Original Article

Dietary carotenoid intake as a predictor of bone mineral density

Naiyana Wattanapenpaiboon PhD[1], Widjaja Lukito PhD[2], Mark L Wahlqvist AO, MD[1], and Boyd JG Strauss PhD, MD[3]

[1] *Asia Pacific Health & Nutrition Centre, Monash Asia Institute, Monash University, Melbourne, Australia*
[2] *SEAMEO-TROPMED Regional Center for Community Nutrition, University of Indonesia, Jakarta, Indonesia*
[3] *Department of Medicine, Faculty of Medicine, Nursing and Health Sciences, Monash University, Melbourne, Australia*

Our understanding of the influence of nutrition on bone health is limited because most studies concentrate on the role of calcium and protein, while other nutrients receive less attention. Recent evidence shows a positive link between fruit and vegetable consumption and bone health. In the present study, the relationships of dietary intakes of preformed retinol and carotenoids, one group of phytonutrients abundant in fruit and vegetables, were examined in an Anglo-Celtic Australian population of 68 men and 137 women. Bone mass of total body and lumbar spine were positively related to lycopene intake in men, and to lycopene and lutein/zeaxanthin intake in premenopausal women. In addition, a positive association of lumbar spine bone mass with dietary β-carotene intake was observed in postmenopausal women. No relationship was found between dietary retinol intake and bone mineral status. The finding of the present study suggests a beneficial effect of fruit and vegetable consumption, as indicated by dietary carotenoid intake, on bone health, possibly via an antioxidant mechanism.

Key Words: bone mineral density, bone, diet, carotenoids, lycopene, β-carotene, fruit, vegetables, phytonutrients

Introduction

It has been hypothesised that a diet with a high ratio of animal to vegetable foods increases the rate of bone loss and the risk of fracture.[1] Long term meat consumption or a high protein diet is known to increase urinary calcium which potentially contributes to a negative calcium balance and a consequent drain on the skeletal calcium levels.[2,3] However, a vegetarian diet, which is high in calcium and phosphate, does not appear to have beneficial effect on bone mass,[4-6] even though some studies confirm the protective effect of vegetarianism.[7, 8] A positive relationship of bone mineral density and the frequency of vegetable consumption has been reported in peri-menopausal women.[9] Furthermore, it has been proposed that fruit and vegetable consumption may be linked to bone health because these two food groups contain a number of nutri-ents other than calcium that are associated with higher bone mass.[10] A number of common vegetables in the human diet have been demonstrated to inhibit bone resorption in the rat.[11]

It has been reported that phytonutrients in certain groups of plant food have favourable effects on bone. Legume isoflavones possess estrogenic activity and have similar effects to estrogen on bone.[12] An increase in bone mineral content is detected following 12 weeks of soy supplementation in postmenopausal women.[13] Intake of fermented soybean increases circulating vitamin K_2 and γ-carboxylated osteocalcin, a biomarker of bone formation.[14] It has also been demonstrated that hesperidin, a citrus flavonoid, inhibits bone loss in ovariectomised mice, an animal model of postmenopausal women.[15]

In addition to cereals in vegetarian diets, and dairy products and eggs in lacto-ovo-vegetarian diets, fruit and vegetables are good sources of provitamin A or carotenoids. While animal studies have shown the association of high intake of vitamin A with bone demineralisation,[16,17] only few cross-sectional studies have reported relationships between vitamin A intake and bone mass.[18,19] However, the effect of dietary carotenoid intake on bone health has been little studied.

The purpose of the present study was to examine the relationships between bone mineral status and dietary intake of carotenoids and preformed retinol. Bone mineral content (BMC) and bone mineral density (BMD) of total body and lumbar spine (L_2-L_4) were used as the indicators of bone mineral status.

