

# No increase in risk of hip fracture at high serum retinol concentrations in community-dwelling older Norwegians: the Norwegian Epidemiologic Osteoporosis Studies<sup>1,2</sup>

Kristin Holvik,<sup>3,4,\*</sup> Luai A Ahmed,<sup>6,8</sup> Siri Forsmo,<sup>9</sup> Clara G Gjesdal,<sup>5,10</sup> Guri Grimnes,<sup>7</sup> Sven Ove Samuelsen,<sup>3,11</sup> Berit Schei,<sup>9,14</sup> Rune Blomhoff,<sup>12</sup> Grethe S Tell,<sup>3,4</sup> and Haakon E Meyer<sup>3,13</sup>

<sup>3</sup>Division of Epidemiology, Norwegian Institute of Public Health, Oslo, Norway; <sup>4</sup>Department of Global Public Health and Primary Care and <sup>5</sup>Department of Clinical Science, University of Bergen, Bergen, Norway; <sup>6</sup>Department of Health and Care Sciences, Faculty of Health Sciences, and <sup>7</sup>Tromsø Endocrine Research Group, Department of Clinical Medicine, UiT the Arctic University of Norway, Tromsø, Norway; <sup>8</sup>Institute of Public Health, College of Medicine & Health Sciences, United Arab Emirates University, Al-Ain, United Arab Emirates; <sup>9</sup>Department of Public Health and General Practice, Norwegian University of Science and Technology, Trondheim, Norway; <sup>10</sup>Department of Rheumatology, Haukeland University Hospital, Bergen, Norway; <sup>11</sup>Department of Mathematics, <sup>12</sup>Department of Nutrition, Institute of Basic Medical Sciences, and <sup>13</sup>Institute of Health and Society, University of Oslo, Oslo, Norway; and <sup>14</sup>Department of Obstetrics and Gynecology, St. Olav's Hospital, Trondheim, Norway

## ABSTRACT

**Background:** Norway has the highest hip fracture rates worldwide and a relatively high vitamin A intake. Increased fracture risk at high intakes and serum concentrations of retinol (s-retinol) have been observed in epidemiologic studies.

**Objective:** We aimed to study the association between s-retinol and hip fracture and whether high s-retinol may counteract a preventive effect of vitamin D.

**Design:** We conducted the largest prospective analysis of serum retinol and hip fracture to date in 21,774 men and women aged 65–79 y (mean age: 72 y) who attended 4 community-based health studies during 1994–2001. Incident hip fractures occurring up to 10.7 y after baseline were retrieved from electronic hospital discharge registers. Retinol determined by high-pressure liquid chromatography with ultraviolet detection in stored serum was available in 1154 incident hip fracture cases with valid body mass index (BMI) data and in a subcohort defined as a sex-stratified random sample ( $n = 1418$ ). Cox proportional hazards regression weighted according to the stratified case-cohort design was performed.

**Results:** There was a modest increased risk of hip fracture in the lowest compared with the middle quintile of s-retinol (HR: 1.41; 95% CI: 1.09, 1.82) adjusted for sex and study center. The association was attenuated after adjustment for BMI and serum concentrations of  $\alpha$ -tocopherol (HR: 1.16; 95% CI: 0.88, 1.51). We found no increased risk in the upper compared with the middle quintile. No significant interaction between serum concentrations of 25-hydroxyvitamin D and s-retinol on hip fracture was observed ( $P = 0.68$ ).

**Conclusions:** We found no evidence of an adverse effect of high serum retinol on hip fracture or any interaction between retinol and 25-hydroxyvitamin D. If anything, there tended to be an increased risk at low retinol concentrations, which was attenuated after control for confounders. We propose that cod liver oil, a commonly used food supplement in Norway, should not be discouraged as a natural source of vitamin D for fracture prevention. *Am J Clin Nutr* 2015;102:1289–96.

**Keywords:** hip fracture, vitamin A, retinol, elderly, Norway, case-cohort

## INTRODUCTION

Norway is a Scandinavian high-latitude country with high fracture rates (1). The highest incidence rate of hip fractures worldwide through the previous 4 decades has been reported in Oslo, Norway (2–5). The reasons for the particularly high fracture rates are not known.

Vitamin A has been linked to bone health and fracture risk (6, 7). Vitamin A is a common designation for organic compounds that have the same biological activity as retinol (8). Humans acquire vitamin A through the diet in form of provitamin A carotenoids from plant foods and preformed retinol mainly from animal fat sources. Retinol is the precursor of retinoic acid, a fat-soluble transcription factor influencing cell differentiation, immune function, and reproduction. Retinoic acid may stimulate osteoclast activity (9) or inhibit osteoblast activity (10). Excess vitamin A has been associated with compromised bone metabolism in animal and in vitro studies (6, 11). Although the evidence of an effect of vitamin A on bone health in humans is inconsistent, several studies have suggested adverse effects on bone mineral

<sup>1</sup> Supported by a grant from the Research Council of Norway. The serum sample analyses in the second Nord-Trøndelag Health Study were partly funded by a grant from Central Norway Regional Health Authority. KH's salary while writing the manuscript was funded by the Norwegian Institute of Public Health.

<sup>2</sup> Supplemental Figures 1 and 2 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at <http://ajcn.nutrition.org>.

\*To whom correspondence should be addressed. E-mail: kristin.holvik@fhi.no.

Received March 3, 2015. Accepted for publication August 19, 2015.

First published online September 16, 2015; doi: 10.3945/ajcn.115.110528.



density, bone turnover, and fracture risk of high retinol intakes (12). In a population-based prospective study from Norway, peri- and postmenopausal women who had taken supplemental cod liver oil rich in vitamin A during childhood had an increased risk of osteoporosis (13). A handful of population-based prospective studies have examined the association between dietary intakes or serum concentrations of vitamin A and risk of hip fracture (14–19). These were recently summed up in a meta-analysis that concluded that retinol seemed to exhibit a U-shaped relation with fractures, including hip fracture (20). Four population-based studies from Sweden and the United States found an increased risk of hip fracture at high intakes (14, 15) or high serum concentrations (16, 17) of retinol (*s*-retinol),<sup>15</sup> whereas 2 other studies did not find any significant association with dietary intakes (18) or serum concentrations (19) in postmenopausal and older women, respectively.

Retinoic acid and the active vitamin D hormone (1,25-dihydroxyvitamin D) both act as transcription factors through specific nuclear receptors. For 1,25-dihydroxyvitamin D to exert its biologic effect, the vitamin D receptor must form a heterodimeric complex with the retinoid X receptor (21). At high concentrations of vitamin A combined with low vitamin D status, the complex may dissociate, leading to reduced vitamin D action (22). In a cross-sectional study on Spanish postmenopausal women, a combination of high *s*-retinol and low serum concentration of 25-hydroxyvitamin D [*s*-25(OH)D] was associated with lower calcaneal bone mineral density measured by quantitative ultrasound (23). As far as we are aware, the net *in vivo* effect of a potential interaction between vitamins A and D on fracture risk has not yet been investigated in a population-based study.

In the Nordic countries, including Norway, vitamin A intake is high, in contrast to the Mediterranean countries (24–26). We hypothesized that high concentrations of vitamin A in the population may be a contributor to the high fracture incidence in Scandinavia by counteracting the bone-protective effects of vitamin D. Our aim was to investigate whether serum retinol concentrations in elderly men and women were independently associated with risk of incident hip fracture and whether high *s*-retinol may interact with the inverse association between high *s*-25(OH)D and hip fracture risk.

