

Impact of Vitamin A Supplementation on Disease Progression in Patients with Multiple Sclerosis

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Abstract

Background: Many studies have shown that active vitamin A derivatives suppress the formation of pathogenic T cells in multiple sclerosis (MS) patients. The aim of the present study is to determine the impact of vitamin A on disease progression in MS patients.

Methods: A total of 101 relapsing-remitting MS (RRMS) patients were enrolled in a 1-yr placebo-controlled randomized clinical trial. The treated group received 25000 IU/d retinyl palmitate for six months followed by 10000 IU/d retinyl palmitate for another six months. The results of the expanded disability status scale (EDSS) and multiple sclerosis functional composite (MSFC) were recorded at the beginning and the end of the study. The relapse rate was recorded during the intervention. Patients underwent baseline and follow-up brain MRIs.

Results: The results showed "Mean \pm SD" of MSFC changes in the treated group was (-0.14 ± 0.20) and in the placebo group was (-0.31 ± 0.19) . MSFC was improved significantly ($p < 0.001$) in the treatment group. There were no significant differences between the "Mean \pm SD" of EDSS changes in the treated (0.07 ± 0.23) and the placebo (0.08 ± 0.23) groups ($p = 0.73$). There were also no significant differences between the "Mean \pm SD" of annualized relapse rate in the treated group (-0.36 ± 0.56) and placebo (-0.53 ± 0.55) groups ($p = 0.20$). The "Mean \pm SD" of enhanced lesions in the treatment (0.4 ± 1.0) and in the placebo (0.2 ± 0.6) groups were not significantly different ($p = 0.26$). Volume of T2 hyperintense lesions "Mean \pm SD" was not significantly different between treatment (45 ± 137) and placebo (23 ± 112) groups after intervention ($p = 0.23$).

Conclusion: Vitamin A improved total MSFC score in RRMS patients, but it did not change EDSS, relapse rate and brain active lesions.

Keywords: Disability evaluation, magnetic resonance imaging, multiple sclerosis, vitamin A

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Introduction

Multiple sclerosis (MS) is an immune-mediated disease of the central nervous system (CNS). Studies have shown that Th1 and Th17 cells are pathogenic T cells. However, Th2 and Foxp3+ regulatory T cells are in reciprocal positions in experimental autoimmune encephalomyelitis (EAE) as well as MS.¹⁻² Some in vitro studies have shown that active vitamin A derivatives, retinoic acids, decrease human lymphocyte prolifera-

tion. Retinoic acids suppress the formation of Th1 and Th17 cells and increase the formation of Th2 and Foxp3+ regulatory T cells from primary T cells.²⁻⁴

One study showed that treatment of relapsing EAE using retinoic acid decreased disease progression and the number of relapses after the onset of symptoms.⁴ It has also been shown that in RRMS patients treated with etretinate (a synthetic retinoid) for 6 months, the function of suppressor T cells increased.⁵

Some human studies have shown that daily supplementation with 25000 IU retinyl palmitate for 6 mo, is a way to elevate circulating retinoic acid concentration, therefore stimulate retinoic acid receptors (RARs) effectively.⁶⁻⁸

The present study assessed the efficiency of vitamin A supplementation for 1-year on the clinical progression, number of relapses, and brain lesions in RRMS patients.

Method and Materials

Enrollment of subjects

Using the 2010 revised McDonald criteria, about 2000 RRMS patients were referred from three neurological disease centers in Tehran to Imam Khomeini Hospital, the Iranian Centre of Neurological Research, between 2010 and 2012. Patients were screened according to the study inclusion criteria, in Iranian center of Neurological Research. Then, 101 RRMS patients were enrolled in a double-blind randomized clinical trial after receiving their informed consent (ClinicalTrials.gov Identifiers: NCT01417273).

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The patients were 20 – 45 year of age. At the beginning of the study, the EDSS of patients ranged from zero to five and no relapses had occurred for at least 3 months before the study. Patients were not in lactation period. All patients who entered in present study received weekly interferon beta-1a injections for at least 3 months before the intervention. The body mass index (BMI) of the patients was between 18.5 and 30. Patients with any type of addiction, alcohol intake, dysphagia, history of myocardial infarction, stroke, allergic reaction to vitamin A, autoimmune diseases such as diabetes and inflammatory bowel disease, or liver, pancreatic, and biliary disorders were excluded from the study.

