Serum retinyl esters are not elevated in postmenopausal women with and without osteoporosis whose preformed vitamin A intakes are high\(^1\)–\(^4\)

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ABSTRACT

Background: Recent observational studies suggest that preformed vitamin A (VA) intakes of 1500–2000 \(\mu\)g/d may increase the risk of osteoporosis and hip fracture. However, few studies have examined associations between biologic indicators of VA and osteoporosis.

Objective: This study characterized VA intake, serum VA, and bone turnover markers in postmenopausal women with and without osteoporosis.

Design: Bone density was measured by dual-energy X-ray absorptiometry. Subjects were separated into those with osteoporosis (\(n = 30\)) and those with normal bone density (\(n = 29\)). Women with osteopenia were excluded. Complete blood chemistries were obtained. Serum was analyzed for retinol, retinyl esters, and metabolites. Assays for 3 bone turnover markers were performed by using commercially available kits. Diet records were quantified. Logistic regression was used to test for an association between dietary and serum variables and osteoporosis.

Results: Dietary VA did not differ significantly between the groups but was nearly twice the Recommended Dietary Allowance in both groups. Body mass index (BMI) and serum triacylglycerols were significantly lower in the osteoporosis group. Retinyl esters were not elevated in either group, but a trend existed for the association of serum retinyl esters as a percentage of total VA with osteoporosis (\(P = 0.070\)) after adjustment for BMI and triacylglycerols in the statistical model. Milk, fruit, and vegetable intakes were below the current recommendations.

Conclusions: Serum retinyl esters were not elevated in these postmenopausal women despite intakes of total VA that were nearly two-fold the Recommended Dietary Allowance. However, retinyl ester concentration (percentage of total VA) was marginally associated with osteoporosis and should be further investigated.


KEY WORDS Vitamin A, osteoporosis, diet analysis, postmenopausal women

INTRODUCTION

Osteoporosis is a disease of low bone mass and skeletal fragility. More than 40% of postmenopausal women (1) and up to 25% of men (2) will sustain osteoporotic fractures, which will result in substantial expense (3, 4), morbidity (5), and mortality (5, 6). The causes of osteoporosis are multiple (7, 8). Nutrition clearly plays a role; nutrient inadequacies, eg, calcium and vitamin D, have received the most emphasis (8–10). However, nutritional excesses may also contribute to the disease. For example, high intakes of dietary sodium and protein from animal sources increase urinary calcium excretion (8, 11, 12) and may promote bone loss (8, 9, 10). Hypervitaminosis A from high intakes of preformed vitamin A (VA) (13–16) may also contribute to bone loss.

Supplement use in the United States approaches 50% by some estimates and is higher within certain subpopulations (17–19). Many multivitamin supplements provide VA as retinyl ester, which is the preformed vitamin that is readily absorbed. As many as 75% of women in the United States currently meet or exceed the VA requirement (20), and much of this VA comes from preformed VA sources. Some have advocated that all adults should take a multivitamin daily, and some support 2 daily multivitamins for the elderly to meet the enhanced need for vitamins B-12 and D (21). Given these considerations, hypervitaminosis A may be an underappreciated problem.

Case reports in humans, particularly in children, suggest that hypervitaminosis A alters the skeleton (22–26). In rats, preformed VA stimulates bone resorption and interferes with vitamin D absorption (27–29). Moreover, other studies conducted in rats report that VA toxicity decreases bone formation and increases resorption (27, 30), with the resultant uncoupling of bone formation and resorption capable of producing bone loss (13). In humans, 4 epidemiologic studies suggest that high preformed VA intakes are associated with lower bone mineral density (BMD) (31–34). However, studies that have examined circulating retinyl ester concentrations, which would suggest hypervitaminosis A if >10%

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of total VA in the fasting state, found no association with bone mineral status (35, 36).

No study has simultaneously evaluated dietary intake of preformed VA, serum retinol + retinyl esters, and serum bone turnover markers in women with and without osteoporosis. Given current population demographics, the number of debilitating osteoporotic fractures will increase dramatically in the foreseeable future (10). Thus, it is important to understand the causes of osteoporosis, especially those that can be manipulated through dietary changes or supplement use, to most effectively address this problem. The Recommended Dietary Allowance (RDA) for VA is 700 and 900 μg/d for women and men, respectively. However, the Percentage Daily Value on food labels retains the 1968 RDA, which was 1500 μg and approximately twice the current RDA. Most Americans are ingesting more than the current RDA for VA on a regular basis (37). The purpose of the present study was to characterize the VA intake, fasting serum retinol and retinyl esters, and skeletal turnover markers in 2 groups of postmenopausal women, half of whom had a diagnosis of osteoporosis.

