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ORIGINAL CONTRIBUTION

Reduced-fat Gouda-type cheese enriched with vitamin D_3 effectively prevents vitamin D deficiency during winter months in postmenopausal women in Greece

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Abstract
Objective The primary aim of the present study was to examine the effectiveness of daily consumption of vitamin D_3-enriched, reduced-fat Gouda-type cheese on 25-hydroxyvitamin D (25(OH)D) concentration in postmenopausal women. Health-related quality of life (HRQL) indices were examined as secondary outcomes.
Design This is a single-blinded (i.e., to study participants), randomized, controlled food-based dietary intervention study.
Methods A sample of 79 postmenopausal women (55–75 years old) was randomized either to a control group (CG: $n = 39$) or an intervention group (IG: $n = 40$) that consumed, as part of their usual diet, 60 g of either non-enriched or vitamin D_3 enriched Gouda-type cheese, respectively, for eight consecutive weeks (i.e., from January to March 2015). Sixty grams of enriched cheese provided a daily dose of 5.7 μg of vitamin D_3.
Results There was a differential response of mean (95 % CI) serum 25(OH)D levels in the IG and CG, with the former increasing and the latter decreasing significantly over the eight weeks of the trial [i.e., by 5.1 (3.4, 6.9) nmol/L vs. −4.6 (−6.4, −2.8) nmol/L, $P < 0.001$, respectively]. The percentages of study participants with 25(OH)D levels <30 (deficiency) and <50 nmol/L (insufficiency) were significantly higher at follow-up in the CG compared to the IG (41.0 vs. 0 %, $P < 0.001$ and 74.4 vs. 47.5 %, $P < 0.001$, respectively). The emotional well-being scale of the HRQL score increased in the IG compared to a decrease in the CG (3.2 vs. −3.8, $P = 0.028$). However, none of the other seven scales of the HRQL score significantly differentiated between study groups ($P > 0.1$).
Conclusions Consumption of 60 g of vitamin D_3-enriched, reduced-fat Gouda-type cheese provided a daily dose of 5.7 μg of additional vitamin D_3 and was effective in increasing mean serum 25(OH)D concentration and in counteracting vitamin D deficiency during winter months in postmenopausal women in Greece.

Keywords Postmenopausal women · Vitamin D · 25-Hydroxyvitamin D · Cheese · Enriched dairy

Introduction

Over the last 15 years, vitamin D has attracted increased attention of the scientific community, the food industry, policy makers and consequently the public [1, 2]. Vitamin D_3 is synthesized endogenously from skin exposure to ultraviolet B (UVB) radiation in sunlight [3, 4]. However, several environmental factors, such as latitude, seasonality

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and prevailing weather conditions, determine whether sunlight (i.e., UVB radiation) of sufficient strength is available to stimulate the conversion of 7-dehydrocholesterol in the skin to vitamin D_3. In this context, it has been calculated that daily skin synthesis of vitamin D in North Americans is 0 IU/day in winter and between 200 and 800 IU/day in summer [5]. Furthermore, there are many sociodemographic, personal and behavioral factors explaining why summertime sun exposure may be inadequate to prevent vitamin D deficiency, even in sunny countries [6]. For example, endogenous synthesis of vitamin D is low in institutionalized elderly people, in highly urbanized regions, especially in those with high air pollution, and in population groups that are not exposed adequately to sun due to religious or other reasons. Thus, regardless of whether the UVB deficit is due to latitude, season, weather or personal and behavioral factors, the absence of sufficient UVB for dermal synthesis places increased importance on the vitamin D food supply.

However, natural food sources of vitamin D are limited, many are not consumed on a regular basis [7] and as such the dietary supply of vitamin D is often insufficient to offset the seasonal deficit of UVB, occurring especially during winter months even in southern European countries [8]. Considering the above and the fact that relying on vitamin D supplement use is not an appropriate public health strategy to increase intakes across the population distribution [9], food fortification with vitamin D has been proposed as a strategy for increasing intake with potentially the widest reach and impact in the population [9–12]. In this context, based on nationally representative data for Irish adults, Black et al. [10] recently showed that even low doses of vitamin D through fortified foods can increase dietary intakes of vitamin D in adults, especially in those with the lowest dietary intakes. Furthermore, two meta-analyses provided evidence at the highest level that food fortification with vitamin D increases serum 25(OH)D levels in randomized controlled trials (RCTs) [9, 13]. Fortified milk was the most common food matrix for providing vitamin to the participants in these RCTs. Regarding other dairy products, there is only a limited number of RCTs [14–18] exploring the effect of vitamin D-enriched cheese on serum 25(OH)D concentration and the results have been quite mixed. This may relate to the quality of some of these studies, as suggested by Black et al. [9] as a limitation, but also to the fact that fortification of cheese with vitamin D has certain technological considerations, particularly for reduced-fat cheese varieties [15].

