

A longitudinal study of serum 25-hydroxyvitamin D and intact parathyroid hormone levels indicate the importance of vitamin D and calcium homeostasis regulation in multiple sclerosis

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► Supplementary fig and table are published online only at <http://jnnp.bmj.com/content/vol79/issue2>

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Received 24 August 2006
Revised 13 April 2007
Accepted 30 May 2007
Published Online First
19 June 2007

ABSTRACT

Background: Past sun exposure and vitamin D3 supplementation have been associated with a reduced risk of multiple sclerosis (MS). There are no previous longitudinal studies of vitamin D in MS.

Objectives: To compare regulation of vitamin D and calcium homeostasis between patients with MS and healthy controls. To study the correlation of parameters of vitamin D metabolism with MS activity.

Methods: We measured 25-hydroxyvitamin D (25(OH)D), parathyroid hormone (PTH), calcium, phosphate, magnesium, chloride, alkaline phosphatase, albumin and thyroid stimulating hormone in serum every 3 months and at the time of relapse over 1 year in 23 patients with MS and in 23 healthy controls. MRI burden of disease and T2 activity were assessed every 6 months.

Results: Vitamin D deficiency (S-25(OH)D ≤ 37 nmol/l) was common, affecting half of the patients and controls at some time in the year. Seasonal variation of 25(OH)D was similar in patients and controls, but 25(OH)D serum levels were lower and intact PTH (iPTH) serum levels were higher during MS relapses than in remission. All 21 relapses during the study occurred at serum iPTH levels >20 ng/l (2.2 pmol/l), whereas 38% of patients in remission had iPTH levels ≤ 20 ng/l. Patients with MS had a relative hypocalcaemia and a blunted PTH response in the winter. There was no correlation between serum 25(OH)D and MRI parameters.

Conclusions: The endocrine circuitry regulating serum calcium may be altered in MS. There is an inverse relationship between serum vitamin D level and MS clinical activity. The role of vitamin D in MS must be explored further.

Multiple sclerosis (MS) is generally believed to be an immune mediated disorder that occurs in genetically susceptible people.¹ The reasons for the variation in prevalence and incidence of multiple sclerosis worldwide are not understood, but genetic and environmental explanations have been offered.¹ In addition to its role in calcium and bone metabolism, vitamin D regulates cell proliferation and differentiation and can regulate immune responses. Receptors for vitamin D are expressed widely on the cells of the immune system. Diminished capacity of vitamin D to regulate immune responses as a consequence of lower serum concentrations of 25-hydroxyvitamin D (25(OH)D) during the winter in tempered climates could be one environmental explanation for the peculiar geographical distribution of MS.^{2,3} A

growing body of evidence supports this hypothesis. Intake of vitamin D is associated with a lower incidence of MS.⁴ The risk of MS is reduced in association with past exposure to sun.⁵ Moreover, a recent retrospective case control study showed that the risk of MS is decreasing with increasing serum vitamin D levels, most notably in those with the highest vitamin D levels (≥ 100 nmol/l).⁶ In an earlier cross sectional study, we showed that newly diagnosed patients with MS have lower serum levels of 25(OH)D during MS relapses than in remission.⁷ There are no previous longitudinal studies on serum 25(OH)D levels in relapsing-remitting MS.

The first hypothesis of this study was that serum concentrations of 25(OH)D would decrease during periods of increased disease activity. Therefore, we measured serum concentrations of 25(OH)D serially every 3 months and at the time of relapse in 23 patients during the first year of the PRISMS Study (Prevention of Relapses and Disability by Interferon beta-1a Subcutaneously in Multiple Sclerosis).⁸ Serum 25(OH)D levels were compared with clinical and MRI disease activity and with the therapy received. The second hypothesis was that patients with MS have vitamin D insufficiency compared with healthy persons. Intact parathyroid hormone (iPTH) is a functional indicator of vitamin D sufficiency and has been used in several studies to define vitamin D deficiency more accurately than measurement of 25(OH)D.^{9,10} Therefore, we included measurement of iPTH, as well as an exploratory analysis of other clinical chemistry parameters closely related to vitamin D metabolism—namely, serum levels of calcium, phosphate, magnesium, chloride, alkaline phosphatase and albumin as well as thyroid stimulating hormone (TSH), in patients with MS and in age and sex matched healthy controls.

