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Gestational Vitamin D and the Risk of Multiple Sclerosis in the Offspring

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Abstract

Background—Vitamin D may have a protective role in the etiology of multiple sclerosis (MS), but the effect of gestational vitamin D on adult onset MS has not been studied.

Methods—In 2001, 35,794 mothers of participants of the Nurses' Health Study II completed a questionnaire inquiring about their experiences and diet during pregnancy with their nurse-daughter. We studied the association of maternal milk intake, maternal dietary vitamin D intake, and predicted maternal serum 25-hydroxyvitamin D (25(OH)D) during pregnancy and their daughters' risk of developing MS.

Results—MS was diagnosed in 199 women. The relative risk (RR) of MS was lower among women born to mothers with high milk or vitamin D intake during pregnancy. The multivariate adjusted RR of MS was 0.62 (95% CI: 0.40–0.95; *p* trend=0.001) for nurses whose mothers consumed 2–3 glasses of milk per day compared with those whose mothers consumed fewer than 3 glasses per month, and 0.57 (95% CI: 0.35–0.91; *p* trend=0.002) for nurses with mothers in the highest quintile of dietary vitamin D intake compared with those in the lowest. The predicted 25(OH) vitamin D level in the pregnant mothers was also inversely associated with the risk of MS

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in their daughters. Comparing extreme quintiles the adjusted RR was 0.59; (95% CI: 0.37–0.92; p trend =0.002).

Conclusion—Higher maternal milk and vitamin D intake during pregnancy may be associated with a lower risk of developing MS in the offspring.

Keywords

Multiple sclerosis; Epidemiology; prenatal; vitamin D

Introduction

Multiple sclerosis (MS) is a complex autoimmune disease, probably caused by a combination of genetic and environmental factors.¹ Results of epidemiologic studies, including studies of childhood sunlight exposure and diet^{2,3}, dietary vitamin D intake⁴, and serum levels of 25-OH vitamin D⁵, support a protective role of vitamin D in MS etiology.

The higher concordance rate of MS among dizygotic twins, who share the same intrauterine environment and time of birth, compared to non-twin siblings suggests a gestational susceptibility for MS.⁶ Further, in studies on time of birth, MS patients were more likely to be born in spring, predominantly the month of May in the Northern hemisphere,⁷ with symmetrical results in Australia, where the highest MS risk was among individuals born in November-December,⁸ possibly due to longer durations of their in utero vitamin D insufficiency during the preceding winter. Although the effect of gestational vitamin D on adult onset MS has not been studied, some epidemiologic studies have assessed early life effects of dietary vitamin D on other autoimmune diseases like type 1 diabetes; the results are inconsistent.^{9, 10, 11}

In this longitudinal study we examined the association between in-utero vitamin D exposure as measured by maternal milk intake, maternal dietary vitamin D intake, and predicted maternal serum 25(OH)D during pregnancy and their daughters' risk of developing MS later in adulthood among 35,372 nurses whose mothers' participated in the Nurses' Mothers' Study.

Methods

Study Population

The Nurses' Health Study (NHS) is a prospective cohort of 121,701 female registered nurses, aged 30 to 55 at study onset in 1976. The Nurses' Health Study II (NHS II), another cohort of 116,430 female registered nurses began in 1989 with women aged 25 to 42 at that time. These nurses completed questionnaires at baseline and at biennial follow-up providing information on demographic, lifestyle and health-related factors, and newly diagnosed diseases, including MS. The Nurses' Mothers' Study¹² began in 2001, when the NHS and NHS II participants, free of cancer, were requested permission to send questionnaires to their mothers regarding their daughters' early life exposures. They were restricted to nurses with living, biological mothers, free of debilitating diseases (N=52,540). The questionnaire inquired about the mothers' experiences during pregnancy with their nurse-daughter and their nurse-daughter's early life exposures. Of the 39,904 (76%) mothers returning questionnaires, the current analyses only includes mothers' of NHS II members (N=35,794), as they comprised 90% of the cohort. NHS II and the Nurses' Mothers' Study were linked, providing data on the nurses' entire lifespan from conception to adulthood, with repeatedly

assessed information on adult life variables. This study was approved by the institutional review board of Brigham and Women's Hospital and the National Cancer Institute.

Outcome assessment

Cases were defined as physician-confirmed MS, as previously described.^{4, 13} In summary, cases identified by self-report on the biennial questionnaires, who provided permission to contact their treating neurologist/internist and to review medical records, were confirmed if the diagnosis was considered definite or probable by the treating neurologist/internist, or by our study neurologist (T.C.) reviewing the medical records. In NHS II, the diagnostic confirmation was extended to cases with disease onset before study enrollment in 1989. These cases were considered prevalent MS cases as opposed to incident cases with disease onset after 1989.

