

1 **Low serum levels of 25-hydroxyvitamin D predict hip fracture in the elderly.**

2 **A NOREPOS study**

3
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1 **Ethical approval**

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

5

6 **Authors' contribution**

7 All study centers providing data are represented among the co-authors. KH, SF, CGG, GG, BS, GST
8 and HEM contributed to developing the protocol and obtain funding. KH prepared the data, performed
9 the statistical analyses and drafted the manuscript. SOS provided statistical advice. All co-authors
10 have critically discussed the results, revised the manuscript and accepted the final version.

11

12 **Disclosure statement**

13 KH, LAA, SF, CGG, GG, SOS, BS and GST have nothing to declare. RB has interests in Bioindex AS
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28

1 **ABSTRACT**

2 **Background:** Despite considerable interest, the relationship between circulating 25-hydroxyvitamin
3 D and risk of hip fracture is not fully established.

4 **Objective:** To study the association between serum 25-hydroxyvitamin D concentrations (s-25(OH)D)
5 and risk of hip fracture in Norway, a high-latitude country that has among the highest hip fracture rates
6 worldwide.

7 **Methods:** N=21,774 men and women aged 65-79 attended four community-based health studies
8 during 1994-2001. Information on subsequent hip fractures were retrieved from electronic hospital
9 discharge registers, with maximum follow-up 10.7 years. Using a stratified case-cohort design, s-
10 25(OH)D was determined by HPLC-APCI-MS in stored serum samples in hip fracture cases (n=1175;
11 307 men, 868 women) and in gender-stratified random samples (n=1438). Cox proportional hazards
12 regression adapted for the case-cohort design was performed.

13 **Results:** We observed an inverse association between s-25(OH)D and hip fracture; those with s-
14 25(OH)D in the lowest quartile (<42.2 nmol/l) had a 38% (95% CI 9-74%) increased risk of hip
15 fracture compared with the highest quartile (\geq 67.9 nmol/l) in a model accounting for age, gender,
16 study center, and BMI. The association was stronger in men than in women: HR 1.65 (95% CI 1.04-
17 2.61) versus HR 1.25 (95% CI 0.95-1.65).

18 **Conclusion:** In this prospective case-cohort study of hip fractures, the largest ever reported, we found
19 an increased risk of hip fracture in subjects in the lowest compared to the highest quartile of serum 25-
20 hydroxyvitamin D. In accordance with findings of previous community-based studies, low vitamin D
21 status was a modest risk factor for hip fracture.

22

1 **INTRODUCTION**

2 The highest incidence rate of hip fractures worldwide has been reported in Oslo, Norway (1, 2). The
3 causes of these high rates in Norway, and in the other Scandinavian countries, are not known (1).

4 Considerable attention has been given to the relation between low vitamin D status and osteoporotic
5 fractures.

6

7 Vitamin D is essential for intestinal calcium absorption and for maintaining calcium homeostasis and
8 skeletal integrity (3). A well-known consequence of vitamin D deficiency is secondary

9 hyperparathyroidism which may lead to bone resorption, osteoporosis, and increased fracture risk (3).

10 Some (4-6) but not other (7, 8) studies have found a positive association between vitamin D status and
11 bone mineral density (BMD) in Caucasian populations. It has therefore been suggested that vitamin D
12 may not be a major determinant of bone health in populations with generally adequate vitamin D and
13 calcium status (7, 8). However, vitamin D may not influence fracture risk through bone metabolism
14 only, but also through muscle function and risk of falling (9).

15

16 Most prospective studies investigating the role of vitamin D status in fracture risk included a low
17 number of hip fractures and analyzed all fractures as outcome (7, 10-14). Some have not been able to
18 establish any association (7, 12) . However, an increased fracture risk has been reported at 25(OH)D
19 levels below 30 nmol/l in the Netherlands (13), and below 40 nmol/l or 50 nmol/l in Sweden (11, 14).

20 The association between s-25(OH)D and hip fracture (as the main outcome) has been studied in three
21 prospective studies from the US: The NHANES III (15), the MrOS study in men (16), and the
22 Women's Health Initiative (17), the latter being the largest prospective study we are aware of with
23 vitamin D status in blood determined at baseline and hip fracture as outcome, including 400 cases.
24 They observed a statistically significant trend of increasing risk of hip fracture through decreasing
25 quartiles of 25(OH)D.

26

27 Meta-analyses of randomized controlled trials conclude that supplementation with vitamin D in
28 combination with calcium has a modest preventive effect on hip fracture, whereas a preventive effect

1 has not been established for vitamin D alone (18-20). In a re-analysis of data from 11 RCTs, only the
2 highest actual intake levels (≥ 792 IU/day) were significantly associated with reduced risk of hip
3 fracture (21).

4

5 The aim of this study was to investigate the association between serum 25-hydroxyvitamin D levels
6 (the sum of $25(\text{OH})\text{D}_2 + 25(\text{OH})\text{D}_3$) and risk of hip fracture during up to 11 years follow-up in
7 community-dwelling older adults in the country with the world's highest hip fracture incidence.

8

1 MATERIALS AND METHODS

2 As part of the Norwegian Epidemiologic Osteoporosis Studies (NOREPOS) research collaboration
3 (www.norepos.no), we performed a gender-stratified case-cohort study (22, 23) using baseline data
4 from four population-based studies in Norway: The Tromsø Study (Tromsø IV) (latitude 69°N) in
5 1994-95 (www.tromsostudy.com), The Nord-Trøndelag Health Study (HUNT 2) (63-65°N) in 1995-
6 97 (www.ntnu.edu/hunt), The Hordaland Health Study (HUSK) (60°N) in 1997-99 (husk-en.b.uib.no),
7 and The Oslo Health Study (HUBRO) (59°N) in 2000-01 (www.fhi.no/hubro-en).

