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Original article

# Vitamin K1 and 25(OH)D are independently and synergistically associated with a risk for hip fracture in an elderly population: A case control study☆

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SUMMARY

*Background & aims:* The incidence of hip fractures in Oslo is among the highest in the world. Vitamin D, as well as vitamin K, may play an important role in bone metabolism. We examined if vitamin K1 and 25(OH)D were associated with an increased risk of hip fracture, and whether the possible synergistic effect of these two micronutrients is mediated through bone turnover markers.
*Methods:* Blood was drawn for vitamin K1, 25(OH)D, and the bone turnover marker osteocalcin upon admission for hip fracture and in healthy controls.
*Results:* Vitamin K1 and 25(OH)D were independently associated with a risk of hip fracture. The adjusted odds ratio (95% CI) per ng/ml increase in vitamin K1 was 0.07 (0.02–0.32), and that per nmol/L increase in 25(OH)D was 0.96 (0.95–0.98). There was a significant interaction between 25(OH)D and vitamin K1 ($p < 0.001$), and a significant correlation between total osteocalcin and vitamin K1 and 25(OH)D (rho = 0.18, $p = 0.01$; rho = 0.20, $p = 0.01$, respectively).
*Conclusions:* Vitamin K1 and 25(OH)D are lower in hip fracture patients compared with controls. Vitamin K1 and 25(OH)D are independently and synergistically associated with the risk of hip fracture when adjusting for confounders. Intervention studies should include both vitamins.


## 1. Introduction

The incidence of hip fractures in Oslo is among the highest in the world[1] and is a major health concern for elderly people and society. Nine thousand patients annually are hospitalized and operated on because of hip fracture in Norway (5 million inhabitants).[2] Low BMI is a frequently reported risk factor for hip fracture. Meyer et al. reported that lean body stature and weight loss increased the risk of hip fracture in Oslo.[3–5] However, whether a low BMI per se or a decrease in specific nutrients leads to an increased fracture risk is unknown. Vitamin D and vitamin K may play an important role in bone metabolism and have an effect on bone mineral density (BMD). Low BMD is a powerful risk factor for hip fracture[6] and BMD in Oslo is reported to be amongst the lowest in Europe.[7]

There are two types of vitamin K, named K1 and K2. The dietary source of vitamin K from green leafy vegetables and some vegetable oils is phylloquinone, known as vitamin K1. Vitamin K2 is a series of vitamers and has been extensively studied by Japanese groups because of the high level of vitamin K2 in the soy product Natto. When not specified, vitamin K refers to both compounds throughout this paper. The active compound of vitamin D is $1,25(OH)_2D$ and is synthesized in the kidneys from 25(OH)D. However $1,25(OH)_2D$ has a short half-life and is unsuitable for measuring vitamin D status.[8] We therefore measured 25(OH)D,

*Abbreviations:* BADL, Barthel activity of daily living; BALP, bone-specific alkaline phosphatase; BMD, bone mineral density; CRP, C-reactive protein; HGS, hand grip strength; IGF-1, insulin-like growth factor-1; K1, phylloquinone; OC, osteocalcin; PTH, parathyroid hormone; RCT, randomized controlled trial; totOC, total osteocalcin; ucOC, undercarboxylated osteocalcin.

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which is regarded to reflect $1,25(OH)_2D$ activity.[8] Vitamin D refers to vitamin D from diet or sun exposure.

Bone turnover markers are helpful for evaluating the physiology and pathophysiology of bone metabolism,[9–11] and in elucidating the pathogenesis of bone disease.[12] The synthesis of the bone matrix protein osteocalcin (OC), a specific marker of osteoblast function and bone formation, depends on vitamin K and probably on $1,25(OH)_2D$.[13]

The association between vitamin D and risk of hip fracture is well described and the role of vitamin K in mineralization of bone has become an area of interest,[10,11,14–17] but few studies have examined the combination of these two nutrients. It has been investigated in two studies in Japan.[18,19] Both studies reported low 25(OH)D and low vitamin K levels in hip fracture patients. However, a possible relation between vitamin K combined with low vitamin D and the risk of fracture has been poorly explored in European populations.

The aim of this case–control study was to determine whether serum concentrations of vitamin K1 are related to the risk of hip fracture in aged patients in Norway, examine whether there is a synergistic effect between vitamin K1 and 25(OH)D and determine whether our findings can be confirmed through analysis of bone turnover markers.

## 2. Materials and methods

### 2.1. Study population

#### 2.1.1. Cases

We enrolled hip fracture patients who took part in a randomized controlled trial (RCT) of orthogeriatric care.[20] They were admitted consecutively to Oslo University Hospital, Ullevål, Norway. Eligible patients were those admitted acutely for a hip fracture as a result of a low energy trauma, defined as a fall from their own height or from a level not higher than 1 m. Cases were enrolled in the period from September 2009 to April 2011. Out of 216 eligible patients, 116 patients were enrolled for preoperative blood analysis. The catchment area for the hospital was the city of Oslo, Norway. Patients were excluded if the hip fracture was part of a multi-trauma. One recent fracture in addition to a hip fracture (e.g. radius or shoulder) was accepted. We also excluded patients who were regarded as moribund at admittance (as judged by the admitting orthopedic surgeon) and patients lacking a valid informed consent or assent.

