Published in final edited form as:

Cancer Causes Control. 2013 February ; 24(2): 343–355. doi:10.1007/s10552-012-0120-x.

Parental nutrient intake and risk of retinoblastoma resulting from new germline *RB1* mutation

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Abstract

Purpose—We conducted a case-control study to examine the role of parents' nutrient intake before their child's conception in the child's risk of sporadic bilateral retinoblastoma, which results from a new germline *RB1* mutation.

Methods—Parents of 206 cases from 9 North American institutions and 269 friend and relative controls participated; fathers of 182 cases and 223 controls and mothers of 202 cases and 260 controls provided useable information in telephone interviews on their diet in the year before the child's conception. We also asked parents about supplements, a significant source of nutrients in users.

Results—Father's intake of dairy-associated nutrients and his use of calcium supplements were associated with decreased risk while his intake of copper, manganese, and vitamin E was associated with increased risk. Mother's use of multivitamins close to conception was associated with lower risk as was her intake of several micronutrients found in these supplements. In analyses to elucidate the primary factor from multiple correlated factors, the most robust findings were for father's calcium intake (adjusted OR=0.46 – 0.63 for 700 mg increase) and calcium supplement use (OR=0.35 – 0.41) and mother's multivitamin use (ORs 0.28 – 0.48).

Conclusions—There are few directly relevant studies but some data indirectly support the biologic plausibility of the inverse associations with father's calcium intake and mother's use of multivitamins; however, we cannot rule out contributions of bias, confounding, or chance. Our findings provide a starting point for further investigation of diet in the etiology of retinoblastoma and new germline mutation generally.

Keywords

germline mutation; diet; retinoblastoma; case-control studies; pediatric cancer

Retinoblastoma, a rare cancer of the embryonal retina, occurs in infants and young children. In about 30% of cases in developed countries, the disease occurs in children who carry a new germline mutation in the retinoblastoma tumor suppressor gene (*RB1*) [1] and is referred to as sporadic germline retinoblastoma. The vast majority of children with this form of retinoblastoma have bilateral disease.[2] Although radiation and many chemicals induce

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The authors declare that they have no conflict of interest.

new germline mutations in animals, research has not confirmed an effect in humans. An emerging consensus explains the apparent contradiction between animal and human data as a result of limitations of the human research rather than human resistance to germline mutation.[3] We undertook to study sporadic germline retinoblastoma in order to better understand the role of environmental exposures in this cancer and in new germline mutation generally.

Food and dietary supplements contain substances that are mutagens and others than inhibit mutagenesis. The dietary anti-oxidants vitamins A, C, and E inhibit the mutagenicity of various compounds as measured in microbial test systems,[4] cell culture,[5], and *in vivo* in rodent and human somatic cells.[6,7] Less is known about the role of diet in germ-cell mutation. The dietary anti-oxidants, zinc, and folate have roles in spermatogenesis and DNA synthesis and repair and have been hypothesized to reduce the risk of germ-cell mutation.[8] The hypothesized role for vitamin C is based on observations that seminal fluid contains high concentrations of vitamin C and a controlled diet that depleted the levels in seminal fluid resulted in increased oxidative damage.[9] Zinc also occurs at high concentrations in seminal fluid and deficiency leads to increased oxidative damage to testicular cell DNA in rats.[10,11] Low folate levels and abnormal metabolism in humans have been associated with aneuploidy in sperm or offspring.[12–14] These findings suggest a role of diet for germ-cell mutation but few if any data exist for the relevant endpoint for retinoblastoma, i.e. single gene mutation. Thus, we explored diet generally in addition to investigating the hypotheses for vitamin C, zinc, and folate.

For this report, we examined the role of nutrients in food and supplements, which contribute substantially to intake of antioxidants and other micronutrients. This report complements our recent publication assessing diet by food groups in the same study population, in which we observed increased risk associated with paternal intake of cured meats and decreased risk associated with paternal intake of dairy foods.[15] The majority of *RB1* mutations that result in sporadic bilateral retinoblastoma occur on the father's allele,[16,17] suggesting a minor role for maternal exposures. However, because of our recent observation that exposure of either parent to medical radiation was associated with sporadic bilateral retinoblastoma in the child[18], we investigated nutrient and supplement intake of both parents.

Materials and Methods

Institutional review boards of all participating institutions approved the study. Participants verbally consented to the telephone interview and gave written consent for the use of DNA.

The methods have been described previously.[18,19] Briefly, eligible patients were diagnosed with sporadic bilateral retinoblastoma from January 1998 – May 2006 and treated at one of nine participating institutions: Children's Hospital of Philadelphia, Wills Eye Institute (Philadelphia), Memorial Sloan-Kettering Cancer Center (New York), University of Illinois – Chicago, Children's Memorial Hospital (Chicago), Children's Hospital of Los Angeles, St. Jude Children's Research Hospital (Memphis), Hospital for Sick Children (Toronto), and Children's Hospital and Regional Medical Center (Seattle).

Controls were friends or relatives of the case matched on birth year (within 2 years of the case's birth year) and without a history of cancer. We chose controls so that the case and control fathers were not biologically related, resulting in a control group consisting of both friends and relatives that was homogeneous in terms of the absence of a biological relationship between case and control fathers. For each case, we attempted to recruit one to two friends and one relative. To be eligible, cases and controls had to reside in North

America, have at least one parent who spoke English or Spanish, and have at least one biological parent available for participation, i.e., not be adopted or in foster care.

Trained interviewers conducted telephone interviews from 2002–2007 to obtain information on diet, vitamin and mineral supplements, and other exposures; it was not practical to blind them to case-control status. The interview included a short Willett food frequency questionnaire (FFQ) [20] modified for use in a telephone interview, to focus on the year before the child's conception, and by the addition of a small number of foods to improve the estimation of zinc and vitamin C, two nutrients with roles in spermatogenesis. The final FFQ queried 71 food items, and 20 supplement items and is provided as an online supplement. For all supplements, we asked the parent about use in the year before the pregnancy began (yes/no) and within that year, use around the time of conception (yes/no). For multivitamins, we asked about frequency of use, type (regular, high-dose, stress type, prenatal (mothers only), other), and brand name. For 9 individual supplements of greater interest because of prevalence of use or possible anti-mutagenicity (calcium, vitamin A, beta-carotene, vitamin C, vitamin E, zinc, folic acid, selenium, iron), we asked whether use occurred "most months, seasonally or only occasionally," and frequency of use. The supplements for which we collected only the minimal information were vitamin D, B vitamins, cod liver oil, omega 3 fatty acids, iodine, copper, brewer's yeast, niacin, magnesium, and antioxidant combination. Parents who were not able or willing to complete the full interview were offered a shortened version of the questionnaire that did not include the FFQ.