8

9 Study population

10 The total cohort included 21,774 home-dwelling men and women aged 65-79 years. The lower age
11 limit was set to define an elderly group, and the higher age limit was set to reduce censoring due to
12 deaths. In Tromsø IV and HUNT 2, age distribution of participants at baseline was 65-79 years, while
13 the participants in HUSK comprised individuals age 70-73, and in HUBRO age 75-77.

14

15 Baseline information

16 Baseline examinations included collection of non-fasting blood samples which were frozen and stored,
17 measurements of weight, height and blood pressure, and self-administered questionnaires. The four
18 studies are part of Cohort of Norway (CONOR) (24), thus standardized questionnaire and background
19 data were available. Questionnaires included information on previous fracture, physical activity,
20 cigarette smoking, chronic diseases, self-perceived health, and use of medications. Body mass index
21 (BMI, kg/m²) was calculated. A question on current health status with four response alternatives was
22 dichotomized into a variable indicating good/very good vs. poor/not so good self-rated health.

23 Participants answering “yes” to the question “Do you currently smoke cigarettes daily?” were defined
24 as smokers. Participants were categorized as physically active or inactive according to their response
25 on two four-graded questions concerning weekly frequency of hard and moderate physical activity
26 during leisure time. Education was assessed as number of years completed education. Questions on
27 whether the participant suffered or had ever suffered from chronic diseases, including osteoporosis, or
28 had experienced adverse events, including fracture of the wrist or hip, were answered with yes or no.

1 Open-ended questions about medication use were included in HUBRO, HUSK, and Tromsø IV, and
2 were coded according to the Anatomical Therapeutic Chemical Classification (ATC) System. Those
3 reporting medications with ATC codes G03 C and F were defined as estrogen therapy users. In HUNT
4 2, only women below 70 years were inquired about estrogen therapy, and those responding “currently”
5 were defined as current users. In addition, HUBRO included the question “Do you or have you used
6 oestrogen (tablets or patches)”, and all those answering "yes, currently" to this question were also
7 included in the estrogen therapy users category. While 99.0% (1162 cases and 1338 non-cases) had
8 valid measurements of weight and height, the response rates on questionnaire data ranged from 98.5%
9 (self-rated health) to 77.6% (physical activity).

10

11 **Deaths and emigration**

12 Dates of death or emigration were obtained from the Norwegian Population Register and follow-up
13 time was calculated. End of follow-up was 31.12.2004 in Tromsø IV and HUNT 2, 31.12.2007 in
14 HUBRO, and 31.12.2008 in HUSK, yielding a maximum follow-up of 10.7 years.

15

16 **Identification of hip fractures**

17 Information about hip fractures was obtained from fracture registers at each study site. A hip fracture
18 was defined as the first fracture of the proximal femur occurring during the observation period. In
19 Tromsø IV and HUNT 2, cases included the diagnoses of fracture of the femoral neck and
20 pertrochanteric fracture, while the case definition in HUSK and HUBRO also included subtrochanteric
21 hip fractures. The discharge diagnoses used to classify a hip fracture were according to the
22 International Classification of Diseases, Ninth Revision (ICD-9): 820-820.9 and Tenth Revision (ICD-
23 10): S72.0, S72.1 and S72.2.

24

25 In Tromsø IV, hip fractures subsequent to the baseline examination until moving out of Tromsø
26 municipality or end of follow-up were retrieved from the radiographic archives and validated against
27 the patient discharge records at the University Hospital of Northern Norway, Tromsø (25). In HUNT
28 2, hip fractures treated in Levanger or Namsos hospitals, the two only hospitals in Nord-Trøndelag,

1 were obtained from the electronic registers of the Nord-Trøndelag Hospital Trust (26). In HUSK, the
2 computerized discharge diagnoses records of the six hospitals serving Hordaland County were
3 searched for diagnosis codes. All admittances with diagnosis codes indicating femoral fracture with a
4 corresponding surgical procedure indicating a primary operation for hip fracture were defined as
5 incident hip fractures (27). In HUBRO, hip fractures were retrieved by linkage to the electronic
6 discharge registers in the five hospitals treating hip fractures in Oslo, by performing a broad search on
7 all diagnosis codes for femur fractures as well as surgical procedure codes for fracture surgery in
8 femur and hip arthroplasty. True primary hip fractures were confirmed by reviewing patients' medical
9 records, except in one hospital where only 42% of the retrieved hospital stays were verified in medical
10 records, as the remainder were no longer electronic accessible. For 84 (16.2%) admissions not
11 reviewed in medical records, a hip fracture diagnosis was confirmed if the hospital stay included a
12 surgical procedure characteristic for a primary operation for hip fracture, corresponding to the method
13 used in HUSK.

14

15 **Selection of cases and the subcohort**

16 All participants who suffered a hip fracture during follow-up were included as cases. Participants with
17 self-reported previous fractures or fractures occurring prior to their date of attendance, but not during
18 the observation period, were treated as non-cases. The gender-specific subcohorts were defined as
19 random unmatched samples of 4.5% of men and 9.0% of women in the study population at baseline.
20 These inclusion fractions were chosen so that the subcohorts within each gender would be
21 approximately the same size as the number of cases.

