at 2–3-mo intervals using a pooled serum sample. The radial BMD at the baseline and follow-up survey was measured using dual-energy X-ray absorptiometry of each participant’s nondominant forearm with an osteometer (model DCS-600EX-III, ALOKA Co., Ltd., Tokyo, Japan), as described previously (27, 28). The measurement of the radial BMD of each participant was performed by a well-trained clinical technologist from the Seirei Preventive Health Care Center (Shizuoka, Japan).

Statistical analysis. Intakes of vitamins C, D, and E and retinol were skewed toward the higher concentrations. These values were log (natural)-transformed to improve the normality of their distribution. Dunnett multiple comparisons testing following one-way ANOVA was used to test the differences among tertiles of vitamin intakes. The paired t-test was used to test differences between the baseline and follow-up survey. All variables were presented as an original scale. The data are expressed as the means (standard deviation), range, or percent.

To assess the relationship between the vitamin intakes at the baseline and the development of osteoporosis after four years, logistic regression analyses were performed after adjusting for age, weight, height, years since menopause, current tobacco use, regular alcohol intake, exercise habits, supplement use, and total energy intake at the baseline. Multivariable adjustment for the intakes of calcium, magnesium, potassium, and vitamin D was further conducted as a sensitivity analysis. In the test for linear trends, the associations among the risks of osteoporosis across three categories assigned by means of vitamin intakes in each tertile were determined by logistic regression analysis.

All statistical analyses were performed using a statistical software package for Windows (SPSS ver. 12.0, SPSS Inc., Chicago, IL) on personal computers.

RESULTS

Characteristics of study subjects at the baseline and at the end of the follow-up survey

Table 1 shows the bone status at the baseline and at the end of the follow-up survey of study subjects according to the tertiles of baseline vitamin intakes. Other characteristics of study subjects, such as body height and weight, total energy and nutrient intake, and lifestyle, including tobacco use, exercise, regular alcohol intake, and dietary supplement use, were described previously (28). Vitamin C and E intakes increased with age, but there was no association between retinol intake and age. The radial BMD at the follow-up survey of each group stratified by tertiles of baseline vitamin intakes was significantly lower than that at the baseline survey. Although the radial BMD and T-scores at the baseline and follow-up survey were not different among the three groups stratified by vitamin intake, newly defined osteoporosis was low in the highest tertile (T3) of vitamin C intake compared with that in the lowest (T1) and middle (T2) groups. In our follow-up survey, two subjects in the normal group at the baseline and fifteen subjects in the
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The osteopenia group at the baseline developed osteoporosis after four years. Furthermore, twenty-five subjects in the normal group at the baseline developed osteopenia. In our survey, three subjects with osteopenia at the baseline were diagnosed as normal during the follow-up survey. The radial BMD in these three subjects seemed to have increased over the four-year follow-up.

In our follow-up survey, the mean of the baseline radial BMD in fallout subjects was significantly higher than that in subjects who participated in the follow-up survey ($p=0.019$).

**Risk of osteoporosis according to tertiles of baseline vitamin intakes**

The odds ratios of osteoporosis associated with the tertiles of three vitamin intakes at the baseline survey after adjusting for confounding factors are shown in Table 2. The odds ratios of osteoporosis in the middle (T2) and highest (T3) groups against the lowest tertile (T1) used for the reference group were calculated. After adjusting for age, weight, height, years since menopause, current tobacco use, regular alcohol intake, exercise habits, supplement use, and total energy intake, a significantly lower odds ratio for osteoporosis was observed in the group with the highest vitamin C intake (T3). This inverse association between baseline vitamin C intake and the development of osteoporosis was also observed after further adjusting for the intakes of calcium, magnesium, potassium, and vitamin D, but this inverse association was not significant. On the other hand, lower odds ratios were observed in groups with

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### Table 1. Bone status at the baseline and the end of follow-up survey of study subjects according to tertile of baseline vitamins intake.

