

Results from an International Case–Control Study of Childhood Brain Tumors: The Role of Prenatal Vitamin Supplementation

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An international case–control study of primary pediatric brain tumors included interviews with mothers of cases diagnosed from 1976 to 1994 and mothers of population controls. Data are available on maternal vitamin use during pregnancy for 1051 cases and 1919 controls from eight geographic areas in North America, Europe, and Israel. Although risk estimates varied by study center, combined results suggest that maternal supplementation for two trimesters may decrease risk of brain tumor (odds ratio [OR] 0.7, 95% confidence interval [CI] 0.5–0.9), with a trend of less risk with longer duration of use (p trend = 0.0007). The greatest risk reduction was among children diagnosed under 5 years of age whose mothers used supplements during all three trimesters (OR 0.5, CI 0.3–0.8). This effect did not vary by histology and was seen for supplementation during pregnancy rather than during the month before pregnancy or while breast feeding. These findings are largely driven by data from the United States, where most mothers took vitamins. The proportion of control mothers who took vitamins during pregnancy varied tremendously: from 3% in Israel and France, 21% in Italy, 33% in Canada, 52% in Spain and 86 to 92% at the three U.S. centers. The composition of the various multivitamin compounds taken also varied: the daily dose of vitamin C ranged from 0 to 600 mg, vitamin E ranged from 0 to 70 mg, vitamin A ranged from 0 to 30,000 IU, and folate ranged from 0 to 2000 μ g. Mothers also took individual micronutrient supplements (e.g., vitamin C tablets), but most mothers who took these also took multivitamins, making it impossible to determine potential independent effects of these micronutrients. — *Environ Health Perspect* 106(Suppl 3):887–892 (1998). <http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-3/887-892preston-martin/abstract.html>

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Abbreviations used: CI, confidence interval; OR, odds ratio; PNET, primitive neuroectodermal tumor.

Introduction

Brain tumors are a leading cause of cancer deaths in children in developed countries (1). Little is known about the causes of these tumors (2). A family history of multiple nervous system tumors, which usually occur in association with predisposing genetic syndromes, appears to be a factor in fewer than 5% of cases (3). Exposure to X-rays and to other forms of ionizing radiation is the only clearly established environmental cause but accounts for only a few percent of cases (4). Many other suggested risk factors have been investigated including head trauma, parental occupational exposures, medication use, and diet (4). This paper looks at maternal use of prenatal vitamin supplements.

An incidental finding in an early case–control study of pediatric brain tumors provided the first indication that prenatal vitamin supplementation might be related to reduced brain tumor risk (5). In this study, mothers were asked about the use of several specific medications during pregnancy; in answer to a final question about any other drugs, more control mothers than case mothers volunteered that they had taken prenatal vitamins (odds ratio [OR] 0.6) (5). More than a decade later, several studies reported similarly decreased risk related to maternal use of prenatal vitamins. These include studies of specific histologic subgroups of cases such as primitive neuroectodermal tumors (PNET) (6) and astrocytoma (2) and studies of all types of pediatric brain tumors combined (7). In the largest study to date, decreased risk related to prenatal vitamin supplementation was apparent for all types of tumors combined as well as for each of the three major subtypes (astroglial tumors, PNET, and other glial tumors) (8). This paper includes data from the Preston-Martin et al. (8) study, which was the U.S. portion of an international collaborative study of childhood brain tumors, as well as data from centers in France, Italy, Spain, Israel, and Canada.

Methods

We investigated whether intake of vitamin supplements by the mother during pregnancy, during the month before pregnancy, or while breast feeding were related to risk of pediatric brain tumors, including each of three major histologic subgroups of these tumors. Dose–response relationships were evaluated. The prevalence of vitamin

intake across the countries in the study and the micronutrient content of supplements used were compared.