Exposure assessment

The Nurses' Mothers' questionnaire in 2001 asked about diet during pregnancy using a semi-quantitative food frequency questionnaire. Mothers reported the frequency of intake of typical serving-sizes of 24 food items during their entire pregnancy. Maternal exposure was assessed in three different ways -- milk intake, dietary vitamin D, and predicted serum 25(OH)D.

Maternal milk intake during pregnancy—In this cohort the main contributor to vitamin D intake from food during pregnancy was fortified milk. Each mother reported their average milk intake by choosing from 7 categories ranging from “never” to “4 or more glasses/day”. The “never” and “1–3 glasses/month” categories of milk were collapsed into one category of “≤3 glasses/month” due to small sample size. Nurses were then categorized into one of six categories of maternal milk intake.

Maternal vitamin D intake from food—The main food items contributing to dietary vitamin D intake were milk, fish, margarine, and eggs. We estimated vitamin D intake from food during pregnancy for each mother by summing up the vitamin D contribution of each of the vitamin D containing foods. Intake was calculated by multiplying the amount of vitamin D in a serving size of those food items by the frequency of consumption. Vitamin D contents of food pertaining to the relevant time period (1945–1965, were derived.^{141516–18} While mothers reported intake of prenatal vitamins during pregnancy, details about type of vitamins, and duration or frequency of intake were not available. Thus, we could only assess vitamin D intake from supplements as yes/no/missing, assuming that all prenatal vitamins contained vitamin D.¹⁹ The validity of maternal diet data recalled from the distant past has not been assessed. Here we have asked mothers to recall data from about 35 to 55 years (average 45 years) prior to completing the questionnaires. When people have recalled diets 11–25 years in the past, average correlations have ranged between 0.28 to 0.38.²⁰²¹ The correlations with historical intake were even lower for recalls of 20–44 years in the past with only some foods such as dairy showing higher correlations ($r=0.55$).²²

Predicted serum 25-hydroxyvitamin D—Because serum 25(OH)D measurements are not available for the Nurses' Mothers', we developed a prediction model, using the mother's characteristics including factors known to influence circulating 25(OH)D concentrations, as predictors of serum 25(OH)D.²³²⁴ We used measured serum 25(OH)D levels from 1,240 nurses who served as controls in three case-control studies nested within the NHS cohort, and were not part of our analyses.²⁴ In a linear regression model, with measured serum 25(OH)D as the dependent variable and race, vitamin D from food, vitamin D from supplements, UV-B flux, age, BMI, season of blood draw, lab-batch, physical activity, alcohol intake, and hormone use as independent predictors of serum 25(OH)D, we obtained

beta-coefficients for each variable. (Table 1) Predicted 25(OH)D “scores” were then estimated for each mother in our cohort using regression coefficients obtained in the NHS sample for race, vitamin D from food, vitamin D from supplements, UV-B flux (based on geographic residence at time of daughter’s birth), mothers’ age and mothers’ pre-pregnancy BMI. This “predicted” maternal serum 25(OH) D, which was used as an approximation of in utero 25(OH)D exposure for NHS II participants, takes into account the combined influence of all of the major determinants of vitamin D status as opposed to just maternal vitamin D intake. The recreational activity question in the mother’s questionnaire asked about distance walked/day as opposed to the physical activity variables in the NHS which were measured as hours/week, making them slightly different. So for the main analysis physical activity was not included in the predicted 25(OH)D score calculations.

Assessment of covariates

Using the NHS II biennial questionnaires, the following risk factors for MS were included as covariates in the multivariate analyses: latitude at birth, ancestry, and pack-years of cigarette smoking. Using state of birth, reported in 1993 we categorized participants latitude at birth into northern, middle and southern tiers as previously described.²⁵ Ancestry was reported in 1989 and women were categorized as Southern-European/Mediterranean, Scandinavian, other Caucasian, and other as previously described.²⁵ Detailed information on smoking was reported at baseline in 1989 and was updated every two years. Pack-years of cigarette smoking was derived from this information and categorized into never, 1–9, 10–24, and ≥ 25 .¹³ Using current height, and weight at age 18 reported in 1989, we calculated nurses’ BMI at age 18 using the World Health Organization’s BMI categories (<18.5, 18.5–25, 25.0–30, and ≥ 30 kg/m²). We used food data from the 1991 questionnaire, to categorize nurses by quintiles of total energy-adjusted vitamin D intake.

