Published in final edited form as:
Int J Vitam Nutr Res. 2008 May ; 78(3): 105–111. doi:10.1024/0300-9831.78.3.105.

Serum Carotenoid Concentrations in Postmenopausal Women from the United States with and without Osteoporosis

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Abstract

Antioxidant defenses may be compromised in osteoporotic women. Little is known about fruit and vegetable or carotenoid consumption among postmenopausal women. The primary carotenoids in human serum are α- and β-carotene, lycopene, β-cryptoxanthin, lutein, and zeaxanthin. This study investigated the interrelationships among serum carotenoid concentrations, fruit and vegetable intake, and osteoporosis in postmenopausal women (n = 59, 62.7 ± 8.8 y). Bone density was assessed by dual energy x-ray absorptiometry and osteoporosis diagnosis was based upon T-scores. Serum samples (n = 53) and 3-day diet records (n = 49) were analyzed. Logistic regression analyzed differences between carotenoids after adjusting for serum retinol; supplement usage; milk, yogurt, fruit, and vegetable intake; and BMI. Pearson statistics correlated carotenoids with specific fruit or vegetable intake. Serum lycopene concentrations were lower in the osteoporosis group than controls (p = 0.03). β-Cryptoxanthin intake was higher in the osteoporosis group (p = 0.0046). Total fruit and vegetable intakes were correlated with serum lycopene and β-cryptoxanthin (p = 0.03, 0.006, respectively). Serum α-carotene concentration was associated with carrot intake, and zeaxanthin and β-cryptoxanthin with lettuce intake. Carotenoids that may have beneficial skeletal effects are lower in women with osteoporosis. Research is needed to identify potential protective mechanisms or utilization of carotenoids during osteoporosis.

Keywords

β-cryptoxanthin; carotenoids; lycopene; osteoporosis; postmenopausal women

Introduction

Osteoporosis is a condition characterized by low bone mass and deterioration of bone tissue, which ultimately leads to bone fragility and increased fracture risk. Osteoporosis results from unbalanced bone remodeling (*i.e.*, inadequate activity of bone-forming osteoblasts and/or excessive activity of bone-resorptive osteoclasts). However, the mechanisms involved in osteoporosis pathogenesis are not fully understood. Increases in oxidative stress caused by reactive oxygen species may be involved in osteoclastogenesis [1] and bone resorption [2]. Antioxidant depletion may negatively impact bone mass because of the increased presence of free radicals, which activate the oxidative stress-responsive transcription factor, NF-κB [3,4]. Oxidative stress also modulates osteoblastic differentiation [5]. Plasma antioxidants derived from the diet and antioxidant enzyme activity (*i.e.*, superoxide dismutase and glutathione

peroxidase) were markedly lower in osteoporotic women compared with women who had normal bone mineral density (BMD) [6].

Carotenoids as antioxidants [7] can quench singlet oxygen and trap peroxyl radicals [8]. The primary carotenoids that circulate in humans are α- and β-carotene, lycopene, β-cryptoxanthin, lutein, and zeaxanthin. Of these, lycopene is the most biologically potent antioxidant [9]. Lycopene's antioxidant activity is twice that of β-carotene and ten times that of α-tocopherol. Numerous studies have examined the association between lycopene and chronic diseases such as cancer and cardiovascular disease [10,11]; the pathogenesis of both may be attributed to oxidative damage. With regard to bone health, lycopene can inhibit mineral resorption by inhibiting osteoclast formation and the production of reactive oxygen species produced by osteoclasts to reduce the burden of free radicals in the body [12]. Additionally, lycopene stimulated proliferation and cell differentiation of osteosarcoma SaOS-2 cells, a human cell line with osteoblastic properties [13]. Thus, lycopene might favorably alter bone remodeling by stimulating bone formation and inhibiting resorption. Similarly, β-cryptoxanthin stimulated bone formation and inhibited bone resorption *in vitro* [14]. In cultured rat femoral tissue, β-cryptoxanthin increased calcium content and alkaline phosphatase activity [15]. Furthermore, oral administration to ovariectomized rats prevented bone loss [16].

