RESEARCH ARTICLE

Effects of Vitamin D3 Fortified Low-fat Dairy Products on Bone Density Measures in Adults with Abdominal Obesity: A Randomized Clinical Trial

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Abstract

Background: Bone disease-related fractures constitute a heavy burden on the healthcare systems and economy. Vitamin D is an important regulator of bone health and its deficiency is a global problem. This study aimed to evaluate the effect of the 1,500 IU nano-encapsulated vitamin D used for fortifying low-fat dairy products (milk and yogurt) on bone health parameters.

Methods: This parallel totally blinded, randomized controlled trial was part of the Ultraviolet Intake by Nutritional Approach study and conducted on 306 individuals with abdominal obesity. Individuals were randomly assigned to four groups, including fortified low-fat milk (1,500 IU nano-encapsulated vitamin D3 per 200 g/d), non-fortified low-fat milk, fortified low-fat yogurt (1,500 IU nano-encapsulated vitamin D3 per 150 g/d), and non-fortified low-fat yogurt, for 10 weeks between January and March 2019. Bone mineral density (BMD) and trabecular bone score (TBS) were measured at the baseline and end of the trial. Trabecular bone score and BMD were defined as primary and secondary outcomes.

Results: There were no significant differences in TBS and BMD between the intervention and control groups at the end of the trial (*P>0.05*).

Conclusion: This trial demonstrated no significant effect of nano-encapsulated vitamin D fortified milk and yogurt on BMD and TBS. There remains a need for longer-term trials regarding bone health outcomes to establish optimal doses of fortification.

Level of evidence: I

Keywords: BMD, Bone health, Fortification, TBS, Vitamin D3

Introduction

Bone health is a major global public health issue with the rising mean age of the world's population. Metabolic bone diseases account for approximately 9 million fractures per year worldwide (1). Osteoporosis, the most prevalent reason for bone fractures, is affecting 9.9 million Americans, and it is estimated that the

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number of adults with osteoporosis or low bone density will increase by 17.2 million from 2010 to 2030 (2, 3). Fragility fractures are the main clinical consequences of osteoporosis and osteopenia and are associated with increased mortality, morbidity, and healthcare costs (1, 4-6).

Bone mineral density (BMD) is a major contributing factor in fragility fractures (3), which is traditionally considered to be associated with vitamin D status. Vitamin D is a fat-soluble vitamin that can be obtained through dietary sources or endogenous production in the skin (7, 8). The classic function of vitamin D is to stimulate the absorption of calcium and phosphate from the gut, which enhances bone mineralization and down-regulates the release of parathyroid hormone which consequently decreases bone reabsorption (7, 8). Vitamin D is an essential nutrient for human health during the life course and supports healthy bone development during childhood and adolescence; it also maintains bone health and muscle strength and reduces falls and fracture risks during late adulthood (9, 10). Vitamin D supplementation is recommended to optimize the peak bone mass, prevent bone loss, improve bone density, and presumably reduce fragility fracture risks (11, 12).

Trabecular bone score (TBS) may be important in the assessment of fracture risk in various causes of secondary osteoporosis. In recent publications, it has been reported that TBS may be useful for risk stratification in osteoporosis, as a surrogate of bone microarchitecture (13).

The high prevalence of vitamin D deficiency and insufficiency is a global concern, and it is estimated that one billion people worldwide suffer from vitamin D deficiency (10). This deficiency is known to cause bone diseases, such as rickets, osteomalacia, and osteoporosis (14) and correlates with fracture, muscle weakness, falls, and osteoarthritis (10, 15). Moreover, a large and growing body of evidence suggests that vitamin D deficiency plays a key role in the development of non-musculoskeletal major health-related diseases and acute or chronic illnesses (10, 14, 16).

Cutaneous synthesis is the main source of vitamin D; however, it is negatively affected by several lifestyle, environmental, and physiological factors (7, 8). Across most societies, dietary intake of vitamin D fails to meet dietary requirements (17). Therefore, policies regarding the prevention of vitamin D deficiency, as well as supplementation and food fortification, are growingly been considered throughout the world. The World Health Organization/Food and Agriculture Organization has indicated food fortification as a potential strategy to reduce micronutrient deficiency, which is cost-effective with a wide and appropriate impact, to ensure that the majority of the population consumes adequate amounts of micronutrients, such as vitamin D (14, 18, 19).

Despite the benefits and cost-effectiveness of vitamin D food fortification, numerous practical obstacles are involved during the food fortification procedure, such as solubility in the food products, stability in the course of processing and storage, homogeneity with the food matrix, physicochemical and photochemical sensitivity, the relative healthiness of the food vehicle, and changes in the flavor, texture, and appearance of the food (20-22). Recent improvements in nanotechnology provide various effective and promising nano-encapsulation techniques to overcome the practical difficulties of food fortification, facilitate nutrient-targeted delivery, and enhance its controlled release and bioavailability (23-26).

In Iran, vitamin D deficiency is highly prevalent (27). Given the heavy economic burden of vitamin D deficiency-related conditions and the cost-effectiveness of preventive strategies, population-based national policies need to be enacted and implemented to ensure the fair access of all society layers to vitamin D sources. This parallel quadruple (totally blind) randomized controlled trial aimed to evaluate the effect of nano-encapsulated vitamin D fortification of low-fat milk (the raw vehicle) and yogurt (a fermented vehicle) as the most two consumable dairy products in Iran and worldwide on bone health among abdominal obese adults.

