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# **Vitamin D Insufficiency Over 5 Years is Associated with Increased Fracture Risk – An Observational Cohort Study of Elderly Women**

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## **Conflict of Interest**

David Buchebner, Fiona McGuigan, Paul Gerdhem, Johan Malm, Martin Ridderstråle and Kristina Åkesson declare that they have no conflict of interest.

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## SUMMARY

This study of elderly Swedish women investigated the association between chronic vitamin D insufficiency and osteoporotic fractures occurring between ages 80-90. The incidence and risk of hip and major osteoporotic fractures was significantly higher in elderly women with low vitamin D levels maintained over 5 years.

## ABSTRACT

**Purpose:** Vitamin D insufficiency among the elderly is common, however relatively little is known about the effects of long-term hypovitaminosis D on fracture. We investigated sequential assessment of serum 25(OH)D at age 75 and 80 to determine if continuously low 25(OH)D levels are associated with increased 10-year fracture incidence.

**Methods:** 1044 Swedish women from the population based OPRA cohort, all 75 years old, attended at baseline (BL); 715 attended at 5 years. S-25(OH)D was available in 987 and 640 respectively and categorized as: <50 (Low); 50-75 (Intermediate); >75 nmol/L (High). Incident fracture data was collected with maximum follow-up to 90 years of age.

**Results:** Hip fracture incidence between age 80-85 was higher in women who had low 25(OH)D at *both* baseline and 5y (22.2% (Low) vs 6.6% (High);  $p=0.003$ ). Between age 80-90 hip fracture incidence was more than double that of women in the high category (27.9% vs 12.3%;  $p=0.006$ ). Within 5-years 50% of women in the continuously low group compared to 34% in the continuously high 25(OH)D group had an osteoporotic fracture ( $p=0.004$ ) while 10-year incidence was higher compared to the intermediate ( $p=0.020$ ) but not the high category ( $p=0.053$ ). The 10-year relative risk of hip fracture was almost 3 times higher and osteoporotic fracture risk almost doubled for women in the lowest 25(OH)D category compared to the high category (HR 2.7 and 1.7;  $p=0.003$  and 0.023 respectively).

**Conclusion:** In these elderly women, 25(OH)D insufficiency over 5-years was associated with increased 10-year risk of hip and major osteoporotic fractures.

**Key words:** Vitamin D, Fracture, Elderly Women, Longitudinal

## INTRODUCTION

Vitamin D, a key player in calcium homeostasis, is essential for normal bone turnover. However there remains considerable controversy over the relationship between low vitamin D status and risk of hip and other osteoporotic fractures. Hence, the clinical utility of vitamin D (25(OH)D) measurement as a risk marker for fracture is unresolved [1-4].

Numerous reports state that fracture, and hip fracture in particular, is associated with low vitamin D levels [5-7], but it has proven difficult to use supplementary intervention to reduce fracture risk [8, 9]. Meta-analyses indicate that vitamin D in combination with calcium may reduce fracture risk, but the number of randomized controlled trials are few and with diverging results [10-13]. Possible benefits have been seen primarily in the elderly but not in the general population [4], which may be in line with the reduced ability to activate vitamin D from skin exposure and reduced exposure and nutritional intake among the elderly and very elderly [14]. The findings are similar in a systematic review where, despite lower serum 25(OH)D in hip fracture patients, neither high nor low doses of supplementation influenced hip fracture risk [15].

An additional complication lies in the difficulty in determining threshold values for vitamin D sufficiency at different ages, together with variation in 25(OH)D assay consistency [16]. Nevertheless, an operational approach is to consider <25 nmol/L as deficient, <50 nmol/L as insufficient, >50 nmol/L as sufficient and >75 nmol/L as a desirable target in the elderly [14], an approach allowing for comparison between studies although the optimal level may lie somewhere between these intervals [17, 18].

Fragility fractures increase with increasing age and particularly from age 70 until end of life there are marked increases in non-vertebral fractures i.e. distal forearm, proximal humerus and hip fractures. Fractures in the elderly are dependant both on bone mineral density (BMD) and the risk of falling and 25(OH)D has been shown to influence muscle strength and falls [19, 20]. Although in Sweden many dairy products are fortified with vitamin D and outdoor walking is a preferred activity among the elderly, fracture rates are among the highest worldwide [21] and the role of hypovitaminosis D is unclear [22].

We showed previously that baseline serum 25(OH)D levels below 50 nmol/L were associated with an increased risk of fragility fracture during a 3-year follow up in the population-based cohort of elderly Swedish women (OPRA) [22], but for how long a 25(OH)D measurement predicts fracture in the long term is unknown.

In the recent NOREPOS study [23] an inverse association between baseline 25(OH)D and hip fractures for up to 11 years was reported, which is in line with our own observations on bone turnover markers and increased fracture risk for up to a decade [24]. However, most studies evaluating the utility of serum 25(OH)D rely on single measurements, which essentially are snap-shots of vitamin D status, providing grounds for assumption, rather than established information, on skeletal health effects. Subsequently, the effect of prolonged hypovitaminosis D on bone metabolism in elderly women has not been fully confirmed.