SUBJECTS AND METHODS

Subjects

Women were recruited by responding to an advertisement in the local newspaper for free bone density screening and possible participation in a research study. Subjects interested in participating were screened at the University of Wisconsin Osteoporosis Clinical Center and Research Program (Madison, WI) to assess eligibility. None had been diagnosed with osteoporosis before entering the study. The study was approved by the University of Wisconsin Health Sciences Institutional Review Board, and all subjects provided written informed consent. Healthy, ambulatory, community dwelling, postmenopausal (≥5 y, natural or surgical) women between 48–83 y of age who had not received estrogen replacement therapy within 1 y were recruited. Additional exclusion criteria included use of any of the following in the year before recruitment: bisphosphonate, calcitriol, >800 IU vitamin D/d, cyclosporine and its derivatives, methotrexate, phenytoin, phenobarbital, estrogen or estrogen-related drugs (ie, tamoxifen or raloxifene), and thyroid-stimulating hormone concentrations outside the normal range. If the subjects were on thyroxine replacement therapy, the dose must have been stable and thyroid-stimulating hormone concentrations within normal range for ≥6 wk before enrollment. A medical history of metabolic bone disease, fracture within preceding year, immobilization (non-weight bearing) for >3 mo at any time, renal disease or serum creatinine concentrations outside the normal range, liver disease, or participation in an investigational drug study within previous 30 d also disqualified potential participants.

Bone densitometry measurements were performed via dual-energy X-ray absorptiometry with the use of either a Lunar DPX-IQ or a Prodigy bone densitometer (GE Healthcare; Madison, WI) in routine clinical fashion. A BMD T score at the lumbar spine or total proximal femur >−1.0 defined the control group. Persons with a lumbar spine or total proximal femur T score ≤−2.5 defined the osteoporotic group.

Study design and procedures

All potential study subjects provided blood samples for screening purposes. After bone status was established by dual-energy X-ray absorptiometry, the subjects were separated into 2 groups, control and osteoporotic; those with osteopenia were excluded. The study subjects were asked to consume their regular diets and to complete a 3-d diet record, reporting their total food and supplement intake from 2 weekdays and 1 weekend day to control for patterns that may differ during the week. The following information was obtained: time of day, meal, type or brand of food item consumed, method of preparation (eg, whether boiled or fried), and amount consumed. A cover page for each diet record provided detailed written instructions for providing food intake. Instructions included how to identify the food items consumed (eg, give brand name where possible, restaurant name, and regular or light), how to measure foods (household measures and use of kitchen scales were the methods of choice), how to explain food preparation methods (eg, fried, baked, or boiled), and the need to list spices, fats, and condiments added to foods during cooking and at the table. The subjects were encouraged to provide food labels for prepackaged food products. Diet record instructions were verbally reviewed with each subject by staff at enrollment. The subjects were provided with the telephone number of the registered dietitian who analyzed the diet records if they had questions.

The subjects who completed the diet records and returned them were given a small incentive. Follow-up phone calls were made to subjects if clarification about foods or supplements was needed. The registered dietitian quantitatively and qualitatively analyzed all diet records for nutrient composition and dietary patterns. Nutrient calculations were performed by using the Nutrition Data System for Research (NDS-R) software version 4.05, which was developed by the Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN (Food and Nutrient Database 33, released July 2002). Study investigators were blinded to the bone density status of the women until analyses of serum and diet records were complete.

Blood collection

After providing blood for the initial screening and confirmed BMD eligibility, the subjects returned to provide fasting (ie, ≥8 h without food) blood samples. Blood was drawn from the antecubital vein by using a standard venipuncture technique. All venipunctures were performed between 0800 and 1100. Blood was placed into a 9-mL serum separator tube and was allowed to clot in the dark for 30 min at room temperature. The blood was then promptly centrifuged at 2190 × g for 20 min at 4 °C. Complete blood chemistry panels and additional serum chemistries were performed in the fully accredited General Medical Laboratories (Madison, WI), and the results were provided to the study investigators. For measurement of serum VA and bone turnover, serum aliquots were quick frozen in liquid nitrogen and maintained at −80 °C until analysis.