Materials and Methods

Study Design and Sampling

This parallel blind randomized controlled trial was conducted as a pilot 10-week study from January 2019 to March 2019 based on the research of Cannell et al. (28). This trial was a part of the Ultraviolet Intake by Nutritional Approach (SUNIVA) study which was a totally blinded trial investigating the development of a practical method to nano-encapsulate vitamin D for dairy products fortification. This SUNIVA study was registered in the Iranian Registry of Clinical Trials (trial registration: IRCT20101130005280N27), and full details of its methods have been published (29). Ethical approval for this trial was obtained from the Ethics Committee of the National Institute for Medical Research Development, Tehran, Iran (protocol ID: IR.NIMAD.REC.1396.027).

A total of 346 individuals, who were the staff and students of the University of Medical Sciences, Khorasan Razavi, Iran, were assessed prior to entry to the trial; however, 40 persons were ineligible to be included in the study. The remaining 306 participants were randomly assigned to four study groups using a stratified block allocation method for the center and gender status with a ratio of 1:1:1:1. The study groups included 1) the fortified milk group (n=76) receiving 1,500 IU Nano encapsulated vitamin D3/serve (200 ml/day), 2) the non-fortified milk group (n=77) receiving simple low-fat milk (200 ml/day), 3) the fortified yogurt group (n=76) taking fortified low-fat yogurt containing 1,500 IU Nano encapsulated vitamin D3/serve (150 g/day), and 4) the non-fortified yogurt group (n=77) administered simple low-fat yogurt (150 g/day) for 10 weeks of trial. Eventually, 289 participants completed the trial. The sample size was calculated based on the confidence interval of 99%, power of 80%, and standardized effect size of 0.25; accordingly, a minimum of 289 people were estimated to be required for comparing variables between groups.

Sealed envelopes containing A or B labels were used for placebo and intervention groups, respectively. In order to ensure the blinding, researchers had no access to the allocation list. Blinding was implemented at four

levels for the subjects, investigators, statistician, and staff responsible for allocation.

Eligibility criteria included middle-aged adults (30-50 years) with abdominal obesity as a sample of "potentially at risk" but "free of chronic diseases" population. "Chronic diseases" referred to the presence of malignancies, renal, or liver diseases. Abdominal obesity was defined based on the International Diabetes Federation description as waist circumferences of ≥ 94 cm for men and ≥ 80 cm for women (30, 31).

Exclusion criteria in this study were deciding to change weight during the study, being pregnant, breastfeeding, having a history of lactose intolerance or sensitivity, and taking supplements containing vitamin D or any medications having interactions with vitamin D (e.g., corticosteroids, anticonvulsants, antidepressant, and sleeping medications) in 3 months before the trial. Moreover, during the trial, participants who withdrew consent, became pregnant, were diagnosed with a disease, developed sensitivity or intolerance to dairy products, were excluded from the study. Prior to the trial commencement, the aims and objectives of the study were explained to all participants, and oral and written informed consents were taken from them.

Nano-encapsulation of vitamin D and dairy products fortification

Nano-capsules were produced using the following materials: precirol as solid lipid, oleic acid as liquid lipid, vitamin D as bioactive fatty core, poloxamer 188 as a surfactant, and deionized water. The physical properties of this vitamin D formulation were then appraised. Nutritional information for 100 g milk and yogurt included 56 kcal, sugar 0 g, protein 7 g, fat 3 g, and trans fatty acids 0.04 g. The low-fat milk and yogurt were fortified at the Salamat pilot dairy product factory under the considerations of the Faculty of Food Sciences and Technology, Mashhad University of Medical Sciences, Mashhad, Iran, and were delivered and consumed on production day or the next day.

Intervention

Patient history was taken from each participant to evaluate the side effects of dairy products with vitamin D intake during the study. A level of at least 1,500 IU Vitamin D for daily consumption was applied due to the possible toxicity for the patients according to the Endocrine Society Clinical Practice Guideline (32, 33).

According to allocations, each participant was provided 200-mL milk or 150-mL yogurt daily portion of dairy products in a plastic container with a specific code labeled to consume once a day at breakfast for 10 weeks. Placebo and fortified products were divided with numbers.

Adherence of participants

Dairy products were distributed daily with a specific code labeled on each container to confirm receipt by the subjects. Products were delivered a day before weekends and holidays to facilitate daily consumption. Participants were asked to return empty containers on the day after the weekend.

Outcome measures

A volume of 20 mL of venous blood was collected from each participant after 12 h of fasting before and after the intervention period and collected in two tubes, including a tube containing ethylenediaminetetraacetic acid for the complete blood count and a gel tube for biochemical and hormonal tests.

Considering the aims of this study, TBS and BMD T-scores and Z-scores were implemented to assess bone quality and density. Owing to the fact that BMD provides limited data on bone quality and microstructure, it has poor value as an independent predictor of fracture risk (34, 35). Trabecular bone score is a non-invasive method that indirectly assesses microarchitecture and quality of the bone tissue through spine Dual-energy X-ray Absorptiometry (DXA) images and it is complementary to the data provided for the measurement of fracture risk and bone health (35). Therefore, TBS was defined as the primary outcome. The lumbar spine, femoral neck, and radius BMD Z-score and T-score from DXA images were defined as the secondary outcomes.

Hormonal measurements, including serum 25(OH)D concentrations, were performed using commercial enzyme-linked immunosorbent assay kits (Pishgaman Sanjesh, Iran), using an Awareness/Stat Fax 2100 analyzer.

