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ORIGINAL COMMUNICATION

Can vitamin D_3 supplementation prevent bone loss in persons with MS? A placebo-controlled trial

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Abstract Multiple sclerosis (MS) is a possible cause of secondary osteoporosis. In this phase II trial we assessed whether a weekly dose of 20,000 IU vitamin D_3 prevents bone loss in ambulatory persons with MS age 18–50 years. ClinicalTrials.gov ID NCT00785473. All patients managed at the University Hospital of North Norway who fulfilled the main inclusion criteria were invited to participate in this double-blinded trial. Participants were randomised to receive 20,000 IU vitamin D_3 or placebo once a week and 500 mg calcium daily for 96 weeks. The primary outcome was the effect of the intervention on percentage change in bone mineral density (BMD) at the hip, the spine, and the ultradistal radius over the study period. Of 71 participants randomised, 68 completed. Mean serum 25-hydroxyvitamin D [25(OH)D] levels in the intervention group increased from 55 nmol/L at baseline to 123 nmol/L at week 96. After 96 weeks, percentage change in BMD did not differ between groups at any site. BMD decreased at the hip, by 1.4% in the placebo group (95% CI −2.3 to −0.4, SD 2.7, $p = 0.006$) and by 0.7% in the treatment group (−1.6 to 0.2, 2.7, $p = 0.118$), difference 0.7% (−1.9 to 0.7, $p = 0.332$). Findings were not altered by adjustment for sex or serum 25(OH)D. Supplementation with 20,000 IU vitamin D_3 a week did not prevent bone loss in this small population. Larger studies are warranted to assess the effect of vitamin D on bone health in persons with MS.

Keywords Multiple sclerosis · Clinical trials randomised controlled (CONSORT agreement) · Osteoporosis · Bone mineral density · 25-Hydroxyvitamin D

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Introduction

Multiple sclerosis (MS), a neuroinflammatory disease, is a possible cause of secondary osteoporosis [17]. In persons with MS, low bone mineral density (BMD) is more prevalent when compared with controls [10, 27, 37, 38] and with reference databases [26, 35, 39].

In individuals, BMD is primarily determined by genetic and hormonal factors, BMI, physical activity, and intake of calcium and vitamin D [22, 29]. Peak bone mass is attained in the third decade of life. Age-related bone loss starts around the age of 40 in both men and women, accelerating around menopause in women [29]. Low bone mass may result from either inadequate peak bone mass acquisition, bone loss, or a combination of these.

In persons with MS, several studies have shown that BMD decreases as disability increasingly limits physical activity [10, 26, 27, 38, 39]. Baseline data from this study showed that one out of four participants had lower than expected BMD for sex and age [35].

The optimal serum 25-hydroxyvitamin D [25(OH)D] value for bone health is not known, but many scientists and clinicians recommend maintaining 25(OH)D levels of at

least 75 nmol/L [4, 12, 24]. This is also the level of optimal intestinal absorption of calcium and the level at which serum parathyroid hormone levels are not further suppressed by increasing vitamin D intake [19].

Most studies in persons with MS have reported mean serum levels of vitamin D between 50 and 65 nmol/L [20]. Low vitamin D levels may contribute to poor bone health, even before disability limits physical activity. If it proves true that vitamin D has a role in MS aetiology and pathogenesis, a shared pathway in the pathogenesis of MS and osteoporosis is conceivable [1, 20]. In this phase II clinical trial, we aimed to assess whether a weekly dose of 20,000 IU vitamin D_3 prevents bone loss in fully ambulatory persons with MS age 18–50 years.

Methods

Trial design

This was a single centre, balanced randomised, double-blinded, placebo-controlled, parallel-group, 96 week study following a pre-defined protocol. The study was conducted at the Department of Neurology, University Hospital of North Norway, the only neurology service in Troms and Finnmark, the northernmost counties of Norway. The study was approved by the Regional Committee for Medical and Health Research Ethics. All participants provided written informed consent. The trial was registered in ClinicalTrials.gov (ID NCT00785473).

