

ASSOCIATION OF SERUM VITAMIN D AND TOTAL CALCIUM LEVELS WITH HIP FRACTURES IN OLDER MEN: A CASE-CONTROL STUDY

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Abstract: Hip fractures in older men are a major cause of morbidity and mortality. Disturbances in bone and mineral metabolism may contribute to fracture risk. There may be various risk factors that contribute in hip fractures, however, this study focuses on the association of serum vitamin D and total calcium concentrations with hip fractures in older men. The aim was to compare serum vitamin D and total calcium concentrations between older men with and without hip fracture and to determine whether these parameters remained associated with hip fracture after adjustment for age. This case-control study included 80 male participants above the age of 65, comprising 40 participants with low-energy hip fracture and 40 controls without a history of hip fracture. Serum 25-hydroxyvitamin D [25(OH)D] and total calcium concentrations were measured in all participants. Vitamin D status was categorized according to the Institute of Medicine criteria. Statistical analysis was performed to evaluate the independent associations of age, serum vitamin D, and total calcium with hip fracture. Patients with hip fractures had significantly lower serum vitamin D concentrations than controls [median 9.24 ng/mL (IQR 4.64–14.19) vs. 18.58 ng/mL (IQR 14.69–23.00); $p < 0.001$] and lower serum total calcium levels (2.09 ± 0.19 vs. 2.31 ± 0.14 mmol/L; $p < 0.001$). Vitamin D deficiency (<12 ng/mL) was present in 67.5% of patients with hip fractures compared with 12.5% of controls ($p < 0.001$). In multivariable logistic regression analysis, increasing age was independently associated with higher odds of hip fracture (adjusted OR 1.18, 95% CI 1.05–1.33; $p = 0.006$), whereas higher serum calcium was independently associated with lower odds of hip fracture (adjusted OR 0.47 per 0.1 mmol/L increase, 95% CI 0.29–0.75; $p = 0.002$). Serum vitamin D was not independently associated with hip fracture after adjustment (adjusted OR 0.93, 95% CI 0.85–1.02; $p = 0.141$). Thus, although vitamin D concentrations differed markedly between groups in unadjusted comparisons, this association was attenuated after adjustment, whereas serum calcium retained an independent inverse association with hip fracture. The fracture group had lower serum vitamin D and total calcium concentrations than controls. After adjustment, increasing age and lower serum calcium remained independently associated with hip fracture, whereas vitamin D did not. Since these measurements were obtained after fracture occurrence, these findings demonstrate association rather than causation. These findings indicate that abnormalities in mineral metabolism are common among older men presenting with hip fracture and may provide additional information regarding their metabolic bone profile. Prospective studies are needed to evaluate their predictive value for fracture-risk assessment.

Keywords: Hip fracture, vitamin D, calcium, older men, case-control study

1. INTRODUCTION

Hip fractures represent a major public health challenge and are one of the most serious injuries among older adults. Their incidence and overall burden are expected to increase further while the population is aging (Feng et al., 2024). Besides the acute injury, hip fractures are associated with increased mortality, reduced quality of life and loss of independence, emphasizing the importance of identifying modifiable risk factors that may improve prevention strategies (Llombart et al., 2024; Manoj et al., 2023; Wang et al., 2024).

Vitamin D promotes intestinal calcium absorption and maintains normal bone mineralization playing a key role in calcium homeostasis. Low levels of vitamin D have also been associated with increased risk of falls and impaired muscle strength, both of which contribute to hip fracture risk (Bouillon et al., 2019; Giustina et al., 2020). Since calcium metabolism is closely regulated by vitamin D, alterations in both biomarkers may reflect an unfavorable metabolic profile in older patients presenting with hip fractures (Cianferotti et al., 2024). Some studies and meta-analyses have demonstrated that lower serum vitamin D concentrations are associated with an increased risk of hip fracture in older adults, although the magnitude and clinical significance of this association is heterogeneous across populations and study designs (Habibi Ghahfarrokhi et al., 2022; Lv et al., 2017; Wang et al., 2020). On the other hand, fewer studies have evaluated serum calcium together with vitamin D and other metabolic biomarkers in patients with hip fractures, and the relationship between these markers and hip fracture remains insufficiently defined (Cianferotti et al., 2024; Kemah et al., 2026). In our previous study, we observed lower testosterone and vitamin D levels in older men with hip fractures, supporting the hypothesis that endocrine and metabolic abnormalities may coexist in this population (Bekteshi et al., 2025). This study aims to compare serum vitamin D and total calcium levels between elderly men with hip fractures and controls without fractures, hypothesizing that men with hip fractures would have lower serum vitamin D and total calcium levels than controls.