Serum vitamin A and bone turnover marker analyses

Vitamin A analysis was performed by using a modification of published procedures with 700 μL serum (38, 39). The CV for this serum extraction method was calculated to be 6.4% for total
On their initial visit, 31 were newly diagnosed with osteoporosis and 29 had normal BMD. The mean (SD) age of the osteoporotic group was 63.3 ± 10.8 (48.5–82.2) years, compared with 62.1 ± 6.2 (51.6–74.7) years in the control group. The mean (SD) BMI (kg/m²) was 23.2 ± 3.2 (18.4–31.8) in the osteoporotic group and 29.6 ± 6.4 (18.9–42.0) in the control group. The mean (SD) BMI > 25 kg/m² as a percentage of total VA was 7 (23) in the osteoporotic group and 9 (31) in the control group. The mean (SD) number of subjects who completed diet record (n) was 27 in the osteoporotic group and 24 in the control group.

Statistical analysis
Statistical analyses were performed with SAS software version 8.2 (SAS Institute, Cary, NC) and R software version 1.71 (available at http://www.r-project.org). Logistic regression models were fitted to analyze the relation between disease condition (osteoporosis compared with control) and subject physical characteristics, diet, serum VA, and bone turnover values. The relation between the intake of different forms of VA (ie, retinol and β-carotene) and the season in which the diet records were completed was also analyzed. Student’s t tests were performed to determine differences in characteristics, nutrient intake data, and serum measurements between the groups. Differences with P < 0.05 were considered significant.

RESULTS

Demographic data
Sixty postmenopausal women aged 48–82 y were recruited. On their initial visit, 31 were newly diagnosed with osteoporosis and 29 had normal BMD. The mean (±SD) T scores of the osteoporotic group were −2.1 ± 1.0 and −2.3 ± 0.5 for the spine and total proximal femur, respectively, with a mean of −2.7 ± 0.4 for the lower of the 2 T score values. The mean T scores of the control group were −0.5 ± 1.1 and −0.2 ± 0.6 for the spine and total proximal femur, respectively, with a mean of −0.4 ± 0.6 for the lower of the 2 values. Fifty-nine subjects provided fasting blood samples (n = 30 and 29 in the osteoporosis and control groups, respectively). Demographic characteristics and compliance with the study protocol were compiled (Table 1). The subjects did not differ significantly with regard to age. Body mass index (BMI; in kg/m²) was significantly different between the groups (P < 0.0001). Logistic regression showed that BMI was associated with osteoporosis (P = 0.01, Wald chi-square test). Both height (P = 0.012) and weight (P < 0.0001) differed significantly between the groups.

Serum biochemistry and bone turnover
Serum creatinine and alanine aminotransferase (Table 2) differed between the groups, but values for both groups were within normal limits. Serum triacylglycerols were also significantly different between the groups (P = 0.0011), and logistic regression analysis showed that they were independently associated with osteoporosis (P = 0.015, Wald chi-square test). Mean (±SD) fasting serum triacylglycerols in the osteoporosis group were 105 ± 41 mg/dL, well within the desirable range, but were 178 ± 103 mg/dL for the control group, which places the group in the borderline high category, defined as 150–199 mg/dL. Elevated fasting serum triacylglycerol concentrations are associated with a high sugar intake, fat intake, or both (41), yet no significant differences in intakes of total carbohydrates or fat were observed between groups. Elevated triacylglycerol concentrations are also associated with hyperlipidemia. However, total cholesterol for the control group was not high (198 ± 37 mg/dL). Because BMI differed between the groups (Table 1), analysis for a correlation between BMI and triacylglycerols was performed. No significant correlation between the 2 variables was found (P > 0.05). No significant differences in markers of serum bone formation or resorption were observed between the groups (Table 2).

Serum vitamin A
Logistic regression analysis uncovered no significant association between osteoporosis and total serum VA and retinol. Serum retinyl esters (sum of retinyl palmitate, oleate, stearate, linoleate, and acetate) in both groups were below the cutoff suggested to indicate hypervitaminosis A, ie, >10% total VA (42), and were not significantly different between groups (Table 2). However, analysis did uncover a trend for the association of serum retinyl esters as a percentage of total VA with osteoporosis (P = 0.070, Wald chi-square test) after adjustment for BMI and triacylglycerols. Serum retinol and total VA was different between groups (Table 2), but neither variable was associated with osteoporosis.