Participants

Patients were eligible for inclusion if they were 18–50 years of age with clinical definite MS according to McDonald criteria and an Expanded Disability Status Scale (EDSS) score $\leq$4.5 [21, 23]. Exclusion criteria were: inability to walk 500 m or more; history of conditions or diseases affecting bone; pregnancy or lactating during the past 6 months; use of bone-active medications other than intravenous methylprednisolone for treatment of relapses; a history of nephrolithiasis during the previous 5 years; menopause defined by not having regular menstruation; unwillingness to use appropriate contraception. A pregnancy test (HCG in serum) was performed in all female participants at screening. The flow of participants through the study is shown in Fig. 1.

Randomisation

A statistician at the Clinical Research Centre, who was not otherwise involved in the study, performed the randomisation by blocks of six, stratified by sex, with a concealed, computer-generated randomisation procedure. An identification number and a randomisation number were created for each participant. All study personnel and participants were blinded to treatment assignment for the duration of the study.

Intervention

Active treatment was 20,000 IU vitamin D_3 (cholecalciferol) once a week, administered as a capsule of Dekristol™ (Mibe GmbH Arzneimittel, Brehna, Germany). Identical placebo capsules were provided by the manufacturer of Dekristol™ (SWISS CAPS AG, Kirchberg, Switzerland). Participants were allowed to continue vitamin D supplements they used at baseline. All participants received 500 mg elemental calcium daily, administered as a chewable tablet of Weifa Kalsium™ (calcium carbonate, Weifa AS, Oslo, Norway). Participants who had gastrointestinal side effects attributed to Weifa calcium discontinued the calcium supplement if they had an estimated dietary calcium intake of $\geq$800 mg/day. If calcium intake was lower, they switched to Calcium Sandoz™ effervescent tablets (calcium lactate-gluconate and calcium carbonate, Sandoz A/S, Odense, Denmark) which were better tolerated.

Adherence and safety

The participants were offered a reminder to take the study medication by weekly SMS. Adherence to study medication was evaluated by capsule counting (Adherence (%) = (number of capsules consumed/number of capsules that should have been consumed) $\times$ 100). The participants were considered adherent if they had taken at least 80% of the study medication. Participants were educated about clinical signs of hypercalcaemia, and ionised serum calcium was determined every 12 weeks. Adverse events were assessed every 12 weeks by phone, at study visits weeks 48 and 96, and in case of hospital admissions. Serious adverse events and unexpected medical events were reported to the Norwegian Medicines Agency and to the Ethical Committee.

Data collection

Study visits were conducted at the University Hospital of North Norway (Clinical Trial Unit and Department of Neurology).

Clinical examination

M.K., L.H.S. or S.I.M. performed a full physical examination and complete neurological status from which Kurtzke's Expanded Disability Status Scale (EDSS) was

