Brain, Behavior, and Immunity 32 (2013) 180–185

Contents lists available at SciVerse ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

The influence of vitamin D supplementation on melatonin status in patients with multiple sclerosis

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ARTICLE INFO

Article history:
Received 23 January 2013
Received in revised form 12 April 2013
Accepted 21 April 2013
Available online 7 May 2013

Keywords:
Chronotherapy
Interferon-β
Light
Melatonin
Multiple sclerosis
Neurohormone
Vitamin D

ABSTRACT

Background: Multiple sclerosis (MS) incidence is higher in geographic regions with less sunlight exposure. Both vitamin D and melatonin are essential mediators of the effect of sunlight in health, and as such are candidates to play a key role in MS. We hypothesized that vitamin D and melatonin may have related influences in patients with MS.

Methods: In a randomized, double blind study of 40 IFN-β treated MS patients, 21 patients were assigned to 800 IU of vitamin D3 per day (low dose), while 19 patients received 4,370 IU vitamin D3 per day (high dose) for one year. Serum 25-hydroxy-vitamin-D (25-OH-D) and nighttime urine melatonin metabolite, 6-sulphatoxy-melatonin (6-SMT), were measured at baseline, 3 months and 1 year from enrolment.

Results: After 3 months supplementation, 25-OH-D levels increased and nighttime melatonin secretion decreased significantly in the high dose group, but not in the low dose group. After 1 year, a decrease in 25-OH-D levels, accompanied by an increase of urine nighttime 6-SMT were observed in the high dose group. Percent change in serum 25-OH-D was significantly and negatively correlated with percent change in urine 6-SMT after 3 months and between 3 months to 1 year. 25-OH-D levels by the end of the study were significantly and negatively correlated to BMI.

Conclusions: Melatonin secretion is negatively correlated with alterations in serum 25-OH-D in IFN-β treated patients with MS. The finding suggests that melatonin should be considered as a potential mediator of vitamin D neuro-immunomodulatory effects in patients with MS.

1. Introduction

Multiple sclerosis (MS) is the most common neurological disease associated with functional disabilities in young adults. It is well known that MS prevalence has an impressive geographical gradient, with significantly higher incidence with increasing latitude (Simpson et al., 2011). Different sunlight exposure and the resulting variation in vitamin D (vit D) production have been advocated as the explanation for this environmental gradient (Disanto et al., 2012; Munger et al., 2006).

Part of these effects is related to vit D mediated immunomodulation such as promotion of an anti-inflammatory cytokine profile and induction of regulatory T cells (Smolders et al., 2008, 2009). In experimental autoimmune encephalomyelitis (EAE), the animal model of MS, vit D is able to suppress the disease process and to abolish EAE induction (Cantorna et al., 1996; Lemire and Archer, 1991).

Recently, it became clear that decreased sunlight exposure remains significantly associated with MS risk even after controlling for vit D levels, thus implying that vit D is not the only mediator of the beneficial influence of sunlight on MS (Hart et al., 2011; Lucas et al., 2011). Melatonin is another sunlight dependent hormone, with suggested immune-regulatory effects, such as controlling the production of cytokines and leukotrienes and regulating the lifespan of leukocytes by interfering with apoptotic processes (Radogna et al., 2010). Moreover, its anti-oxidative properties promote scavenging of oxidative stress in inflamed tissues (Radogna et al., 2010).

Both vit D and melatonin are candidate intermediates for the influence of sunlight on MS (Mehta, 2010). We hypothesized that these two hormones might have mutual relationship because they

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http://dx.doi.org/10.1016/j.bbi.2013.04.010

are both light-dependent mediators and share immunomodulatory properties. Thus, homeostatic mechanisms might exist to keep them both coordinated. Accordingly, we aimed to assess the effects of vit D supplementation on melatonin secretion in patients with MS.