2. MATERIALS AND METHODS

Study design

This case-control study was conducted at the University Clinic for Surgical Diseases “St. Naum Ohridski”, Skopje, North Macedonia. The study included men with hip fractures admitted between January 2024 – January 2026 and male controls without a history of hip fracture who were treated at the same institution.

Study population

Cases

The fracture group consisted of men aged 65 years or older who were admitted with a low-energy hip fracture. Hip fractures included femoral neck, intertrochanteric, and subtrochanteric fractures confirmed by standard radiographic examination. Blood samples were obtained on the morning following hospital admission.

Controls

The control group consisted of men aged 65 years or older without a history of hip fracture who were admitted to the same institution for elective minor surgical procedures unrelated to bone or mineral metabolism.

Inclusion and Exclusion Criteria

Inclusion criteria

- Male participants 65 years old or older
- Low-energy hip fracture confirmed radiographically (case group)
- No history of hip fracture (control group)
- Written informed consent

Exclusion criteria

- Pathological fractures
- Metastatic bone disease
- Chronic kidney disease (stage 4 or 5)
- Chronic liver disease
- High-energy trauma
- Primary hyperparathyroidism
- Malabsorption syndromes
- Current vitamin D or calcium supplementation
- Other metabolic bone disorders affecting vitamin D or calcium metabolism

Blood Sampling and Laboratory Analysis

Venous blood samples were collected from all participants. In the case group, blood samples were obtained on the morning following hospital admission and prior to surgical treatment. In the control group, blood samples were collected before the scheduled surgical procedure. Serum concentrations of 25-hydroxyvitamin D [25(OH)D] and total calcium were determined in the University Clinic for Clinical Biochemistry, Skopje. All biochemical analyses were performed in the same accredited laboratory according to standardized laboratory procedures and institutional quality-control protocols. All samples from both groups were analyzed using the same analytical methods.

Vitamin D Classification

Serum 25-hydroxyvitamin D [25(OH)D] concentrations were categorized according to the Institute of Medicine (now the National Academy of Medicine) criteria as deficiency (<12 ng/mL), inadequacy (12 to <20 ng/mL), and sufficiency (≥20 ng/mL) (Institute of Medicine, 2011).

Outcome Measures

The primary outcome of the study was the association between serum vitamin D and total calcium concentrations and the presence of hip fracture. Secondary analyses evaluated the distribution of vitamin D categories and the independent associations of age, serum vitamin D, and serum calcium with hip fracture using multivariable logistic regression.

Statistical Analysis

The statistical analysis of the data was conducted according to the distribution and type of variables. In the cases when continuous data were normally distributed, they were expressed as mean $\pm$ standard deviation (SD), whereas the variables that deviated from a normal distribution were summarized using the median and interquartile range (IQR). On the other hand, categorical data were reported as absolute numbers with corresponding percentages. In order to determine if the continuous variables meet the assumption normality, the Shapiro-Wilk test was used.

As it was already mentioned, the choice of statistical test was based on the distribution of the variables. Normally distributed continuous variables were compared using the independent-samples *t*-test, whereas for not normally distributed variables the Mann-Whitney *U* test was applied. Pearson's chi-square test was used to examine whether the distribution of vitamin D categories differed between the groups.

A multivariable logistic regression model was subsequently fitted to examine the independent relationship between selected clinical and biochemical variables and the presence of hip fracture. Hip fracture status was treated as the outcome variable, with age, serum 25-hydroxyvitamin D [25(OH)D], and total serum calcium entered as explanatory variables. Age was modeled continuously, with the effect expressed for each additional year of age. Serum 25(OH)D was likewise analyzed as a continuous measure, with the odds ratio corresponding to a 1 ng/mL increase. For total serum calcium, the odds ratio was calculated for every 0.1 mmol/L increase. The regression results were presented as adjusted odds ratios (ORs) together with their 95% confidence intervals (CIs). All statistical tests were performed using a two-sided approach, with statistical significance defined as a *p*-value < 0.05 . The analyses were carried out in Python version 3.13.5 using the SciPy 1.17.0 and statsmodels 0.14.6 packages.

3. RESULTS

80 male participants were included in the study, comprising 40 patients with hip fractures and 40 controls without a history of hip fracture. Patients in the fracture group were older than controls [median 76.0 years (IQR 71.0–82.3) vs. 71.5 years (IQR 67.0–74.0); $p = 0.0002$]. Serum vitamin D was lower in the fracture group [median 9.24 ng/mL (IQR 4.64–14.19) vs. 18.58 ng/mL (IQR 14.69–23.00); $p < 0.001$], as was mean total serum calcium (2.09 ± 0.19 vs. 2.31 ± 0.14 mmol/L; $p < 0.001$). These characteristics are summarized in Table 1.