22

23 **Serum sample analyses**

24 Frozen serum samples were sent to AS Vitas, Oslo, Norway, where they were analyzed in 2011.
25 Samples from cases and non-cases at each study center were analyzed simultaneously. Biobank staff
26 and laboratory staff were blinded with regard to case status. 25-hydroxyvitamin D₂ and 25-
27 hydroxyvitamin D₃ in serum were determined by HPLC-APCI- MS (28). One hundred and fifty µL of
28 human plasma was diluted with 450 µL 2-propanol containing BHT as an antioxidant. After thorough

1 mixing (15 min) and centrifugation (10 min, 4000 g at 10 °C), an aliquot of 35 µL was injected from
2 the supernatant into the HPLC system. HPLC was performed with a HP 1100 liquid chromatograph
3 (Agilent Technologies, Palo Alto, CA, USA) interfaced by atmospheric pressure chemical ionization
4 (APCI) to a HP mass spectrometric detector (MS) operated in single ion monitoring mode (SIM).
5 Vitamin D analogues were separated on a 4.6 mm x 50 mm reversed phase column with 1.8 µM
6 particles. The column temperature was 80°C. A two-point calibration curve was made from analysis of
7 albumin solution enriched with known vitamin D concentration. Recovery is 95%, the method is linear
8 from 5-400 nM at least and the limit of detection is 1-4 nM. CVs for interassay analyses were 7.6% at
9 s-25(OH)D 47.8 nmol/l and 6.9% at s-25(OH)D 83.0 nmol/l.

10

11 **Statistical analyses**

12 Data management was done in PASW Statistics 17 / IBM SPSS 20, and main statistical analyses were
13 performed in R (29). We performed Cox proportional hazards regression adjusted for a gender-
14 stratified case-cohort design (22, 23), using the case-cohort function 'cch' in the R package 'survival'
15 (30). Age is accounted for through the flexible baseline in Cox regression. We initially performed Cox
16 regression with penalized splines of s-25(OH)D (31) to examine the trends in hip fracture hazard over
17 the full distribution of s-25(OH)D. In addition, we examined hazard ratios according to quartiles of s-
18 25(OH)D. Quartile limits were based on distribution of 25(OH)D in the subcohort. Gender-specific
19 quartile limits were used in the gender-specific analyses. Additional analyses were performed using
20 pooled month-specific quartiles of s-25(OH)D to account for seasonal variation (32). We also
21 performed a subgroup analysis including only those who did not report prevalent osteoporosis at
22 baseline examination, and we performed additional study center-specific analyses where we
23 scrutinized the association between s-25(OH)D and hip fracture within each study center. Potential
24 confounders considered were BMI, cigarette smoking, physical inactivity, education, and self-rated
25 health. Tests and plots of Schoenfeld residuals (31) revealed non-proportional hazards for study
26 center, suggesting a time-dependent effect of study center on hip fracture hazard. Using strata allowing
27 for different baseline hazards by study center yielded virtually identical results as when including the
28 variable as a factor. Study center is therefore included as an adjustment variable.

1 RESULTS

2 Incident hip fractures

3 During a median observation time of 8.2 years, 1232 individuals (340 men (3.4%) and 892 women
4 (7.5%)) suffered a hip fracture. The randomly sampled subcohorts included 1502 individuals of whom
5 93 became cases. Intact frozen serum samples were obtained and successfully analyzed for 2526
6 (95.6% of those selected) participants. Missing serum was equally distributed among cases and non-
7 cases. The number included in the presented analyses are 2500 with valid BMI data (94.7% of those
8 selected), of whom 1162 hip fracture cases (Fig. 1).

10 Characteristics of the study population

11 Baseline characteristics according to whether participants suffered a hip fracture or not during the
12 observation period are shown in Table 1. Cases were on average older at baseline, and a higher
13 proportion of cases reported previous fractures. Among women, cases also had significantly lower
14 BMI, poorer self-rated health, higher smoking prevalence, and a higher prevalence of osteoporosis.
15

16 Serum 25-hydroxyvitamin D

17 Overall median (25,75-percentile) s-25(OH)D in the randomly sampled subcohort was 53.5 (42.2,
18 67.8) nmol/l. S-25(OH)D was slightly higher in men than in women (median 55.5 vs. 52.5 nmol/l,
19 $p=0.07$). It was similar across study centers except in HUBRO where it was higher (median 61.6
20 nmol/l, $p<0.001$ for HUBRO vs. the other study centers). Baseline characteristics in the subcohorts
21 according to quartiles of s-25(OH)D are shown in Table 2. In men, participants were older in the
22 highest s-25(OH)D quartile, and the prevalence of smoking and physical inactivity was lower at higher
23 s-25(OH)D although this was not statistically significant. In women there was a clear inverse
24 relationship between BMI and s-25(OH)D (Spearman's $\rho = -0.11$ ($p=0.001$)).

26 Risk of hip fracture according to serum 25-hydroxyvitamin D

27 We observed an inverse association between s-25(OH)D and hazard of hip fracture (Fig. 2), with
28 increasing risk estimates across decreasing quartiles of s-25(OH)D (Table 3). Cox regression with

