

ORIGINAL ARTICLE

## Serum Retinol Levels and the Risk of Fracture

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### ABSTRACT

#### BACKGROUND

Although studies in animals and epidemiologic studies have indicated that a high vitamin A intake is associated with increased bone fragility, no biologic marker of vitamin A status has thus far been used to assess the risk of fractures in humans.

#### METHODS

We enrolled 2322 men, 49 to 51 years of age, in a population-based, longitudinal cohort study. Serum retinol and beta carotene were analyzed in samples obtained at enrollment. Fractures were documented in 266 men during 30 years of follow-up. Cox regression analysis was used to determine the risk of fracture according to the serum retinol level.

#### RESULTS

The risk of fracture was highest among men with the highest levels of serum retinol. Multivariate analysis of the risk of fracture in the highest quintile for serum retinol ( $>75.62$   $\mu\text{g}$  per deciliter [ $2.64$   $\mu\text{mol}$  per liter]) as compared with the middle quintile ( $62.16$  to  $67.60$   $\mu\text{g}$  per deciliter [ $2.17$  to  $2.36$   $\mu\text{mol}$  per liter]) showed that the rate ratio was 1.64 (95 percent confidence interval, 1.12 to 2.41) for any fracture and 2.47 (95 percent confidence interval, 1.15 to 5.28) for hip fracture. The risk of fracture was further increased within the highest quintile for serum retinol. Men with retinol levels in the 99th percentile ( $>103.12$   $\mu\text{g}$  per deciliter [ $3.60$   $\mu\text{mol}$  per liter]) had an overall risk of fracture that exceeded the risk among men with lower levels by a factor of seven ( $P<0.001$ ). The level of serum beta carotene was not associated with the risk of fracture.

#### CONCLUSIONS

Our findings, which are consistent with the results of studies in animals, as well as in vitro and epidemiologic dietary studies, suggest that current levels of vitamin A supplementation and food fortification in many Western countries may need to be reassessed.

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VITAMIN A IN HIGH DOSES STIMULATES bone resorption and inhibits bone formation. These effects are demonstrated by *in vitro* data and by the occurrence of spontaneous fractures in studies in animals.<sup>1</sup> In addition, a high dietary intake of vitamin A increases the risk of skeletal deformities in human fetuses.<sup>2</sup> There are substantial differences among countries in the average dietary intake of vitamin A. In a study of dietary patterns in Europe, the intake of vitamin A in Scandinavia was up to six times as high as the intake in southern Europe.<sup>3</sup> The risk of a hip fracture in a Swedish man is approximately twice that in a woman in England or the Netherlands<sup>4</sup> — an observation that cannot readily be explained by lifestyle, genetic factors, climate, or longevity.<sup>4,5</sup> Three reports — one by us<sup>6</sup> and two by groups in the United States<sup>7,8</sup> — have indicated an increased risk of hip fracture<sup>6,7</sup> and low bone density<sup>6,8</sup> in women with a high dietary intake of vitamin A, though one study also reported increased bone loss at low levels of intake.<sup>8</sup> However, biologic markers of retinol status have so far not been evaluated with respect to the risk of fracture. We used data from a longitudinal, population-based cohort study to investigate the relation between serum retinol levels and the subsequent risk of fracture among men.

#### METHODS

From 1970 to 1973, we invited all 2841 men born between 1920 and 1924 and living in the municipality of Uppsala, Sweden, to participate in a health survey, the Uppsala Longitudinal Study of Adult Men. A total of 2322 men (82 percent) agreed to participate. The base-line evaluation included a medical and lifestyle questionnaire and interview, tests of serum samples obtained after an overnight fast, and anthropometric measurements.<sup>9,10</sup> At 60 years of age, 1860 men (80 percent of the total cohort) took part in a second evaluation, and at 70 years, 1221 men (53 percent) took part in a third evaluation.