Correspondence address: Dr N Wattanapenpaiboon, Asia Pacific Health & Nutrition Centre, Monash Asia Institute Building 11A, Monash University VIC 3800, Australia.
Tel: +61 3 9905 8145; Fax: +61 3 9905 8146
E-mail: tikky.w@adm.monash.edu.au
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Subjects and methods

The present study used a sub-population of 68 men (aged 27–78 years) and 137 women (aged 26–86 years) recruited for an Anglo-Celtic Nutrition and Health Study which was carried out to investigate the association between food intakes, lifestyle and health status in Anglo-Celtic Australians. Participants recruited for this study were apparently healthy individuals, aged 25 years or over. Other entry criteria included being born in Australia, of English, Irish, Scottish or Welsh ancestry. Pregnant or lactating women were excluded. A total of 510 adults were recruited from the South-Eastern region of Melbourne metropolitan area in close proximity to Monash Medical Centre in such a way as to be representative of the Greater Melbourne Statistical Division. The method of subject sampling using the telephone directory was adopted from a method used in the Melbourne Chinese Health Study.[20] The study protocol was presented to and approved by the Monash University Standing Committee on Ethics in Research on Humans.

Information on health status, selected lifestyles, and menopausal status of women was obtained from a self-administered questionnaire. Women were grouped as either premenopausal if they had regular or irregular menstruation in the past six months, or postmenopausal if they did not have menstruation in the past six months. A semi-quantitative food frequency questionnaire was used to estimate daily food and dietary nutrient intakes in the past 12 months. To obtain information on dietary carotenoid intakes, data on daily food intake were linked to a USDA-NCI Carotenoid Food Composition Database.[21,22]

A technique of dual energy X-ray absorptiometry (DEXA) technique using a Lunar DPX densitometer with Lunar software version 3.6z (Lunar Radiation, Madison, WI, USA) was employed for the determination of BMC and BMD in total body and lumbar spine (L_2-L_4). The DPX uses a constant potential X-ray generator at 78 keV and a K-edge filter to produce effective energy levels of 40 keV and 70 keV. The scan speed was medium, except for obese subjects, where the speed was slow. Measurements of bone mineral status and anthropometry (weight, height, circumferences and skinfold thickness) were performed on each subject at the Body Composition Laboratory, Clinical Nutrition and Metabolism Unit, Monash Medical Centre, Melbourne, Australia.

Statistical analyses

Statistical Analysis System (SAS) package software version 8.2 (SAS Institute, Cary, NC, USA) was used for statistical analyses. Relationships between dietary factors and bone mineral density were initially assessed by Spearman correlation analysis. To derive independent dietary determinants of bone mineral status, a stepwise regression analysis was performed. The term 'determination' or 'determinant' was used to describe a statistical relationship between the predictor and the dependent variable. These analyses also included the estimation of the variance of the dependent variable which is accounted for by the predictor(s) or 'determinant(s)'.

Results

BMC and BMD of total body and lumbar spine (L_2-L_4) of subjects after adjustment for age and BMI, are presented in Table 1. At a given age and BMI, men had higher BMC and BMD both in total body and lumbar spine than did women. As expected, menopausal status had an effect on bone mineral status; postmenopausal women had lower bone mineral status than premenopausal women.

Relationships of BMC and BMD with dietary carotenoid and retinol Intake

Partial Spearman correlation coefficients (r_S) describing associations of dietary carotenoid and retinol intake with BMC and BMD are shown in Table 2. Since menopausal status was strongly related to bone mineral status, premenopausal and postmenopausal women were considered separately. In men, BMC and BMD of total body were positively related to lycopene intake (r_S = 0.30 and 0.24, P<0.05), adjusted for age and BMI. Similar relationships were also observed in premeno-pausal women (r_S = 0.47, P<0.01 and r_S = 0.31, P<0.05). In addition, lutein/zeaxanthin intake was related to BMC and BMD of lumbar spine and total body in premenopausal women. Significant relationships were also observed between total body BMC and β-cryptoxanthin intake (r_S = 0.33, P < 0.05); and between lumbar spine BMD and lutein/zeaxanthin intake (r_S = 0.35, P < 0.05). In contrast, none of these carotenoids were associated with BMC or BMD, either of total body or of lumbar spine in postmenopausal women. No relationships were observed between retinol intake and bone mineral status in men or in women.

Table 1. Bone mineral content (BMC) and bone mineral density (BMD) of total body and lumbar spine (L_2-L_4) [§]

		Total body		Lumbar spine (L_2 - L_4)	
	N	BMC (g)	BMD (g/cm^2)	BMC (g)	BMD (g/cm^2)
Men	68	3075 ± 364 ****	1.19 ± 0.08****	59.2 ± 10.4 ****	1.18 ± 0.17*
Women	137	2438 ± 363	1.10 ± 0.08	47.9 ± 10.4	1.13 ± 0.17
Premenopausal	47	2543 ± 432	1.13 ± 0.08	51.1 ± 12.4	1.19 ± 0.20
Postmenopausal	90	2382 ± 406	1.09 ± 0.09*	46.1 ± 11.6 *	1.09 ± 0.19**

[§]Mean ± SD. Adjusted for age and BMI. Significantly different from women: *, P < 0.05; ****, P < 0.0001.
Significantly different from premenopausal women: *, P < 0.05; **, P < 0.01.

