RESEARCH LETTER

Supplementation with vitamins D_3 and K_2 increases serum levels of DKK-2, a biochemical marker of fragility fracture, in postmenopausal women: a single-arm study

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Introduction The Wnt protein family, consisting of at least 19 distinct members, exerts its influence through canonical (Wnt/β-catenin) and noncanonical signaling pathways.[1] Accumulating evidence supports the crucial role of Wnt signaling in bone mineralization and overall musculoskeletal health.[2] Three Wnt signaling inhibitors, dickkopf-related protein (DDK)-1, DKK-2, and sclerostin (SOST), regulate osteoblast-mediated bone formation.[3] Research has demonstrated that serum concentrations of DKK-1 and SOST increase with age and correlate with reduced bone mass.[3,4] However, the relationship between circulating levels of DKK-1 and SOST and the occurrence of low-impact fractures remains uncertain.[5,6] In contrast, lower serum concentrations of DKK-2, a molecule capable of acting as both a Wnt agonist and antagonist depending on cellular context,[7] have been linked to an increased risk of low-impact fractures in postmenopausal women.[3] Intriguingly, a recent genome-wide association study[8] revealed that 2 single nucleotide polymorphisms (SNPs) (rs2051756 and rs2866908) within an intron of the *DKK2* gene are associated with peak bone mass in women,[8] further emphasizing the critical role of DKK-2 in maintaining bone health.

Although a sufficient daily intake of vitamins D_3 and K_2 may reduce the risk of fragility fractures,[9] it remains unclear whether the underlying mechanisms partially involve circulating inhibitors of Wnt signaling. Vitamin D_3 deficiency can lead to bone structural defects resembling osteoporosis and decreased calcium availability.[10] Moreover, vitamin K_2 plays a crucial role in the γ-carboxylation of osteocalcin, which is essential for maintaining skeletal health by facilitating calcium integration into the bone.[11] Serum assays for biochemical markers of bone health are vital for monitoring preventative and therapeutic interventions, such as supplementation of vitamins D_3 and K_2. However, traditional biomarkers of bone remodeling have limitations, including within-subject variability and modest associations with low-impact fracture risk.[12] As a result, recent research has explored serum levels of secreted Wnt inhibitors to determine their usefulness in predicting future fractures in elderly women.[3]

In this pilot, 60-day, single-arm study, we examined the potential impact of short-term daily supplementation with vitamins D_3 (2000 IU) and K_2 (37.5 μg) on serum levels of DKK-1, DKK-2, and SOST in postmenopausal women.

Patients and methods A convenience sample of 22 postmenopausal women aged over 50 years was identified from a primary prevention program of low-impact fragility fractures. The research was part of a broader health survey, CardioTest Lecco, which aimed to examine the role of diverse genetic, biochemical, and psychological risk factors in developing age-related diseases and to initiate suitable primary prevention interventions for at-risk individuals. All participants had experienced no menstrual periods for at least 12 months and had no prior history of low-impact fractures. Exclusion criteria included bone mineral density (BMD) in the total spine and/or femoral neck T-score values of –1.5 or lower (SD), ongoing osteoporosis treatment, bone metabolic disorders (eg, Paget disease), use of medications

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TABLE 1 Changes in serum levels of dickkopf-related protein-1, dickkopf-related protein-2, and sclerostin in postmenopausal women before and after 60 days of vitamin D_3 (2000 UI) and K_2 (37.5 µg) supplementation

Serum biomarker	Baseline	60 days	Crude change	Relative change	*P* value
DKK-1, pmol/l	115 (54–224)	119 (59–218)	+4	+3.5%	0.76[a]
DKK-2, ng/ml	6.4 (5.9–7.1)	7.1 (6.6–8)	+0.7	+7.6%	0.03[b]
SOST, pmol/l	24 (17–42)	25 (19–40)	+1	+4.2%	0.68[a]

Serum levels of DKK-1, DKK-2, and SOST are presented as medians (interquartile ranges).

a Wilcoxon test; **b** *t* test

Abbreviations: DKK, dickkopf-related protein; SOST, sclerostin

impacting bone metabolism, known history of major diseases, documented intolerance to any components of the supplement, and inability to provide signed informed consent. The participants received a 60-day supplementation of vitamins D_3 (2000 UI) and K_2 (37.5 µg). The oral supplement used in this study (Bone plus D3K2, Golden Wave, Cittadella, Italy) was provided in the form of capsules.

Venous blood samples were obtained using serum separator tubes at baseline and after 60 days. The blood was allowed to clot at room temperature for 30 minutes and then was centrifuged at 1000 × g for 15 minutes. The resulting serum was carefully extracted, divided into aliquots, and preserved at –80 °C until analysis. The measurement of circulating Wnt inhibitor concentrations was carried out using commercially available kits from Biomedica Medizinprodukte (Vienna, Austria) (for SOST and DKK-1) or Elabscience (Wuhan, China) (for DKK-2), according to the manufacturers' instructions. The intra- and interassay coefficients of variation were below 7% and below 10%, respectively.