## METHODS

We performed a multicenter case-cohort analysis by using baseline data from 4 population-based studies in Norway: the fourth survey of the Tromsø Study, 1994; the second Nord-Trøndelag Health Study, 1995–97; the Hordaland Health Study, 1997–99; and the Oslo Health Study, 2000–01. The data material is described in detail elsewhere (27). Briefly, a cohort of 21,774 men and women aged 65–79 y participated in baseline examinations during 1994–2001 and were followed up with regard to hip fracture for up to 10.7 y. Fat-soluble vitamins were determined in stored sera of cases and sex-stratified random samples of 4.5% of men and 9.0% of women in the cohort.

<sup>15</sup> Abbreviations used: REC, Regional Committees for Medical and Health Research Ethics; *s*-retinol, serum concentration of retinol; *s*-25(OH)D, serum concentration of 25-hydroxyvitamin D; *s*- $\alpha$ -tocopherol, serum concentration of  $\alpha$ -tocopherol; 25(OH)D, 25-hydroxyvitamin D.

## Baseline information

The baseline examination included collection of nonfasting blood samples, which were frozen and stored; anthropometric measurements (weight and height); blood pressure measurements; and questionnaire data. The 4 health studies are part of Cohort of Norway (28), and thus common questionnaire and background data were available. The data collected included information about self-reported previous fracture, physical activity, cigarette smoking, use of medication, chronic diseases, and self-rated health.

## Deaths and emigration

Dates of death and emigration were obtained from the Norwegian Population Register for the calculation of observation time. End of follow-up was 31 December 2004 in the fourth survey of the Tromsø Study and the second Nord-Trøndelag Health Study, 31 December 2007 in the Oslo Health Study, and 31 December 2008 in the Hordaland Health Study, yielding a maximum follow-up of 10.7 y.

## Case identification and selection of subcohort

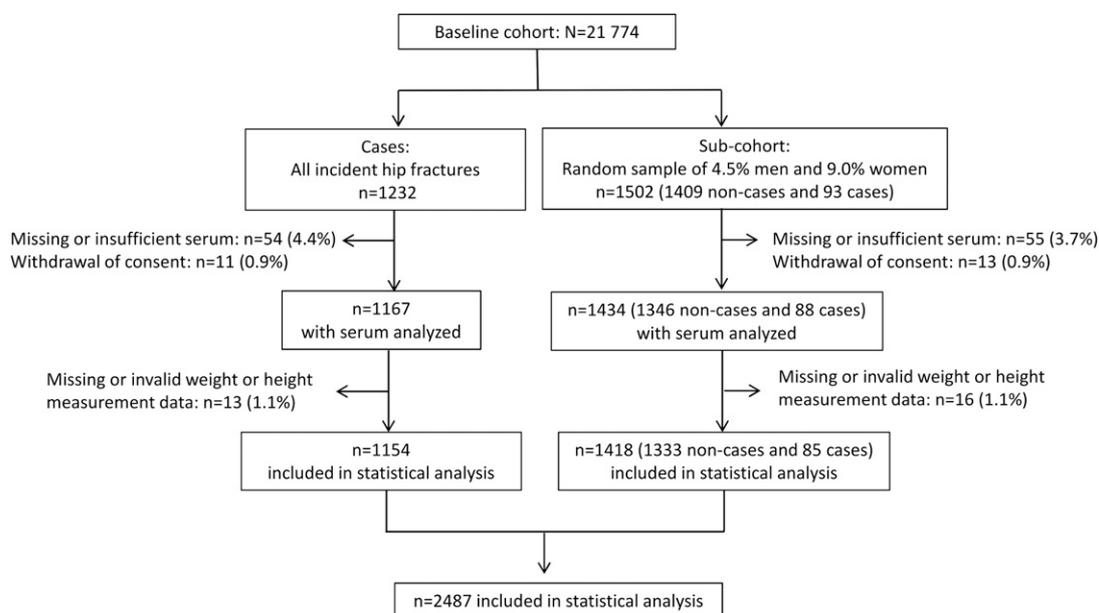
Information on incident hip fractures after the baseline examinations was retrieved from hip fracture registers at each of the 4 study sites, as previously described (27). Case identification was based on diagnosis codes and surgical procedure codes in the hospitals' electronic patient administrative systems. A hip fracture was defined as the first fracture of the proximal femur occurring during the observation period. The discharge diagnoses used to classify a hip fracture were according to the International Classification of Diseases, Ninth Revision (820–820.9) and Tenth Revision (S72.0–S72.2).

To approximate the anticipated cumulative incidences of hip fracture and allowing for the possibility of missing or insufficient serum, the sex-specific subcohorts were defined as random unmatched samples of 4.5% of men and 9.0% of women in the study population at baseline (Figure 1).

## Biochemical measurements

Frozen serum samples were sent to AS Vitas, where they were analyzed in 2011. Samples from cases and noncases at each study center were analyzed simultaneously. Biobank staff and laboratory staff were blinded with regard to case status. Retinol was determined by reverse-phase HPLC with UV detection. In total, 100  $\mu$ L serum was diluted with 300  $\mu$ L 2-propanol containing the internal standard trimethylmethoxyphenyl-retinol and butylated hydroxytoluene as an antioxidant. After thorough mixing (15 min) and centrifugation (10 min, 4000  $\times$  *g* at 10°C), an aliquot of 7  $\mu$ L was injected from the supernatant into the HPLC system. HPLC was performed with an HP 1100 liquid chromatograph (Agilent Technologies) with an HP 1100 single-wavelength ultraviolet detector operated at 325 nm. Retinol was separated from the matrix and internal standard on a 4.6-mm  $\times$  25-mm reverse-phase column. Two-point calibration curve was made from analysis of plasma calibrators with known retinol concentrations. The column temperature was 40°C. Recovery was >95%, the method was linear from 0.1 to 10  $\mu$ mol/L at





**FIGURE 1** Inclusion of participants to the case and subcohort groups. All hip fractures in the subcohort are duplicates of those in the case group.

least, and the limit of detection was  $0.01 \mu\text{mol/L}$ . CV was 4.9% ( $1.2 \mu\text{mol/L}$ ) and 5.8% ( $1.7 \mu\text{mol/L}$ ).

25-Hydroxyvitamin D [25(OH)D] was determined by HPLC–atmospheric pressure chemical ionization–mass spectrometry, and  $\alpha$ -tocopherol was determined by HPLC–fluorescence detection, as previously described (27, 29).

### Statistical analysis

Statistical analyses were performed with R open-access statistical software (30). Forest plots were produced with STATA 13 (StataCorp LP). The usual method for analyzing stratified case-cohort data is by a weighted Cox proportional hazards regression where weighting is by the inverse of the sampling probabilities (31, 32). We performed Cox regression modified for the sex-stratified case-cohort design by using the function “cch” in the R library “survival” (33). Age was used as time variable, left-truncated at age at baseline examination. Risk of hip fracture was examined across quintiles of s-retinol and across the distribution of s-retinol by plots by using Cox proportional hazards regression with penalized splines of s-retinol, inversely weighted by inclusion probabilities in the case-cohort sample (34). All analyses were adjusted for sex and study center. In additional analysis, s-retinol was entered as a continuous variable to test a possible dose-response relation. Statistical interaction was tested by the Wald  $\chi^2$  test for interaction between s-retinol and, respectively, study center, BMI, sex, age at baseline, and s-25(OH)D. In addition, the relation between s-25(OH)D and hip fracture was studied within strata of s-retinol to explore whether an association between serum 25(OH)D and risk of hip fracture varied across concentrations of s-retinol.