Serum carotenoids are used as biomarkers for fruit and vegetable consumption [17,18]. Lifestyle and physiological factors such as smoking and alcohol consumption are associated with decreased serum carotenoid concentrations [19]. Little is known about the determinants of serum carotenoids from individual fruit and vegetable intake. Data on the correlation of carotenoids with osteoporosis among postmenopausal women are lacking in the literature. As an extension of a prior study [20], the main aim of this follow-up study was to investigate whether individual serum carotenoids are associated with osteoporosis and to assess the correlations between serum carotenoid concentrations and specific fruit and vegetable intake among postmenopausal women.

Subjects and Methods

Study subjects

This study received approval from the Health Sciences Institutional Review Board at University of Wisconsin-Madison. Subjects were recruited from potential research participants being screened for other osteoporosis or bone metabolism studies at the University of Wisconsin-Osteoporosis Clinical Research Program, Madison, WI, USA. BMD was measured during their examination using either a DPX-IQ or Prodigy bone densitometer (GE Healthcare Lunar, Madison, WI, USA) in routine clinical fashion. BMD T-scores at the lumbar spine or total proximal femur were used to distinguish groups: ≥ -1.0 (normal group) or < -2.5 (osteoporotic group). Subjects with osteopenia (-1.0 to -2.5) were not enrolled. Postmenopausal women with (n = 31) and without (n = 29) osteoporosis were invited to participate in the primary study to evaluate vitamin A status [20]. This evaluation was an extension of that study. Osteoporosis was defined by the lower of the T-score values which were -2.7 ± 0.4 and -0.4 ± 0.6 for the women with and without osteoporosis, respectively. After receiving information concerning study requirements, qualified participants signed an informed consent form. The enrollment criteria were: 1) ambulatory, community dwelling female; 2) postmenopausal ≥ 5 y (natural or surgical); and 3) not currently receiving estrogen therapy. If estrogen therapy was in the subject's history, this must have been discontinued for at least one year prior to study enrollment. Blood samples for serum chemistry determination were obtained in 59 subjects, 30 with osteoporosis and 29 with normal BMD. Three-day diet records and detailed instructions on how to complete them were given to the participants. Participants were instructed to include two weekdays and one weekend day in the record.

Serum carotenoid analysis

β-*Apo*-8'-carotenyl decanoate solution (50 μL) was added to 200 μL serum as an internal standard. Ethanol (250 μL, 0.1% butylated hydroxytoluene) was added to denature proteins. Samples were vortexed for 30 s. Carotenoids were extracted three times using hexanes (250 μL). The extracts were pooled and dried under argon; redissolved in 100 μL 50:50 dichloromethane:methanol and 50 μL was injected into the HPLC system. A gradient HPLC system was used, including a Waters 600 multisolvent delivery system (Milford, MA, USA) at a flow rate of 2 mL/min, a Waters 2487 dual wavelength absorbance detector set at 450 nm, and a Shimadzu C-R7A Chromatopac data processor (Kyoto, Japan) recorded and calculated peak areas. The stationary phase was a Waters Sunfire™ 5-μm, reverse-phase, C-18 column, 4.6 × 250 mm (Milford, MA, USA) equipped with a precolumn to protect the analytical column from particulate matter. Mobile phase consisted of 95:5 (v:v) acetonitrile:water as solvent A and 80:10:10 (v:v:v) acetonitrile:methanol:dichloroethane as solvent B. The gradient procedure was as follows: 1) 100% A for 3 min, 2) 7-min linear gradient to 100% B, 3) 22-min hold, 4) 2-min linear gradient back to 100% A. Standard curves were generated for each carotenoid using HPLC purified standards and serum concentrations were calculated and corrected for the extraction efficiency of the internal standard (85 ± 10%). Coefficient of variation for the assay was 3.9% for the same sample run 5 times.

