

Assessment of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D₃ concentrations in male and female multiple sclerosis patients and control volunteers

MS Barnes¹, MP Bonham¹, PJ Robson¹, JJ Strain¹, AS Lowe-Strong², J Eaton-Evans¹, F Ginty³ and JMW Wallace¹

Populations with insufficient ultraviolet exposure and who consume diets low in vitamin D have low vitamin D status (plasma 25-hydroxyvitamin D (25(OH)D) concentrations) and a reported higher incidence of multiple sclerosis (MS). The active form of vitamin D, 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃), is an effective anti-inflammatory molecule. No research to date has assessed 1,25(OH)₂D₃ concentrations in individuals with MS. In this study, plasma concentrations of 25(OH)D, 1,25(OH)₂D₃ and parathyroid hormone (PTH) were measured in 29 individuals with MS and 22 age- and sex-matched control volunteers. There were no significant differences in plasma PTH, 25(OH)D and 1,25(OH)₂D₃ concentrations between individuals with MS and control volunteers. Women with MS had significantly higher 25(OH)D and 1,25(OH)₂D₃ concentrations than men with MS (79.1 ± 45.4 versus 50.2 ± 15.3 nmol/L, $P=0.019$ and 103.8 ± 36.8 versus 70.4 ± 28.7 pmol/L, $P=0.019$, respectively). There was a significant positive correlation between 25(OH)D and 1,25(OH)₂D₃ concentrations in all subjects ($r=0.564$, $P=0.000$), but secondary analysis revealed that the correlation was driven by women with MS ($r=0.677$, $P=0.001$). Significant sex differences in vitamin D metabolism were observed and were most marked in individuals with MS, suggesting that vitamin D requirements may differ between the sexes, as well as by underlying disease state. *Multiple Sclerosis* 2007; 13: 670–672. <http://msj.sagepub.com>

Key words: 1,25-dihydroxyvitamin D₃; 25-hydroxyvitamin D; multiple sclerosis; vitamin D

Introduction

Multiple sclerosis (MS) may occur as the result of an underlying genetic susceptibility in combination with as yet unproven environmental factors; one proposed factor is low vitamin D status.

Epidemiological studies have indicated that countries at high latitudes, where the intensity of UV irradiation on the skin and hence cutaneous vitamin D synthesis is insufficient for much of the year, report a high incidence of MS [1]. Others

have reported that total dietary intake of vitamin D from diet and supplements is inversely associated with risk of MS [2]. Low vitamin D status (plasma 25-hydroxyvitamin D (25(OH)D) < 50 nmol/L) has also been observed in individuals with MS [3], and one case-control study has shown that vitamin D status is lower in individuals at time of diagnosis of MS compared to healthy controls [4].

The hormonally active form of vitamin D, 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃), formed

¹ Northern Ireland Centre for Food and Health (NICHE), University of Ulster, Coleraine BT52 1SA, Northern Ireland, UK

² Health and Rehabilitation Sciences Research Institute, University of Ulster, Jordanstown BT37 0QB, Northern Ireland, UK

³ MRC Human Nutrition Research, Elsie Widdowson Laboratory, Fulbourn Road, Cambridge CB1 9NL, UK

Author for correspondence: Julie MW Wallace, Northern Ireland Centre for Food and Health (NICHE), University of Ulster, Coleraine BT52 1SA, Northern Ireland, UK. E-mail: j.wallace@ulster.ac.uk

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by hydroxylation of 25(OH)D, is a potent immunoregulator that increases production of anti-inflammatory cytokines and inhibits proinflammatory cytokines [5]. This may reduce inflammation associated with MS. In murine experimental autoimmune encephalomyelitis (EAE), the animal model of MS, 1,25(OH)₂D₃ has been reported to decrease the onset and progression of the disease [6].

Taken together, evidence suggests that perturbations in vitamin D metabolism influence immune function and may affect MS onset and progression. This study aimed to investigate vitamin D metabolism by measuring 25(OH)D, 1,25(OH)₂D₃ and parathyroid hormone (PTH) concentrations in adults with MS and healthy age- and sex-matched controls.