Among population groups, most vulnerable for vitamin D insufficiency, postmenopausal women are of specific interest as the prevalence of osteoporosis is relatively high in this group. Osteoporosis and susceptibility to osteoporosis-related fractures are part of the health issues associated with low vitamin D status [19]. Hip and vertebral fractures have important functional consequences, thereby also reducing health-related quality of life (HRQL) [20]. Beyond its contribution to physical impairment, vitamin D deficiency has also been suggested to play a role in disorders related to mental health, such as stress and depression [21], and as such sufficient vitamin D status can be important for the overall health, HRQL and well-being of postmenopausal women.

The primary objective of the present RCT was to examine whether consumption of vitamin D_3-enriched, reduced-fat Gouda-type cheese could counteract the expected decrease in serum 25(OH)D levels during winter months in postmenopausal women and prevent vitamin D deficiency. In addition, a secondary objective was to investigate any potential effect of the intervention on HRQL indices in these postmenopausal women.

Subjects and methods

Study design

The current study was a single-blinded (i.e., blinded only to study participants), randomized controlled dietary intervention study, testing the effect of vitamin D_3-enriched, reduced-fat Gouda-type cheese on serum 25(OH)D levels as the primary outcome measure and on HRQL indices, as secondary outcome measures.

Sampling

The sampling procedures followed in the present study are graphically presented in a flow diagram (Fig. 1). According to a sample size estimation, a sample of 37 women per study group was adequate to provide statistical power greater than 80 % ($\alpha = 0.05$, two-tailed) for detecting an increase in serum 25(OH)D levels of ~6 nmol/L from baseline to follow-up at probability of Type I error <0.05. The mean difference of 6 nmol/L in serum 25(OH)D concentrations was based on a previous clinical trial conducted with postmenopausal women in Greece, who were supplemented with a similar (to the current study) dose of vitamin D_3 through fortified milk [22]. To account for potential drop out, the number of women was increased to 40 per group.

Screening

The study was initiated with two screening phases in October 2014, when volunteers were invited to participate via informational brochures and posters distributed in public buildings and community centers in two municipalities within the wider district of Athens, namely Kallithea and Tavros-Moschato. The selection procedure