Diet analysis

Nutritionist Pro™ nutrient analysis software, produced by First Databank (Houston, TX, USA), was used to analyze the 3-day diet records. Nutritionist Pro™ provided information on servings of individual fruits and vegetables, and carotenoid content from the fruits and vegetables. A serving size by this program was defined as 1 cup leafy vegetables, ½ cup other raw or cooked vegetables, one medium fruit, ½ cup cut fruit, or ¾ cup juice.

Statistical analysis

The Statistical Analysis System statistical package (SAS Institute Inc., Version 8.2, Cary, NC, USA; 2001) was used for all analyses. For continuous variables, Student's t-test was conducted to test the difference between the osteoporosis and control groups. In addition, multiple regression models were fitted for group comparisons adjusted for other variables. Logistic regression was used to evaluate the difference of individual carotenoid concentrations between osteoporosis and control groups after adjusting for serum retinol, supplements, fruit and vegetable intake, milk and yogurt consumption, and BMI. Pearson correlation statistics were computed for the correlation between serum carotenoid concentrations and specific fruit and vegetable intake. Values presented are means ± SD. Differences were considered significant at $p < 0.05$ for all analyses.

Results

The characteristics of the participants by osteoporosis group are described in Table I. As previously reported [20], BMI was significantly lower in the osteoporotic group than control group ($p < 0.0001$), even though energy intakes were similar (*i.e.*, 1725 ± 666 and 1767 ± 326 kcal/d for the osteoporotic and control groups, respectively). Serum retinol concentrations were also lower in the osteoporotic group ($p = 0.034$). No other statistical differences were noted with age, supplement usage, milk and yogurt consumption, or fruit and vegetable intake between groups. Furthermore, calcium intakes from all sources did not differ (*i.e.*, 1641 ± 480 and 1500 ± 675 mg/d for the osteoporotic and control groups, respectively).

After the primary analyses for retinol and retinyl esters and differences in blood chemistries were performed [20], 53 serum samples (27 from the osteoporotic group and 26 from the control group) were available for serum carotenoid concentration analysis. Mean serum lycopene

concentration was significantly lower in the osteoporotic group (1.22 ± 1.21 μmol/L, median 0.93 μmol/L) than in the control group (1.97 ± 1.01 μmol/L, median 1.83 mol/L) (p = 0.03) (Table II). Using a t-test, β-cryptoxanthin concentration was higher in the control group (0.43 ± 0.36 μmol/L, median 0.34 μmol/L) than in the osteoporotic group (0.27 ± 0.18 μmol/L, median 0.24 μmol/L) (p = 0.04). To determine how carotenoid concentrations in this study compared to the general public in the USA, serum carotenoids were compared with reference values in healthy female elderly participants from the Framingham Heart Study [19] and subjects from NHANES III [21] (Table III). Serum carotenoid concentrations were consistently higher than the values obtained in those two larger, more diverse population studies.

Each subject was asked to complete a 3-d diet record within two weeks of enrollment. Forty-nine records (93%) were returned for analysis of carotenoid intake. Fruit and vegetable intakes were compared with serum carotenoids. Serum lycopene and β-cryptoxanthin were positively correlated with total fruit and vegetable intake (p = 0.03, p = 0.006, respectively). Carrot consumption correlated with serum α-carotene concentration (p = 0.04). Lettuce consumption correlated with serum β-cryptoxanthin and zeaxanthin (p = 0.02 and 0.009, respectively). No other correlations were found and this may be due to the time difference in blood sampling and diet record. The subjects consumed 3.9 ± 2.1 (range 0.7 to 8.7) fruit and vegetable servings per day (Table I). The median intake was 3.3 servings daily. The most frequently consumed fruits were banana (54%), apple (42%), orange juice (32%), oranges (22%), berries (21%), and apple juice (14%). Frequently consumed vegetables included lettuce (58%), tomato and tomato sauce (58%), sweet-red and green peppers (38%), and carrots (28%).