1 penalized splines revealed a linear association between s-25(OH)D and HR of hip fracture (p=0.008).
2 When entering s-25(OH)D as a continuous variable, HR (95% CI) was 1.13 (1.03, 1.25) per 25 nmol/l
3 lower s-25(OH)D in a model accounting for age, gender, study center, and BMI (p=0.014).
4
5 Those with s-25(OH)D in the lowest quartile (<42.2 nmol/l) had a statistically significant 38% (95%
6 CI 9-74%) increased risk of hip fracture compared with the highest quartile (>=67.9 nmol/l) in a
7 model accounting for age, gender, study center, and BMI (Table 3). The trend across quartiles was
8 statistically significant (p=0.009). The association was statistically significant in men (HR 1.65, 95%
9 CI 1.04-2.61) but not in women (HR 1.25, 95% CI 0.95-1.65).
10
11 The interaction term between gender and s-25(OH)D on HR of hip fracture was not statistically
12 significant. The association in the genders combined analysis persisted after adjustment for calendar
13 month of blood sample (HR 1.34, 95% CI 1.05-1.70). Further adjustment for cigarette smoking,
14 physical inactivity and self-rated health yielded similar results. In additional analysis using month-
15 specific quartiles of s-25(OH)D as exposure (32), HR was 1.32 (95% CI 1.04-1.67) in men and women
16 combined in a model including age, gender, study center, and BMI.
17
18 Excluding those with self-reported osteoporosis at baseline strengthened the association somewhat:
19 HR 1.49 (95% CI 1.16-1.91) for Q1 vs. Q4 in genders combined, and HR 1.34 (95% CI 1.00-1.81) in
20 women, in the model including age, study center, and BMI. The association in men was not influenced
21 by excluding those with osteoporosis due to the very low prevalence of osteoporosis in men.
22
23 In additional analyses restricted to each study center, a similar association between s-25(OH)D and
24 risk of hip fracture was observed within all study centers. The risk estimates were higher in men than
25 in women, except in Tromsø where the risk estimate was higher in women. However, the only
26 significant associations were seen in HUBRO (180 hip fractures) where the overall HR (95% CI) for
27 Q1 vs. Q4 was 2.03 (1.12-3.69) in a BMI-adjusted model.
28

1 **DISCUSSION**

2 In this large case-cohort study we observed a modest inverse association between s-25(OH)D and hip
3 fracture during up to 10.7 years of follow-up. In our community-dwelling elderly population, there
4 was a 38% (95% CI 9-74%) increased risk of hip fracture in the lowest (<42.2 nmol/l) compared with
5 the highest quartile (\geq 69.7 nmol/l) of 25-hydroxyvitamin D after accounting for the effects of age,
6 gender, study center, and BMI.

7
8 In order to appraise our results relative to previous studies, we have plotted relative risks with 95%
9 confidence intervals for hip fracture at 25-hydroxyvitamin D concentrations below 50 nmol/l in
10 prospective studies including our own (Fig. 3). In addition to our study, we identified published data
11 from five prospective studies on baseline s-25(OH)D in a total of 843 Caucasian subjects with hip
12 fracture (216 men and 627 women) (14-17, 33). Variations in biochemical assays, statistical
13 adjustments, age differences and other discrepancies between populations may hamper comparisons
14 between studies. However, all published studies reporting relative risk estimates for hip fractures
15 found estimates in favour of an increased risk at lower 25-hydroxyvitamin D, although with varying
16 precision.

17
18 There is a large variation in 25-hydroxyvitamin D concentrations from different assays and
19 laboratories in the same blood samples (34, 35). We therefore chose to present hazard ratios according
20 to relative distribution (quartiles) rather than absolute limits. In our data, the 25- and 75-percentile
21 (42.2 and 67.8 nmol/l) fell close to the commonly used cutoffs for defining vitamin D insufficiency or
22 sufficiency; 50 and 75 nmol/l. As expected, in additional analyses with these commonly accepted
23 limits, the results were similar, with a statistically significant 35% increased risk in subjects with
24 levels <50 compared with >75 nmol/l when adjusting for gender, study center and BMI. These results
25 suggest a preventive effect of levels above 75 nmol/l compared with levels below 50 nmol/l. Hip
26 fracture risk decreased continuously with increasing s-25(OH)D, with the steepest and most consistent
27 decrease at levels in the magnitude of 40-60 nmol/l (Fig. 2). Additional analyses yielded significantly

1 increased hazard ratios for hip fracture at levels <50 compared with 50-75 nmol/l (HR 1.21, 95% CI
2 1.01-1.45), but not at levels 50-75 nmol/l compared with >75 nmol/l (HR 1.12, 95% CI 0.88-1.42).

3
4 Excluding those reporting osteoporosis at baseline strengthened the association between low 25-
5 hydroxyvitamin D and risk of hip fracture somewhat. Current guidelines for treatment of osteoporosis
6 recommend daily vitamin D supplementation in combination with calcium, due to its well-documented
7 fracture prevention effect (18, 19). Women with diagnosed osteoporosis may have improved their
8 vitamin D status. The prevalence of osteoporosis was as high in the highest as in the lowest quartile of
9 s-25(OH)D (Table 2B). The finding of a U-shaped association between 25(OH)D status and frailty in
10 older women participating in the Study of Osteoporotic Fractures (36) also supports this notion.

11
12 Osteoporosis is a multifactorial disorder, and vitamin D may not be a major determinant of bone
13 strength in populations with generally adequate vitamin D and calcium status. In spite of living at high
14 latitude, the population in Norway has earlier been shown to have a relatively good vitamin D status
15 (37), likely owing to the country's coastal location with strong traditions of fatty fish and cod liver oil
16 consumption, as well as an active sun-seeking attitude. Nutritional factors other than vitamin D may
17 also affect fracture risk and may differ between populations. Fatty fish and cod liver oil are rich in
18 retinol, a substance that may reduce the effect of the active vitamin D hormone through competition
19 for the nuclear receptor. As retinol intake is high in Scandinavian countries, it would be of interest to
20 study whether high retinol influences the observed association between serum 25(OH)D and hip
21 fracture.

22

23 **Strengths and limitations**

24 The strengths of this study include the population-based approach, inviting age-specific samples of the
25 general population, and the prospective design with up to 10.7 years follow-up. The representativeness
26 is, however, limited by the participation rates. These varied between the study centers. In the age
27 groups included in this study, the overall attendance rates were 82% in Tromsø IV, 81% in HUNT 2,
28 77% in HUSK, and 53% in HUBRO.

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As is common in cohort studies, the categorization of exposure was based on a single baseline measurement. However, data from the Tromsø study suggest a satisfactory tracking of individual 25(OH)D levels in serum over 14 years, similar to that of blood pressure and serum lipids (38).

