

PRENATAL VITAMIN SUPPLEMENTATION AND RISK OF CHILDHOOD BRAIN TUMORS

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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 1,051 cases and for 1,919 controls from 8 geographic areas in North America, Europe and Israel. While risk estimates varied by study center, combined results suggest that maternal supplementation for 2 trimesters decreased 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 3 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. Our 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 markedly from 3% in Israel and in France, 21% in Italy, 33% in Canada and 52% in Spain to 86–92% at the 3 U.S. centers. The composition of the various multivitamin compounds taken also varied: daily dose of vitamin C ranged from 0 to 600 mg; vitamin E from 0 to 70 mg; vitamin A from 0 to 30,000 IU; and folate from 0 to 2,000 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 the potential independent effects of these micronutrients. *Int. J. Cancer Supplement 11:17–22, 1998.*

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Brain tumors are the leading cause of cancer deaths in children in developed countries (McKendry *et al.*, 1989). Little is known about the causes of these tumors (Bunin *et al.*, 1994). 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 (Bondy *et al.*, 1991). Exposure to X-rays and to other forms of ionizing radiation is the only clearly established environmental cause and also accounts for only a few percent of cases (Preston-Martin and Mack, 1996). Many other suggested risk factors have been investigated including head trauma, parental occupational exposures, medication use and diet (Preston-Martin and Mack, 1996). This report 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 (Preston-Martin *et al.*, 1982). In this study, mothers were asked about use during pregnancy of several specific medications and, 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; Preston-Martin *et al.*, 1982]. More than a decade later, several studies reported similarly decreased risk related to maternal use of prenatal vitamins; these included studies of specific histologic subgroups of cases such as primitive neuroectodermal tumors (PNET; Bunin *et al.*, 1993) and astrocytoma (Bunin *et al.*, 1994) and studies of all types of pediatric brain tumor combined (Sarasua and Savitz, 1994). 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 3 major subtypes (astroglial tumors, PNET and other tumors; Preston-Martin *et al.*, 1996). The present report includes data from this 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.

SUBJECTS AND METHODS

We investigated whether intake of vitamin supplements by the mother during pregnancy, during the month before pregnancy or while breast-feeding was related to risk of pediatric brain tumors, including each of 3 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.

Selection of cases and controls

All cases who had a primary tumor of the brain, cranial nerves or cranial meninges (ICD-O site codes 191, 192.1 and 192.2; World Health Organization, 1976) were eligible. 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 9 centers (Paris, France; Milan, Italy; Valencia, Spain; Tel-Hashomer, Israel; Winnipeg, Canada; Los Angeles, San Francisco and Seattle, USA; Sydney, Australia) collaborated to develop the international protocol, design a standard questionnaire and make decisions regarding study conduct

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and analysis. This study was coordinated by the International Agency for Research on Cancer (IARC) in Lyon, France, where data from the centers were compiled and merged into a combined data set that includes 1,218 cases and 2,223 controls. Of these, maternal vitamin supplementation data were available for 1,051 cases and 1,919 controls. 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).

Cases were diagnosed during a range of years from 1976 to 1994; in Tel-Hashomer, cases were diagnosed during 1976–1992; in Paris, 1985–1987; in Milan, 1984–1988; in Valencia, 1983–1990; in Los Angeles, 1984–1991; in Seattle and San Francisco, 1984–1990. Diagnosis years in Winnipeg were 1980–1994. 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 Tel-Hashomer and Winnipeg; 16 years in Milan; 15 years in Paris; and 14 years in 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 4 centers (Seattle, Winnipeg, Valencia and Milan), geographic region. A “reference age” and a “reference date” were defined for each control. In the United States, these were the age and date when the control reached the age of diagnosis of a similar case; in other centers, these were the age of the control at the time of selection for the study and the date of selection. Further details of control selection and other study design features at each of the participating centers are available from earlier reports from individual centers (Cordier *et al.*, 1994; Filippini *et al.*, 1994; Peris-Bonet *et al.*, 1996; Preston-Martin *et al.*, 1996).