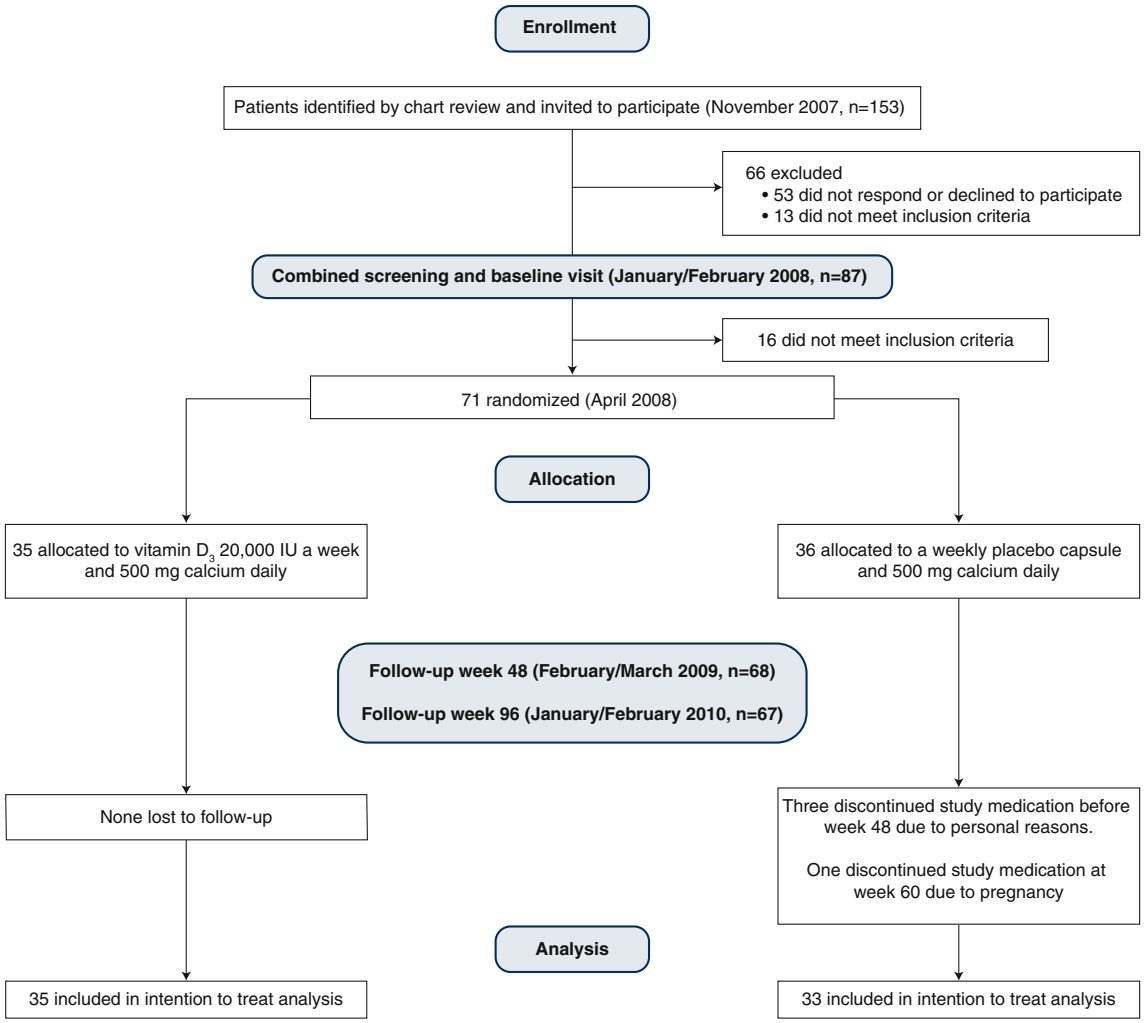

Fig. 1 Flow of the participants through the study

determined at each study visit [21]. Standing height and body weight were measured with participants wearing lightweight clothing and no shoes.

Dual-energy X-ray absorptiometry (DXA)

Measurement of BMD at the hip (mean of left and right total hip), the spine (anterior–posterior spine L1–L4), and the non-dominant ultradistal (UD) radius by DXA was performed by trained technicians using a Lunar Prodigy advanced densitometer (Lunar Radiation Corp., Madison, WI, USA.). The long-term precision was 0.26–0.28%, obtained by daily calibration of the densitometer.

Blood tests

Every 12 weeks, routine blood tests including ionised calcium were performed. Serum was collected for measurement of 25(OH)D. At week 96 serum testosterone and sex hormone-binding globulin (SHBG) were measured in men and FSH in women. FSH values of $\geq$40 IU/L were defined as a marker for perimenopause [16, 32]. All laboratory tests except serum 25(OH)D, were performed at the Department of Clinical Chemistry, University Hospital of North Norway. Batch analysis of serum 25(OH)D concentrations by mass spectroscopy [15] was performed at the Hormone Laboratory, Haukeland University Hospital, Bergen, Norway.

Outcomes

We assessed the effect of the intervention on percentage change in BMD at the hip, the spine, and the UD radius over the 96 week study period. Occurrence of fractures was registered every 3 months. Analyses were carried out according to a pre-established analysis plan. The modified intention-to-treat population included all participants who had received at least one dose of the study medication and

presented at one follow up visit. In the patient who discontinued the study medication because of pregnancy, the last measurements (week 48) were carried forward.

Statistical analysis

Distribution and variance of BMD and vitamin D values were assessed by appropriate tests. Interactions between sex and treatment group were tested for. Differences in baseline variables between the groups were assessed by independent T-test for continuous variables and by Chi-Square test for categorical variables. The main outcome, percentage of change in BMD, was analysed by independent sample T-test. Changes in BMD from baseline to week 96 were analysed by paired samples T-test. A preplanned multiple regression model included the variables age, BMI, baseline BMD, baseline 25(OH)D, percentage change in 25(OH)D, and perimenopausal FSH values. Subgroup analyses were performed by sex and by treatment group. All statistical analyses were conducted using SPSS 16.0 for Windows. p values <0.05 were considered statistically significant.