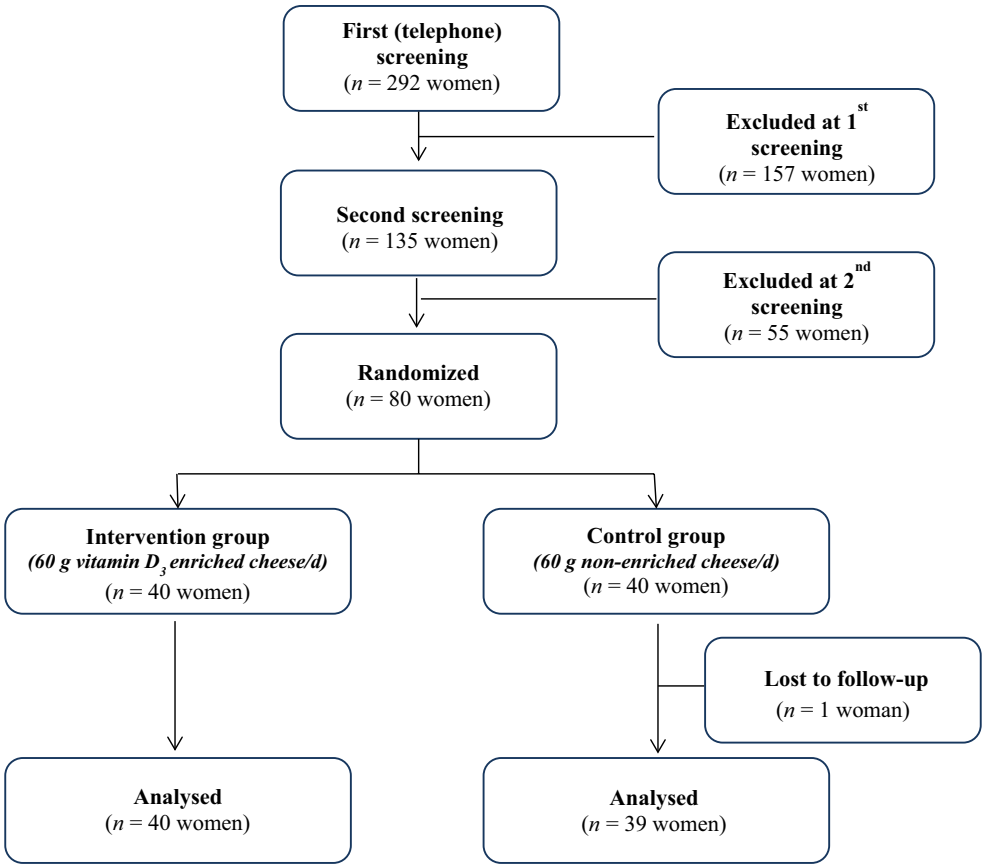

Fig. 1 Flow of participants throughout the study

followed in these screening phases required the recording and assessment of information on women's medical history, history of supplements' use, demographics (i.e., date of birth, age of menopause, educational level), anthropometric data (i.e., body weight and height) and dietary habits through a Food Frequency Questionnaire (FFQ) that was completed by study participants and was used to assess habitual cheese consumption, as well as calcium and vitamin D intakes. Women eligible to participate in the study were those that had no disease/pathology that interacts with vitamin D metabolism; those not requiring or taking medications, including hormone replacement therapy that interact with vitamin D metabolism or vitamin D supplements for medical reasons (e.g., osteoporosis); those not having a cow's milk allergy or a history of drug and/or alcohol abuse; those who used to consume cheese daily; those that had not a planned vacation to a sunny holiday destination during the intervention period; those who were >5 years postmenopause (i.e., not experiencing any menstrual flow, excluding all cases of hysterectomy, pregnancy or lactation [23]); and those with a body mass index (BMI) outside the range 20–33 kg/m^2. As approximately two-thirds of Greek adult women are overweight or obese [24], the current study attempted to reflect these prevalence rates by including normal weight, overweight and obese study participants. Reflecting the weight status of the general population of women in Greece and also excluding women with such increased adiposity levels that could adversely affect vitamin D metabolism (e.g., due to sequestration of vitamin D in the adipose tissue, hypo-hydroxylation of vitamin D due to fatty infiltration of liver) were the two main reasons for setting the upper BMI threshold at 33 kg/m^2.

Through the first screening phase, 135 women (aged 55–75 years) satisfying the inclusion criteria were identified and were invited to participate in the second screening (December 2014) of the study. The second screening was carried out via scheduled meetings and personal sessions with each one of the 135 eligible women at the Metabolic Unit of the Laboratory of Nutrition and Clinical Dietetics (LNCD) at Harokopio University. At this second screening phase, 80 eligible women were finally identified having for the most part homogenous characteristics at baseline.

Study groups and intervention

These 80 eligible women were randomly assigned to an intervention group (IG) and a control group (CG) using a random sampling approach. More specifically the "Select cases; Random sample of cases" command in the SPSS statistical software version 22.0 were used to generate two random study groups of 40 cases each. Study participants were blinded to the treatment arm to which they were randomized. Although those research team members that distributed the two different types of cheese to study participants were not blinded to treatment, the researchers undertaking, and subsequently reporting, the biochemical outcome measures were masked to all subjects' allocation scheme. Four weeks prior to the initiation of the intervention, dietary supplement users among study participants were instructed to discontinue any intake of vitamin D supplements (i.e., one woman in the CG and one woman in the IG). This washout period was included prior to the initiation of the intervention so as to avoid or minimize any possible effect on 25(OH)D concentrations at baseline derived from any supplemental use of vitamin D. Study participants were provided with, and were also instructed to consume (as part of their usual diet), 60 g (provided as 2 slices in a pack) of either non-enriched reduced-fat Gouda-type cheese (CG; $n = 40$) or vitamin D_3-enriched, reduced-fat Gouda-type cheese (IG; $n = 40$) for 8 weeks during winter (i.e., from January to March 2015). Sixty grams of the vitamin D_3-enriched, reduced-fat Gouda-type cheese provided 5.7 μg of vitamin D per day, which was independently verified using a liquid chromatography–mass spectrometry-based method, which has been described in detail elsewhere for pork [25]. In brief, samples were saponified followed by liquid–liquid extraction and then normal-phase solid-phase extraction. The analytes were derivatized with 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) to improve the ionization efficiency by electrospray ionization, and labeled internal standards were used for quantification.

All study participants were instructed to equally replace their habitual consumption of cheese with the corresponding amount of the experimental cheese, so as to compensate for additional calories. In addition, they received detailed guidance by the research group members to report any signs of illness, use of medication and any deviations from the study protocol. Furthermore, study participants were advised not to change their general dietary habits (especially for the habitual consumption of meat, eggs, milk and yogurt), level of physical activity, smoking and alcohol consumption habits. Study participants were also advised to maintain their body weight stable (body weight at baseline ±3 kg). Meetings with participants were held biweekly within the settings of the LNCD of Harokopio University, to supply participants with cheese for the next two weeks. Compliance to the intervention scheme (i.e., adherence to daily consumption of the 60 g of experimental cheese) was also assessed via information obtained at the biweekly meetings, as well as from biweekly telephone communication with study participants to confirm the planned meetings and ask for any illnesses, adverse effects or medications used during the intervention. In order to remind and motivate participants to consume daily the recommended slices of cheese, they were also provided with and instructed to keep a logbook, where they had to record their consumption of cheese on a daily basis. During the 8-week intervention period, study participants should have received and consumed a total of 112 slices of cheese. When study participants for any reason did not manage to consume the requested slices of cheese on one day, they were asked to compensate the missed portions the next day.