Table 2. Partial Spearman correlation of daily intake of carotenoids and retinol to bone mineral content (BMC) and bone mineral density (BMD)[§]

Dietary intake	Total body		Lumbar spine (L_2 - L_4)	
(μg/d)	BMC (g)	BMD (g/cm^2)	BMC (g)	BMD (g/cm^2)
Men *(N = 69)*				
Dietary carotenoids (μg)				
Lutein/zeaxanthin	0.05	–0.07	–0.15	–0.18
β-Cryptoxanthin	0.07	0.13	0.01	0.18
Lycopene	0.30*	0.24*	0.05	0.05
α-Carotene	–0.03	–0.07	–0.06	–0.04
β-Carotene	–0.13	–0.12	–0.14	–0.10
Retinol (μg)	–0.01	0.07	0.05	0.16
Premenopausal women *(N = 46)*				
Dietary carotenoid (μg)				
Lutein/zeaxanthin	0.34*	0.40**	0.29	0.35*
β-Cryptoxanthin	0.33*	0.14	0.22	0.22
Lycopene	0.47**	0.31*	0.26	0.24
α-Carotene	–0.08	0.01	0.05	0.17
β-Carotene	0.15	0.13	0.18	0.23
Retinol (μg)	0.03	–0.18	–0.07	–0.21
Postmenopausal women *(N =90)*				
Dietary carotenoid (μg)				
Lutein/zeaxanthin	0.11	0.12	0.20	0.18
β-Cryptoxanthin	–0.05	–0.05	–0.01	–0.03
Lycopene	0.18	0.15	0.05	0.01
α-Carotene	0.07	0.07	0.15	0.18
β-Carotene	0.13	0.14	0.16	0.14
Retinol (μg)	–0.05	–0.02	–0.12	–0.11

[§] Adjusted for age and BMI. Significantly different from zero: *, $P < 0.05$; **, $P < 0.01$.

Table 3. Determination of BMC and BMD by dietary carotenoid intake in 69 men[§]

Determinants	Regression Coefficient		Partial R^2
	Parameter estimate	Standard error	(x 100)
Total body BMC (g)			
Age (years)	–7.0166*	3.2233	4.4*
Cigarettes smoked per day	–17.3082	9.1536	3.3
Alcohol intake (g/d)	8.2001*	3.9476	6.2*
Dietary lycopene intake (μg/d)	0.0620***	0.0160	10.2**
Dietary α-carotene intake (μg/d)	0.1114	0.0569	3.6
Other nutrient intake[a]	–	–	17.3
% VARIANCE EXPLAINED BY THE MODEL	R^2 (x 100) = 45%		
Total body BMD (g/cm^2)[b]			
Age (years)	–0.0015	0.0009	2.7
BMI (kg/m^2)	–0.0141**	0.0049	9.1*
Alcohol intake (g/d)	0.0027*	0.0011	7.8*
Dietary lycopene intake (μg/d)	0.1546**	0.0458	7.7*
Dietary α-carotene intake (μg/d)	0.2795	0.1630	2.5
Other nutrient intake[a]	–	–	14.1
% VARIANCE EXPLAINED BY THE MODEL	R^2 (x 100) = 44%		
Lumbar spine BMC (g)			
BMI (kg/m^2)	0.7175	0.4612	2.8
Dietary lycopene intake (μg/d)	0.0009*	0.0004	4.5
Other nutrient intake[a]	–	–	19.2
% VARIANCE EXPLAINED BY THE MODEL	R^2 (x 100) = 27%		
Lumbar spine BMD (g/cm^2)[b]			
BMI (kg/m^2)	0.0122	0.0074	3.4
Dietary β-cryptoxanthin intake (μg/d)	3.7472	2.0248	4.5
Other nutrient intake[a]	–	–	12.5
% VARIANCE EXPLAINED BY THE MODEL	R^2 (x 100) = 20%		