In this study, a DXA scan was performed for each participant before and after the intervention using a Hologic Discovery Wi (S/N 93045M) device (Chicago, USA) at Ghaem Academic Hospital, Mashhad, Iran. Total femur least significant change (LSC), lumbar spies LSC, and forearm LSC were reported at 0.030, 0.025, and 0.024, respectively.

Statistical analysis

Collected data were analyzed in SPSS software (version18; SPSS Inc., Chicago, IL, USA). The Kolmogorov-Smirnov test was used to assess the normality of quantitative data. Quantitative variables were described as means ± standard deviation, while the qualitative variables were expressed as percentage and frequency and were compared using the Chi-square test. Repeated measurement was used to compare the effects of intervention in groups. A p-value of less than 0.05 was considered significant.

Results

Data of 289 participants were analyzed, and the baseline characteristics are presented in Table 1. Accordingly, age, gender, serum 25(OH)D, TBS, T-score, and Z-score were similar in all groups.

Based on the results, the mean TBS T-score was compared in the four groups, namely fortified milk (intervention), simple milk (control), fortified yogurt (intervention), and simple yogurt (control) by ANOVA test [Table 2]. Significant differences were observed in the mean TBS T-score between the four groups. Subsequently, multiple comparison Bonferroni-adjusted t-tests were applied for post hoc analysis to the ANOVA at a statistically significant level of 0.05. Bonferroni test showed a statistically significant difference between fortified milk and fortified yogurt groups.

Table 1. Clinical and biological characteristics of the participants at the baseline

		Total		Milk		Yogurt	
		Intervention	Control	Intervention	Control	Intervention	Control
Age (years)		41.82±7.74	41.82±7.91	40.42±8.03	40.26±8.27	43.47±7.21	43.19±7.25
P-value		0.99		0.86		0.66	
Gender	Male	69 (23.87%)	74 (25.60%)	36 (48.6%)	38 (51.4%)	33 (47.8%)	36 (52.2%)
	Female	71 (24.56%)	75 (25.95%)	34 (49.3%)	35 (50.7%)	37 (47.3%)	40 (52.7%)
P-value		0.48		0.45		0.51	

Data are expressed as mean ± standard deviation for the two-sample independent t-test.

Table 2. Effects of intervention on bone indices according to the milk and yogurt consumption

Variables	Type	Groups	Before intervention	After intervention	*P-value*[1]	Mean difference or mean rank difference	*P-value*[2]
TBS	Milk	Intervention	1.406±0.09	1.401±0.12	0.82	-0.05±0.31	0.296
		Control	1.41±0.09	1.41±0.08	0.80	-0.004±0.49	
	Yogurt	Intervention	1.40±0.20	1.41±0.11	0.30	0.016±0.07	0.176
		Control	1.41±0.19	1.39±0.08	0.57	0.05±0.05	
TBS T-score	Milk	Intervention	-0.48±0.80	-0.61±0.89	0.29	-0.13±5.07	0.037 [a]
		Control	-0.34±0.92	-0.35±0.82	0.86	-0.01±0.06	
	Yogurt	Intervention	-0.45±1.12	-0.49±0.98	0.28	0.16±0.55	0.044 [a]
		Control	-0.47±0.91	-0.45±0.89	0.93	0.04±0.47	
Lumbar T-score	Milk	Intervention	-0.63±1.35	-0.66±1.13	0.99	-0.03±0.8	0.421
		Control	-0.36±1.12	-0.27±1.09	0.91	0.09±0.24	
	Yogurt	Intervention	-0.27±1.09	-0.22±1.05	0.59	0.54±0.41	0.87
		Control	-0.51±1.01	-0.49±1.02	0.98	0.16±0.23	
Lumbar Z-score	Milk	Intervention	-0.45±1.15	-0.044±1.03	0.56	0.038±0.97	0.663
		Control	-0.066±1.11	-0.008±1.20	0.76	0.075±0.22	
	Yogurt	Intervention	0.21±1.17	0.24±1.14	0.97	0.024±0.3	0.13
		Control	-0.016±1.01	-0.017±1.15	0.73	-0.012±0.59	
NFemor T-score	Milk	Intervention	-0.49±0.78	-0.43±0.85	0.81	0.061±0.45	0.981
		Control	-0.2±0.93	-0.16±0.91	0.91	-0.045±0.37	
	Yogurt	Intervention	-0.24±0.84	-0.16±0.83	0.59	0.035±0.41	0.235
		Control	-0.28±0.95	-0.22±1.03	0.83	0.058±0.38	
NFemor Z-score	Milk	Intervention	-0.12±0.77	-0.09±0.87	0.40	0.03±0.51	0.315
		Control	0.21±0.81	0.21±0.90	0.90	-0.013±0.27	
	Yogurt	Intervention	0.31±0.87	1.1±4.09	0.26	0.043±0.4	0.571
		Control	0.19±0.96	0.28±1.00	0.66	-0.044±0.63	