Additional covariates using the Nurses’ Mothers’ Questionnaire included mothers’ smoking during pregnancy, maternal and paternal education level, paternal occupation (proxies for early life socioeconomic-status), mothers’ pre-pregnancy BMI, the daughter’s preschool intake of cod-liver oil or multivitamins, and mothers’ recreational physical activity (a proxy for sun-exposure) during pregnancy (in five categories ranging from highly active to inactive).

Statistical analysis

Incident (diagnosed after 1989) and prevalent MS cases (diagnosed before 1989) were included in the study. Follow up for the incident analyses started in 1989 and women contributed person time from the return date of the baseline questionnaire (1989) to the date of diagnosis of MS, death, or end of follow-up (June, 2005), whichever came first. In the prevalent analyses person-time was calculated from age 19 to the time of cohort recruitment (1989) or the date of MS diagnosis, whichever came first. For each analysis, person-time was analyzed in two-year follow-up intervals, and for person-time accrued after cohort recruitment these intervals corresponded to the time period between questionnaire dates.

We used Cox proportional hazards models, stratified by age in months and calendar year to compute hazard ratios to estimate the rate ratios (RR) and 95% confidence intervals for the age adjusted effect of maternal milk intake, quintiles of maternal vitamin D intake from food, and quintiles of predicted serum 25(OH)D on MS. Vitamin D from food and predicted serum 25(OH)D were also assessed as continuous variables in increments of 400IU/day and 10nmol/L respectively. In our multivariable analysis we adjusted for latitude at birth, ethnicity and pack years of smoking which are known MS risk factors. Smoking, the only time varying covariate, was also updated in the prevalent analyses for consistency. Tests for linear trend were conducted by using the median values of categories of milk, quintiles of

maternal dietary vitamin D, and quintiles of predicted serum vitamin D. We used the missing indicator method when controlling for covariates with missing data and excluded those missing in the primary exposures.²⁶ Analyses were conducted separately in the incident and prevalent cases and rate ratio estimates were pooled using inverse-variance weighting, and a Q test for heterogeneity was conducted.²⁷ The Nurses' Mothers' Questionnaire was completed in 2001, when more than 80% (n=118) of the incident MS cases had already occurred. There were too few cases (n=29) to conduct a fully prospective analysis; therefore, our analyses were primarily retrospective. Analyses were conducted using SAS version 9. A p value <0.05 was considered statistically significant.

Results

We had 147 incident cases (diagnosed after 1989) and 52 prevalent cases (diagnosed before cohort recruitment) available for analysis. The relation between maternal milk intake and potential risk factors for MS is shown in Table 2. One notable observation is that compared to mothers with low milk intake, those who had higher milk intake were also more highly educated and physically active during pregnancy, and more likely to breast feed. (Table 2).

Maternal milk intake during pregnancy was inversely associated with their daughter's risk of MS. The risk of MS among the nurse-daughters was 38% lower if their mothers consumed 2–3 glasses of milk per day compared to mothers who consumed 3 or fewer glasses of milk per month. The pooled multivariate adjusted RR was 0.62 (95%CI=0.40–0.95); p trend=0.001. (Table 3)

The mother's vitamin D intake from food during pregnancy was also inversely associated with the risk of MS in their daughters – with a greater than 40% lower risk when comparing the highest with the lowest quintile of vitamin D consumption. (Table 3) As a continuous variable, maternal vitamin D intake from food was associated with a 58% reduced risk of MS for each 400IU/day increment. (Table 3)

Similarly, predicted 25(OH)D levels in the pregnant mothers was inversely associated with the risk of MS in their daughters (Figure 1A). Using predicted 25(OH)D as continuous, the pooled adjusted RR was 0.69 (95%CI= 0.56–0.86) for a 10nmol/L increment.

None of these multivariate adjusted RRs, adjusted for the nurse-daughters' pack-years of smoking, latitude of birth, and ethnicity, were materially different from the age adjusted results. Additionally adjusting for nurses' baseline vitamin D intake and their BMI at age 18 as well as mother's education, father's profession, mother's exercise, mother's breast feeding and mother's smoking did not materially change our results. (Table 3) Maternal intake of prenatal vitamins was not associated with the risk of MS in this analysis. Maternal recreational activity was inversely associated with the risk of MS when comparing the most active to the inactive group. The multivariate adjusted RR was 0.44 (95%CI=0.25–0.75); p trend=0.003.

In a sensitivity analysis where we included mothers' recreational activity in the predicted 25(OH)D calculations, the significant inverse association with MS risk became stronger (p trend <0.001) (Figure 1B).