Mutation detection

RB1 mutation analysis was performed for 190 of the cases as described previously.[21,22] When a *RB1* mutation was found, we screened the child's parents for the same mutation; 359 parents provided DNA for this purpose. Of cases with a detected mutation and samples from both parents, 6.5% were found to be ineligible because they had familial retinoblastoma (a parent carried the mutation found in the child) or were mosaic for the mutation (the mutation was not germline). Therefore, of the 37 cases without complete sets of samples, we estimate that 2 (6.5%) did not have a new germline *RB1* mutation. We included these 37 cases in our study because of the low level of misclassification.

Statistical analysis

Demographic and other characteristics of cases and controls were compared by chi-squared tests for categorical variables and t-tests for continuous variables.

The Nurses' Health Study (NHS) calculated nutrient content from the FFQ using its database of food and supplement contents.[23] Nutrient intake was calculated by multiplying the number of servings per day by the nutrient content of a standard portion size of each food and summing across all foods and adding intake from supplements. For micronutrients, dietary intake, i.e. from food alone, and total intake, i.e. from food and supplements combined, were calculated. For vitamin C, we used reported dose or when not known, 500 mg. For the other 18 individual supplements, we used standard doses because we and/or the NHS did not ask parents to recall dose or at least 40% of parents reporting use in our study did not know the dose. For the 9 individual supplements with detailed information, we used the full dose when reported use was in 'most months' with frequency at least 4 times/week (calcium 200 mg, vitamin A 10,000 IU, beta-carotene 6,000 IU, vitamin E 180 mg, zinc 25 g, folic acid 400 g, selenium 150, iron 60 mg). When individual supplement use was 'seasonal' and daily, the dose category that was approximately half the standard dose was used. When use was 'occasional,' 'seasonal' and less than daily, or 'most months' and less than 4 times per week, the supplement dose was not considered. For the individual supplements for which only yes/no use was collected, the standard doses of the

NHS were used. The content of multivitamins was based on the NHS database and the frequency of use. Multivitamin use less than 3 times per month was not considered.

We assessed the association between diet and risk of retinoblastoma in two ways. We used logistic regression to compare all cases and all controls ('Complete population') as the most inclusive analysis. However, as a substantial proportion of cases did not have controls and the cases with controls and those without controls differed demographically, the comparison of all cases and controls might be biased. Therefore, we also conducted analyses restricted to the matched case-control sets ('Case-control sets') using conditional logistic regression.

We considered 73 dietary factors, including calories, different types of fats, proteins, and sugars, all B vitamins, vitamins A, C, D, E, and K, minerals, trace minerals, and carotenoids. A full list of the factors is available on request. For vitamins and minerals we considered both total intake and intake from food sources only.

We performed analyses using each nutrient as a continuous variable and considered for further analysis nutrients with $P < 0.05$ in either the complete population or the matched sets. For comparison, we also analyzed the nutrients by quartiles of intake. As results using intake as a continuous variable and as a quartiled variable were similar, only the continuous results are presented.

In analyses of supplement use, we considered any use in the year prior to the child's conception, regular use (≥ 4 times/week) in the year prior, any use around the time of conception, and, if numbers permitted, regular use around the time of conception.

Individual nutrients and supplements were analyzed for mothers and fathers separately and adjusted for a minimal set of possible confounders, namely child's birth year (the matching factor), race/ethnicity (non-Hispanic white, other), education level (not a college graduate, college graduate) and energy intake (total calories). For highly correlated nutrients significantly associated with disease, we did additional analyses considering some or all of the nutrients simultaneously to try to determine the primary factor of interest. When supplements contributed a substantial proportion of the intake of a nutrient associated with risk, we performed analyses that included both total intake of the nutrient and use of the nutrient supplement in order to assess the supplement's contribution to risk. For these additional analyses, we present a minimally adjusted model that includes the relevant parent's race/ethnicity, educational level, energy intake, and child's birth year, as well as a 'fully adjusted' model that includes some or all of the significant nutrients/supplements, the covariates in the minimally adjusted model, and additional covariates selected by stepwise procedure from among the following: the other parent's educational level, father's smoking (non-smoker, 1–10 cigarettes/day, ≥ 11 cigarettes/day), mother's smoking (non-smoker, 1–10 cigarettes/day, ≥ 11 cigarettes/day), father's dose of gonadal medical radiation before the child's conception (0, 1–49 mGy, ≥ 50 mGy), mother's dose of gonadal medical radiation before the child's conception (0, 1–24 mGy, ≥ 25 mGy), father's intake (servings/day) of: dairy foods, fruit, cured meats, sweets, alcohol, father's age at the child's birth (<35 years, ≥ 35 years), father's body mass index (BMI, continuous), mother's BMI, and mother's alcohol intake (servings/day).

We used STATA/IC version 10.0 to perform conditional logistic regression and SPSS version 16.0 for unconditional logistic regression of nutrients or supplements considered one at a time. For the simultaneous analyses of multiple nutrients and supplements (minimally adjusted model and fully adjusted model using stepwise selection), we used SAS version 9.2. Other analyses were performed using SPSS. Statistical significance was defined as 2-sided $P < 0.05$.

