Alpha-tocopherol and MRI Outcomes in Multiple Sclerosis – Association and Prediction

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Abstract

Objective: Alpha-tocopherol is the main vitamin E compound in humans, and has important antioxidative and immunomodulatory properties. The aim of this study was to study alpha-tocopherol concentrations and their relationship to disease activity in Norwegian multiple sclerosis (MS) patients.

Methods: Prospective cohort study in 88 relapsing-remitting MS (RRMS) patients, originally included in a randomised placebo-controlled trial of omega-3 fatty acids (the OFAMS study), before and during treatment with interferon beta. The patients were followed for two years with repeated 12 magnetic resonance imaging (MRI) scans and nine serum measurements of alpha-tocopherol.

Results: During interferon beta (IFNB) treatment, each 10 μmol/L increase in alpha-tocopherol reduced the odds (CI 95%) for simultaneous new T2 lesions by 36.8 (0.5–59.8) %, p = 0.048, and for combined unique activity by 35.4 (1.6–57.7) %, p = 0.042, in a hierarchical regression model. These associations were not significant prior to IFNB treatment, and were not noticeably changed by gender, age, body mass index, HLA-DRB1*15, treatment group, compliance, or the concentrations of 25-hydroxyvitamin D, retinol, neutralising antibodies against IFNB, or the omega-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid. The corresponding odds for having new T1 gadolinium enhancing lesions two months later was reduced by 65.4 (16.5–85.7) %, p = 0.019, and for new T2 lesions by 61.0 (12.4–82.6) %, p = 0.023.

Conclusion: During treatment with IFNB, increasing serum concentrations of alpha-tocopherol were associated with reduced odds for simultaneous and subsequent MRI disease activity in RRMS patients.

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Introduction

Vitamin E is an essential nutritional factor found in vegetable oils and margarines, vegetables, fruits, nuts and to some extent fish [1]. Natural vitamin E comprises eight different compounds, of which alpha-tocopherol is the most abundant in human blood and has the highest biological potency [2]. Vitamin E has antioxidative and immunomodulatory properties and is considered one of the most important antioxidative factors against reactive oxygen
species (ROS) overload and damages from oxidative stress [3]. ROS and oxidative stress have been incriminated in the pathogenesis of several diseases, including neurodegenerative disease and multiple sclerosis (MS) [4].

Vitamin E is shown to affect different immune cells. In mice, vitamin E enhanced naïve T cell function by increasing division and interleukin-2 production, and by reducing T cell suppressive prostaglandin E2 from macrophages [3]. Moreover, appropriate function and interaction between CD4+ T cells, dendritic cells, and T regulatory cells in response to viral infection depend on adequate vitamin E levels [5]. In murine microglia cultures, vitamin E has been shown to induce morphological changes and down regulate different adhesions molecules, both associated with deactivation [6]. Treatment with vitamin E also inhibited demyelination caused by ethidium bromide [7], increased subsequent remyelination [7], and has been shown to exert dose-dependent effects in a murine lupus model [8].

The effect of interferon beta (IFNB) in MS is only partial, and antioxidant therapy might potentially be an adjuvant. IFNB treatment was associated with higher concentrations of alphatocopherol in plasma of MS patients compared to controls [9], and also with normalization of the alpha-tocopherol levels in erythrocytes [10].

In spite of relevant biological properties, the relationship between vitamin E and disease activity in MS has not been investigated. To chart the vitamin E levels and their relationship to disease activity in Norwegian MS patients, we have measured alphatocopherol in serum samples from 88 relapsing-remitting MS (RRMS) patients who participated in a randomised placebo-controlled trial of supplementation with omega-3 fatty acids or interferon beta-1a (IFNB) at study month 6. The MRI scans were included in the regression model. The same statistical model was used to analyse the association between alphatocopherol and MRI outcomes lagged by 1 and 2 study months after the alphatocopherol measurements. Gender, age, body mass index (BMI), HLA-DRB1*15 status, treatment group (omega-3 or placebo), compliance (intake of study medication in percentage of the total dosage) and serum concentrations of 25-hydroxyvitamin D, retinol, EPA, DHA and HLA-DRB1*15 has been described previously [11,12,13].