2. Methods

2.1. Patients

The study took place at north Israel, latitude 32.8° north. Relapsing remitting MS (RRMS) patients who attended our clinic for routine follow-up from November 3rd, 2010 to March 4th, 2011 were offered to participate. Inclusion criteria were: at least 18 years of age, IFN-β treatment of at least 4 months duration, 25-hydroxy-vitamin-D (25-OH-D) blood levels below 75 nmol/l, EDSS score up to 7 and signed informed consent.

Exclusion Criteria consisted of abnormalities of vit D related hormonal system other than low dietary intake or decreased sun exposure. Participation after enrolment was terminated in case of withdrawal of IFN-β treatment from any reason, pregnancy, hypercalcemia or patient's decision. The study was approved by the Helsinki Committee of Carmel Medical Center, Haifa, Israel. All participants signed informed consent. The trial was registered at ClinicalTrials.gov ID: NCT01005095.

2.2. Study groups and randomization

There were two interventional groups. The "high dose" group was orally treated with a bottle containing 75,000 IU of cholecalciferol (vit D3) solution every 3 weeks plus 800 IU of vit D3 by daily tablets (total of 4370 IU/d). The "low dose" group received 800 IU of vit D3 by daily tablets, along with the content of a bottle of placebo solution every 3 weeks (total of 800 IU/d).

After cholecalciferol (vit D3) is ingested, part of it is stored in fat tissue and is subsequently gradually released for about 3 months. Part of the ingested and storage released cholecalciferol undergo 25-hydroxylation in the liver (Bringhurst et al., 2008). The half-life of 25-OH-D is about 2–3 weeks (Bringhurst et al., 2008). Basic pharmacology suggests that circulating half life is a suitable dosing interval for a drug and that after five half lives serum levels are at steady state (Buxton, 2006). Because of the long half life of 25-OH-D it is expected that after 3 months supplementation, at steady state, administration of 75,000 IU every 3 weeks plus 800 IU daily of vit D3 will produce the same 25-OH-D serum levels as if 4370 IU per day of vit D3 had been given. The equivalence of daily and monthly vit-D3 administration has been shown empirically (Binkley et al., 2011; Ish-Shalom et al., 2008).

The assignment to groups was randomly set in advance, according to recruitment order. vit D supplementation was double-blind in this study – both participants, physicians and investigators were unaware of the ingredients of the solution bottles.

2.3. Evaluations

Patients' characteristics including age, gender, ethnicity, BMI (Body mass index), MS disease characteristics and use of anti depressants were registered at recruitment. Health related quality of life (HRQoL) was evaluated at baseline and at the completion of the study, using the 'Functional assessment of MS' questionnaire (FAMS), which is divided to six subscales: mobility, symptoms, emotional well-being (depression), general contentment, thinking/fatigue, and family/social well-being (Cella et al., 1996). The depression score runs between 0 and 28. High score denotes decreased mood.

Serum 25-OH-D and calcium levels were measured at baseline and after 3 months and 1 year from enrolment. Total vit D was measured by chemiluminescent immunoassay (CLIA) technology and was tested on the Liaison analyzer (DiaSorin S.p.A., Italy) for the quantitative determination of 25-hydroxy-vitamin-D.

Urine was collected over a 12-h nighttime (19:00–07:00) period at baseline, after 3 months and by the end of 1 year. Aliquots were frozen at −80 °C until analyzed. Nighttime Melatonin metabolite 6-sulphatoxy-melatonin (6-SMT) levels were measured using a highly specific ELISA assay (IBL International, Biotest Ltd., Israel).

Night durations at each date of urine collection were calculated as the time between sunset and sunrise at the city of Haifa, which were obtained by using the 'suncalc.net' application. (Agafonkin, 2009).