Subjects and methods

This case-control study was approved by the Research Ethics Committee of the University of Ulster, and all participants gave written informed consent. Twenty-nine individuals (10 male, 19 female) with MS were recruited from MS support groups in late February/early March, over a two-week period. Twenty-two (10 male, 12 female) apparently healthy age- and sex-matched controls were recruited from the same area and sampled concurrently. Subjects were not taking vitamin D-containing dietary supplements, did not normally expose themselves to artificial sources of UV light, eg, sunbeds, and had not spent any time outside Ireland or the UK in the preceding six months. Individuals were also not taking any medications known to affect calcium or vitamin D metabolism.

Non-fasting venous blood samples were collected by a trained phlebotomist and frozen at -80°C until batch analysis at the end of the study. Plasma 25(OH)D concentrations were measured using the OCEIA 25(OH)D enzyme immunoassay kit and 1,25(OH)₂D₃ concentrations were measured using the GAMMA-B 1,25(OH)₂ vitamin D₃ radioimmunoassay kit (both from Immunodiagnostic Systems Limited, UK), following manufacturers' instructions. Analysis of intact plasma PTH was performed at the Clinical Chemistry Laboratory, Antrim Area Hospital. Standardized control material provided by manufacturers was included with each assay.

Statistical analyses were performed using SPSS (SPSS Inc., version 11.0, Chicago, IL). A *P*-value <0.05 was considered significant in all analyses.

Results

There were no significant differences (independent samples *t*-test) in plasma PTH, 25(OH)D or

1,25(OH)₂D₃ concentrations between cases and controls (Table 1). However, when stratified by sex, women had higher plasma 25(OH)D and 1,25(OH)₂D₃ concentrations than men, reaching statistical significance in the MS group only. There were no significant differences in either metabolite between men with MS and control men or between women with MS and control women.

Spearman's rank order correlation analyses revealed a significant positive correlation between plasma 25(OH)D and 1,25(OH)₂D₃ concentrations in all subjects ($r=0.564$, $P=0.000$) and when each group was analysed separately (MS, $r=0.608$, $P=0.000$; controls, $r=0.456$, $P=0.033$). When each group was stratified by sex, the correlation remained significant only in females with MS ($r=0.677$, $P=0.001$).

Discussion

Results of the current study suggest that plasma PTH, 25(OH)D and 1,25(OH)₂D₃ concentrations are not significantly different between individuals with MS and healthy controls in spring, the nadir for vitamin D status. At the group level, vitamin D status was adequate in both groups (25(OH)D concentrations >50 nmol/L). However, it is important to note that the 50 nmol/L cut-off is based on evidence from studies of bone health. It is suggested that plasma 25(OH)D concentrations >100 nmol/L may be required for optimal immune function [7]. Although no significant differences in circulating 1,25(OH)₂D₃ concentrations were observed, it is still possible that differences in the active metabolite may be evident at local sites of inflammation, as reported in a recent EAE study in which 1,25(OH)₂D₃ was increased in the CNS with no difference evident in the circulation [6]. Furthermore, 1,25(OH)₂D₃ has a short half-life of only 4–6 h in the circulation, thus multiple measures from individuals may be required to accurately assess the concentration of the active metabolite.

Significant sex differences in the concentrations of vitamin D metabolites were observed and this was most marked in the MS group, with women having higher 25(OH)D and 1,25(OH)₂D₃ concentrations than men, although this may be due to the small sample size. Studies have shown that oestrogen regulates 1,25(OH)₂D₃ synthesis, with elevated concentrations reported during puberty [8] and interestingly during pregnancy [9], a time when MS symptomatology is improved in most individuals. Sex differences in vitamin D metabolism have also been observed in EAE, with dietary vitamin D delaying the onset and severity of the disease in female but not male mice [6]. Our results suggest that in humans, sex differences in vitamin D

Table 1 Clinical and biochemical variables measured in adults with MS and healthy control volunteers^a