While the association between a single vitamin D measurement and hip fracture has been investigated in a number of studies [25-28], the effect of prolonged vitamin D insufficiency in this age group has not been adequately investigated. The primary objective of this study was therefore to investigate the association between vitamin D insufficiency sustained over 5 years with hip and major osteoporotic

fractures in a population-based cohort of elderly women. To do this we have assessed serum 25(OH)D measurements at two consecutive visits 5 years apart and followed incident fractures for up to 10 years and made comparison with a single 25(OH)D measurement at age 75 and 80.

## **MATERIAL AND METHODS**

### *Subjects*

The Malmö Osteoporotic Prospective Risk Assessment (OPRA) cohort is a longitudinal population-based cohort of elderly women aged 75 years ( $75.2 \pm 0.1$ ; range 75.0-75.9) who were randomly selected from the population files, Malmö, Sweden, between 1995 and 1999. No exclusion criteria were applied. A total of 1604 women were invited (33% of all women in this age group living in the city during the study period) and 1044 (65%) attended the baseline (BL) investigation [29]. The women returned at 5 years ( $n=715$ ) and 10 years ( $n=382$ ) for follow-up. The investigation included bone mineral density (BMD) measurements, anthropometrics, detailed questionnaire on health, nutrition, medication and lifestyle, and blood and urine samples were collected at all visits.

Participants gave written informed consent and the Regional Ethical Review Board in Lund approved the study, which was performed according to the principles of the Helsinki declaration.

### *Vitamin D measurements*

Blood samples were collected (non-fasting) before noon and serum was stored at  $-80^{\circ}\text{C}$  until analysis. Serum concentration of 25(OH)D, which is stable in stored serum [30], was assessed by liquid chromatography mass spectrophotometry (LC-MS), with an inter-assay co-efficient of variation of 3-6%. Assays were performed according to accredited methods at the Department of Clinical Chemistry, Malmö, Skåne University Hospital Sweden, which also participates in DEQAS, a Vitamin D External Quality Assessment Scheme. For this study serum samples were available for 1011 and 640 at baseline and 5y, respectively.

### *Incident Fractures*

Information on fractures was continuously registered through the X-rays files at the Radiology Department, Malmö, Skåne University Hospital, by using the personal identification number based on birth date allotted to every Swedish citizen. This department, where all X-ray files have been saved since the beginning of the last century, serves the Department of Orthopaedics which is the only unit treating adult and paediatric fractures in the catchment area, and hence loss to follow-up is exceptionally low [31]. Prevalent fractures (i.e. prior to inclusion in the study at age 75) were registered, as previously

reported [32], although here we report incident fracture data collected until October 31st 2012, providing a maximum follow-up for fracture of 17.2 years (mean 13.1 years).

In this report our primary outcomes were hip fractures and the major osteoporotic fractures i.e. hip, vertebra, distal radius, shoulder, which are used in the FRAX fracture risk assessment tool (<http://www.shef.ac.uk/FRAX>) as a group and per fracture. Fractures resulting from pathology and high energy were excluded.

### *Statistical analyses*

Vitamin D data was normally distributed (Shapiro-Wilks test). For the purposes of analysis, women were categorized according to vitamin D status at each time-point using the currently accepted values for vitamin D sufficiency and insufficiency: Low (<50 nmol/L) ; Intermediate ( 50-75 nmol/L) and High (>75 nmol/L) [33, 34].

Figure 1 illustrates the strategies used for the analysis of fracture incidence in relation to 25(OH)D. To reflect vitamin D status over an extended time period, which may not be captured by a single 'snap-shot' 25(OH)D measurement, we classified women according to whether they remained in the same 25(OH)D category at both baseline and 5 year follow-up (n=339), on the assumption that they had maintained that status throughout the 5 years between visits. Women who changed category from age 75 to 80 years were excluded from the analyses. Fracture incidence was determined from age 80 onwards and women who had fractured between baseline were also excluded from the analyses in order to rule out fracture as a potential confounder for 25(OH)D status.

To enable comparison we also analysed the relationship between a single 25(OH)D measurement and fracture incidence over 5- and 10-years. For these analyses we first assessed the association between s - 25(OH)D measured at age 75 and fractures occurring between age 75-80, and between age 75-85. We then 'reset' the baseline, taking age 80 as the starting point, to determine if the association between s - 25(OH)D and 5-year fracture incidence could be replicated at this age i.e. between age 80-85. Additionally we have classified the cohort according to quintiles of 25(OH)D for this purpose of fully describing 25(OH)D levels over follow-up.