Table 2. Continued

TFemor T-score	Milk	Intervention	-0.34±0.75	-0.24±0.80	0.48	0.1±0.43	0.921
		Control	-0.12±0.73	-0.07±0.76	0.88	0.04±0.31	
	Yogurt	Intervention	-0.013±0.77	-0.01±0.76	0.75	0.003±0.3	0.34
		Control	-0.23±0.86	-0.21±0.88	0.88	0.023±0.19	
TFemor Z-score	Milk	Intervention	1.05±-0.18	1.10±-0.1	0.28	0.08±0.43	0.632
		Control	0.15±0.62	0.114±0.77	0.74	-0.002±0.15	
	Yogurt	Intervention	0.31±0.83	0.35±0.79	0.92	0.04±0.4	0.537
		Control	0.12±1.30	0.07±1.50	0.81	-0.04±0.63	
Radius1.3 T-score	Milk	Intervention	-0.92±1.15	-0.94±1.15	0.78	-0.02±0.54	0.516
		Control	-0.92±1.1	-0.8±1.12	0.61	0.11±0.37	
	Yogurt	Intervention	-0.85±1.09	-0.75±0.83	0.22	0.1±0.36	0.288
		Control	-0.98±0.78	-0.96±0.82	0.95	0.01±0.37	
Radius1.3 Z-score	Milk	Intervention	-0.85±1.05	-0.857±1.03	0.17	-0.007±0.61	0.682
		Control	-0.57±0.96	-0.52±1.05	0.83	0.04±0.4	
	Yogurt	Intervention	1.40±-0.34	1.40±-0.27	0.48	0.06±0.29	0.305
		Control	-0.501±0.86	-0.5±0.89	0.92	0.001±0.38	

TBS: Trabecular bone score; NFemor: Femoral neck; TFemor: Total femur Kruskal-Wallis test, one-way ANOVA test, and Bonferroni correction were used to compare TBS T-scores in groups. Data are expressed as mean ± standard deviation for the two-sample independent t-test and median (interquartile range) for the Mann-Whitney U test. The p-value1 refers to the comparison before and after the intervention and the p-value2 refers to the comparison of the differences in groups. a: significant difference between groups

A repeated-measures ANOVA test was used to evaluate the effectiveness of vitamin D intervention on bone health factors. The results of repeated-measures ANOVA showed that there was no significant (*P>0.05* for all) time × vitamin D treatment [Table 3]. Therefore, vitamin D intervention was not effective.

Table 3. Comparison of bone indicators within and between time points and groups

Variables	Tests of between and within-subjects effects						
	Source	Sum of squares	Df	Mean square	F	P-value	Eta
TBS	Group	0.007	1	0.007	0.273	0.602	0.002
	Time	0.002	1	0.002	0.146	0.703	0.001
	Time*group	0.008	1	0.008	0.665	0.416	0.004
TBS T-score	Group	0.229	1	0.229	0.142	0.707	0.001
	Time	0.027	1	0.027	0.172	0.679	0.001
	Time*group	<0.001	1	<0.001	0.002	0.967	<0.001
Lumbar T-score	Group	0.079	1	0.079	0.034	0.853	<0.001
	Time	0.121	1	0.121	1.046	0.307	0.004
	Time*group	0.047	1	0.047	0.406	0.524	0.002

Table 3. Continued

	Group	0.322	1	0.322	0.126	0.723	0.001
Lumbar Z-score	Time	0.007	1	0.007	0.042	0.838	<0.001
	Time*group	0.014	1	0.014	0.080	0.778	<0.001
	Group	1.199	1	1.199	0.764	0.383	0.003
NFemor T-score	Time	0.293	1	0.293	3.569	0.060	0.015
	Time*group	<0.001	1	<0.001	0.005	0943	<0.001
	Group	0.739	1	0.739	0.177	0.674	0.001
NFemor Z-score	Time	4.502	1	4.502	1.593	0.209	0.009
	Time*group	2.805	1	2.805	0.992	0.321	0.006
	Group	0.042	1	0.042	0.033	0.855	<0.001
TFemor T-score	Time	0.213	1	0.213	4.134	0.043	0.018
	Time*group	0.007	1	0.007	0.140	0.708	0.001
	Group	<0.001	1	<0.001	<0.001	0.994	<0.001
TFemor Z-score	Time	0.025	1	0.025	0.235	0.629	0.001
	Time*group	0.178	1	0.178	1.676	0.197	0.009
	Group	0.375	1	0.375	0.223	0.637	0.001
Radius1.3 T-score	Time	0.278	1	0.278	3.137	0.078	0.014
	Time*group	0.015	1	0.015	0.173	0.678	0.001
	Group	0.095	1	0.095	0.052	0.819	<0.001
Radius1.3 Z-score	Time	0.057	1	0.057	0.615	0.434	0.004
	Time*group	0.004	1	0.004	0.039	0.844	<0.001

TBS: Trabecular bone score; NFemor; Femoral neck, TFemor: Total femur

Discussion

Following the 10-week intervention with nano-encapsulated vitamin D fortified milk and yogurt, it was revealed that there was no significant effect on the primary and secondary outcomes regarding bone health parameters; however, there were small but statistically significant effects on TBST-score. Nano-encapsulation of vitamin D for food and beverage fortification has been evaluated previously in several *in vitro* studies (24, 36-39); nevertheless, to the best of our knowledge, this study was the first clinical trial evaluation.