2.4. Statistical analysis

Data analysis was performed using the SPSS statistical package (SPSS Inc., Chicago, IL, USA). Comparisons of patients' characteristics between study groups were done by Student's *t*-test or Mann–Whitney test, according to data distribution. Categorical variables were compared using Chi square or Fisher's exact test. Comparisons of serum 25-OH-D and urine 6-SMT at various times were done by mixed model ANOVA with Bonferroni adjustment for multiple comparisons. This procedure takes into account the intra-correlation of repeated measurements carried out at the same subject and does not exclude subjects with incomplete data at follow-up. Changes in FAMS depression scores in each dosage group were analyzed by Wilcoxon Signed Rank test. Changes in night duration between baseline and 3 months were compared with paired Student's *t*-test. Spearman's Correlation analysis was used to estimate the association between percent change in serum 25-OH-D and percent change in overnight urine 6-SMT. Pearson's Correlation analysis was used to estimate the associations between BMI and 25-OH-D, BMI and 6-SMT, age and 6-SMT and between FAMS depression scores and 6-SMT. Summary statistics are given as mean ± standard deviation and median and (range).

3. Results

Flow chart of the study is shown in Fig. 1. Patients' characteristics at recruitment are provided in Table 1. There were no significant differences between baseline characteristics of the dosage groups. 14 patients dropped out ('censored') before the completion

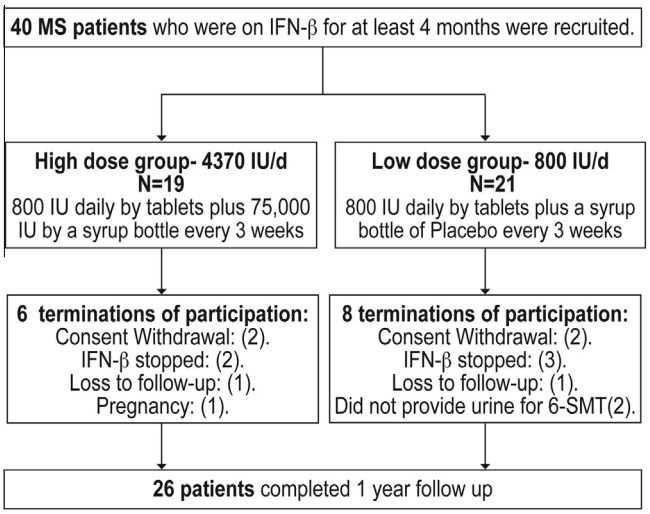

Fig. 1. Study flow chart.

Table 1
Patients' baseline characteristic[a,b].

	Low dose group 800 IU/d $N = 21$			High dose group 4370 IU/d $N = 19$		
	All $N = 21$	Complete follow up $N = 13$	Censored $N = 8$	All $N = 19$	Complete follow up $N = 13$	Censored $N = 6$
Age	44.7 ± 10.7 43.6 (27–64)	46.3 + 9.2 44.6 (32–59)	42.1 ± 13 40.1 (27–64)	46.5 ± 11 46.9 (23–64)	47.7 + 11.6 47.5 (23–64)	44.1 ± 10.1 42.5 (32–61)
Gender (M = Male, F = Female)	8 M 13 F	2 M[c] 11 F	6 M[c] 2 F	5 M 14 F	3 M 10 F	2 M 4 F
Ethnicity	Jews 16 Arabs 5	Jews 10 Arabs 3	Jews 6 Arabs 2	Jews 18 Arabs 1	Jews 13 Arabs 0	Jews 5 Arabs 1
Time from MS diagnosis (years)	9.3 ± 7.4 8.1 (0.4–32.9)	9.9 ± 8.3 8.3 (0.7–32.9)	8.4 ± 6 7.2 (0.4–19)	6.9 ± 6 4.6 (0.3–19.1)	8.7 ± 6.3[d] 8.3 (1.7–19.1)	3.0 ± 2.7[d] 2.3 (0.3–7)
BMI[e]	26.8 ± 6.6 25.7 (18.3–45.8)	26.2 ± 7.4 24.2 (18.3–45.8)	27.8 ± 5.3 28.7 (19.2–36.3)	25.1 ± 5.8 24.6 (17.4–41.9)	25.2 ± 6.2 24.6 (17.4–41.9)	25.0 ± 5.6 24.9 (17.4–33.8)
IFN-β treatment duration (months)	61.3 ± 46 59.1 (3.1–144.1)	58.5 ± 47.2 53 (6.2–143)	65.8 ± 46.8 60.9 (3.1–144.1)	44.6 ± 33.8 33.3 (3.0–96.7)	51.6 ± 34.5 54.6 (7.5–96.7)	29.7 ± 29.6 18.8 (3–69.6)
EDSS	3.6 ± 2.2 4.5 (0–6.5)	3.8 ± 2 4.5 (1–6.5)	3.2 ± 2.4 3.3 (0–6)	3.3 ± 2.1 3 (0–7)	3.4 ± 2.4 3 (0–7)	3.1 ± 1.5 2.8 (1–5)
Depression score (FAMS)	20.1 ± 5.5 21 (9–28)	20.9 ± 5.7 22 (12–28)	19 ± 5.5 20 (9–25)	20.4 ± 7 22 (5–28)	19.3 ± 7.3 22 (5–27)	22.7 ± 6.2 25.5 (13–28)
Number using anti depressants	3	3	0	4	3	1
Night duration (hours)	13.6 ± 0.4 13.7 (12.4–14)	–	–	13.7 ± 0.3 13.8 (13.1–14)	–	–

