Vitamin A: Is It a Risk Factor for Osteoporosis and Bone Fracture?

Judy D. Ribaya-Mercado, ScD, and Jeffrey B. Blumberg, PhD

Results from observational studies of the association between vitamin A intake or serum concentration and bone mineral density or fracture are mixed. The inconsistencies may be due, in part, to difficulties in obtaining an accurate assessment of vitamin A intake or status. Serum retinol is a poor measure of vitamin A status because it is subject to homeostatic control. Stable-isotope-dilution methodology gives a validated assessment of the total-body and liver vitamin A stores and is recommended in future studies on vitamin A status and osteoporosis. The potential for exacerbating an already serious public health problem with intakes of vitamin A currently considered safe indicates further research into this matter is warranted.

Key words: vitamin A, retinol, bone, osteoporosis, fracture

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INTRODUCTION

Vitamin A, i.e., all-trans-retinol and its metabolites of retinyl esters, retinal, and retinoic acid, is obtained from foods of animal origin, particularly liver and dairy products. Further, preformed vitamin A is increasingly found in fortified foods as well as dietary supplements. Vitamin A is also formed in vivo from provitamin A carotenoids, particularly β-carotene, α-carotene, and β-cryptoxanthin, found in orange and leafy green plant foods, e.g., carrots, pumpkin, spinach, and collards. Vitamin A is essential for the normal development of cells and tissues, integrity of membranes, growth, vision, and immune function. Vitamin A deficiency can result in impaired cellular differentiation, reduced resistance to infection, anemia, xerophthalmia, and ultimately, blindness and death. In many developing nations, vitamin A deficiency is highly prevalent among children and pregnant women.

One recent study showed that low liver concentrations of vitamin A (assessed by stable-isotope-dilution methodology) may also be common among certain elderly populations.

There is a potential for excessive vitamin A intake and toxicity in countries where vitamin A supplements and fortified foods are readily available. In the United States, an over-the-counter multivitamin can provide 5000 IU (circa 1500 μg) of preformed vitamin A. Vitamin A is found in fortified milk and cereals. The Dietary Reference Intakes provide recommended dietary allowances (RDA) of vitamin A at 900 and 700 μg/d for adult men and non-pregnant, non-lactating women, respectively.

The tolerable upper intake level (UL), defined as “the highest level of daily vitamin A intake that is likely to pose no risk of adverse health effects in almost all individuals” is 3000 μg of preformed retinol for women and men aged ≥19 years.

Case reports in children and adults have indicated that at chronic high intakes (>75,000 IU), vitamin A induces signs and symptoms of toxicity including bone abnormalities, but some epidemiological studies suggest that much lower doses may also be associated with an increased risk of bone loss and fracture. Osteoporosis, an age-related condition characterized by low bone mass and increased risk of fracture, is a major public health problem among older adults, particularly among postmenopausal white women.

Fractures of the hip and spine increase sharply in the fourth decade of life; continue to increase exponentially with age, and often contribute substantially to disability and increased risk of death.

VITAMIN A INTAKE, BONE MINERAL DENSITY, AND BONE FRACTURE

Some observational studies have found that intake of retinol at levels only slightly greater than the RDA and
considerably lower than the UL are associated with poor bone mineral density (BMD) and increased risk of hip fracture.7-9 Promislow et al.10 found both high and low intakes of retinol are associated with low BMD. However, several studies found little or no association between vitamin A intake and bone health,11-19 while others suggested a protective effect of vitamin A against bone loss.20-23 To date, one small clinical trial of short duration has been conducted.24 Table 1 provides a summary of these studies, including the dietary assessment methods used.

Melhus et al.7 conducted a nested case-control study among 247 women in central Sweden who had a first hip fracture within 2–64 months after enrollment and 873

Table 1. Studies examining relationships of vitamin A intake and bone mineral density or fractures in humans