Several short-term trials have been conducted previously to investigate the effect of vitamin D or calcium on different bone markers (40-43). Lerchbaum et al. (2019) conducted a 12-week randomized control trial to examine the effect of 20,000 IU/week of vitamin D supplementation on BMD, TBS, and bone turnover markers (BTMs). They found that there was no significant treatment effect on BTMs, BMD, or TBS (44). They reported no significant change in BMD, TBS, and BTMs, which was consistent with our findings. In another study, Grønborg et al. carried out a randomized control trial in 12 weeks to investigate the effect of vitamin D fortified yogurt, cheese, eggs, and bread on serum vitamin D and BTMs. Based on the results of the mentioned study, there was no significant change in BTMs, despite a significant rise in serum vitamin D (45), which was in line with our findings. Contrary to these studies, the results of a 10-week trial of vitamin-D-fortified cheese indicated a significant decrease in parathyroid hormone (PTH) level among those who received a high dose (28,000 IU) of vitamin-D3-fortified cheese weekly rather than those taking a low dose (200 IU) of vitamin D (46). Since PTH

serum level is assumed as a bone turnover indicator (10, 47), the results of this trial were inconsistent with our findings. This discrepancy can be explained partially by the fact that our cases received a lower dose of vitamin D (1,500 IU daily).

On the other hand, the results of some long-term clinical trials have reported the benefits of vitamin D food fortification with or without calcium on bone health (48-52), which are inconsistent with our findings. In a randomized control trial by Manios et al., the effects of fortified dairy products were evaluated on bone metabolism, the results of which revealed a significantly greater improvement in pelvis, spine, and total-body BMD among participants who received calcium plus vitamin D fortified dairy products rather than the control and calcium supplemented groups (53), which was not in line with the findings of our study. This discrepancy can be justified by concurrent calcium fortification, longer intervention duration (20 months), and a high-risk population with low base-line BMD in the study by Manios et al. since it is generally believed that calcium and/or vitamin D supplementation might be more beneficial among individuals with low BMD or osteoporosis (48). A recent randomized double-blind clinical trial was conducted on 78 menopausal women to assess the effect of fortified yogurt with calcium, vitamin D, vitamin K, vitamin C, zinc, and magnesium on bone health parameters (54). After a 24-month intervention, BMD decreased significantly in the control group, which was in agreement with the results of previous studies and contrary to ours.

Despite the promising results of these studies, those of some long-term trials have demonstrated no significant effect on BMD or BTMs (55, 56), which are consistent with our findings. Aloia et al. conducted a 2-year trial on 208 calcium-replete healthy black postmenopausal women to investigate the effect of vitamin D supplementation on bone loss and reported no significant effect of vitamin D supplementation on BMD and BTMs (57). Bischoff-Ferrari et al. conducted a three-year double-blind randomized clinical trial to investigate the effect of vitamin D and Omega-3 supplementation and exercise on various health outcomes among 2157 low-risk old adults. The results of their trial showed that the 2,000 IU/d vitamin D supplementation failed to prevent nonvertebral fractures (58). In addition, the results of several meta-analysis studies have indicated that vitamin D supplementation seems to be inefficient in osteoporosis reduction (59), fracture prevention, and BMD improvement (60, 61), which was in line with our findings.

Although extensive observational data are available identifying the positive relationship between vitamin D serum level and BMD (62), there is uncertainty about the efficacy of vitamin D supplementation in improving BMD or fracture risk, especially among healthy adults, and mixed results are concluded by several studies (9, 60). These controversies depend on vitamin D type, dosage, administration modalities, treatment duration, and concomitant calcium treatment. Some researchers believe that vitamin D alone is not effective and calcium is required alongside (2, 63, 64), and some have indicated a higher dose of vitamin D (65).

Hence, fortification is considered to be the most effective method for improving vitamin D status in a population where widescale, sustained, and gradual impacts of fortification are desired (14). The present study did not provide evidence for the positive impact of nano-encapsulated vitamin D fortification on bone density or quality that might be explained by the limitations of this study. Apart from bone density, bone turnover is another important factor contributing to bone health (66). However, BTMs, such as a specific marker of bone resorption (carboxy-terminal collagen crosslinks) and bone formation (procollagen type 1 amino-terminal propeptide) were not assessed in the current study. The investigation of these elements is appropriate for studies with a shorter duration (45) and those that provide data on the ongoing bone remodeling (67). The other limitations of the present study were its relatively short-term follow-up and small sample size. Another limitation was related to the fact that calcium intake was not determined. Furthermore, the homogeneous distribution of vitamin D nano-capsules into the fortified milk and yogurt matrix was not appraised.

However, this was the first clinical trial that examined the effects of nano-encapsulated vitamin D fortified milk and yogurt on bone health. Moreover, this study was carried out during the winter season which eliminated confounding from vitamin D obtained from cutaneous production. In this study, bone health assessment between intervention and control groups was quantified through BMD and TBS, which was a strength, since TBS is a recently-developed method that evaluates bone texture and microarchitecture and provides complementary data on bone health in addition to BMD (35).

Although several recent studies have highlighted the cost-effectiveness and beneficial effect of consuming vitamin D fortified foods on bone health, the results of this trial highlighted the need for additional long-term trials covering all bone health indicators to establish optimal doses of fortification.

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Ethical considerations: Prior to data collection, the research protocol was approved by the Ethics Committee of the National Institute for Medical Research Development (protocol ID: IR.NIMAD.REC.1396.027). Moreover, the Ethical Committee of Mashhad University of Medical Sciences approved the research (code: IR.MUMS.MEDICAL.REC.1397.698), and informed written consent was received from all participants.

Conflicts of interest: The authors declare that there is no conflict of interest.