Descriptive data are reported as mean and standard deviation (SD) or number and percentage. Independent predictors of fracture were identified using regression analysis including 25(OH)D, BMD, body mass index (BMI), bisphosphonate use, smoking and physical activity category in the model. The Pearson Chi<sup>-2</sup> test was used to compare categories of 25(OH)D and fracture incidence. Hazard ratios (HR) and 95% confidence intervals (CI) were calculated from Cox proportional hazard models testing

association between fracture and 25(OH)D categories taking the lowest 25(OH)D category as the referent group and taking mortality into account. HR's were calculated unadjusted and adjusted for smoking, bisphosphonate use and physical activity level at age 80. To make direct comparison between 25(OH)D measured at a single and two time-points, fracture rates per 1000 person years were calculated, using 3 categories and quintiles of 25(OH)D. Incidence and rate ratio with 95% CI were estimated by Poisson distribution. *A priori* power analyses were performed based on the assumption of 0.13 g/cm<sup>2</sup> SD in BMD. The study has >80% power to detect a 0.056 g/cm<sup>2</sup> difference between equal groups at the 5% significance level.

All statistical analyses were performed using SPSS version 20.0 (SPSS, Inc., Chicago, IL). P-values of <0.05 were considered nominally significant.

## RESULTS

### *Characteristics of participants*

The clinical characteristics of the OPRA cohort participants are presented in Table 1 for the population overall at age 75 and 80.

In Table 2 the participants are stratified according to 25(OH)D category. Of the 284 women in the low 25(OH)D category at age 75, 105 were lost at follow-up due to death or unwillingness/inability to attend. Sixty eight remained in the 'low' category at age 80, while 96 women changed category. Of the 236 women in the high 25(OH)D category at age 75, 60 were lost at follow-up. 130 remained 'high' whereas 25 women changed group downwards. Overall, the population mean 25(OH)D level was higher at age 80 compared to age 75 driven by elevated 25(OH)D levels in the 'high' category at 80y (102.3 v 88.1).

During the full follow-up period, 349 women suffered 452 fractures at major osteoporotic sites (hip n=130, vertebra n=152, distal radius n=100, shoulder n=70). The majority of fractures occurred between the ages of 80 and 85 (hip 61%; major osteoporotic fractures 60%). Not unexpectedly the mean baseline 25(OH)D level was not statistically different between women without hip fracture compared to women who sustained a hip fracture at some point time during the remaining study time of almost 17 years (data not shown).

Prevalent osteoporotic fracture (i.e. before baseline at age 75) was higher among vitamin D supplemented women (29.2% vs. 15.5%; p=0.005) as was fracture incidence within 5-years of follow-up (30.1% vs. 20.2%; p=0.013). However, among the women taking supplements 57% of fractures sustained between ages 75-80 occurred in women categorized as 'low'. Fifty-five women, 5.3% of the study population, had a prevalent hip fracture and their 25(OH)D levels at 75y did not differ from the



population mean (62 nmol/L) or from women without hip fracture (62 nmol/L). Only 7 of these women were taking vitamin D supplements at baseline.

*Prolonged low 25(OH)D for 5 years - Fracture incidence using duplicate 25(OH)D measurements*

To investigate whether persistent low 25(OH)D levels are associated with increased fracture incidence, women who remained in the same 25(OH)D category at age 75 and 80 were considered to have maintained that level throughout the intervening period. Fracture incidence was evaluated in the short term i.e. 5-years (from age 80 to 85), and in the long term i.e. 10-years (80-90).

Women who had continuously low 25(OH)D levels had a higher incidence of hip fracture during the following 5-years compared to women with higher levels (low 22.2%; intermediate 10.5%; high 6.6%,  $p=0.028$  and  $p=0.003$ ). Similarly, the proportion who suffered a major osteoporotic fracture was also higher (low 50%; Intermediate 28%; high 34%;  $p=0.004$  and  $p=0.029$ ) (Table 3A).

Women who had continuously low 25(OH)D levels also had a higher incidence of fracture in the long term (80-90y), with a hip fracture incidence that was more than double that of women in the high category (27.9% v 12.3%;  $p=0.006$ ). Even women who would be considered vitamin D sufficient with intermediate levels (50-75nmol/L), had a higher fracture incidence (21.3% v 12.3%;  $p=0.035$ ; Table 3A). The incidence of major osteoporotic fractures was also higher among women who had continuously low compared to intermediate 25(OH)D levels (50% vs. 34%;  $p=0.020$ ), but did not reach statistical significance compared with the high category (50% vs. 36.9%;  $p=0.053$ ; Table 3A).