Results

Figure 1 shows the flow of participants through the study. Sixteen persons who were examined at the combined screening and baseline visit did not fulfil the inclusion criteria: EDSS >4.5 (5), history of nephrolithiasis (4), lactating or planning pregnancy (4), primary progressive MS (1), rheumatoid arthritis (1), and menopause (1). Of 71 persons who were randomised, 35 completed in the intervention group (24 women, 11 men), and 33 completed in the placebo group (24 women, 9 men). Three participants withdrew from the study for personal reasons before the first follow up examination.

Adherence to study medication use and adverse events

By capsule count, all subjects were ≥80% (mean 98%, range 80–100%) adherent. One participant in the placebo group discontinued the study medication at week 94 because of nephrolithiasis. One participant in each treatment arm experienced an unexpected medical event that was not related to the study medication.

Baseline characteristics of study participants

Baseline demographic and clinical characteristics did not differ between groups (Table 1).

BMD measurements

Table 2 shows BMD values at baseline, BMD at week 96, and change in BMD over the study period. The values were normally distributed. Analysis of variance (ANOVA) showed no interactions between treatment group and sex. Percentage change in BMD did not differ between participants who had received vitamin D_3 and those who had received placebo. This result was not altered by adjustment for baseline serum 25(OH)D or change in serum 25(OH)D over the study period. At the hip, BMD decreased by 1.4% in the placebo group (95% CI −2.3 to −0.4, SD 2.7, $p = 0.006$) and by 0.7% in the treatment group (95% CI −1.6 to 0.2, SD 2.7, $p = 0.118$). Women in the placebo group had the highest bone loss at the hip. This result persisted when women in presumed late menopausal transition (FSH ≥40 nmol/L) at the end of the study were excluded. Respective changes at the spine were a decrease

Table 1 Baseline demographical and clinical characteristics

	Placebo $n = 33$	Vitamin D_3 $n = 35$
Sex (% women)	72.7	68.6
Age (years), mean (range)	41.0 (26–50)	39.7 (21–50)
BMI (kg/m^2), mean (range)	26.4 (18.4–39.9)	25.9 (21.0–40.7)
Duration of MS from first symptom, years, mean (range)	10.0 (2.0–26.0)	10.9 (1.0–27.0)
EDSS, median (range)	2.0 (0–4.5)	2.5 (0–4.5)
Intravenous methylprednisolone*		
Ever treated (%)	39.4	37.1
Cumulative dose (g), mean (range)	7.9 (3.0–33.0)	13.8 (3.0–43.0)
Disease modifying treatment* interferon beta (%)	45.5	45.7
Vitamin D intake as recommended* (≥7.5 μg from diet and supplements), %	57.6	48.6
Calcium intake as recommended* (≥800 mg from diet and supplements), %	39.4	40.0

* As described earlier [35]

Table 2 Bone mineral density (BMD) at baseline and at week 96, and change in BMD

	Placebo $n = 33$, mean (SD)	Vitamin D_3 $n = 35$, mean (SD)	Difference (95% CI)	p-value
Total hip				
BMD (mg/cm^2) baseline	969 (120)	1,019 (99)	−50 (−103 to 3)	0.064
BMD (mg/cm^2), week 96	956 (124)	1,012 (106)	−56 (−112 to 0)	0.050
BMD change (mg/cm^2)	−13 (25)	−7 (27)	−6 (−18 to 7)	0.361
BMD change (%)	−1.4* (2.7)	−0.7 (2.7)	−0.7 (−1.9 to 0.7)	0.332
Lumbar spine				
BMD (mg/cm^2), baseline	1,166 (136)	1,205 (118)	−39 (−101 to 22)	0.203
BMD (mg/cm^2), week 96	1,165 (143)	1,202 (121)	−37 (−101 to 27)	0.255
BMD change (mg/cm^2)	−1 (42)	−3 (31)	3 (−15 to 21)	0.769
BMD change (%)	−0.1 (3.6)	−0.3 (2.7)	0.2 (−1.3 to 1.7)	0.793
UD radius				
BMD (mg/cm^2), baseline	473 (81)	485 (67)	−12 (−48 to 24)	0.503
BMD (mg/cm^2), week 96	479 (85)	496 (73)	−17 (−55 to 22)	0.395
BMD change (mg/cm^2)	6 (26)	11 (28)	−4 (−17 to 9)	0.501
BMD change (%)	1.3 (5.7)	2.3 (5.7)*	−1.0 (−3.7 to 1.8)	0.506