METHODS

Patients

In the PRISMS Study, patients were randomly assigned to receive subcutaneous recombinant interferon-beta-1a (IFNB-1a; Rebif) 22 µg, 44 µg or placebo, three times a week for 2 years.⁸ In the PRISMS-4 study, patients initially receiving placebo were randomised to IFNB-1a 22 or 44 µg, while the other patients continued with their original dose.¹¹ In the present study, 23 Finnish patients (17 women, six men) from Turku University Hospital district (60.13° northern and

22.19° eastern latitude) participating in the PRISMS and PRISMS-4 studies were included. Ethics approval was obtained from the Commission of Ethics of the Turku University and the Turku University Central Hospital. All patients gave written informed consent. MRI was assessed every 6 months. The MRI parameters studied were burden of disease (BOD mm³) and T2 activity (mean active new and/or enlarging lesions/scan).¹¹ The patients had neurological examinations every 3–6 months and within 7 days of relapse. Morning blood samples were collected within 2 weeks before and 4 weeks after exacerbation onset, and were determined as “relapse” and others as “remission” samples. Three of 21 relapses were treated with intravenous methylprednisolone.

At the PRISMS study baseline, mean (SEM) age of the patients with MS was 34.1 (1.5) years and the mean Expanded Disability Status Scale (EDSS) was 2.3 (range 0–5). Time from diagnosis of definite MS ranged from 6 months to 15 years (mean 5.6 years). Mean number of relapses during the preceding 2 years was 2.6 (range 2–6). Eight patients were treated with placebo, seven with Rebif 22 µg and eight with Rebif 44 µg. Blood was collected at PRISMS study baseline and at weeks 12, 26, 36, 52 and 64, as well as within 7 days of the onset of a relapse. Serum was separated rapidly from red cells by centrifuging at 1000 g for 10 min after clotting and stored frozen in aliquots of 1 ml at –40°C until analyses.

Controls

Morning blood samples from apparently healthy laboratory personnel living in the same area as the MS patients (60.13° northern and 22.19° eastern latitude) were collected during the same months of the year as samples from the patients with MS and were processed similarly and stored until the analyses.¹² Mean (SEM) age of the controls was 32.0 (2.2) years; 17 were women and six were men.

25(OH) D analysis

Serum samples were stored at –40°C and protected from direct exposure to sunlight until the analysis. For quantitative determination of 25(OH)D in serum samples, a commercially available 25-hydroxyvitamin D ¹²⁵I RIA Kit (DiaSorin Catalogue No 68100E; Stillwater, Minnesota, USA) was used according to the manufacturer's instructions. All determinations were performed at Turku University Hospital Central Laboratory. Two quality control samples were included in each assay series and the specimens and controls were assayed in duplicate. Using

this method, values less than 20 nmol/l (8 µg/l) indicate severe hypovitaminosis D, 20–37 nmol/l (8–15 µg/l) moderate hypovitaminosis D and levels above 37 nmol/l (15 µg/l) adequate vitamin D stores.⁹

Intact parathyroid hormone analysis

The biologically active form of PTH, iPTH, was measured from serum samples using the Elecsys immunochemiluminometric assay and Modular E 170 analyser (Roche, Mannheim, Germany). The reference values for iPTH using this assay method are 15 ng/l (1.7 pmol/l) (lower limit) and 65 ng/l (7.2 pmol/l) (upper limit).

Analysis of albumin, calcium, magnesium, alkaline phosphatase, chloride, phosphate and thyroid stimulating hormone

Analyses of serum albumin, calcium, magnesium, alkaline phosphatase, chloride and phosphate were performed with a Modular P800 analyser and TSH analysis with a Modular E170 analyser (Roche). The laboratory reference values for each parameter are shown in table 1.