Potential confounding variables were known risk factors for hip fracture, which also correlated with s-retinol: BMI, daily cigarette smoking, physical inactivity, educational level, concentrations of s-25(OH)D and  $\alpha$ -tocopherol, and self-rated health (to account for potential unmeasured confounding by health behavior).

### Ethical approval

The 4 regional parts of this study were approved by the respective Regional Committees for Medical and Health Research Ethics (REC): REC West (reference 067.09), REC Central (reference 2009-714/2), REC North (reference 31/94), and REC South East (reference 08/2037-4).

### RESULTS

#### Hip fractures

During a median observation time of 8.2 y, 1232 individuals [340 men (3.4%) and 892 women (7.5%)] had a hip fracture, and the randomly sampled subcohorts included 1502 individuals, of whom 93 were cases (27). The flow of participants is shown in Figure 1. After exclusion of 104 persons with missing or insufficient serum (3.9% of the original sample), 23 who withdrew their consent during the study (0.9%), and 26 with missing height or weight measurements (1.1%), the sample included in the main statistical analysis constituted 1154 cases and 1333 noncases. Missing serum, withdrawals, and missing BMI information were equally distributed in cases and noncases (Figure 1).

#### Serum retinol

Mean  $\pm$  SD s-retinol in the subcohort was  $2.93 \pm 1.04 \mu\text{mol/L}$ . s-Retinol varied by study center ( $P < 0.001$ ), with highest mean  $\pm$  SD concentrations in the 2 northernmost study centers of Tromsø ( $3.31 \pm 1.09 \mu\text{mol/L}$ ) and Nord-Trøndelag ( $3.16 \pm 1.01 \mu\text{mol/L}$ ) and lower concentrations in the southern centers of Hordaland ( $2.44 \pm 0.71 \mu\text{mol/L}$ ) and Oslo ( $2.23 \pm 0.91 \mu\text{mol/L}$ ). Concentrations were higher in men than in women; mean  $3.06$  vs.  $2.87 \mu\text{mol/L}$  ( $P = 0.002$ ). Seventeen individuals in the subcohort (1.2%) had s-retinol  $< 0.70 \mu\text{mol/L}$ .

There were only modest differences in baseline characteristics according to s-retinol quintiles (Table 1). Mean age at baseline

TABLE 1

Baseline characteristics of the subcohorts according to quintiles of serum retinol concentrations<sup>1</sup>

	Valid <i>n</i>	Q1	Q2	Q3	Q4	Q5	<i>P</i> value <sup>2</sup>	<i>P</i> value <sup>3</sup>
<b>Men, <i>n</i></b>	414	83	83	81	84	83		
s-Retinol range, $\mu\text{mol/L}$	414	0.20–2.24	2.25–2.66	2.67–3.10	3.10–3.80	3.80–7.76		
s-Retinol, $\mu\text{mol/L}$	414	1.80 $\pm$ 0.40 <sup>4</sup>	2.44 $\pm$ 0.12	2.85 $\pm$ 0.12	3.41 $\pm$ 0.19	4.78 $\pm$ 0.89		
s-25(OH)D, nmol/L	410	53.1 $\pm$ 21.6	55.4 $\pm$ 18.8	58.7 $\pm$ 18.0	58.9 $\pm$ 19.4	60.1 $\pm$ 22.7	0.15	0.013
s- $\alpha$ -Tocopherol, $\mu\text{mol/L}$	414	25.0 $\pm$ 9.9	28.0 $\pm$ 9.6	29.2 $\pm$ 10.8	29.4 $\pm$ 9.9	36.3 $\pm$ 15.4	<0.001	<0.001
Age at baseline, y	414	73.4 $\pm$ 3.1	71.6 $\pm$ 3.7	71.9 $\pm$ 4.3	71.9 $\pm$ 4.0	70.7 $\pm$ 3.9	<0.001	<0.001
BMI, kg/m <sup>2</sup>	414	25.5 $\pm$ 4.3	26.4 $\pm$ 3.8	26.6 $\pm$ 3.7	26.8 $\pm$ 4.0	26.3 $\pm$ 2.9	0.20	0.12
Education, y	378	8.9 $\pm$ 4.4	9.6 $\pm$ 4.4	8.4 $\pm$ 3.6	8.7 $\pm$ 3.9	8.6 $\pm$ 3.9	0.47	0.34
Self-rated health good or very good, <i>n</i> (%)	411	46 (56.8)	50 (60.2)	44 (54.3)	52 (61.9)	49 (59.8)	0.87	0.66
Current daily cigarette smokers, <i>n</i> (%)	398	19 (23.2)	18 (22.5)	19 (24.1)	17 (20.7)	19 (25.3)	0.57	0.10
Inactive during leisure time, <i>n</i> (%)	345	4 (5.4)	3 (4.1)	2 (2.7)	6 (8.8)	2 (3.5)	0.57	0.87
Previous hip fracture, <i>n</i> (%)	368	3 (4.2)	4 (5.2)	1 (1.3)	0 (0)	1 (1.4)	0.20	0.06
Previous forearm fracture, <i>n</i> (%)	370	10 (13.3)	7 (9.1)	9 (12.0)	6 (8.2)	4 (5.7)	0.55	0.15
Self-reported osteoporosis, <i>n</i> (%)	354	1 (1.4)	1 (1.4)	1 (1.4)	0 (0)	1 (1.4)	1.00	0.75
<b>Women, <i>n</i></b>	1004	201	201	200	201	201		
s-Retinol range, $\mu\text{mol/L}$	1004	0.24–2.08	2.08–2.52	2.52–2.94	2.95–3.60	3.60–8.07		
s-Retinol, $\mu\text{mol/L}$	1004	1.66 $\pm$ 0.43	2.31 $\pm$ 0.12	2.72 $\pm$ 0.12	3.26 $\pm$ 0.19	4.40 $\pm$ 0.75		
s-25(OH)D, nmol/L	998	55.6 $\pm$ 22.1	53.5 $\pm$ 18.1	56.0 $\pm$ 21.2	58.4 $\pm$ 22.4	55.2 $\pm$ 20.4	0.36	0.28
s- $\alpha$ -Tocopherol, $\mu\text{mol/L}$	1004	28.1 $\pm$ 12.3	31.0 $\pm$ 11.4	33.6 $\pm$ 14.2	32.5 $\pm$ 15.0	35.8 $\pm$ 16.8	<0.001	<0.001
Age at baseline, y	1004	73.1 $\pm$ 3.4	72.8 $\pm$ 3.6	72.7 $\pm$ 3.9	71.8 $\pm$ 4.0	71.1 $\pm$ 3.9	<0.001	<0.001
BMI, kg/m <sup>2</sup>	1004	26.6 $\pm$ 4.6	27.0 $\pm$ 4.3	28.2 $\pm$ 4.8	27.3 $\pm$ 4.5	27.4 $\pm$ 4.2	0.005	0.040
Education, y	849	7.8 $\pm$ 3.4	7.8 $\pm$ 3.2	7.6 $\pm$ 3.3	7.1 $\pm$ 2.3	7.4 $\pm$ 3.0	0.18	0.21
Self-rated health good or very good, <i>n</i> (%)	987	113 (57.4)	106 (53.5)	107 (54.0)	100 (50.0)	98 (50.5)	0.59	0.13
Current daily cigarette smokers, <i>n</i> (%)	959	42 (21.6)	49 (25.7)	22 (11.4)	31 (16.7)	33 (16.9)	0.005	0.038
Inactive during leisure time, <i>n</i> (%)	765	23 (13.8)	15 (9.8)	19 (12.8)	28 (17.6)	17 (12.4)	0.36	0.54
Previous hip fracture, <i>n</i> (%)	811	11 (6.5)	10 (6.0)	8 (5.0)	8 (4.9)	9 (6.0)	0.97	0.69
Previous forearm fracture, <i>n</i> (%)	838	55 (31.6)	63 (36.2)	47 (28.0)	40 (24.2)	31 (19.7)	0.010	0.001
Self-reported osteoporosis, <i>n</i> (%)	799	28 (16.9)	20 (12.0)	22 (13.3)	16 (10.3)	11 (7.5)	0.13	0.013
Current systemic estrogen use, <i>n</i> (%)	1004	19 (9.5)	10 (5.0)	16 (8.0)	12 (6.0)	22 (10.9)	0.16	0.51