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References

1. Johnell O, Kanis JA. An estimate of the worldwide prevalence and disability associated with osteoporotic fractures. Osteoporos Int. 2006;17(12):1726-33.
2. Nowson CA. Prevention of fractures in older people with calcium and vitamin D. Nutrients. 2010;2(9):975-84.
3. Cosman F, de Beur SJ, LeBoff MS, Lewiecki EM, Tanner B, Randall S, et al. Clinician's Guide to Prevention and Treatment of Osteoporosis. Osteoporosis International. 2014;25(10):2359-81.
4. Cauley JA, Chalhoub D, Kassem AM, Fuleihan Gel H. Geographic and ethnic disparities in osteoporotic fractures. Nat Rev Endocrinol. 2014;10(6):338-51.
5. Hernlund E, Svedbom A, Ivergård M, Compston J, Cooper C, Stenmark J, et al. Osteoporosis in the European Union: medical management, epidemiology and economic burden. A report prepared in collaboration with the International Osteoporosis Foundation (IOF) and the European Federation of Pharmaceutical Industry Associations (EFPIA). Arch Osteoporos. 2013;8(1-2):136.
6. Burge R, Dawson-Hughes B, Solomon DH, Wong JB, King A, Tosteson A. Incidence and economic burden of osteoporosis-related fractures in the United States, 2005-2025. J Bone Miner Res. 2007;22(3):465-75.
7. Polzonetti V, Pucciarelli S, Vincenzetti S, Polidori P. Dietary Intake of Vitamin D from Dairy Products Reduces the Risk of Osteoporosis. Nutrients. 2020;12(6):1743.
8. Laird E, Ward M, McSorley E, Strain JJ, Wallace J. Vitamin D and bone health: potential mechanisms. Nutrients. 2010;2(7):693-724.
9. Bischoff-Ferrari HA, Bhasin S, Manson JE. Preventing Fractures and Falls: A Limited Role for Calcium and Vitamin D Supplements? Jama. 2018;319(15):1552-3.
10. Holick MF. Vitamin D deficiency. N Engl J Med. 2007;357(3):266-81.
11. Cranney A, Horsley T, O'Donnell S, Weiler H, Puil L, Ooi D, et al. Effectiveness and safety of vitamin D in relation to bone health. Evid Rep Technol Assess (Full Rep). 2007(158):1-235.
12. Lips P, Bouillon R, van Schoor NM, Vanderschueren D, Verschueren S, Kuchuk N, et al. Reducing fracture risk with calcium and vitamin D. Clin Endocrinol (Oxf). 2010;73(3):277-85.
13. Harvey N, Glüer C, Binkley N, McCloskey E, Brandi M-L, Cooper C, et al. Trabecular bone score (TBS) as a new complementary approach for osteoporosis evaluation in clinical practice. Bone. 2015;78:216-24.
14. Roth DE, Abrams SA, Aloia J, Bergeron G, Bourassa MW, Brown KH, et al. Global prevalence and disease burden of vitamin D deficiency: a roadmap for action in low- and middle-income countries. Ann N Y Acad Sci. 2018;1430(1):44-79.
15. Girgis CM. Vitamin D and Skeletal Muscle: Emerging Roles in Development, Anabolism and Repair. Calcif Tissue Int. 2020;106(1):47-57.
16. Wacker M, Holick MF. Vitamin D - effects on skeletal and extraskeletal health and the need for supplementation. Nutrients. 2013;5(1):111-48.
17. Cesari M, Incalzi RA, Zamboni V, Pahor M. Vitamin D hormone: a multitude of actions potentially influencing the physical function decline in older persons. Geriatr Gerontol Int. 2011;11(2):133-42.