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<tr>
<td>Nested case-control study: 247 with a first hip fracture and 873 controls from a cohort of 66,651 Swedish women, age 40–76 y</td>
<td>FFQ with 60 food items for intakes during the past 6 mo, and four 1-wk diet records</td>
<td>Hip fracture, based on hospital discharge records</td>
<td>Increased risk for retinol intake &gt;1500 μg/d vs ≤500 μg/d (OR: 2.1); no risk associated with β-carotene intake</td>
<td>Melhus et al. (1998)7</td>
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<tr>
<td>Cross-sectional study in 175 randomly selected Swedish women, age 28–74 y</td>
<td>FFQ with 60 food items for intakes during the past 6 mo, and four 1-wk diet records</td>
<td>BMD at the femoral neck, Ward triangle, trochanter region of proximal femur, lumbar spine, and total body, by DXA</td>
<td>BMD was reduced by 6–14% at all skeletal sites for retinol intake &gt;1500 μg/d vs ≤500 μg/d</td>
<td>Melhus et al. (1998)7</td>
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<tr>
<td>Prospective study nested within a longitudinal cohort study of Swedish men (N=2,322) with 30-y follow-up; dietary data was obtained at the 3rd evaluation at age 70 y from 1221 men of whom 111 had a subsequent first fracture</td>
<td>7-d Diet record</td>
<td>Hip or any fracture, based on hospital, radiographic, and outpatient records</td>
<td>Increased risk for retinol intake &gt;1500 μg/d vs &lt;530 μg/d (rate ratio: 2.00); no risk associated with β-carotene intake</td>
<td>Michaelsson et al. (2003)8</td>
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<td>Prospective-analysis among postmenopausal women in the Nurses’ Health Study; N=72,337 with 603 incident hip fractures during 18-y follow-up; mean age, ~60 y at end of follow-up</td>
<td>Average of 5 FFQs with 61-130 food items, taken over 18 y</td>
<td>Hip fracture, self-reported</td>
<td>Increased risk for total vitamin A intake ≥3000 μg RE/d vs &lt;1250 μg RE/d (RR: 1.48), and for retinol intake (food + supplements) ≥2000 μg/d vs &lt;500 μg/d (RR: 1.89). Among nonusers of supplements, increased risk for food retinol ≥1000 μg/d vs &lt;400 μg/d (RR: 1.69); no risk associated with β-carotene intake</td>
<td>Feskanich et al. (2002)9</td>
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<tr>
<td>Prospective analysis</td>
<td>FFQ</td>
<td>BMD at total hip, femoral neck and spine at baseline and after 4 y, by DXA</td>
<td>Both high and low retinol intakes from food + supplements were associated with low BMD at baseline, low BMD at 4 y, and increased bone loss at all sites. Maximum BMD occurred at retinol intakes of 2000–2800 IU/d (~600–840 µg/d). Among women, use of retinol supplements was associated with increased bone loss</td>
<td>Promislow et al. (2002)¹⁰</td>
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<td>Among 570 women and 288 men in the Rancho Bernardo Study (CA) with 4-y follow-up; mean age at follow-up, 71 y</td>
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<td>Observational studies showing little or no relations</td>
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<td>Prospective analysis in a cohort of postmenopausal women from the Iowa Women's Health Study (N=34,703) with incident hip fractures (N=525) or all fractures (N=6502) during mean 9.5 y of follow-up</td>
<td>FFQ with 127 food items</td>
<td>Self-reported hip and non-hip fractures (upper arm, forearm, wrist, ribs, vertebrae)</td>
<td>No association of vitamin A intake (from food, supplements, or both) with the incidence of any fracture. Compared with nonusers, supplement users had a small increased risk of hip fracture (RR: 1.18), but there was no dose-response relationship</td>
<td>Lim et al. (2004)¹¹</td>
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<td>Longitudinal study in a subset of mainly premenopausal women, 45–55 y at baseline (N=891) from the Aberdeen Prospective Osteoporosis Screening Study in Scotland, with 5–7-y follow-up</td>
<td>FFQ with 98 food items for intakes over the previous 12 mo, obtained at baseline and after 5 y</td>
<td>BMD at lumbar spine and femoral neck at baseline and after 5–7 y, by DXA</td>
<td>Vitamin A from food sources appeared to worsen bone loss, but no association was seen when retinol from supplements (mostly as cod liver oil) was added to that from food sources</td>
<td>MacDonald et al. (2004)¹²</td>
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<td>Cross-sectional study of 70-y-old Caucasian women in Reykjavik, Iceland (N=232)</td>
<td>FFQ with 130 food items for intakes during the past 3 mo</td>
<td>BMD in total body, lumbar spine, femoral neck and total hip, by DXA</td>
<td>No association of retinol intake (mostly from cod liver oil, meat, and multivitamins) and BMD at any site</td>
<td>Sigurdsson et al. (2001)¹³</td>
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age-matched controls from a mammography study cohort of 66,651 women who were aged 40–76 years. Increased intake of retinol was associated with increased risk of hip fracture in a multivariate analysis that adjusted for osteoporosis risk factors including age, energy intake, body mass index (BMI), smoking, diabetes, menopausal status, menopausal age, hormone replacement therapy (HRT), oral cortisone and contraceptive use, lifetime physical activity, and previous non-hip fracture. For every 1-mg increase in daily intake of

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Table 1. (Cont’d) Studies examining relationships of vitamin A intake and bone mineral density or fractures in humans