[§] Variables included in the analysis were age, BMI, cigarette smoking, alcohol consumption and nutrient intake. Significant level was set at 0.15 for variables to be entered into the model. [a] Nutrient intake other than dietary carotenoids, listed in Table 6.
[b] Parameter estimate and standard error values for BMD are to be multiplied by 10,000.
Significant level for F-test at which values are different from zero: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Dietary carotenoid intake as a determinant of bone mineral status

Results of stepwise regression analyses of dietary carotenoid intake on BMC and BMD of total body and lumbar spine for men are listed in Table 3. Other variables included in the regression model were age, BMI, cigarettes smoking, alcohol consumption and dietary intake of retinol and other nutrients.

Dietary lycopene intake was found to have a beneficial effect on bone mineral status. About 10% of the variance of total body BMC was explained by lycopene intake. Neither other carotenoids nor retinol were found to predict bone mineral status at the 0.05 significance level. Lutein/zeaxanthin intake had a favourable effect on bone mineral status in premenopausal women (Table 4). About 12% and 8% respectively, of variance of BMD of total body and lumbar spine were accounted for by lutein/ zeaxanthin intake alone. In contrast, α-carotene was found to have adverse effect on total body bone mineral status. Its contributions on BMC and BMD were 10% and 7%. As in men, bone mineral in premenopausal women was not related to retinol. In postmenopausal women, neither dietary carotenoids nor retinol were found to be determinants of total body BMC or BMD. Nevertheless, dietary β-carotene intake had a favourable effect on bone mineral status of the lumbar spine (Table 5).

Dietary factors as determinants of bone mineral status

In addition to dietary carotenoids, other nutrients were found to contribute, either positively or negatively, to BMC and BMD. These nutrients are listed in Table 6. Results from the stepwise regression analysis show that 45–55% of the variance of total body bone mass in men and premenopausal women was explained by age, BMI, cigarette smoking, alcohol consumption and nutrient intake. For postmenopausal women, only 30% of the variance was attributable to these factors. Nutrient intake, including dietary carotenoid intake, accounted for about 35% of the variance of bone mass of total body in premenopausal women. However, no effect of dietary factors was observed in postmenopausal women. In addition, nutrient intake contributed 30–50% of the variance of bone mass of lumbar spine in premenopausal women, whereas it contributed only 10% in post-menopausal women.

Discussion

Research on the influence of dietary factors on bone health has concentrated on calcium.[23-25] Associations of bone mass and intakes of other nutrients, such as protein, zinc, magnesium, potassium and phosphorus, have also been reported.[3,26,27] Because a substantial lag phase occurs

Table 4. Determination of BMC and BMD by dietary carotenoid intake in 46 premenopausal women[§]

Determinants	Regression Coefficient		Partial R^2 (x 100)
	Parameter estimate	Standard error	
Total body BMC (g)			
BMI (kg/m^2)	34.8783**	11.9643	16.4**
Dietary lutein/zeaxanthin intake (μg/d)	0.2393**	0.0722	5.7
Dietary lycopene intake (μg/d)	0.0240*	0.0106	4.6
Dietary α-carotene intake (μg/d)	–0.1534*	0.0633	10.4*
Other nutrient intake[a]	–	–	17.9
% VARIANCE EXPLAINED BY THE MODEL	R^2 (x 100) = 55%		
Total body BMD (g/cm^2)[b]			
BMI (kg/m^2)	0.0089 **	0.0028	14.3**
Dietary lutein/zeaxanthin intake (μg/d)	0.7696****	0.1611	11.7*
Dietary α-carotene intake (μg/d)	–0.3813*	0.1437	7.1*
Other nutrient intake[a]	–	–	16.2
% VARIANCE EXPLAINED BY THE MODEL	R^2 (x 100) = 49%		
Lumbar spine BMC (g)			
Dietary lutein/zeaxanthin intake (μg/d)	0.0047**	0.0013	8.8*
Dietary lycopene intake (μg/d)	0.0005	0.0003	4.5
Other nutrient intake[a]	–	–	20.2
% VARIANCE EXPLAINED BY THE MODEL	R^2 (x 100) = 33%		
Lumbar spine BMD (g/cm^2)[b]			
# Cigarettes smoked per day	–0.0084	0.0043	3.2
Dietary lutein/zeaxanthin intake (μg/d)	0.6977***	0.1922	8.4*
Dietary lycopene intake (μg/d)	0.0754	0.0405	4.1
Other nutrient intake[a]	–	–	39.1
% VARIANCE EXPLAINED BY THE MODEL	R^2 (x 100) = 55%		