[a] Summary statistics are mean ± standard deviation and median (range).
[b] There were no significant differences between baseline characteristics of the dosage groups. Baseline characteristics of censored patients and those with complete follow up in each dosage group were not statistically different except:
[c] Significantly different gender distribution in the low dose group ($P = 0.02$).
[d] Significantly different times from MS diagnosis in high dose group ($P = 0.05$).
[e] BMI = Body mass index, calculated as body weight (kg) divided by the square of height (m). All participants' 25-OH-D levels by the end of the study and 6-SMT at baseline were negatively correlated to BMI [Pearson's $r = -0.45$, $P = 0.015$ and Pearson's $r = -0.39$, $P = 0.014$, respectively].

of the intended 12 months follow up. Notably, all premature withdrawals took place after the 3 months' evaluation. Censored patients in the high dose group were closer to the time of MS diagnosis. Censored patients in the low dose group were mainly males whereas those with complete follow up were mainly females. Apart from these, no other significant differences in baseline characteristics were noted between the censored and uncensored patients in the two intervention groups.

Serum 25-OH-D levels at baseline, 3 months and 1 year in the two dosage regimens are presented in Fig. 2. Serum 25-OH-D levels at both 3 month and 1 year were significantly higher in the high dose group [$F(1,35.4) = 21.6$, $P < 0.0001$]. In patients receiving the high dose, 25-OH-D levels were significantly above baseline at all time points [$t(18) = 9.29$, $P < 0.0001$ at 3 months, $t(15.5) = 3.91$, $P = 0.003$ at 1 year], and within 3 month reached levels above 75 nmo/l, considered sufficient by the American society of endocrinology (Holick et al., 2011). Notably, a significant decrease in serum 25-OH-D was found between 3 months and 1 year of follow up [$t(13.5) = 2.66$, $P = 0.038$]. 5 out of 13 patients (38%) assigned to the high dose group, reached the end of the study with 25-OH-D levels below 75 nmol/l.

Low dose vit D supplementation resulted in significantly increased 25-OH-D levels compared to baseline only at 3 months [$t(20) = 5.72$, $P < 0.0001$], however average level did not reach 75 nmo/l. A trend towards reduced 25-OH-D levels at 1 year compared to levels after 3 months, was also apparent in this dosage group, albeit not statistically significant [$t(18.7) = 2.17$, $P = 0.09$].

All participants' 25-OH-D levels by the end of the study were significantly negatively correlated to BMI [Pearson's $r = -0.45$, $P = 0.015$].