<table>
<thead>
<tr>
<th>Tertiles of vitamin intake</th>
<th>Lowest</th>
<th>Middle</th>
<th>Highest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$n$</td>
<td>61</td>
<td>64</td>
<td>62</td>
</tr>
<tr>
<td>Age (years)</td>
<td>57.3 (5.1)</td>
<td>60.6 (5.8)</td>
<td>61.6 (5.6)</td>
</tr>
<tr>
<td>Dietary intake (mg/d)</td>
<td>108 (47–139)</td>
<td>169 (140–208)</td>
<td>256 (214–625)</td>
</tr>
<tr>
<td>Baseline survey in 2005</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone mineral density (g/cm²)</td>
<td>0.588 (0.082)</td>
<td>0.564 (0.075)</td>
<td>0.564 (0.062)</td>
</tr>
<tr>
<td>T-Score (%)</td>
<td>91.1 (12.6)</td>
<td>87.4 (11.6)</td>
<td>87.3 (9.7)</td>
</tr>
<tr>
<td>Follow-up survey in 2009</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone mineral density (g/cm²)</td>
<td>0.553 (0.085)</td>
<td>0.538 (0.075)</td>
<td>0.542 (0.061)</td>
</tr>
<tr>
<td>T-Score (%)</td>
<td>85.6 (13.1)</td>
<td>83.2 (11.6)</td>
<td>84.0 (9.5)</td>
</tr>
<tr>
<td>Newly defined osteoporosis</td>
<td>7 (11.5)</td>
<td>8 (12.5)</td>
<td>2 (3.2)</td>
</tr>
<tr>
<td>Vitamin E</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$n$</td>
<td>61</td>
<td>62</td>
<td>64</td>
</tr>
<tr>
<td>Age (years)</td>
<td>58.3 (5.3)</td>
<td>59.6 (5.8)</td>
<td>61.6 (5.7)</td>
</tr>
<tr>
<td>Dietary intake (mg/d)</td>
<td>6.0 (3.2–7.1)</td>
<td>7.9 (7.2–9.0)</td>
<td>10.5 (9.1–16.5)</td>
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<tr>
<td>Baseline survey in 2005</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone mineral density (g/cm²)</td>
<td>0.587 (0.077)</td>
<td>0.573 (0.071)</td>
<td>0.556 (0.070)</td>
</tr>
<tr>
<td>T-Score (%)</td>
<td>90.9 (12.0)</td>
<td>88.8 (11.0)</td>
<td>86.2 (10.9)</td>
</tr>
<tr>
<td>Follow-up survey in 2009</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone mineral density (g/cm²)</td>
<td>0.555 (0.081)</td>
<td>0.542 (0.068)</td>
<td>0.535 (0.073)</td>
</tr>
<tr>
<td>T-Score (%)</td>
<td>85.8 (12.6)</td>
<td>84.0 (10.4)</td>
<td>82.9 (11.3)</td>
</tr>
<tr>
<td>Newly defined osteoporosis</td>
<td>7 (11.5)</td>
<td>4 (6.5)</td>
<td>6 (9.4)</td>
</tr>
<tr>
<td>Retinol*</td>
<td></td>
<td></td>
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<tr>
<td>$n$</td>
<td>62</td>
<td>62</td>
<td>63</td>
</tr>
<tr>
<td>Age (years)</td>
<td>61.2 (5.9)</td>
<td>59.2 (5.4)</td>
<td>59.1 (5.8)</td>
</tr>
<tr>
<td>Dietary intake (mg/d)</td>
<td>138 (29–199)</td>
<td>265 (200–349)</td>
<td>538 (351–2,320)</td>
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<tr>
<td>Baseline survey in 2005</td>
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<td></td>
<td></td>
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<tr>
<td>Bone mineral density (g/cm²)</td>
<td>0.568 (0.068)</td>
<td>0.586 (0.077)</td>
<td>0.562 (0.075)</td>
</tr>
<tr>
<td>T-Score (%)</td>
<td>88.0 (10.5)</td>
<td>90.8 (11.9)</td>
<td>87.0 (11.6)</td>
</tr>
<tr>
<td>Follow-up survey in 2009</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone mineral density (g/cm²)</td>
<td>0.541 (0.071)</td>
<td>0.557 (0.070)</td>
<td>0.535 (0.080)</td>
</tr>
<tr>
<td>T-Score (%)</td>
<td>83.7 (11.0)</td>
<td>86.2 (10.9)</td>
<td>82.8 (12.3)</td>
</tr>
<tr>
<td>Newly defined osteoporosis</td>
<td>6 (9.7)</td>
<td>3 (4.8)</td>
<td>8 (12.7)</td>
</tr>
</tbody>
</table>

The data are expressed as the means (standard deviation), range, or percent.

* Preformed retinol.

† $p<0.01$ vs baseline by paired t-test.
the middle (T2) and highest (T3) levels of vitamin E intake, but these were not significant. Furthermore, a significant inverse association between baseline retinol intake and the development of osteoporosis was not observed. In this study population, although four post-menopausal female subjects were currently using female hormones, such as estrogen, the associations of the radial BMD with serum carotenoid concentrations did not change after excluding these four subjects.