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<td>Cross-sectional, longitudinal, and nested case-control studies in perimenopausal Danish women in the DOPS study (N=2016); 163 fracture cases were identified after 5-y follow-up, each case was matched to 6 controls</td>
<td>4-d or 7-d Food records at baseline</td>
<td>BMD at the lumbar spine and femoral neck at baseline and after 5 y, by DXA; vertebral fractures by X-ray of the spine; self-reported fractures validated by hospital discharge records</td>
<td>No association of retinol or β-carotene from foods, or retinol from foods + supplements and BMD, change in BMD, or fractures in vertebrae or the appendicular skeleton; OR: 1.03 comparing retinol intakes &gt;1500 μg/d vs &lt;500 μg/d</td>
<td>Rejnmark et al. (2004)14</td>
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<td>Prospective study of white men (N=470) and women (N=474) in the Norfolk, England branch of the EPIC study; age 67-79 y at baseline</td>
<td>7-d Food records</td>
<td>BMD at the total hip region by DXA, done 2 times, an average of 3 y apart (range: 2-5 y)</td>
<td>No association of vitamin A or β-carotene intake with BMD loss</td>
<td>Kaptoge et al. (2003)15</td>
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<tr>
<td>Cross-sectional study in 50-79-y-old women (N=11,068), enrolled in the WHI study at 3 clinics (Pittsburgh, PA; Birmingham, AL; and Tucson, AZ)</td>
<td>FFQ with 122 food items for intakes during the past 3 mo</td>
<td>BMD of the total body, lumbar spine, and total hip with subregions of the femoral neck and trochanter, by DXA</td>
<td>No association of vitamin A or total vitamin A (retinol + provitamin A carotenoids) from foods alone, or from foods + supplements and BMD at any site</td>
<td>Wolf et al. (2005)16</td>
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<tr>
<td>Cross-sectional study in 324 postmenopausal women, age 55-80 y, in 2 rural communities in Iowa</td>
<td>24-h Recall and supplement use interview</td>
<td>Radial bone mass by single photon absorptiometry</td>
<td>No association of current vitamin A intake from supplements and/or diet with radial bone mass</td>
<td>Sowers et al. (1985)17</td>
</tr>
<tr>
<td>Cross-sectional study in 246 postmenopausal women, of Northern European descent, age 55-80 y, in a rural Iowa community</td>
<td>24-h Recall and supplement use interview</td>
<td>Radial bone mass by single photon absorptiometry; self-reported fractures at spine, hip, wrist, and other sites</td>
<td>No association of current vitamin A intake from supplements and/or diet with radial bone mass or fractures</td>
<td>Sowers and Wallace (1990)18</td>
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<tr>
<td>Case-control study in postmenopausal women, age 48–83 y, with osteoporosis (N=27) or without osteoporosis (N=24)</td>
<td>3-d Food records from 2 weekdays and 1 weekend day</td>
<td>Osteoporosis, defined as a BMD T-score ≤ -2.5 at the lumbar spine or total proximal femur, by DXA</td>
<td>No significant difference in current vitamin A intake (from foods + supplements) of women with osteoporotic vs normal bone density</td>
<td>Pennistöm et al. (2006)19</td>
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retinol, the risk for hip fracture increased by 68% [95% confidence interval (95%CI), 18–140%; \( P_{\text{trend}} = 0.006 \)]. The odds ratio (OR) for hip fracture was 2.1 (95%CI, 1.1–4.0) with dietary retinol intakes >1500 compared with those ≤500 μg/d. Intakes of β-carotene were not associated with risk of hip fracture. Melhus et al.7 also conducted a cross-sectional examination in Uppsala, Sweden, of 175 randomly selected women aged 28-74 years. Using multivariate analysis, they found retinol intake was inversely associated with BMD at all of the
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<td>C. Observational studies showing certain protective relations</td>
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<td>Nested case-control study (312 cases, 934 controls) from a cohort of &gt;75-y-old women in the UK (N=2,606) with mean follow-up of 3.7 y</td>
<td>Detailed medical and lifestyle history at baseline</td>
<td>Hip fracture and other osteoporotic fractures ascertained by medical records and home visits by nursing staff</td>
<td>Multivitamin or cod liver oil supplementation was associated with a significantly lower risk of any fracture (HR: 0.76)</td>
<td>Barker et al. (2005)</td>
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<td>Cross-sectional and longitudinal analysis of 3-y data from pre-menopausal (N=17) and post-menopausal (N=67) female participants in a clinical trial of calcium supplementation in Madison, WI</td>
<td>Up to 72 daily food records over 3 y</td>
<td>BMC measurements in arm (radius, ulna, humerus) by single photon absorptiometry every 6 mo for 3 y</td>
<td>Cross-sectional analysis showed no correlation of vitamin A with BMC; longitudinal analysis in pre-menopausal women not taking calcium supplements showed a beneficial relation of vitamin A intake in slowing the rate of humerus bone loss</td>
<td>Freudenheim et al. (1986)</td>
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<tr>
<td>Longitudinal study of 66 Caucasian women (age 28–39 y) who participated in a clinical trial of resistance exercise training and calcium supplementation in Tucson, AZ</td>
<td>Up to 12 daily food records over 18 mo</td>
<td>BMD of total body, spine, and 3 femur sites including neck, Ward’s triangle and trochanter at baseline, 5, 12, and 18 mo, by DXA</td>
<td>Vitamin A or carotene from foods was associated with slowing the annual rate of total body bone loss</td>
<td>Houtkooper et al. (1995)</td>
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<td>Cross-sectional study of elderly Japanese residents (1208 men, 912 women) in Hawaii</td>
<td>24-h Recall interview, and previous week's consumption of supplements</td>
<td>BMC at 5 sites (distal and proximal radius and ulna, and the os calcis) using a modification of single photon absorptiometry</td>
<td>In women, but not men, correlations of vitamin A intake and BMC at the distal radius and ulna were positive but very, modest. BMD was not different in users vs nonusers of vitamin A supplements</td>
<td>Yano et al. (1985)</td>
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<td>D. Short-term clinical trial</td>
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<td>Randomized clinical study in 18–58-y-old men in Wisconsin who ingested 25,000 IU retinyl palmitate (N=40) or placebo (N=40) daily for 6 wk</td>
<td>Serum markers of skeletal turnover (BSAP, osteocalcin, and NTx)</td>
<td>No effect of retinyl palmitate supplementation on serum markers of skeletal turnover</td>
<td>Kawahara et al. (2002)</td>
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Abbreviations: BMC, bone mineral content; BMD, bone mineral density; BSAP, bone specific alkaline phosphatase; DOPS, Danish Osteoporosis Prevention Study; DXA, dual energy x-ray absorptiometry; EPIC, European Prospective Investigation into Cancer; FFQ, food frequency questionnaire; HR, hazard ratio; IU, international units; NTx, N-Telopeptide of type I collagen; OR, odds ratio; RE, retinol equivalents; RR, relative risk; WHI, Women’s Health Initiative
skeletal sites examined. No significant change in BMD was found with retinol intakes of up to 1500 μg/d, but for intakes >1500 compared with those ≤500 μg/d, BMD was reduced by 10% at the femoral neck (P=0.05), 13% at the Ward triangle (P=0.01), 9% at the trochanter region of the proximal femur (P=0.03), 14% at the lumbar spine (P=0.001), and 6% for total body BMD (P=0.009). No associations between site-specific BMD and potential confounders such as intakes of calcium, vitamin D, or alcohol were observed.