Statistics
As the MRI outcomes were skewed towards none or one lesion, they were dichotomised as present or absent. We addressed our research question by using a hierarchical logistic regression model as previously described [12]. This model takes into account the repeated MRI scans and serum measurements within a patient. The SAS GLIMMIX procedure was used to fit the model with random intercepts for patients and fixed effects of alphatocopherol. Only paired measurements of alphatocopherol and MRI scans were included in the regression model. The same statistical model was used to analyse the association between alphatocopherol and MRI outcomes lagged by 1 and 2 study months after the alphatocopherol measurements. Gender, age, body mass index (BMI), HLA-DRB1*15 status, treatment group (omega-3 or placebo), compliance (intake of study medication in percentage of the total dosage) and serum concentrations of 25-hydroxyvitamin D, retinol, EPA and DHA and NAb against IFNB (categorized as negative, low to moderate and high concentration) were included as possible predictors to the logistic regression model. The Pearson’s correlation coefficient (r) was calculated to examine the association between baseline values of alphatocopherol and the cumulative number of new T1Gd+ and T2 lesions and CUA. Independent samples t-test was used for the comparison of means. Mean (SD) values are presented unless otherwise stated. The statistical analyses were conducted using SAS version 9.2 and SPSS version 15.0. Findings with p<0.05 were considered significant.

Missing Values
Twelve alphatocopherol, 25-hydroxyvitamin D and retinol values were missing (one at baseline, three during study months 1–6 and eight during study months 7–24). Twenty-three MRI scans were missing (14 during study months 1–6, nine during study months 7–24). We defined MRI scans and serum samples collected within an interval of one month as paired, and 11 MRI/alpha-tocopherol measurements that exceeded this limit were excluded from analysis. EDTA-blood for HLA-DRB1 typing was missing for four, and BMI was missing for two patients. EDSS scores were missing for two patients at month 24. Missing values were not replaced.
**Ethics Statement**

The study was approved by the Regional Committee for Medical and Health Research Ethics in Western Norway Regional Health Authority. All participants gave written informed consent.

**Results**

**Patient Population**

Eighty-eight RRMS patients, 57 (65%) women and 31 (35%) men, 58 (66%) HLA-DRB1*15 positive, age 38.9 (8.3) years, BMI 25.7 (4.3), disease duration 1.9 (3.1) years, and EDSS score at baseline of 1.9 (0.8) were included in the study.

**Alpha-tocopherol Status**

From the whole study period (study months 0–24) there were 780 measurements of alpha-tocopherol. The mean baseline concentration of alpha tocopherol was 29.5 (7.3) μmol/L and the mean concentration during the rest of the study was 32.4 (0.76) μmol/L (p<0.001 for difference). The mean difference between concentrations at baseline and through the rest of the study period was 2.0 (3.9) μmol/L for the patients treated with omega-3 fatty acids (n = 46) and 4.5 (3.8) μmol/L for the placebo group (n = 41) (p = 0.003 for difference). There were no significant differences before and during IFNB treatment or between genders, or any seasonal variation (Figure 1). The mean ratio between the highest and the lowest concentration of alpha-tocopherol (baseline not included) in each patient was 1.34 (0.22). The intraclass correlation coefficient was 0.788, which implies that 21.2% of the total variance in alpha-tocopherol concentrations was explained by intra-individual variation. The reference range for the total variance in alpha-tocopherol concentrations was 0.788, which implies that 21.2% of

**MRI Outcomes**

From the whole study period, a total of 587 paired MRI scans and alpha-tocopherol measurements (interval 3.4 (3.3) days), of which 254 were collected before and 333 after initiation of IFNB treatment, were available for analysis (table 1). During IFNB treatment, the odds (CI 95%) for new T2 lesions and CUA were significantly reduced by 36.8 (0.5–59.8) % and 35.4 (1.6–57.7) % with each 10 μmol/L increase in alpha-tocopherol, whereas no significant association was found before IFNB treatment or for the whole study period. It has previously been reported higher concentrations of alpha-tocopherol in women compared to men, and an inverse relationship with BMI [14]. Adjusting for gender, age and BMI did not noticeably change our results.