Results

Recruitment and characteristics of cases and controls

The details of participant recruitment have been published.[18,19] Briefly, participating institutions identified 236 patients of whom 18 were ineligible (did not have sporadic bilateral retinoblastoma on genetic testing, biologic parent not available or did not speak English or Spanish), 9 refused, and 3 could not be located or were not contacted at physician request. The mother (n=204) and/or father (n=203) of the remaining 206 patients were interviewed for the study. Case families nominated 1 to 3 friends and relatives for a total of 374 potential controls, although some case families were unable or unwilling to nominate any controls. Parents of 72 refused, 22 could not be interviewed before the study ended, and 12 were ineligible. Of the 268 participating control children, mothers of 263 (70%) and fathers of 247 (66%) completed interviews. We recruited at least one control parent for 146 (71%) of the 206 case families. For a small proportion of participants, the other parent provided the information (Table 1).

Exclusions from those interviewed resulted from parents who did not provide diet information because they completed the shorter questionnaire (19 case fathers, 17 control fathers, 3 control mothers), had improbable energy intake (> 6000 or < 500 kcal/day) calculated from the FFQ (2 case fathers, 2 case mothers), or for the matched sets, did not have a corresponding control at all (53 case fathers, 62 case mothers), or did not have a corresponding case or control with diet information (2 case mothers, 7 case fathers, 7 control fathers). The analyses reported here include 182 case fathers and 223 control fathers forming the 'complete population' and a subset of these fathers, 122 case fathers and 223 control fathers forming the 122 matched case-control sets. There were 202 case and 260 control mothers in the mothers' complete population and 140 case and 260 control mothers in 140 matched sets.

Case and control children were similar in birth year, the matching factor. Control parents were more likely to be non-Hispanic white, have at least a college education, have a higher income, be married, and be a non-smoker compared to cases. Control fathers were more likely to have taken calcium supplements close to the child's conception and control mothers were more likely to have taken multivitamin supplements. When only the case-control sets were considered, case parents and control parents were similar in race/ethnicity, marital status, age at the index child's birth, and income (Table 1), but they differed in father's educational level and mother's smoking and multivitamin use.

Father's supplement use and nutrient intake

Father's intake of lactose and intake of dietary (excluding supplemental) calcium, phosphorous, vitamin B₂, retinol, and vitamin D were associated with significantly decreased risk in the matched analyses and, for calcium and phosphorous, in the complete population as well. Total intake, i.e. from food and supplements combined, of copper, manganese, vitamin E and phosphorous was significantly associated with increased risk in either the matched analyses or both the matched and complete analyses (Table 2).

Among fathers, the most commonly used supplements were multivitamins, calcium, vitamin C, vitamin E, zinc, and B vitamins. Ten or fewer participants used other supplements. Increased risk was significantly associated with the father's regular use of multivitamin (OR=1.76, 95% CI 1.05–2.95) and any use of vitamin C supplements (OR=2.03, 95% CI 1.17–3.53) during the year before the child's conception in the matched sets (Table 2), with somewhat smaller, non-significant ORs in the complete population. Any use of calcium supplements around the time of conception was significantly associated with decreased risk (OR=0.37, 95% CI 0.17–0.80) in the complete population; a somewhat attenuated

association was observed in the matched sets. Use of vitamin E supplements was associated with elevated risk, but none of the ORs was statistically significant. We observed no significant associations with risk for zinc or B vitamin supplements, although few fathers reported using them.

We conducted additional analyses to explore in greater detail the association with calcium. Besides calcium, five other nutrients found in dairy foods were also associated with decreased risk (lactose, vitamin D, retinol, phosphorous, vitamin B₂) and consequently, these nutrients were highly correlated with each other (average correlation = 0.68, range 0.36 – 0.93). Including all 6 nutrients simultaneously in one model produced attenuated and non-significant effect estimates for all nutrients (results not shown). We also explored whether dietary and supplemental calcium (any use at conception) were independently associated with risk (Table 3). In these analyses with mutual adjustment, the ORs for both dietary calcium and calcium supplement use remained, with one exception, statistically significant or nearly so and similar in magnitude to those from the analyses of each alone.

Total manganese, copper, and vitamin E were intercorrelated to some extent ($r=0.62$ for manganese and copper, 0.44 for manganese and vitamin E, and 0.20 for copper and vitamin E), and multivitamins were the major supplemental source for all three of these nutrients. To explore associations for these nutrients in greater detail, we performed analyses that also considered regular use of multivitamins in the year prior. Phosphorous was not explored here because supplements contributed only about 1% of total intake of this mineral. Among the other three nutrients, in models with the individual micronutrient and multivitamin use, only total manganese intake remained consistently associated with risk, with p-values that were statistically significant or approached significance (Table 3). When the three nutrients and multivitamins were included together in models, nearly all ORs were attenuated compared to the models including each singly, and none was statistically significant. Only the OR for manganese in the fully adjusted, matched model was not attenuated and approached significance (OR=1.91, 95% CI 1.00, 3.65, $p=0.04$).

Mother's supplement use and nutrient intake

Mother's total intake of iron, copper, niacin, manganese, zinc, and folate was significantly associated or nearly so with decreased risk in both the complete population and matched sets (Table 4). For all these nutrients, the ORs for dietary (excluding supplemental) intake were not significant although they were often farther from 1.0 (Table 4) compared to those for total intake.

With respect to supplements, mothers most commonly used multivitamin, calcium, vitamin C, folic acid, iron, and vitamin E supplements (Table 4). Fewer than 10 mothers used the other supplements. Multivitamin use was consistently associated with reduced risk, with ORs (0.45 – 0.74) that became more pronounced as the definition of use narrowed from any use during the year before conception to regular use during this year and to use around the time of conception. Use of calcium supplements was consistently associated with lower risk, but the ORs were not statistically significant. Use of vitamin C supplements was associated with decreased risk but only for any use at conception in the complete population was the OR significant. We observed no clear patterns or statistically significant associations for folic acid, iron, and vitamin E supplements, but the number of users of the latter two was small.

We further explored the extent to which the associations for total iron, copper, niacin, manganese, zinc, and folate were due to intake of multivitamins, the major supplemental source of these nutrients. The 6 micronutrients were substantially correlated with a mean r of 0.55 and a range of 0.26 – 0.83. In analyses that included an individual micronutrient and

any use of multivitamins at conception, the effect of multivitamin use remained strong and statistically significant while the nutrient effect was attenuated and not significant. The results for iron and folate are shown as examples (Table 5).