Data collection

The common study questionnaire asked mothers about several exposures they may have had during index pregnancies 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, 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, *e.g.*, a mother’s use of vitamin supplements was asked about in the section on her diet during pregnancy so no information is available on use during the month before or while breast-feeding. Each center provided micronutrient content for each supplement brand reported. One center, Winnipeg, did not have information on brand name of supplements used. 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. Micronutrient analyses were restricted to vitamins C, E, A and folate.

Statistical methods

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 (Breslow and Day, 1980). Four centers (Seattle, Winnipeg, Valencia and Milan) also used geographic region as a matching variable. Unconditional risk estimates for Seattle and Winnipeg were also adjusted for geographic region. For the other 2 centers that matched on region (Milan and Valencia), there were too many levels of region 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, 15–19 years). Since 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 purposes of analysis; parents in the upper level had at least some college education. Risk estimates and CIs from random effects models (with center as the random effect) are reported for exposure effects that differed by center (DerSimonian and Laird, 1986); otherwise, results from fixed effects models are reported. Other factors that were considered 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 (World Health Organization, 1976) as: 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, vital status of the cases and interview quality also were evaluated as potential sources 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 cutpoints were tertiles of exposure among all exposed mothers. Hypothesis testing was 2-sided with a significance level of $p = 0.05$. Analyses were performed using Epilog Plus statistical software (version 3.99, Epicenter Software, Pasadena, CA).

RESULTS

Approximately one third of children with brain tumors for whom vitamin supplement data were available were less than 5 years of age, 54% were male and 50% had astroglial tumors (Table I). Reported use of vitamins during the prenatal period varied considerably by study center. Among control mothers, reported use varied from 3% in Tel-Hashomer and in Paris to 86–92% at the 3 US centers (Table II). Intermediate levels of use were reported by control women from the other centers (21% in Milan, 33% in Winnipeg, and 52% 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 non-significant elevations in risk were observed with data from Tel-Hashomer and Paris, the study centers with the lowest reported levels of vitamin use. The remaining 2 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). Refinement of the exposure period was attempted by calculating risk estimates associated with maternal vitamin use during the month prior to the pregnancy (as a surrogate for use during the 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 III). Mothers could be in none, 1, 2 or all 3 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 2 trimesters (OR = 0.7; CI = 0.5–0.9) or throughout all 3 trimesters (OR = 0.6; CI = 0.5–

TABLE I – 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 = 1,051) | | Controls (n = 1,919) | |
|---------------------------------------|-------------------|-----|----------------------|-----|
| | Number | % | Number | % |
| Age at diagnosis (years) ¹ | | | | |
| <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 | 1,068 | 56 |
| Year of diagnosis ¹ | | | | |
| 1976–1979 | 3 | 0.3 | 6 | 0.3 |
| 1980–1984 | 167 | 16 | 270 | 14 |
| 1985–1989 | 685 | 65 | 1,256 | 65 |
| 1990–1994 | 196 | 19 | 387 | 20 |
| Morphologic subgroup | | | | |
| Astroglial | 529 | 50 | — | — |
| PNET | 232 | 22 | — | — |
| Other tumors | 282 | 27 | — | — |
| Unknown | 8 | 1 | — | — |
| Study center | | | | |
| Paris | 67 | 6 | 107 | 6 |
| Milan | 80 | 8 | 314 | 16 |
| Valencia | 57 | 5 | 116 | 6 |
| Tel-Hashomer | 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 |

¹Varied 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.

TABLE II – 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 | Number of cases (%) | Number of controls (%) | OR (95% CI) ¹ |
|---------------|---------------------|------------------------|--------------------------|
| Tel-Hashomer | 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) |

¹Adjusted for sex and age group; Seattle and Winnipeg, also adjusted for geographic region.

0.8; *p* trend = 0.0007; Table III). The suggested decreased risk of childhood brain tumors with increasing duration of vitamin use during pregnancy was seen for each of the 3 major morphologic tumor subtypes: astroglial (*p* trend = 0.009), PNET (*p* trend = 0.05) and other tumors (*p* trend = 0.01) (Table IV). This effect was apparent among children of all ages, but was somewhat more marked among children who were less than 5 years of age at diagnosis (Fig. 1).