All study participants signed written informed consent forms. The study was approved by the Ethics Committee of Harokopio University (Approval code: 43/23-07-2014) and was conducted in accordance with the code of ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. The study protocol registration number was ClinicalTrials.gov: NCT02543671.

The effectiveness of the intervention was assessed with the assessment of dietary intake and clinical examinations conducted at baseline and follow-up using the same procedures and methodology. The dietary data obtained at the second screening of the study were used as baseline dietary intake data. The follow-up examination took place in mid-March 2015, after 8 weeks of intervention.

Measurements

An existing quantitative FFQ validated for calcium intake [26] was updated to also include vitamin D dietary sources (such as fish/seafood, beef, pork, mushrooms, eggs and margarine) and was used to assess habitual dietary calcium and vitamin D intakes. The mean content of the foods listed in the FFQ in calcium and vitamin D was multiplied to their daily frequency of consumption, thus providing the amount of habitual, mean daily calcium and vitamin D intakes.

Body weight and standing height were measured by using a digital scale (Type SECA 813, Hamburg, Germany) with an accuracy of ±100 g and a commercial stadiometer (Leicester Height Measure, Invicta Plastics Ltd, Oadby, UK) to the nearest 0.5 cm, respectively. Study participants were measured while wearing light clothing and no shoes. Body mass index (in kg/m^2) was calculated by dividing body weight to standing height squared.

Early-morning venous blood samples were obtained from each study participant following a 12-h overnight fast at baseline and follow-up examination. Blood was collected in vacutainers without added anticoagulant and was

kept at room temperature for ≈2 h, where it was allowed to clot. Centrifugation for serum separation was conducted at 3000 rpm for 15 min. Aliquots of 1.5 mL serum were then stored at −80 °C. Serum concentrations of total 25(OH)D (i.e., 25(OH)D_2 plus 25(OH)D_3) in all serum samples were measured by the Cork Centre for Vitamin D and Nutrition at University College Cork, using a liquid chromatography-tandem mass spectrometry method, described in detail elsewhere [27]. The intra-assay CV of the method was <5 % for all 25(OH)D metabolites, while the inter-assay CV was <6 %. The Cork Centre for Vitamin D and Nutrition Research at University College Cork is a member in the Vitamin D Standardization Program [28] and is certified by the Centers for Disease Control and Prevention's Vitamin D Standardization Certification Program [29]. The change in serum concentrations of total 25(OH)D from baseline to follow-up examination was examined as the primary outcome measure in the present study. The Institute of Medicine (IOM) Dietary Reference Intake Committee's recent thresholds were used for defining vitamin D deficiency. More specifically, the risk of deficiency was defined at serum 25(OH)D concentration below 30 nmol/L, while 50 nmol/L are consistent with that needed by 97.5 % of individuals aged >1 year in terms of bone health [30].

HRQL was assessed using the Short Form Health Survey questionnaire (SF-36), which is a self-administered tool, also validated for Greek adult population [31]. It includes 36 questions that can be scored and used to assess respondents' mental and/or physical health during the past 4 weeks, as well as their overall health perceptions which are grouped and scored in eight health scales, i.e., physical functioning, role limitations due to physical health, role limitations due to emotional problems, vitality, emotional well-being, social functioning, bodily pain and general health. The changes in these HRQL scales from baseline to follow-up examination were examined as secondary outcome measures in the present study.

Statistical analysis

Data were reported as mean and standard deviations (SD) or as mean change and 95 % confidence intervals (CI). The Kolmogorov–Smirnov test was used to determine normality of distribution of the examined variables. Differences in mean values of baseline characteristics among the two groups of women were derived from Student's *t* test or the nonparametric Mann–Whitney Test, whenever appropriate. Repeated-measures analysis of variance (RM-ANOVA) was used to examine the significance of the differences between groups regarding the changes of dietary intakes of cheese, calcium and vitamin D, serum 25(OH)D concentrations and HRQL indices as well as the significance of these changes within groups. Adjustments were made for three potential confounders, i.e., for years of education, age and BMI levels. In this regard, considering the variance/range in years of education (4–20 years), age (55–75 years) and BMI levels (20–33 kg/m^2) among study participants, as well as the known effect of these factors on vitamin D status (e.g., educational level affects dietary habits and subsequently dietary intake of vitamin D; age and/or BMI have an effect on vitamin D ingestion, endogenous synthesis and metabolism) and HRQL, all RM-ANOVAs performed in the current study were adjusted for these three potential confounders. The Chi-square test was also used to examine the differences in the percentages of subjects with serum 25(OH)D levels below 30 and 50 nmol/L between groups at baseline and follow-up examination, as well as from baseline to follow-up examination within each group. The SPSS statistical analysis software for Windows, version 22.0 (SPSS Inc., Chicago, IL, USA) was used to perform all statistical analyses. All statistical tests were two-tailed and the level of statistical significance was set at $P < 0.05$. The "rate constant" value, which reflects the change (i.e., rise or fall) in 25(OH)D levels per 1 μg of additional vitamin D intake, was also calculated by subtracting the net changes in 25(OH)D levels observed in the intervention and control groups, further divided by the administered dose of vitamin D (i.e., 5.7 μg/day in the present study). The approach followed in the present study for calculating "rate constant" was the same as the one also used by Whiting et al. [32] in a systematic review of vitamin D supplementation studies.