Discussion

We observed that father's intake of nutrients found in dairy products was associated with decreased risk as were calcium supplements. In addition, father's intake of several micronutrients and use of multivitamins were associated with increased risk but it was not clear which of these was the primary factor. We did not find support for our hypotheses of protective effects of the anti-oxidant vitamins (A, C, E), zinc, or folate, which play roles in spermatogenesis and DNA synthesis and repair. In mothers, multivitamin use close to the child's conception seemed to be protective.

The inverse association with father's intake of nutrients found in dairy products is not surprising, given that dairy products as a group appeared protective in our examination of food groups in the same study population.[15] The protective association with calcium supplements as well as dairy foods suggests that calcium might be the nutrient that explains the findings. However, calcium does not directly inhibit mutation, the mechanism that leads to sporadic bilateral retinoblastoma. Calcium protects against colon cancer but the effect is thought to be through mechanisms unrelated to mutation, such as inhibition of cell proliferation.[24] Possibly, calcium has an effect on germ-cell mutation through a mechanism not relevant to somatic mutation. Another possibility is that the association with calcium supplements resulted from chance as it was based on relatively few users and there were too few regular users to allow analysis of a group with greater exposure. If the calcium supplement association occurred by chance, another component of dairy might be responsible for the observed effect. Whey protein, a heterogeneous group of proteins that account for 20% of the protein in milk, has been observed to decrease colon and breast tumors in animals. These effects may be due to whey's ability to increase synthesis of glutathione, which participates in the destruction of reactive oxygen species, detoxification of some carcinogens, and other processes that could reduce mutation.[25]. Interpretation of the association for dairy-related nutrients will require further research to replicate or refute our observations.

We observed increased risk associated with father's total intake of vitamin E, manganese, and copper. The findings for manganese were more robust than those for the other two nutrients. However, for all three micronutrients, their intercorrelation and the substantial contribution to intake from multivitamins made it difficult to discern the primary factor and to distinguish real from chance findings.

We found no evidence that fathers' intake of vitamins A, C, and E, folate, or zinc protected against the occurrence of a new germ-cell mutation despite their roles in spermatogenesis, DNA synthesis and repair, and reduction of oxidative damage to DNA. The rationale for a protective effect is perhaps strongest for vitamin C and zinc, because the high concentrations in seminal fluid compared to blood suggest an important role and deficiencies result in increased oxidative damage in sperm.[26,10,11,9] In direct conflict with the hypotheses about these micronutrients, vitamin C and multivitamin supplements were actually associated with increased risk in some analyses. However, for vitamin C, as the definition narrowed from any use to regular use or use at conception, the odds ratios became closer to 1 and not significant, suggesting that this was not a real effect. The interpretation of the multivitamin finding is less clear as the association was stronger with regular use in the year before the pregnancy but not present with use close to conception. We may have missed

protective associations with these micronutrients because of measurement error in assessing their intake or because the endpoints studied in sperm are not relevant to *RB1* mutation.

The protective effect we observed for mother's multivitamin use corroborates a finding from a small previous study for multivitamin use in the first trimester,[27] a related but not identical time period. As about 85% of new germline *RB1* mutations are thought to arise on the father's allele, an effect of mother's supplement use is surprising. Multivitamins might act to reduce risk of mutations of maternal origin through actions of anti-oxidants or other nutrients. Another possibility is that maternal nutrition may impact mutations of paternal origin by influencing the likelihood that pre-mutations present at conception get repaired rather than become mutations. Maternal genotype in mice influenced the incidence of chromosomal aberrations in the zygote after radiation treatment of males,[28] demonstrating that maternal factors can affect at least some types of paternally derived mutation. Thus, there is biologic plausibility for an influence of maternal multivitamin use on *RB1* mutations of maternal and/or paternal origin. One nutrient found in supplements, folate plays an important role in DNA methylation and synthesis and its deficiency can lead to mutagenesis. A study of retinoblastoma in Mexico observed a protective effect of maternal folate intake from fruit and vegetables;[29] however, the relevance of this study to our results is unclear as only about 30% of the retinoblastoma cases had the sporadic bilateral form of the disease and the study also observed a protective effect of maternal vegetable intake that we did not observe.[15] Further research into the role of maternal diet and supplement use in retinoblastoma and other conditions resulting from new germline mutation is needed. However, it will remain difficult to determine the specific nutrient(s) that explain the effect because multivitamins contain multiple nutrients.

Our study's major strength lies in the restriction of its case group to patients with new germline *RB1* mutations. With a case group homogeneous in the mechanism of disease causation, our power to identify risk factors was increased. An important limitation of our study is measurement error in assessing diet 1 to 15 (median 4.5) years in the past using a FFQ. FFQs can measure past adult diet reasonably well in this interval but not as well as current diet. [20] Even for current diet, nutrient intake as measured by FFQ is subject to measurement error.[30] If the error in measuring diet was non-differential, ie did not differ between cases and controls, the study's power would be reduced and thus, we may have missed some associations. The assessment of nutrient quantities in supplements seems particularly subject to error as it relies on parents' memory of particular products, when there are hundreds of multivitamin supplements available. When parents didn't remember their brand or used a brand for which NHS did not have a code, the nutrient content of a typical multivitamin was assigned. Several of the nutrients associated with risk were ones with substantial contributions from multivitamin supplements. Thus, we find the results of analyses of supplement use itself more convincing than those of total micronutrient intake, which relied on possibly inaccurate estimates of dosage.

Other limitations of the study may have led to spurious and/or missed associations. Recall or interviewer bias resulting from the beliefs of the participants and/or the (non-blinded) interviewers may have created associations with components of healthy or unhealthy diets. In addition, selection bias may have occurred if parents of cases chose more health-conscious friends and relatives to nominate as controls or more health conscious individuals chose to participate as controls. If any of these biases occurred, we might expect a pattern of associations with many nutrients of healthy diets and/or those of unhealthy diets. Such patterns were not observed. We examined over 80 nutrients and supplements and some results are likely to have occurred by chance alone, especially because we did not correct significance levels for multiple comparisons. Given the lack of research in this area, we wanted to consider all possible associations and were less concerned with false positives.