Subjects in this study participated in the international population-based case-control study conducted to investigate risk factors for primary brain tumors in children. Investigators from nine centers (Paris, France; Milan, Italy; Valencia, Spain; Israel; Winnipeg, Canada; Los Angeles and San Francisco, California; Seattle, Washington; and Sydney, Australia) collaborated to develop the international protocol, design a standard questionnaire, and make decisions regarding study conduct and analysis. This study was coordinated by the International Agency for Research on Cancer in Lyon, France, where data from the centers were compiled and merged into a combined data set that includes 1218 cases and 2223 controls. Of these, maternal vitamin supplementation data were available for 1051 cases (86%) and 1919 controls (86%). Analyses excluded subjects for whom vitamin supplementation data were not collected (all Sydney subjects and 35 cases [78%] and 58 controls [70%] from Winnipeg) and for whom information on vitamin supplementation was unknown (12 cases and 10 controls from Milan, Paris, and Valencia).

Controls were frequency matched to cases in all U.S. centers and in Paris; otherwise they were individually matched. Matching variables were sex, age, and at five centers (Seattle, Winnipeg, Valencia, Milan, and Israel), geographic region. Cases were diagnosed during a range of years from 1976 to 1994. In Israel, cases were diagnosed between 1976 and 1992; in Paris, 1985 and 1987; in Milan, 1984 and 1988; in Valencia, 1983 and 1990; in Los Angeles, 1984 and 1991; and in Seattle and San Francisco, 1984 and 1990. Diagnosis years in Winnipeg were between 1980 and 1994; however, questions concerning maternal vitamin use were added to that questionnaire only in the final years of the study. The range of age at diagnosis covered birth to 19 years overall, with some variation in the upper age by study center. The maximum age included was 19 years in all U.S. centers and in Israel and Winnipeg, 16 years in Milan, 15 years in Paris, and 14 years in Valencia. A reference age and reference date was defined for each control. In the United States, this was the age and date when the control reached the age of diagnosis of a similar case; in other centers, this was the age of the control at the time of selection for the study and the date of selection. Approximately one-third

of children with brain tumors for whom vitamin supplement data were available was younger than 5 years of age; 54% were male, and 50% had astroglial tumors (Table 1). All cases that had a primary tumor of the brain, cranial nerves, or cranial meninges (*International Classification of Diseases—Oncology* [World Health Organization, Geneva]) site codes 191, 192.1, and 192.2) were eligible (8). Further details of control selection and other study design features at each of the participating centers are available from earlier reports from individual centers (9–12).

The common study questionnaire asked mothers about several exposures they may have had during the index pregnancy, including use of specific medications. The final questions in this section of the questionnaire asked about use of vitamin supplements. Mothers were asked specifically about intake of multivitamins and of vitamin C and vitamin E supplements. Detailed data were collected on timing (month before pregnancy, specific trimesters, use during breast feeding), brand or type, frequency, and duration of vitamin supplementation.

At some centers, vitamin use was not queried in the standard manner. In Paris,

for example, a mother's use of vitamin supplements was queried in the section on her diet during pregnancy; therefore, no information is available on use during the month before pregnancy or while breast feeding. Each center provided micronutrient content for each supplement brand reported. Neither the U.S. centers nor Winnipeg had information on the brand name of supplements used; for U.S. centers, however, the specific type of multivitamins taken was queried (e.g., regular, high potency, prenatal). Where type but not specific brand of vitamin was reported, market surveys were conducted to determine average levels of micronutrients in various types of supplements. The percentage of respondents from each center (other than the United States and Winnipeg) who reported vitamin use but did not report specific brands was 72% in Israel, 67% in Milan, and 57% in Valencia. Micronutrient analyses were restricted to vitamins C, E, A, and folate.

Maximum likelihood estimates of ORs and 95% confidence intervals (CIs) were computed using both conditional and (to minimize the problem of missing data within strata) unconditional logistic regression stratified by center, sex, and age group (13). Five centers (Seattle, Winnipeg,

Table 1. Characteristics of cases and controls with maternal vitamin supplementation data available; international collaborative case-control study of childhood brain tumors, 1976–1994.