Results

One of the 80 women initially assigned to participate in the study was lost to follow-up due to medical reasons. Consequently, the number of subjects in each group with full baseline and follow-up data was 39 in the control group and 40 in the intervention group (Fig. 1). Compliance to treatment (i.e., consumption of 112 slices of cheese per study participant) during the intervention period, as assessed by logbook entries by subjects, was very high at 100 and 97.5 % in the intervention and control group, respectively.

The baseline characteristics of the 79 study participants with full data at baseline and follow-up examination are summarized in Table 1. The mean (SD) age of postmenopausal women participating in the current study was 62.6 (6.4) years and the mean (SD) elapsed time since their menopause was 13.8 (6.8) years. No statistically significant differences were observed between the study groups, thus indicating homogeneity in substantial baseline demographic and anthropometric indices, as well as with regard to alcohol consumption and smoking habits at baseline.

The means (SDs) at baseline and follow-up examination, as well as mean (95 % CI) changes from baseline

Table 1 Baseline descriptive characteristics of study participants completing the trial

	Control group ($n = 39$)	Intervention group ($n = 40$)	P value*
	Mean (SD)		
Age (years)	63.2 (5.9)	62.6 (6.0)	0.670
Years since menopause	14.2 (7.3)	13.9 (5.7)	0.805
Education (years)	12.7 (3.5)	12.3 (3.8)	0.594
Body mass index (kg/m^2)	29.0 (2.9)	28.0 (3.8)	0.190
Alcohol consumption (portions/week)	1.31 (2.17)	1.65 (2.55)	0.863[§]
	% (n)		
Smokers[†]	28.2 (11)	30.0 (11)	0.861[‡]

* P values were derived from Student's t test, unless stated otherwise

[§] P values were derived from the nonparametric Mann–Whitney test

[†] Currently smoking or quitted smoking for less than 5 years (definition provided by Sohl et al. [42])

[‡] P values were derived from Chi-square test

Table 2 Changes in cheese consumption, dietary calcium and vitamin D intakes and total serum 25(OH)D levels from baseline to follow-up by study group

	Baseline[§] Mean (SD)	Follow-up Mean (SD)	8-week change Mean change (95 % CI)	P value*
Total cheese consumption (g/day)[†]				0.741
Control group ($n = 39$)	48.1 (35.7)	63.9 (27.0)	15.9 (6.40 to 25.4)	
Intervention group ($n = 40$)	56.2 (31.5)	69.9 (24.0)	13.7 (4.30 to 23.0)	
Calcium intake (mg/day)				0.590
Control group ($n = 39$)	919 (350)	1085 (320)	157 (67 to 246)	
Intervention group ($n = 40$)	951 (369)	1133 (359)	191 (103 to 279)	
Vitamin D intake (μg/day)				<0.001
Control group ($n = 39$)	2.05 (1.51)	1.41 (1.13)	−0.64 (−1.06 to −0.22)	
Intervention group ($n = 40$)	1.70 (1.24)	6.81 (0.89)[‡]	5.12 (4.70 to 5.53)	
Body mass index (kg/m^2)				0.052
Control group ($n = 39$)	29.4 (3.5)	29.5 (3.5)	0.04 (−0.15 to 0.22)	
Intervention group ($n = 40$)	28.5 (4.6)	28.8 (4.7)	0.29 (0.11 to 0.48)	
Total serum 25(OH)D (nmol/L)				<0.001
Control group ($n = 39$)	42.9 (17.7)	38.3 (18.9)	−4.59 (−6.36 to −2.81)	
Intervention group ($n = 40$)	47.3 (15.2)	52.5 (12.0)[‡]	5.14 (3.39 to 6.89)	

* P values are those of treatment × time effects on mean values in repeated-measures analysis of variance; adjustments were made for age (in all tested variables) and BMI at baseline (in all tested variables except from the BMI variable); in the case of dietary calcium and vitamin D intakes, the analyses were also adjusted for the changes in cheese consumption from baseline to follow-up; in the case of 25(OH)D levels, the analysis was additionally adjusted for the changes in dietary calcium and vitamin D intakes from baseline to follow-up

[§] There was no significant difference in baseline parameters between the two groups; $P > 0.25$ in all cases