The large number of events is a major strength. As far as we are aware, this is the largest existing study with hip fracture outcome and biochemical measurements at baseline, with serum sample analyses and a large number of covariates available for 1175 subjects (868 women and 307 men) who suffered a hip fracture during the observation period. We thus had sufficient statistical power to detect a risk increase of 30% in the lowest quartile of 25-hydroxyvitamin D compared with the three higher quartiles.

Another strength is that the measures of exposure and outcome were objective and retrieved from independent sources outside the baseline data collection settings. All serum samples were analyzed in the same laboratory, by HPLC-APCI-MS, a highly valid 25(OH)D assay (34, 39). Samples from cases and non-cases were handled and analyzed simultaneously, biobank and laboratory personnel being blinded to case status.

Heterogeneity of different health studies included in a multicenter study may introduce limitations. In this study, HR of hip fracture did not differ significantly according to study center (not shown), and analyses stratified by study center revealed the same trend in the association between s-25(OH)D and hip fracture in all regions.

The four health studies are part of the CONOR collaboration (24) and were therefore coordinated to a large extent. Some variables were collected in a standard way, such as blood samples, anthropometric measurements, and common questions including physical activity, education, chronic diseases, and self-rated health. A limitation is that we had poor data on diet and supplement use, and were unable to assess the influence of dietary calcium intake. However, results from previous studies (14, 15, 17)

1 suggest that taking into account variations in dietary calcium intake in a population of home-dwelling
2 elderly men and women in an observational cohort study would not influence the observed
3 associations substantially.

4

5 **Conclusion**

6 In this prospective case-cohort study, the largest of its kind, we found an increased risk of hip fracture
7 in subjects in the lowest quartile of serum 25-hydroxyvitamin D compared with the highest quartile. In
8 accordance with findings of previous community-based studies, low vitamin D status was a modest
9 risk factor for hip fracture in our population.

10

1 **TABLE 1.** Baseline characteristics comparing participants who experienced a hip fracture with participants who were free of hip fracture until
 2 end of follow-up. A NOREPOS study

	Men				Women			
	n	No hip fracture ¹ n=400	Hip fracture n=307	p value ²	n	No hip fracture ¹ n=951	Hip fracture n=868	p value ²
Age, mean (SD) years	707	71.8 (3.9)	73.1 (3.2)	<0.001	1819	72.2 (3.9)	73.5 (3.4)	<0.001
s-25(OH)D, mean (SD) nmol/l	707	57.8 (20.5)	55.9 (19.8)	0.20	1819	55.7 (21.1)	55.2 (21.7)	0.64
Body mass index, mean (SD) kg/m ²	701	26.4 (3.8)	25.9 (3.8)	0.12	1799	27.4 (4.5)	25.8 (4.4)	<0.001
Body height, mean (SD) cm	703	173.7 (5.8)	174.5 (6.4)	0.065	1801	159.9 (5.8)	160.2 (6.1)	0.45
Education, mean (SD) years	639	8.9 (4.1)	8.3 (3.5)	0.070	1526	7.5 (3.1)	7.5 (3.1)	1.00
Good or very good self-rated health, n (%)	700	235 (59.2)	163 (53.8)	0.18	1788	506 (54.1)	393 (46.1)	0.001
Daily cigarette smoker, n (%)	681	88 (22.8)	82 (27.8)	0.16	1745	165 (18.3)	202 (24.0)	0.004
Inactive during leisure time, n (%)	591	20 (6.0)	21 (8.2)	0.36	1368	96 (13.1)	98 (15.4)	0.27
History of hip fracture, n (%)	616	10 (2.8)	18 (6.9)	0.026	1455	39 (5.1)	71 (10.4)	<0.001
History of wrist fracture, n (%)	616	32 (8.9)	40 (15.6)	0.016	1511	220 (27.6)	285 (39.9)	<0.001
Osteoporosis, n (%)	597	5 (1.5)	3 (1.2)	1.00	1453	84 (11.1)	164 (23.6)	<0.001
Estrogen therapy, n (%)	-	-	-	-	1819	79 (8.3)	71 (8.2)	0.99

3

4 ¹ Subcohort excluding cases (16 men, 71 women)

5 ² ANOVA for continuous variables, chi square test for factor variables. Fisher's Exact test for osteoporosis in men due to low cell counts.

1 **TABLE 2A.** Baseline characteristics of male subcohort across quartiles of serum 25-hydroxyvitamin D. A NOREPOS study
 2