Table 1. Demographic and biochemical characteristics of the study population

Variable	Fracture Group (n=40)	Control Group (n=40)	P value
Age (years)	76.0 (71.0–82.3)	71.5 (67.0–74.0)	0.0002
Vitamin D (ng/mL)	9.24 (4.64–14.19)	18.58 (14.69–23.00)	<0.001
Calcium (mmol/L)	2.09 ± 0.19	2.31 ± 0.14	<0.001

*Values are presented as median (IQR) for age and serum vitamin D and as mean $\pm$ SD for serum calcium. P values were calculated using the Mann-Whitney *U* test for age and serum vitamin D and the independent-samples *t*-test for serum calcium.

Source: Authors' research

The distribution of serum 25-hydroxyvitamin D status according to predefined clinical categories is presented in Table 2.

Table 2. Distribution of serum 25-hydroxyvitamin D categories in the study population

Vitamin D category*	Fracture Group (n=40)	Control Group (n=40)	P value†
Deficiency (<12 ng/mL)	27 (67.5%)	5 (12.5%)	
Inadequacy (12 to <20 ng/mL)	8 (20.0%)	19 (47.5%)	
Sufficiency (≥ 20 ng/mL)	5 (12.5%)	16 (40.0%)	
Total	40 (100%)	40 (100%)	<0.001

* Vitamin D categories were defined according to the Institute of Medicine criteria. Values are presented as number (%). The overall distribution differed significantly between groups (Pearson $\chi^2 = 25.37$, $df = 2$; $p < 0.001$).

Source: Authors' research

The independent associations between age, serum vitamin D, calcium concentrations, and hip fracture are presented in Table 3.

Table 3. Multivariable logistic regression analysis of factors associated with hip fracture

Variable	Adjusted OR (Odds Ratio)	95% CI (Confidence Interval)	P value
Age (per 1 year increase)	1.18	1.05 – 1.33	0.006
Vitamin D (per 1 ng/mL increase)	0.93	0.85 – 1.02	0.141
Calcium (per 0.1 mmol/L increase)	0.47	0.29 – 0.75	0.002

*OR, odds ratio; CI, confidence interval. Adjusted ORs were obtained from a multivariable logistic regression model with hip fracture status as the dependent variable and age, serum vitamin D, and serum calcium as independent variables.

Source: Authors' research

4. DISCUSSION

The present study evaluated the association of serum vitamin D and total calcium concentrations with hip fracture in men aged 65 years and older. Patients with hip fractures had significantly lower vitamin D and total calcium concentrations than controls, and vitamin D deficiency was substantially more frequent in the fracture group. After adjustment in the multivariable logistic regression model, increasing age and lower serum calcium remained independently associated with hip fracture, whereas serum vitamin D was no longer independently associated. These findings support a multifactorial metabolic pattern among older men with hip fractures but, given the case-control design and post-fracture blood sampling, should not be interpreted as evidence that the measured biomarkers caused or prospectively predicted the fracture.

Vitamin D

In the present study, patients with hip fractures had significantly lower serum 25-hydroxyvitamin D [25(OH)D] concentrations than controls, and vitamin D deficiency was considerably more frequent in the fracture group. These findings are consistent with previous evidence demonstrating an association between low serum vitamin D levels and hip fracture in older adults. A systematic review and meta-analysis including more than 61,000 older participants found that lower serum 25(OH)D concentrations were associated with increased odds of hip fracture (Habibi Ghahfarrokhi et al., 2022). In our multivariable analysis, however, vitamin D was no longer independently associated with hip fracture after adjustment for age and serum calcium. LeBoff et al. (1999) also reported lower vitamin D levels in postmenopausal women with acute hip fracture compared with women undergoing elective joint replacement, with comparisons adjusted for age and estrogen intake. Differences in sex, study design and baseline vitamin D status suggest that vitamin D should be interpreted in the context of the broader clinical and metabolic profile rather than as an isolated causal marker.

Calcium

Our study demonstrated significantly lower total serum calcium concentrations in patients with hip fractures than in controls. Lower calcium remained independently associated with hip fracture after adjustment for age and serum vitamin D. A recent retrospective study by Kemah et al. (2026) likewise reported lower serum calcium, albumin, and total protein in older patients with femoral neck or intertrochanteric fractures compared with controls. Li et al. (2024) found that preoperative serum calcium was independently associated with postoperative mortality among older adults with hip fractures; although that study addressed prognosis after fracture rather than fracture occurrence, it further illustrates the clinical relevance of calcium homeostasis in this population. Importantly, total serum calcium is influenced by albumin and acute physiological changes. Because albumin-corrected or ionized calcium was not available in the present analysis, the observed association should be interpreted cautiously and should not be considered evidence that low calcium caused the fracture.