Total carotenoid intake of this population was 4.2 ± 6.8 mg/day. Dietary β-cryptoxanthin was significantly higher in the osteoporotic group (0.16 ± 0.19 mg/d) than in the control group (0.09 ± 0.15 mg/day, *P* = 0.0046) (Table IV). These results are interesting as serum β-cryptoxanthin concentrations had the reverse relationship, *i.e.*, 0.27 ± 0.18 μmol/L in the osteoporotic group and 0.43 ± 0.36 μmol/L in the control group.

Discussion

In these postmenopausal women, serum lycopene was lower in the osteoporotic than in the control group, despite no difference in intake. Interestingly, β-cryptoxanthin serum concentrations of the women with osteoporosis were lower even though dietary intake was significantly higher. *In vitro* studies demonstrate osteoblastic stimulation and osteoclastic inhibition [12–15,22] of both lycopene and β-cryptoxanthin. Oxidative stress may be important in the development of osteoporosis [2,5,6]. Others have documented an association of antioxidant depletion with osteoporosis through measurement of various plasma antioxidants in women with and without osteoporosis [6]. Increased 8-isoprostaglandin F2α, a biomarker of oxidative stress, was associated with reduced bone density in both women and men [2]. Enhanced malondialdehyde, an oxidative stress marker, and decreased superoxide dimutase and glutathione peroxidase were correlated with diminished alkaline phosphatase activity in postmenopausal osteoporotic women [23].

Antioxidants can donate electrons to free radicals, thus destroying reactive species. This process provides a rationale for the possible beneficial effect of antioxidants in the prevention of disease, such as osteoporosis, with putative oxidative stress origins. Epidemiological studies have suggested that vitamin C, vitamin E and β-carotene reduce the risk of osteoporosis [24–26]. This study further suggests that lycopene and β-cryptoxanthin, or the foods that contain them, may have beneficial implications in osteoporosis prevention. However, these studies cannot exclude that the beneficial effects are from other nutrients or phytochemicals present in fruits and vegetables, the synergy between nutrients and phytochemicals, or lifestyles that correlate with high intakes.

Reference ranges for serum concentrations of selected carotenoids were established for adults in the Framingham study [19] and NHANES III [21]. The subjects in the present study had generally higher serum carotenoids. Subjects were recruited from one urban area, while the prior two surveys recruited subjects nationwide who were more diverse. Values from NHANES III were representative for females aged 51–70 y, and values from Framingham represented subjects ≥ 65 y in both gender groups. Values from the present study are in females aged 48 to 82 y. Serum carotenoid concentrations are associated with different physiologic and lifestyle factors, including sex, age, BMI, smoking, and alcohol consumption [17,18,27,28]. Dietary habits and lifestyle are diverse regionally and ethnically, which may account for the differences between the studies. Also, consumption of supplements, which was ~61% in this study, has increased during the past decade. Supplement use is higher among different subpopulations including women, older adults, the more educated, and Asian Americans [29]. This diversity and other dietary and lifestyle factors should be further examined in larger prospective studies to assess population nutritional differences and health.

Another reason for higher carotenoid serum concentrations in the current study was that the vegetable choices of the women were more deeply colored than general consumption patterns in the US. Starchy vegetables made up 40% of all vegetables consumed in the US in 1994–1996, 80% of which was white potatoes [30]. Typical white potatoes are low in carotenoids; these were not among the frequently consumed vegetables in this study. Carrot consumption has increased by 50% in the US in the past decade, and this has been attributed to the introduction of "cut & peel" carrots [31]. α-Carotene concentrations were associated with carrot consumption and were ~200% higher than prior assessments of serum α-carotene concentrations among more diverse groups [19,21].