#### SERUM ANALYSES

In 1986, we measured retinol and beta carotene levels in serum samples that had been obtained at base line from 2047 subjects and stored in liquid nitrogen at  $-196^{\circ}\text{C}$ . The serum was protected from light and was not thawed before analysis. Measurements were performed with the use of high-performance liquid chromatography (with an octadecyl silica col-

umn and a methanol mobile phase<sup>11</sup>). The light absorption of the compounds was measured with a diode-array detector at a wavelength of 305 nm for retinol (coefficient of variation, 3.1 percent) and 460 nm for beta carotene (coefficient of variation, 6.5 percent). Retinol remains stable for at least 15 years, especially when stored at a temperature of  $-70^{\circ}\text{C}$  or lower.<sup>12</sup> In a five-year study, with annual measurement of serum retinol, the average level varied by less than 10 percent throughout the study period.<sup>13</sup>

Serum calcium, albumin, creatinine, cholesterol, and triglycerides, as well as the erythrocyte sedimentation rate, were analyzed by standard methods between 1970 and 1973.<sup>10</sup> A blood sample was obtained for measurement of  $\gamma$ -glutamyltransferase in a subgroup of 777 participants between 1980 and 1983, and another blood sample was obtained for aspartate aminotransferase and alanine aminotransferase measurements in 1189 men between 1990 and 1993.

#### DIETARY ASSESSMENT

We performed a dietary assessment in a subgroup of 1138 men, using a seven-day dietary record, in conjunction with the third evaluation (between 1990 and 1993). The daily intake of calories, vitamin A, and alcohol was calculated with the use of a data base from the Swedish National Food Administration. Information about vitamin A-containing supplements, including the type of preparation and the dose but not the duration of use, was also collected.

#### MATCHING TO NATIONAL REGISTERS

All hospital admissions in the Uppsala health care region have been reported to the Hospital Discharge Register since 1965, and since 1987 this register has covered all public inpatient care in Sweden. The register is updated yearly and has a high validity for identifying cases of fracture.<sup>14</sup> The Uppsala Longitudinal Study of Adult Men cohort has been matched to this register every year for all diagnoses, with the use of personal identification numbers, which are given to all inhabitants of Sweden. We also linked the subjects to national census data bases for 1960, 1970, 1980, and 1990, which enabled us to categorize the participants according to socioeconomic status.

#### IDENTIFICATION OF CASES OF FRACTURE

We sought to identify all fractures that occurred in study participants after enrollment. We matched the study cohort to the Hospital Discharge Register to

identify cases treated on an inpatient basis. All orthopedic records at the local hospitals in areas where the participants in the initial investigation resided were reviewed to identify fractures according to the type and circumstances of the injury. Fractures were also confirmed by linkage, with use of the personal identification number, to radiographic records and county outpatient registries. We excluded seven cases of fracture caused by metastatic cancer.

#### STATISTICAL ANALYSIS

We used Cox proportional-hazards models to estimate rate ratios, with 95 percent confidence intervals calculated as measures of association. For each man, the number of years of follow-up was calculated from the date of enrollment (i.e., the date of the first investigation) until the date of a first fracture, the date of death (in the case of 989 participants), the date of a move from the county of residence (in the case of 130 men), or the end of the follow-up period (December 31, 2001). Dates of deaths and of moves were based on data from the continuously updated Swedish National Population Register.

Serum retinol levels were evaluated both as a continuous variable and as a categorical variable, in quintiles. Separate analyses were performed for fractures specifically designated as osteoporotic (i.e., fractures of the hip, pelvis, spine, distal forearm, and proximal humerus).<sup>15</sup> The results were similar whether or not we included the seven cases of fracture due to suspected high-impact trauma, and these cases were therefore retained in the analyses. The nonlinear risk in the highest quintile of the retinol level was determined by inclusion of retinol as a quadratic term in the model together with retinol as a continuous variable. We then estimated the trend in the risk of fracture by a restricted cubic-spline Cox regression analysis<sup>16</sup> with eight "knots" (serum retinol percentiles 1, 5, 20, 40, 60, 80, 95, and 99), which enabled us to investigate extreme retinol values. The results of this analysis are presented as smoothed plots with 95 percent confidence intervals for both the overall risk of fracture and the risk of hip fracture.