Age

Increasing age remained independently associated with hip fracture in our multivariable analysis, consistent with its established role as a major non-modifiable correlate of osteoporotic fractures. Large epidemiological studies demonstrate that fracture incidence rises progressively with advancing age owing to age-related bone loss, loss of muscle mass and strength, impaired balance, and an increased propensity to falls (Center et al., 1999; Cummings & Melton, 2002). The persistence of the age association after adjustment for serum vitamin D and calcium further highlights the multifactorial nature of hip fracture in older men.

5. CLINICAL IMPLICATIONS

Overall, our findings indicate that vitamin D and calcium abnormalities are common among older men presenting with hip fracture and may provide useful information about their metabolic status. These results support biochemical assessment as part of the clinical evaluation of patients with hip fracture. However, because

the biomarkers were measured after fracture occurrence, the present study cannot establish their value for prospective fracture-risk prediction or prevention. Prospective studies with pre-fracture measurements are required to determine whether these biomarkers add predictive value beyond established clinical risk factors.

6. STRENGTHS AND LIMITATIONS

This study has several strengths. Relatively few studies have evaluated serum vitamin D and total calcium simultaneously in older men with hip fractures. By focusing exclusively on a male population, the study addresses a group that has received less attention than postmenopausal women in osteoporosis research. Both cases and controls underwent biochemical analyses in the same accredited laboratory using standardized methods, minimizing analytical variability. In addition, multivariable logistic regression allowed simultaneous adjustment for age, vitamin D, and calcium, providing a more robust assessment of their independent associations with hip fracture status.

Several limitations should be acknowledged. First, this was a single-center case-control study with a modest sample size, which may limit generalization. Second, the fracture group was significantly older than the control group; although age was included in the multivariable model, residual confounding remains possible. Third, biochemical parameters were measured after fracture occurrence, so reverse causation and acute physiological changes related to trauma, hospitalization, hydration, or inflammatory response cannot be excluded. Fourth, only total serum calcium was analyzed; serum albumin was not available for albumin-corrected calcium, and ionized calcium was not included. Finally, potentially important factors such as bone mineral density, nutritional status, season of vitamin D measurement, physical activity, frailty, and other medications or comorbidities were not included in the regression model. The observed associations therefore should not be interpreted as causal.

7. CONCLUSION

In conclusion, older men with hip fractures had lower serum vitamin D and total calcium concentrations than controls, and vitamin D deficiency was substantially more frequent in the fracture group. After adjustment for age, vitamin D, and calcium, increasing age and lower serum calcium remained independently associated with hip fracture, whereas vitamin D did not. These findings describe a multifactorial clinical and metabolic profile associated with hip fracture but do not establish causation or prospective predictive utility. Larger prospective studies incorporating pre-fracture biomarker measurements, bone mineral density, albumin or ionized calcium, and other established fracture determinants are warranted.