[§]Variables included in the analysis were age, BMI, cigarette smoking, alcohol consumption and nutrient intake. Significant level was set at 0.15 for variables to be entered into the model. [a] Nutrient intake other than dietary carotenoids, listed in Table 6;
[b]Parameter estimate and standard error values for BMD are to be multiplied by 10,000.
Significant level for F-test at which values are different from zero: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

Table 5. Determination of BMC and BMD by dietary carotenoid intake in 90 postmenopausal women[§]

Determinants	Regression Coefficient		Partial R^2 (x 100)
	Parameter estimate	Standard error	
Total body BMC (g)			
Age (years)	–7.2219**	2.2233	6.0**
BMI (kg/m^2)	34.8783**	11.9643	20.2****
Other nutrient intake[a]	–	–	8.7
% VARIANCE EXPLAINED BY THE MODEL	R^2 (x 100) = 35%		
Total body BMD (g/cm^2)[b]			
Age (years)	–0.0010*	0.0005	4.1*
BMI (kg/m^2)	0.0089**	0.0028	19.8****
Other nutrient intake[a]	–	–	6.0
% VARIANCE EXPLAINED BY THE MODEL	R^2 (x 100) = 28%		
Lumbar spine BMC (g)			
BMI (kg/m^2)	0.8779**	0.3014	9.4**
Dietary β-carotene intake (μg/d)	0.0007**	0.0003	3.3
Other nutrient intake[a]	–	–	6.1
% VARIANCE EXPLAINED BY THE MODEL	R^2 (x 100) = 19%		
Lumbar spine BMD (g/cm^2)[b]			
BMI (kg/m^2)	0.0133*	0.0057	6.5*
Dietary β-carotene intake (μg/d)	0.1364**	0.0499	4.4*
Other nutrient intake[a]	–	–	4.8
% VARIANCE EXPLAINED BY THE MODEL	R^2 (x 100) = 16%		

[§]Variables included in the analysis were age, BMI, cigarette smoking, alcohol consumption, and nutrient intake. Significant level was set at 0.15 for variables to be entered into the model. [a] Nutrient intake other than dietary carotenoids, listed in Table 6.
[b] Parameter estimate and standard error values for BMD are multiplied by 10,000.
Significant level for F-test at which values are different from zero: *, $P < 0.05$; **, $P < 0.01$; ****, $P < 0.0001$.

Table 6. Dietary factors, other than carotenoids, as determinants of bone mineral status of total body and lumbar spine[§]

Bone mineral status	Men (*N* = 69)	Premenopausal women (*N* = 46)	Postmenopausal women (*N* = 90)
Total body			
Positive effect	dietary fibre*†	dietary cholesterol*	zinc**
Negative effect	riboflavin**	zinc**	dietary cholesterol**
		calcium*	
		phosphorus	
Lumbar spine			
Positive effect	carbohydrate*	dietary cholesterol****	
Negative effect	dietary fibre**	zinc****	dietary fat*
	zinc*	saturated fatty acids**	saturated fatty acids*
		monounsaturated fatty acids	

[§]Significant level was set at 0.15 for variables to be entered into the multiple regression model.
Significant determinants: *, $P < 0.05$; **, $P < 0.01$; ****, $P < 0.0001$.

between nutrition and its expression in skeletal mass,[28] dietary intake of carotenoids, which is likely to reflect a long term practice, may be a more appropriate parameter than serum concentration to use in the present study. Indeed, significant associations between bone mineral status and serum concentration of any carotenoids were not observed in the study population (data not shown).