	<i>n</i>	<i>1st Quartile</i> 9.9-43.3 nmol/l <i>n=104</i>	<i>2nd Quartile</i> 43.6-55.4 nmol/l <i>n=104</i>	<i>3rd Quartile</i> 55.6-69.1 nmol/l <i>n=104</i>	<i>4th Quartile</i> 69.2-156.4 nmol/l <i>n=104</i>	<i>p value</i> ¹
S-25(OH)D, mean (SD) nmol/l	416	34.0 (7.5)	50.0 (7.5)	61.7 (4.1)	83.7 (15.9)	<0.001
Age, mean (SD) years	416	71.9 (4.0)	72.1 (3.8)	71.0 (3.6)	72.5 (4.0)	0.044
Study center:						
Tromsø (Tromsø IV), n (%)	416	11 (10.6)	15 (14.4)	13 (12.5)	8 (7.7)	
Nord-Trøndelag (HUNT 2), n (%)		68 (65.4)	61 (58.7)	65 (62.5)	58 (55.8)	
Hordaland (HUSK), n (%)		19 (18.3)	14 (13.5)	16 (15.4)	14 (13.5)	
Oslo (HUBRO), n (%)		6 (5.8)	14 (13.5)	10 (9.6)	24 (23.1)	0.041
Season of blood sample:						
Summer; Apr-Sep, n (%)	416	35 (33.7)	44 (42.3)	51 (49.0)	48 (46.2)	
Winter; Oct-Mar, n (%)		69 (66.3)	60 (57.7)	53 (51.0)	56 (53.8)	0.13
Body mass index, mean (SD) kg/m ²	412	27.0 (4.3)	26.1 (3.4)	25.9 (4.0)	26.3 (3.4)	0.22
Body height, mean (SD) cm	414	173.0 (5.9)	173.8 (5.6)	174.0 (5.6)	174.0 (6.1)	0.56
Education, mean (SD) years	381	8.6 (4.0)	9.1 (4.1)	9.1 (4.3)	8.6 (3.9)	0.69
Self-rated health, good or very good, n (%)	413	55 (53.4)	62 (59.6)	67 (65.0)	58 (56.3)	0.36
Daily cigarette smoker, n (%)	401	26 (25.5)	26 (26.8)	25 (25.5)	15 (14.4)	0.12
Inactive during leisure time, n (%)	349	8 (9.9)	6 (6.9)	3 (3.2)	3 (3.4)	0.21
History of hip fracture, n (%)	372	0	4 (4.3)	3 (3.3)	3 (3.2)	0.22
History of wrist fracture, n (%)	374	7 (7.6)	11 (11.8)	6 (6.3)	12 (12.8)	0.36
Osteoporosis, n (%)	357	1 (1.1)	1 (1.2)	1 (1.1)	2 (2.2)	0.94

3
4

¹ ANOVA for continuous variables, Chi Square test for factor variables. Fisher's Exact test for physical inactivity, history of hip fracture, and osteoporosis in men due to low cell counts.

1 **TABLE 2B.** Baseline characteristics of female subcohort across quartiles of serum 25-hydroxyvitamin D. A NOREPOS study
 2

	<i>n</i>	<i>1st Quartile</i> 4.5-41.4 nmol/l <i>n=256</i>	<i>2nd Quartile</i> 41.6-52.5 nmol/l <i>n=255</i>	<i>3rd Quartile</i> 52.5-67.0 nmol/l <i>n=255</i>	<i>4th Quartile</i> 67.0-193.9 nmol/l <i>n=256</i>	<i>p value</i> ¹
S-25(OH)D, mean (SD) nmol/l	1022	32.2 (6.4)	47.1 (3.0)	59.5 (4.2)	84.2 (15.1)	
Age, mean (SD) years	1022	72.5 (3.9)	71.8 (4.0)	72.4 (3.7)	72.6 (3.7)	0.10
Study center:						
Tromsø (Tromsø IV), n (%)	1022	24 (9.4)	41 (16.1)	30 (11.8)	21 (8.2)	
Nord-Trøndelag (HUNT 2), n (%)		161 (62.9)	149 (58.4)	149 (58.4)	132 (51.6)	
Hordaland (HUSK), n (%)		45 (17.6)	31 (12.2)	37 (14.5)	45 (17.6)	
Oslo (HUBRO), n (%)		26 (10.2)	34 (13.3)	39 (15.3)	58 (22.7)	<0.001
Season of blood sample:						
Summer; Apr-Sep, n (%)	1022	92 (35.9)	105 (41.2)	107 (42.0)	128 (50.0)	
Winter; Oct-Mar, n (%)		164 (64.1)	150 (58.8)	148 (58.0)	128 (50.0)	0.014
Body mass index, mean (SD) kg/m ²	1010	27.9 (4.8)	27.6 (4.3)	27.0 (4.6)	26.7 (4.3)	<0.001
Body height, mean (SD) cm	1011	159.8 (6.0)	160.2 (5.8)	160.1 (6.0)	159.9 (5.5)	0.80
Education, mean (SD) years	861	7.3 (2.8)	7.4 (3.0)	7.6 (3.2)	7.7 (3.2)	0.32
Self-rated health, good or very good, n (%)	1005	120 (47.8)	137 (54.2)	128 (51.4)	147 (58.3)	0.11
Daily cigarette smoker, n (%)	972	47 (19.3)	44 (18.1)	42 (17.4)	49 (20.0)	0.88
Inactive during leisure time, n (%)	779	32 (17.4)	29 (15.5)	22 (11.2)	23 (10.9)	0.16
History of hip fracture, n (%)	829	14 (7.0)	7 (3.3)	15 (7.5)	12 (5.6)	0.25
History of wrist fracture, n (%)	857	53 (25.6)	59 (26.8)	64 (30.6)	65 (29.4)	0.65
Osteoporosis, n (%)	817	26 (13.1)	27 (12.7)	17 (8.8)	28 (13.2)	0.47
Estrogen therapy, n (%)	1022	19 (7.4)	15 (5.9)	22 (8.6)	25 (9.8)	0.41