Urine melatonin metabolite levels in the two intervention groups are shown in Fig. 3. One patient, in the low dose group, had 414% increases in urine melatonin metabolite at 1 year compared to 3 months. This increment was higher than the median change in urine 6-SMT at that period, by 10.9 times the inter-quartile range. This patient had severe limb ataxia, which might have hampered the ability to collect urine accurately. Therefore, 6-SMT data from this patient was excluded from analysis, as an outlier.

Despite randomization, significantly higher baseline 6-SMT levels were found in the high dose vit-D group. [$t(37) = 2.06$, $P = 0.05$]. None of the registered characteristics at baseline, as shown in table 1, could account for the difference in baseline 6-SMT levels between the groups. No correlation was observed between baseline depression score and baseline 6-SMT levels [Pearson's $r = 0.045$, $P = 0.8$]. However, baseline 6-SMT levels from all participants, were significantly and negatively correlated with age and BMI [Pearson's $r = -0.51$, $P = 0.001$ and $r = -0.39$, $P = 0.014$, respectively].

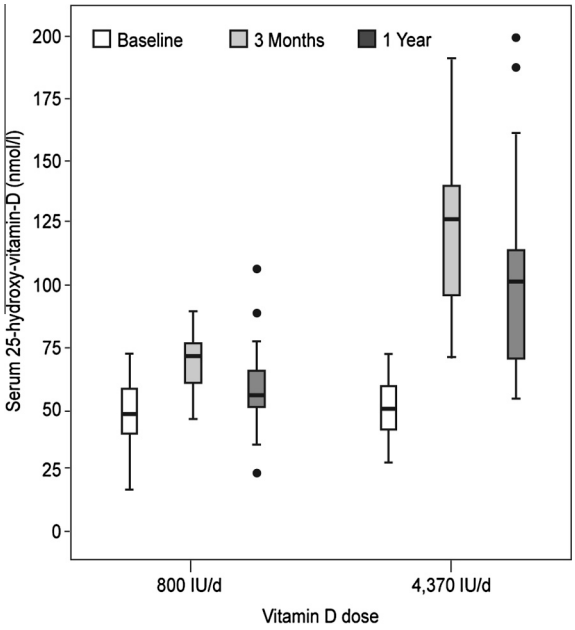

Fig. 2. Serum 25-hydroxy-vitamin-D levels. With high dose, 25-OH-D levels were significantly above baseline at all time points. Notably, a significant decrease in serum 25-OH-D was found between 3 months and 1 year of follow up ($P = 0.038$). Low dose supplementation resulted in significantly increased 25-OH-D levels compared to baseline only at 3 months.

Urine 6-SMT was significantly decreased after 3 months of high dose vit D supplementation [$t(18) = -3.33$, $P = 0.008$], however by the end of the study nighttime 6-SMT secretion returned to baseline levels [$t(13.4) = 0.89$, $P = 0.78$]. In contrast, melatonin metabolite levels were not changed after 3 and 12 months follow up in the low dose group [$t(19) = -1.12$, $P = 0.55$ and $t(16.1) = 0.24$, $P = 1.0$, respectively].

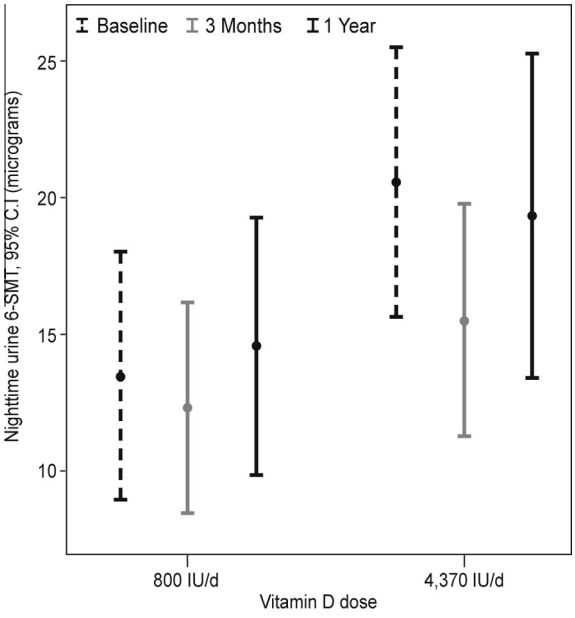

Fig. 3. Nighttime urine 6-sulphatoxy-melatonin (6-SMT). Nighttime (12 h) urine 6-SMT levels were significantly decreased after 3 months of high dose vit D supplementation ($P = 0.008$), however by the end of the study nighttime 6-SMT secretion returned to be insignificantly different from baseline levels. Melatonin metabolite levels were not significantly changed in the low dose group.