18. Dary O, Hurrell R. Guidelines on food fortification with micronutrients. World Health Organization, Food and Agricultural Organization of the United Nations: Geneva, Switzerland. 2006:1-376.
19. Pilz S, März W, Cashman KD, Kiely ME, Whiting SJ, Holick MF, et al. Rationale and Plan for Vitamin D Food Fortification: A Review and Guidance Paper. Front Endocrinol (Lausanne). 2018;9:373-.
20. Al Khalifah R, Alsheikh R, Alnasser Y, Alsheikh R, Alhelali N, Naji A, et al. The impact of vitamin D food fortification and health outcomes in children: a systematic review and meta-regression. Systematic Reviews. 2020;9(1):144.
21. Maurya VK, Bashir K, Aggarwal M. Vitamin D microencapsulation and fortification: Trends and technologies. The Journal of Steroid Biochemistry and Molecular Biology. 2020;196:105489.
22. Walia N, Dasgupta N, Ranjan S, Chen L, Ramalingam C. Fish oil based vitamin D nanoencapsulation by ultrasonication and bioaccessibility analysis in simulated gastro-intestinal tract. Ultrasonics Sonochemistry. 2017;39:623-35.
23. Ezhilarasi PN, Karthik P, Chhanwal N, Anandharamakrishnan C. Nanoencapsulation Techniques for Food Bioactive Components: A Review. Food and Bioprocess Technology. 2013;6(3):628-47.
24. Park SJ, Garcia CV, Shin GH, Kim JT. Development of nanostructured lipid carriers for the encapsulation and controlled release of vitamin D3. Food Chem. 2017;225:213-9.
25. McClements DJ, Öztürk B. Utilization of Nanotechnology to Improve the Handling, Storage and Biocompatibility of Bioactive Lipids in Food Applications. Foods. 2021;10(2):365.
26. Jafari SM. An overview of nanoencapsulation techniques and their classification. Nanoencapsulation technologies for the food and nutraceutical industries. 2017:1-34.
27. Vatandost S, Jahani M, Afshari A, Amiri MR, Heidarimoghadam R, Mohammadi Y. Prevalence of vitamin D deficiency in Iran: A systematic review and meta-analysis. Nutr Health. 2018;24(4):269-78.
28. Cannell J, Hollis B, Zasloff M, Heaney R. Diagnosis and treatment of vitamin D deficiency. Expert opinion on pharmacotherapy. 2008;9(1):107-18.
29. Sharifan P, Bagherniya M, Bajgiran MM, Safarian M, Vatanparast H, Eslami S, et al. The efficacy of dairy products fortified with nano-encapsulated vitamin D3 on physical and mental aspects of the health in obese subjects; the protocol of the SUVINA trial. Translational Metabolic Syndrome Research. 2021;4:1-9.
30. Alberti KG, Zimmet P, Shaw J. Metabolic syndrome--a new world-wide definition. A Consensus Statement from the International Diabetes Federation. Diabet Med. 2006;23(5):469-80.
31. Sharifan P, Ziaee A, Darroudi S, Rezaie M, Safarian M, Eslami S, et al. Effect of low-fat dairy products fortified with 1500IU nano encapsulated vitamin D3 on cardiometabolic indicators in adults with abdominal obesity: a total blinded randomized controlled trial. Current Medical Research and Opinion. 2021;37(4):579-88.
32. Pramyothin P, Holick MF. Vitamin D supplementation: guidelines and evidence for subclinical deficiency. Curr Opin Gastroenterol. 2012;28(2):139-50.
33. Amiri Z, Nosrati M, Sharifan P, Saffar Soflaei S, Darroudi S, Ghazizadeh H, et al. Factors determining the serum 25-hydroxyvitamin D response to vitamin D supplementation: Data mining approach. BioFactors. 2021.
34. McClung MR. Do current management strategies and guidelines adequately address fracture risk? Bone. 2006;38(2 Suppl 2):S13-7.
35. Silva BC, Leslie WD. Trabecular Bone Score: A New DXA-Derived Measurement for Fracture Risk Assessment. Endocrinol Metab Clin North Am. 2017;46(1):153-80.
36. Loewen A, Chan B, Li-Chan ECY. Optimization of vitamins A and D(3) loading in re-assembled casein micelles and effect of loading on stability of vitamin D(3) during storage. Food Chem. 2018;240:472-81.
37. Maurya VK, Aggarwal M. Fabrication of nano-structured lipid carrier for encapsulation of vitamin D(3) for fortification of 'Lassi'; A milk based beverage. J Steroid Biochem Mol Biol. 2019;193:105429.
38. Syama MA, Arora S, Gupta C, Sharma A, Sharma V. Enhancement of vitamin D2 stability in fortified milk during light exposure and commercial heat treatments by complexation with milk proteins. Food Bioscience. 2019;29:17-23.
39. Zhou H, Liu J, Dai T, Muriel Mundo JL, Tan Y, Bai L, et al. The gastrointestinal fate of inorganic and organic nanoparticles in vitamin D-fortified plant-based milks. Food Hydrocolloids. 2021;112:106310.
40. Ferrar L, van der Hee RM, Berry M, Watson C, Miret S, Wilkinson J, et al. Effects of calcium-fortified ice cream on markers of bone health. Osteoporos Int. 2011;22(10):2721-31.
41. Neyestani TR, Hajifaraji M, Omidvar N, Nikooyeh B, Eshraghian MR, Shariatzadeh N, et al. Calcium-vitamin D-fortified milk is as effective on circulating bone biomarkers as fortified juice and supplement but has less acceptance: a randomised controlled school-based trial. J Hum Nutr Diet. 2014;27(6):606-16.
42. Bonjour JP, Benoit V, Pourchaire O, Ferry M, Rousseau B, Souberbielle JC. Inhibition of markers of bone resorption by consumption of vitamin D and calcium-fortified soft plain cheese by institutionalised elderly women. Br J Nutr. 2009;102(7):962-6.
43. Jafari T, Faghihimani E, Feizi A, Iraj B, Javanmard SH, Esmaillzadeh A, et al. Effects of vitamin D-fortified low fat yogurt on glycemic status, anthropometric indexes, inflammation, and bone turnover in diabetic postmenopausal women: A randomised controlled clinical trial. Clin Nutr. 2016;35(1):67-76.
44. Lerchbaum E, Trummer C, Theiler-Schwetz V, Kollmann M, Wölfler M, Pilz S, et al. Effects of Vitamin D Supplementation on Bone Turnover and Bone Mineral Density in Healthy Men: A Post-Hoc Analysis of a Randomized Controlled Trial. Nutrients. 2019;11(4):731.