We may also have missed true associations. For example, the unintentional close matching of cases and their friend/relative controls on socioeconomic status and parental age may have reduced our power to detect some associations. Finally, although we controlled for potential confounding, there may be other factors we did not consider and residual confounding for factors despite their inclusion in the regression model.

In summary, our main findings were a protective association for father's intake of nutrients found in dairy products in the year before the child's conception and a protective association of mother's use of multivitamin supplements around the time of the child's conception. Few previous studies have considered diet in relation to conditions resulting from new germline mutation. Our findings provide a starting point for further investigation of diet in the etiology of new germline mutation.

Acknowledgments

This work was supported by grants from the US National Institutes of Health (R01CA081012 and R01CA118580). We are grateful to the families of the patients and their relatives and friends for their participation. We thank Drs. Debra Friedman, Carol Shields, Kim Nichols, Ann Leahey, Ira Dunkel, Rima Jubran, Carlos Rodriguez-Galindo, Mary Lou Schmidt, Joanna Weinstein, Stewart Goldman, David Abramson, Matthew Wilson, Brenda Gallie, Helen Chan, and Michael Shapiro for enrolling and caring for the patients and Drs. Avital Cnaan and Larry Kushi for their assistance in the design of the study. We also thank the clinical research staff at the participating centers and study staff at Children's Hospital of Philadelphia for their diligent efforts particularly Bethany Barone, Jaclyn Bosco, Greta Anschuetz, Sheila Kearney, and the late Jean Rodwell.

Abbreviations

CI	confidence interval
FFQ	food frequency questionnaire
NHS	Nurses' Health Study
OR	odds ratio

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Table 1
Demographic and other characteristics of sporadic bilateral retinoblastoma cases and controls

	Fathers			Mothers		
	All cases (n=182) # (%)	Cases in case-control sets (n=122) # (%)	Controls (n=223) # (%)	All cases (n=202) # (%)	Cases in case-control sets (n=140) # (%)	Controls (n=260) # (%)
Race/ethnicity^{a, b}						
Non-Hispanic White	125 (68)	100 (82)	192 (86)	131 (65)	104 (74)	208 (80)
Non-Hispanic African American	18 (10)	6 (5)	7 (3)	28 (14)	16 (11)	21 (8)
Hispanic	23 (13)	10 (8)	16 (7)	30 (15)	14 (10)	22 (8)
Other	16 (9)	6 (5)	8 (4)	13 (6)	6 (4)	9 (3)
Educational level^{a, b}						
No college degree	107 (59)	64 (52)	88 (39)	120 (59)	70 (50)	108 (42)
College degree or higher	75 (41)	58 (48)	135 (61)	82 (41)	70 (50)	152 (58)
Annual income^c						
< \$25,000	22 (13)	6 (5)	15 (8)	34 (21)	15 (13)	31 (14)
\$25 – 35,000	21 (13)	12 (11)	15 (8)	19 (12)	13 (11)	18 (8)
\$35 – 50,000	32 (19)	24 (21)	33 (17)	31 (20)	24 (21)	48 (21)
\$50 – 75,000	39 (23)	25 (22)	49 (25)	35 (21)	26 (23)	46 (20)
> \$75,000	54 (32)	46 (41)	85 (43)	43 (26)	37 (32)	85 (37)
Marital status at interview^{a, b}						
Married	167 (92)	117 (96)	216 (97)	162 (80)	122 (87)	239 (92)
Not married	15 (8)	5 (4)	7 (3)	40 (20)	18 (13)	21 (8)
Age at birth of child						
< 20	0 (0)	0 (0)	0 (0)	6 (3)	3 (2)	8 (3)
20 – 24	12 (7)	3 (2)	11 (5)	25 (12)	14 (10)	29 (11)

	Fathers			Mothers		
	All cases (n=182)	Cases in case-control sets (n=122)	Controls (n=223)	All cases (n=202)	Cases in case-control sets (n=140)	Controls (n=260)
	# (%)	# (%)	# (%)	# (%)	# (%)	# (%)
25 – 29	45 (24)	28 (23)	62 (28)	63 (31)	41 (29)	77 (30)
30 – 34	56 (30)	42 (34)	76 (34)	63 (31)	50 (36)	96 (37)
35 – 39	53 (29)	37 (30)	51 (23)	41 (20)	29 (21)	42 (16)
40 +	16 (9)	12 (10)	23 (10)	4 (2)	3 (2)	8 (3)
Proxy interview						
Yes	15 (8)	8 (7)	26 (12)	2 (1)	1 (1)	3 (1)
No	167 (92)	114 (93)	197 (88)	200 (99)	139 (99)	256 (99)
Smoked in year before index pregnancy^{a, b}						
Yes	59 (32)†	28 (23)	45 (20)	49 (24)	32 (23)	40 (15)
No	123 (68)	94 (77)	178 (80)	153 (76)	108 (77)	220 (85)
Body mass index						
< 25	55 (30)	35 (29)	76 (34)	137 (68)	100 (71)	167 (66)
25	126 (70)	87 (71)	147 (66)	65 (32)	40 (29)	87 (34)
Father's alcohol intake in year before index pregnancy (drinks/day)						
0.00	52 (29)	36 (29)	53 (24)			
0.08 – 0.30	62 (34)	40 (33)	73 (33)			
0.31 – 0.99	38 (21)	27 (22)	72 (32)			
1.00 – 5.80	30 (16)	19 (16)	25 (11)			
Mother's alcohol intake in year before index pregnancy (drinks/day)						
0.00				104 (51)	68 (49)	128 (49)
0.08 – 0.50				78 (39)	56 (40)	95 (37)
0.51 – 2.95				20 (10)	16 (11)	37 (14)
Supplement use^d						
Multivitamin^b						
Any use	71 (39)	52 (43)	79 (35)	79 (39)	62 (44)	137 (53)
Regular use ^e	58 (32)	44 (36)	60 (27)	62 (31)	47 (34)	121 (46)
Any use close to conception	53 (30)	38 (32)	72 (33)	50 (25)	40 (29)	120 (46)