**Clinical Disease Activity**

The mean concentration of alpha-tocopherol was 30.95 (1.22) μmol/L in the 23 patients who experienced at least one relapse during the study period and 32.85 (0.93) μmol/L in the 65 who did not (p = 0.28). In the 26 patients who progressed at least one EDSS point the mean concentration was 31.68 (1.39) μmol/L and 32.74 (0.94) μmol/L in the 60 stable patients (p = 0.54). We did not detect any significant association between the mean concentration of alpha-tocopherol and the occurrence of relapses when stratified by IFNB treatment.

**Discussion**

We found that during IFNB treatment, increasing serum concentrations of alpha-tocopherol were associated with reduced odds for simultaneous and subsequent MRI disease activity. The results were not noticeably influenced by gender, age, BMI, HLA-DRB1*15 status, treatment group or compliance (omega-3 fatty acids or placebo), or the concentrations of NAb against IFNB, 25-hydroxyvitamin D, retinol, EPA or DHA.
Studies regarding vitamin E and MS are relatively few. In small cross-sectional studies, lower concentrations of both vitamin E and vitamin E/cholesterol-ratio have been reported in stable MS patients compared to controls [18], and also in MS patients during exacerbation compared to stable MS patients with or without IFNB treatment, and controls [9,19,20]. In a prospective study, the risk of developing MS was not associated with total or dietary intake of vitamin E [21]. To our knowledge, there are no prospective studies addressing the relationship between vitamin E and MRI disease activity in MS.

Our patient cohort comprises well characterized RRMS patients examined with repeated MRI scans and serum measurements, allowing eight paired MRI/alpha tocopherol assessments, and is well suited for a prospective study of the relationship between vitamin E and MS disease activity. Moreover, simultaneous measurements of vitamin A, vitamin D, DHA, EPA and NAb against IFNB in the same patients, combined with records of the compliance of study medication, enabled us to adjust for potential confounding. However, our study also has limitations. Although several paired MRI scans and serum measurements of alpha-tocopherol were conducted in each patient, the cohort might have been too small to detect minor, but nevertheless potentially important associations with relapse rate and EDSS progression. The dietary habits including use of vitamin supplements were not recorded. Moreover, the patients received either approximately 13 or 22 mg alpha-tocopherol from the omega-3 or placebo preparations. This constitutes 2–3 times the intake of vitamin E estimated in a Finnish study [22]. Only the baseline values are therefore representative for the habitual vitamin E status of the patients. Accordingly, there was an increase in the mean concentration of alpha-tocopherol from baseline to the rest of the study period, and the supplementation might therefore have evened out both the inter- and intra-individual variation. Even so, we found a mean ratio between the highest and the lowest concentration of alpha-tocopherol in each patient of 1.34 and 22.8% of the total variation was accounted for by intra-individual variation. The serum concentration of alpha-tocopherol is correlated to the concentration of lipoproteins [2,24]. Unfortunately, we did not measure lipoproteins or total cholesterol, and can therefore not exclude that lipoproteins might have confounded our results. However, in a previous study T2 lesion volume was not associated with lipoprotein concentrations [25], and the association with new T2 lesions was not influenced by BMI or omega-3 fatty acids in our study. It is therefore less likely that lipoprotein status have confounded the results.

Vitamin E has both antioxidative, immunomodulatory and neuroprotective properties [3,7,26]. Our results are therefore biologically plausible. Immune cells produce ROS, which may contribute to neuroinflammation in experimental allergic encephalomyelitis [27] and MS [28,29]. Vitamin E has also been shown to have other properties including regulation of enzymatic activity and gene transcription [2] that might be relevant in MS [30,31], and to have immunomodulatory properties in animal models of rheumatoid arthritis [26] and systemic lupus erythematosus [8].