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Demographic data for postmenopausal women with and without osteoporosis who were recruited for a study of vitamin A intake and status assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>63.3 ± 10.8 (48.5–82.2)²</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.2 ± 3.2 (18.4–31.8)⁴</td>
</tr>
<tr>
<td>BMI &gt; 25 kg/m² [% (%)]</td>
<td>7 (23)</td>
</tr>
<tr>
<td>No. of subjects who competed diet record (n)</td>
<td>27</td>
</tr>
</tbody>
</table>

¹ Age was calculated from birth date and date of initial blood draw.
² ± SD; range in parentheses (all such values).
³ Calculated from height and weight measurements obtained at enrollment.
⁴ Significantly different from control group, P < 0.0001 (Student’s t test).
TABLE 2
Serum biochemistry profile and bone turnover markers and vitamin A (VA) values of postmenopausal women with and without osteoporosis

<table>
<thead>
<tr>
<th></th>
<th>Osteoporosis group (n = 30)</th>
<th>Control group (n = 29)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>13.8 ± 0.85(^2)</td>
<td>13.7 ± 0.86</td>
<td>NS</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.7 ± 0.13</td>
<td>0.8 ± 0.15</td>
<td>0.0067(^3)</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>215 ± 36</td>
<td>198 ± 37</td>
<td>0.077</td>
</tr>
<tr>
<td>Triglycerols (mg/dL)</td>
<td>105 ± 41</td>
<td>178 ± 103</td>
<td>0.0011</td>
</tr>
<tr>
<td>Alanine transaminase (U/L)</td>
<td>20 ± 7</td>
<td>29 ± 21</td>
<td>0.031(^4)</td>
</tr>
<tr>
<td>Aspartate transaminase (U/L)</td>
<td>24 ± 5</td>
<td>27 ± 10</td>
<td>NS</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>4.4 ± 0.31</td>
<td>4.3 ± 0.22</td>
<td>NS</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>9.6 ± 0.44</td>
<td>9.5 ± 0.34</td>
<td>NS</td>
</tr>
<tr>
<td>Alkaline phosphatase (U/L)</td>
<td>83 ± 23</td>
<td>84 ± 22</td>
<td>NS</td>
</tr>
<tr>
<td>Osteocalcin (ng/mL)</td>
<td>19.0 ± 5.0</td>
<td>19.6 ± 5.8</td>
<td>NS</td>
</tr>
<tr>
<td>Bone-specific alkaline phosphatase (U/L)</td>
<td>33.8 ± 10.1</td>
<td>35.1 ± 13.6</td>
<td>NS</td>
</tr>
<tr>
<td>NTXs (nmol bone collagen equivalents/L)</td>
<td>15.4 ± 3.8</td>
<td>15.7 ± 4.6</td>
<td>NS</td>
</tr>
<tr>
<td>Retinol (μmol/L)</td>
<td>2.11 ± 0.50 (1.29–3.89)(^d)</td>
<td>2.43 ± 0.62 (1.45–3.95)</td>
<td>0.034</td>
</tr>
<tr>
<td>Retinyl esters (nmol/L)</td>
<td>54.2 ± 35.5 (19.9–181) NS</td>
<td>55.7 ± 40.7 (19.0–204)</td>
<td>NS</td>
</tr>
<tr>
<td>Total VA (μmol/L)(^5)</td>
<td>2.16 ± 0.52 (1.33–3.98)</td>
<td>2.48 ± 0.63 (1.49–3.99)</td>
<td>0.038</td>
</tr>
<tr>
<td>Retinyl esters (% of total VA)</td>
<td>2.45 ± 1.30 (0.91–6.25)</td>
<td>2.26 ± 1.39 (0.69–6.16)</td>
<td>NS</td>
</tr>
</tbody>
</table>

\(\text{Fasting serum was collected at the initial visit to the clinic or on a follow-up visit shortly after enrollment. Clinical chemistry panel was analyzed at General Medical Laboratories (Madison, WI). NTXs, cross-linked N-telopeptides of type 1 bone collagen in human urine.}\)

\(\text{2} \bar{x} \pm \text{SD (all such values).}\)

\(\text{3} \text{Although statistically different with a Student's} \, t \text{test, the values for both groups are within the normal clinical range.}\)

\(\text{4} \bar{x} \pm \text{SD; range in parentheses (all such values).}\)