Dietary antioxidants may salvage and save carotenoids in redox reactions [32]. This study demonstrated a positive correlation between fruit and vegetable intake and serum lycopene and β-cryptoxanthin. In another study, reported daily intake of fruits and vegetables correlated most strongly with plasma β-cryptoxanthin and β-carotene among women, and with plasma α- and β-carotene among men [33]. In the regression analysis, dietary β-cryptoxanthin was significantly lower in the control group compared with the osteoporosis group. Serum lycopene was strikingly higher in the control group. Because carotenoids are potent antioxidants, lycopene and β-cryptoxanthin may have a higher utilization rate in women with osteoporosis. These findings warrant further investigation.

In this study, BMI was higher in the control group and was significant in the model. Although we did not measure body composition in these women, one could reasonably assume a higher body fat percentage in the control group for these postmenopausal women. Higher adipose tissue could lead to accumulation of specific carotenoids in the adipose, possibly due to high intake of preformed vitamin A [20] leading to less conversion to vitamin A and more provitamin A carotenoid storage [34]. A relatively high preformed vitamin A intake was indeed observed in both groups [20].

Three-day dietary records may not be the most sensitive tool to analyze carotenoid intakes. From the diet records, subjects consumed 3.9 ± 2.1 (median 3.3, range 0.7 to 8.7) servings of fruit and vegetables per day. Currently, Dietary Reference Intakes are not established for carotenoids [21,35]. The National Cancer Institute's "Five a Day for Better Health" program indicated that 5 servings (*i.e.*, 2.5 cups) would provide approximately 5.2 to 6.0 mg carotenoids/d [36]. The total intake of carotenoids in this study (4.2 ± 6.8 mg/d) was lower than that, consistent with ~4 servings/d. Considering that current Dietary Guidelines for Americans [37] are 3.5 cups (7 servings) per day for a 1600 calorie diet, women should be encouraged through nutrition education to increase their consumption.

Results of the present study cannot be generalized to all postmenopausal US women because our participants were recruited from a highly educated, urban environment. A more diverse study with more subjects is needed. This study is supported by another cross-sectional study in Italy where decreased carotenoid and retinol concentrations were found in severely osteoporotic women compared with controls [38]. Furthermore, in a recent study in post-menopausal Canadian women, lycopene consumption was associated with a lower concentration of bone turnover markers (N-telopeptides of type I collagen) [39].

The significance of these findings should spawn interest in and studies of the relationship of carotenoids to the prevention or treatment of osteoporosis with more comprehensive measures of dietary intake. The temporality of fruit and vegetable intake should be investigated. Additional study of antioxidant depletion and its relevance to osteoporosis pathogenesis is indicated.

Acknowledgments

This research was supported by USDA-NRI Cooperative State Research, Education and Extension Service, grant number 2003-35200-05377, the UW-Graduate School 135-2146, and NIHNIDDK 61973. The authors do not have any conflicts of interest with the funding agencies of this study.

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Table I

Study population characteristics[1]

Variable	Osteoporosis	Control
Age (y)	63.3 ± 10.8	62.1 ± 6.2
BMI (kg/m^2)	23.2 ± 3.2	29.6 ± 6.4[2]
Bone mineral T scores[3]	−2.7 ± 0.4	−0.4 ± 0.6
Serum retinol (μmol/L)	2.11 ± 0.50	2.43 ± 0.62[4]
Milk consumption (cups/d)	0.98 ± 1.04	0.93 ± 0.81
Yogurt consumption (cups/d)	0.26 ± 0.32	0.30 ± 0.45
Fruit and vegetables (servings/d)[5]	3.6 ± 1.77	4.27 ± 2.46
Supplement use (%)	66.7	55.6

[1] Values are means ± SD. n = 30 and 29 for physical characteristics; n = 27 and 24 for the dietary parameters for the osteoporosis and control groups, respectively.

[2] BMI was significantly different between groups, $p < 0.0001$.