Characteristic	Cases, n=1051		Controls, n=1919	
	n	%	n	%
Age at diagnosis, years ^a				
< 5	372	35	579	30
5–9	315	30	594	31
10–14	227	22	448	23
15–19	137	13	298	16
Male	564	54	1068	56
Year of diagnosis ^a				
1976–1979	3	0.3	6	0.3
1980–1984	167	16	270	14
1985–1989	685	65	1256	65
1990–1994	196	19	387	20
Morphologic subgroup				
Astroglial	529	50	—	—
Primitive neuroectodermal	232	22	—	—
Other glial	282	27	—	—
Unknown	8	1	—	—
Study center				
Paris	67	6	107	6
Milan	80	8	314	16
Valencia	57	5	116	6
Israel	300	29	573	30
Winnipeg	14	1	21	1
Los Angeles	300	29	307	16
San Francisco	101	10	200	10
Seattle	132	13	281	15

^aVaried by study center. For non-U.S. controls, age and year of diagnosis are the age and year of selection. For U.S. controls, age and year are the age at diagnosis of a similar case and the year in which the control attained the case's diagnosis age.

Valencia, Milan, and Israel) also used geographic region as a matching variable. Unconditional risk estimates for Seattle and Winnipeg were also adjusted for geographic region. For the other centers that matched on region (Valencia, Milan, and Israel), there were too many regional levels to allow for adjustment in unconditional analyses. For individually matched studies, strata for conditional analyses were defined by matched sets; for frequency matched studies, strata were defined by center, sex, and age group (0–1, 2–3, 4–5, 6–8, 9–11, 12–14, or 15–19 years of age). Because estimates were similar using both conditional and unconditional methods, only results from unconditional analyses are reported. Birth year, parents' education, and child's use of vitamin supplements were considered potential confounders. Parents' education was defined as the highest level attained by either parent and was dichotomized for analysis; parents in the upper level had at least some college education. Race was not considered a confounder because of its homogeneity within each center except Los Angeles (race as a potential confounder in the U.S. data is addressed in "Discussion"). Risk estimates and CIs from random effects models (with center as the random effect) are reported for exposure effects that differed by center (14); otherwise, results from fixed effects models are reported. Other factors considered as possible effect modifiers were gender, birth year, and parents' education. For tumor-specific analyses, cases within each tumor group were compared to all controls. Morphologic subgroups were defined by the World Health Organization (8): astroglial (9380–9384, 9400–9421, 9424–9442), PNET (9470–9473, 9501), and all other tumors (8000–8004, 8010, 8800, 8801, 8850, 8900–8910, 8940–8990, 9060–9085, 9150–9161, 9350–9364, 9390–9394, 9450, 9451, 9480, 9490, 9500, 9503–9505, 9530–9538, 9540–9570). A series of analyses restricted to histologically confirmed cases (91% of total) was performed. Length of time between pregnancy and interview was evaluated as a potential source of bias. Multiple logistic regression was used to assess independent effects of multiple exposures. Dose–response trend tests for individual micronutrient intake were performed using log-transformed data; for categorical analyses, unexposed mothers were the reference group and cut points were tertiles of exposure among all exposed mothers. Hypothesis testing was two-sided, with a significance level of 0.05. Analyses were

performed using Epilog Plus statistical software (Version 3.99, Epicenter Software, Pasadena, CA).

Results

Reported use of vitamins during the prenatal period varied considerably by study center. Among control mothers, reported use varied from 3% in Israel and Paris to 86 to 92% at the three U.S. centers (Table 2). Intermediate levels of use were reported by control women from the other centers (21% in Milan, 33% in Canada, and 47% in Valencia). A significantly decreased risk of childhood brain tumor associated with any reported use of vitamins during pregnancy was observed with the Los Angeles data (OR 0.5, CI 0.3–0.8, adjusted for age at diagnosis and gender). Decreased risks that did not reach statistical significance were observed in San Francisco, Valencia, and Winnipeg. Statistically nonsignificant elevations in risk were observed with data from Israel and Paris, the study centers with the lowest reported levels of vitamin use. The remaining two study centers, Milan and Seattle, had risk estimates of 1.0.