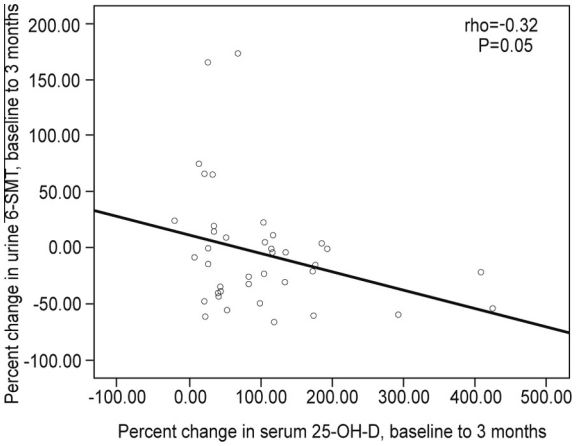

Fig. 4. Correlation between increase in serum 25-OH-D and decrease in urine melatonin metabolite, baseline to 3 months. The percent increase in serum 25-OH-D was significantly correlated to the percent decrease in urine 6-SMT after 3 months.

The duration of the nights, in which urine were collected, at 3 months was 11.7 ± 1.2 h, significantly shorter compared to their duration at baseline (13.7 ± 0.3 h) and at 1 year (13.6 ± 0.4 h) [$t(38) = 10.5$, $P < 0.0001$].

The percent increase in serum 25-OH-D was significantly correlated to the percent decrease in urine 6-SMT after 3 months (Spearman's rho = −0.32, $P = 0.05$) (Fig. 4). Interestingly, percent decrease in 25-OH-D between 3 months and 1 year was significantly correlated to a corresponding increase in urine 6-SMT secretion (Spearman's rho = −0.4, $P = 0.05$) (Fig. 5). Vitamin D and melatonin data from both dosage groups were used for correlation analysis.

Depression scores after 1 year, in both dosage groups, were not significantly changed from baseline. No gender effect was observed on either 25-OH-D or 6-SMT levels. There was no significant difference in either 25-OH-D or 6-SMT levels between males and females at all time points, and regardless of supplementation schedule.

4. Discussion

The effect of vit D on MS disease activity in IFN-β treated patients is gaining increasing attention in recent years, but has yet

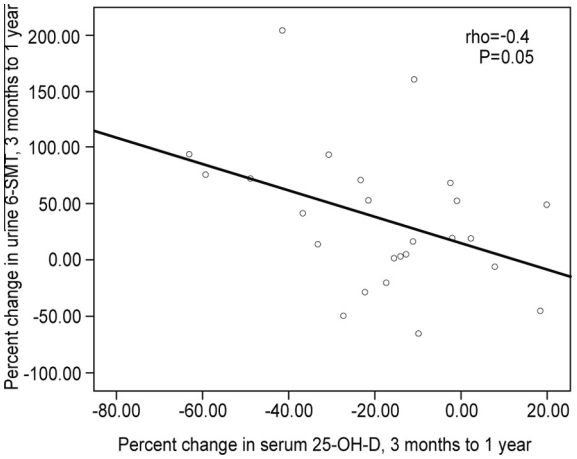

Fig. 5. Correlation between decrease in serum 25-OH-D and increase in urine melatonin metabolite, 3 months to 1 year. The percent decrease in 25-OH-D between 3 months and 1 year was significantly correlated to a corresponding increase in urine 6-SMT secretion.