Statistical analysis To assess normality of distribution of continuous variables, we employed the Shapiro–Wilk test. The *t* test and the Wilcoxon test were utilized to test for paired differences in normally distributed and skewed data, respectively. The analyses were performed using SPSS 20.0 package (IBM, Armonk, New York, United States) and GraphPad Prism 7.0 package (GraphPad Inc., San Diego, California, United States). Two-tailed *P* values below 0.05 were considered significant.

Ethics This single-arm study was approved by the local ethics committee (2021/29), and all participants provided written informed consent.

Results A total of 22 postmenopausal women, of a mean (SD) age of 59.1 (4.2) years, received supplementation with vitamins D_3 and K_2 for 60 days as a potential primary preventative measure against low-impact fractures. The participants successfully completed the study without any withdrawals, and the supplement was found to be well-tolerated. Following the 60-day period, there were no significant changes in serum concentrations of DKK-1 and SOST (TABLE 1). Nonetheless, a notable impact on serum DKK-2 levels was observed. The values exhibited a 7.6% increase, as compared with the baseline measurements, rising from a median of 6.4 ng/ml (interquartile range [IQR], 5.9–7.1 ng/ml) to a median of 7.1 ng/ml (IQR, 6.6–8 ng/ml), with a *P* value of 0.03 (TABLE 1).

Discussion In this pilot study, we explored the possible changes in serum levels of DKK-1, DKK-2, and SOST after administering vitamin D_3 and K_2 supplements to postmenopausal women. It is noteworthy that after 60 days of supplementation, there was a significant increase in circulating levels of DKK-2. This finding suggests that the secretion of this molecule is dependent on the availability of sufficient concentrations of vitamins D_3 and K_2. However, serum levels of DKK-1 and SOST remained unaltered, which is consistent with the results of a recent investigation.[13] The study conducted by Rodrigues et al[3] revealed that while reduced serum levels of DKK-2 were predictive of fragility fractures, no association was observed with lumbar spine and hip BMD. Notably, this predictive ability was specific to DKK-2, as serum concentrations of DKK-1 and SOST did not demonstrate any significant association with fragility fractures. Unlike conventional biomarkers of bone remodeling, decreased levels of DKK-2 can potentially indicate disorganized bone nanoarchitecture. These results support a hypothesis that providing postmenopausal women with vitamin D_3 and K_2 supplements might potentially reduce bone fragility, regardless of their influence on total bone mass. Upcoming clinical research may delve deeper into this hypothesis with enhanced precision. From a clinical standpoint, our insights on DKK-2 levels could assist in pinpointing postmenopausal women with a heightened risk of fragility fractures who might gain advantages from vitamin D_3 and K_2 supplementation. Consequently, this would enable the implementation of early and focused preventive strategies.

This study is the first, to our knowledge, that investigates the impact of vitamin D_3 and K_2 supplementation on serum levels of secreted Wnt inhibitors. However, it is important to acknowledge several limitations. First, we employed a simplistic single-arm design, which may be deemed suitable for obtaining preliminary, proof-of-concept evidence of efficacy.

A single-arm study could also be preferable when there is a limited patient pool, making it less than ideal to randomize participants to a control arm. It should be noted that single--arm studies cannot differentiate between treatment effects, placebo effects, and the influence of natural history of osteoporosis. Nonetheless, this design might be deemed appropriate when a spontaneous improvement in the outcome measure (eg, DKK-2 levels) among participants is unlikely. Rodrigues et al[3] found that mean (SD) DKK-2 levels in Portuguese women aged 65 years and above were 6.86 (2.39) ng/ml and 7.75 (2.75) ng/ml in those with and without fragility fractures, respectively. This interestingly suggests that higher DKK-2 levels may be associated with lower fracture risk in this population. Our study indicated that the supplementation led to an increase in DKK-2 levels, which rose from a median of 6.4 ng/ml (IQR, 5.9–7.1 ng/ml) to a median of 7.1 ng/ml (IQR, 6.6–8 ng/ml). The observed difference in DKK-2 levels is therefore similar in magnitude to that reported previously in older women with and without fragility fractures,[3] suggesting that it is unlikely to be due to natural history of osteoporosis alone. Second, the limited sample size and short study duration could have restricted the external validity of the findings, necessitating confirmation in other cohorts with larger participant numbers and extended follow-up periods. Moreover, the participant count might not have been sufficiently large to identify significant differences concerning other biomarkers. An additional limitation is the inability to measure estrogen levels due to financial constraints. Hence, further investigation is necessary to explore the possible correlations between hormone status, bone health, and serum DKK-2 levels. While DKK-2 has been identified as a first-in-class beige fat adipokine,[14] and beige fat is known to have anabolic effects on the skeleton,[15] additional research is also required to gain a more comprehensive understanding of the relationships between these variables. Finally, it would be interesting to explore the impact of vitamin D_3 and K_2 supplementation on serum DKK-2 levels in a secondary prevention setting.

In summary, our findings suggest that the positive effects of vitamin D_3 and K_2 supplementation on skeletal health may, in part, be associated with an increase in serum levels of DKK-2. This molecule might function as an early biochemical indicator of response to supplementation.

ARTICLE INFORMATION

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