When data for all sites were combined, the risk of brain tumor associated with any

maternal prenatal vitamin use was 0.8 (CI 0.6–1.0) adjusted for age, sex, and study center; with center as a random effect, the OR was 1.0 (CI 0.4–2.4), and when data from U.S. centers were excluded the OR was 1.1 (CI 0.7–1.5). Refinement of the exposure period was attempted by computing risk estimates associated with maternal vitamin use during the month prior to the pregnancy (as a surrogate for use during very early pregnancy), during the pregnancy, and during breast feeding immediately after the child's birth, with simultaneous adjustment for any use during all periods (Table 3). Mothers could be in none, one, two, or all three of these exposure groups. Results suggest that any decreased risk may be restricted to vitamin use during pregnancy (OR 0.7, CI 0.6–1.0). Relative to women who did not use vitamins during the index pregnancy, decreased risks for childhood brain tumor were observed for those who used vitamins for two trimesters (OR 0.7, CI 0.5–0.9), or throughout all three trimesters (OR 0.6, CI 0.5–0.8, *p* trend = 0.0007, Table 3). The suggested decreased risk of childhood brain tumors with increasing duration of vitamin use during pregnancy was seen for

Table 2. Risk of childhood brain tumor by maternal vitamin supplementation during pregnancy by study center; international collaborative case–control study of childhood brain tumors, 1976–1994.

Study center	Cases, no. (%)	Controls, no. (%)	OR (95% CI) ^a
Israel	9 (3)	15 (3)	1.2 (0.5, 2.7)
Los Angeles	229 (76)	263 (86)	0.5 (0.3, 0.8)
Milan	17 (21)	65 (21)	1.0 (0.5, 1.9)
Paris	6 (9)	3 (3)	4.3 (0.8, 22.2)
San Francisco	89 (88)	183 (92)	0.7 (0.3, 1.5)
Seattle	116 (88)	249 (89)	1.0 (0.5, 1.8)
Valencia	27 (47)	45 (52)	0.6 (0.3, 1.4)
Winnipeg	3 (27)	7 (33)	0.6 (0.1, 2.8)

^aAdjusted for sex and age group; Seattle and Winnipeg are also adjusted for geographic region.

Table 3. Risk of childhood brain tumor by exposure period and duration of daily maternal vitamin supplementation during pregnancy; international collaborative case–control study of childhood brain tumors, 1976–1994.

	Cases, no. (%)	Controls, no. (%)	OR (95% CI) ^a
Exposure period			
During the month pre-pregnancy	49 (5)	66 (3)	1.2 (0.8, 1.8)
During pregnancy	496 (47)	839 (44)	0.7 (0.6, 1.0)
While breast feeding	202 (19)	351 (18)	0.9 (0.7, 1.2)
Trimester of exposure			
First	410 (42)	687 (38)	1.0 (0.7, 1.4)
Second	457 (46)	772 (43)	0.8 (0.5, 1.3)
Third	451 (46)	760 (42)	0.9 (0.6, 1.5)
Duration			
Never took daily	579 (56)	1107 (58)	1.0
Less than two trimesters	83 (8)	143 (8)	0.8 (0.6, 1.2)
Two trimesters	174 (17)	267 (14)	0.7 (0.5, 0.9)
All three trimesters	207 (20)	389 (20)	0.6 (0.5, 0.8)

^aAdjusted for center, sex, and age group; for exposure period and trimester of exposure analyses, each exposure period is also adjusted for the other two.

Table 4. Risk of childhood brain tumor by duration of daily maternal vitamin supplementation during pregnancy by morphologic subgroup^a; international collaborative case-control study of childhood brain tumors, 1976–1994.

Duration	Cases, no. (%)	Controls, no. (%)	OR (95% CI) ^b
Astroglial			
Never took daily	263 (50)	1107 (58)	1.0
Less than two trimesters	43 (8)	144 (8)	0.8 (0.5, 1.3)
Two trimesters	108 (21)	270 (14)	0.8 (0.6, 1.1)
All three trimesters	112 (21)	389 (20)	0.6 (0.4, 0.9)
PNET			
Never took daily	138 (60)	1107 (58)	1.0
Less than two trimesters	19 (8)	143 (8)	0.8 (0.5, 1.5)
Two trimesters	25 (11)	267 (14)	0.4 (0.2, 0.8)
All three trimesters	49 (21)	389 (20)	0.6 (0.4, 1.1)
Other glial			
Never took daily	171 (62)	1107 (58)	1.0
Less than two trimesters	20 (7)	143 (8)	0.7 (0.4, 1.2)
Two trimesters	41 (15)	267 (14)	0.6 (0.4, 1.0)
All three trimesters	46 (17)	389 (20)	0.5 (0.3, 0.9)

^aAll controls were used for each subset of cases. ^bAdjusted for center, sex, and age group.

each of the three major morphologic tumor subtypes: astroglial (p trend = 0.009), PNET (p trend = 0.05), and other glial (p trend = 0.01) (Table 4). This effect was apparent among children of all ages, but was somewhat more marked among children who were younger than 5 years of age at diagnosis (Figure 1). When data from U.S. centers were excluded, there was no trend relating duration of use to risk.