All analyses were performed at Turku University Hospital Central Laboratory according to the Finnish Accreditation Service (FINAS) standard EN ISO/IEC 17025.

Statistical analyses

Longitudinal data were analysed using analysis of variance for repeated measurements (the mixed procedure ANOVA) using a confidence interval of 95%. Because of the positively skewed distributions, 25(OH)D, PTH and TSH values were log transformed for statistical analysis. The Student's t test was used for comparison between relapse and remission samples. Pearson analysis was used for testing correlations. The SAS System for Windows (V.9.1; SAS Institute Inc., Cary, North Carolina, USA) was used for the analyses.

RESULTS

Comparison of serum levels of 25(OH)D and iPTH between patients with MS and healthy controls

To examine the hypothesis that patients with MS have vitamin D insufficiency in comparison with healthy persons, we examined differences in summer, autumn, winter and spring 25(OH)D and iPTH serum levels between patients with MS and healthy controls. Interaction between time and group was not significant in a variance analysis of serum 25(OH)D between patients with MS and controls ($p = 0.1507$). Figure 1A shows

Table 1 Exploratory analysis of clinical chemistry parameters related to vitamin D metabolism

	Albumin (g/l)	Calcium (mmol/l)	Phosphate (nmol/l)	AFOS (U/l)	Magnesium (mmol/l)	Chloride (mmol/l)	TSH (mU/l)
Normal values	36–48	2.15–2.51	0.76–1.41 (female) 0.71–1.53 (male)	35–105	0.7–1.1	100–108	0.3–4.2
MS patients	44.36 (3.12)	2.28 (0.11)	1.01 (0.16)	43.00 (11.31)	0.85 (0.05)	104.82 (1.75)	2.21 (1.04)
Controls	42.64 (3.48)	2.36 (0.09)	1.19 (0.21)	58.50 (14.46)	0.81 (0.05)	106.18 (1.72)	2.55 (1.40)
Time×group interaction	0.0035*	0.0149*	0.011*	0.0778 NS	0.0922 NS	0.5677 NS	0.285 NS
p Value for overall difference				0.0002*	0.0009*	0.0016*	0.257 NS
Summer	0.0086*	0.1699	<0.0001*				
Autumn	0.1553	0.1670	0.0644				
Winter	0.6733	<0.0001*	0.0030*				
Spring	0.0276*	0.0013*	0.0103*				

Values are mean (SD) of 23 patients with MS and 23 age and sex matched healthy controls during the whole year.

For each patient and control, four 3 monthly samples were included.

When the interaction between time and group was statistically significant (*), the p values for each time point are given. When the interaction between time and group was not significant, the p values for the overall difference between patients with MS and controls during the whole year are given.

AFOS, alkaline phosphatase; MS, multiple sclerosis; TSH, thyroid stimulating hormone.