Prospectively following a cohort of 2322 men for 30 years in Uppsala, Michaelsson et al. examined dietary data obtained from 1221 subjects when they turned 70 years of age. Among these men, 111 had a subsequent first fracture. The highest quintile of daily retinol intake (>1500 μg) was associated with a rate ratio of 2.00 (95% CI, 1.00–3.99) for fracture at any site when compared with the lowest quintile (<500 μg). Dietary β-carotene was not associated with the risk of fracture.

In the USA, Feskanich et al. found similar results in a prospective analysis of vitamin A intake and hip fractures among 72,337 postmenopausal women, aged 34–77 years, in the Nurses’ Health Study. During 18 years of follow-up, data on dietary and supplemental intakes were obtained using five food frequency questionnaires administered every 2–4 years. There were 603 self-reported incident hip fractures resulting from low or moderate trauma during this time period; they occurred at a mean age of 64 years. In a multivariate analysis, women in the highest quintile of daily total vitamin A intake (≥3000 μg retinol equivalents) had an elevated relative risk (RR) of hip fracture (RR, 1.48; 95% CI, 1.05–2.07, P_trend=0.003) compared with those in the lowest quintile of intake (<1250 μg retinol equivalents). The increased risk was attributable primarily to preformed retinol intake from diet plus supplements (RR, 1.89; 95% CI, 1.33–2.68, P_trend <0.001 comparing ≥2000 vs. <500 μg/d). Women currently using vitamin A supplements had a non-significant 40% increased risk of hip fracture compared to those not using supplements. Among those not using supplements, increased retinol intake from unfortified and fortified foods was associated with risk of hip fracture (RR, 1.69; 95% CI, 1.05–2.74; P_trend=0.05 comparing ≥1000 vs. <400 μg/d). These risk estimates were adjusted for age, BMI, HRT, and thiazide diuretics, smoking, hours of leisure-time activity, and intakes of calcium, protein, vitamins D and K, alcohol, caffeine, and energy. Intakes of β-carotene were not associated with an increased risk of hip fracture.

From the Rancho Bernardo Study in California, USA, Promislow et al. reported that either too much or too little retinol intake was associated with poor BMD at the hip, femoral neck, and spine. The study subjects, 570 women and 288 men, aged 55–92 years, were tested for BMD at study entry and again 4 years later. The highest BMD at both time points (and the slowest loss of BMD) was associated with daily retinol intakes of 2000–2800 IU (circa 600–840 μg). Lower BMD at all of the skeletal sites was observed at intakes higher or lower than this range. Among women, the associations of retinol with both BMD and the BMD change over 4 years were negative for supplement users and positive for non-users at all skeletal sites. The risk estimates were adjusted for age, BMI, calcium intake, energy intake, years menopausal, diabetes, current exercise, weight change, and current use of estrogen, steroids, cigarettes, alcohol, thiazides, and thyroid hormones.

In contrast to studies suggesting an adverse association between vitamin A intake and BMD or fracture, other reports found little or no evidence of such a relationship. In a prospective study of 34,703 predominantly white postmenopausal women from the Iowa Women’s Health Study, Lim et al. documented 525 hip fractures and 6502 total fractures (including those in the upper arm, forearm, wrist, ribs, and vertebrae) during an average 9.5 years of follow-up. There was no association of vitamin A intake from food, supplements, or food plus supplements with increased risk of hip or total fractures. Compared to non-users of vitamin A supplements, supplement users had a slightly increased risk of hip fracture (RR, 1.18; 95% CI, 0.99–1.41), but a dose-response relationship was not evident; similarly, no increased risk was noted for total fractures in supplement users.

In a longitudinal study of mainly pre-menopausal women in the Aberdeen Prospective Osteoporosis Screening Study in Scotland, MacDonald et al. found that a BMD change at the femoral neck after 5–7 years of follow-up was inversely correlated with intake of retinol or total vitamin A (retinol plus β-carotene) from food sources, suggesting that vitamin A from food sources worsens bone loss. However, when the contribution of retinol from supplements (mostly as cod liver oil) was added to dietary retinol, the adverse association was no longer seen. The authors speculated that covariance between nutrients may have occurred because cod liver oil also contains vitamin D and long-chain n-3 fatty acids.

In a cross-sectional study of 232 70-year-old women in Reykjavik, Iceland, Sigurdsson et al. found that more than half of preformed retinol intake was derived from cod liver oil and multivitamins, but no adverse association with BMD was seen in the lumbar spine, hip, femoral neck, or total body. Daily intakes ranged from 300–7300 μg (mean intake, 2300 μg) with no associations to mean or median BMD at any site. Intakes of β-carotene were also unrelated to BMD.

Interestingly, in a case-control study of 312 incident osteoporotic fracture cases and 934 controls, nested in a prospective study of 2606 British women aged >75
years, with a mean follow-up of 3.7 years, Barker et al.\textsuperscript{20} found that use of cod liver oil or multivitamin supplements was associated with a significantly lower risk of any fracture [hazard ratio (HR), 0.76; 95\%CI, 0.60–0.95; \(P=0.02\)]. Most multivitamin supplements in the United Kingdom contain retinol at a dose of 800 \(\mu\)g and cod liver oil contains approximately 100 \(\mu\)g/g oil.

Rejnmark et al.\textsuperscript{14} analyzed data from 2016 perimenopausal Caucasian women, aged 45–58 years, in the Danish Osteoporosis Prevention Study—a prospective study on the effect of HRT on BMD and fracture risk. Cross-sectional analysis at baseline and after 5 years showed no association between intake of retinol or \(\beta\)-carotene from food or food plus supplements and BMD at the lumbar spine or femoral neck. Multiple regression analyses showed no association between baseline vitamin A intake and BMD change. Similarly, no differences in BMD changes were found between the lowest 5\% versus the highest 5\% intake of vitamin A. During the 5 years of follow-up, 163 women sustained a fracture in the appendicular skeleton and/or vertebrae; each case subject was matched to six control subjects, based on HRT treatment. Multivariate analysis revealed no increase in fracture risk comparing subjects in the highest (>1500 \(\mu\)g) versus the lowest (<500 \(\mu\)g) categories of daily retinol intake from foods plus supplements (odds ratio, 1.03; 95\%CI, 0.56–1.89).