We considered two separate models: a univariate model and a multivariate model. Age, weight, height, serum beta carotene, serum calcium, and serum albumin at enrollment in the study were included as continuous variables. For smoking status at base line, the men were categorized as never hav-

ing smoked, as former smokers, or as current smokers. Marital status at base line was categorized as married (or living with a partner) or single. Social class, physical activity at work, and leisure physical activity were all evaluated in three categories. The Michigan Alcoholism Screening Test<sup>17</sup> was used at the second evaluation (at 60 years of age) to identify cases of alcohol abuse; the answers were used to categorize alcohol use as none, normal use, or suspected dependence. The estimates remained similar when we also included cholesterol, triglycerides, creatinine, the sedimentation rate, tocopherol (all at the age of 50 years),  $\gamma$ -glutamyltransferase (at the age of 60 years), and aspartate aminotransferase, alanine aminotransferase, dietary energy intake, and alcohol intake (all at the age of 70 years) in the model. Consequently, these variables were omitted from the reported analyses. We further modeled the association between dietary vitamin A intake in quintiles, estimated according to the reported intake at the age of 70 years, and the subsequent risk of fracture.

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#### RESULTS

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Characteristics of the participants according to the quintile for serum retinol are shown in Table 1. There was a tendency toward higher weight and body-mass index as well as higher serum lipid values in higher quintiles for serum retinol. Serum calcium and alcohol consumption as estimated on the basis of the Michigan Alcoholism Screening Test were also positively associated with serum retinol (data not shown). During a total of 56,281 person-years of observation, 266 men had one or more fractures (Table 2), and data on serum retinol were available for 241 of them. The average follow-up was 24 years, with a median of 24 years for subjects with fractures and 29 years for those without fractures.

The overall risk of fracture increased by 26 percent for every increase of 1 SD in serum retinol (multivariate rate ratio, 1.26; 95 percent confidence interval, 1.13 to 1.41) (Table 3). The corresponding rate ratio was 1.38 (95 percent confidence interval, 1.13 to 1.69) for subjects with two or more fractures during follow-up. However, a Wald chi-square test indicated a nonlinear association ( $P=0.02$ ). The increment was thus mainly concentrated in the highest quintile for retinol: multivariate rate ratio for any fracture, as compared with the middle quintile, 1.64 (95 percent confidence interval, 1.12 to 2.41) (Table 3), with an estimated population at-

