Excessive Dietary Intake of Vitamin A Is Associated with Reduced Bone Mineral Density and Increased Risk for Hip Fracture

▶Hakan Melhus, MD; Karl Michaelsson, MD; Andreas Kindmark, MD; Reinhold Bergstrom, PhD; Lars Holmberg, MD; Hans Mallmin, MD; Alicja Wolk, PhD; and Sverker Ljunghall, MD

Background: The highest incidence of osteoporotic fractures is found in northern Europe, where dietary intake of vitamin A (retinol) is unusually high. In animals, the most common adverse effect of toxic doses of retinol is spontaneous fracture.

Objective: To investigate whether excessive dietary intake of vitamin A is associated with decreased bone mineral density and increased risk for hip fracture.

Design: A cross-sectional study and a nested case-control study.

Setting: Two counties in central Sweden.

Participants: For the cross-sectional study, 175 women 28 to 74 years of age were randomly selected. For the nested case-control study, 247 women who had a first hip fracture within 2 to 64 months after enrollment and 873 age-matched controls were selected from a mammography study cohort of 66 651 women 40 to 76 years of age.

Measurements: Retinol intake was estimated from dietary records and a food-frequency questionnaire. Bone mineral density was measured with dual-energy x-ray absorptiometry. Hip fracture was identified by using hospital discharge records and was confirmed by record review.

Results: In multivariate analysis, retinol intake was negatively associated with bone mineral density. For every 1-mg increase in daily intake of retinol, risk for hip fracture increased by 68% (95% CI, 18% to 140%; P for trend, 0.006). For intake greater than 1.5 mg/d compared with intake less than 0.5 mg/d, bone mineral density was reduced by 10% at the femoral neck (P = 0.05), 14% at the lumbar spine (P = 0.001), and 6% for the total body (P = 0.009) and risk for hip fracture was doubled (odds ratio, 2.1 [CI, 1.1 to 4.0]).

Conclusion: High dietary intake of retinol seems to be associated with osteoporosis.

Age-adjusted rates of hip fracture incidence vary more than sevenfold in Europe; the highest rates are found in northern Europe, particularly Sweden and Norway [1,2]. Rates are higher among residents of Scandinavia than among comparable persons in North America [2] and, remarkably, are higher among Swedish men than among women in countries such as the United Kingdom, the Netherlands, and Switzerland [1]. Known risk factors [3] cannot explain these observations; this indicates that hitherto unknown environmental factors contribute to the development of osteoporosis.

When dietary patterns in Europe were compared, a large variation in vitamin A intake was found: Median intake was up to sixfold higher in Scandinavian countries than in southern Europe [4]. The most prominent features of hypervitaminosis A in laboratory animals are accelerated bone

resorption, bone fragility, and spontaneous fractures [5-7]. Anecdotal reports suggest that accidental vitamin A poisoning impairs bone remodeling [6-8], and recent studies have shown that osteoporosis is a toxic effect of long-term therapy with etretinate, a synthetic retinoid [9,10].

We previously did a large, nested case-control study of diet and risk for hip fracture [11] and a cross-sectional study of diet and bone mineral density [12], but we did not analyze dietary intake of retinol in these studies. We therefore decided to investigate whether excessive dietary intake of vitamin A is associated with decreased bone mineral density and increased risk for hip fracture.

Methods

Bone Mineral Density Study

Participants

We previously investigated the relation between nutrient intake and bone mineral density in a population-based, cross-sectional study [12]. Briefly, a random sample of women who were living in the county of Uppsala, Sweden, and were 28 to 74 years of age at study entry was selected from the population register. A total of 175 women were included. Diet was assessed by review of four 1-week dietary records for each woman.

Outcome Measures

Bone mineral density was measured by using dual-energy x-ray absorptiometry (DPX-L, Lunar Co., Madison, Wisconsin) at the lumbar spine, the total body, and three regions of the proximal femur (the neck, the Ward triangle, and the greater trochanter).