After further adjusting for nurses own adult vitamin D intake, which was associated with lower risk of MS in previous analyses⁴, the inverse association between maternal vitamin D from food and daughter's MS was only slightly attenuated (multivariate RR for extreme quintiles=0.64, 95%CI=0.37–1.09; p trend=0.024). Knowing that blacks have a lower risk of MS than whites, because of genetic factors²⁸, and lower 25(OH)D levels, mostly because of darker skin pigmentation²⁹, restricting to white nurses did not change the results. The

associations from our main analysis persisted in additional sensitivity analyses where we adjusted for other potential covariates such as mothers' prenatal vitamin intake, mothers' pre-pregnancy BMI, the nurses' preschool intake of cod-liver oil or multivitamins. The observed associations between MS and maternal vitamin D from food and maternal predicted serum 25(OH)D were attenuated and no longer significant after controlling for maternal milk intake (p trends=0.83 and p trend=0.2, respectively). However, when the maternal predicted 25(OH)D score including mothers' recreational activity was adjusted for maternal milk intake, the results did not change and remained significant with p trend <0.002.

Discussion

In this large cohort study, with more than 35,000 mothers and 199 MS cases, we observed a 38% lower risk of MS in women whose mothers drank 2–3 glasses of milk daily during pregnancy as compared to those whose mothers drank very little or no milk. We also observed that daughters of mothers with higher dietary vitamin D intake during pregnancy and higher predicted serum 25(OH)D levels had a lower risk of MS than those whose mothers were in the lowest quintiles.

Milk is an important dietary source of vitamin D in the United States since the 1930s when vitamin D fortification (400IU/quart) was introduced in all varieties of milk, including low fat milk.³⁰ Although the association of maternal dietary milk and MS has not been studied before, a number of studies have considered maternal milk consumption in association with other autoimmune diseases and found no association with type 1 diabetes and an inverse association with asthma.^{31, 32}

The biological mechanism explaining how maternal milk intake could reduce MS risk in the offspring is not known. One hypothesis is that the high vitamin D content in milk mediated this effect -- nurses in this study had all been born between 1945 and 1965, a period when milk was being fortified with vitamin D. However, an effect of other nutrients and biological compounds in milk cannot be excluded.

In the NHS and NHS II, earlier studies did not find evidence for a protective role for adulthood milk intake and risk of MS.³³ Of interest, milk intake has been considered a risk factor for MS in a number of studies^{34, 35}. This association has been attributed to molecular mimicry between myelin oligodendrocyte glycoprotein (MOG) and butyrophilin, a bovine milk protein.³⁶ Nonetheless, no data support the occurrence of this cross reaction between maternal ingestion of cow's milk and fetal MOG.

The protective role of vitamin D during adult life and MS risk is supported by studies of dietary vitamin D intake⁴ and serum 25(OH)D⁵. Studies suggesting a season of birth effect⁷⁸ and a direct association of maternal pre-pregnancy BMI with MS³⁷ indirectly support a potential role for in-utero vitamin D insufficiency in the pathogenesis of MS. However, no previous studies have directly examined whether in-utero vitamin D influences the pathogenesis of MS. Human studies have shown that the fetus depends entirely on the mother for its supply of 25(OH)D.³⁸ If a mother has low vitamin D levels, the fetus will have low vitamin D exposure during its developmental period.³⁹ Many studies have shown that vitamin D levels in the US population are low, particularly in women of childbearing age.⁴⁰ As an immunomodulator, vitamin D may enhance regulatory T cell function which may be one mechanism through which higher maternal vitamin D levels could be protective for MS in their offspring.

Alternatively in-utero vitamin D deficiency may alter brain development through changes in gene regulation leading to long-lasting changes in structure and function of the adult brain as

seen in animal studies of maternal vitamin D deprivation.^{41,42, 43} However, the relevance of these observations for MS etiology is uncertain.

While the strengths of our study include access to a unique data set with more than 35,000 reports on pregnancy diet for mothers of adult women participating in a large cohort study, thorough case ascertainment, confirmation of self-reported diagnosis, and comprehensive data on other established risk factors for MS, there are also several limitations to consider. Most mothers in this cohort reported their diet during pregnancy after their daughters had been diagnosed with MS, thus recall bias cannot be excluded. The chance of a differential recall of the intake of milk or other vitamin D containing foods however appears, to be very low as the potential link between maternal consumption of milk and other dietary components during pregnancy and MS risk in the offspring were not widely known at that time. Comparing nurses whose mothers' drank 1 or more glasses per day to those whose mother's drank less than 1 glass per day, we observed a similar risk of MS in the prospective cases (n=29, RR: 0.69, 95%CI: 0.33–1.45) and the retrospective cases (n=123, RR:0.62, 95%CI: 0.44– 0.86).