Age, gender and duration of the disease were recorded for each patient. A neurologist measured the Kurtzke EDSS. The MSFC including a timed 25 foot walk (T25FW) for lower limb function assessment, nine-hole peg test (9HPT) for upper limb function assessment and paced auditory serial addition test (PASAT3) for cognitive function assessment were administered at the beginning and the end of the study to all participants by a trained physician using a standard protocol.⁹

Patients were assigned to blocks, based on gender. Then, within each block, Patients were randomly assigned to treatment or placebo group. For this design, 13 men and 38 women got the treatment, and 12 men and 38 women got the placebo. The treatment group received 25000 IU/d retinyl palmitate (Zahravi Pharmaceutical Co., Iran) for the first 6 months and 10000 IU/d for the second 6 mo.

The present study, similar to Johnson et al.¹⁰ calculated the mean relapse rate for 2 years before the intervention. These measurements were compared with the relapse rate during the 1-year study. Patients filled-out three 24-hour food records at the beginning and end of the study. Anthropometric characteristics were also recorded at these times.

All patients underwent a baseline and a 1-yr follow-up brain MRI at the end of the trial using a GE Signa 1.5T MRI scanner (General Electric Medical Systems, Milwaukee, WI). The whole-brain MRI protocol at baseline consisted of:

- i. Axial high-resolution T1-weighted 3D IR-SPGR (TR/TE = 13/4 ms, TI= 400ms, slice thickness= 1.2 mm, gap= 0 mm, matrix size= 512×512, in-plane pixel spacing= 0.5×0.5 mm, flip angle= 30°, FOV= 26 cm, number of averages = 1);
- ii. Axial T2-weighted FSE (TR/TE = 6000/100 ms, slice thickness = 1.5 mm, gap = 0 mm, FOV= 24 cm);
- iii. Axial PD-weighted FSE (TR/TE = 3000/20 ms, slice thickness= 1.5 mm, gap= 0 mm, FOV= 24 cm);
- iv. Axial FLAIR FSE (TR/TE/TI = 7000/100/2200 ms, slice thickness= 5 mm, gap= 0 mm, FOV= 24 cm).

During the follow-up scan, all patients received an intravenous dose of gadoteric acid (Dotarem/Guerbet, France, 0.1 mmol/kg body wt). A post-gadolinium (post-Gd) T1-weighted FSE axial image (TR/TE = 660/20 ms, slice thickness= 1.5 mm, gap= 0 mm, matrix size= 512×512, in-plane pixel spacing= 0.5×0.5 mm, FOV= 24 cm) was obtained in addition to the baseline protocol with a scan delay of 10 min. The total acquisition time was approximately 30 min at baseline, and extended to 40 min for the follow-up.

Preprocessing was performed using FSL (FMRIB Software Library, www.fmrib.ox.ac.uk/fsl).¹¹ For each patient, the baseline PD-weighted and FLAIR images, follow-up T2- and PD-weighted and FLAIR images were registered to the baseline T2-weighted scan using 7-parameter rigid body transformation implemented

in FLIRT (FMRIB Linear Image Registration Tool; www.fmrib.ox.ac.uk/fsl/flirt).¹² Then, patients underwent noise reduction using SUSAN (Smallest Univalued Segment Assimilating Nucleus; http://fsl.fmrib.ox.ac.uk/fsl/susan).¹³ Lesions were segmented using a semi-automatic manual tracing pipeline (Amira, Visage Imaging GmbH, www.amira.com). Baseline and follow-up T2 hyperintensities were segmented using the co-registered FLAIR and PD-weighted images. New T2 lesions were segmented from follow-up T2-weighted scans using the co-registered baseline T2-weighted images. Follow-up Gd-enhancing lesions were segmented from post-Gd images after registration to the follow-up T1-weighted scans.