<sup>1</sup>Quintile limits based on the sex-specific subcohorts. Q, quintile; s-retinol, serum concentration of retinol; s-25(OH)D, serum concentration of 25-hydroxyvitamin D; s- $\alpha$ -tocopherol, serum concentration of  $\alpha$ -tocopherol.

<sup>2</sup>ANOVA for continuous variables,  $\chi^2$  test for factor variables. Exception: Fisher exact test because of low cell counts for the following variables in men: physical inactivity, previous hip fracture, and self-reported osteoporosis.

<sup>3</sup>Test for linear trend across quintiles.

<sup>4</sup>Mean  $\pm$  SD (all such values).

was 72 y, and s-retinol was inversely related to age, with lower concentrations in the oldest. BMI was lower at low s-retinol in women. Among women, there were also a higher proportion of daily cigarette smokers and a higher proportion reporting osteoporosis and previous forearm fracture at low s-retinol. Serum concentration of  $\alpha$ -tocopherol (s- $\alpha$ -tocopherol) was higher at higher s-retinol. Spearman  $\rho$  for the correlation between s-retinol and s- $\alpha$ -tocopherol in the subcohort was 0.20 ( $P < 0.001$ ), whereas that between s-retinol and s-25(OH)D was 0.05 ( $P = 0.041$ ).

### Risk of hip fracture according to serum retinol

There was a statistically significant 41% higher risk of hip fracture in the lowest quintile of s-retinol ( $<2.12 \mu\text{mol/L}$ ) than in the middle quintile (2.56–2.97  $\mu\text{mol/L}$ ), after adjustment for age and study center. This association was attenuated after further adjustment for BMI and s- $\alpha$ -tocopherol (Table 2, Figure 2).

Correspondingly, entering s-retinol as a continuous variable, there was a linear inverse association between s-retinol and hip fracture, with an HR of 0.90 (95% CI: 0.82, 0.98) per 1  $\mu\text{mol/L}$  higher s-retinol when adjusted for age, sex, and study center. This association was not materially affected by daily cigarette

smoking or s-25(OH)D, but it was attenuated after including either of the variables BMI or s- $\alpha$ -tocopherol, with an HR of 0.98 (95% CI: 0.88, 1.08) per 1  $\mu\text{mol/L}$  in the model, including age, sex, study center, BMI, smoking, s-25(OH)D, and s- $\alpha$ -tocopherol.

In a multivariable-adjusted model adjusting for age, study center, sex, BMI, smoking, educational level, self-rated health, s-25(OH)D, and s- $\alpha$ -tocopherol, there was no association between s-retinol and hip fracture, with HRs (95% CIs) of 0.99 (0.88, 1.10) per 1  $\mu\text{mol/L}$  higher s-retinol, 1.11 (0.82, 1.51) for the lowest vs. middle s-retinol quintile, and 1.08 (0.78, 1.49) for the highest vs. middle quintile. The associations were not affected by adjustment for time since last meal (data not shown). Nor were the results affected by excluding those reporting use of estrogen, bisphosphonates, or glucocorticoids at baseline ( $n = 212$ ) in sensitivity analysis. Survival plots and spline analysis stratified by use of cod liver oil supplements indicated that there was no time-dependent effect of s-retinol on hip fracture (Supplemental Figures 1 and 2).

The interaction term between s-25(OH)D and s-retinol on hip fracture was not statistically significant ( $P = 0.68$ ) when entered as a continuous variable and adjusted for age, sex, study center, and BMI. The association between s-25(OH)D and hip fracture

**TABLE 2**  
HRs for hip fracture according to quintiles of serum retinol concentration<sup>1</sup>

s-Retinol quintiles	s-Retinol range, $\mu\text{mol/L}$	Hip fractures, <i>n</i>	Model 1 <sup>2</sup>		Model 2 <sup>3</sup>		Model 3 <sup>4</sup>	
			HR (95% CI)	<i>P</i> value	HR (95% CI)	<i>P</i> value	HR (95% CI)	<i>P</i> value
All <sup>5</sup>								
Q1	$\leq 2.12$	294	1.41 (1.09, 1.82)	0.009	1.26 (0.97, 1.64)	0.09	1.16 (0.88, 1.51)	0.29
Q2	2.12–2.55	247	1.21 (0.94, 1.56)	0.15	1.11 (0.86, 1.45)	0.41	1.09 (0.84, 1.42)	0.51
Q3	2.56–2.97	210	1.00 (reference)	—	1.00 (reference)	—	1.00 (reference)	—
Q4	2.98–3.63	230	1.17 (0.90, 1.52)	0.24	1.11 (0.85, 1.45)	0.44	1.10 (0.84, 1.43)	0.49
Q5	$> 3.63$	173	1.02 (0.77, 1.34)	0.89	0.95 (0.72, 1.26)	0.75	1.00 (0.75, 1.32)	0.97
Men								
Q1	$\leq 2.24$	81	1.35 (0.80, 2.25)	0.23	1.33 (0.79, 2.21)	0.28	1.18 (0.70, 2.01)	0.53
Q2	2.25–2.66	61	1.07 (0.65, 1.78)	0.87	1.04 (0.63, 1.71)	0.89	1.00 (0.60, 1.65)	1.00
Q3	2.67–3.10	55	1.00 (reference)	—	1.00 (reference)	—	1.00 (reference)	—
Q4	3.10–3.80	60	1.14 (0.68, 1.92)	0.72	1.09 (0.66, 1.82)	0.74	1.08 (0.65, 1.80)	0.77
Q5	$> 3.80$	44	1.08 (0.63, 1.86)	0.97	0.99 (0.58, 1.70)	0.97	1.08 (0.62, 1.87)	0.80
Women								
Q1	$\leq 2.08$	216	1.38 (1.03, 1.86)	0.034	1.21 (0.89, 1.65)	0.21	1.13 (0.82, 1.54)	0.45
Q2	2.08–2.52	183	1.14 (0.85, 1.53)	0.39	1.04 (0.77, 1.41)	0.81	1.01 (0.74, 1.37)	0.97
Q3	2.52–2.94	161	1.00 (reference)	—	1.00 (reference)	—	1.00 (reference)	—
Q4	2.95–3.60	171	1.09 (0.81, 1.47)	0.58	1.01 (0.74, 1.38)	0.95	1.00 (0.73, 1.37)	1.00
Q5	$> 3.60$	122	0.93 (0.67, 1.28)	0.65	0.87 (0.62, 1.21)	0.40	0.90 (0.64, 1.25)	0.52

<sup>1</sup>Cox proportional hazards regression adapted for the case-cohort design (function “cch”), with middle quintile as reference. Quintile cutoffs based on limits in subcohort. Q, quintile; s-retinol, serum concentration of retinol.