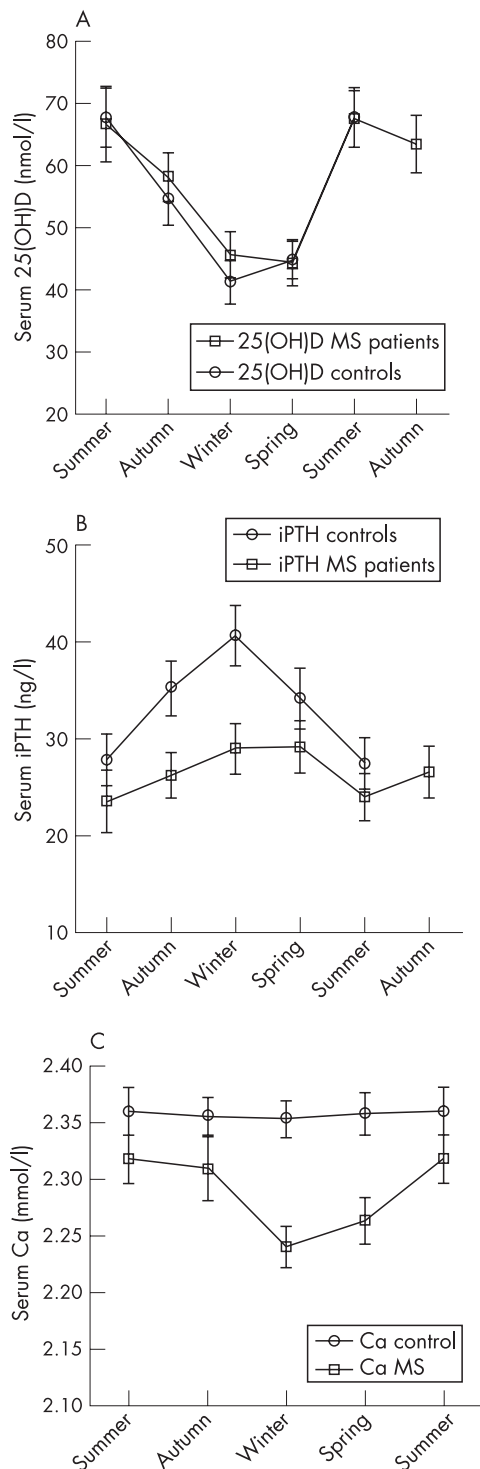


Figure 1 Seasonal variation in 25-hydroxyvitamin D (25(OH)D), intact parathyroid hormone (iPTH) and calcium (Ca) in serum in 23 patients with multiple sclerosis (MS) and in 23 healthy controls. (A) Mean serum concentration of 25(OH)D was similar in patients with MS and in healthy controls throughout the year (p value for overall difference between groups 0.81; p value for interaction between time and group 0.1507, NS). (B) There was a blunted PTH response in patients with MS in the autumn and most notably in the winter (p value for interaction between time and group 0.0385*; p value for the difference between MS and control values in the winter 0.0042* and in the autumn 0.0141*). (C) There was relative winter and spring hypocalcaemia in patients with MS in comparison with healthy controls (p value for the interaction between time and group 0.0149*; p value for the difference between MS and control values in the winter <0.0001* and in the spring 0.0013*). Error bars represent SEM.

that seasonal variation of serum 25(OH)D levels was almost identical in patients with MS and in healthy controls, with the lowest levels in winter and spring (December to May) and highest in summer (June to August). The mean (SD) level of serum 25(OH)D during the whole year in patients with MS was 57.6 (20.5) nmol/l (23.0 (8.2) µg/l) and in healthy controls 55.3 (22.4) nmol/l (22.1 (8.9) µg/l; p value for overall difference between groups 0.81, NS). Interaction between time and group was significant (p = 0.0385) in a variance analysis of serum iPTH between patients with MS and controls, demonstrating a blunted PTH response in patients with MS in the autumn and most notably in the winter (fig 1B). The mean (SD) winter level of iPTH in patients with MS was 29.1 (12.9) ng/l (3.2 (1.4) pmol/l) and in healthy controls 40.7 (14.9) ng/l (4.5 (1.6) pmol/l; p = 0.0042). The mean (SD) autumn level of iPTH in patients with MS was 26.8 (11.1) ng/l (2.9 (1.2) pmol/l) and in controls 35.2 (13.4) ng/l (3.8 (1.5) pmol/l; p = 0.0141). The mean summer and spring iPTH values in patients with MS and controls did not differ significantly (fig 1B). The mean (SD) summer level of iPTH in patients with MS was 23.5 (10.8) ng/l (2.6 (1.2) pmol/l) and in controls 27.9 (12.3) ng/l (3.1 (1.3) pmol/l; p = 0.3418). The mean (SD) spring level of iPTH in patients with MS was 24.0 (11.6) ng/l (2.6 (1.2) pmol/l) and in controls 27.5 (12.4) ng/l (3.0 (1.3) pmol/l; p = 0.2379).