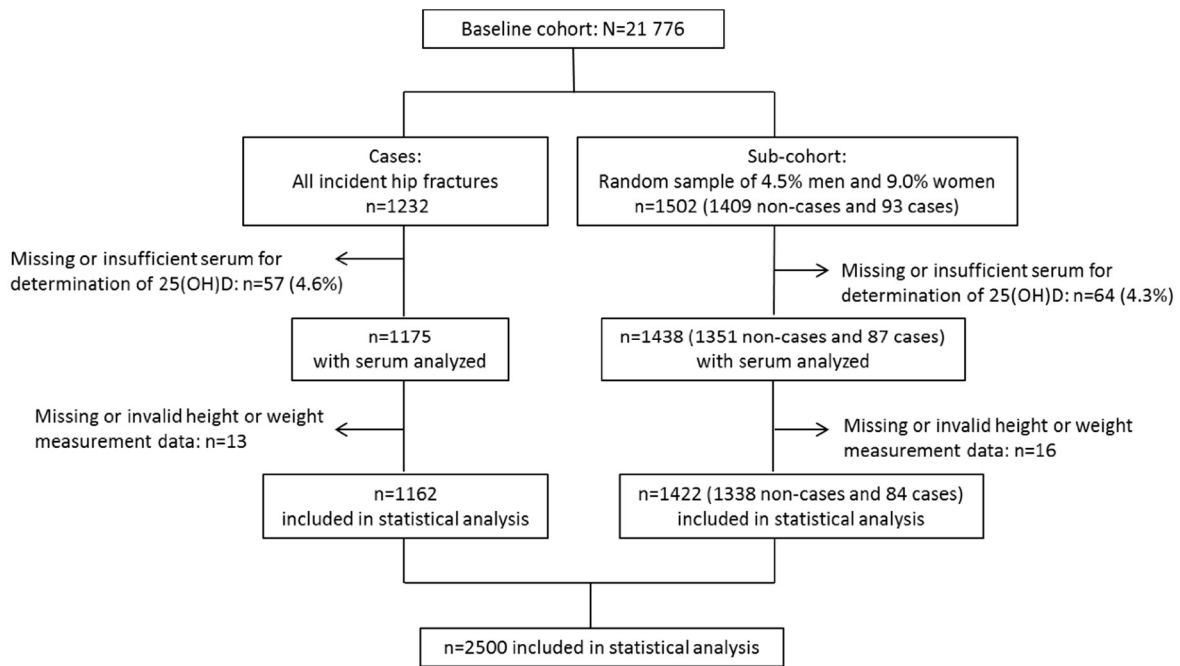
3
 4 ¹ ANOVA for continuous variables, chi square test for factor variables.
 5

1 **TABLE 3.** Hazard ratios for hip fracture according to quartiles of serum 25-hydroxyvitamin D concentration (nmol/l) in NOREPOS.
 2 Cox proportional hazards regression adjusted for a gender-stratified case-cohort design. A NOREPOS study ^{a)}
 3

	25(OH)D range, nmol/l	# hip fx	HR¹	95% CI¹	HR²	95% CI²	HR³	95% CI³
All								
Q1	4.5-42.1	317	1.27	1.01-1.59	1.38	1.09-1.74	1.34	1.05-1.70
Q2	42.2-53.5	294	1.13	0.90-1.43	1.16	0.92-1.46	1.13	0.90-1.44
Q3	53.5-67.8	272	1.13	0.90-1.42	1.12	0.88-1.41	1.10	0.87-1.39
Q4	67.9-250.0	279	1.00	(ref.)	1.00	(ref.)	1.00	(ref.)
Men								
Q1	9.9-43.3	82	1.56	0.99-2.46	1.65	1.04-2.61	1.55	0.95-2.51
Q2	43.6-55.4	74	1.29	0.82-2.01	1.27	0.81-1.99	1.24	0.77-1.98
Q3	55.6-69.1	69	1.10	0.69-1.74	1.06	0.66-1.69	1.07	0.66-1.73
Q4	69.2-156.4	79	1.00	(ref.)	1.00	(ref.)	1.00	(ref.)
Women								
Q1	4.5-41.5	227	1.14	0.87-1.48	1.25	0.95-1.65	1.23	0.92-1.63
Q2	41.6-52.5	208	1.06	0.81-1.38	1.10	0.84-1.45	1.09	0.82-1.44
Q3	52.5-67.0	212	1.12	0.86-1.47	1.16	0.88-1.52	1.14	0.86-1.49
Q4	67.0-250.0	211	1.00	(ref.)	1.00	(ref.)	1.00	(ref.)

4
 5 a) Quartiles based on distribution in the subcohorts. Data set restricted to those with valid BMI measurement (1162 cases, 1338 noncases in subcohort)
 6 1) age, gender, and study center
 7 2) age, gender, study center, and BMI
 8 3) age, gender, study center, BMI, month of blood sample