**Table 1.** Base-Line Characteristics of the Study Participants According to the Quintile of Serum Retinol.\*

| Characteristic   | Quintile 1<br>( $<1.95 \mu\text{mol/liter}$ ) | Quintile 2<br>( $1.95\text{--}2.16 \mu\text{mol/liter}$ ) | Quintile 3<br>( $2.17\text{--}2.36 \mu\text{mol/liter}$ ) | Quintile 4<br>( $2.37\text{--}2.64 \mu\text{mol/liter}$ ) | Quintile 5<br>( $>2.64 \mu\text{mol/liter}$ ) |
|--|---|---|---|---|---|
| No. of participants  | 412   | 411   | 407   | 409   | 408   |
| Age — yr   | 49.7 $\pm$ 0.6                                | 49.6 $\pm$ 0.6  | 49.7 $\pm$ 0.6  | 49.7 $\pm$ 0.6  | 49.7 $\pm$ 0.6                                |
| Weight — kg  | 75.3 $\pm$ 10.4                               | 77.5 $\pm$ 11.1   | 77.4 $\pm$ 10.8   | 79.6 $\pm$ 12.0   | 79.1 $\pm$ 10.8                               |
| Height — cm  | 176.6 $\pm$ 6.1                               | 175.8 $\pm$ 5.8   | 176.4 $\pm$ 6.1   | 176.4 $\pm$ 5.9   | 175.7 $\pm$ 5.9                               |
| Body-mass index  | 24.1 $\pm$ 3.0                                | 25.1 $\pm$ 3.3  | 24.9 $\pm$ 3.2  | 25.5 $\pm$ 3.4  | 25.6 $\pm$ 3.1                                |
| Duration of smoking — yr                                   | 20 $\pm$ 13                                   | 19 $\pm$ 13   | 19 $\pm$ 13   | 19 $\pm$ 13   | 20 $\pm$ 13                                   |
| Serum retinol — $\mu\text{mol/liter}$                      | 1.72 $\pm$ 0.20                               | 2.07 $\pm$ 0.07   | 2.27 $\pm$ 0.06   | 2.49 $\pm$ 0.08   | 2.97 $\pm$ 0.38                               |
| Serum beta carotene — $\mu\text{mol/liter}$                | 0.31 $\pm$ 0.20                               | 0.31 $\pm$ 0.21   | 0.32 $\pm$ 0.22   | 0.30 $\pm$ 0.25   | 0.28 $\pm$ 0.21                               |
| Serum calcium — mmol/liter                                 | 2.34 $\pm$ 0.09                               | 2.35 $\pm$ 0.09   | 2.36 $\pm$ 0.09   | 2.37 $\pm$ 0.09   | 2.37 $\pm$ 0.09                               |
| Serum cholesterol — mmol/liter                             | 6.5 $\pm$ 1.2                                 | 6.8 $\pm$ 1.2   | 6.9 $\pm$ 1.3   | 7.0 $\pm$ 1.2   | 7.4 $\pm$ 1.4                                 |
| Serum triglycerides — mmol/liter                           | 1.56 $\pm$ 0.56                               | 1.72 $\pm$ 0.88   | 1.83 $\pm$ 0.76   | 2.05 $\pm$ 1.07   | 2.42 $\pm$ 1.82                               |
| Serum albumin — mmol/liter                                 | 44.8 $\pm$ 3.0                                | 46.2 $\pm$ 3.2  | 46.1 $\pm$ 3.0  | 46.5 $\pm$ 2.9  | 46.5 $\pm$ 3.1                                |
| Smoking status — no./total no. (%)                         |   |   |   |   |   |
| Never smoked   | 100/408 (25)                                  | 108/408 (26)  | 111/407 (27)  | 104/409 (25)  | 92/408 (23)                                   |
| Former smoker  | 76/408 (19)                                   | 100/408 (25)  | 90/407 (22)   | 103/409 (25)  | 110/408 (27)                                  |
| Current smoker   | 232/408 (57)                                  | 200/408 (49)  | 206/407 (51)  | 202/409 (49)  | 206/408 (50)                                  |
| Married or living with a partner — no./total no. (%)       | 337/391 (86)                                  | 324/385 (84)  | 345/396 (87)  | 333/389 (86)  | 319/396 (81)                                  |
| Socioeconomic class — no./total no. (%)                    |   |   |   |   |   |
| High   | 61/407 (15)                                   | 49/407 (12)   | 60/407 (15)   | 62/407 (15)   | 59/406 (15)                                   |
| Middle   | 153/407 (38)                                  | 163/407 (40)  | 187/407 (46)  | 153/407 (38)  | 163/406 (40)                                  |
| Low  | 193/407 (47)                                  | 195/407 (48)  | 160/407 (39)  | 192/407 (47)  | 184/406 (45)                                  |
| Physical activity at work — no./total no. (%) <sup>†</sup> |   |   |   |   |   |
| None or minimal (sedentary)                                | 133/412 (32)                                  | 130/411 (32)  | 174/407 (43)  | 149/409 (36)  | 142/408 (35)                                  |
| Strenuous  | 63/412 (15)                                   | 73/411 (18)   | 48/407 (12)   | 56/409 (14)   | 61/408 (15)                                   |
| Leisure physical activity — no./total no. (%) <sup>†</sup> |   |   |   |   |   |
| None or minimal (sedentary)                                | 197/412 (48)                                  | 171/411 (42)  | 204/407 (50)  | 202/409 (49)  | 213/408 (52)                                  |
| $\geq 3$ Hr of sports or gardening per week                | 175/412 (42)                                  | 199/411 (48)  | 201/407 (49)  | 191/409 (47)  | 167/408 (41)                                  |

\* Plus-minus values are means  $\pm$ SD. To convert values for retinol to micrograms per deciliter, divide by 0.03491. The body-mass index is the weight in kilograms divided by the square of the height in meters. To convert values for beta carotene to micrograms per deciliter, divide by 0.01863; to convert values for calcium to milligrams per deciliter, divide by 0.250; to convert values for cholesterol to milligrams per deciliter, divide by 0.02586; to convert values for triglycerides to milligrams per deciliter, divide by 0.01129. CI denotes confidence interval.