[3] The mean bone mineral T scores were the midpoint of the lowest two values for the spine and total proximal femur.

[4] Serum retinol concentration was significantly different between groups, $p = 0.034$.

[5] The nutrition program (Nutritionist Pro™) defined a serving as 1/2 cup fresh or cooked fruit or vegetable, 1 cup leafy vegetables and 3/4 cup of juice.

Table II

Serum carotenoid concentrations in postmenopausal women with and without osteoporosis[1]

Carotenoid (μmol/L)	Osteoporosis n = 27	Control n = 26
Lutein	0.62 ± 0.31	0.49 ± 0.41
Zeaxanthin	0.10 ± 0.11	0.06 ± 0.04
β-Cryptoxanthin	0.27 ± 0.18	0.43 ± 0.36[2]
Lycopene	1.22 ± 1.21	1.97 ± 1.01[3]
α-Carotene	0.36 ± 0.34	0.27 ± 0.22
β-Carotene	1.55 ± 1.15	1.10 ± 1.11

[1] Values presented are means ± SD. Using multiple linear regression, the model was adjusted for age because of the large age range in the sample and BMI because of significant differences between groups.

[2] Using Student's t-test, β-cryptoxanthin concentration is higher in the control group ($p = 0.04$); however, after adjusted for BMI and age, group difference is no longer significant in the regression analysis.

[3] Lycopene concentrations are significantly higher in the control group as compared with the osteoporosis group ($p = 0.03$) after adjusting for age and BMI.

Table III

Comparison of serum carotenoid concentrations in postmenopausal women with two representative population studies

Carotenoid (μmol/L)	Present study[1] n = 53	Framingham study[2] n = 408	NHANES III[3] n = 1938–1956
Lutein[4]	0.63 ± 0.40	0.51 ± 0.26	0.43 ± 0.40
	(0.18 – 1.25)	(0.19 – 0.99)	(0.13 – 1.23)
β-Cryptoxanthin	0.35 ± 0.29	0.26 ± 0.17	0.19 ± 0.22
	(0.08 – 0.87)	(0.07 – 0.54)	(0.04 – 0.63)
Lycopene	1.59 ± 1.17	0.59 ± 0.36	0.39 + 0.31
	(0.47 – 3.98)	(0.13 – 1.3)	(0.08 – 0.93)
α-Carotene	0.32 ± 0.28	0.11 ± 0.08	0.11 ± 0.18
	(0.07 – 0.79)	(0.03 – 0.25)	(0.02 – 0.43)
β-Carotene	1.33 ± 1.14	0.49 ± 0.32	0.49 ± 0.66
	(0.20 – 4.20)	(0.14 – 1.14)	(0.07 – 1.23)

[1] Values presented are means ± SD. The range presented is the 5th and 95th percentile for these women.

[2] The range is the 5th and 95th percentile for both men and women.

[3] The values were for female aged from 51–70 y. Range is the 1st and 99th percentile.

[4] Serum lutein and zeaxanthin were combined in the Framingham study [19] and NHANES III [21].

Table IV

Dietary carotenoid intake for women with and without osteoporosis[1]

Carotenoid (mg/d)	Osteoporotic group (n = 25)	Control group (n = 24)
Lutein/zeaxanthin	1.27 ± 2.09	0.82 ± 1.14
β-Cryptoxanthin	0.16 ± 0.19	0.09 ± 0.15[2]
Lycopene	0.59 ± 1.08	1.57 ± 3.21
α-Carotene	0.19 ± 0.33	0.70 ± 2.52
β-Carotene	1.02 ± 1.38	2.09 ± 5.16

[1] Values are means ± SD.

[2] In the multiple linear regression model, after adjusting for age, BMI, supplement use, fruit and vegetable consumption, and milk intake, β-cryptoxanthin intake is significantly higher in the osteoporosis group as compared with the control group ($p = 0.0046$) and relates to a difference in fruit intake ($p < 0.0001$) in the same model.