* Change in BMD significantly different from zero, $p < 0.05$

by 0.1% (95% CI −1.3 to 1.2, SD 3.6), and 0.3% (95% CI −1.2 to 0.7, SD 2.7) and at the UD radius an increase by 1.3% (95% CI −0.7 to 3.3, SD 5.7) and 2.3% (95% CI 0.3–4.2, SD5.7). BMD at the hip in women was the only baseline characteristic that differed between treatment arms (mean (SD), placebo: 965 mg/cm^2 (132), vitamin D_3: 1,039 mg/cm^2 (80), $p = 0.023$). No fractures occurred during the study period.

Intercorrelations among independent variables

There were no significant associations between percentage change in BMD and possible predictors of BMD (sex, age, BMI, baseline BMD, baseline serum 25(OH)D, change serum 25(OH)D, serum testosterone in men at week 96, and EDSS). Percent change in BMD indicated significantly higher bone loss in women with FSH values ≥40 IU/L indicating perimenopause, compared with women with FSH <40 IU/L: hip FSH ≥40 IU/L: −4.4% (SD 2.3), hip FSH <40 IU/L: −0.7% (SD 2.3), difference −3.7%, 95% CI −1.7 to −5.7, $p < 0.001$; spine FSH ≥40 IU/L: −3.5% (SD 3.0), spine FSH <40 IU/L: 0.2% (SD 2.8), difference −3.7%, 95% CI −1.3 to −6.1, $p = 0.003$; UD radius FSH ≥40 IU/L: −2.3% (SD 4.5), UD radius FSH <40 IU/L: 2.5% (SD 6.0), difference −4.8%, 95% CI −0.1 to −9.6, $p = 0.047$. Baseline serum 25(OH)D did not differ between sexes and did not correlate with BMI or age.

Blood tests

Table 3 shows values of serum 25(OH)D and ionised calcium at baseline and at week 96. An unexplained decrease of serum 25(OH)D occurred in one participant treated with vitamin D_3 (from 127 nmol/L at baseline to 48 nmol/L at week 96). All measured values of PTH and ionised calcium were within the respective reference ranges (1.1–6.8 pmol/L and 1.10–1.34 mmol/L). ALP values were unchanged. FSH in women and testosterone and SHBG in men did not differ between groups. At week 96, seven women (five in the placebo group and two in the vitamin D_3 group) probably were (peri) menopausal, having FSH values ≥40 IU/L.

Discussion

A weekly dose of 20,000 IU vitamin D_3 administered for 96 weeks did not affect BMD in fully ambulatory persons with MS age 18–50. Findings did not vary by sex and were not altered after adjustment for serum 25(OH)D. Optimal 25(OH)D levels of ≥75 nmol/L were reached in 32 out of 35 participants who received vitamin D_3.

Serum 25(OH)D levels, at least up to 75 nmol/L, are strongly associated with higher BMD in young, healthy individuals [5]. There is also good evidence that vitamin D supplementation increases 25(OH)D concentrations and improves bone health [6, 11]. A meta-analysis concluded that the effect of vitamin D supplementation on bone health was greater in persons with low (<25 nmol/l) serum 25(OH)D than those whose serum level was normal [36]. Only three individuals in the intervention group of this study were overtly vitamin D deficient at baseline (25(OH)D <25 nmol/L), and participants were allowed to continue vitamin D supplements they used at baseline, both of which could have contributed to the negative findings in this trial. In women, baseline BMD at the hip was significantly higher in the intervention than in the placebo group,