Since most mothers took multivitamin compounds, it is difficult to determine the effects of individual micronutrients. Nonetheless, among children who were less than 5 years old at diagnosis, there is a suggestion of a decreasing risk of tumor with increasing daily dose of each of 4 micronutrients, analyzed individually (Table V). In general, there is a progressive reduction of risk across the 4 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 some differences among the various micronutrients are noteworthy. For vitamin C, risk reduction is progressive across exposure levels, and

TABLE III – 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

| | Number of cases (%) | Number of controls (%) | OR (95% CI) ¹ |
|-----------------------|---------------------|------------------------|--------------------------|
| Exposure period | | | |
| Month pre-pregnancy | 49 (5) | 66 (3) | 1.2 (0.8, 1.8) |
| Pregnancy | 496 (47) | 839 (44) | 0.7 (0.6, 1.0) |
| 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) | 1,107 (58) | 1.0 |
| <2 trimesters | 83 (8) | 143 (8) | 0.8 (0.6, 1.2) |
| 2 trimesters | 174 (17) | 267 (14) | 0.7 (0.5, 0.9) |
| All 3 trimesters | 207 (20) | 389 (20) | 0.6 (0.5, 0.8) |

¹Adjusted for center, sex and age group; for exposure period and trimester of exposure analyses, each exposure period is also adjusted for the other 2.

TABLE IV – RISK OF CHILDHOOD BRAIN TUMOR BY DURATION OF DAILY MATERNAL VITAMIN SUPPLEMENTATION DURING PREGNANCY BY MORPHOLOGIC SUBGROUP: INTERNATIONAL COLLABORATIVE CASE-CONTROL STUDY OF CHILDHOOD BRAIN TUMORS, 1976–1994¹

| Duration | Number of cases (%) | Number of controls (%) | OR (95% CI) ² |
|------------------|---------------------|------------------------|--------------------------|
| Astroglial | | | |
| Never took daily | 263 (50) | 1,107 (58) | 1.0 |
| <2 trimesters | 43 (8) | 144 (8) | 0.8 (0.5, 1.3) |
| 2 trimesters | 108 (21) | 270 (14) | 0.8 (0.6, 1.1) |
| All 3 trimesters | 112 (21) | 389 (20) | 0.6 (0.4, 0.9) |
| PNET | | | |
| Never took daily | 138 (60) | 1,107 (58) | 1.0 |
| <2 trimesters | 19 (8) | 143 (8) | 0.8 (0.5, 1.5) |
| 2 trimesters | 25 (11) | 267 (14) | 0.4 (0.2, 0.8) |
| All 3 trimesters | 49 (21) | 389 (20) | 0.6 (0.4, 1.1) |
| Other tumors | | | |
| Never took daily | 171 (62) | 1,107 (58) | 1.0 |
| <2 trimesters | 20 (7) | 143 (8) | 0.7 (0.4, 1.2) |
| 2 trimesters | 41 (15) | 267 (14) | 0.6 (0.4, 1.0) |
| All 3 trimesters | 46 (17) | 389 (20) | 0.5 (0.3, 0.9) |

¹All controls were used for each subset of cases.—²Adjusted for center, sex and age group.

risk among those who took at least 100 mg/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, 5,000 IU or more for vitamin A and 400 μ g or more for folate.

While 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 were generally consistent with each other. For example, results from dose-response analyses of the 4 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 1965–1969 to 57% for birth years 1985 and later. However, birth year was neither a confounder nor an effect modifier, and risk estimates were similar for each gender. No sources of bias were identified among the factors evaluated (histological confirmation,