Exposure misclassification is likely, as we are asking the mothers to recall dietary intakes for pregnancies that occurred on average 45 years prior to enrollment; however, for milk intake as a continuous variable, this misclassification is likely to be non-differential with respect to MS risk in the daughters, and would tend to attenuate any association between diet and MS risk, although the possibility of differential misclassification cannot be ruled out. As we did not have direct serum measurements, we used a predictive model to estimate the serum 25(OH)D levels in these mothers which may not be a good approximation of the actual serum levels which is mostly derived from sunlight exposure. Additionally the measured serum vitamin D values were obtained from a group of non-pregnant nurses, and applied to calculating predicted serum levels for pregnant women, not accounting for possible physiological differences. Finally, we cannot completely exclude unknown or residual confounding as an explanation of these results. It is also possible that these findings may be due to chance. Further studies are needed to replicate these findings.

In summary, our results suggest that high intake of maternal milk during pregnancy and increasing exposure to vitamin D during intrauterine life may reduce the risk of MS in the offspring. If this inverse association reflected a true protective effect of milk, 33% of the MS cases among mothers who drank less than 2 glasses of milk per day could have been hypothetically prevented by increasing milk intake to more than 2 glasses per day. Because of the numerous limitations, these results should be interpreted cautiously and should be confirmed in other populations. On the other hand, even a modest beneficial effect of vitamin D could translate into important interventions because vitamin D supplementation in pregnancy could be safely increased above the levels currently recommended.

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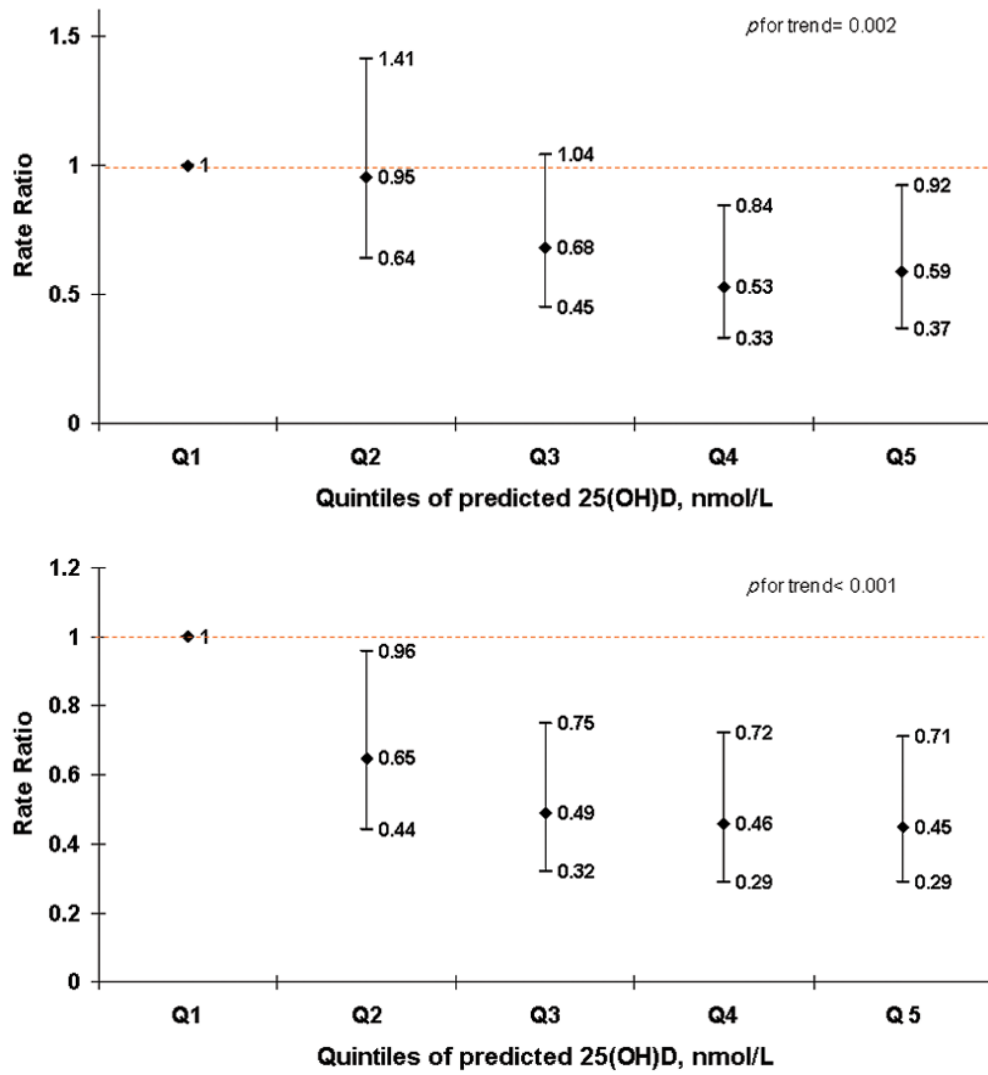


Figure 1.