[‡] Significantly different from mean of control group at follow-up; $P < 0.001$

[†] The increases observed in daily cheese intake in both study groups were attributed to the consumption of 60 g of the experimental Gouda-type cheese

to follow-up examination, for both treatment groups with respect to serum 25(OH)D concentrations (primary outcome measure), cheese consumption, the dietary intakes of calcium and vitamin D and BMI are shown in Table 2. There were no significant differences between the two groups in any of the four variables at baseline. Regarding changes within groups, the replacement of habitual cheese consumption by the 60 g of experimental cheese resulted in significant increases in mean cheese consumption by 13.7 g (95 % CI 4.3–23.0) and 15.9 g (95 % CI 6.4–25.4) from baseline to follow-up in the IG and the CG, respectively, which did not differ between the two groups ($P = 0.7$). Likewise, mean calcium intake significantly increased by 191 mg (95 % CI 103–279) in the IG and 157 mg (95 % CI

67, 247) in the CG over the eight weeks, but there was no difference between groups ($P = 0.6$). Mean dietary vitamin D intake increased significantly from baseline to follow-up only in the IG, while decreased significantly in the CG over the same period, and these changes differentiated significantly between groups [5.1 μg (95 % CI 4.7–5.5) vs. −0.6 μg (−1.1 to −0.2) $P < 0.001$, respectively]. Likewise, mean serum 25(OH)D concentration significantly increased in the IG while decreased in the CG over the 8-week intervention period [5.1 nmol/L (3.4–6.9) vs. −4.6 nmol/L (−6.4 to −2.8) $P < 0.001$, respectively]. Using the measured changes in serum 25(OH)D concentrations in the two groups, a rate constant was estimated as 1.7 nmol/L per 1 μg of the administered vitamin D dose [i.e., net change in the IG minus net change in the CG/administered vitamin D dose: +5.14 − (−4.59)/5.7) = 1.7]. Regarding BMI, although a significant increase of 0.29 kg/m^2 (95 % CI 0.11–0.48) was observed over the intervention period in the IG, no significant difference was found compared to the respective BMI change observed in the CG ($P = 0.052$). The above-mentioned findings were similar when the analysis was performed without any adjustment for years of education, age and BMI levels (data not shown).

The differences between study groups and changes within study groups from baseline to follow-up examination in the percentages of study participants with 25(OH)D levels <30 and <50 nmol/L, respectively, are shown in Fig. 2. While there were no differences between groups at baseline, the percentages of women with 25(OH)D concentrations <30 and <50 nmol/L were significantly lower at follow-up in the IG compared to the CG (0 vs. 41.0 %, $P < 0.001$ and 47.5 vs. 74.4 %, $P = 0.015$, respectively). Furthermore, the percentage of study participants with 25(OH)D concentrations <30 nmol/L decreased

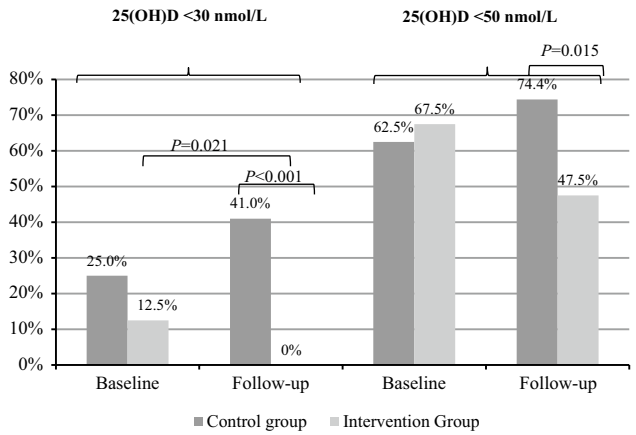

Fig. 2 Differences between study groups and changes within study groups from baseline to follow-up in the percentage of study participants with serum 25(OH)D concentrations <30 nmol/L and <50 nmol/L. *P* values derived from Chi-square tests

significantly from baseline to follow-up in the IG (i.e., from 12.5 % at baseline to 0 % at follow-up, $P = 0.021$), but not in the CG ($P > 0.1$). No other statistically significant changes within groups or differences between groups were observed.

Table 3 summarizes the mean changes within groups and the differences between groups from baseline to follow-up in the eight HRQL scales (secondary outcome measures). RM-ANOVA showed that the emotional well-being scale improved in the IG as compared to the CG (3.20 vs. −3.79, $P = 0.028$), but no other statistically significant differences were observed in the remaining HRQL indices.

Discussion

This dietary intervention study has shown that daily consumption of 60 g of vitamin D-enriched, reduced-fat Gouda-type cheese, providing a daily dose of 5.7 μg of vitamin D_3, in addition to the usual dietary intake of ~2 μg/day vitamin D, significantly increased mean serum 25(OH)D concentrations and prevented wintertime vitamin D deficiency in postmenopausal women in Greece. Although the prevalence of vitamin D deficiency was higher in the control group than in the intervention group at baseline (25.0 vs. 12.5 % at mid-January 2015, which is a typical winter month in Greece), not a single woman who received vitamin D_3-enriched cheese had serum 25(OH)D concentration below 30 nmol/L at endpoint (i.e., in March 2015), which coincided with the end of the winter period. However, 41 % of women in the control group, who received the equivalent non-enriched cheese, were vitamin D deficient at endpoint. In relation to meeting the criteria for vitamin D adequacy (serum 25(OH)D > 50 nmol/L; [30]) and recognizing that the combined total vitamin D intake from usual diet and enriched cheese (~7 μg/day) was much lower than the 15 μg/day suggested by the IOM [30], the data showed that less than half (47.5 %) of women in the intervention group had a 25(OH)D concentration <50 nmol/L at endpoint compared to three-quarters of women (74.4 %) in the control group.