Examining subjects in the Norfolk, England, branch of the European Prospective Investigation into Cancer study, Kaptoge et al.\textsuperscript{15} found no relationship between intakes of vitamin A or \(\beta\)-carotene and loss of hip bone during 2–5 years of follow-up in 470 white men and 474 white women who were 67–79 years of age.

In a cross-sectional analysis of baseline data from 11,068 women, aged 50–79 years, who were participants in the Women's Health Initiative, Wolf et al.\textsuperscript{16} found in a multivariate analysis that intakes of retinol or of total vitamin A (retinol plus provitamin A carotenoids) from food and/or supplements, were unrelated to BMD at the femoral neck, trochanter, total hip, lumbar spine, and total body. Further, no associations were observed between BMD at these skeletal sites and serum retinol. Interestingly, total \(\beta\)-carotene intake from foods plus supplements (but not from foods only) was inversely associated with BMD in the femoral neck and total body (\(P=0.03\) after multiple adjustments). However, no associations were found between serum \(\beta\)-carotene and any site-specific BMD.

Examining two cross-sectional studies comprised of 324 and 246 postmenopausal women of northern European descent residing in rural communities in Iowa, Sowers et al.\textsuperscript{17} and Sowers and Wallace\textsuperscript{18} found no significant relationship between current vitamin A intake from foods and/or supplements and radial bone mass or fractures of the spine, wrist, hip, and ribs. However, in both studies, dietary intake was assessed with a single 24-h food recall, which is a limited approach for determining usual intakes of vitamin A. Penniston et al.\textsuperscript{19} also found no significant difference in current total vitamin A intake between 27 postmenopausal women with osteoporosis and 24 without evidence of this condition.

As part of a 4-year intervention trial with 500 mg/d calcium supplementation in Madison, Wisconsin, USA, Freudenheim et al.\textsuperscript{21} conducted a cross-sectional analysis at study entry and found no relationship between bone mineral content (BMC) of the radius, humerus, or ulna and total vitamin A intakes in 17 pre-menopausal and 67 postmenopausal women. However, longitudinal analysis of 3-year data showed a beneficial association of vitamin A intake with the slope of loss of humerus BMC (\(r=0.821\), \(P=0.007\)) in pre-menopausal women not receiving calcium supplements. In contrast, one postmenopausal woman receiving calcium supplements who had also used a high-dose vitamin A supplement (14,624 IU/d) showed an accelerated loss of ulna BMC compared to other subjects. Thus, this study suggests complex interrelationships between vitamin A intake and bone loss dependent on skeletal site, menopausal status, and use of calcium supplements.

Among a pooled sample of 66 pre-menopausal Caucasian women, aged 28–39 years, in Tucson, Arizona, USA, participating in the exercise or sedentary arms of an 18-month randomized clinical trial of resistance exercise training and calcium supplementation (500 mg/d), Houtkooper et al.\textsuperscript{22} found that vitamin A and carotene from food were associated with a slower annual rate of total body bone loss, although no interaction was observed with changes of BMD of the spine or femur.

Cross-sectional data obtained by Yano et al.\textsuperscript{23} from 912 elderly Japanese-American women (but not from 1208 men) in Hawaii showed a positive but very modest correlation between total vitamin A intake from diet plus supplements and BMC in two of five skeletal sites examined; the standardized regression coefficient were 0.071 and 0.063 for distal radius and distal ulna, respectively (\(P<0.05\)). No difference in BMC was noted between users and nonusers of vitamin A supplements. A limitation of this study was the use of a single 24-h food recall to assess dietary intake.

To date, only one clinical study of the effect of vitamin A supplementation on bone has been reported. Kawahara et al.\textsuperscript{24} conducted a 6-week, randomized, placebo-controlled, single-blind study with 25,000 IU/d retinyl palmitate in 80 healthy men aged 18–58 years. They found the supplementation did not alter serum markers of skeletal turnover, i.e., bone-specific alkaline phosphatase, osteocalcin, and N-Telopeptide of type I collagen.
SERUM OR PLASMA RETINOL AND RETINYL ESTERS, BONE MINERAL DENSITY, AND BONE FRACTURE

Like studies of the relationship between vitamin A intake and BMD or fracture, those evaluating serum or plasma retinol status showed similar mixed results. One study revealed an adverse association, another showed both high and low serum retinol linked to adverse outcomes, several reports indicated little or no association between serum retinol or retinyl esters and bone health, and two studies suggested a protective relationship. Studies relating serum or plasma concentrations of retinol or retinyl esters with BMD or fracture are summarized in Table 2.

In the longitudinal Swedish cohort described above, Michaelsson et al. analyzed 266 incident fractures obtained over 30 years of follow-up and found the risk was greatest among men with the highest levels of serum retinol at baseline. Multivariate analyses comparing the highest quintile of serum retinol (>2.64 \( \mu \)mol/L) with the middle reference quintile (2.17–2.36 \( \mu \)mol/L) showed a rate ratio of 2.47 (95% CI, 1.15–5.28) for hip fracture and 1.64 (95% CI, 1.12–2.41) for any fracture. The risk was concentrated in the highest quintile with an exponential increase, such that men with serum retinol in the 99th percentile (>3.60 \( \mu \)mol/L) had an overall risk of fracture that was 7-fold greater than in those with lower concentrations. The analysis was adjusted for age, weight, height, smoking status, marital status, socioeconomic class, physical activity at work, leisure physical activity, alcohol consumption, and serum \( \beta \)-carotene, calcium, and albumin. Serum \( \beta \)-carotene was not associated with risk of fracture.