Dietary carotenoid intake and bone mineral status

Total body BMC and BMD were positively related to β-carotene intake in postmenopausal women, and to lycopene and lutein/zeaxanthin intake in premenopausal women. In addition, a positive association of bone mass of the lumbar spine with dietary lycopene intake was also observed in men. Since lycopene does not possess provitamin A activity, it is not likely that they would be through the provitamin A activity. This conjecture is supported by the demonstration in a cell line study that that β-carotene has at a physiological concentration is able to stimulate the differentiation of osteoblasts.[29] The antioxidant property of these carotenoids may in part explain the findings of the present study. Experimental studies have shown that oxygen-derived free radicals are a contributory factor to bone resorption.[30,31] The production of oxygen-derived free radicals, such as hydrogen peroxide, is related to the regulation of osteoclast

differentiation[32,33] and is necessary for a normal osteoclastic function.[34] Superoxide generated by an interferon gamma treatment in osteoporotic patients was reported to be associated with an increase in bone resorption.[34]

Further studies on relationships between carotenoid status and bone metabolic activity are needed to support the findings of the present study. In addition, investigations at cellular levels may help to clarify the effect of carotenoids on bone mass. The reason for the opposing effect of α-carotene to other carotenoids on bone mineral status in premenopausal women, however, remains unclear. It is worth noted that fruit and vegetables are a good source of not only carotenoids, but also other antioxidant phytonutrients, such as flavonoids and polyphenols. It has been shown in an animal model that various vegetables eaten by humans, such as onion, tomato, lettuce, can inhibit bone resorption as assessed by urinary excretion of previously administered radiolabelled tetracycline.[11] In addition, vitamin K present in green leafy vegetable is essential for the activation of osteocalcin, a vitamin K-dependent calcium binding protein, which is the most abundant non-collagenous protein in bone.[35,36] It is possible that the findings of the present study may be due to those phytonutrients, or their combinations (and possible synergies) with carotenoids.

Dietary retinol intake and bone mineral status

A detrimental effect of hypervitaminosis A on bone has been reported in animal studies,[16,17] and retinoic acid, the major metabolite of retinol, is shown to be a contributory factor to this effect.[17] However, evidence of a direct relationship between bone mass and dietary vitamin A is inconsistent. A negative relationship be-tween vitamin A intake and mid-radial bone density has been reported from a cross-sectional study of diet and bone in 324 postmenopausal women.[37] In a four-year longitudinal study of calcium supplementation in 99 women aged 35–65 years, vitamin A was found to accelerate the rate of ulnar BMC reduction in the supplement group of postmenopausal women but retard the loss of humeral BMC in premenopausal women.[19]

In the present study, no relationship was found between dietary preformed retinol intake and bone mineral status. The disparities in results obtained from the present study and other studies may be due to differences in the definition of vitamin A or retinol. Most studies considered dietary carotenoids in fruit and vegetables only as a source of vitamin A and their content in food was expressed as retinol equivalents or units. In the present study, retinol and carotenoids were separately defined according to their biological forms. It could be speculated that positive relationships between vitamin A and bone mass reported in some studies may be attributable to the action of carotenoids. In addition, the inconsistency in relationships between vitamin A and bone mass observed by other investigators may result from the variations in contents of carotenoids which were defined as vitamin A precursors. Therefore, re-analysis of the dietary retinol and carotenoid data may help to clarify the effect of these compounds on bone mass.

Dietary factors, other than carotenoids and retinol, and bone mineral density

Genetic factors have been shown to be major determinants of bone mass in the lumbar spine.[38,39] However, results of the present study supported the evidence that dietary intake is also important to bone mass.[19,40] Calcium intake, however, was not found to be a contributor to bone mineral status in lumbar spine either in premenopausal or postmenopausal women. The lack of the relationship between calcium and bone mass is similar to some studies,[23,37,39] but not with others.[18,41] One of the possible explanations is that effects of nutritional status in early life on bone mass are greater than those of present intake.[42,43] A history of higher calcium intake was shown to be associated with larger bones in a Yugoslavian population.[41] Different bones have been found to respond to dietary factors differently.[19] Since the present study is limited to bone mineral status of total body and lumbar spine, further investigations are needed to examine effects of these dietary factors on bone mass of the femoral neck which is the site of fracture with the greatest morbidity and mortality.[44]

It is concluded that the present study provides evidence of a link between fruit and vegetable consumption, as represented by dietary carotenoid intake, and bone health. It is proposed that antioxidant property of carotenoids (or other antioxidant phytonutrients) in part explains these findings. Investigations at cellular levels may help to clarify the effect of carotenoids on bone mass. It would be advisable to include an appropriate amount of fruit and vegetables in diet, as it is an effective and inexpensive way to reduce the incidence of osteoporosis.

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