In contrast, a significant inverse association of baseline vitamin C intake with the risk of developing osteopenia after four years was not observed (data not shown). Risk of osteoporosis according to vitamin C intake and serum β-cryptoxanthin concentration level

Next, study subjects were divided into two groups by median values of vitamin C intake and/or serum β-cryptoxanthin. Then the subjects were divided into four groups as follows: group 1, lower intake of vitamin C (47–168 mg/d) with a lower serum level of β-cryptoxanthin (0.24–1.84 μM); group 2, lower intake of vitamin C (47–168 mg/d) with a higher serum level of β-cryptoxanthin (1.88–10.53 μM); group 3, higher intake of vitamin C (169–625 mg/d) with a lower serum level of β-cryptoxanthin (0.24–1.84 μM); group 4, higher intake of vitamin C (169–625 mg/d) with a higher serum level of β-cryptoxanthin (1.88–10.53 μM). In neither group of higher vitamin C intake with a lower serum level of β-cryptoxanthin or lower vitamin C intake with a higher serum level of β-cryptoxanthin, were significantly lower odds ratios observed against the group of lower vitamin C intake with a lower serum level of β-cryptoxanthin used for the reference group (Table 3). In contrast, a significantly lower odds ratio was observed in the group with higher vitamin C intake and a higher serum level of β-cryptoxanthin 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 3). However, this significantly lower odds ratio became insignificant after further adjustments for calcium, magnesium, potassium, and vitamin D intakes (data not shown). Furthermore, significantly lower odds ratios were not observed in the groups with higher vitamin C intake and higher serum levels of carotenoids other than β-cryptoxanthin, such as lutein, lycopene, α-carotene, β-carotene, and zeaxanthin (data not shown).

**DISCUSSION**

Although analytical data on the serum concentra-
tions of six major types of carotenoids have been collected in our Mikkabi cohort study, no measurement has been made of the serum concentrations of vitamins. Thus, data from a validated simple food-frequency questionnaire developed especially for the Japanese were used to estimate the daily intake of three types of vitamins (retinol, vitamin C, and vitamin E) in each subject to assess how the intake of these vitamins may affect BMD. The results showed that a higher intake of vitamin C was significantly associated with a lower risk of developing osteoporosis. Our finding might be consistent with previous reports (25, 26). Sahni et al. have found that high vitamin C intake was inversely associated with bone loss (25) and the risk of hip fracture (26) from the Framingham Osteoporosis Study. Vitamin C is an essential cofactor for the formation of collagen and the synthesis of hydroxyproline and hydroxylysine (32). 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. Furthermore, in our data analysis, a higher intake of vitamin C with a higher serum level of β-cryptoxanthin was associated with a lower risk of developing osteoporosis 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 multiple adjustments (data not shown). This result agrees with our previous finding from cross-sectional analysis (29). Therefore, we concluded that minerals and/or vitamin D might be more valuable nutrients for maintaining bone health rather than vitamin C and β-cryptoxanthin, although there is no denying the possibility of multicollinearity among these nutrients because these micronutrients were also abundant in fruit and vegetables. From these results, it is conceivable that vitamin C intake combined with the intake of carotenoids such as β-cryptoxanthin might be a beneficial dietary pattern for the maintenance of bone health in post-menopausal female subjects. Further studies on the complicated interactions of antioxidant vitamins and carotenoids related to bone health will be required.

Our Mikkabi study showed that serum β-cryptoxanthin levels in post-menopausal women were weakly but significantly associated with BMD measured at the radius (27) and revealed a strong negative association between a β-cryptoxanthin dietary pattern, with a heavy intake of β-cryptoxanthin and vitamin C, and the risk of low bone mineral density values (29). Based on our present study and our previous findings, we suggest that a high intake of nutrients such as vitamin C and minerals together with β-cryptoxanthin has additional beneficial effects for the prevention of bone loss in post-menopausal Japanese women. In our study population, the intake of fruit, especially Japanese mandarin oranges, might provide benefits to maintain bone health, since the subjects in this survey were residents of an area in which the Japanese mandarin orange is considerably more popular than in the rest of Japan. Beta-cryptoxanthin is a carotenoid pigment that is particularly abundant in the Japanese mandarin orange (33, 34). Therefore, the serum concentrations of β-cryptoxanthin in this study population were widely distributed. One normal-sized Japanese mandarin orange (about 100 g) is expected to contain 1.2 mg of β-cryptoxanthin and 25 mg of vitamin C (35, 36). In view of this, to prevent menopause-related bone loss in post-menopausal Japanese female subjects, vitamin C needs to be consumed not only from Japanese mandarin oranges but also from other food sources.