Sample size calculation:

Maximum sample size was obtained according to EDSS changes in reference article and mentioned formula:¹⁴

$$d = \left(\frac{D_1 - D_2}{\sqrt{2} \times \sigma_d} \right) = \frac{1}{\sqrt{2} \times 1.75} = 0.396$$

$$n = \left(\frac{Z_{1-\alpha/2} + Z_{1-\beta}}{d} \right)^2 = \left(\frac{1.96 + 0.84}{0.396} \right)^2 = 50$$

Statistical analysis

Data was analyzed using SPSS 18 software (SPSS Inc, Chicago, IL). The normality of the data was evaluated using the Kolmogorov-Smirnov test. Then non-normal distribution data (Age, Disease duration, Vitamin A dietary intake, EDSS, MSFC, T2 lesion volume, Gd-enhancing lesion number and volume, Annualized relapse rate, Micronutrient intakes and Laboratory test endpoints) were then analyzed using a nonparametric test (Mann-Whitney). Normal distribution data (BMI) was analyzed using a parametric test (independent sample t-test). P-values of <0.05 were defined as significant.

Results

Out of the original 101 RRMS patients enrolled, four patients in each group did not complete the course. One male and three female patients in the treatment group and four female patients in the placebo group were excluded from the study due to changes in taking interferon, use of multivitamins and major changes in diet.

There were no significant differences between groups for baseline characteristics and clinical parameters (Table 1). After intervention, significant differences between groups were found for total MSFC ($p < 0.001$) and its subscales of 9HPT ($p = 0.01$) and PASAT3 ($p = 0.03$) (Table 2). Changes in lower limb function (MSFC subscale) as measured by T25FW were not significantly different between groups ($p = 0.09$) (Table 2).

The EDSS ($p = 0.73$) and annualized relapse rates ($p = 0.20$) were not significantly different between groups (Table 2). The results showed that there were no significant differences between groups for the percentage of patients with stable EDSS ($p = 0.48$) and patients with no relapse during the intervention ($p = 0.21$) (Table 2). The total lesion volume of brain white matter between groups before ($p = 0.16$) and after ($p = 0.40$) intervention were not significantly different. The number of enhanced lesions ($p = 0.26$) and the volume of T2 hyperintense lesions ($p = 0.23$) post-

Table 1. Baseline characteristics and clinical signs of MS patients

Characteristics	Treatment group (N = 47) ^a	Placebo group (N = 46) ^a	P-value
Age (year)	30.4 ± 6.9	32.3 ± 5.9	0.16 ^e
Disease duration (year)	4.3 ± 4.0	5.4 ± 4.0	0.24 ^e
Vitamin A dietary intake ^b	737.4 ± 483.78	744.8 ± 541.91	0.84 ^e
BMI	23.9 ± 3.1	24.5 ± 4.3	0.41 ^f
EDSS	1.30 ± 0.97	1.40 ± 1.05	0.10 ^e
MSFC	-0.05 ± 0.7	0.13 ± 0.9	0.27 ^e
T2 lesion volume ^c	10136.55 ± 1521.54	15547.95 ± 2787.40	0.16 ^e
Gender	12/35 ^d	12/34 ^d	0.95 ^e

^aMean ± SD; ^bμg/d; ^cmm³; ^dthe number of male to female; ^eMann-Whitney test; ^fIndependent samples t-test; ^gChi square test; BMI: Body Mass Index; EDSS: Expanded Disability Status Scale; MSFC: Multiple Sclerosis Functional Composite

Table 2. Clinical endpoints

Clinic-cognitive endpoints	Treatment group (N = 47)	Placebo group (N = 46)	P-value
Annualized relapse rate	-0.36 ± 0.56 ^a -1-1 ^b	-0.53 ± 0.55 ^a -1-1 ^b	0.20 ^c
Percent with no relapse	76.6	86.9	0.21 ^d
Change in EDSS	0.07 ± 0.23 ^a 0-1 ^b	0.08 ± 0.23 ^a 0-1 ^b	0.73 ^c
Percent with stable EDSS	91.5	86.9	0.48 ^d
Change in MSFC score	-0.14 ± 0.20 ^a -0.8-0.2 ^b	-0.31 ± 0.19 ^a -0.9-0.01 ^b	< 0.001 ^c
Change in 25FW	0.53 ± 0.54 ^a -0.55-2.04 ^b	0.71 ± 0.44 ^a 0.01-1.86 ^b	0.09 ^c
Change in 9HPT	-0.0015 ± 0.0014 ^a -0.0045-0.0017 ^b	-0.0023 ± 0.0015 ^a -0.0084-(-0.0004) ^b	0.01 ^c
Change in PASAT3	3.17 ± 4.22 ^a -3-12 ^b	1.11 ± 2.00 ^a -4-9 ^b	0.03 ^c
Change in PASAT2	2.72 ± 5.26 ^a -20-12 ^b	1.61 ± 1.78 ^a -2-6 ^b	0.02 ^c