The risk of sustaining a hip fracture during the 10 remaining years of follow-up (80-90y) was almost 3 times higher for women who had five years of continuously low, compared to women who had continuously high, levels of 25(OH)D (HR 2.7, 95% CI 1.4-5.3,  $p=0.003$ ; Figure 2A). The incidence of hip fractures per 1000 person-years was 20.7 [95% CI 12.3-32.8] in the continuously low group compared to 8.7 [95% CI 4.9-14.3] in the continuously high group (Rate Ratio 2.39; [95% CI 1.14-5.09]; Figure 3A). Similarly, the risk of sustaining a major osteoporotic fracture was nearly twice as high compared to the other groups (low vs. high: HR 1.7, 95% CI 1.1-2.6,  $p=0.023$ ; low vs. Intermediate, HR 1.8, 95% CI 1.2-2.8,  $p=0.008$ ; Figure 2B). Among women who were continuously low for 5 years, the incidence of major osteoporotic fractures was 53.0 per 1000 person-years [95% CI 38.8-70.7] compared to 27.8 per 1000 person-years [95% CI 20.5-36.9] among women who were continuously high (Rate Ratio 1.91; [95% CI 1.24-2.89]; Figure 3B).

### *Snap-shot measurement of 25(OH)D - Fracture incidence using single 25(OH)D measurement*

We first analysed s-25(OH)D measured at age 75 and 5- and 10-year fracture incidence, then 'reset' the baseline, using s-25(OH)D measured at age 80 as the starting point, to determine the 5-year fracture incidence at this age.

In general, a smaller proportion of women with high 25(OH)D levels at age 75 fractured within the following 5-year period compared to women with lower 25(OH)D (Table 3B). Although not statistically different for hip or major osteoporotic fractures, vertebral fracture incidence was more than double in the lowest compared to the highest 25(OH)D category ( $p=0.018$ ).

Resetting the baseline to age 80, we found that in the 5-year period between age 80-85, hip fracture incidence among women with the lowest 25(OH)D levels was double that of the higher categories (low vs. high  $p=0.010$  and low vs. intermediate  $p=0.019$ ; Table 3B). With the exception of the radius the incidence of other fracture types was generally lower among women with high 25(OH)D levels.

Over the 10 year period, from age 75 to 85, there was a stepwise reduction in hip fracture incidence from low to high 25(OH)D category, although not statistically different. There was no significant difference in fracture incidence per 1000 person-years (low: 13.4 [95% CI 9.1-18.1]; intermediate: 10.4 [95% CI 8.0-13.4]; high: 8.1 [95% CI 5.1-12.2]).

Women with both the lowest and highest 25(OH)D levels had a higher incidence of major osteoporotic fractures (Table 3B), although vertebral fracture incidence did not differ with 25(OH)D category (data not shown). The incidence per 1000 person-years for major osteoporotic fractures did not differ between the three categories (low: 51.4 [95% CI 43.7-59.9]; intermediate: 45.7 [95% CI 40.4-51.6]; high: 37.0 [95% CI 30.3-44.8]). To more fully explore this we also compared quintiles of s-25(OH)D and found a dose-dependent reduction in fracture incidence per 1000 person years from the lowest to highest quintiles. Risk ratio for Q1 v Q5, 1.53 [95% CI 1.16-2.01]; Q1 v Q4, 1.46 [95% CI 1.12-1.96].

The incidence of radius and shoulder fractures was not associated with elderly women's 25(OH)D status, based on either a single time-point or consecutive measurements (data not shown).

## DISCUSSION

The importance of 25(OH)D in bone metabolism has been demonstrated in several studies [1, 22, 23], however, questions remain regarding the long-term effects of elevated or low levels. Especially in older age groups, the effects of chronic, prolonged hypovitaminosis D on bone health are still unclear and a consensus has yet to be reached concerning the optimal level of s-25(OH)D for bone health in the elderly. This study, a longitudinal population based study involving 75 year old Swedish women followed until over age 90, aimed to address these questions. We therefore investigated the association between prolonged exposure to ranges of 25(OH)D and risk of incident hip and major osteoporotic fractures over an extended time period using both single and consecutive 25(OH)D measurements at age 75 and 80.

The main finding of this study was that elderly women who maintained 25(OH)D levels above 50 nmol/L between age 75 and 80 had a significantly lower incidence of both hip and major osteoporotic fractures within the upcoming five years (age 80-85). However, in order to reduce the incidence of hip fractures over a ten year period, considerably higher 25(OH)D levels appear to be necessary.

Based on a single 25(OH)D measurement at age 75 we found no association between 25(OH)D level and incident hip or major osteoporotic fractures during the subsequent 5 year period, possibly explained by the relatively small number of fractures that occurred during this time. At the age of 80 however, women with 25(OH)D levels above the recommended 50 nmol/L did have fewer hip fractures in the following 5 years between age 80 and 85. Although an association with vertebral fractures within the first 5 years, but not thereafter was observed in women with high 25(OH)D at age 75, this should be interpreted cautiously due to the relatively small number of fractures reported and spine radiographs were not standard in the study protocol. None the less, the incidence of major osteoporotic fractures per 1000 person-years was higher in the lowest quintiles of 25(OH)D at age 75, compared to the highest.