Vitamin D deficiency (25(OH)D ≤ 37 nmol/l (14.8 µg/l)) was detected in 43% of patients with MS and in 53% of controls. When a higher cut-off value for vitamin D deficiency was used (50 nmol/l (20 µg/l)),¹³ only 17% of patients with MS and 22% of controls had sufficient vitamin D levels throughout the year. Elevation of iPTH above the upper limit of the laboratory reference range of 65 ng/l (7.2 pmol/l) was detected in 15% of patients with MS and 17% of controls.

Correlation of serum levels of 25(OH)D and iPTH with clinical and MRI activity of MS

To examine the hypothesis that serum concentrations of 25(OH)D would decrease during periods of increased disease activity, we examined differences in 25(OH)D serum concentration levels between periods of relapse and remission. Figure 2 shows that serum levels of 25(OH)D were significantly lower and serum levels of iPTH significantly higher during MS relapses than in periods of remission. Mean (SD) concentration of 25(OH)D at relapse was 47.4 (14.4) nmol/l (18.9 (5.8) µg/l) and at remission 60.0 (21.8) nmol/l (24.0 (8.5) µg/l; p value for difference 0.012). Mean (SD) concentration of iPTH at relapse was 33.1 (11.9) ng/l (3.6 (1.3) pmol/l) and at remission 26.4 (11.6) ng/l (2.9 (1.3) pmol/l; p value for difference 0.009). All relapses occurred at PTH levels above 20 ng/l (2.2 pmol/l) whereas 38% of patients in remission had PTH levels ≤ 20 ng/l (fig 2). None of the relapses but 11% of remissions occurred at serum concentrations of 25(OH)D above 85 nmol/l (9.4 pmol/l) (fig 2). There was no correlation with serum 25(OH)D or iPTH levels and MRI BOD or T2 activity (not shown). Gadolinium enhanced images were not included in the study. Serum levels of 25(OH)D or iPTH were not significantly different in patients treated with placebo in comparison with patients treated with either dose of IFNβ-1a (not shown).

When examining longitudinal patterns of 25(OH)D and iPTH and relapse, in individual patients, we found a pattern of 14 of 21 relapses occurring at the peak of increasing levels of iPTH and 16 of 21 relapses occurring at the lowest or decreasing levels of 25(OH)D in 10 of the 12 relapsing patients (see supplementary fig online). Only 4 of 21 relapses in two patients (panels H and L