In a prospective analysis of the National Health and Nutrition Examination Survey I Follow-up Study on 2799 women, aged 50–74 years at baseline, Optowosky and Bilezikian found that both high and low serum vitamin A (retinol plus retinyl esters) are associated with an increased risk of hip fracture. Analyzing 172 incident hip fractures over 22 years of follow-up, fracture risk was higher among subjects in the highest quintile of serum vitamin A (\( \geq 2.56 \mu \)mol/L) (HR, 2.1; 95% CI, 1.2–3.6) and the lowest quintile (\( \leq 1.61 \mu \)mol/L) (HR, 1.9; 95% CI, 1.1–3.3) compared with those in the middle quintile (1.90–2.13 \( \mu \)mol/L). The multivariate model included age, weight, serum albumin and cholesterol, alcohol use, recreational and non-recreational physical activity, HRT, history of previous fracture, calcium intake, and race.

In 11,068 women, aged 50–79 years, who participated in the Women's Health Initiative study, Wolf et al. found no significant association of serum retinol or individual provitamin A carotenoids (\( \beta \)-carotene, \( \alpha \)-carotene, and \( \beta \)-cryptoxanthin) with BMD at the lumbar spine, femoral neck, trochanter, total hip, or total body. These results are consistent with their finding that intake of retinol or total vitamin A (retinol plus provitamin A carotenoids) are unrelated to BMD in this cohort.

Ballew et al. analyzed data from National Health and Nutrition Examination Survey III for cross-sectional associations of serum retinyl esters and BMD at the femoral neck, trochanter, intertrochanter, and total hip. Complete data were available from 5790 participants, aged 20 to >90 years, including non-Hispanic white (84%), non-Hispanic black (11%), and Mexican-American (5%) men and women. No significant associations between fasting serum retinyl esters and BMD or osteoporosis/osteoporosis were found, even though the prevalence of high fasting serum retinyl esters and low BMD were substantial in this cohort. High retinyl esters in fasting serum is thought to be indicative of excess vitamin A intake. Generally, <5% of total circulating vitamin A is in the form of retinyl esters and most of the remainder is in the form of retinol bound to retinol-binding protein. The amounts of circulating retinyl esters increase when the storage capacity in liver or the capacity of the carrier protein to transport vitamin A is exceeded.

A nested case-control study in the UK was conducted by Barker et al. within the placebo arm of a cohort participating in a prospective study of hip fracture in elderly women. After a mean follow-up of 3.7 years, 312 incident osteoporotic fracture cases were identified and matched to 934 controls. Multivariate analysis showed no association of serum retinol, retinyl palmitate, or \( \beta \)-carotene with fracture risk.

The relationship between serum retinol concentrations, radial bone mass, and fracture history was studied by Sowers and Wallace in a cross-sectional study of 246 post-menopausal women, aged 55–80 years, of Northern European descent in a rural community in Iowa. No significant relationships were observed between serum retinol and radial bone mass or fractures.

In a case-control study of 30 post-menopausal women with osteoporosis (BMD T-score ≤−2.5 at the lumbar spine or total proximal femur) and 29 women with normal BMD, Penniston et al. found serum retinyl esters were not different between the groups and were not elevated despite intakes of total vitamin A that were nearly 2-fold the RDA. Serum retinyl esters did not significantly correlate with vitamin A intake. Serum retinol was lower in women with osteoporosis (\( P=0.03 \), but no significant association was seen between osteoporosis and serum retinol. Similarly, no association was seen between osteoporosis and serum total vitamin A.
Table 2. Studies examining relationships of serum or plasma vitamin A concentration and bone mineral density or fractures in humans

<table>
<thead>
<tr>
<th>Study design and subjects</th>
<th>Exposure measure</th>
<th>Outcome studied</th>
<th>Results</th>
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<tr>
<td>A. Observational studies showing adverse relations</td>
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<tr>
<td>Longitudinal cohort study in Swedish men (N=2322) age 49–51 y at baseline; 266 men had fractures in 30 y of follow-up</td>
<td>Serum retinol and β-carotene</td>
<td>Hip fracture or any fracture based on hospital, radiographic, and outpatient records</td>
<td>Increased risk of hip fracture (RR: 2.47) or any fracture (RR: 1.64) comparing highest (&gt;2.64 μmol/L) vs middle quintile (2.17–2.36 μmol/L) of serum retinol; no risk associated with serum β-carotene</td>
<td>Michaelsson et al. (2003)8</td>
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<tr>
<td>Prospective analysis of the NHANES I Follow-up Study; N=2799 women, age 50–74 y at baseline, with 172 incident hip fractures in 22 y of follow-up</td>
<td>Serum vitamin A (retinol + retinyl esters)</td>
<td>Hip fracture</td>
<td>Both high and low serum vitamin A are associated with increased risk; HR: 2.1 for highest quintile (≥2.56 μmol/L) and HR: 1.9 for lowest quintile (≤1.61 μmol/L) compared to middle quintile (1.90–2.13 μmol/L)</td>
<td>Opotowsky and Bilezikian (2004)25</td>
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<tr>
<td>B. Observational studies showing little or no relations</td>
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<td>Cross-sectional study in 50–79-y-old women (N=11,068) enrolled in the WHI study at 3 clinics (Pittsburgh, PA; Birmingham, AL; and Tucson, AZ)</td>
<td>Serum retinol, β-carotene, α-carotene, and β-crypoxanthin</td>
<td>BMD of the total body, lumbar spine, and total hip with subregions of the femoral neck and trochanter, by DXA</td>
<td>No association of serum retinol or carotenoids and BMD at any site</td>
<td>Wolf et al. (2005)16</td>
</tr>
<tr>
<td>Cross-sectional analysis of NHANES III data from 5790 participants, age 20 to &gt;90 y</td>
<td>Serum retinyl esters</td>
<td>BMD at the femoral neck, trochanter, intertrochanter, and total hip, by DXA; osteopenia, osteoporosis</td>
<td>No association of serum retinyl esters and any measure of bone mineral status</td>
<td>Ballew et al. (2001)26</td>
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<tr>
<td>Nested case-control study (312 cases, 934 controls) from a cohort of &gt;75-y-old women in the UK (N=2606) with mean 3.7 y of follow-up</td>
<td>Serum retinol, retinyl palmitate, and β-carotene at baseline</td>
<td>Hip fracture and other osteoporotic fractures based on medical records and home visits</td>
<td>No association of serum retinol, retinyl palmitate, or β-carotene with fracture risk</td>
<td>Barker et al. (2005)20</td>
</tr>
<tr>
<td>Cross sectional study in 246 postmenopausal women, age 55–80 y, of Northern European descent, in a rural community in Iowa</td>
<td>Serum retinol</td>
<td>Radial bone mass by single photon absorptiometry; self-reported fractures at spine, hip, wrist, and other sites</td>
<td>No association of serum retinol and radial bone mass or fractures</td>
<td>Sowers and Wallace (1990)18</td>
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</tbody>
</table>
Table 2. (Cont'd) Studies examining relationships of serum or plasma vitamin A concentration and bone mineral density or fractures in humans