\(\text{5} \text{Total VA represents the sum of retinol and retinyl esters (retinyl palmitate, oleate, stearate, linoleate, and acetate).}\)

with a logistic regression analysis. Serum retinol was lower in women with osteoporosis (\(P = 0.034\)) and they had a slightly higher percentage of total circulating retinyl esters (2.45 ± 1.30 compared with 2.26 ± 1.39% for the osteoporosis and control groups, respectively). Polar metabolites of VA (eg, retinoic acid and other derivatives) were not observed in these samples, although the method used for measuring serum VA is capable of detecting them in monkeys whose daily intake of preformed VA exceeded the 700 μg RDA (39). Retinyl esters and retinol esters (retinyl palmitate, oleate, stearate, linoleate, and acetate) were included in order of their relative retinol contribution: fortified milk and soymilk, cheese, ready-to-consume breakfast cereals and eggs (same relative contribution), ice cream, and butter or margarine (Table 4).

**Nutrient intake**

Fifty-one subjects completed and returned diet records (\(n = 27\) and 24 in the osteoporosis and control groups, respectively). The results were interpreted by the registered dietitian, and the results for VA intake were subsequently mailed to the subjects who completed diet records (\(n = 51\)). The subjects were offered appropriate recommendations to optimize their VA intakes. T-tests showed no significant differences between the groups for any of the nutrient variables analyzed (Table 3). All subjects were recruited in <1 y, and the season in which the diet records were completed did not correlate significantly with intake of total preformed VA or β-carotene (data not shown). Intake of total VA exceeded the 700 μg RDA for women by nearly 2-fold in both the osteoporosis and control groups at 1336 ± 631 and 1424 ± 661 μg Retinol Activity Equivalents (RAE/d) and was consistent with VA intakes of women reported by others (20, 43). The range of total VA intake was 485–2560 and 250–2567 μg RAE/d for the osteoporosis and control groups, respectively. Vitamin A intake at the 25th percentile for both groups exceeded the RDA (ie, 837 and 746 μg RAE/d for the osteoporosis and control groups, respectively). The number of subjects in each group whose total VA intake exceeded 1500 μg RAE/d was 9 (33%) and 13 (54%) for the osteoporosis and control groups, respectively.

Preformed VA intake at the 25th percentile was 539 and 536 μg retinol for the osteoporosis and control groups, respectively. The number of subjects whose retinol intake exceeded 1500 μg/d, which is the concentration associated with an increased risk for hip fracture and osteoporosis in some studies, was 8 (30%) and 7 (29%) for the osteoporosis and control groups, respectively. The range of preformed retinol intake was 219–2061 and 255–2329 μg/d for the osteoporosis and control groups, respectively. Retinol intake accounted for 77% and 78% of total VA intake in the osteoporosis and control groups, respectively. This is consistent with the VA intake pattern of persons in developed nations, as previously reported (20). The foods that contributed most to the dietary retinol intake were the following, in order of their relative retinol contribution: fortified milk and soymilk, cheese, ready-to-consume breakfast cereals and eggs (same relative contribution), ice cream, and butter or margarine (Table 4).

Total fat intake was at the upper limit for current recommendations in both groups at 55% of total kcal (Table 3). Fiber intake, at a mean of 18 and 19 g for the osteoporosis and control groups, respectively, was lower than the recommended intake. Sodium intake was not excessive, as reported by the subjects, which included salt added at the table. Potassium intake in either group did not meet recommendations, falling nearly 2000 mg short of the Adequate Intake of 4700 mg. Calcium intake was numerically, but not statistically, higher in the osteoporosis group.

**Dietary and supplement patterns**

Qualitative analysis of the dietary patterns (Table 4) showed that 56% of the women with osteoporosis and 67% of those...
TABLE 3
Nutrient intakes of postmenopausal women with and without osteoporosis from both food and reported intake of supplements