Table 3 Serum 25(OH)D and ionised calcium at baseline and at week 96

	Baseline		Week 96	
	Placebo, $n = 33$	Vitamin D_3, $n = 35$	Placebo, $n = 33$	Vitamin D_3, $n = 35$
25(OH)D, nmol/L				
Mean (SD)	57.3 (21.8)	55.6 (29.0)	61.8 (25.2)	123.2** (34.2)
Range	17.9–101.0	19.9–143.0	19.1–106.0	47.5–205.0
Deficient (<25 nmol/L), %	9	9	12	0
Insufficient (25–49 nmol/L), %	33	45	24	3
Marginal (50 to <75 nmol/L), %	33	29	33	6
Optimal (≥75 nmol/L), %	24	17	30	91
Ionised calcium, mmol/L, mean (SD)	1.2 (0.0)	1.2 (0.0)	1.2 (0.0)	1.2 (0.0)

** Significant change from baseline, $p < 0.01$

which may also have influenced the result of the study. The major limitation of this study, however, is the small number of participants. Data from BMD measurements in a comparable group of persons with MS were not available when the trial was planned. Based on change in BMD from this study, a trial with approximately 250 persons in each treatment arm would have been needed to confirm or reject the hypothesis that a weekly dose of 20,000 IU vitamin D_3 administered for 96 weeks prevents bone loss in persons with MS (two tailed test, power 80%, risk of type I error 5%, drop out rate 10%).

Supplementing 20,000 IU vitamin D_3 per week increased serum 25(OH)D by 68 nmol/L, and 32 out of 35 participants in the intervention group reached desired vitamin D levels of ≥75 nmol/L. The study medication was tolerated without clinical or biochemical side effects, and even higher doses of vitamin D_3 are probably safe [7, 33]. Supplementation with vitamin D can be achieved equally well with daily as with weekly dosing frequencies [9].

At baseline, one quarter of our study population had lower than expected BMD for sex and age [35]. Over the 96 week study period, BMD at the hip decreased in both treatment arms, the change being statistically significant in the placebo group. This finding indicates that MS may be a cause of secondary osteoporosis. In healthy individuals, studies have reported no significant bone loss at the hip in women before the perimenopausal years [2, 3, 8, 18, 25, 32], while men may or may not have a small bone loss before age 50 [2, 14, 18]. The general bone loss at the hip, a weight bearing site, may result from decreased physical activity even in fully ambulatory persons with MS [30], due to fatigue, visual problems, and impaired balance. We observed no change in BMD at the lumbar spine. This finding is in agreement with two recent studies reporting unchanged BMD at the lumbar spine over a period of respectively 96 weeks and 39 months in interferon beta-treated persons with MS [31, 34]. At the non-dominant UD radius, BMD increased significantly in the intervention group, but not in the placebo group. In healthy individuals, BMD at this site has been reported to be stable or increasing up to age 40 in both sexes [8, 13, 18]. BMD at the UD radius, especially in the non-dominant arm, is not dependent on weight bearing activity and therefore is likely to be less affected by mild to moderate MS-related disability.

No fractures occurred during this study, while 22% of more disabled persons with MS experienced low impact fractures during a 2-year observation period in another study [10].

The strengths of this study are its double-blinded, population-based design and its long follow-up. All eligible persons with MS from a homogeneous and stable population in a defined geographical area who were willing to participate were included. Only three out of 71 participants were lost to follow-up and adherence to treatment was excellent. Confounding by seasonal variations in 25(OH)D and BMD was avoided by conducting study visits at the end of winter. We included both women and men, because we considered it relevant from a clinical point of view, but there are considerable sex differences in bone metabolism, and findings in a mixed population must be interpreted with care.

Multiple sclerosis (MS) is the most common disease that causes neurological disability among young Caucasians, onset being before age 30 in half of the patients. Persons with MS gradually experience reduced muscle strength, problems with balance and coordination, vision problems and fatigue, all of which are likely to result in limited opportunity for high-impact physical activity, also early in the course of the disease, and thus interfere with maintenance of good bone health. Evidence is accumulating that hypovitaminosis D may play a role in the aetiology and pathogenesis of MS [28], which by itself might put persons with MS at risk of low peak bone mass. We also found that bone loss at the hip was higher than expected in healthy individuals.

Supplementation with vitamin D and calcium are the basis of osteoporosis prevention and treatment. Given that

MS is a cause of secondary osteoporosis, vitamin D deficiency in a person with MS would represent an additional risk factor. Much larger studies are needed to assess whether optimising intake of vitamin D and calcium is beneficial for bone health in persons with MS.

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Conflict of interest The authors declare that they have no conflict of interest.

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