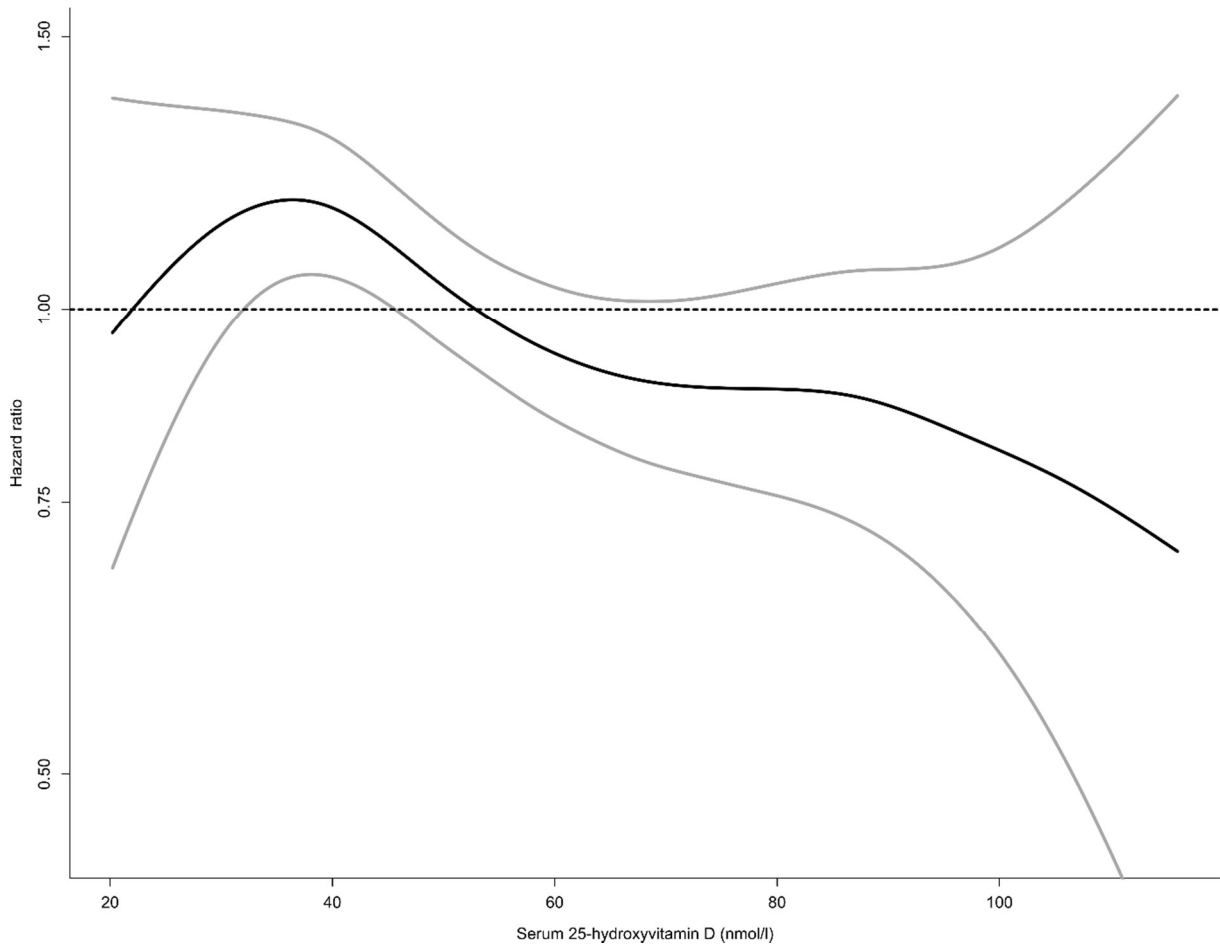
1 **FIG. 1.** Inclusion of participants to the case and subcohort groups. A NOREPOS study¹
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 6 ¹ The cases present in the subcohort (n=93 originally sampled; n=84 included in statistical analysis) are
 7 duplicates of hip fracture patients occurring in the case group
 8

9

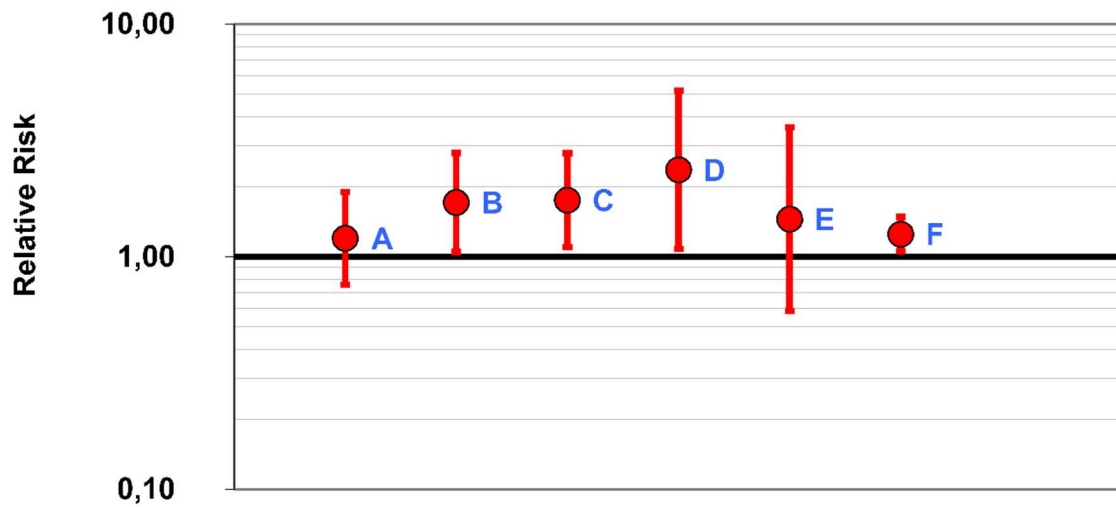
1 **FIG. 2.** Distribution of hazard ratio (solid line) with 95% CI (dotted lines) for hip fracture
 2 across the distribution of serum 25-hydroxyvitamin D (nmol/l). A NOREPOS study¹
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¹ Based on Cox proportional hazards regression with penalized splines of s-25(OH)D in a model including age, gender, study center, and BMI, with robust variance estimates and inverse probability weighting for sampling fraction to the subcohort. HR=1 represents average hazard in the data. S-25(OH)D range from 1- to 99-percentile in the subcohort is included.

1 **FIG. 3.** Relative risk estimates with 95% confidence intervals for hip fracture at plasma 25-
 2 hydroxyvitamin D < 50 nmol/l in prospective studies ¹
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4
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 7 ¹ Diagram made using Rothman's Episheet (40). A: Study of Osteoporotic Fractures, women, 133 hip fractures,
 8 RIA, <47.5 nmol/l vs. >=47.5 nmol/l (33); B: Women's Health Initiative, women, 400 hip fractures, RIA, <=
 9 47.5 nmol/l (25-percentile) vs. >=70.7 nmol/l (75-percentile) (17); C: NHANES III, men and women, 156 hip
 10 fractures, RIA, (15); D: mrOS, men, 81 hip fractures, LC-MS/MS, <= 47.5 nmol/l (25-percentile) vs. >=70.7
 11 nmol/l (75-percentile) (16); E: Uppsala Longitudinal Study of Adult Men, men, 73 hip fractures, HPLC-APCI-
 12 MS, 25(OH)D <40 nmol/l (5-percentile) vs. >= 40 nmol/l (14); F: NOREPOS, men and women, HPLC-APCI-
 13 MS, 1162 hip fractures.

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