Table 1

Factors contributing to serum 25 (OH) D in a prediction model

Factor	Change in 25(OH)D, ng/ml
Intercept	25.4
Race	
White	0 (referent)
Black	-11.16
Dietary vitamin D	
< 100IU/day	0 (referent)
100–199 IU/day*	0.47
200–299 IU/day	1.98
300–399 IU/day	3.36
≥ 400 IU/day	3.87
Vitamin D from supplements	
No	0 (referent)
Yes	2.92
Season of blood draw	
Fall (Sept., Oct., Nov.)	0 (referent)
Winter (Dec, Jan., Feb.)	-3.63
Spring (March, Apr., May)	-2.79
Summer (June, July, Aug.)*	1.03
UV-B flux	
>113	0 (referent)
113	-2.83
<113*	-1.22
Physical activity	
Quintile5	0 (referent)
Quintile4*	1.51
Quintile3	-2.94
Quintile2*	-1.5
Quintile1	-3.58
Alcohol intake	0.12
Hormone use	
pre-men/ missing men	0 (referent)
never/past PMH user*	-0.27
current PMH user	2
Age*	0.10
Body mass index	
<21kg/m ²	0 (referent)
21–23, kg/m ² *	3.01
23–24.9 kg/m ² *	0.61

Factor	Change in 25(OH)D, ng/ml
25–28.9 kg/m ²	–0.59
>29 kg/m ²	–3.11

Plasma 25(OH)D prediction model developed among 1240 NHS participants who served as controls in previous nested case-control studies of breast cancer, colon polyps, and colon cancer.

* Not statistically significant

PMH: Post Menopausal Hormone

R²= 0.32

Table 2
 Baseline age-adjusted characteristics of participants in NHS II according to servings of maternal milk intake*

Characteristics	NHS II					
	≤3/mo	1–4/wk	5–6/wk	1/day	2–3/day	4+/day
Mothers' milk intake (glasses) †						
Number of nurses (%)	5243 (14.8)	4361 (12.3)	22001 (5.7)	9748 (27.6)	12716 (36.0)	1303 (3.7)
Nurses' characteristics						
Nurses age in '89, mean	34.4	34.0	34.1	34.3	34.2	34.0
Ever Smoker in '89, %	32.4	32.2	32.6	31.7	31.3	29.4
Smoking status '89, %						
Never	67.5	67.7	67.3	68.2	68.6	70.5
Past	20.1	21.3	21.6	20.8	21.4	19.8
Current	12.3	10.8	10.9	10.9	9.9	9.5
Tier of birth, %						
North	28.2	30.2	29.3	30.8	34.7	40.4
Middle	49.3	47.7	48.1	46.9	45.2	39.9
South	12.0	13.0	14.3	13.2	11.3	10.3
Ethnicity, %						
Southern European	16.0	15.4	13.9	13.6	11.4	11.5
Scandinavian	3.4	3.8	4.0	4.6	5.7	5.9
Other Caucasian	72.6	73.7	76.9	75.7	77.4	76.8
Other	8.0	7.2	5.1	6.1	5.5	5.8
Nurses' baseline diet, mean						
Whole milk, servings/d	0.06	0.07	0.06	0.07	0.07	0.09
Skim milk, servings/d	0.87	0.97	1.00	1.02	1.24	1.37
Total milk, servings/d	0.93	1.04	1.06	1.09	1.31	1.46
Total Vitamin D, IU	371.0	381.1	390.9	390.7	414.6	428.8
Vitamin D w/o supp., IU	236.6	247.2	248.9	253.8	271.9	284.8
Preschool vitamin intake, %	62.9	66.2	69.6	68.1	71.0	73.1
Preschool codliver oil intake, %	12.0	10.9	10.4	12.1	11.6	15.0