<sup>†</sup> Data on a third category, moderate physical activity, are not shown here.

tributable risk of 12 percent. For fractures at sites that are typical of osteoporotic fractures (accounting for 79 percent of all the fractures), the multivariate rate ratio was 1.78 (95 percent confidence interval, 1.17 to 2.70). When we restricted the analysis to all fractures in the first 20 years of follow-up, the risk was slightly higher in the highest quintile (multivariate rate ratio, 2.18; 95 percent confidence interval, 1.14 to 4.16). For hip fractures, the multivariate rate ratio for the highest quintile as compared with the third quintile was 2.47 (95 percent confidence interval, 1.15 to 5.28) (Table 3). The serum

beta carotene level was not associated with the risk of fracture: multivariate rate ratio per 1 SD increase, 0.95 (95 percent confidence interval, 0.81 to 1.11).

The risk of fracture was further increased in the highest quintile for serum retinol ( $P=0.06$  for a quadratic term of retinol). With the median value of 64.74  $\mu\text{g}$  per deciliter (2.26  $\mu\text{mol}$  per liter) as the reference value, there was an especially steep rise in the rate-ratio curve for men with serum levels above the 95th percentile (i.e., 88.80  $\mu\text{g}$  per deciliter [3.1  $\mu\text{mol}$  per liter]) (Fig. 1). These crude estimates were not substantially altered after multivariate adjust-

**Table 2. Type and Number of Fractures in the 2322 Study Participants.**

| Fracture          | No. of Men (%) |
|-------------------|----------------|
| Any               | 266 (11.5)     |
| Two or more       | 81 (3.5)       |
| Site of fracture* |                |
| Hip               | 84 (3.6)       |
| Distal forearm    | 46 (2.0)       |
| Proximal humerus  | 41 (1.8)       |
| Spine or pelvis   | 46 (2.0)       |
| Other             | 58 (2.5)       |

\* Some men had fractures at more than one site.

ment. A complementary analytic approach showed that the subjects with retinol levels in the 99th percentile (i.e., >103.12 µg per deciliter [3.60 µmol per liter]) had an overall risk of fracture that was seven times the risk among those with lower levels (univariate rate ratio, 6.85 [95 percent confidence interval, 3.38 to 13.90]; multivariate rate ratio, 7.14 [95 percent confidence interval, 3.43 to 14.86]; P<0.001). Analysis of the risk of hip fractures showed a pattern similar to that for the overall risk of fracture: a small increase in the risk ratio between the 80th and 95th percentiles for serum retinol and a substantial increase in the highest percentiles (Fig. 2).

Only 111 of the 1221 men for whom dietary data were available (i.e., those who participated in the third evaluation at the age of 70 years) had a subsequent first fracture. Of the 49 men (4 percent) who reported the use of vitamin A-containing supplements, 6 had a subsequent fracture. The highest quintile for estimated retinol intake (>1.50 mg per day) was associated with an energy-adjusted rate ratio of 2.00 (95 percent confidence interval, 1.00 to 3.99) for any fracture, as compared with the lowest quintile (<0.53 mg per day). With vitamin A-containing supplements included in the nutrient calculation, the rate ratio for the overall risk of fracture was 1.99 (95 percent confidence interval, 0.98 to 4.01). We found only a weak association between energy-adjusted dietary intake of vitamin A at the age of 70 years and the serum retinol level 20 years earlier (r=0.05, P=0.08). Dietary beta carotene intake was not associated with the risk of fracture (data not shown).