To our knowledge, this is the only study of vitamin D-enriched cheese to report the impact on prevalence of vitamin D deficiency as the primary outcome measure. More specifically, the present study was designed to test the effectiveness of vitamin D-enriched cheese during winter months, when UVB-induced dermal synthesis of vitamin D would be negligible. It also used a level of vitamin D addition to the cheese (i.e., 5.7 μg per 60 g), which not only is allowable under legislation, but which would be expected (combined with habitual dietary intakes) to reduce the prevalence of vitamin D deficiency. A number of previous intervention studies have provided vitamin D-enriched cheese

Table 3 Changes in health-related quality of life scales (SF-36) from baseline to follow-up by study group

	Baseline Mean (SD)	Follow-up Mean (SD)	8-week change Mean change (95 % CI)	*P* value*
Physical functioning				0.958
Control group ($n = 38$)	79.9 (15.6)	77.6 (18.1)	−2.59 (−7.25 to 2.07)	
Intervention group ($n = 40$)	76.5 (19.5)	73.8 (19.4)	−2.42 (−6.96 to 2.13)	
Role limitations due to physical health				0.721
Control group ($n = 39$)	78.2 (34.0)	74.4 (35.6)	−4.57 (−20.4 to 11.3)	
Intervention group ($n = 40$)	68.8 (37.4)	67.5 (38.9)	−0.54 (−16.2 to 15.1)	
Role limitations due to emotional problems				0.974
Control group ($n = 39$)	76.9 (32.6)	70.1 (37.3)	−7.46 (−18.8 to 3.90)	
Intervention group ($n = 40$)	68.3 (33.7)	60.0 (40.8)	−7.73 (−18.9 to 3.49)	
Vitality				0.440
Control group ($n = 39$)	69.5 (16.7)	69.1 (20.8)	−0.73 (−5.39 to 3.93)	
Intervention group ($n = 40$)	68.6 (16.2)	65.0 (19.0)	−3.29 (−7.89 to 1.31)	
Emotional well-being				0.028
Control group ($n = 39$)	71.0 (15.5)	67.4 (18.8)	−3.79 (−8.20 to 0.62)	
Intervention group ($n = 40$)	67.4 (21.4)	70.4 (22.6)	3.20 (−1.16 to 7.54)	
Social functioning				0.400
Control group ($n = 39$)	77.6 (22.2)	78.5 (23.5)	0.24 (−7.84 to 8.31)	
Intervention group ($n = 40$)	80.9 (20.2)	75.6 (24.8)	−4.61 (−12.6 to 3.36)	
Bodily pain				0.084
Control group ($n = 39$)	74.7 (19.9)	73.2 (23.0)	−2.13 (−10.1 to 5.80)	
Intervention group ($n = 40$)	67.4 (25.4)	74.4 (26.8)	7.70 (−0.13 to 15.5)	
General health				0.818
Control group ($n = 37$)	60.8 (14.9)	63.0 (17.8)	1.72 (−2.88 to 6.32)	
Intervention group ($n = 39$)	62.6 (15.6)	64.6 (17.0)	2.47 (−2.00 to 6.95)	

* *P* values are those of treatment × time effects on mean values in repeated-measures analysis of variance; adjustments were made for age, BMI at baseline and years of education

§ There was no significant difference in baseline parameters between the two groups; $P > 0.19$ in all cases

to adults and reported changes in mean serum 25(OH)D concentration as the primary [14, 15] or secondary outcome measure [16–18]. However, the findings of these studies were mixed and likely due to very different study design characteristics.

In summary, Johnson et al. [14] unexpectedly showed in their partially double-blind, 2-month RCT conducted with older individuals (≥60 years) that daily consumption of 85 g vitamin D-enriched processed cheese (supplying 15 μg/day) led to a significant mean decrease in serum 25(OH)D of 6 nmol/L, whereas the groups who received non-enriched cheese or no cheese had either a significant increase (3.5 nmol/L) or no change in serum 25(OH)D levels, respectively. However, there were differences in baseline serum 25(OH)D concentration across these three groups, with the vitamin D-enriched cheese group having higher baseline 25(OH)D levels compared to the other two non-enriched cheese groups, respectively, thus probably explaining the observed changes. In three other RCTs, Bonjour et al. showed that consumption of 200 g enriched soft plain cheese (made from semi-skimmed milk), providing 2.5 μg/day of vitamin D_3 for 1–1½ month, led to increases in serum 25(OH)D concentrations, ranging from 2 to ~5 nmol/L in institutionalized elderly (>65 years) women with low vitamin D status at entry [16, 17] and of 9 nmol/L in postmenopausal women (aged 50–65 years) [18] in France during winter and summer months, respectively. Furthermore, Wagner et al. [15] performed a trial in which young adults (mean ~28 years) in Canada were randomized to weekly servings of enriched cheddar cheese (34 g/day) or enriched low-fat (7 %) cheese (41 g/day), or a placebo cheddar cheese (as well as three further study groups receiving a liquid vitamin D supplement, taken with food or without food, or a placebo supplement) over 8 weeks (between January and April). The weekly treatments contained 28,000 IU vitamin D_3, equivalent to 100 μg/day. In the placebo groups, mean serum 25(OH)D concentration decreased over the 8 weeks of winter by 4.3 nmol/L, whereas in the enriched cheddar cheese and the enriched low-fat cheese groups these increased by over