Because most mothers took multivitamin compounds, it is difficult to determine effects of individual micronutrients. Nonetheless, among children who were younger than 5 years of age at diagnosis, there is a suggestion of a decreasing risk of tumor with increasing daily dose of each of four micronutrients when analyzed individually (Table 5). In general, there is a progressive reduction of risk across the four levels of exposure for each of the micronutrients (p trend = 0.01, 0.004, 0.002, and 0.002 for vitamins C, E, A, and folate), but

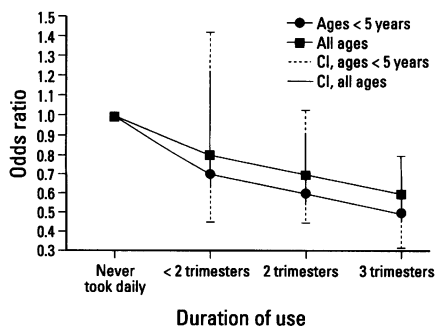


Figure 1. Duration of daily use of prenatal vitamins and risk of childhood brain tumors for children younger than 5 years of age at diagnosis compared to all children; international collaborative case-control study of childhood brain tumors, 1976–1994. Ages < 5 years, p trend = 0.003. All ages, p trend = 0.0007.

some differences among the various micronutrients are noteworthy. For vitamin C, risk reduction is progressive across exposure levels, and risk among those who took at least 100 mg per day is 0.5 (CI 0.3–0.9). For vitamins E and A and for folate, there is a sharp reduction of risk among those who took a small level of daily supplement compared to those who took none. For vitamin E, risk was similarly reduced (OR 0.5) among those in the third (10.3–13.2 mg) and fourth (≥ 13.3 mg) exposure levels of daily use. The highest levels of exposure were 100 mg or more for vitamin C, 13.3 mg or more for vitamin E, 5000 IU or more for vitamin A, and 400 μ g or more for folate. Average levels of nutrients

contained in supplements reported by each center varied considerably across centers: vitamin C from 67 to 203 mg/dose, vitamin E from 8 to 46 mg/dose, vitamin A from 3738 to 25,000 IU/dose, and folate from 100 to 1250 μ g/dose (Table 6).

Although parents' education was not a confounder, reduced brain tumor risk from maternal vitamin supplementation was somewhat more evident among children with more highly educated parents. However, results when education was dichotomized generally were consistent with each other. For example, results from dose-response analyses of the four micronutrients stratified by education level were essentially the same. Risk estimates were unchanged after adjusting for children's use of vitamin supplements. Among controls, prevalence of vitamin supplement use during pregnancy gradually increased over time, from 49% in the birth years from 1965 to 1969 to 57% for birth years 1985 and later, and risk estimates were generally lower among subjects born in 1980 or later (though birth year was not a confounder). Risk estimates were similar for each gender and also did not differ by histologic confirmation. Interview quality was considered a potential source of bias; however, only 6.5% of all interviews were deemed questionable or unsatisfactory by the interviewer.

Discussion

Intake of vitamin supplements during pregnancy was associated with an apparent

Table 5. Dose response for maternal selected micronutrient supplementation during pregnancy for subjects younger than 5 years of age at diagnosis; international collaborative case-control study of childhood brain tumors, 1976–1994.