to be clarified. Recently, two prospective observational studies addressed this question, reaching conflicting results. Stewart et al. found that increasing 25-OH-D was related to lower relapse rate only in patients that were on IFN-β treatment (Stewart et al., 2012). Løken-Amsrud et al. reported decreased odds for new MRI lesions with increasing 25-OH-D levels only in MS patients that were not on IFN-β (Løken-Amsrud et al., 2012). Interestingly, the first study is from Tasmania, latitude 40–44 south, while the later is from Norway, latitude 58–63 north, implying that sunlight dependent mediators, other than vit D, may confound the association between 25-OH-D and MS disease activity in IFN-β treated patients (Ascherio and Marrie, 2012). One possible such mediator is melatonin, which is both sunlight-dependent (Brzezinski, 1997) and an immunomodulator (Radogna et al., 2010).

The exact role of melatonin in MS is yet to be elucidated. Prior reports have documented melatonin levels to be inversely correlated with MS disease activity (Sandyk and Awerbuch, 1992, 1993a), and we have recently reported that IFN-β treatment increases melatonin levels in treatment naïve patients with MS (Melamud et al., 2012) as well as proposed application of Melatonin-related chronobiological concepts to MS care (Glass-Marmor et al., 2007, 2009).

Melatonin has both anti-inflammatory and pro-inflammatory effects, depending on the context, such as the stage of inflammation (Radogna et al., 2010), making its potential influence on MS complex to predict. Among melatonin's pro-inflammatory properties, with potential negative consequences in MS, is enhancement of inflammatory cytokine production, e.g. IL-2, IL-6 and (IFN)-γ, which was demonstrated in cultured human mononuclear cells (Garcia-Maurino et al., 1997). This finding was attributed to the upregulation of cytokine gene expression (Liu et al., 2001). On the other hand, melatonin has also anti-oxidative properties (Reiter et al., 2008), which may exert favorable neuroprotective effects in diseases such as MS, where axonal loss is part of the pathogenesis and seems to be induced, at least partially, by oxidative stress (Gonsette, 2008). Neuroprotective influence of melatonin was recently demonstrated in hypoxic-ischemic models of brain injury (Robertson et al., 2013).

Our data show that the high dosage regimen is more effective than low dosage in increasing 25-OH-D serum levels, at least for short term. Our patients' average EDSS score was about 3.5, implying that most of them were not housebound and could have been influenced by outdoor sun exposure. Therefore, the decline in 25-OH-D at 12 months in both dosage groups can be partly attributed to winter at that time (baseline and 12 months examinations were carried out at November to March). Annual variation in 25-OH-D levels, with decreased levels at winter, has been reported in Israel (Saliba et al., 2012). Additionally, attrition in patients' compliance with the vit D supplementation regimen cannot be ruled out.

BMI was found to be associated with decreased 25-OH-D levels at 1 year, despite supplementation. This finding is in line with previous reports of diminished response to vit D supplementation in obese patients (Saliba et al., 2013; Gallagher et al., 2012). Our patients' BMI was also negatively correlated with melatonin secretion as was their age. Both findings in agreement with prior research (Wetterberg et al., 1999; Schernhammer et al., 2006).

In this study an inverse association was found between changes in serum 25-OH-D levels and changes in urine melatonin metabolite. The overnight secretion of melatonin decreased after 3 months of high dose vit D supplementation in IFN-β treated MS patients. Thereafter, along with a decrease in serum 25-OH-D levels, an accompanying increase of melatonin secretion at night was observed. In fact, the course of changes in nighttime melatonin secretion was the mirror image of the trend in serum 25-OH-D levels. These findings are derived from a clinical trial, in which vit D supplementation preceded melatonin decreased secretion, thus implying that the observed association may be causal.