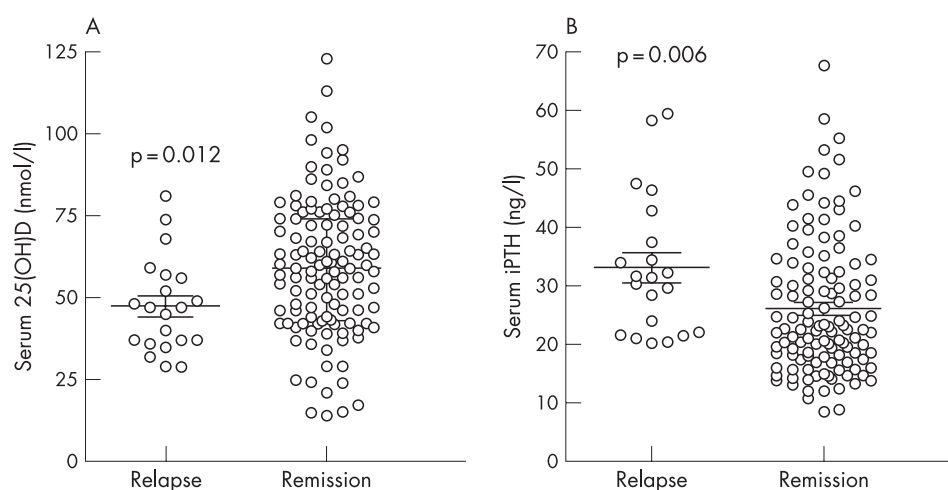


Figure 2 Serum levels of 25-hydroxyvitamin D (25(OH)D) and intact parathyroid hormone (iPTH) during periods of relapse and remission of multiple sclerosis (MS). Serum levels of 25(OH)D and iPTH were measured every 3 months and at the time of relapses over 1 year in 23 patients with MS. Samples collected within 2 weeks before and 4 weeks after exacerbations were determined as “relapse” and others as “remission” samples. (A) Mean (SD) concentration of 25(OH)D at relapse was 47.4 (14.4) nmol/l (18.9 (5.8) μ g/l) and at remission 60.0 (21.8) nmol/l (24 (8.5) μ g/l; p value for difference 0.012*). None of the 21 relapses but 13/22 (11%) cases of remission occurred at serum 25(OH)D concentrations above 85 nmol/l (34 μ g/l). (B) Mean (SEM) concentration of iPTH at relapse was 33.1 (2.6) ng/l and at remission 26.4 (1.1) ng/l ($p = 0.009^*$). All relapses occurred at PTH levels above 20 ng/l (2.2 pmol/l) whereas 38% of patients in remission had PTH levels ≤ 20 ng/l.

in the supplementary fig) occurred at the peak or increasing serum 25(OH)D levels.

Patients were then grouped into quintiles by their winter, spring, summer and autumn serum 25(OH)D levels. The pre-study relapse rate, relapse rate during the study and confirmed EDSS progression during the study in these vitamin D quintiles were determined (see supplementary table online). The increase in EDSS during the study and the relapse rate before and during the study were lower in the highest quintile than in the lowest quintile, but there was no statistically significant inverse correlation between vitamin D nutrition and EDSS or relapse rate ($p = 0.065$ for EDSS, $p = 0.660$ for relapse rate during the study and $p = 0.119$ for pre-study relapse rate).

Exploratory analysis of clinical chemistry parameters related to vitamin D metabolism in patients with MS and controls

To examine the hypothesis that patients with MS have vitamin D insufficiency in comparison with healthy persons, that is reflected in clinical chemistry parameters related to vitamin D metabolism, we measured serum albumin, calcium, magnesium, alkaline phosphatase, chloride, phosphate and TSH levels in patients with MS and healthy controls. All of these values in both patients and controls were within the laboratory reference range (table 1). Analysis of variance for repeated measurements revealed a seasonal pattern in serum calcium, phosphate and albumin such that serum calcium and phosphate levels were significantly lower in winter and spring in patients with MS in comparison with controls, whereas serum albumin levels were significantly higher in patients with MS in the summer and spring (fig 1C, table 1). Serum alkaline phosphatase and serum chloride levels were significantly lower and serum magnesium levels were significantly higher in patients with MS than in controls throughout the year whereas serum TSH was similar in patients and controls at all times (table 1).

There were no significant differences in any of the clinical chemistry parameters or TSH levels between MS periods of relapse and remission (data not shown). However, there was a trend towards lower serum calcium concentrations in the

relapse samples in comparison with the remission samples (0.24 (0.02) mmol/l vs 0.28 (0.01) mmol/l; $p = 0.06$). There were no significant differences in any of the clinical chemistry parameters or TSH between patients treated with placebo and patients treated with IFNB-1a (data not shown).

DISCUSSION

The serum concentration of 25-hydroxyvitamin D (25(OH)D, calcidiol) is a reflection of the intake of vitamin D in food and its synthesis from pro-vitamins in the skin under the influence of UV light.⁹ The cellular effects of vitamin D are mediated through the binding of the active metabolite of vitamin D, 1,25-dihydroxyvitamin- D_3 (1,25 (OH) $_2D_3$, vitamin D hormone) into the vitamin D receptor expressed on a wide variety of cell types, including cells of the immune system and neuronal and glial cells in the human brain.^{14 15} Conversion of calcidiol into the vitamin D hormone takes place in the kidney, and serum levels of calcidiol closely reflect levels of the hormonally active form of vitamin D in subjects with normal kidney function.¹⁶