	Individuals with MS (<i>n</i> = 29)	Control volunteers (<i>n</i> = 22)	<i>P</i> -value ^b
Age (years)	46.7 ± 11.3	46.9 ± 9.38	0.941
Height (m)	1.9 ± 0.1	1.7 ± 0.1	0.444
Weight (kg)	75.3 ± 18.3	83.0 ± 13.0	0.102
Body mass index (kg/m ²)	26.9 ± 7.4	28.7 ± 5.2	0.346
Plasma 25(OH)D (nmol/L)			
All	69.1 ± 40.0	67.1 ± 28.8	0.831
Males <i>n</i> = 20	50.2 ± 15.3	59.5 ± 19.8	0.253
Females <i>n</i> = 31	79.1 ± 45.4 ^c	73.4 ± 34.2	0.713
Plasma 1,25(OH) ₂ D ₃ (pmol/L)			
All	92.3 ± 37.4	80.3 ± 26.1	0.353
Males <i>n</i> = 20	70.4 ± 28.7	67.5 ± 14.4	0.778
Females <i>n</i> = 31	103.8 ± 36.8 ^c	91.0 ± 29.3 ^d	0.319
Plasma PTH (pg/mL)	39.9 ± 17.0	38.4 ± 12.6	0.928

^aMean ± standard deviation; ^b*P*-values calculated by independent samples *t*-tests; ^csignificantly higher than males with MS (*P* = 0.019); ^dsignificantly higher than male control volunteers (*P* = 0.032).

metabolism may be evident in the circulation. If such differences are confirmed, and are mirrored in the CNS, they may partially explain sex differences in MS. However, future studies should also assess dietary intakes, lifestyle habits (such as sun avoidance) and disease state, factors that influence 25(OH)D and 1,25(OH)₂D₃ concentrations not assessed in the current study.

The importance of optimal vitamin D status is highlighted by our observation that the concentration of plasma 1,25(OH)₂D₃ is positively associated with the concentration of plasma 25(OH)D, the status marker of vitamin D; an association reported by others [10]. Subsequent analysis of the current data revealed that the association between 25(OH)D and 1,25(OH)₂D₃, in keeping with previous research [10], was only evident in women, and indeed was most marked in women with MS.

The current study, together with recent animal research, raises the possibility that vitamin D requirements may differ between the sexes, as well as with underlying disease state; an area that clearly requires further investigation.

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References

1. Kurtzke JF. Geography in multiple sclerosis. *J Neurol* 1977; **215**: 1–26.
2. Munger KL, Zhang SM, O'Reilly E, Hernan MA, Olek MJ, Willett WC *et al.* Vitamin D intake and incidence of multiple sclerosis. *Neurology* 2004; **62**: 60–65.
3. Nieves J, Cosman F, Herbert J, Shen V, Lindsay R. High prevalence of vitamin D deficiency and reduced bone mass in multiple sclerosis. *Neurology* 1994; **44**: 1687–92.
4. Soilu-Hanninen M, Airas L, Mononen I, Heikkila A, Viljanen M, Hanninen A. 25-Hydroxyvitamin D levels in serum at the onset of multiple sclerosis. *Mult Scler* 2005; **11**: 266–71.
5. Lemire JM. Immunomodulatory actions of 1,25-dihydroxyvitamin D₃. *J Steroid Biochem Mol Biol* 1995; **53**: 599–602.
6. Spach KM, Hayes CE. Vitamin D₃ confers protection from autoimmune encephalomyelitis only in female mice. *J Immunol* 2005; **175**: 4119–26.
7. Vieth R. Why the optimal requirement for Vitamin D₃ is probably much higher than what is officially recommended for adults. *J Steroid Biochem Mol Biol* 2004; **89–90**: 575–79.
8. Aksnes L, Aarskog D. Plasma concentrations of vitamin D metabolites in puberty: effect of sexual maturation and implications for growth. *J Clin Endocrinol Metab* 1982; **55**: 94–101.
9. Kumar R, Cohen WR, Silva P, Epstein FH. Elevated 1,25-dihydroxyvitamin D plasma levels in normal human pregnancy and lactation. *J Clin Invest* 1979; **63**: 342–44.
10. Yan L, Prentice A, Zhang H, Wang X, Stirling DM, Golden MM. Vitamin D status and parathyroid hormone concentrations in Chinese women and men from north-east of the People's Republic of China. *Eur J Clin Nutr* 2000; **54**: 68–72.

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