Nondietary Information

Weight and height were measured with a scale and a stadiometer soon after bone density measurements. Participants were asked to complete a questionnaire about major risk factors for osteoporosis [12]. They were asked whether they were former or current smokers and how many cigarettes they smoked each day; how often they had engaged in physical activity in their leisure time (never, occasionally, <1 hour per week, 1 or 2 hours per week, or >or=to2 hours per week) during three periods of life (as a teenager, between 18 and 30 years of age, and in recent years); whether they had diabetes and, if so, what type of treatment they received for it; whether they used cortisone, hormone replacement therapy, or oral contraceptives and, if they did, how long they had used these treatments and when they had used them; whether they were menopausal and their menopausal age; whether they had formerly engaged in athletic activity and, if so, what type of activity they had engaged in and when they had engaged in it; and whether they had had a fracture of the hip, ankle, spine, or lower or upper arm after age 40 years. The question about physical activity had been used and validated in a large European study of hip fracture [13]. No association between potential confounding factors, such as intake of calcium, vitamin D, or alcohol, and site-specific bone mineral density was found in these women [12].

Data Analysis

A linear regression model was used in the analyses of bone mineral density. In multivariate modeling, we introduced the variables used in the hip fracture analysis (see below). Body mass index, energy intake, and current and menopausal age were continuous variables, and the following variables were categorical: current and former smoking, any use of cortisone, any use of hormone replacement therapy and oral contraceptives, menopausal status, history of fracture after age 40 years, diabetes mellitus and type of treatment received (oral treatment or insulin treatment compared with no diabetes), and lifetime physical activity (the sum of the scores of physical activity for the three periods of life, expressed as being above or below the median lifetime score). In these linear regression analyses, retinol intake was considered both in original continuous and in categorized form, with cut-off values identical to those used in the hip fracture study. We tested the linear relation between retinol intake and bone mineral density by adding a quadratic term of retinol intake to the multivariate model, which included retinol in continuous form. The P values for the quadratic term were between 0.25 and 0.90 in different models, indicating that there was no obvious nonlinear relation between retinol intake and bone mineral density. The main analysis included retinol intake divided into four (<0.5, 0.51 to 1.0, 1.01 to 1.5, and >1.5 mg/d) or two (<or=to1.5 and >1.5 mg/d) categories in models that also included the covariates described above. Test results and CIs were computed with the standard method used in regression analysis [14]. The estimated average bone mineral density for each category was computed from the estimated regression models. In multivariate models, the covariate values were set at their means.

Hip Fracture Study

Study Sample

The Swedish Mammography Cohort [15,16] and our study design have been described in detail elsewhere [11]. Briefly, from 1987 to 1990, all women who were 40 to 76 years of age and were resident in two counties in central Sweden (source population, 90 303) were mailed a validated [16] food-frequency questionnaire; 66 651 (74%) of the women replied.

Dietary Questionnaire

The questionnaire asked women to report their usual intake of 60 foods during the past 6 months. In addition to requesting information on dietary habits, it also asked for participants' self-reported current weight and height, marital status, parity, and educational level.

Case-Patients and Controls

Women who had a first hip fracture within 2 to 64 months after entry into the cohort were defined as case-patients in a nested case-control analysis of hip fracture. For each case-patient, four controls (individually matched to the case-patient by age and county of residence) were selected from the cohort.

Hip fracture was defined as a cervical, trochanteric, or subtrochanteric femoral fracture. By using hospital discharge records from the six hospitals of the two counties included in the study, we identified possible cases of hip fracture. Hospital records for these possible cases were scrutinized, and we excluded women with incorrect diagnoses and women with fractures due to cancer or high-energy trauma. Of the hip fractures included in the study, 62% were located in the cervical portion of the femur.

Additional Information

A second questionnaire on potentially confounding factors not included in the first questionnaire was mailed to controls and case-patients [11]. The following information was requested: self-reported age at menopause, duration and dates of exposure to postmenopausal hormone replacement therapy and oral cortisone, duration of oral contraceptive use, smoking status (with number of cigarettes) in each decade of life, current use of vitamin or mineral supplementation (with number of tablets taken per week and weeks of use per year), diabetes mellitus with current type of treatment received and age at first appearance, physical activity during leisure time (on a five-point scale) at four different periods of life (as a teenager, at 30 years of age, 5 years earlier, and currently), athletic participation (with type and duration of activity), and previous fractures other than hip fracture after age 40 years.

Of those who were eligible, 92.5% of case-patients and 89.1% of controls returned the second questionnaire, and 247 case-patients and 873 controls were included in the analysis. No signs of liver disease were noted in the hospital records of the women in the study. Previous analyses of these data identified several established risk factors for hip fracture. However, no statistically significant differences were seen between case-patients and controls in the use of multivitamin or calcium supplements, alcohol, caffeine, or thiazides [11].