	Fathers			Mothers		
	All cases (n=182) # (%)	Cases in case-control sets (n=122) # (%)	Controls (n=223) # (%)	All cases (n=202) # (%)	Cases in case-control sets (n=140) # (%)	Controls (n=260) # (%)
Calcium^{a,b}						
Any use	19 (10)	14 (11)	36 (16)	34 (17)	51 (20)	24 (17)
Regular use	4 (2)	4 (3)	6 (3)	8 (4)	5 (4)	19 (7)
Any use close to conception	10 (6)	8 (7)	30 (13)	19 (9)	11 (8)	39 (15)
Vitamin C						
Any use	47 (26)	38 (31)	49 (22)	21 (10)	16 (12)	45 (17)
Regular use	15 (8)	12 (10)	21 (9)	6 (3)	5 (4)	15 (6)
Any use close to conception	25 (14)	19 (16)	33 (15)	7 (3)	7 (5)	24 (9)
Vitamin E						
Any use	16 (9)	13 (11)	13 (6)	-- ^f		
Regular use	7 (4)	7 (6)	8 (4)			
Any use close to conception	9 (5)	9 (7)	10 (4)			
Zinc						
Any use	7 (4)	5 (4)	7 (3)	-- ^f		
Folic acid						
Any use	-- ^f			17 (8)	13 (9)	30 (11)
Regular use				13 (6)	10 (7)	22 (8)
Any use close to conception				14 (7)	12 (9)	25 (10)
Iron						
Any use				10 (5)	5 (4)	15 (6)
Regular use	-- ^f			7 (3)	3 (2)	6 (2)
Any use close to conception				9 (4)	4 (3)	9 (3)
B vitamins						
Any use	5 (3)	5 (4)	7 (3)	-- ^f		
Any use close to conception	4 (2)	4 (3)	6 (3)			
Child's birth year^g						

	Fathers			Mothers		
	All cases (n=182) # (%)	Cases in case-control sets (n=122) # (%)	Controls (n=223) # (%)	All cases (n=202) # (%)	Cases in case-control sets (n=140) # (%)	Controls (n=260) # (%)
1991 – 1997				15 (7)	6 (4)	28 (11)
1998				25 (12)	18 (13)	19 (7)
1999				27 (13)	18 (13)	30 (11)
2000				29 (14)	21 (15)	43 (16)
2001				27 (13)	20 (14)	38 (15)
2002				23 (11)	14 (10)	29 (11)
2003				21 (10)	16 (11)	36 (14)
2004 – 2006				35 (17)	27 (19)	37 (14)

^aDifferences between fathers of all cases and fathers of controls were statistically significant for race/ethnicity ($P<0.001$), educational level ($P<0.001$), marital status ($P=0.02$), smoking ($P=0.006$), and any use of calcium supplements close to conception ($p=0.008$). The difference between fathers of cases in case-control sets and fathers of controls was statistically significant for educational level ($P=0.02$).

^bDifferences between mothers of all cases and mothers of controls were statistically significant for race/ethnicity ($P=0.004$), educational level ($P<0.001$), marital status ($P=0.02$), regular multivitamin use ($p<0.001$), multivitamin use at conception ($p<0.001$) Differences between mothers of cases in case-control sets and mothers of controls were statistically significant for regular multivitamin use ($p=0.01$), multivitamin use at conception ($p<0.001$) and calcium supplement use at conception ($p=0.04$).

^cIncome missing for 14 case fathers, 26 control fathers, 40 case mothers, and 32 control mothers

^dSupplement use in year before the child's conception or as specified

^eRegular use defined as 4 times/week 'most months' or 'seasonally'

^fData not shown because 10 or fewer parents used this supplement at any time during the year before the pregnancy

^gChild's birth year shown only for mothers.

Table 2

Adjusted odds ratios (ORs)^a for sporadic bilateral retinoblastoma in the child associated with father's nutrient intake and supplement use in the year before his child's conception

	Complete population			Matched sets		
	OR	95% CI ^c	P	OR	95% CI	P
<i>Nutrients^b</i>						
<i>Lactose</i>	0.84	0.67, 1.06	0.15	0.70	0.50, 0.96	0.03
<i>Calcium</i>						
Total	0.71	0.46, 1.10	0.13	0.64	0.36, 1.13	0.13
Dietary	0.61	0.38, 0.97	0.04	0.48	0.26, 0.88	0.02
<i>Phosphorous</i>						
Total	0.42	0.15, 1.19	0.10	0.26	0.07, 0.95	0.04
Dietary	0.32	0.11, 0.92	0.03	0.16	0.04, 0.65	0.01
<i>Copper</i>						
Total	1.31	1.06, 1.61	0.01	1.30	1.01, 1.66	0.04
Dietary	1.44	0.92, 2.24	0.11	1.02	0.53, 1.99	0.94
<i>Manganese</i>						
Total	1.45	1.07, 1.96	0.02	1.61	1.12, 2.32	0.01
Dietary	1.34	0.78, 2.30	0.29	1.31	0.71, 2.40	0.39
<i>Vitamin B₂</i>						
Total	1.03	0.99, 1.07	0.15	1.04	1.00, 1.08	0.06
Dietary	0.54	0.27, 1.10	0.09	0.33	0.13, 0.81	0.02
<i>Retinol</i>						
Total	1.00	0.91, 1.10	0.96	1.00	0.89, 1.12	0.98
Dietary	0.85	0.65, 1.10	0.23	0.65	0.44, 0.96	0.03
<i>Vitamin D</i>						
Total	1.03	0.90, 1.16	0.70	1.04	0.90, 1.21	0.60
Dietary	0.76	0.57, 1.00	0.05	0.61	0.42, 0.88	0.01
<i>Vitamin E</i>						
Total	1.02	1.00, 1.05	0.09	1.03	1.00, 1.05	0.03
Dietary	0.84	0.52, 1.35	0.47	0.81	0.45, 1.44	0.47