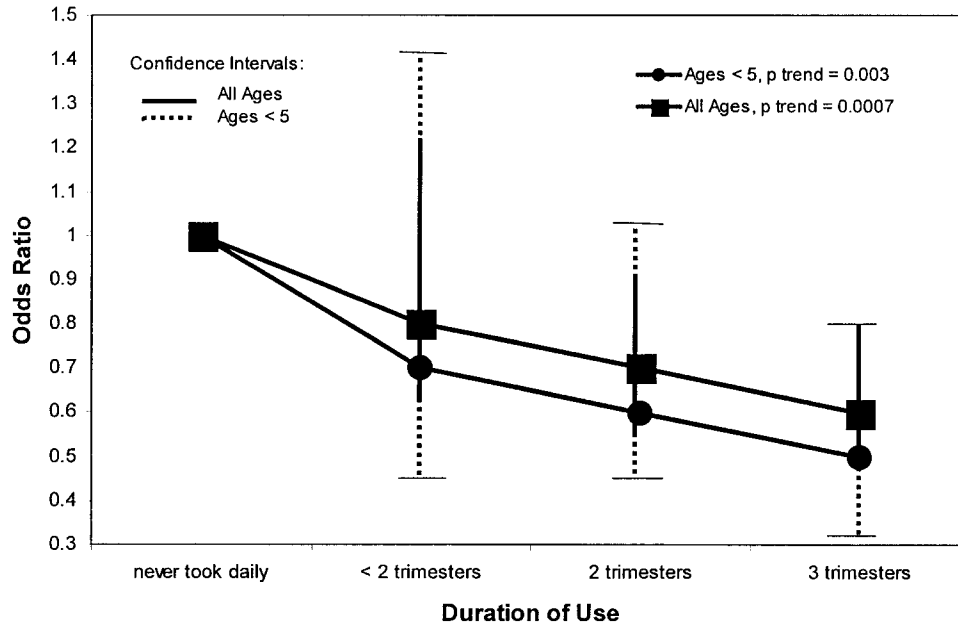


FIGURE 1 – Duration of daily use of prenatal vitamins and risk of childhood brain tumors for children less than 5 years old at diagnosis compared to all children: international collaborative case-control study of childhood brain tumors, 1976–1994.

TABLE V – DOSE-RESPONSE ANALYSES FOR MATERNAL SELECTED MICRONUTRIENT SUPPLEMENTATION DURING PREGNANCY FOR SUBJECTS <5 YEARS OF AGE AT DIAGNOSIS: INTERNATIONAL COLLABORATIVE CASE-CONTROL STUDY OF CHILDHOOD BRAIN TUMORS, 1976–1994

| Daily dosage ¹ | Number of cases (%) | Number of controls (%) | OR (95% CI) ² |
|---------------------------|---------------------|------------------------|--------------------------|
| Vitamin C (mg) | | | |
| 0.0 | 176 (47) | 277 (48) | 1.0 |
| <75.9 | 66 (18) | 70 (12) | 0.8 (0.5, 1.5) |
| <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 |
| <10.3 | 68 (18) | 77 (13) | 0.6 (0.3, 1.1) |
| <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 |
| <3,900 | 61 (16) | 77 (13) | 0.6 (0.3, 1.0) |
| <5,000 | 33 (9) | 41 (7) | 0.6 (0.3, 1.2) |
| ≥5,000 | 97 (26) | 181 (31) | 0.4 (0.2, 0.8) |
| Folate (µg) | | | |
| 0 | 180 (49) | 277 (48) | 1.0 |
| <313 | 65 (18) | 78 (14) | 0.6 (0.3, 1.1) |
| <400 | 34 (9) | 40 (7) | 0.6 (0.3, 1.3) |
| ≥400 | 92 (25) | 179 (31) | 0.5 (0.3, 0.8) |

¹Exposure categories are mutually exclusive. ²Adjusted for center, sex and age group. Risk estimates are not adjusted for other micronutrients in the table.

length of time between pregnancy and interview, vital status of the case and interview quality).

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 3,738 to 25,000 IU/dose; folate, from 100 to 1,250 µg/dose (Table VI).