On the other hand, in our previous study (29), we also found that vitamin E intake was inversely associated with the risk for low BMD, but this was insignificant cross-sectionally. In this longitudinal cohort study, lower odds ratios were also observed in groups with the middle (T2) and highest (T3) vitamin E intakes; however, these were not significant. Recently, Michaëlsson et al. found that low intake and low serum concentrations of α-tocopherol are associated with an increased rate of fracture in elderly Swedish women and men (37). In that study, they examined the association of vitamin E intake and the risk of fracture, comparing the highest quintile intake (median: 6.8 mg/d) with the lowest quintile intake (median: 4.3 mg/d). In contrast, in our study population, the median values of vitamin E intake in the highest and lowest tertiles were 10.5 mg/d and 6.0 mg/d, respectively. The subjects in the lowest vitamin E intake tertile in our study population seem to consume sufficient amounts of vitamin E from foods. The vitamin E intakes of Japanese subjects seemed to be higher than those of non-Japanese people; therefore, it is conceivable that an inverse association of vitamin E intake and the risk of osteoporosis might be difficult to determine in Japanese female subjects.

On the other hand, some epidemiological studies have reported that excessive intake of retinol may have adverse effects on BMD (38–40). The recommended daily intake of retinol activity equivalents is 450–500 μgRE/d for Japanese women, with a tolerable upper intake of 2,700 μgRE/d (41). In our previous report (29), a significant positive association between retinol intake and the risk of low BMD was observed cross-sectionally; however, these were not significant. Recently, Michaëlsson et al. found that low intake and low serum concentrations of α-tocopherol are associated with an increased rate of fracture in elderly Swedish women and men (37). In that study, they examined the association of vitamin E intake and the risk of fracture, comparing the highest quintile intake (median: 6.8 mg/d) with the lowest quintile intake (median: 4.3 mg/d). In contrast, in our study population, the median values of vitamin E intake in the highest and lowest tertiles were 10.5 mg/d and 6.0 mg/d, respectively. The subjects in the lowest vitamin E intake tertile in our study population seem to consume sufficient amounts of vitamin E from foods. The vitamin E intakes of Japanese subjects seemed to be higher than those of non-Japanese people; therefore, it is conceivable that an inverse association of vitamin E intake and the risk of osteoporosis might be difficult to determine in Japanese female subjects.

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examined the associations of vitamins and carotenoids with the risk for developing osteoporosis among current non-smokers and non-drinkers. As a result, a lower odds ratio was observed in the highest group of basal vitamin C intake; however, this was not significant (OR=0.23, 95% CI: 0.04–1.50). Similarly, a lower odds ratio was observed in the group of higher vitamin C intake with a higher serum level of β-cryptoxanthin against the group of lower vitamin C intake with a lower serum level of β-cryptoxanthin used for the reference group (OR=0.11, 95% CI: 0.01–1.13). For this reason, we concluded that the sample size of current non-smokers and non-drinkers (n=157) was too small, and statistical power would be reduced.

Furthermore, some studies reported a seasonal change in serum concentration and/or dietary intake of vitamins and carotenoids. In our previous study, we found that serum concentration of beta-cryptoxanthin showed notable seasonal change (43). In such a case, we have no clear idea whether the seasonal variation caused by dietary intake of carotenoid-rich foods would have an effect on the associations of basal serum carotenoid concentrations and the risk for developing osteoporosis. This point is an issue to be resolved in the future.

This study had some limitations. First, we could not evaluate the association of the blood levels of vitamin C with the radial BMD. It would be necessary to measure the blood levels of vitamin C in order to examine the associations of serum vitamin C concentration with the incidence of osteoporosis. Second, in this report, we evaluated the radial BMD at 1/3 of the forearm length measured from the styloid process on the ulna. Therefore, an analysis of the association of serum carotenoids with BMD in cancellous bone, such as the femoral neck or lumbar spine, will be required. Finally, in our study, the sample size of post-menopausal female subjects was not large and thus has less statistical power. Further study on a large scale will be required.

In conclusion, this longitudinal cohort study among post-menopausal Japanese female subjects shows that a high intake of vitamin C with high serum concentration of β-cryptoxanthin inversely is associated with the risk of developing osteoporosis. Our findings lead us to conclude that a high intake of nutrients such as vitamin C and minerals together with β-cryptoxanthin has additional beneficial effects for the prevention of bone loss in post-menopausal Japanese women. To determine whether antioxidant vitamins and carotenoids are beneficial to bone health, further cohort or intervention studies are required.

Authors’ contributions

M.S. was responsible for study design, data collection, and data management and carried out the data analysis and wrote the manuscript. M.N. was responsible for study design, data collection, and data management and assisted in manuscript preparation. K.O., Y.I., and M.Y. were involved in the data collection and assisted in manuscript preparation.

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