We showed that seasonal variation of 25(OH)D in serum is similar in Finnish patients with MS and age and sex matched healthy persons. This suggests that vitamin D may be functioning as a disease modifier in genetically susceptible individuals, rather than as a disease determinant in the general population. Vitamin D deficiency (serum 25(OH)D ≤ 37 nmol/l) was common in this longitudinal study, affecting half of the patients and controls at some time of the year. A similar high prevalence of vitamin D deficiency has been detected previously in Finnish medical inpatients and outpatients and healthy army recruits during the winter.^{10 17} In our earlier cross sectional study, 30% of newly diagnosed Finnish patients with MS had vitamin D deficiency.⁷

Experimentally, vitamin D deficiency results in an increased incidence of autoimmune diseases.¹⁸ The mechanism is likely to be related to the development of self-tolerance as vitamin D hormone regulates T helper cell and dendritic cell function and induces regulatory T cells resulting in a decrease in Th1 driven autoimmune responses and tolerance instead of vigorous

immune responses.^{18 19} In a careful epidemiological study, the risk of MS was shown to be higher in persons born in the month of May.²⁰ One explanation for this could be lower levels of circulating vitamin D available to the fetus during winter pregnancies. Immunological tolerance to self-antigens is a process that occurs during development, rather than being genetically preprogrammed.²¹

In Finland, the incidence of MS is among the highest and type I diabetes the highest in the world. The incidence of both diseases has been increasing in parallel with a decrease in the recommended dose of vitamin D supplementation to infants from 4000–5000 IU until 1964, to 2000 IU until 1975 and to 1000 IU until 1992, when it was reduced to the current level of 400 IU/day.^{22 23} Vitamin D supplementation to infants in Finland at our latitude of 60° to 70° north is stopped at the age of 3 years. It has been suggested that at our latitude, vitamin D supplementation should be continued for longer as prophylaxis for osteoporosis.¹⁷

There are two alternative interpretations of our results showing lower levels of circulating vitamin D during MS relapse compared with periods of remission: either increasing circulating vitamin D reduces the risk of MS relapse or MS relapse reduces serum vitamin D levels. In a recent pilot study with 2.5 µg/day of oral calcitriol (vitamin D hormone) in 15 relapsing–remitting patients with MS, the on-study exacerbation rate was less than baseline.²⁴ This is in favour of the first interpretation. We also found a trend for a correlation between increasing serum vitamin D status and less relapses and less EDSS progression, but possibly because of the sample size of our study, statistical significance was not reached. Our results also suggest that the previously observed increased risk of MS relapse in the spring could be related to lower serum levels of vitamin D after the winter.²⁵ Unfortunately, gadolinium enhanced images were not included in the PRISMS trial and therefore we could not evaluate the previously presented hypothesis that serum vitamin D levels inversely correlate with gadolinium enhanced brain images in patients with MS 2 months later.^{26 27}

The current adult recommendation for vitamin D (200–600 IU/day) is very low when one considers that 10–15 min of whole body exposure to peak summer sun will generate and release up to 20 000 IU vitamin D₃ into the circulation.²⁸ Using functional indicators of vitamin D sufficiency, such as calcium absorption, bone mineral density and iPTH, several studies have more accurately defined vitamin D deficiency as circulating levels of 25(OH)D ≤ 80 nmol/l (32 µg/l).²⁸ Recent studies reveal that current dietary recommendations for adults are not sufficient to maintain circulating 25(OH)D levels at or above this level, especially in pregnancy and lactation, as there is hardly any vitamin D₃ in the diet.²⁸ In our study, none of the 21 serum samples that were collected during MS relapse but 13/122 samples collected in remission reached a serum 25(OH)D level of 85 nmol/l. Only four of the 23 patients with MS reached 85 nmol/l of serum vitamin D in the summer and two in the autumn or spring, but none in the winter. There was considerable overlap in the distribution of serum vitamin D values in participants in remission versus those who relapsed. Intact PTH was a better predictor of MS relapse than vitamin D such that all patients in relapse had serum iPTH levels above 20 ng/l (2.2 pmol/l) whereas 38% of patients in remission had iPTH less than 20 ng/l (2.2 pmol/l).