100 %, respectively; responses that although differed from the placebo groups did not differ from the vitamin D_3 liquid supplement groups [15]. Notably, this level of addition of vitamin D_3 far exceeds that allowed in Europe or North America.

The differences in serum 25(OH)D response to vitamin D-enriched cheese in the available RCTs are likely to be related to differences in age of participants, administered dose of vitamin D, the frequency of administration (i.e., daily or weekly), the duration and adherence to the intervention and other parameters such as latitude, seasonality and different baseline 25(OH)D levels. Differences associated with the bioavailability of vitamin D from the different cheeses could also potentially have a role, even though a recent review suggested that the food matrix apparently has little effect on vitamin D bioavailability [33]. These factors are also likely to explain the higher rate constant value evident in the present study and that of Bonjour et al. [17] (1.7 and 2.0 nmol/L serum 25(OH)D per 1 μg additional vitamin D, respectively) compared to that in the other studies (range −0.6 to 0.7 nmol/L per μg; [14–16, 18]).

While the HRQL was included as a secondary outcome measure in the present trial, the finding that the score of the "emotional well-being" scale increased significantly in the IG compared to the CG was interesting, even though no other significant differences were observed between study groups with respect to the changes in the other HRQL scales. Since "emotional well-being" is also a surrogate measure of depression and/or depressive symptoms, the positive results of the present study on this specific HRQL scale highlight the need for further research on this particular health outcome. This is particularly the case in light of the inconclusive findings derived from four recent systematic reviews on this topic [34–37]. A beneficial effect of increased vitamin D intake on HRQL, if substantiated, could be of public health importance, particularly if the increase is within the normal dietary range. Nevertheless, significant effects on secondary outcome measures, even in an RCT, can only be hypothesis-generating and do not prove the concept that this is a vitamin D-mediated effect. In addition, we cannot exclude the possibility that the effect may have been due to a Type 1 error. In addition, the limited number of available studies demonstrating significant improvements in "emotional well-being" [38–40] used higher doses of vitamin D (i.e., up to 100 μg/day with or without calcium) and the tools used to quantify HRQL were not validated.

The findings of the present study should be interpreted in light of its potential strengths and limitations. Strengths of the study include the detailed study protocols and procedures, which were tightly followed to assure the correct implementation of the intervention, the independent verification of the vitamin D content of foods, the high adherence of participants to treatment as also confirmed by the procedures followed to record cheese consumption, the blinded nature of the study design and the inclusion of a control group using a placebo product. One potential limitation of the present study could be the significant increase in BMI observed for women in the intervention group that were consuming the vitamin D-enriched cheese. However, in order to control for the possible confounding effect of this significant increase in BMI, the statistical analysis examining the differences in serum 25(OH)D concentrations between study groups was adjusted for BMI. It is worth noting, however, if anything, the increase in BMI in the intervention group would be more likely to attenuate the response of serum 25(OH)D to increased vitamin D intake [41]. It is also worth noting that while there was a modest decrease in dietary vitamin D intake in the control group over the 8-week winter intervention period (i.e., −0.6 μg/day), this is likely to have only contributed in a relatively minor way to the expected seasonal-decline in serum 25(OH)D concentration observed in this group. Lastly, the fact that there was no treatment allocation concealment in the present study is also a limitation.

In conclusion, the present RCT demonstrated that a daily dose of 5.7 μg of vitamin D_3 provided to postmenopausal women in addition to the usual dietary intake (~2 μg/day) for two winter months by reduced-fat, Gouda-type cheese was sufficient for increasing serum 25(OH)D concentrations and preventing vitamin D deficiency. These findings highlight consumption of low-fat vitamin D-enriched cheese as a potentially important food-based solution for increasing population vitamin D intake. Lastly, more research is required to further investigate and confirm any potential beneficial effect of vitamin D-fortified products on HRQL indices in older women.

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Compliance with ethical standards

Conflict of interest C.S.P. and E.vdH. are employees at Friesland-Campina. None of the other authors have any potential conflict of interest to declare. Any opinions, findings, conclusions or recommendations expressed in the current study are those of the authors and do not necessarily reflect the views of FrieslandCampina.

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