45. Grønborg IM, Tetens I, Andersen EW, Kristensen M, Larsen REK, Tran TLL, et al. Effect of vitamin D fortified foods on bone markers and muscle strength in women of Pakistani and Danish origin living in Denmark: a randomised controlled trial. Nutr J. 2019;18(1):82-.
46. Al-Khalidi B, Chiu W, Rousseau D, Vieth R. Bioavailability and Safety of Vitamin D3 from Pizza Baked with Fortified Mozzarella Cheese: A Randomized Controlled Trial. Can J Diet Pract Res. 2015;76(3):109-16.
47. Dagli M, Kutlucan A, Abusoglu S, Basturk A, Sozen M, Kutlucan L, et al. Evaluation of bone mineral density (BMD) and indicators of bone turnover in patients with hemophilia. Bosn J Basic Med Sci. 2018;18(2):206-10.
48. Daly RM, Brown M, Bass S, Kukuljan S, Nowson C. Calcium- and vitamin D3-fortified milk reduces bone loss at clinically relevant skeletal sites in older men: a 2-year randomized controlled trial. J Bone Miner Res. 2006;21(3):397-405.
49. Liu C, Kuang X, Li K, Guo X, Deng Q, Li D. Effects of combined calcium and vitamin D supplementation on osteoporosis in postmenopausal women: a systematic review and meta-analysis of randomized controlled trials. Food Funct. 2020;11(12):10817-27.
50. Reyes-Garcia R, Mendoza N, Palacios S, Salas N, Quesada-Charneco M, Garcia-Martin A, et al. Effects of Daily Intake of Calcium and Vitamin D-Enriched Milk in Healthy Postmenopausal Women: A Randomized, Controlled, Double-Blind Nutritional Study. J Womens Health (Larchmt). 2018;27(5):561-8.
51. Moschonis G, Manios Y. Skeletal site-dependent response of bone mineral density and quantitative ultrasound parameters following a 12-month dietary intervention using dairy products fortified with calcium and vitamin D: the Postmenopausal Health Study. Br J Nutr. 2006;96(6):1140-8.
52. Mocanu V, Stitt PA, Costan AR, Voroniuc O, Zbranca E, Luca V, et al. Long-term effects of giving nursing home residents bread fortified with 125 microg (5000 IU) vitamin D(3) per daily serving. Am J Clin Nutr. 2009;89(4):1132-7.
53. Manios Y, Moschonis G, Panagiotakos DB, Farajian P, Trovas G, Lyritis GP. Changes in biochemical indices of bone metabolism in post-menopausal women following a dietary intervention with fortified dairy products. J Hum Nutr Diet. 2009;22(2):156-65.
54. Morato-Martínez M, López-Plaza B, Santurino C, Palma-Milla S, Gómez-Candela C. A Dairy Product to Reconstitute Enriched with Bioactive Nutrients Stops Bone Loss in High-Risk Menopausal Women without Pharmacological Treatment. Nutrients. 2020;12(8):2203.
55. Aspray TJ, Chadwick T, Francis RM, McColl E, Stamp E, Prentice A, et al. Randomized controlled trial of vitamin D supplementation in older people to optimize bone health. Am J Clin Nutr. 2019;109(1):207-17.
56. Hosseinpanah F, Rambod M, Hossein-nejad A, Larijani B, Azizi F. Association between vitamin D and bone mineral density in Iranian postmenopausal women. J Bone Miner Metab. 2008;26(1):86-92.
57. Aloia JF, Talwar SA, Pollack S, Yeh J. A randomized controlled trial of vitamin D3 supplementation in African American women. Arch Intern Med. 2005;165(14):1618-23.
58. Bischoff-Ferrari HA, Vellas B, Rizzoli R, Kressig RW, da Silva JAP, Blauth M, et al. Effect of Vitamin D Supplementation, Omega-3 Fatty Acid Supplementation, or a Strength-Training Exercise Program on Clinical Outcomes in Older Adults: The DO-HEALTH Randomized Clinical Trial. Jama. 2020;324(18):1855-68.
59. Reid IR, Bolland MJ, Grey A. Effects of vitamin D supplements on bone mineral density: a systematic review and meta-analysis. Lancet. 2014;383(9912):146-55.
60. Bolland MJ, Grey A, Avenell A. Effects of vitamin D supplementation on musculoskeletal health: a systematic review, meta-analysis, and trial sequential analysis. Lancet Diabetes Endocrinol. 2018;6(11):847-58.
61. Winzenberg TM, Powell S, Shaw KA, Jones G. Vitamin D supplementation for improving bone mineral density in children. Cochrane Database Syst Rev. 2010(10):Cd006944.
62. Mason RS, Rybchyn MS, Brennan-Speranza TC, Fraser DR. Is it reasonable to ignore vitamin D status for musculoskeletal health? Fac Rev. 2020;9:19-.
63. Avenell A, Mak JC, O'Connell D. Vitamin D and vitamin D analogues for preventing fractures in post-menopausal women and older men. Cochrane Database Syst Rev. 2014;2014(4):Cd000227.
64. Yao P, Bennett D, Mafham M, Lin X, Chen Z, Armitage J, et al. Vitamin D and Calcium for the Prevention of Fracture: A Systematic Review and Meta-analysis. JAMA Netw Open. 2019;2(12):e1917789.
65. Bischoff-Ferrari HA, Willett WC, Wong JB, Stuck AE, Staehelin HB, Orav EJ, et al. Prevention of nonvertebral fractures with oral vitamin D and dose dependency: a meta-analysis of randomized controlled trials. Arch Intern Med. 2009;169(6):551-61.
66. Garnero P, Delmas PD. Contribution of bone mineral density and bone turnover markers to the estimation of risk of osteoporotic fracture in postmenopausal women. J Musculoskelet Neuronal Interact. 2004;4(1):50-63.
67. Shetty S, Kapoor N, Bondu JD, Thomas N, Paul TV. Bone turnover markers: Emerging tool in the management of osteoporosis. Indian J Endocrinol Metab. 2016;20(6):846-52.