Data Analysis

Because of the matched study design, the basic model used in the hip fracture study was conditional logistic regression [17]. To assess risk for hip fracture, we estimated odds ratios and 95% CIs from the model. Retinol intake was analyzed both in continuous form and in four categories (based on the daily intake of 0.5 mg of retinol equivalents recommended by the Food and Agricultural Organization and the World Health Organization [18]): <or=to0.5 mg/d, 0.51 to 1.0 mg/d, 1.01 to 1.5 mg/d, and >1.5 mg/d. The lowest category was considered the reference category. No women in the lowest category had intake less than 0.27 mg/d, which is the basal level sufficient to meet all physiologic needs [18]. The P value for a quadratic term of retinol intake in the multivariate model with retinol intake in continuous form was 0.31, indicating that a linear relation between retinol and hip fracture risk was a reasonable possibility. Thus, tests for trend were performed with retinol intake in both original continuous form and in categorized form by introducing a new variable obtained by assigning the four categories of retinol intake into consecutive integers (0, 1, 2, and 3) in the model. Data were analyzed in a multivariate model that included the following covariates categorized into quartiles on the basis of distribution of controls: body mass index (kg/m^2), energy intake, age at menopause, and lifetime physical activity during leisure time (established by summing the scores for the four periods of life). In the model, we also introduced cigarette smoking and hormone replacement therapy (no, former, or current use), diabetes mellitus (no diabetes, oral treatment of diabetes, or insulin treatment of diabetes), and the following dichotomized variables: any use of oral contraceptives or cortisone (any or never), previous osteoporotic fracture of the distal forearm or the proximal humerus, menopause at the time of the second questionnaire (yes or no), and former athletic activity (ever or never). The odds ratios for retinol intake remained almost unchanged if we also considered duration of smoking estimated in pack-years (1 pack-year is the equivalent of smoking one pack of cigarettes each day for 1 year) or duration of hormone replacement therapy and use of cortisone in the model instead of the simpler categorization.

Using current height, using body weight instead of body mass index, or classifying educational level as only primary school or a higher educational level did not change our estimates of retinol intake in either the hip fracture study or the bone mineral density study.

In addition to considering the variables listed in Table 1 and Table 7 and Table 2, we considered models that made further adjustment for certain nutrient variables, such as intake of iron, magnesium, vitamin C, and calcium (and vitamin D in the bone mineral density study). Because some of these variables were highly correlated among themselves and with energy intake, we did a detailed analysis of multicollinearity. Thus, we compared estimates and SEs obtained in models

that included different variables. We also computed such characteristics as variance inflation factors [19,20], which indicate the degree of multicollinearity. The effects on the estimates of retinol intake, including the effects of the additional nutrient variables, were moderate. However, because multicollinearity may have been a problem, at least in the hip fracture study, we chose to present results from models that did not include the additional nutrient variables.

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Table 1. **beta-Coefficients Obtained in Linear Regression Models of Covariates and Bone Mineral Density***

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Table 7. **Table 1 Continued**

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Table 2. **Odds Ratios for Covariates in the Hip Fracture Study***

Role of the Study Sponsor

This study was supported by grants from the Swedish Medical Research Council, a government council that played no role in the design, conduct, or reporting of the study.

Results

The major characteristics of the participants in the case-control study and the cross-sectional study are shown in Table 3.

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Table 3. **Characteristics of Case-Patients and Controls in the Diet and Hip Fracture Study and of the Participants in the Diet and Bone Mineral Density Study***

Retinol Intake and Bone Mineral Density

To investigate whether subclinical hypervitaminosis A is associated with a reduction in bone mineral density, we analyzed the relation between dietary intake of retinol and bone mineral density. The effect of covariates on bone mineral density is shown in Table 1 and Table 7. As Table 4 and Table 8 shows, retinol intake was negatively associated with bone mineral density at all five of the sites examined, and the association was significant in multivariate analysis. We then studied the relation between retinol intake in categories and bone mineral density (Table 5 and Table 9). In multivariate analysis, we found no significant changes in bone mineral density at intake up to 1.5 mg/d. However, when intake greater than 1.5 mg/d was compared with intake less than 0.5 mg/d, significant reductions in adjusted bone mineral density were found: 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 the total body (P = 0.009).