NHS II						
Characteristics	Mothers' milk intake (glasses) †					
	≤3/mo	1-4/wk	5-6/wk	1/day	2-3/day	4+/day
Mother's characteristics						
Mother's age at birth, mean	26.2	26.2	26.1	26.5	26.3	26.2
Prenatal vitamin users, %	61.2	66.7	69.9	68.3	73.5	74.5
Smoke during pregnancy, %	29.4	28.8	27.6	25.2	24.7	22.0
Alcohol use (pregnancy), %	30.0	34.0	34.1	31.2	31.7	24.5
Breast Feeding, %	37.4	41.6	43.1	45.0	50.6	55.9
Own Home	52.9	52.8	53.2	51.3	51.6	51.4
Mothers' BMI, mean	21.3	21.4	21.3	21.4	21.14	21.01
Mother's leisure physical activity, %						
Highly active	9.9	8.0	8.5	9.0	10.8	18.3
Somewhat active	17.5	19.1	20.4	18.8	21.6	22.5
Active	35.6	38.9	40.6	41.9	40.4	36.5
Mostly inactive	21.6	22.3	21.8	20.6	18.5	15.2
Inactive	14.9	11.5	8.3	9.2	8.3	7.1
Mother's education, %						
Less than high school	19	15	12	13	11	12
High School	53	52	51	51	47	44
College	28	32	37	35	42	44
Father's education, %						
Less than high school	21.7	19.5	19.5	17.8	15.2	16.3
High School	39.7	37.3	36.3	37.6	35.4	31.6
College	33.4	38.4	40.7	40.4	45.7	48.3
Father high paying job, %	22.7	25.6	27.6	28.0	31.2	33.3

* Each category is directly standardized to the baseline age distribution of the cohort

† In the Nurses Mother's Study, 421 women were missing "mothers' milk intake"

Table 3
RRs and 95% CIs of MS by maternal dietary factors during pregnancy in NHS II

	Maternal Milk Intake ^{***}							P trend	P heterogeneity ^{†††}
	< 3/mo	1-4/wk	5-6/wk	1/day	2-3/day	4+/day			
Median, glasses	3/mo	2.5/wk	5.5/wk	1/day	2.5/day	4/day			
cases ^{§§}	28	26	10	37	41	3			
person-year	81,521	67,800	31,114	151,604	197,994	20,305			
Incident cases (n=147)									
Age adjusted [*]	Ref	1.13 (0.66-1.94)	0.97 (0.47-2.00)	0.70 (0.43-1.15)	0.60 (0.37-0.97)	0.47 (0.13-1.44)	0.0067		
Multivariate 1 [†]	Ref	1.14 (0.67-1.95)	0.98 (0.48-2.04)	0.71 (0.43-1.17)	0.61 (0.38-1.00)	0.48 (0.15-1.60)	0.0094		
Multivariate 2 [‡]	Ref	1.12(0.66-1.92)	0.99(0.48-2.04)	0.71(0.43-1.16)	0.62(0.38-1.02)	0.51(0.15-1.68)	0.015		
Multivariate 3 [§]	Ref	1.16(0.67-1.99)	1.04(0.50-2.17)	0.73(0.44-1.20)	0.66(0.40-1.09)	0.54(0.16-1.80)	0.028		
Prevalent cases (n=52)									
Age adjusted [*]	Ref	1.7(0.69-4.23)	1.0(0.27-3.78)	1.02(0.43-2.39)	0.63(0.26-1.53)	0.52(0.06-4.12)	0.047		
Multivariate 1 [†]	Ref	1.74(0.70-4.31)	1.03(0.27-3.88)	1.05(0.44-2.47)	0.65(0.26-1.59)	0.54(0.07-4.33)	0.056		
Multivariate 2 [‡]	Ref	1.81(0.73-4.50)	1.09(0.29-4.1)	1.08(0.46-2.55)	0.68(0.28-1.67)	0.56(0.07-4.52)	0.067		
Multivariate 3 [§]	Ref	1.83(0.73-4.58)	1.02(0.27-3.87)	1.04(0.44-2.47)	0.61(0.24-2.01)	0.47(0.06-3.84)	0.032		
Pooled cases (n=199)									
Age adjusted [*]	Ref	1.26 (0.79-2.00)	0.98 (0.52-1.84)	0.77 (0.50-1.18)	0.60 (0.39-0.92)	0.48 (0.17-1.35)	0.001	0.75	
Multivariate 1 [†]	Ref	1.27 (0.80-2.02)	0.99 (0.53-1.88)	0.78 (0.51-1.20)	0.62 (0.40-0.95)	0.50 (0.18-1.40)	0.001	0.73	
Multivariate 2 [‡]	Ref	1.27(0.80-2.02)	1.01(0.53-1.91)	0.79 (0.51-1.21)	0.64 (0.41-0.98)	0.52 (0.18-1.47)	0.002	0.73	
Multivariate 3 [§]	Ref	1.30(0.82-2.08)	1.04(0.55-1.97)	0.80(0.52-1.23)	0.65(0.42-1.00)	0.52 (0.18-1.48)	0.003	0.45	
Maternal Vitamin D from food^{†††}									
Continuous [†]	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5				
Median, IU/d (range)	187.5 (0-757)	132.6	187.5	294	350.3				
cases ^{§§}	147	35	30	22	22				
person-year	442,616	110,569	110,613	110,716	110,737				