Previous small studies have reported an increase of alpha-tocopherol in erythrocytes and plasma of MS patients treated with IFNB [9,10], as well as normalisation of ROS production in mononuclear cells [32]. The finding that the odds for new MRI disease activity were only significantly reduced during IFNB

### Table 1. Odds ratio for MRI outcomes for each 10 μmol/L increase in alpha-tocopherol.

| MRI outcomes       | Whole study period (88 patients)* | Prior to IFNB (88 patients)a | During IFNB (88 patients)a |
|--------------------|----------------------------------|-----------------------------|---------------------------|
|                    | Odds ratio (CI 95%) p-value      | Odds ratio (CI 95%) p-value | Odds ratio (CI 95%) p-value |
| **CUA**            | 0.803 (0.584–1.103) 0.176        | 0.732 (0.461–1.164) 0.186    | 0.646 (0.423–0.984) 0.042  |
| **New T2 lesions** | 0.737 (0.537–1.011) 0.059        | 0.682 (0.450–1.034) 0.071    | 0.632 (0.402–0.995) 0.048  |
| **New T1Gd+ lesions** | 0.838 (0.598–1.174) 0.305 | 0.787 (0.511–1.212) 0.275    | 0.651 (0.403–1.053) 0.080  |

*587 alpha-tocopherol/MRI pairs, a524 alpha-tocopherol/MRI pairs, b333 alpha-tocopherol/MRI pairs.
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### Table 2. Odds ratio for lagged MRI outcomes for each 10 μmol/L increase in alpha-tocopherol.

| MRI outcomes       | Whole study period* | Prior to IFNBb | During IFNBc |
|--------------------|---------------------|---------------|--------------|
|                    | Odds ratio (CI 95%) p-value | Odds ratio (CI 95%) p-value | Odds ratio (CI 95%) p-value |
| **CUA**            | 0.835 (0.564–1.235) 0.365 | 0.730 (0.450–1.185) 0.202 | 0.878 (0.535–1.435) 0.596 |
| **New T2 lesions** | 0.855 (0.582–1.258) 0.426 | 0.907 (0.562–1.463) 0.688 | 0.659 (0.366–1.188) 0.163 |
| **New T1Gd+ lesions** | 0.779 (0.541–1.122) 0.179 | 0.681 (0.426–1.088) 0.107 | 0.840 (0.508–1.390) 0.493 |
| **New T1Gd+ lesions** | 0.773 (0.535–1.116) 0.169 | 0.917 (0.607–1.386) 0.680 | 0.390 (0.174–0.876) 0.023 |
| **New T1Gd+ lesions** | 0.738 (0.495–1.100) 0.135 | 0.632 (0.379–1.053) 0.078 | 0.824 (0.490–1.386) 0.462 |
| **New T1Gd+ lesions** | 0.731 (0.473–1.074) 0.105 | 0.834 (0.525–1.325) 0.440 | 0.346 (0.143–0.835) 0.019 |

*Whole study period: Lag 1: 88 patients, 419 observations, Lag 2: 88 patients, 420 observations.
aPrior to IFNB: Lag 1: 88 patients, 250 observations, Lag 2: 88 patients, 251 observations.
bDuring IFNB: Lag 1: 88 patients, 169 observations, Lag 2: 87 patients, 169 observations.
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treatment could indicate an interaction between IFNB and vitamin E. However, the difference in odds reduction before and during IFNB treatment was modest, and adjusting for NAb against IFNB did not alter our results. Thus, there is not sufficient evidence to draw any conclusion, and a possible interaction between vitamin E and IFNB treatment should be studied in a larger cohort.

In conclusion, we have shown an association between increasing alpha-tocopherol concentrations and simultaneous and subsequent MRI disease activity in RRMS patients during treatment with IFNB. The relation between vitamin E and MS should be further investigated in epidemiological and experimental studies.

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Author Contributions
Conceived and designed the experiments: KMM SJB AGB KS BTH HH FL RM TP TH. Analyzed the data: KILA JSB TH. Contributed reagents/materials/analysis tools: KSB. Wrote the paper: KILA TH. Contributed by interpretation of the data and revising the manuscript: KMM SJB AGB KS BTH HH FL RM TP OT SW.

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