^aMean ± SD; ^bminimum-maximum; ^cMann-Whitney test; ^dChi-Square Tests; 25FW: Timed 25-Foot Walk; 9HPT: Nine-Hole Peg Test; PASAT3: Paced Auditory Serial Addition Test; PASAT2: Paced Auditory Serial Addition Test

Table 3. Imaging endpoints

MRI endpoints	Treatment group (N = 46)	Placebo group (N = 46)	P-value
Change in T2 lesion volume, mm ³			
Mean ± SD	905 ± 3458	-396 ± 3866	0.40 ^a
Median (range)	323(-5409-15275)	307(-17670-5063)	
Percent with decreased or stable lesion volume	33	47	0.17 ^b
Percent with no new T2 lesion	44	58	0.20 ^b
New T2 lesion number			
Mean ± SD	2.5 ± 4.2	0.9 ± 1.9	0.10 ^b
Median (range)	1 (0-18)	0 (0-5)	
Percent with no Gd-enhancing lesion	80	87	0.40 ^b
Gd-enhancing lesion number			
Mean ± SD	0.4 ± 1.0	0.2 ± 0.6	0.26 ^a
Median (range)	0(0-6)	0(0-3)	
Gd-enhancing lesion volume, mm ³			
Mean ± SD	45 ± 137	23 ± 112	0.23 ^a
Median (range)	0(0-733)	0(0-737)	

^aMann-Whitney test; ^bChi-Square Tests; mm³: millimeter³; Gd: Gadolinium

Table 4. Vitamin A and carotenoids daily dietary intake of MS patients

Dietary intake	Treatment group (N = 47)	Placebo group (N = 46)	P-value
Change in carotenoids ($\mu\text{g}/\text{d}$)	-77.9 ± 714.7^a $-2796.47-1824.7^b$	-54.4 ± 637.68^a $-1831.41-1281.35^b$	0.73 ^c
Change in vitamin A ($\mu\text{g}/\text{d}$)	-11.2 ± 68.04^a $-188.95-133.45^b$	17.8 ± 90.85^a $-161.06-247.03^b$	0.08 ^c

^aMean \pm SD; ^bminimum-maximum; ^cMann-Whitney test; $\mu\text{g}/\text{d}$: microgram per day

Table 5. Comparison between two groups of study before and after intervention

Micronutrients		Treatment group (N = 47)	Placebo group (N = 46)	P-value ^e
Vitamin B9 ($\mu\text{g}/\text{d}$)	Before	282.6 ± 74.0^a $139.2-433.9^b$	277.7 ± 82.4^a $89.9-502.3^b$	0.92
	After	255.6 ± 57.4^a $83.3-356.6^b$	267.3 ± 69.9^a $115.9-411.6^b$	0.82
Vitamin B12 ($\mu\text{g}/\text{d}$)	Before	2.1 ± 1.0^a $0.5-4.8^b$	2.2 ± 1.2^a $0.4-5.2^b$	0.28
	After	2.3 ± 1.0^a $0.5-4.9^b$	2.1 ± 1.0^a $0.3-4.7^b$	0.72
Magnesium (mg/d)	Before	243.7 ± 64.5^a $143.7-68.4^b$	237.6 ± 53.4^a $128.6-414.4^b$	0.85
	After	248.2 ± 84.0^a $67.7-464.6^b$	228.0 ± 55.6^a $119.4-456.1^b$	0.24
Zinc (mg/d)	Before	7.6 ± 2.0^a $4.8-14.2^b$	7.9 ± 2.1^a $4.4-15.6^b$	0.59
	After	8.1 ± 2.0^a $3.7-14.1^b$	7.5 ± 1.7^a $4.7-13.2^b$	0.23
Calcium (mg/d)	Before	598.4 ± 283.4^a $155.9-1325.8^b$	592.9 ± 297.2^a $151.2-1734.7^b$	0.82
	After	581.2 ± 224.0^a $234.8-1230.4^b$	568.4 ± 211.3^a $230.9-1102.1^b$	0.95
Iron (mg/d)	Before	12.1 ± 3.7^a $6.9-23.8^b$	11.8 ± 3.4^a $5.8-20.9^b$	0.99
	After	12.1 ± 3.7^a $6.9-20.9^b$	23.0 ± 64.5^a $5.8-387.6^b$	0.91
MUFA (g/d)	Before	26.7 ± 7.6^a $15.1-52.7^b$	26.7 ± 7.4^a $16.0-44.0^b$	0.93
	After	27.3 ± 7.3^a $11.9-51.4$	27.3 ± 7.5^a $13.4-45.5$	0.82
PUFA (g/d)	Before	17.7 ± 7.1^a $8.4-45.7^b$	17.8 ± 4.4^a $8.9-29.6^b$	0.56
	After	18.8 ± 6.5^a $5.9-33.2^b$	17.9 ± 5.2^a $9.3-32.3^b$	0.95