<table>
<thead>
<tr>
<th></th>
<th>Osteoporosis group (n = 27)</th>
<th>Control group (n = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal/d)</td>
<td>1725 ± 666</td>
<td>1767 ± 326</td>
</tr>
<tr>
<td>Fat (g/d)</td>
<td>67 ± 37</td>
<td>68 ± 23</td>
</tr>
<tr>
<td>Carbohydrates (g/d)</td>
<td>216 ± 82</td>
<td>213 ± 47</td>
</tr>
<tr>
<td>Protein (g/d)</td>
<td>67 ± 25</td>
<td>76 ± 16</td>
</tr>
<tr>
<td>Animal protein</td>
<td>44 ± 21</td>
<td>49 ± 18</td>
</tr>
<tr>
<td>Vegetable protein</td>
<td>22 ± 9.0</td>
<td>26 ± 9.0</td>
</tr>
<tr>
<td>Fiber (g/d)</td>
<td>18 ± 8.0</td>
<td>19 ± 8.0</td>
</tr>
<tr>
<td>Vitamin D (µg/d)</td>
<td>14 ± 9.0</td>
<td>11 ± 7.0</td>
</tr>
<tr>
<td>Calcium (mg/d)</td>
<td>1641 ± 480</td>
<td>1500 ± 675</td>
</tr>
<tr>
<td>Sodium (mg/d)</td>
<td>2886 ± 1241</td>
<td>2936 ± 898</td>
</tr>
<tr>
<td>Potassium (mg/d)</td>
<td>2720 ± 1107</td>
<td>2766 ± 904</td>
</tr>
<tr>
<td>Magnesium (mg/d)</td>
<td>341 ± 263</td>
<td>359 ± 137</td>
</tr>
<tr>
<td>Phosphorus (mg/d)</td>
<td>1147 ± 446</td>
<td>1265 ± 241</td>
</tr>
<tr>
<td>Vitamin A-12 (µg/d)</td>
<td>7.2 ± 6.7</td>
<td>8.8 ± 8.7</td>
</tr>
<tr>
<td>Folate (µg DFE/µd)</td>
<td>557 ± 333</td>
<td>729 ± 460</td>
</tr>
<tr>
<td>ß-Carotene (µg/µd)</td>
<td>3275 ± 2935</td>
<td>3527 ± 2785</td>
</tr>
<tr>
<td>Retinol (µg/µd)</td>
<td>1029 ± 595</td>
<td>1105 ± 620</td>
</tr>
<tr>
<td>Total vitamin A (µg RAE/µd)</td>
<td>1336 ± 631</td>
<td>1424 ± 661</td>
</tr>
</tbody>
</table>

All values are ± SD. Dietary information, including total food and supplement intakes, was obtained from 3-d diet records completed by subjects. Data was analyzed with the NUTRITION DATA SYSTEM for RESEARCH software [version 4.05 (2002), Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN]. No significant differences in the intake of any nutrient were observed between the groups (t-test).

DISCUSSION

The present study was unique in that it investigated the relations of VA intake (as well as other nutrients), biochemical indicators of VA status, and serum bone turnover markers in postmenopausal women with and without osteoporosis. Previous studies have investigated associations between VA intake and bone mineral status and concluded that a relation exists. One of these studies used serum retinol as an indicator of VA status. However, serum retinol is not a good indicator of VA status, except in deficiency. Women in developed nations, such as the United States and northern Europe, where the incidence of osteoporosis is high, generally have adequate VA status. Fast ing serum retinyl ester concentrations are considered a better measure of VA status and may be an especially good indicator of subchronic hypervitaminosis A. Nonetheless, given the recent large epidemiologic studies that found an association between VA intake and osteoporosis or hip fracture and several reviews that link VA toxicity with skeletal deformations in humans, more studies are clearly needed.

We hypothesized that postmenopausal women would have a high intake of total VA and preformed VA and that those with the highest preformed VA intake would be more represented in the osteoporosis group and have higher fasting serum retinyl esters. Although it is usually preferable to have at least 7-d data for dietary records to capture variability in provitamin A sources of VA, intake of preformed VA is more consistent in developed countries. Therefore, total VA intake may have been underestimated in the present study due to the use of 3- and not 7-d records, but preformed intake is probably accurate. Consistent with this, the VA intake of these women was nearly twice the RDA at ≈1400 µg RAE, with >75% coming from preformed VA. However, the VA intake was not significantly different between the groups. The retinol intake of nearly one-third of the women in each group exceeded 1500 µg/d, which has been associated with an increased risk of bone loss, hip fracture, or both (31–34).
However, no significant association between VA intake, either as total VA or as retinol, and osteoporosis was observed. An association between VA intake and bone turnover markers was also not found.