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Table 4. **beta-Coefficients Obtained in Linear Regression Models of Retinol Intake and Bone Mineral Density***

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Table 8. **Table 4 Continued**

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Table 5. **Bone Mineral Density within Categories of Retinol Intake***

Table 9. **Table 5 Continued**

Retinol Intake and Risk for Hip Fracture

The effect of confounding variables on risk for hip fracture is shown in Table 2. The relation between retinol intake in categories and risk for hip fracture is shown in Table 6. A graded increase in relative risk for hip fracture (P for trend = 0.006) was seen with increasing retinol intake. We found a doubled risk for hip fracture (odds ratio, 2.1 [95% CI, 1.1 to 4.0]) with dietary intake of retinol greater than 1.5 mg/d compared with intake less than 0.5 mg/d. No evidence of attenuation of the association in multivariate analyses with adjustment for major risk factors for osteoporosis was seen. When we introduced a dichotomous variable, no or current use of vitamin supplements, risk for hip fracture was unchanged. No increased risk was seen with excessive dietary intake of beta-carotene (data not shown).

Table 6. **Daily Dietary Intake of Retinol Associated with Risk for Hip Fracture**

When we adjusted for intake of iron, magnesium, vitamin C, and calcium, the association of retinol with hip fracture decreased but remained significant (odds ratio, 1.54 [CI, 1.06 to 2.24]; P = 0.02). Similarly, adjustment for intake of these nutrients did not negate the association of retinol intake and bone mineral density. After inclusion of these nutrients in the multivariate model, the beta-coefficients (+/- SE) were -0.095 +/- 0.03 (P = 0.002) for retinol intake in continuous form and bone mineral density of the spine, -0.070 +/- 0.028 (P = 0.01) for continuous retinol and bone mineral density of the femoral neck, and -0.040 +/- 0.016 (P = 0.01) for continuous retinol and bone mineral density of the total body.

Discussion

We found that dietary intake of retinol greater than 1.5 mg/d was associated with reduced bone mineral density and increased risk for hip fracture. The relation was dose-dependent, and no attenuation of the risk for hip fracture was seen in multivariate analyses that adjusted for major risk factors for osteoporosis. There was a consistency between the two outcome measures-bone mineral density and risk for hip fracture-even though two different study designs were used. At an intake greater than 1.5 mg/d compared with a lower intake, the reduction in bone mineral density

at the femoral neck was close to 1 SD, a reduction that has been reported to approximately double the risk for hip fracture [21].

Furthermore, our results are consistent with extensive experimental data from both in vitro and in vivo studies. Animal studies [5,22,23] have shown the importance of vitamin A in the bone remodeling process. Vitamin A deficiency results in retarded bone growth [22], but the most prominent features of hypervitaminosis A are accelerated bone resorption, bone fragility, and spontaneous fracture [5]. These findings have been confirmed in several mammalian species by using highly purified crystalline forms of the vitamin and its derivatives (retinoids) [6-8,24,25]. More recent studies [26,27] have shown that both osteoblasts and osteoclasts express the nuclear receptors for retinoic acid (retinoic acid receptors and retinoid X receptors). Retinoic acid inhibits osteoblast activity [28], stimulates osteoclast formation [29], and induces bone resorption [28-30]. In addition, toxic doses of retinoids have been reported to produce musculoskeletal symptoms (such as pain, tenderness, and stiffness of muscles or joints) and neurologic symptoms (including fatigue, headaches, and dizziness) [7] that may influence the occurrence of fracture.

The food-frequency questionnaire in our study was validated by comparison with dietary records. Between the food-frequency questionnaire and four 1-week dietary records, the validity measure (expressed as a Pearson correlation coefficient) for energy-adjusted retinol intake was 0.5, a value similar to that reported by others [31]. Mean retinol intake was 0.79 mg/d according to the food-frequency questionnaire and 0.81 mg/d according to the dietary records. The mean daily intake of total vitamin A (retinol intake plus one sixth of beta-carotene intake) in our study groups (Table 1 and Table 7) was more than three times the 0.5-mg daily dose of retinol equivalents recommended by the Food and Agricultural Organization and the World Health Organization [18]. This is in agreement with a previous national nutrition survey [32], which found that the mean dietary intake of vitamin A in the Swedish adult population was 1.3 to 1.6 mg of retinol equivalents per day. About half of the vitamin A intake was in the form of preformed retinol. In Norway, which has some of the highest incidence rates of hip fracture ever reported [33,34], mean intake in the adult population is even higher: 1.5 to 2.0 mg of retinol equivalents per day [35].