<sup>2</sup>Model 1: age and study center.

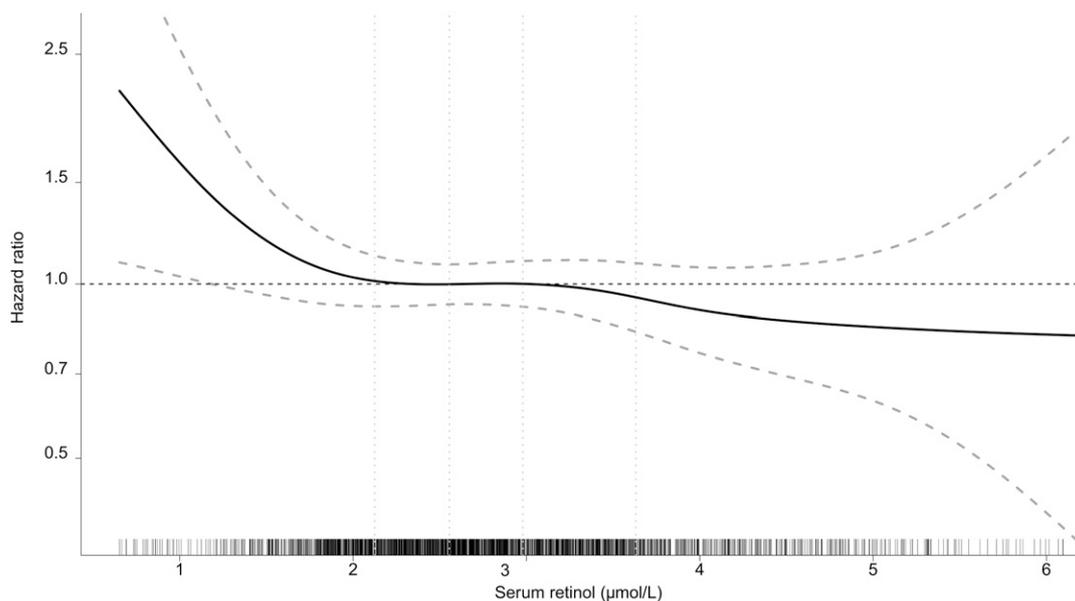
<sup>3</sup>Model 2: age, study center, and BMI.

<sup>4</sup>Model 3: age, study center, BMI, and serum  $\alpha$ -tocopherol.

<sup>5</sup>Adjusted for sex.

was not materially different in 2 strata of s-retinol divided at the median (not shown). An interaction term between sex and s-retinol on hip fracture was not significant:  $P = 0.34$  with s-retinol as a continuous variable adjusted for age and study center ( $P = 0.44$  with additional adjustment for BMI). Nor was there any statistical interaction between age at baseline and s-retinol on hip fracture

( $P = 0.49$ ) or between BMI and s-retinol on hip fracture ( $P = 0.12$ ). In Norway, the retinol content in cod liver oil was reduced in 1999, but we observed no statistical interaction between study center and serum retinol concentrations on hip fracture (age and sex adjusted,  $P = 0.34$ ), implying similar risk patterns across the participating study centers.



**FIGURE 2** HR (solid line) with 95% CI (dashed lines) for hip fracture across the distribution of s-retinol. Based on Cox proportional hazards regression with penalized splines of s-retinol in a model including age, sex, study center, BMI, and serum  $\alpha$ -tocopherol concentrations, with robust variance estimates and inverse probability weighting for sampling fraction to the subcohort. An HR of 1 represents average hazard in the data. s-Retinol range from the 1st to the 99th percentile is included. Dotted vertical lines represent quintile limits. Ticks along the horizontal axis represent individual s-retinol concentrations. s-retinol, serum concentration of retinol.

## DISCUSSION

In this large case-cohort study, we observed an inverse association between *s*-retinol and risk of hip fracture, which was statistically significant in women but not in men. The association was attenuated after adjustment for BMI and *s*- $\alpha$ -tocopherol. There was no evidence of an increased fracture risk at high retinol concentrations. In post hoc analyses, the results were unaltered by taking into account body height, serum total cholesterol concentrations, or frequency of alcohol consumption (not shown).

To our knowledge, only 3 prospective studies have specifically examined the association between serum retinol concentrations and risk of incident hip fractures in individuals from the general population, all reported in the recent meta-analysis by Wu et al. (20). For 2 of these studies and our present data, we have summed up the HRs for hip fracture in the fifth vs. third quintile and the first vs. third quintile of *s*-retinol, respectively, in **Figure 3**. The third study (19) is not included in the figure because it is unclear how the numbers were arrived at. The distributions of serum retinol concentrations in our data were considerably higher than in the previous studies. A possible reason is a higher intake of fish and cod liver in our population, particularly in northern Norway.

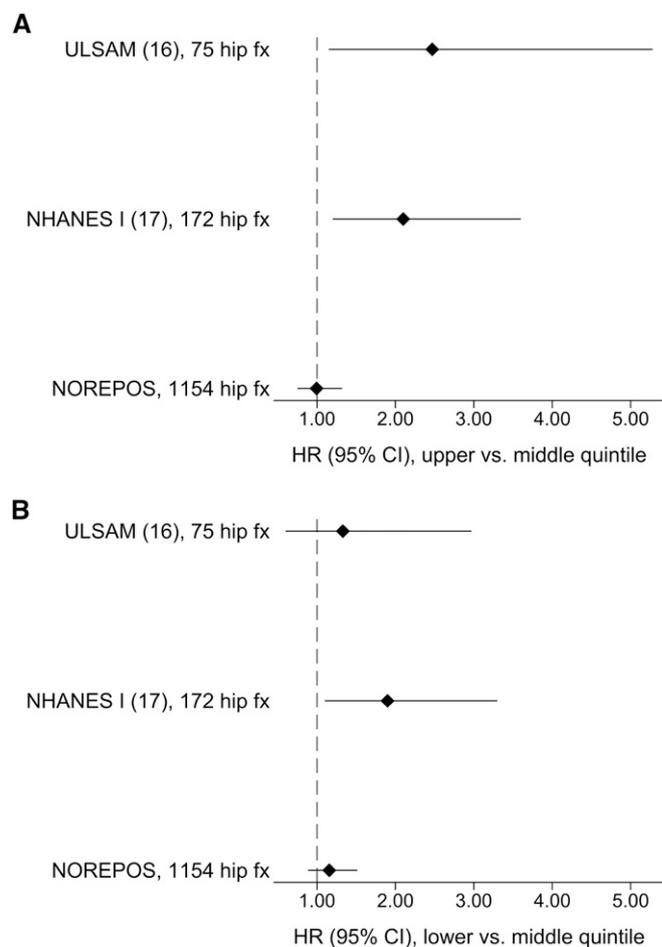
In the Uppsala Longitudinal Study of Adult Men (16), 2322 men aged 49–51 y were followed for up to 30 y with regard to fractures. The risk was increased only in the highest quintile ( $>2.64 \mu\text{mol/L}$ ), with a multivariate RR for hip fracture in quintile 5 vs. quintile 3 of 2.47 (95% CI: 1.15, 5.28).