	Maternal Vitamin D from food ^{††}					P trend	P heterogeneity ^{§§}
	Continuous [¶]	Quintile 1	Quintile 2	Quintile 3	Quintile 4		
Incident cases n=147							
Age adjusted [*]	0.45 (0.25–0.82)	Ref	0.98 (0.62–1.56)	0.81 (0.50–1.31)	0.59 (0.35–1.01)	0.61 (0.36–1.03)	0.012
Multivariate 1 [†]	0.46 (0.25–0.85)	Ref	0.99 (0.62–1.57)	0.82 (0.51–1.34)	0.61 (0.36–1.04)	0.62 (0.37–1.06)	0.017
Multivariate 2 [‡]	0.49(0.26–0.89)	Ref	0.99 (0.62–1.57)	0.83 (0.51–1.34)	0.62 (0.36–1.06)	0.64 (0.38–1.10)	0.026
Multivariate 3 [§]	0.51(0.27–0.95)	Ref	1.01 (0.63–1.62)	0.85 (0.52–1.38)	0.65 (0.38–1.12)	0.67 (0.39–1.16)	0.04
Prevalent cases (n=52)							
Age adjusted [*]	0.30 (0.11–0.84)	Ref	1.16(0.55–2.44)	0.77(0.34–1.76)	0.70(0.30–1.63)	0.39(1.4–1.08)	0.036
Multivariate 1 [†]	0.30 (0.11–0.86)	Ref	1.16(0.55–2.44)	0.78 (0.34–1.78)	0.71 (0.30–1.66)	0.390(0.14– 1.11)	0.041
Multivariate 2 [‡]	0.33 (0.12–0.96)	Ref	1.18(0.56–2.49)	0.81(0.35–1.84)	0.74(0.31–1.73)	0.41(0.15–1.16)	0.055
Multivariate 3 [§]	0.27(0.09–0.77)	Ref	1.15(0.54–2.42)	0.75(0.33–1.73)	0.66(0.28–1.56)	0.36(0.12–1.02)	0.025
Pooled cases (n=199)							
Age adjusted [*]	0.41 (0.24–0.68)	Ref	1.03 (0.69–1.52)	0.80 (0.53–1.21)	0.62 (0.32–0.97)	0.55 (0.35–0.89)	0.57
Multivariate 1 [†]	0.42 (0.25–0.70)	Ref	1.03 (0.70–1.53)	0.81 (0.53–1.23)	0.63 (0.40–1.00)	0.57 (0.35–0.91)	0.002
Multivariate 2 [‡]	0.44(0.26–0.74)	Ref	1.04(0.70–1.54)	0.82(0.54–1.25)	0.65(0.41–1.03)	0.59(0.36–0.94)	0.001
Multivariate 3 [§]	0.43(0.24–0.75)	Ref	1.05(0.71–1.56)	0.82(0.54–1.25)	0.65(0.41–1.03)	0.58(0.34–0.98)	0.003

* Adjusted for nurses' age (in months) and time interval

[†] Multivariate 1=Adjusted for nurses' age (in months), latitude of birth (north, middle, south), ethnicity (S. European, Scandinavian, other Caucasian, other), smoking (never smoker, 1–9, 10–24, and >25 pack-years)

[‡] Multivariate 2= Multivariate 1 + adjusted for nurses' baseline vitamin D intake in quintiles and nurses' BMI at age 18 (using the World Health Organization's BMI categories (<18.5, 18.5–25, 25.0–30, and ≥ 30)

[§] Multivariate 3= Multivariate 2 + adjusted for mother's education, father's profession, mother's exercise, mother breast feeding and mother's smoking

[¶] Maternal vitamin D from food includes: milk, fish, margarine, eggs, white bread and rice, processed meats, butter, bakery products and other dairy foods (cheese, yogurt, and ice cream). It was calculated by multiplying the amount of vitamin D in a serving size of these food items by the frequency of consumption

^{¶¶} RRs are for 400 IU increments of dietary vitamin D intake as a continuous variable

** In the Nurses Mother's Study, 421 women were missing maternal milk intake

^{††} In the Nurses Mother's Study, 238 women were missing maternal vitamin D from food

^{††}The test of heterogeneity is comparing the heterogeneity of the incident and prevalent effect estimates for the p for trends

^{§§}The mean age of onset of MS in this population is 36 years, ranging between 16–52 years.