	Complete population			Matched sets		
	OR	95% CI ^c	P	OR	95% CI	P
<i>Nutrients^b</i>						
<i>Supplements</i>						
<i>Multivitamins</i>						
Any use	1.36	0.88, 2.08	0.12	1.48	0.91, 2.43	0.12
Regular use ^d	1.45	0.92, 2.28	0.11	1.76	1.05, 2.95	0.03
Any use at conception	1.02	0.65, 1.61	0.92	1.14	0.68, 1.90	0.63
<i>Calcium</i>						
Any use	0.62	0.33, 1.14	0.12	0.67	0.35, 1.29	0.23
Regular use	0.72	0.19, 2.81	0.64	1.50	0.40, 5.63	0.55
Any use at conception	0.37	0.17, 0.80	0.01	0.47	0.21, 1.07	0.07
<i>Vitamin C</i>						
Any use	1.53	0.94, 2.48	0.08	2.03	1.17, 3.53	0.01
Regular use	1.12	0.54, 2.31	0.76	1.51	0.67, 3.39	0.32
Any use at conception	1.16	0.64, 2.10	0.62	1.25	0.65, 2.41	0.51
<i>Vitamin E</i>						
Any use	2.10	0.95, 4.66	0.07	2.15	0.93, 4.99	0.07
Regular use	1.76	0.61, 5.07	0.30	2.31	0.77, 6.94	0.14
Any use at conception	1.77	0.68, 4.57	0.24	2.08	0.79, 5.51	0.14
<i>Zinc^e</i>						
Any use	1.53	0.51, 4.57	0.44	1.35	0.41, 4.45	0.62
<i>B vitamins^e</i>						
Any use	1.23	0.37, 4.13	0.73	1.81	0.49, 6.71	0.38

^a adjusted for father's race/ethnicity, educational level, total energy, and child's birth year

^b ORs for increases in intake of the following amounts: 15 g lactose, 2mg manganese, 1 mg copper, 2mg phosphorous, 1200 mg calcium, 700 mg calcium, 1200 mg phosphorous, 2 mg vitamin B2, 1200 IU retinol, 150 IU vitamin D, 5 mg vitamin E

^c confidence interval

^d Regular use defined as 4 times/week 'most months' or 'seasonally'

^e Results not shown for regular use and any use at conception because there were 10 or fewer users

Table 3

Adjusted odds ratios (ORs) for father's micronutrient intake, with simultaneous adjustment for dietary and supplemental sources, in relation to sporadic bilateral retinoblastoma in his child

Nutrient or supplement	Complete population			Matched sets					
	Minimal model ^a			Full model ^b			Minimal model ^a		
	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P
<i>Models with individual micronutrient intake^d and supplement use</i>									
<i>Dietary calcium</i>	0.63	0.39,1.01	0.06	0.59	0.36,0.98	0.04	0.46	0.24,0.89	0.02
							0.58	0.26,1.30	0.19
<i>Calcium supplement^e</i>	0.35	0.16,0.77	0.009	0.35	0.15,0.77	0.01	0.41	0.18,0.96	0.04
							0.40	0.15,1.03	0.06
<i>Total copper</i>	1.28	1.00,1.64	0.05	1.24	0.94,1.62	0.12	1.19	0.89,1.58	0.24
							1.02	0.72,1.44	0.90
<i>Multivitamin^f</i>	1.10	0.64,1.89	0.72	1.52	0.84,2.76	0.17	1.48	0.80,2.76	0.21
							2.14	0.94,4.86	0.07
<i>Total manganese</i>	1.41	0.99,2.00	0.06	1.55	1.05,2.29	0.03	1.46	0.98,2.19	0.06
							1.55	0.96,2.50	0.08
<i>Multivitamin</i>	1.13	0.66,1.92	0.67	1.49	0.83,2.69	0.18	1.37	0.75,2.52	0.30
							1.44	0.67,3.09	0.35
<i>Total vitamin E</i>	1.02	0.99,1.04	0.21	1.02	0.99,1.05	0.12	1.02	0.99,1.05	0.12
							1.02	0.99,1.06	0.23
<i>Multivitamin</i>	1.32	0.81,2.14	0.26	1.72	1.01,2.92	0.046	1.53	0.87,2.71	0.14
							1.85	0.89,3.88	0.10
<i>Model with 3 nutrients and multivitamin</i>									
<i>Total copper</i>	1.21	0.91,1.61	0.20	1.05	0.76,1.44	0.76	1.09	0.78,1.53	0.62
							0.76	0.49,1.19	0.23
<i>Total manganese</i>	1.17	0.77,1.80	0.46	1.41	0.88,2.28	0.16	1.30	0.79,2.14	0.31
							1.91	1.00,3.65	0.04
<i>Total vitamin E</i>	1.01	0.98,1.04	0.39	1.01	0.98,1.05	0.37	1.02	0.99,1.05	0.24
							1.02	0.98,1.05	0.33
<i>Multivitamin</i>	0.97	0.91,1.61	0.91	1.39	0.74,2.64	0.31	1.21	0.62,2.34	0.58
							1.56	0.67,3.65	0.30

^a Adjusted for father's race/ethnicity (non-Hispanic white, other), educational level (< college graduate, college graduate), energy intake, child's birth year

^b Variables in addition to those in minimal model were chosen by a stepwise procedure as described in the text. The variables in each model are: calcium/calcium supplement: mother's multivitamin use and mother's medical radiation dose; copper/multivitamin: father's calcium supplement, father's fruit intake, mother's multivitamin, mother's radiation dose; manganese/multivitamin: father's calcium supplement, father's fruit intake, mother's multivitamin, mother's radiation dose, mother's smoking; vitamin E/multivitamin: father's calcium supplement, father's dairy food intake, mother's multivitamin, mother's radiation dose, mother's smoking; 3 nutrients/multivitamins: father's calcium supplement, father's fruit intake, father's dairy food intake, mother's multivitamin, mother's radiation dose, mother's smoking

^c Variables in addition to those in minimal model were chosen by a stepwise procedure as described in the text. The variables in each model are: calcium/calcium supplement: father's sweets intake, father's cured meat intake, mother's multivitamin use, mother's medical radiation dose; copper/multivitamin: father's cured meat intake, father's sweets intake, father's radiation dose, mother's multivitamin use, mother's radiation dose; manganese/multivitamin: father's cured meat intake, father's sweets intake, mother's multivitamin, mother's radiation dose; vitamin E/multivitamin: father's cured meat intake, father's sweets intake, father's radiation dose, mother's multivitamin, mother's radiation dose; 3 nutrients/multivitamins: father's sweets intake, father's cured meat intake, mother's multivitamin, mother's radiation dose