In the NHANES I cohort (17), 2799 women aged 50–74 y were followed for up to 22 y, during which time 172 women had a hip fracture. There was no linear relation between serum vitamin A (retinol and retinyl esters) and risk of hip fracture. Analysis by quintiles revealed a U-shaped relation, with significantly higher risk of hip fracture in the lowest (HR: 1.9; 95% CI: 1.1, 3.3) and highest (HR: 2.1; 95% CI: 1.2, 3.6) quintiles than in the middle quintile.

The association between *s*-retinol and hip fracture was also studied in a nested case-control analysis in the placebo arm of a randomized trial of clodronate in older British women. The analysis included 92 hip fracture cases (312 any-fracture cases) followed for a mean of 3.2 y and 3 controls per case. They found no association between *s*-retinol or other retinoids and risk of hip or any fracture (19).

In addition, a recent follow-up of middle-aged Australian men and women who had previously participated in a vitamin A supplementation trial found no relation between plasma retinol concentrations and self-reported osteoporotic fractures occurring during up to 17 y of follow-up (35).

Others have reported lower serum retinol concentrations in patients with osteoporosis compared with normal controls (36, 37). In our study, we also observed lower retinol concentrations in those reporting prevalent osteoporosis, women, the oldest, those with a low BMI, and daily cigarette smokers—in other words, those with the anticipated highest fracture risk. Dietary sources rich in preformed retinol are generally foods associated with a high energy and protein density, which is anticipated to be bone protective through nutritional status, muscle mass, and body weight (38). We may speculate that the discrepancy in findings between studies could be attributed to variations in



**FIGURE 3** Forest plots of HRs with 95% CIs for hip fracture in the upper (A) and lower (B) vs. middle quintile of serum retinol concentrations in 3 prospective studies. ULSAM (16): men only. Included covariates: age, weight, height, serum  $\beta$ -carotene, serum calcium, serum albumin, smoking status, marital status, socioeconomic status, physical activity at work, leisure physical activity, and alcohol consumption. NHANES I (17): women only. Included covariates: age, weight, serum albumin, serum cholesterol, alcohol use, recreational and nonrecreational physical activity, hormone replacement use, history of previous fracture, dietary calcium intake, and race. NOREPOS: both sexes. Included covariates: age, sex, study center, BMI, and serum  $\alpha$ -tocopherol. fx, fracture; NOREPOS, Norwegian Epidemiologic Osteoporosis Studies; ULSAM, Uppsala Longitudinal Study of Adult Men.

sources providing vitamin A in different populations. Older Norwegians have a high retinol intake from a diet traditionally rich in fatty fish (39). Traditional meals rich in cod liver and cod liver oil are more common in northern Norway, and a higher reported fish consumption in northern Norway compared with other regions (40) may reflect this. Overall, the quantitatively most important dietary sources of retinol in the Norwegian diet are meat, high-fat dairy products, and margarine (40), the latter being the only vitamin A–fortified food marketed in Norway. It is likely that a low proportion of retinol consumed in this population stems from water-miscible emulsified preparations, which may be metabolized differently and consequently are more toxic (41). No evidence of a harmful effect on bone of retinol supplementation, predominantly from fish oil, could be found in a study in older nursing home residents in 2 cities in Norway (42).

Several studies have examined the relation between dietary vitamin A intake estimated from food-frequency questionnaires

and bone mineral density or fracture risk, with divergent findings (14, 15, 18, 43–46). A review summarizing 20 studies published up to 2004 on the role of vitamin A intake in osteoporosis (12) concluded that although it was not possible to set a specific intake level of vitamin A above which an adverse effect on bone may be anticipated, vitamin A-containing supplements should not be used with the express goal of improving bone health. In the current study, total vitamin A intake calculated from a food-frequency questionnaire was available only in the Hordaland Health Study substudy (207 hip fracture cases and a subcohort of 192). We found no association between estimated daily retinol intake and hip fracture in this subset (not shown).

An inverse association has recently been demonstrated between *s*- $\alpha$ -tocopherol and hip fracture (29, 47). In our study,  $\alpha$ -tocopherol, 25(OH)D, and retinol were measured simultaneously in serum from the same individuals. This allowed us to adjust adequately for possible confounding. Without this adjustment, we found an inverse association between *s*-retinol and hip fracture (Table 2). In the analysis including all 3 vitamins (not shown),  $\alpha$ -tocopherol showed a clear inverse association with risk of hip fracture, whereas 25(OH)D showed a borderline inverse association with risk of hip fracture. Strengths of our study include the community-based prospective design and the large number of events, retrieved from patient administrative systems and subsequently confirmed in medical records and X-ray archives.

A weakness pertaining to all studies using measurements of retinol as exposure is the limited validity of this biochemical marker. Retinol reflects enzymatic conversion of various retinoids and is regarded as the best biochemical indicator of vitamin A intake and status (48). However, unlike circulating 25(OH)D, which reflects vitamin D supply from diet and sun exposure in the course of the previous month (49), a corresponding established biochemical indicator of nutritional vitamin A status is not available. This is due to the metabolism of vitamin A, which is mainly stored in hepatic stellate cells and is released into the circulation to provide adequate circulating concentrations (8, 48).

A specific limitation to this study is that other retinoids or carotenoids were not measured. Precursors of retinol may have different physiologic effects. One of these is  $\beta$ -carotene, which appears to be beneficial to bone (50–52). Possible opposing actions of  $\beta$ -carotene and preformed retinol could not be disentangled in this study.

It is a limitation that the questions concerning diet and supplements posed in the 4 substudies were not readily comparable and that only 52% responded to questions concerning cod liver oil supplement use. Another limitation is the long-term storage of the serum samples, varying between 10 and 17 y depending on study center. There is scarce information concerning long-term stability of retinol in stored serum samples. A literature review concluded that retinol appears to remain stable for at least 15 y if kept frozen at below  $-70^{\circ}\text{C}$  and even at higher temperatures (53). We believe that degradation of retinol has not influenced our results substantially and that subjects have been adequately classified according to distribution of serum retinol concentrations. Samples from cases and noncases at each study center were analyzed simultaneously. We observed expected and plausible associations between serum retinol and covariates, as well as with the outcome in unadjusted analysis, which was attenuated when including confounders. The distribution of serum retinol con-

centrations did not depend directly on storage time in our study: concentrations were highest in Tromsø, with the longest storage time, and lowest in Oslo, with the shortest storage time. In general, the distribution of serum retinol in our study was higher than those previously reported in other populations (16, 17, 19, 54).