By combining consecutive measurements we demonstrated that in the short term, women who maintained a 25(OH)D level above 50 nmol/L over a prolonged period had significantly lower fracture rates for both hip and major osteoporotic fractures during the following five years (age 80-85). Over ten years this prolonged level was also associated with a lower incidence of major osteoporotic fractures, while a significant reduction of hip fractures could only be seen at serum levels above 75 nmol/L. The almost two times lower incidence of major osteoporotic fractures in the high compared to the low 25(OH)D category, mirrored the ~2 times lower risk for women who maintained a 25(OH)D level above 50 nmol/L although there was no further risk reduction from 25(OH)D levels above 75 nmol/L. On the contrary, fracture risk was slightly higher in that category.

Our observations of a long term association between 25(OH)D status and fracture confirm the results from other studies. A recent study in Swedish men with a similar follow-up time, reported that 25(OH)D levels below 40 nmol/L were associated with increased fracture risk [35] and another in which 25(OH)D predicted fracture risk for up to 10 years after being measured [36]. In elderly white women the association between 25(OH)D and fracture risk has also been previously observed [37].

Most importantly, our results also indicate the necessity of identifying and adequately treating women at risk for hypovitaminosis D in order to avoid chronic insufficiency and increased risk of fracture. Assessment of 25(OH)D in elderly women could potentially complement the recommended Fracture Risk Assessment Tool [38].

What still remains controversial is the definition of vitamin D deficiency. Previously it was defined as a serum concentration below 25 nmol/L [39] and several studies have shown an inverse relationship between PTH and 25(OH)D, reaching a plateau at concentrations between 75-100 nmol/L [40-42]. This is consistent with the suggested optimal threshold of 75-110 nmol/L for hip and non-vertebral fracture prevention [43]. The 2011 Institute of Medicine report [34] redefined vitamin D deficiency as concentrations below 50 nmol/L, whereas the Endocrine Society suggests that definitions of insufficiency and deficiency should be based on the normalization of PTH [33]. Our study provides further evidence that a 25(OH)D level of at least 50 nmol/L should be maintained in women aged 75-80 in order to reduce their risk of osteoporotic fracture, but 25(OH)D levels above 75 nmol/L seem to be required in order to achieve a significant risk reduction of hip fracture in the very elderly.

The strengths of this study include the long follow-up. The number of women with fractures is substantial, and all incident fractures have been confirmed radiographically with a satisfactory external validity [44]. These have made it possible to evaluate the relative importance of serum 25(OH)D measurements at different ages and for long and short time-periods. Generalization of the results with regard to other populations may not be possible however since this large well-characterized cohort consists of identically aged and socio-geographically similar participants. Although to our knowledge this is the largest study with repeat measurements of 25(OH)D in elderly women, an undoubted limitation of such a study is that the length of follow-up and high age of the participants also reduces the number at each follow-up. As in all epidemiological studies, confounding factors cannot be fully excluded, although being an observational study it did not interfere in the women's medication. A small number of women were on anti-resorptive treatment at baseline, and the number increased during the study period, but the results did not differ with their inclusion/exclusion. During follow-up the mean s-25(OH)D levels increased. We have ruled out inter-assay error, therefore at least part of this increase over time could be explained by the higher number of women taking vitamin D supplements at age 80 and 85 (although

duration and compliance are unknown) or a higher loss to follow-up (from unwillingness, inability or death) amongst women with low s-25(OH)D levels at baseline.

In conclusion, in this cohort of Swedish women, recruited at age 75 and followed until age 90, we found that prolonged hypovitaminosis D is associated with higher incidence of hip fractures and major osteoporotic fractures. By maintaining 25(OH)D levels above 50 nmol/L, the incidence of major osteoporotic fractures could be reduced for up to a decade but in very elderly women, 25(OH)D levels above 75nmol/L might be needed in order to achieve a significant risk reduction of hip fractures.

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**TABLE 1.** Descriptive information for the OPRA cohort at baseline and 5y follow-up

	<b>Age 75 (Baseline)</b> <b>n=1044</b>	<b>Age 80 (5 year)</b> <b>n=715</b>
Height (cm)	160.5 (5.7)	159 (5.7)
Weight (kg)	67.8 (11.7)	66.0 (11.6)
BMI (kg/m <sup>2</sup> )	26.3 (4.2)	26.1 (4.2)
Current Smokers ( <i>Number(%)</i> )	145 (14)	85 (10)
Vitamin D supplement use ( <i>Number(%)</i> )	91 (9)	113 (16)
Calcium supplement use ( <i>Number(%)</i> )	91 (9)	181 (25)
Bisphosphonate use ( <i>Number(%)</i> )	33 (3)	58 (8)
S-25(OH)D (nmol/L)	62 (19)	78 (30)
S-PTH (pmol/L)	4.7 (2.1)	4.4 (3.2)
S-Calcium (mmol/L)	2.40 (0.07)	2.41 (0.13)
S-Creatinine (umol/L)	70 (19)	74 (20)

\* Values are mean (SD) except for smoking, vitamin D, calcium and bisphosphonate use which are number (%)