DISCUSSION

In this prospective, population-based cohort study of men, the overall risk of fracture was substantially increased among the men with high levels of serum retinol. The risk was concentrated in the highest quintile for serum retinol, with an exponential increase within this category. A recent review of the

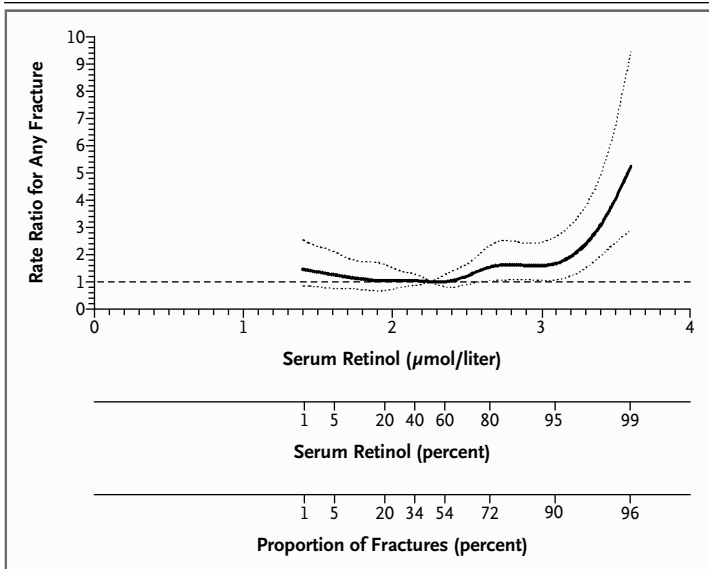
**Table 3. Rate Ratio for Any Fracture and for Hip Fracture, According to the Base-Line Serum Retinol Level.\***

| Retinol Quintile          | Median Retinol Level<br>µmol/liter | Any Fracture |                        |                           | Hip Fracture |                        |                           |
|---------------------------|------------------------------------|--------------|------------------------|---------------------------|--------------|------------------------|---------------------------|
|                           |                                    | No. of Men   | Univariate RR (95% CI) | Multivariate RR (95% CI)† | No. of Men   | Univariate RR (95% CI) | Multivariate RR (95% CI)† |
| 1 (<1.95 µmol/liter)      | 1.78                               | 48           | 1.08 (0.72–1.62)       | 0.93 (0.62–1.41)          | 17           | 1.63 (0.74–3.59)       | 1.33 (0.60–2.97)          |
| 2 (1.95–2.16 µmol/liter)  | 2.07                               | 33           | 0.80 (0.51–1.26)       | 0.78 (0.50–1.23)          | 13           | 1.38 (0.60–3.15)       | 1.44 (0.62–3.30)          |
| 3 (2.17–2.36 µmol/liter)‡ | 2.26                               | 45           | 1.00                   | 1.00                      | 10           | 1.00                   | 1.00                      |
| 4 (2.37–2.64 µmol/liter)  | 2.48                               | 47           | 0.96 (0.64–1.45)       | 0.91 (0.60–1.38)          | 13           | 1.17 (0.51–2.67)       | 1.14 (0.49–2.62)          |
| 5 (>2.64 µmol/liter)      | 2.88                               | 68           | 1.72 (1.18–2.51)       | 1.64 (1.12–2.41)          | 22           | 2.57 (1.22–5.43)       | 2.47 (1.15–5.28)          |
| Per 1 SD increase         |                                    |              | 1.22 (1.10–1.35)       | 1.26 (1.13–1.41)          |              | 1.22 (1.01–1.48)       | 1.30 (1.05–1.60)          |
| P value                   |                                    |              | <0.001                 | <0.001                    |              | 0.04                   | 0.01                      |

\* RR denotes rate ratio, and CI confidence interval. To convert values for retinol to micrograms per deciliter, divide by 0.03491. P values are for a 1 SD increase in the retinol level.