In conclusion, in this community-based case-cohort study of older home-dwelling Norwegians, the largest of its kind, we could not demonstrate any adverse effect of high serum concentrations of retinol on hip fracture. Thus, retinol is not responsible for the high incidence of hip fractures in Norway. If anything, there tended to be an increased risk at low serum concentrations, which was attenuated after controlling for confounders. We found no evidence that a protective effect of high 25(OH)D on risk of hip fracture is counteracted by high retinol concentrations. We propose that cod liver oil should not be discouraged as a natural source of vitamin D supplementation for fracture prevention.

We thank the people involved in carrying out the data collection in the fourth survey of the Tromsø Study, the second Nord-Trøndelag Health Study, the Hordaland Health Study, and the Oslo Health Study; those involved in establishing and maintaining the 4 respective hip fracture follow-up registers; those involved in data management; those involved in biobanks and blood sample handling; and the laboratory AS Vitas, Oslo, Norway, for performing the serum sample analyses.

The authors' responsibilities were as follows—KH, SF, CGG, GG, BS, GST, and HEM: developed the protocol and obtained the funding; KH, LAA, SF, CGG, GG, BS, GST, and HEM: collected the data; KH: prepared the data, performed the statistical analyses, drafted the manuscript, and took responsibility for the integrity of the data analysis; RB: provided scientific advice; SOS: provided statistical advice; and all authors: critically discussed the results, revised the manuscript, and accepted the final version. RB has interests in Vitas AS, a company established by Oslo Innovation Center. The rest of the authors declared no conflicts of interest related to this study.

## REFERENCES

- Omsland TK, Holvik K, Meyer HE, Center JR, Emaus N, Tell GS, Schei B, Tverdal A, Gjerdal CG, Grimnes G, et al. Hip fractures in Norway 1999–2008: time trends in total incidence and second hip fracture rates: a NOREPOS study. *Eur J Epidemiol* 2012;27:807–14.
- Maggi S, Kelsey JL, Litvak J, Heyse SP. Incidence of hip fractures in the elderly: a cross-national analysis. *Osteoporos Int* 1991;1:232–41.
- Lofthus CM, Osnes EK, Falch JA, Kaastad TS, Kristiansen IS, Nordstletten L, Stensvold I, Meyer HE. Epidemiology of hip fractures in Oslo, Norway. *Bone* 2001;29:413–8.
- Cheng SY, Levy AR, Lefavre KA, Guy P, Kuramoto L, Sobolev B. Geographic trends in incidence of hip fractures: a comprehensive literature review. *Osteoporos Int* 2011;22:2575–86.
- Støen RO, Nordstletten L, Meyer HE, Frihagen JF, Falch JA, Lofthus CM. Hip fracture incidence is decreasing in the high incidence area of Oslo, Norway. *Osteoporos Int* 2012;23:2527–34.
- Ahmadieh H, Arabi A. Vitamins and bone health: beyond calcium and vitamin D. *Nutr Rev* 2011;69:584–98.
- Nieves JW. Skeletal effects of nutrients and nutraceuticals, beyond calcium and vitamin D. *Osteoporos Int* 2013;24:771–86.
- Blomhoff R, Blomhoff HK. Overview of retinoid metabolism and function. *J Neurobiol* 2006;66:606–30.
- Saneshige S, Mano H, Tezuka K, Kakudo S, Mori Y, Honda Y, Itabashi A, Yamada T, Miyata K, Hakeda Y, et al. Retinoic acid directly stimulates osteoclastic bone resorption and gene expression of cathepsin K/OC-2. *Biochem J* 1995;309:721–4.
- Ohishi K, Nishikawa S, Nagata T, Yamauchi N, Shinohara H, Kido J, Ishida H. Physiological concentrations of retinoic acid suppress the osteoblastic differentiation of fetal rat calvaria cells in vitro. *Eur J Endocrinol* 1995;133:335–41.