**TABLE 2.** Descriptive information based on serum 25(OH)D categories at 75y and 80y

	25(OH)D Category <sup>#</sup>		
	LOW	INTERMEDIATE	HIGH
<b>AGE 75</b>	n=284 (28%)	n=491 (49%)	n=236 (23%)
S-25(OH)D (nmol/L)	40 (8)	62 (7)	88 (12)
Height (cm)	160.6 (6.0)	160.1 (5.5)	161.4 (5.5)
Weight (kg)	68.7 (12.4)	68.1 (11.0)	66.2 (11.7)
BMI (kg/m <sup>2</sup> )	26.6 (4.6)	26.5 (3.9)	25.4 (4.0)
Current Smokers ( <i>Number(%)</i> )	43 (15)	59 (12)	40 (17)
Vitamin D use ( <i>Number(%)</i> )	7 (3)	32 (6)	22 (9)
Calcium use ( <i>Number(%)</i> )	14 (5)	33 (7)	20 (8)
Bisphosphonate use ( <i>Number(%)</i> )	6 (2)	12 (2)	13 (5)
S-PTH (pmol/L)	5.2 (2.2)	4.6 (2.0)	4.1 (1.8)
S-Calcium (mmol/L)	2.39 (0.07)	2.41 (0.07)	2.41 (0.08)
<b>AGE 80</b>	n=101 (16%)	n=233 (36%)	n=306 (48%)
S-25(OH)D (nmol/L)	38 (10)	63 (7)	102 (22)
Height (cm)	158.8 (5.3)	158.7 (5.9)	159.3 (5.8)
Weight (kg)	66.4 (12.5)	66.7 (10.6)	65.8 (11.6)
BMI (kg/m <sup>2</sup> )	26.3 (4.5)	26.1 (3.8)	25.9 (4.2)
Current Smokers ( <i>Number(%)</i> )	18 (18)	18 (8)	36 (12)
Vitamin D use ( <i>Number(%)</i> )	4 (4)	18 (8)	74 (24)
Calcium use ( <i>Number(%)</i> )	5 (5)	34 (15)	114 (37)
Bisphosphonate use ( <i>Number(%)</i> )	2 (2)	7 (3)	27 (9)
S-PTH (pmol/L)	5.4 (3.9)	4.6 (3.0)	4.0 (3.1)
S-Calcium (mmol/L)	2.39 (0.13)	2.40 (0.13)	2.42 (0.12)

\*Values are mean (SD)

<sup>#</sup> 25(OH)D categories are Low (<50 nmol/L); Intermediate (50-75 nmol/L); High (>75 nmol/L)

**TABLE 3** Hip and major osteoporotic fracture incidence using A) Consecutive measurements and B) Single measurements of 25(OH)D

		25(OH)D CATEGORY <sup>#</sup>			p <sup>a</sup>	p <sup>b</sup>
		Low	Intermediate	High		
<b>A) Consecutive measurements*</b>						
5-year fracture incidence (80-85y)		<b>n= 58-66</b>	<b>n= 118-136</b>	<b>n= 110-127</b>		
	<b>Hip</b>	14 (22.2%)	13 (10.5%)	8 (6.6%)	0.003	0.028
	<b>Major osteoporotic</b>	29 (50.0%)	33 (28.0%)	37 (33.6%)	0.029	0.004
10-year fracture incidence (80-90y)		<b>n= 68</b>	<b>n= 141</b>	<b>n= 130</b>		
	<b>Hip</b>	19 (27.9%)	30 (21.3%)	16 (12.3%)	0.006	0.186
	<b>Major osteoporotic</b>	34 (50.0%)	48 (34.0%)	48 (36.9%)	0.053	0.020
<b>B) Single measurement</b>						
5-year fracture incidence (75y-80y) <sup>1)</sup>		<b>n=284</b>	<b>n=491</b>	<b>n=236</b>		
	<b>Hip</b>	14 (4.9%)	24 (4.9%)	9 (3.8%)	0.346	0.346
	<b>Major osteoporotic</b>	50 (17.6%)	81 (16.5%)	33 (14.0%)	0.158	0.381
5-year fracture incidence (80y-85y) <sup>2)</sup>		<b>n=101</b>	<b>n=233</b>	<b>n=306</b>		
	<b>Hip</b>	15 (14.8%)	16 (6.9%)	20 (6.5%)	<b>0.010</b>	<b>0.019</b>
	<b>Major osteoporotic</b>	23 (26.7%)	37 (18.0%)	57 (21.6%)	0.199	0.064
10-year fracture incidence (75y-85y) <sup>1)</sup>		<b>n=284</b>	<b>n=491</b>	<b>n=236</b>		
	<b>Hip</b>	42 (14.8%)	61 (12.4%)	23 (9.7%)	0.054	0.204
	<b>Major osteoporotic</b>	100 (35.2%)	156 (31.8%)	78 (33.1%)	0.336	0.184

Major osteoporotic fractures i.e. occurring at the hip, vertebra, distal radius, shoulder