^aMean \pm SD; ^bminimum-maximum; ^cMann-Whitney test; $\mu\text{g}/\text{d}$: microgram per day; mg/d: milligram per day; g/d: gram per day.

Table 6. Laboratory tests endpoints of MS patients

Laboratory tests	Treatment group (N = 47)	Placebo group (N = 46)	P-value ^e
Change in Chol ^d	-3.60 ± 16.37^a $-67-41^b$	-10.74 ± 31.92^a $-121-22^b$	0.88
Change in TG ^d	-9.02 ± 28.9^a $-95-37^b$	-0.48 ± 30.03^a $-130-45^b$	0.16
Change in LDL ^d	-0.30 ± 15.92^a $-51-40^b$	-2.11 ± 21.06^a $-96-33^b$	0.93
Change in HDL ^d	2.02 ± 11.46^a $-30-40^b$	2.02 ± 11.32^a $-45-35^b$	0.60
Change in FBS ^d	0.45 ± 8.08^a $-16-19^b$	1.43 ± 7.53^a $-10-22^b$	0.54
Change in ALT ^c	0.57 ± 9.44^a $-38-21^b$	-0.85 ± 7.91^a $-29-9^b$	0.53
Change in AST ^c	0.15 ± 1.85^a $-9-3^b$	0.24 ± 1.48^a $-4-4^b$	0.99

^aMean \pm SD; ^bminimum-maximum; ^cU/L: units per liter; ^dmg/dl: Milligrams per deciliter; ^eMann-Whitney test; Chol: Cholesterol; TG: Triglyceride; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; FBS: Fasting blood sugar; ALT: Alanine transaminase; AST: Aspartate aminotransferase

treatment were not significantly different between groups. In addition, there were no significant differences between groups in the percentage of patients with decreased or stable lesion volume ($p = 0.17$), no Gd-enhanced lesions ($p = 0.40$), and no new T2 lesions ($p = 0.20$) (Table 3).

There was also no significant difference in daily dietary intake of vitamin A ($p = 0.07$) and carotenoids ($p = 0.83$) between groups (Table 4). Daily dietary intakes of nutrients that may have affected the results of the study were measured in all patients and no significant differences between groups were found (Table 5).

In all patients, the results of lipid profile tests, liver function tests, and BMI measurements showed no significant differences between groups; the values did not increase in the pathologic range after intervention (Table 6).

Discussion

To the best of our knowledge, no study has been found so far to show the impact of vitamin A on disease progression in MS patients. The present study evaluated the effect of vitamin A (25000 IU/d retinyl palmitate for 6 mo and 10000 IU/d for next 6 months) on the clinical status, relapse rate, and brain lesions in MS patients.

In the present study, we measured and compared clinical status of patients with two scores, MSFC and EDSS. MSFC measures the progression of disability as indicated by cognitive, lower and upper limb function, but EDSS determines lower limb function dominantly. Thus, MSFC is more sensitive and comprehensive for estimation of disease progression in short-term studies in comparison with EDSS.¹⁵ It has been reported that EDSS is strongly correlated with lower limb component of MSFC.¹⁶ Results of the MSFC score subscales showed that vitamin A is able to restrain progression of upper limb and cognitive disabilities but does not inhibit progression of lower limb disability in MS patients in the present study. Overall, MSFC score was significantly improved in the group supplemented by vitamin A while the EDSS was not changed significantly.