11. Binkley N, Krueger D. Hypervitaminosis A and bone. *Nutr Rev* 2000; 58:138–44.
12. Crandall C. Vitamin A intake and osteoporosis: a clinical review. *J Womens Health (Larchmt)* 2004;13:939–53.
13. Forsmo S, Fjeldbo SK, Langhammer A. Childhood cod liver oil consumption and bone mineral density in a population-based cohort of peri- and postmenopausal women: the Nord-Trøndelag Health Study. *Am J Epidemiol* 2008;167:406–11.
14. Melhus H, Michaëlsson K, Kindmark A, Bergström R, Holmberg L, Mallmin H, Wolk A, Ljunghall S. Excessive dietary intake of vitamin A is associated with reduced bone mineral density and increased risk for hip fracture. *Ann Intern Med* 1998;129:770–8.
15. Feskanich D, Singh V, Willett WC, Colditz GA. Vitamin A intake and hip fractures among postmenopausal women. *JAMA* 2002;287:47–54.
16. Michaëlsson K, Lithell H, Vessby B, Melhus H. Serum retinol levels and the risk of fracture. *N Engl J Med* 2003;348:287–94.
17. Opatowsky AR, Bilezikian JP. Serum vitamin A concentration and the risk of hip fracture among women 50 to 74 years old in the United States: a prospective analysis of the NHANES I follow-up study. *Am J Med* 2004;117:169–74.
18. Lim LS, Harnack LJ, Lazovich D, Folsom AR. Vitamin A intake and the risk of hip fracture in postmenopausal women: the Iowa Women's Health Study. *Osteoporos Int* 2004;15:552–9.
19. Barker ME, McCloskey E, Saha S, Gossiel F, Charlesworth D, Powers HJ, Blumsohn A. Serum retinoids and beta-carotene as predictors of hip and other fractures in elderly women. *J Bone Miner Res* 2005;20:913–20.
20. Wu AM, Huang CQ, Lin ZK, Tian NF, Ni WF, Wang XY, Xu HZ, Chi YL. The relationship between vitamin A and risk of fracture: meta-analysis of prospective studies. *J Bone Miner Res* 2014;29:2032–9.
21. Dusso AS, Brown AJ, Slatopolsky E. Vitamin D. *Am J Physiol Renal Physiol* 2005;289:F8–28.
22. Johansson S, Melhus H. Vitamin A antagonizes calcium response to vitamin D in man. *J Bone Miner Res* 2001;16:1899–905.
23. Mata-Granados JM, Cuenca-Acevedo R, Luque de Castro MD, Sosa M, Quesada-Gómez JM. Vitamin D deficiency and high serum levels of vitamin A increase the risk of osteoporosis evaluated by Quantitative Ultrasound Measurements (QUS) in postmenopausal Spanish women. *Clin Biochem* 2010;43:1064–8.
24. Cruz JA, Moreiras-Varela O, van Staveren WA, Trichopoulou A, Roszkowski W. Intake of vitamins and minerals. Euronut SENECA investigators. *Eur J Clin Nutr* 1991;45(Suppl 3):121–38.
25. Freisling H, Fahey MT, Moskal A, Ocké MC, Ferrari P, Jenab M, Norat T, Naska A, Welch AA, Navarro C, et al. Region-specific nutrient intake patterns exhibit a geographical gradient within and between European countries. *J Nutr* 2010;140:1280–6.
26. Jenab M, Salvini S, van Gils CH, Brustad M, Shakya-Shrestha S, Buijsse B, Verhagen H, Touvier M, Biessy C, Wallström P, et al. Dietary intakes of retinol, beta-carotene, vitamin D and vitamin E in the European Prospective Investigation into Cancer and Nutrition cohort. *Eur J Clin Nutr* 2009;63(Suppl 4):S150–78.
27. Holvik K, Ahmed LA, Forsmo S, Gjesdal CG, Grimnes G, Samuelsen SO, Schei B, Blomhoff R, Tell GS, Meyer HE. Low serum levels of 25-hydroxyvitamin D predict hip fracture in the elderly: a NOREPOS study. *J Clin Endocrinol Metab* 2013;98:3341–50.
28. Næss Ø, Sjøgaard AJ, Arnesen E, Beckstrøm AC, Bjertness E, Engeland A, Hjort PF, Holmen J, Magnus P, Njølstad I, et al. Cohort profile: cohort of Norway (CONOR). *Int J Epidemiol* 2008;37:481–5.
29. Holvik K, Gjesdal CG, Tell GS, Grimnes G, Schei B, Apalset EM, Samuelsen SO, Blomhoff R, Michaëlsson K, Meyer HE. Low serum concentrations of alpha-tocopherol are associated with increased risk of hip fracture: a NOREPOS study. *Osteoporos Int* 2014;25:2545–54.
30. The R Project for Statistical Computing. [Internet]. Vienna (Austria): R Foundation for Statistical Computing. [cited 2015 May 28]. Available from: <http://www.R-project.org>.
31. Borgan Ø, Samuelsen SO. Nested case-control and case-cohort studies. In: Klein JP, van Houwelingen HC, Ibrahim JG, Scheike TH, editors. *Handbook of survival analysis*. Boca Raton (FL): CRC Press, 2013. p. 343–67.
32. Cologne J, Preston DL, Imai K, Misumi M, Yoshida K, Hayashi T, Nakachi K. Conventional case-cohort design and analysis for studies of interaction. *Int J Epidemiol* 2012;41:1174–86.
33. Breslow N. cch: fits proportional hazards regression model to case-cohort data. Function in Therneau T: "Package 'survival': Survival analysis, including penalised likelihood" [Internet]. [cited 2015 May 28]. Available from: <http://cran.r-project.org/web/packages/survival/survival.pdf>.
34. Therneau TM, Grambsch PM. *Modeling survival data: extending the Cox model*. New York: Springer Science; 2000.
35. Ambrosini GL, Alfonso H, Reid A, Mackerras D, Bremner AP, Beilby J, Olsen NJ, Musk AW, de Klerk NH. Plasma retinol and total carotenoids and fracture risk after long-term supplementation with high doses of retinol. *Nutrition* 2014;30:551–6.
36. Maggio D, Polidori MC, Barabani M, Tufi A, Ruggiero C, Cecchetti R, Aisa MC, Stahl W, Cherubini A. Low levels of carotenoids and retinol in involutional osteoporosis. *Bone* 2006;38:244–8.
37. Penniston KL, Weng N, Binkley N, Tanumihardjo SA. Serum retinyl esters are not elevated in postmenopausal women with and without osteoporosis whose preformed vitamin A intakes are high. *Am J Clin Nutr* 2006;84:1350–6.
38. Mithal A, Bonjour JP, Boonen S, Burckhardt P, Degens H, El Hajj Fuleihan G, Josse R, Lips P, Morales Torres J, Rizzoli R, et al. Impact of nutrition on muscle mass, strength, and performance in older adults. *Osteoporos Int* 2013;24:1555–66.
39. Norwegian Nutrition Council. NORKOST 1997: nationwide dietary survey among men and women aged 16–79 years. Oslo (Norway): Norwegian Directorate of Health; 1999 (in Norwegian).
40. Norwegian Directorate of Health. Norkost 3: a nationwide dietary survey among men and women in Norway aged 18–70 years. Norwegian Directorate of Health; Oslo (Norway): 2012 (in Norwegian).
41. Myhre AM, Carlsen MH, Bøhn SK, Wold HL, Laake P, Blomhoff R. Water-miscible, emulsified, and solid forms of retinol supplements are more toxic than oil-based preparations. *Am J Clin Nutr* 2003;78:1152–9.
42. Meyer HE, Smedshaug GB, Kvaavik E, Falch JA, Tverdal A, Pedersen JI. Vitamin A intake and osteoporotic fractures. *J Bone Miner Res* 2004;19(Suppl 1):S294 (abstr).
43. Promislow JH, Goodman-Gruen D, Slymen DJ, Barrett-Connor E. Retinol intake and bone mineral density in the elderly: the Rancho Bernardo Study. *J Bone Miner Res* 2002;17:1349–58.
44. Wolf RL, Cauley JA, Pettinger M, Jackson R, Lacroix A, Leboff MS, Lewis CE, Nevitt MC, Simon JA, Stone KL, et al. Lack of a relation between vitamin and mineral antioxidants and bone mineral density: results from the Women's Health Initiative. *Am J Clin Nutr* 2005;82:581–8.
45. Rejnmark L, Vestergaard P, Charles P, Hermann AP, Brot C, Eiken P, Mosekilde L. No effect of vitamin A intake on bone mineral density and fracture risk in perimenopausal women. *Osteoporos Int* 2004;15:872–80.
46. Caire-Juvera G, Ritenbaugh C, Wactawski-Wende J, Snetselaar LG, Chen Z. Vitamin A and retinol intakes and the risk of fractures among participants of the Women's Health Initiative Observational Study. *Am J Clin Nutr* 2009;89:323–30.
47. Michaëlsson K, Wolk A, Byberg L, Arnlöv J, Melhus H. Intake and serum concentrations of alpha-tocopherol in relation to fractures in elderly women and men: 2 cohort studies. *Am J Clin Nutr* 2014;99:107–14.
48. Sauberlich HE, Dowdy RP, Skala JH. Laboratory tests for the assessment of nutritional status. *CRC Crit Rev Clin Lab Sci* 1973;4:215–340.
49. Lips P. Relative value of 25(OH)D and 1,25(OH)2D measurements. *J Bone Miner Res* 2007;22:1668–71.
50. Zhang J, Munger RG, West NA, Cutler DR, Wengreen HJ, Corcoran CD. Antioxidant intake and risk of osteoporotic hip fracture in Utah: an effect modified by smoking status. *Am J Epidemiol* 2006;163:9–17.
51. Sahni S, Hannan MT, Blumberg J, Cupples LA, Kiel DP, Tucker KL. Protective effect of total carotenoid and lycopene intake on the risk of hip fracture: a 17-year follow-up from the Framingham Osteoporosis Study. *J Bone Miner Res* 2009;24:1086–94.
52. Tanumihardjo SA. Vitamin A and bone health: the balancing act. *J Clin Densitom* 2013;16:414–9.
53. Comstock GW, Alberg AJ, Helzlsouer KJ. Reported effects of long-term freezer storage on concentrations of retinol, beta-carotene, and alpha-tocopherol in serum or plasma summarized. *Clin Chem* 1993;39:1075–8.
54. Ballew C, Bowman BA, Sowell AL, Gillespie C. Serum retinol distributions in residents of the United States: third National Health and Nutrition Examination Survey, 1988–1994. *Am J Clin Nutr* 2001;73:586–93.