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<tr>
<td>Case-control study of postmenopausal women, age 48–83 y, with osteoporosis (N=30) or no osteoporosis (N=29)</td>
<td>Serum retinyl esters and retinol</td>
<td>Osteoporosis, defined as a BMD T-score ≤ -2.5 at the lumbar spine or total proximal femur, by DXA</td>
<td>No association between osteoporosis and total serum vitamin A (retinol + retinyl esters) or serum retinol; however, retinyl ester concentration (i.e., percentage of total vitamin A) tended to be associated with osteoporosis (P=0.07)</td>
<td>Penniston et al. (2006)²⁹</td>
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C. Observational studies showing certain protective relations

<table>
<thead>
<tr>
<th>Study design and subjects</th>
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<tbody>
<tr>
<td>Case-control study of ≥60-y-old Italian women with severe osteoporosis (N=75) or no osteoporosis (N=75)</td>
<td>Plasma retinol</td>
<td>Severe osteoporosis, defined as a BMD T-score ≤ -3.5 at the femoral neck, by DXA</td>
<td>Plasma retinol was positively correlated with femoral neck BMD (r=0.36, P&lt;0.01)</td>
<td>Maggio et al. (2003)²⁷</td>
</tr>
<tr>
<td>Case-control study of ≥60-y-old Italian women with severe osteoporosis (N=45) or no osteoporosis (N=45)</td>
<td>Plasma retinol, β-carotene, α-carotene, and β-cryptoxanthin</td>
<td>Severe osteoporosis, defined as a BMD T-score ≤ -3.5 at the femoral neck, by DXA</td>
<td>Plasma retinol, but not carotenoids, was positively correlated with femoral neck BMD (r=0.4, P&lt;0.01)</td>
<td>Maggio et al. (2006)²⁸</td>
</tr>
</tbody>
</table>

Abbreviations: BMD, bone mineral density; DXA, dual energy x-ray absorptiometry; HR, hazard ratio; NHANES, National Health and Nutrition Examination Survey; RR, relative risk; WHI, Women’s Health Initiative.

However, serum retinyl esters (as a percentage of total vitamin A) tended to be positively associated with osteoporosis (P=0.07). No differences in biomarkers of bone turnover (bone specific alkaline phosphatase, osteocalcin, and N-Telopeptide of type I collagen) were observed between the groups.

A beneficial bone-sparing relationship between vitamin A and BMD has been reported by Maggio et al.²⁷ in a study of ≥60-year-old women in Perugia, Italy, with severe osteoporosis (BMD T-score ≤ -3.5 at the femoral neck; N=75) or without osteoporosis (N=75). Plasma vitamin A was positively correlated with BMD at the femoral neck (r=0.36, P<0.01) when age and BMI were used as covariates in the analysis. Mean plasma vitamin A was lower in the osteoporotic women than in controls (P<0.001). In another study by Maggio et al.,²⁸ a similar group of 45 Italian women with severe osteoporosis were observed to have lower plasma retinol and carotenoids compared to 45 healthy controls. While plasma retinol was positively correlated with femoral BMD (r=0.4, P<0.01), no associations were noted for individual plasma carotenoids.

ANIMAL STUDIES, CASE REPORTS, AND STUDIES OF HUMANS ON SYNTHETIC RETINOID TREATMENTS

Animal studies support the notion that both vitamin A excess³¹⁻³⁷ and deficiency³²⁻³⁴⁻³⁸ have profound adverse effects on bone. In rats fed oral doses of retinyl esters at 25,000–75,000 IU/d, the most characteristic lesion found was spontaneous bone fracture,³¹⁻³³⁻³⁶ an outcome that occurred more consistently in young than in older rats.³¹ Lower doses of retinyl esters at approximately 9000 IU/d when ingested by young rats for 12 weeks also resulted in thinner and 'weaker bones.³³ In senescent rats, 14 months of moderately high vitamin A feeding at 300 IU/d resulted in no adverse effects on bone remodeling, and intake at 120 IU/d, about 2-fold the daily requirement, showed a beneficial 15% increase in trabecular bone.⁴⁰ Vitamin A deficiency in animals results in shortness of the long bones due to failure of endochondral bone growth and bone remodeling.³²⁻³⁸⁻¹.