Disease progression and activity were detected by measurement of total white matter lesions and Gd-enhancing lesions number or volume in brain MRI results. We found no significant changes in white matter and Gd-enhancing lesions between two groups of study. The present study could not investigate changes in brain gray matter volume because of some limitations in the MRI protocol. Vitamin A did not change the relapse rate in MS patients significantly.

Bjartmar et al. stated that a major reason for irreversible disability in MS patients is axonal loss and gray matter atrophy.¹⁷ Rudick, et al. showed that the rate of gray matter atrophy correlated with disease progression as measured by the MSFC.¹⁸ Evangelou, et al. reported that severe axonal loss may occur in the normal-appearing white matter area of the CNS in MS patients.¹⁹

This could be a possible reason that there were no significant changes in the volume of white matter lesions between groups despite significant improvements in MSFC. The present findings suggest that vitamin A may be more effective on the neurodegenerative features of MS (upper limb function and cognitive function) rather than the inflammatory features (relapse rate and white matter active lesions). According to present results, vitamin A supplementation does not affect on disease activity, but may inhibit disease progression in MS patients. We should follow up patients

at least one more year to detect the actual effect of vitamin A on relapse rate, similar to some trials in MS.^{10,20}

Our previous publications reported no adverse effects on lipid profile or liver laboratory tests for 6 month supplementation with 25000 IU vitamin A in MS patients.^{8,21} One study has reported that a dose of 25000 IU/d of vitamin A for 1 year is not toxic.²² The present study found no changes in lipid profile and liver laboratory tests in the pathologic range after 1 yr of intervention. There were no significant differences in laboratory tests between groups. It should be noted that none of the patients were excluded from the study due to adverse effects of vitamin A supplementation.

Very few studies have focused on the effect of RA on disease progression in EAE or MS. To the best of our knowledge, Qu, et al. study administered etretinate (a synthetic retinoic acid) to MS patients for 6 mo. This study reported enhanced activity of suppressor T cells and detected many serious side effects. Qu et al. detected no significant differences in terms of EDSS, fatigue and depression in treated patients, due to possible positive effects that were covered by side effects.⁵

Vitamin A supplementation effects on different outcomes (molecular, cellular, and clinical) in MS patients are the conversion of vitamin A (retinyl palmitate) to retinoic acids and increasing level of circulating retinoic acids. After 6 months of supplementation with 25000 IU/day retinyl palmitate, circulating retinoic acid level were elevated up to 3 – 4 ng/dl.⁷ Retinoic acids connect to RAR- α and γ on peripheral blood mononuclear cells⁸ and alter their expression. Retinoic acids altered the Th1/Th2 balance to Th2 and induced production of Foxp3⁺ T cells instead of Th17 phenotypes from primary T cells. In this way, retinoic acids regulated the immune response in MS patients.^{2,23,24} Our previous cellular assessments have shown that vitamin A supplementation in MS patients suppressed the proliferation of inflammatory T helper cells.^{25,26} Our previous molecular assessment has shown that vitamin A supplementation in MS patients down-regulated gene expression of proinflammatory cytokines and their transcription factors.²⁷ Finally, present study showed that vitamin A decreased the progression of upper limb and cognitive disability in RRMS patients, probably due to the above mentioned mechanism.

Factors affecting the power of the present study are effective dose of vitamin A (according to literature review), appropriate inclusion criteria, large sample size and long duration of the study. In the present study, we treated patients with vitamin A instead of retinoids to avoid side effects and obtained some expected results. Future studies with MRI protocol for detection of gray matter volume, axonal loss, and spinal lesions are recommended.

In conclusion, the present study treated patients with 25000 IU/day vitamin A (retinyl palmitate) instead of active vitamin A derivatives (retinoic acids) to avoid their side effects and obtained some expected results. The results showed that supplementation with vitamin A decreased the progression of upper limb and cognitive disability in RRMS patients. Vitamin A did not affect the lower limb function; white matter total lesion volume and number, as well as relapse rate significantly. Future studies with gray matter volume measurement and long-term follow-up to detect delayed effects of vitamin A supplementation could be helpful.

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