Daily dosage ^a	Cases, no. (%)	Controls, no. (%)	OR (95% CI) ^b
Vitamin C, mg			
0.0	176 (47)	277 (48)	1.0
> 0.0 < 75.9	66 (18)	70 (12)	0.8 (0.5, 1.5)
≥ 75.9 < 100.0	38 (10)	55 (10)	0.6 (0.3, 1.1)
≥ 100.0	93 (25)	177 (31)	0.5 (0.3, 0.9)
Vitamin E, mg			
0.0	180 (49)	279 (48)	1.0
> 0.0 < 10.3	68 (18)	77 (13)	0.6 (0.3, 1.1)
≥ 10.3 < 13.3	28 (8)	44 (8)	0.5 (0.2, 1.0)
≥ 13.3	95 (26)	176 (31)	0.5 (0.3, 0.8)
Vitamin A, IU			
0	180 (49)	277 (48)	1.0
> 0 < 3900	61 (16)	77 (13)	0.6 (0.3, 1.0)
≥ 3900 < 5000	33 (9)	41 (7)	0.6 (0.3, 1.2)
≥ 5000	97 (26)	181 (31)	0.4 (0.2, 0.8)
Folate, μg			
0	180 (49)	277 (48)	1.0
> 0 < 13	65 (18)	78 (14)	0.6 (0.3, 1.1)
> 13 < 400	34 (9)	40 (7)	0.6 (0.3, 1.3)
≥ 400	92 (25)	179 (31)	0.5 (0.3, 0.8)

^aExposure categories are mutually exclusive. ^bAdjusted for center, sex, and age group. Risk estimates are not adjusted for other micronutrients in the table.

Table 6. International variation in micronutrient content of vitamin supplements; international collaborative case-control study of childhood brain tumors, 1976-1994.

Center	Average micronutrient content ^a			
	Vit C, mg	Vit E, mg	Vit A, IU	Folate, µg
Paris	325	20	25,000	250
Milan	128	46	14,300	100
Valencia	67	8	3738	333
Israel	162	40	5750	1250
Winnipeg ^b	175	40	5000	400
United States ^c	203	23	4500	380

Vit, vitamin. ^aOf vitamin brands and types reported that contain that micronutrient. ^bSpecific brands and types not reported; micronutrient content based on typical vitamin for the geographical area. ^cLos Angeles, San Francisco, and Seattle.

reduction of risk in earlier studies (2,5-7,9) and in this largest case-control study of childhood brain tumors to date. Risk reduction appeared to relate only to use during pregnancy rather than use during the month before pregnancy or during breast feeding, and the greatest risk reduction was observed when vitamins were taken during the entire pregnancy. The reduction of risk was greatest among children diagnosed at younger ages (<5 years at diagnosis), but also was seen among older children.

This international study has a number of limitations that must be considered. The small number of cases in most centers (<100 cases in all but the U.S. and Israeli studies) and the low prevalence of vitamin use in some geographic areas (e.g., 3% among control mothers in Israel and Paris) resulted in varied center-specific risk estimates and combined risk estimates that were dominated by findings in the United States, where vitamins were taken by the majority of mothers. In fact, the non-U.S. data added only a 14% increase in number of exposed cases and a 19% increase in number of exposed controls, despite increases of 126 and 178% in total numbers of cases and controls, respectively. This may suggest that the U.S. findings are the result of an unknown confounder, such as quality of prenatal care, that is related to reduced brain tumor risk, or that vitamin compounds in the United States differ in ways that make them more effective in reducing risk. Although supplements used in all geographic areas contained at least some of each of the four micronutrients, average levels of nutrients contained in supplements reported by each center varied considerably across centers, as indicated in Table 6.

There is a suggestion that an increasing reduction in risk occurs with increasing daily intake of each micronutrient evaluated (vitamins C, E, A, and folate), but because most mothers took a multivitamin compound, intake of these four was highly correlated. In addition, specific brand names of vitamins taken were not known or not recorded for many mothers (though U.S. centers asked about specific types of multivitamins taken). As in any retrospective case-control study, the possible influence of recall bias is a concern. We did not have the necessary data to perform a validation analysis against medical records. However, in studies of childhood cancer, recall bias is usually associated with case mothers trying harder than control mothers to remember medication use and other exposures during the index pregnancy. If such bias is present in relation to an apparently protective exposure such as vitamin use, it would have had the effect of biasing our risk estimates toward the null. Further, there is some evidence to suggest that recall bias does not exist in studies of adverse reproductive outcomes that use mothers as respondents (15). Though the lower risk estimates we observed for later birth years may be due to increasing prevalence of supplementation over time, they may also suggest nondifferential poor recall among mothers whose pregnancies were in the distant past.