DISCUSSION

Intake of vitamin supplements during pregnancy is associated with a reduction of risk in earlier studies (Preston-Martin *et al.*,

TABLE VI – INTERNATIONAL VARIATION IN MICRONUTRIENT CONTENT OF VITAMIN SUPPLEMENTS: INTERNATIONAL COLLABORATIVE CASE-CONTROL STUDY OF CHILDHOOD BRAIN TUMORS, 1976–1994

| Center | Average micronutrient content ¹ | | | |
|----------------------------|--|----------------|----------------|-------------|
| | Vitamin C (mg) | Vitamin E (mg) | Vitamin A (IU) | Folate (µg) |
| Paris | 325 | 20 | 25,000 | 250 |
| Milan | 128 | 46 | 14,300 | 100 |
| Valencia | 67 | 8 | 3,738 | 333 |
| Tel-Hashomer | 162 | 40 | 5,750 | 1,250 |
| Winnipeg ² | 175 | 40 | 5,000 | 400 |
| United States ³ | 203 | 23 | 4,500 | 380 |

¹Of vitamin brands/types reported that contain that micronutrient. ²Specific brands/types not reported; micronutrient content based on typical vitamin for the geographical area. ³Los Angeles, San Francisco and Seattle.

1982; Bunin *et al.*, 1993, 1994; Sarasua and Savitz, 1994; Preston-Martin *et al.*, 1996) 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 in France) 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. This may suggest that the U.S. findings are the result of an unknown confounder, such as quality of prenatal care, which 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 4 micronutrients, average levels of nutrients contained in supple-

ments reported by each center varied considerably across centers, as indicated in Table VI.

There is a suggestion that an increasing reduction in risk occurs with increasing daily intake of each micronutrient evaluated (vitamins C, E and A and folate), but because most mothers took a multivitamin compound, intake of these 4 was highly correlated. In addition, specific brand names of vitamins taken were not known or not recorded for many mothers. As in any retrospective case-control study, the possible influence of recall bias cannot be ruled out, although in studies of childhood cancer such bias is usually thought to result in 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.

In the U.S. portion of the study, findings relating to vitamin use remained after controlling for all factors considered here as well as mother's education, social class (an index considering education and occupation of head of household), ethnicity (Hispanic, other white, black and other), and mother's diet during pregnancy (Preston-Martin *et al.*, 1996). It is possible that respondents differed from non-respondents in these factors or in the exposures we studied (although participation rates at the U.S. centers was approximately 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 our study. We are processing the dietary data from each center to allow a combined analysis of micronutrient intake from diet and supplements, as well as analysis of modifying effects of supplement intake in relationship to other dietary constituents such as nitrite from cured meats. This latter is important to consider because both vitamins C and E are effective inhibitors of nitrosation (see 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 40–50 foods that account for 90% of population intake of nitrite, nitrate and vitamins C and E in each geographic area under study (Howe *et al.*, 1986). 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).

Nitrite from cured meats is an important precursor of carcinogenic N-nitroso compounds commonly formed in the gut after ingestion of precursor compounds (Magee *et al.*, 1976; National Research Council, 1981). One group of these compounds, the nitrosoureas, has been shown to cause nervous system tumors in experimental animals (Ivankovic and Druckrey, 1968; Ivankovic and Preussman, 1970; Ivankovic *et al.*, 1973; Rice *et al.*, 1989; Lijinsky, 1992). 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 (Mirvish, 1981). 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 (Preston-Martin *et al.*, 1996). This synergism also was seen in a small earlier study (Sarasua and Savitz, 1994). 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 (Patterson *et al.*, 1997). Antioxidants (*e.g.*, vitamins C and E) can prevent oxidative damage to DNA by scavenging free radicals (Frei *et al.*, 1988). Some micronutrients such as vitamins A and D have been shown to have a role in cell differentiation and proliferation (Bostick *et al.*, 1995). Supplementation may prevent deficiencies, such as of folic acid, which may lead to malignant transformation of normal cells by weakening chromosomal structure and altering gene expression (Butterworth, 1991). Presence of micronutrients in the gut or bladder can prevent endogenous formation of carcinogens (such as N-nitroso compounds; see above) 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 (Rajewsky *et al.*, 1976).

Our findings of a very marked variation in prevalence of use of prenatal vitamins 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 of other pediatric cancers.

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