The most important function of vitamin D in bone metabolism is maintenance of an adequate calcium and phosphate supply to the bone by increasing their absorption

from the gut.²⁹ We hypothesise that the observed lower winter calcium and phosphate levels in patients with MS compared with controls indicates a relative vitamin D deficiency in MS. Neither patients with MS nor controls reported intake of vitamin D supplements. The serum albumin concentration has an effect on the serum concentration of total calcium such that an increase of 10 g/l in serum albumin concentration leads to an increase of 0.2 mmol/l in serum calcium concentration.²⁹ As serum albumin levels were higher in patients with MS than in controls, the corrected difference between serum concentrations of calcium in patients with MS and in controls would be even greater than that reported (0.12 mmol/l instead of 0.08 mmol/l). The observed winter hypocalcaemia and a blunted PTH response in patients with MS compared with controls raises the possibility that the endocrine circuitry regulating serum calcium is altered in patients with MS either as a cause or a consequence of their disease.

There were no statistically significant differences in any of the clinical chemistry parameters between the relapse and remission samples or between the IFNB-1a and placebo treatment arms. Hence the differences cannot be explained by IFNB-1a or corticosteroid therapy. Higher magnesium levels in patients with MS than in controls could be explained by frequent use of supplements containing magnesium by patients with MS.³⁰ Alkaline phosphatase is stimulated by mobility and hence could be lower in patients with MS as a consequence of less physical activity. Whatever the explanation for the observed differences in the clinical chemistry parameters closely related to vitamin D metabolism, our results suggest that regulation of vitamin D and calcium homeostasis is likely to be important in MS. Our findings also raise the concern of bone health in patients with MS. It is important to recognise that the proinflammatory cytokines IFN-γ, interleukin 1-α and tumour necrosis factor-α, that are pathogenic in MS,¹ are also strong stimulators of osteoclastic bone resorption and inhibitors of bone formation.^{29 31} The mechanism of the inflammation induced bone loss has been shown to be cytokine induced activation of the inducible nitric oxide pathway in bone cells.³² In a recent study, dietary calcium and vitamin D hormone treatment directly and indirectly inhibited the tumour necrosis factor-α pathway and suppressed inflammatory bowel disease in vitamin D deficient knockout mice.³²

Oral vitamin D₃ supplementation is a safe and cost effective way to increase circulating vitamin D levels. The risk of vitamin D₃ supplementation is hypercalcaemia. It is usually asymptomatic at serum calcium concentrations below 2.8 mmol/l but at higher serum calcium concentrations may provoke disorientation, muscle weakness and cardiac arrhythmias. However, the risk of severe hypercalcaemia only arises when high amounts of vitamin D (>1000 µg/day or >40 000 IU/day) are consumed.³⁰ There are also new vitamin D hormone analogues that effectively regulate the immune system without increasing serum calcium.¹⁸ The therapeutic utility of vitamin D₃ or vitamin D hormone analogues in MS should be addressed in randomised clinical trials. Based on our results, we suggest choosing relapse rate reduction as a primary outcome measure for these trials. Analysis of other serum parameters of vitamin D metabolism, including iPTH and calcium as well as measurement of bone density, would be useful. Our results indicate that the dose of vitamin D₃ or its analogue should target at suppressing iPTH levels to a minimum (<20 ng/l or 2.2 pmol/l). It remains to be determined whether vitamin D treatment in MS proves beneficial both for the health of the brain, the immune system, the muscles and the bones of these patients.

Acknowledgements: We thank Tero Vahlberg, MS, consulting biostatistician from the University of Turku, Department of Biostatistics, for assistance with the statistical analysis. Ms Tuula Laukkanen and Ms Heli Virta are acknowledged for technical assistance.

Funding: The study was supported by research grants from the Finnish Medical Foundation Duodecim and from the Finnish Foundation of Neurology (MS-H) and EVO-grant from Turku University Central Hospital (IM). The donors of the grants had no role in the study design or analysis.

Competing interests: None.

Ethics approval: The study was approved by the local research ethics committee.

Patient consent: Informed consent was obtained from all patients.

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