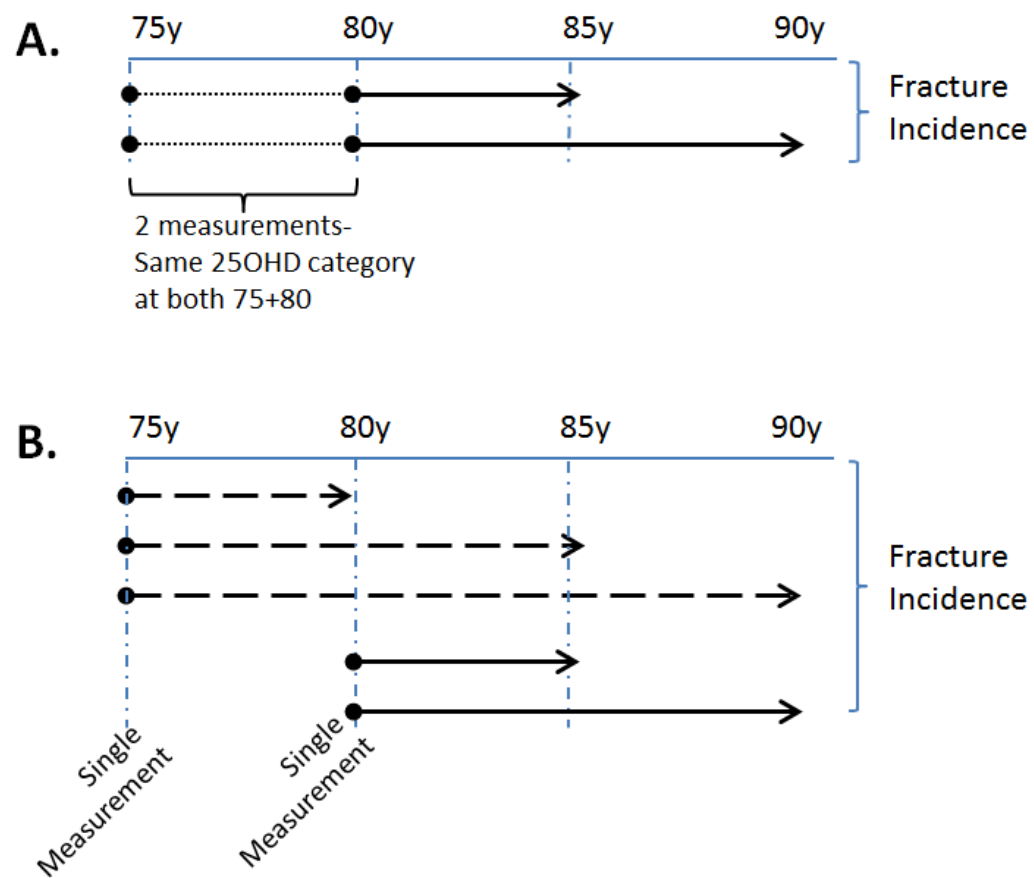
<sup>#</sup>Serum concentrations of 25(OH)D in nmol/L for each category: Low <50; Intermediate 50-75; High >75

\*Individuals 25(OH)D category at BOTH 75y &amp; 80y

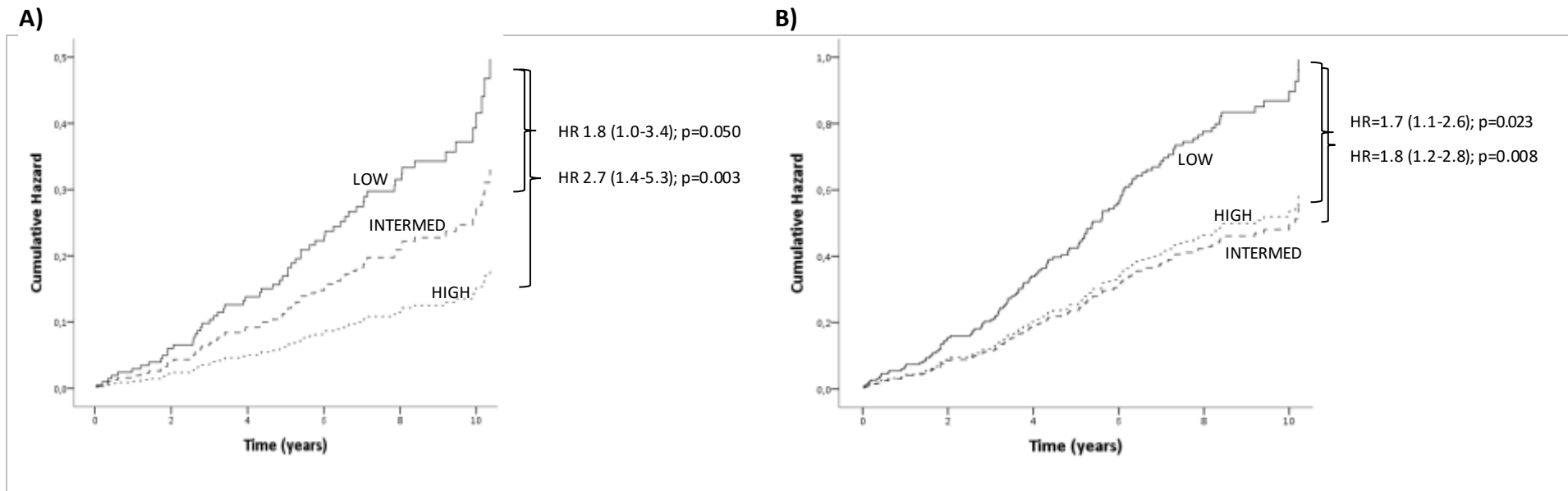
<sup>1)</sup> Single measurement at baseline, age 75; <sup>2)</sup> Single measurement at age 80, i.e. resetting baselinep<sup>a</sup> Low v High category and p<sup>b</sup> Low v Intermediate category