Why is the consumption of retinol excessive in northern Europe? A possible explanation is a high consumption of cod liver oil [35] and milk and other dairy products [4,36]. In Nordic countries, cod liver oil has been consumed for centuries [37]. Because of its high levels of vitamin A and D, it has been used extensively since World War I, especially in Norway and Sweden [35,38], to prevent rickets [39] and, to a lesser degree, xerophthalmia [40]. Margarine is fortified with vitamins A and D in many countries, but Sweden is the only European country that fortifies low-fat milk products with both vitamins. Fortified milk in Sweden contains 0.45 mg of retinol per L-almost twice the level found in normal, 3% fat milk. In our previous study of diet and risk for hip fracture [11], we found an unexpected increased risk for hip fracture in the highest quartile of calcium intake. However, the odds ratio approaches 1.0 after adjustment for retinol intake. In that study, we identified iron, magnesium, and vitamin C intake as risk factors. Adjustment for these factors and for calcium intake does not significantly affect the impact of retinol on risk for hip fracture or bone mineral density found in the current study.

Why is excessive dietary intake of beta-carotene not associated with increased risk for hip fracture? Carotenoids do not cause hypervitaminosis A even when ingested in large amounts [41]. Several studies have shown that beta-carotene is safe for humans and that it is not teratogenic [42]. It is therefore not surprising that the general assumption that dietary carotenoids can improve vitamin A status and the commonly used conversion factors (one sixth for beta-carotene and one twelfth for other carotenoids) have been questioned [43]. Previous studies showing a positive effect have had serious weaknesses [44], and lack of response in persons with normal retinol concentrations indicates that absorption of carotenoids and their conversion to retinol may be determined by the body's needs for the vitamin [45]. For this reason, we analyzed beta-carotene and other provitamin A carotenoids separately in our analyses of vitamin A intake.

For substances such as vitamin A, for which the liver has a large storage capacity [46], the chronicity of exposure is crucial. Thus, the daily dose needed to produce chronic toxicity is considerably lower than that required to produce acute toxicity [6]. A recent study showing the teratogenicity of a daily intake of retinol greater than about 3 mg/d [47] suggests that in young pregnant women with an increased need for retinol, the liver's capacity to remove excess retinyl esters from the bloodstream is exceeded at levels lower than expected. Elderly persons may have an even lower tolerance for excessive retinol intake.

A limitation of our study is the possibility of information bias resulting from our questioning of case-patients after hip fracture had occurred, as was done for some covariates, such as physical activity. Data on thyroid hormone therapy and family history of osteoporosis, which are possible confounding factors, were not available. We cannot exclude the possibility of an unidentified dietary confounder. Finally, our study probably has a high degree of random error in the assessment of retinol intake, but this nondifferential misclassification would lead to an underestimation of the true risk for hip fracture associated with excessive retinol intake.

We conclude that excessive dietary intake of vitamin A is associated with osteoporosis. Previous experimental work showing the harmful effects of retinol strengthens this conclusion. Our findings may provide one explanation for the high incidence of osteoporotic fractures in Sweden and Norway.

Requests for Reprints: Hakan Melhus, MD, Department of Internal Medicine, University Hospital, S-751 85 Uppsala, Sweden; e-mail, Hakan.Melhus@medicin.uu.se.

Current Author Addresses: Drs. Melhus, Kindmark, and Ljunghall: Department of Internal Medicine, University Hospital, S-751 85 Uppsala, Sweden.

Dr. Holmberg: Department of Surgery, University Hospital, S-751 85 Uppsala, Sweden.

Drs. Michaelsson and Mallmin: Department of Orthopaedics, University Hospital, S-751 85 Uppsala, Sweden.

Dr. Bergstrom: Department of Statistics, Uppsala University, Box 513, S-751 20 Uppsala, Sweden.

Dr. Wolk: Department of Medical Epidemiology, Karolinska Institute, S-171 77 Stockholm, Sweden.

Author and Article Information

From University Hospital, Uppsala, Sweden; and Karolinska Institute, Stockholm, Sweden.

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