Missing patients were included in the RCT. Age and BMI was registered, and a pre-surgical blood test was performed according to standard protocol in the missing patients.

#### 2.1.2. Controls

The control group comprised individuals with no previous hip fracture, and was drawn at random by Statistics Norway from home-dwelling inhabitants aged 60–100 years (median age, 82 years) in the census files of Oslo in 2005. The control subjects were approached by letter and followed up by two phone calls. A total of 73 control subjects (66% women) were recruited.

### 2.2. Data collection

The data were collected by designated project staff for patients and controls. In patients, weight was measured using a class 3 chair scale. Patients wore light clothing on the first possible day after the operation. Height was either measured using a tape measure against a wall or calculated from measured knee height.[21] The knee was flexed so that it was bent at 90° and measurements were taken from the anterior surface of the thigh near the patella to under the heel. Because weight was measured shortly after the operation, it was anticipated to reflect the nutritional background before the fracture.

In the control group, standing height was measured with a tape measure towards a wall. Weight was measured using a class 4 standing scale wearing light clothing.

Civil status was categorized as: widowed or non-widowed, (never married or divorced were grouped together with non-widowed because these subjects probably have a social network that is independent of a spouse); residence in home dwelling or institutionalized; education level in elementary school or higher than elementary school; smoking habits in current smokers or non-current smokers; and alcohol consumption in total abstainers and non-abstainers. The number of drugs used was recorded and activity of daily living was measured using the Barthel activity of daily living index (BADL).[22] Hand grip strength (HGS) was examined by hand dynamometry (Jamar, Germany; three repetitions per examination) in the dominant arm. Patients were examined daily throughout the duration of the hospital stay. The best hand grip test was used for analysis. In controls, the best result of three repetitions was used.

### 2.3. Preparation and analysis of blood samples

In patients, blood was collected by venipuncture shortly after admission for hip fracture and prior to operation. Standard preoperative blood analyses for C-reactive protein (CRP) and albumin levels were carried out in all patients by a multianalyzer Cobas Integra 800 from September 2009 to October 2011, and thereafter by the Cobas 800 (both from Roche Diagnostics, Mannheim, Germany) in the Department of Clinical Chemistry at Oslo University Hospital, Ullevål.

In the controls, blood was collected in the morning by venipuncture following an overnight fast. All samples were clotted for 30 min at room temperature and serum was separated by centrifugation. Aliquots were immediately stored at −80 °C, and later analyzed for assays of phylloquinone (vitamin K1) and 25-hydroxycholecalciferol (25(OH)D), total osteocalcin (totOC), undercarboxylated osteocalcin (ucOC), bone specific alkaline phosphatase (BALP), parathyroid hormone (PTH), and insulin-like growth factor 1 (IGF-1). CRP and albumin levels were assayed with a Modular P 800 multianalyzer (Roche Diagnostics) in the Department of Clinical Chemistry at Oslo University Hospital, Aker.

Vitamin K1 and ucOC were analyzed in serum by Vitas AS Norway (www.vitas.no). Vitamin K1 was measured by high pressure liquid chromatography with on-line electrochemical reduction and fluorescent detection. UcOC was measured by the glu-OC EIA kit (cat# MK118, Takara Bio Inc., Japan). The following analyses were performed by the endocrinological laboratory at Oslo University Hospital: A radioimmunoassay was used to measure 25(OH)D (DiaSorin, Stillwater, MN, USA). BALP was measured by an enzyme activity immune extraction kit (Metra Biosystems Inc., Sand Diego, CA USA. BALP was quantified in E/L, with $1E = 1$ μmol hydrolyzed p-NPP/min, where p-NPP is a monoclonal anti-bone-ALP antigen. TotOC and PTH were analyzed by a non-competitive immunoluminometric assay using the Immulite 2500 kit (Siemens Healthcare Diagnostics, Los Angeles, CA, USA) and IGF-1 was measured using a non-competitive immunoluminometric assay (Immulite 2000 from Siemens Healthcare Diagnostics).

The coefficients of variation for the analysis were as follows: vitamin K1, 16%; ucOC, 20%, 25(OH)D, 16%; BALP, 2%; totOC, 7%; PTH, 8%; and IGF-1, 12%. The coefficients of variation remained stable over time. None of the methods were changed, and we used the same laboratories for analyzing patients and controls.

### 2.4. Sample size and statistical analysis

Reference samples for vitamin K1 and 25(OH)D in healthy home-dwelling citizens of Oslo were established in 2005. Estimated

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