Case reports of children⁴¹⁻⁴³ and adults⁴⁻⁴⁵ who ingested excessive daily doses of vitamin A (75,000–
300,000 IU) for months or years describe tender swelling in the feet, ankles, arms, hands and wrists, multiple cortical hyperostosis in the long bones, abnormal bone turnover, decreased BMD, and vague skeletal pains which reversed upon vitamin A withdrawal.

Long-term treatment for skin disorders, with synthetic analogues of retinoic acid, such as isotretinoin or etretinate, has been reported to result in skeletal radiographic changes and increased risk of osteoporosis. One possible mechanism for the adverse effect of hypervitaminosis A on bone may involve a direct effect on the bone remodeling process through retinoic acid, which has been shown in vitro to inhibit osteoblast activity and stimulate osteoclast formation. Retinoic acid receptors and retinoid X receptors are found in osteoblasts and osteoclasts. Hypervitaminosis A may have an indirect adverse effect on bone through antagonism of the action of vitamin D in normalizing serum calcium levels and bone mineralization. All retinoid receptors and the receptor for 1,25-dihydroxyvitamin D₃ are members of the steroid and thyroid hormone receptor superfamily, so interactions can readily be hypothesized.

**RECOMMENDATION FOR FUTURE STUDIES: USE OF STABLE-ISOTOPE-DILUTION METHOD FOR ASSESSMENT OF VITAMIN A STATUS**

During the past decade, stable-isotope-dilution techniques have emerged as powerful tools for the biochemical assessment of vitamin A status. The method is based on the principle that when an individual's vitamin A status is poor, less dilution of an oral dose of labeled vitamin A by endogenous vitamin A occurs, resulting in a relatively higher ratio of labeled-to-non-labeled retinol in serum. Conversely, in vitamin A subtoxic states, an oral dose of labeled vitamin A is diluted by endogenous vitamin A. The deuterated retinol dilution method (DRD) employs deuterium-labeled vitamin A, and has been shown to provide a quantitative estimate of the total-body vitamin A stores (also called total-body vitamin-A pool size) and of liver vitamin A concentration in young and older adults and children. The procedure has been used in field studies and in clinical settings. It has been validated in two studies by comparison of the calculated vitamin A values with those obtained by direct measurements of liver biopsies; from generally healthy adult surgical patients with low to adequate vitamin A status; correlation coefficients of 0.88 and 0.75 were obtained.

Serum or plasma retinol, which has been used in the studies described above, is a poor measure of vitamin A status because it is subject to homeostatic control over a wide physiologic range of liver vitamin A concentrations; hence, serum retinol may not reflect liver stores. The normal physiologic range of liver vitamin A is between 0.07 and 1.05 μmol/g liver (20–300 μg/g). Serum retinol values tend to fall when liver vitamin A concentrations are <0.07 μmol/g and tend to increase steeply when liver concentrations are >1.05 μmol/g. However, within usual physiological ranges, circulating retinol concentrations respond with a very shallow slope to increments in liver content and are not sensitive indicators of vitamin A status. Further, a variety of clinical conditions can alter serum retinol levels, e.g., an acute-phase response to infection can result in a transient decrease and lead to misclassification of vitamin A status.

Studies that employed both the DRD procedure and serum retinol measurements show that DRD, but not serum retinol, can distinguish differences in the vitamin A status of population groups. For example, in Guatemala, where a national program of sugar fortification of vitamin A is in place, the mean total-body vitamin A stores in elders was about four times higher than in Filipino elders, yet their mean serum retinol concentrations were not significantly different. The DRD procedure can detect changes in total-body stores of vitamin A in response to dietary interventions, whereas the serum retinol response is dependent on serum retinol concentration at pre-intervention; in general, an improvement in serum retinol is seen in studies with low baseline values (0.57–0.76 μmol/L) but not in studies with higher baseline values (0.84–2.31 μmol/L). In a study among the elderly, total-body vitamin A stores correlated with their dietary intakes of total vitamin A, preformed retinol, β-carotene, fat, and protein; in contrast, their serum retinol concentrations showed no correlation with any of these dietary intakes. One year after the initiation of the national program of sugar fortification with vitamin A in Nicaragua, none of the study participants had detectable retinyl esters in plasma; however, 9 of the 21 study participants had liver vitamin A concentrations that were >1.05 μmol/g, values that are considered subtoxic. Subtoxic status without clinical signs of toxicity may be a growing concern in populations in which vitamin A supplements and vitamin A-fortified foods are readily available. The DRD methodology provides a more accurate, less confounded, and more meaningful assessment of vitamin A status, from deficiency to subtoxicity, than measurements of serum or plasma retinol concentrations.

**SUMMARY**

While some data suggest an adverse impact of high intakes of vitamin A or high serum retinol status on bone
health, the results in the literature are inconsistent and a firm conclusion cannot be drawn about these relationships. These inconsistencies may be due, in part, to the lack of an accurate assessment of dietary vitamin A and vitamin A status. Further confounding the results are the collinearity of vitamin A intake with intake of other nutrients that influence bone health and differences between studies in skeletal sites examined, menopausal status, and variables used for adjusting risk estimates. Nonetheless, the potential for exacerbating an already serious public health problem with intakes of vitamin A currently considered safe indicates further research into this matter is warranted. Utilization of the DRD method should provide a better assessment of the relationship between vitamin A and bone health.

REFERENCES