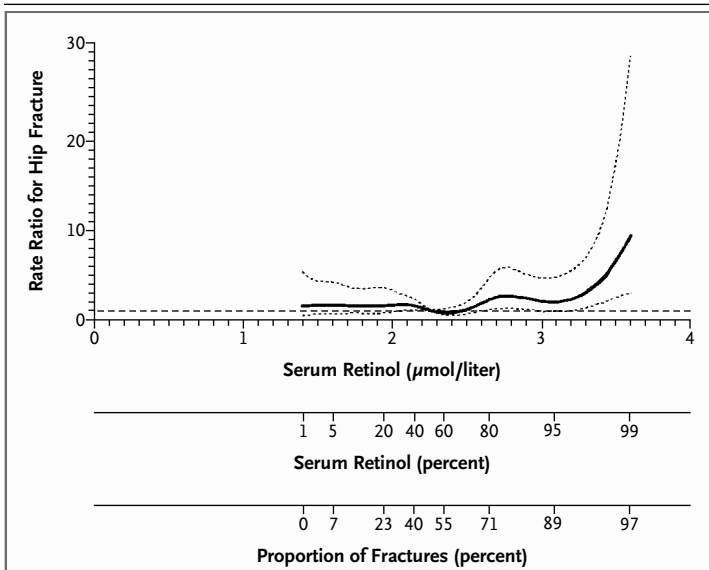
† The analysis was adjusted for age, weight, height, and serum beta carotene, calcium, and albumin values (all continuous variables); smoking status (never smoked, former smoker, or current smoker); marital status (married or living with a partner vs. single); socioeconomic class (low, middle, or high); and physical activity at work, leisure physical activity, and alcohol consumption (all in three categories).

‡ This was the reference group.



**Figure 1. Smoothed Plot of Rate Ratios for Any Fracture According to the Serum Retinol Level.**

The rate ratios (solid line) and 95 percent confidence intervals (dotted lines) were estimated by restricted cubic-spline Cox regression analysis, with the median serum retinol level, 2.26  $\mu\text{mol}$  per liter, as the reference value. To convert the values for retinol to micrograms per deciliter, divide by 0.03491.



**Figure 2. Smoothed Plot of Rate Ratios for Hip Fracture According to the Serum Retinol Level.**

The rate ratios (solid line) and 95 percent confidence intervals (dotted lines) were estimated by restricted cubic-spline Cox regression analysis, with the median serum retinol level, 2.26  $\mu\text{mol}$  per liter, as the reference value. To convert the values for retinol to micrograms per deciliter, divide by 0.03491.

effects of hypervitaminosis A on bone concluded that the question is not whether, but rather at what levels, retinol increases bone fragility.<sup>1</sup> Our data suggest that serum levels higher than 86  $\mu\text{g}$  per deciliter (3  $\mu\text{mol}$  per liter) may increase the risk of fracture. The normal level of serum retinol appears to be highly regulated within a range of 20.1 to 80.2  $\mu\text{g}$  per deciliter (0.7 to 2.8  $\mu\text{mol}$  per liter).<sup>18</sup> The median serum retinol value in our study (64.74  $\mu\text{g}$  per deciliter) is similar to the median value (63.02  $\mu\text{g}$  per deciliter [2.20  $\mu\text{mol}$  per liter]) in men of similar age in a recent large study in the United States.<sup>19</sup>

Our findings are consistent with the results of two previous prospective epidemiologic investigations that examined dietary retinol intake and the risk of hip fracture in women.<sup>6,7</sup> Our study, in which retinol was used as a biologic marker together with the overall risk of fracture, corroborates the detrimental effect of excess retinol on human bone. Serum retinol has been positively associated with both dietary vitamin A intake and use of supplemental vitamin A in most studies<sup>12,20-23</sup> but not all.<sup>24</sup> As in the two previous epidemiologic dietary studies, we compared the risk of fracture among subjects who had an estimated dietary vitamin A intake of more than 1.5 mg per day with the risk among those whose intake was less than 0.5 mg per day. All three studies showed that the risk was increased by a factor of approximately two among subjects in the highest category of vitamin A intake.