Although fasting serum retinol and retinyl esters were well within normal limits for both groups, an interesting, yet subtle, relation between the presence of osteoporosis and retinyl esters as a percentage of total serum VA was observed. Although clearly not near the >10% of total VA cutoff indicating hypervitaminosis A, it is notable that a trend existed for an association with osteoporosis and warrants further investigation. The osteoporotic women had lower serum triacylglycerols than did the control women. Elevated retinyl esters could be associated with higher blood lipids, but the present study was not large enough to look at this association within a group.

Total VA intake was not significantly different between the groups, nor was the intake of preformed VA. Serum retinyl esters did not significantly correlate with VA intake. The use of supplements containing preformed VA was slightly higher in the control than in the osteoporosis group. Women in the osteoporosis group may have made changes in their diet or supplement intake after being diagnosed with osteoporosis, indicating a potential weakness of the present study—the inability to capture the temporality of nutrient intake. Perhaps the women with osteoporosis had higher exposure to preformed VA before their diagnoses. Alternatively, it may be that the same intake of VA, particularly as preformed VA, affected the women differently, perhaps due to genetic differences in tolerance.

Slightly higher circulating retinyl ester concentrations as a percentage of total VA, with a concomitant reduction in the concentration of circulating retinol, were observed in the osteoporosis group. This does not conflict with other reports that suggest serum retinol concentrations are homeostatically controlled and may remain static or decline to compensate for higher circulating esters (22, 42). Circulating retinol that is not bound to retinol-binding protein, ie, as retinyl esters or other forms, could potentially enter the cells and be readily transformed to retinoic acid, the hormone form of vitamin A. Future studies could investigate intracellular concentrations of retinoic acid or measure the differential effect of VA on osteoblast and osteoclast activity in women with and without osteoporosis. Delayed chylomicron clearance after a meal or supplement containing VA has been observed in the elderly (42). Vitamin A antagonizes calcium metabolism perhaps by competing for absorption with vitamin D (45). Persons with delayed chylomicron clearance would not only have higher concentrations of circulating retinyl esters, but may have altered calcium metabolism, manifested either by decreased intestinal absorption or increased bone resorption.

BMI was significantly lower in the osteoporosis group than in the control group, and height and weight were different between the groups. Although no significant difference in energy intake was found, nor in the intake of other macronutrients, the women with osteoporosis were leaner. Some reports have linked BMI with osteoporosis, suggesting that a heavier frame, especially lean body mass, may protect against bone loss (8, 46). Yet, other reports suggest that body mass, and fat mass in particular, is not protective against bone loss (42).

Milk intake was surprisingly low in the subjects. Only 2 subjects (7%) in the osteoporosis group consumed 3 or more cups of milk/d; no subjects in the control group did so. Recent research suggests milk intake may improve vitamin D status and slow bone loss (47). Given widespread attention to calcium and vitamin D and to their necessity in building and maintaining optimal bone mass, milk intake did not meet the current recommendations. The intake of yogurt was also low in both groups at less than one-half cup/d. Although calcium intake appeared slightly higher in the osteoporosis group than in the control group, changes in calcium intake may have occurred after diagnosis of osteoporosis. Because the diet records were collected shortly after diagnosis, some persons may have immediately increased their calcium intakes. Although calcium and vitamin D intakes in both groups met or exceeded their respective RDAs (Table 3), much of this came from calcium supplements, other foods containing calcium, and multivitamins, which generally provided 400 IU vitamin D. The RDA for vitamin D may be too low (48). Because the RDA for vitamin D increases to 15 μg (600 IU)/d for persons aged >71 y, women whose intake is less will need to significantly increase their intake as they age. Without consuming adequate milk and other food sources of vitamin D, additional strategies are required if the rate of bone mineral loss is to be slowed.

The intake of fruit and vegetables in both groups was low compared with current recommendations. Fruit and vegetables are rich in carotenoids, both provitamin A and other, which have antioxidant and immune-enhancing effects that may be of general benefit to health (49). Fruit and vegetables are also rich in potassium and magnesium and have been associated with greater BMD in the elderly (50). This partially explains the low potassium intake in these women.

In the present study, a trend existed for circulating retinyl ester concentrations to be associated with osteoporosis. Osteoporosis is clearly a multifactorial disease in which nutrition plays an important role. Further evaluation of a potential role of excess VA in osteoporosis pathogenesis is necessary. Application of stable isotope methods (51) to assess total body reserves of VA in women with and without osteoporosis is needed to discern any possible linkages of excess VA with the disease. Moreover, understanding the role of the whole diet in osteoporosis pathogenesis is a paramount issue.

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