REFERENCES

- Bekteshi, L., Nikolikj, K., Trpeski, S., & Nastov, N. (2025). Low levels of testosterone as a risk factor of hip fractures in men above the age of sixty-five: A case series study. *Eurasia Proceedings of Health, Environment and Life Sciences*, 18, 1–4. <https://doi.org/10.55549/ephels.157>
- Bouillon, R., Marcocci, C., Carmeliet, G., Bikle, D., White, J. H., Dawson-Hughes, B., Lips, P., Munns, C. F., Lazaretti-Castro, M., Giustina, A., & Bilezikian, J. (2019). Skeletal and extraskeletal actions of vitamin D: Current evidence and outstanding questions. *Endocrine Reviews*, 40(4), 1109–1151. <https://doi.org/10.1210/er.2018-00126>
- Center, J. R., Nguyen, T. V., Schneider, D., Sambrook, P. N., & Eisman, J. A. (1999). Mortality after all major types of osteoporotic fracture in men and women: An observational study. *The Lancet*, 353(9156), 878–882. [https://doi.org/10.1016/S0140-6736\(98\)09075-8](https://doi.org/10.1016/S0140-6736(98)09075-8)
- Cianferotti, L., Bifulco, G., Caffarelli, C., Mazziotti, G., Migliaccio, S., Napoli, N., Ruggiero, C., & Cipriani, C. (2024). Nutrition, vitamin D, and calcium in elderly patients before and after a hip fracture and their impact on the musculoskeletal system: A narrative review. *Nutrients*, 16(11), 1773. <https://doi.org/10.3390/nu16111773>
- Cummings, S. R., & Melton, L. J. (2002). Epidemiology and outcomes of osteoporotic fractures. *The Lancet*, 359(9319), 1761–1767. [https://doi.org/10.1016/S0140-6736\(02\)08657-9](https://doi.org/10.1016/S0140-6736(02)08657-9)
- Feng, J. N., Zhang, C. G., Li, B. H., Zhan, S. Y., Wang, S. F., & Song, C. L. (2024). Global burden of hip fracture: The Global Burden of Disease Study. *Osteoporosis International*, 35(1), 41–52. <https://doi.org/10.1007/s00198-023-06907-3>
- Giustina, A., Adler, R. A., Binkley, N., Bollerslev, J., Bouillon, R., Dawson-Hughes, B., Ebeling, P. R., Feldman, D., Formenti, A. M., Lazaretti-Castro, M., Marcocci, C., Rizzoli, R., Sempos, C. T., & Bilezikian, J. P. (2020). Consensus statement from 2nd International Conference on Controversies in Vitamin D. *Reviews in Endocrine and Metabolic Disorders*, 21(1), 89–116. <https://doi.org/10.1007/s11154-019-09532-w>
- Habibi Ghahfarrokhi, S., Mohammadian-Hafshejani, A., Sherwin, C. M. T., & Heidari-Soureshjani, S. (2022). Relationship between serum vitamin D and hip fracture in the elderly: A systematic review and meta-analysis. *Journal of Bone and Mineral Metabolism*, 40(4), 541–553. <https://doi.org/10.1007/s00774-022-01333-7>

- Institute of Medicine. (2011). *Dietary reference intakes for calcium and vitamin D*. National Academies Press. <https://doi.org/10.17226/13050>
- Kemah, B., Söylemez, M. S., Erinc, S., Ozkan, K., & Poyanli, O. (2026). Comparison of vitamin D, parathyroid hormone (PTH), and bone metabolism markers in hip fracture patients by fracture type and control group. *Ulus Travma Acil Cerrahi Derg*, 32(3), 367–373. <https://doi.org/10.14744/tjtes.2025.71736>
- LeBoff, M. S., Kohlmeier, L., Hurwitz, S., Franklin, J., Wright, J., & Glowacki, J. (1999). Occult vitamin D deficiency in postmenopausal US women with acute hip fracture. *JAMA*, 281(16), 1505–1511. <https://doi.org/10.1001/jama.281.16.1505>
- Li, D. Y., Zhang, K., Wang, H., Zhuang, Y., Zhang, B. F., & Zhang, D. L. (2024). Preoperative serum calcium level predicts postoperative mortality in older adult patients with hip fracture: A prospective cohort study of 2333 patients. *Journal of the American Medical Directors Association*, 25(4), 655–660. <https://doi.org/10.1016/j.jamda.2023.08.004>
- Llombart, R., Mariscal, G., Barrios, C., de la Rubia Ortí, J. E., & Llombart-Ais, R. (2024). Impact of vitamin D deficiency on mortality in patients with hip fracture: A meta-analysis. *Journal of the American Geriatrics Society*, 72(1), 268–279. <https://doi.org/10.1111/jgs.18601>
- Lv, Q. B., Gao, X., Liu, X., Shao, Z. X., Xu, Q. H., Tang, L., Chi, Y. L., & Wu, A. M. (2017). The serum 25-hydroxyvitamin D levels and hip fracture risk: A meta-analysis of prospective cohort studies. *Oncotarget*, 8(24), 39849–39858. <https://doi.org/10.18632/oncotarget.16337>
- Manoj, P., Derwin, R., & George, S. (2023). What is the impact of daily oral supplementation of vitamin D3 (cholecalciferol) plus calcium on the incidence of hip fracture in older people? A systematic review and meta-analysis. *International Journal of Older People Nursing*, 18(1), e12492. <https://doi.org/10.1111/opn.12492>
- Wang, K., Xia, C., Zhou, L., Zheng, Y., Wang, X., & Cheng, L. (2024). The association between vitamin D deficiency and the risk of mortality after hip fractures: A systematic review and meta-analysis. *Journal of Nutritional Science and Vitaminology*, 70(2), 89–97. <https://doi.org/10.3177/jnsv.70.89>
- Wang, Q., Yu, D., Wang, J., & Lin, S. (2020). Association between vitamin D deficiency and fragility fractures in Chinese elderly patients: A cross-sectional study. *Annals of Palliative Medicine*, 9(4), 1660–1665. <https://doi.org/10.21037/apm-19-610>