^d ORs for nutrients are for increases in intake of 700 mg calcium, 1 mg copper, 2 mg manganese, 5 mg vitamin E

^e Any use of calcium supplements at conception

^f Regular multivitamin use in year prior to child's conception

Table 4

Adjusted odds ratios (ORs)^a for sporadic bilateral retinoblastoma in the child associated with mother's nutrient intake and supplement use in the year before her child's conception

	Complete population			Matched sets		
	OR	95% CI ^c	P	OR	95% CI	P
Nutrients^b						
Iron						
Total	0.86	0.74, 1.00	0.05	0.81	0.68, 0.97	0.02
Dietary	0.73	0.42, 1.29	0.28	0.77	0.40, 1.47	0.43
Copper						
Total	0.82	0.68, 0.99	0.03	0.75	0.59, 0.94	0.01
Dietary	0.93	0.63, 1.38	0.73	0.67	0.35, 1.28	0.22
Niacin						
Total	0.74	0.56, 0.97	0.03	0.72	0.52, 1.00	0.05
Dietary	0.57	0.25, 1.34	0.20	0.65	0.24, 1.72	0.38
Manganese						
Total	0.71	0.51, 0.98	0.04	0.60	0.39, 0.92	0.02
Dietary	0.72	0.44, 1.17	0.18	0.64	0.35, 1.17	0.15
Zinc						
Total	0.80	0.66, 0.96	0.02	0.81	0.65, 0.996	0.046
Dietary	0.69	0.43, 1.10	0.12	0.71	0.40, 1.27	0.25
Folate						
Total	0.79	0.65, 0.96	0.02	0.79	0.64, 0.99	0.04
Dietary	0.70	0.38, 1.27	0.24	0.70	0.35, 1.40	0.32
Supplement Use						
Multivitamins						
Any use	0.69	0.46, 1.03	0.07	0.74	0.47, 1.17	0.19
Regular use ^d	0.60	0.40, 0.89	0.01	0.55	0.25, 2.83	0.02
Any use at conception	0.46	0.30, 0.71	<0.00	0.45	0.27, 0.75	0.00
Calcium						

	Complete population			Matched sets		
	OR	95% CI ^c	P	OR	95% CI	P
Nutrients^b						
Any use	0.88	0.54, 1.44	0.61	0.86	0.50, 1.48	0.58
Regular use	0.53	0.22, 1.26	0.15	0.39	0.13, 1.18	0.09
Any use at conception	0.62	0.34, 1.12	0.11	0.51	0.25, 1.05	0.07
Vitamin C						
Any use	0.63	0.35, 1.11	0.11	0.66	0.34, 1.28	0.21
Regular use	0.56	0.21, 1.51	0.25	0.51	0.15, 1.66	0.26
Any use at conception	0.38	0.16, 0.92	0.03	0.50	0.19, 1.31	0.16
Folic acid						
Any use	0.80	0.42, 1.51	0.48	0.71	0.34, 1.50	0.37
Regular use	0.82	0.39, 1.70	0.60	0.81	0.37, 1.77	0.59
Any use at conception	0.81	0.40, 1.63	0.56	0.87	0.40, 1.89	0.72
Iron						
Any use	0.67	0.28, 1.58	0.36	0.45	0.14, 1.44	0.18
Regular use	1.14	0.36, 3.60	0.82	-.5		
Any use at conception	1.05	0.40, 2.81	0.92	0.67	0.19, 2.32	0.53
Vitamin E^e						
Any use	0.57	0.18, 1.78	0.34	0.64	0.18, 2.24	0.48

^a adjusted for mother's race/ethnicity, educational level, total energy, and child's birth year

^b ORs for increases in intake of the following amounts: 12 mg iron, 1 mg copper, 20 mg niacin, 10 mg manganese, 10 mg zinc, 400 µg folate

^c Confidence interval

^d Regular use defined as 4 times/week 'most months' or 'seasonally'

^e OR not calculated for regular use of iron and for regular use and any use at conception for vitamin E because <10 users

Adjusted odds ratios (ORs) for mother's micronutrient intake, with simultaneous adjustment for dietary and supplemental sources, in relation to sporadic bilateral retinoblastoma in her child

Table 5

	Complete population						Matched sets					
	Minimal model ^a			Full model ^b			Minimal model ^a			Full model ^c		
	OR	95 % CI	P	OR	95 % CI	P	OR	95 % CI	P	OR	95 % CI	P
Iron^d	0.93	0.77,1.12	0.43	0.91	0.75,1.10	0.33	0.94	0.76,1.16	0.54	0.86	0.68,1.07	0.18
Multivitamin^e	0.46	0.28,0.76	0.002	0.46	0.27,0.77	0.003	0.36	0.20,0.67	0.001	0.30	0.15,0.59	<0.001
Folate^d	0.90	0.71,1.15	0.41	0.91	0.71,1.17	0.47	0.95	0.73,1.25	0.73	0.95	0.70,1.30	0.75
Multivitamin^e	0.4	0.28,0.83	0.009	0.46	0.26,0.81	0.007	0.36	0.18,0.70	0.003	0.28	0.13,0.59	<0.001

^a Adjusted for mother's race/ethnicity (non-Hispanic white, other), mother's educational level (less than college graduate, college graduate), mother's energy intake, child's birth year

^b Variables in addition to those in minimal model were chosen by a stepwise procedure as described in the text. The variables in each model are: iron/multivitamin: mother's medical radiation dose, father's calcium supplement use, father's cured meat intake; folate/multivitamin: mother's medical radiation dose, father's calcium supplement use, father's cured meat intake.

^c Variables in addition to those in minimal model were chosen by a stepwise procedure as described in the text. The variables in each model are iron/multivitamin: mother's medical radiation dose, father's cigarette smoking, father's dairy food intake, father's cured meat intake; folate/multivitamin: medical radiation dose, father's dairy food intake, father's cured meat intake, father's sweets intake.

^d ORs for nutrients are for increases in intake of 12 mg iron, 400 µg folate.

^e Any multivitamin use at conception