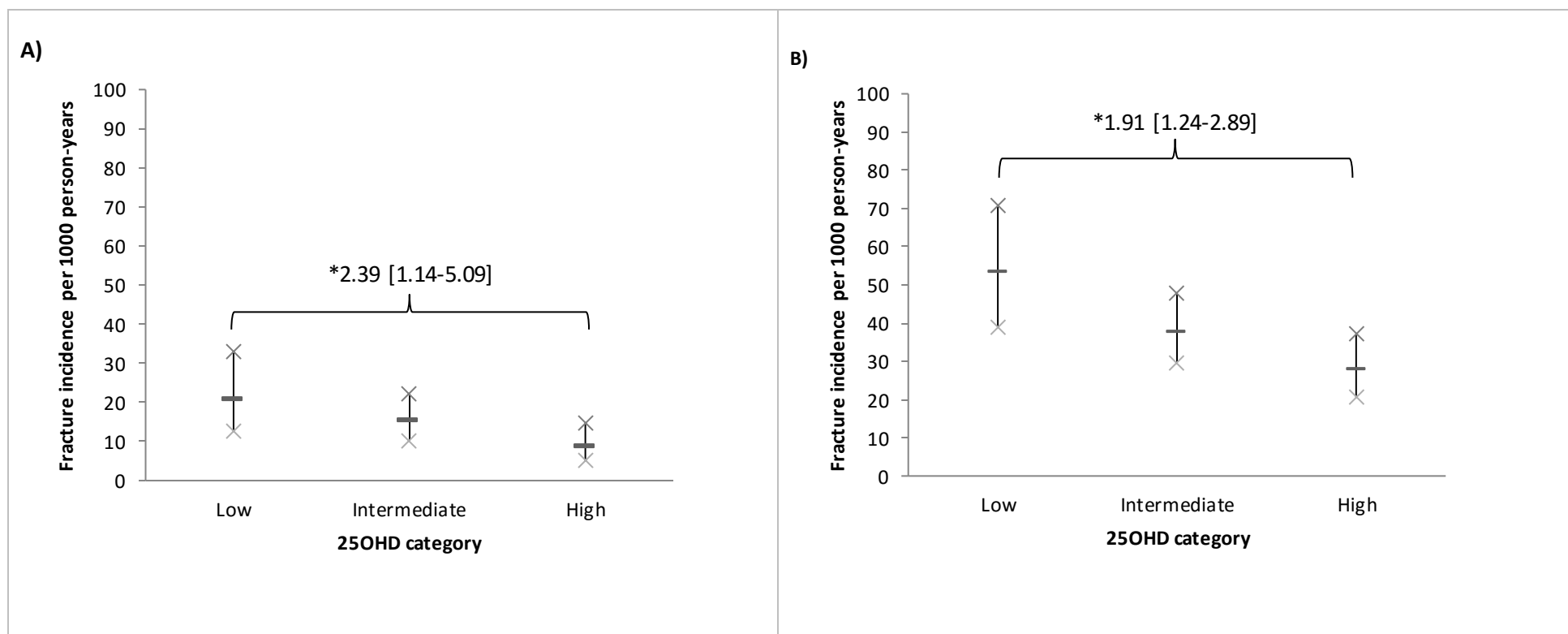
**FIGURE 1** Strategies used for the assessment of fracture incidence



**FIGURE 2** 10 year risk (i.e between 80y-90y) associated with continuously low s-25(OH)D of A) hip fracture and B) major osteoporotic fracture



**FIGURE 3** Incidence and risk of A) hip fracture and B) major osteoporotic fracture per 1000 person-years between age 80y-90y based on consecutive 25(OH)D measurements



\*Rate Ratios [95% CI]

A and B) Low: 53.0 nmol/L [38.8-70.7]; Inter: 37.5 nmol/L [29.2-47.5]; High: 27.8 nmol/L [20.5-36.9]

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