Nights in which urine was collected, were on average 2 h shorter after 3 months. It is unlikely that night shortening was the reason for the observed decreased 6-SMT excretion, because it should have influenced both dosage groups to the same extent, and not only the high dose group. Furthermore, most prior studies found that nighttime melatonin levels in humans are stable throughout the year, regardless of the photoperiod (Bojkowski and Arendt, 1988; Kennaway and Royles, 1986; Wetterberg et al., 1999). Seasonal variation in night hours in Israel is modest, much unlike the major fluctuations in polar region. Indeed, studies which have reported seasonal variation in nighttime melatonin secretion were generally conducted at polar or sub-polar latitudes (Ruhayel et al., 2007; Stokkan and Reiter, 1994).

The synthesis and release of melatonin from the pineal gland is stimulated by darkness and inhibited by light. Photic information from the retina is transmitted to the pineal gland through the suprachiasmatic nucleus of the hypothalamus (Brzezinski, 1997). It is tempting to hypothesize that 25-OH-D can bring a 'message of light' to the pineal gland and consequently decrease melatonin synthesis. Such a biological cascade and interconnectivity between the vit D and the neuro-endocrine/hormonal (melatonin) system is yet to be depicted.

Vit D receptor protein is expressed throughout the human brain, including the hypothalamus, and found on most neurons and on some glia cells (Eyles et al., 2005). 1α-Hydroxylase, the enzyme responsible for the formation of the active vit D from 25-OH-D, is also present in the brain, and is strongly expressed in the supraoptic nucleus of the hypothalamus (Eyles et al., 2005; Harms et al., 2011). These findings support the possibility that 25-OH-D and the pineal gland of the central nervous system could indeed influence each other, as part of a vit D – related neuro-endocrine-immune network (Fernandes de Abreu et al., 2009).

Our results must be interpreted with caution. We did not fully control for potential confounders of the alleged association between vit D and melatonin. Depressed mood is known to be associated with decreased melatonin synthesis in MS (Akpinar et al., 2008; Sandyk and Awerbuch, 1993b). Although no significant difference was observed in the depression subscale of the FAMS between the dosage groups, this cannot be regarded as full evaluation of an affective disorder. Furthermore, we did not evaluate for circadian rhythm disorders, which may shift the melatonin secretion curve and influence the nighttime 6-SMT levels (Pandi-Perumal et al., 2007). Randomization in clinical trials usually results in balanced representation of confounders, but the small sample size and high dropout rate in this trial, increases the possibility for unbalanced representation of unrecognized confounders between the two intervention groups.

Another concern is the higher baseline 6-SMT level, which was observed in the high dose group. Baseline characteristics, including age, BMI and depression scores, were similar for the two dosage groups. Therefore, the most plausible explanation is a chance occurrence of unbalanced randomization, which was caused by small sample size and the known high inter-individual variability of human 6-SMT levels (Wetterberg et al., 1999). Because of this natural variability, the change in 6-SMT level within an individual is more important than the difference between groups of individuals. The importance of using subjects as their own control when studying changes in melatonin excretion was advocated by others (Bojkowski and Arendt, 1988).

Repeated measurement in the same subjects tends to give lower values if they were initially high. This phenomenon, 'regression towards the mean', probably did not contribute to the described results because an increase in 6-SMT, back towards baseline levels,

significantly correlated to the decrease in 25-OH-D, was noted after the initial decrease in 6-SMT.

Despite the caveats the observation of decreased melatonin secretion following vit D supplementation and the apparently mirror trends of vit D and melatonin are novel and important, as both hormones are light-dependent and have immunomodulatory properties. If this observation is verified in larger trials, alteration in melatonin level should be taken into account as a potential mediator of the neuro-immunomodulatory effects of vit D in patients with MS. As many other factors can influence melatonin secretion, this may potentially explain some of the variation in the clinical and immunologic response to vit D in MS patients. The mutual effects of melatonin and vit D on immune-competence and neuroprotection should be further assessed.

Acknowledgments

This study was supported by Merck Serono – Israel and Bayer Schering – Israel. Vitamin D supplements and placebos were donated by CTS – Israel and Altman – Israel.

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