In the U.S. portion of the study, findings relating to vitamin use remained after controlling for all factors considered in the pooled analysis, as well as the mother's education, social class (an index considering education and occupation of head of household), ethnicity (Latino, other white, black, and other), and the mother's diet during pregnancy (9). The U.S. data were also evaluated with respect to whether pregnancies were planned, which did not confound the U.S. results. It is possible that respondents differed from nonrespondents in these factors or in the exposures we studied (although participation rates at the U.S. centers were 70% or higher), or that controls targeted for participation (through random-digit dialing) were not representative of the population. However, these potential biases are not quantifiable in this study. We are processing the dietary data from each center to allow a combined analysis of micronutrient intake from diet and supplements, which will be particularly useful for centers in which prevalence of supplementation was low (e.g., Israel and France) and thus a minor contributor to population intake of micronutrients.

The dietary analysis will also allow us to examine the modifying effects of supplement intake in relationship to other dietary constituents such as nitrite from cured meats (an important consideration because both vitamins C and E are effective inhibitors of nitrosation, as described below). However, the dietary data from this study have their own set of limitations. The focus of this study was investigation of the *N*-nitroso hypothesis. Therefore, the questionnaire asked only about those 40 to 50 foods that account for 90% of population intake of nitrite, nitrate, and vitamins C and E in each geographic area under study (16). The list of foods queried varied considerably across centers. In addition, these lists were not designed to assess intake of most micronutrients (e.g., folate and vitamin A) or macronutrients (e.g., fat or animal protein). The Israel study is an exception, however, in that relatively complete dietary data (i.e., not just foods most correlated with intake of nitrite, nitrate, and vitamins C and E) were collected.

Our data suggest an increasing use of prenatal vitamin supplements over time, which is inconsistent with the modest increase in U.S. incidence of pediatric brain tumors over the past 20 years (17). However, increased incidence is likely due to the simultaneous effects of several different factors, such as improved diagnosis and possible secular changes in environmental exposures that may increase risk. The effects of these potential influences on pediatric brain tumor incidence cannot be disentangled when comparing the trends in incidence and prevalence of this single exposure.

Nitrite from cured meats is an important precursor of carcinogenic *N*-nitroso compounds commonly formed in the gut after ingestion of precursor compounds (18,19). One group of these compounds, the nitrosoureas, causes nervous system tumors in experimental animals (20-24). When exposure is transplacental, only low doses of precursors such as sodium nitrite and ethyl urea in the food and drinking water of the pregnant rats are required for 100% tumor induction in offspring. This effect can be blocked if ascorbate (vitamin C) and/or alpha-tocopherol (vitamin E) are added to the dams' diet (25). In the U.S. portion of this study, we found risk of brain tumors to be substantially higher for children of mothers who consumed above-average quantities of cured meats during their pregnancies and did not take vitamins compared with those who did (9). This synergism also was seen in a small earlier study (7). The hypothesis that

childhood brain tumors relate to maternal exposure to *N*-nitroso compounds during the pregnancy was the primary focus of this international study.

Various possible mechanisms have been suggested by which vitamin and mineral supplementation may reduce cancer risk (26). Antioxidants (e.g., vitamins C and E) can prevent oxidative damage to DNA by scavenging free radicals (27). Some micronutrients such as vitamins A and D have a role in cell differentiation and proliferation

(28). Supplementation may prevent deficiencies, such as folic acid deficiency, that may lead to malignant transformation of normal cells by weakening chromosomal structure and altering gene expression (29). Presence of micronutrients in the gut or bladder can prevent endogenous formation of carcinogens (such as *N*-nitroso compounds) or alter metabolism of mutagens. It is possible that the developing brain may be more susceptible to some of these effects because of the higher rate of brain cell

proliferation during gestation and early childhood and the fetal brain's lower ability to rid itself of mutagenic compounds (30).

Our findings of a huge variation in prevalence of prenatal vitamin use and of the content of vitamin compounds across countries may be of interest to clinicians and public health workers. In addition, we hope that our findings will stimulate investigators to consider vitamin supplementation in future epidemiologic studies of childhood brain tumors and other pediatric cancers.

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