The main dietary sources of retinoids are fish, liver, and dairy products, together with fortified foods (in Sweden, margarine and low-fat dairy products). A small proportion of carotenoids from vegetables and fruits is also converted to retinol.<sup>25</sup> Dietary vitamin A is absorbed from the intestine and transported to the liver by chylomicrons. Vitamin A is stored in the liver in the form of retinyl esters but is mobilized from the liver as retinol, normally bound to retinol-binding protein. Retinol is released in target cells and converted to retinoic acid, which exerts its effects by binding to specific nuclear receptors.<sup>26</sup> Retinoid receptors have been identified in both osteoblasts<sup>27</sup> and osteoclasts.<sup>28,29</sup> Retinoic acid suppresses osteoblast activity and stimulates osteoclast formation in vitro.<sup>29,30</sup>

Only a small proportion of circulating vitamin A is normally in the form of retinyl esters.<sup>31</sup> In a large cross-sectional study, a linear analysis showed no association between serum retinyl esters in the fasting state and bone density.<sup>32</sup> However, serum retinyl esters may simply reflect a temporary excess in

vitamin A intake rather than long-term vitamin A intake and storage. Studies of plasma kinetics have shown that the clearance of serum retinyl esters varies substantially from one person to another,<sup>33-35</sup> with an average increase in clearance of more than 50 percent over a 12-hour period after a moderate intake of vitamin A (1.0 to 1.5 mg).<sup>36</sup> Furthermore, in patients with vitamin A toxicity, serum retinyl esters decrease much faster than serum retinol after discontinuation of vitamin A supplements.<sup>31</sup>

Serum retinol has been positively associated with age, weight, serum lipids, socioeconomic status, and renal failure and has been negatively associated with smoking, alcohol consumption, infections, and chronic liver diseases.<sup>18,19,23,37,38</sup> With the exception of serum lipids and infections, all these factors also influence the risk of fracture.<sup>39-43</sup> When we controlled for these possible covariates, only small effects were found. Men with high serum levels of retinol had elevated circulating lipid levels, a well-known side effect of treatment with vitamin A<sup>44</sup> or retinoids,<sup>45</sup> as well as high serum calcium levels, which may have been attributable to the mobilization of calcium from bone.

Long-term ingestion of large amounts of vitamin A can lead to hypercalcemia.<sup>46</sup> Serum calcium might thus be regarded as having a role in the development of osteoporosis.<sup>46,47</sup> However, since serum vitamin D was not measured in our study, we cannot rule out the possibility that a concurrent excessive intake of vitamin D contributed to the increase in serum calcium. Exclusion of serum calcium from our multivariate analysis resulted in a somewhat stronger association between serum retinol and the risk of fracture. There was no association between a high serum level of beta carotene and

an increased risk of fracture. Dietary intake of beta carotene influences serum levels of beta carotene but not serum retinol levels.<sup>48,49</sup>

Our longitudinal, population-based, prospective study involved a cohort of men who were similar in age, and we used hospital-record verification for complete ascertainment of cases of fracture. We also used a biologic marker of retinol status, rather than dietary assessments alone, as previous studies have done. However, serum retinol was measured only once, and the interval between measurement and follow-up was long. One would expect that the usefulness of a single serum retinol measurement in predicting the risk of fracture would be attenuated as the period of observation increased, which was indicated by our analysis. There was only a weak association between serum retinol at base line and dietary vitamin A intake 20 years later, which may be explained in part by the 20-year interval between the evaluations. In addition, a one-week dietary record may not reflect vitamin A intake accurately,<sup>50</sup> leading to a weakening of the associations identified. Nevertheless, the dietary data, obtained from only half the original study population, appeared to reveal an increased risk of fracture with a high dietary vitamin A intake, although the small number of cases and borderline significance of the association limit the interpretation of this finding.

The results of our study suggest that subclinical hypervitaminosis A may increase the risk of fracture. Johansson et al. have reported that subclinical hypervitaminosis A increases the risk of fracture in rats<sup>51</sup>; our clinical data support this finding.

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