Risks of orofacial clefts in children born to women using multivitamins containing folic acid periconceptionally

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Summary

Women are advised to take folic acid before they conceive as a precaution against neural-tube defects. However, the use of folic acid in preventing orofacial clefts is unknown. We investigated whether a woman's periconceptional use of multivitamins containing folic acid was associated with a reduced risk of orofacial clefts.

We derived data from a population-based case-control study of fetuses and liveborn infants with orofacial anomalies among a 1987–89 cohort of births in California. We interviewed 731 (84·7%) of eligible mothers with orofacial cleft case infants and 734 (78·2%) mothers with non-malformed control infants. We found a reduced risk of orofacial clefts if the mother had used multivitamins containing folic acid during the period from one month before through two months after conception. The odds ratios ranged from 0·50–0·73 depending on cleft phenotype. Controlling for the potential influence of other variables did not substantially alter the results. Maternal daily consumption of cereal containing folic acid was also associated with a reduced risk of orofacial clefts. Women who used multivitamins containing folic acid periconceptionally had a 25–50% reduction in risk for offspring with orofacial clefts compared to women who did not use such vitamins. However, this association may not be attributable to folic acid specifically, but may be a consequence of other multivitamin supplement components, or behaviours, that are highly correlated with the use of multivitamins containing folic acid.

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Introduction

Over the past 20 years, many studies[1–9] have reported that supplementation with folic acid and other vitamins prevents neural-tube defects in the unborn child. However, few researchers have investigated whether periconceptional multivitamin/folic acid use is associated with reduced risks for other congenital anomalies,[10–13] despite the fundamental importance of folate in the synthesis of nucleotides necessary for DNA replication. More than 15 years ago, Tolarova[12,13] observed a six-fold reduction in recurrence risk for having a child with an orofacial cleft in women who already had a child with an orofacial cleft if the mothers took multivitamin pills and 10 mg per day of folic acid. To investigate whether maternal multivitamin use reduced the occurrence risk of delivering offspring with orofacial clefts we did a large population-based case-control study of California infants born with or without orofacial clefts.

Patients and methods

Patients

The California Birth Defects Monitoring Program[14] reviewed medical records at all hospitals and genetic centres in a known geographic population base to find case infants or fetuses with orofacial clefts. Infants and fetuses with these defects who were diagnosed within a year after birth were eligible for the study. 552 601 births and fetal deaths occurred between January, 1987 and December, 1989 to women residing in most countries in California. We reviewed the diagnostic information from medical records, necropsies, and surgical reports of all infants and fetuses with orofacial anomalies. However, we restricted our study to infants with cleft of the palate (CP) or lip or both (CL±P) and excluded infants who had trisomy 21 or Turner syndrome (45,X) (n=81). Overall, 891 infants or fetuses (93% were liveborn infants) were eligible.

Methods

We phenotypically subgrouped the cases as: isolated CL±P, multiple CL±P, isolated CP, multiple CP, and clefts whose aetiology is known, nearly all of which were monogenic conditions. A medical geneticist (MMT) classified each case as an isolated defect or multiple defect on the basis of the type of accompanying congenital anomalies. CP and CL±P cases with no other anomaly or with anomalies thought to be minor (such as low-set ears) or not true malformations (such as undescended testicles) were classified as isolated. CP and CL±P cases with at least one accompanying major anomaly or with a combination of phenotypic features suggesting a known syndrome were classed as multiple CP or CL±P cases (eg, Robin sequence with flat face, hypotonia, and characteristic ophthalmologic findings was evidence for Stickler syndrome).

Control infants were born during 1987–89; and the infant had a mother who was resident in the same counties in which cases were ascertained; had no reportable birth defect[14] before the first birthday. Using a pseudo-random number generator among eligible liveborn infants (n=548 844), 972 control infants were electronically selected from California vital records.

Maternal vitamin use	Cases	Controls
None in period 1 month before through 3 months after conception	195	137
Any in period 1 month before through 2 months after conception	418	507
Daily	384	453
Less than daily	27	46
Frequency not stated	7	8
Any in period 1 month before through 1 month after conception	203	244
Any in period 1 month before conception	105	139
Any starting third month after conception	103	84
Unknown*	15	6

*Includes women who did not know if they took vitamins (n=11), and women who took a vitamin with unknown folic acid content (n=10).

Table 1: **Use of multivitamins containing folic acid by 731 case and 734 control mothers**

We interviewed all mothers by telephone in English (91%) or Spanish. Women who only spoke a language other than English or Spanish (25 cases and 33 controls) and 3 case mothers who died before interview contact were excluded, yielding 863 cases and 939 eligible controls. Interviews were completed an average 3·5 years after the date of delivery for cases and 3·6 years for controls. At the start of the interview, each interviewer established a 4-month time frame from 1 month before conception to 3 months afterwards. The interview took 40 min and we elicited information on maternal vitamin or mineral supplements use, cereal intake, medical history, and other exposures during pregnancy.

Women were asked about the types of vitamin supplements they used (prenatal vitamins, multivitamins, vitamin A, folic acid, and other types). For each supplement and for each month during the time frame, women were asked about the frequency of use and quantity taken. Folic acid concentration in a supplement was assumed to be 0·8 mg in prenatal vitamins and 0·4 mg in any other vitamin supplements that contained folic acid.

If a woman used a vitamin preparation whose folic acid content could not be determined, the folic acid intake from that preparation was taken as zero. We estimated a woman's average daily folic acid intake from all supplements she reported using. Fortified cereal consumption (based on 1 serving size) is 25% of the folic acid content of a unit dose in a multivitamin supplement. Women were asked if they ate cold cereals in the 4-month period, and, if so, which brands and how frequently. Presence of folic acid in a cereal was determined by comparing cereals to a nutrient database[15] or from manufacturers' information.

The odds ratio along with its 95% confidence interval was used to estimate risk. Estimates, including those derived from logistic regression models, were computed using EGRET.[16] For each phenotypic case group, analyses were performed for multivitamins containing folic acid intake for the period 1 month before conception through 2 months afterwards. We chose the latter endpoint for the period because it encompasses the most relevant period of lip/palate morphogenesis which is usually completed by 60 days. Women who started vitamin use in the third month after conception were excluded from comparisons. Folic acid intake was analysed as a dichotomous variable (use or no use) or as a polytomous variable (no use, <0·4, 0·4–0·9, and >1·0 mg per day). These categories approximated doses of less than, about the same, and more than the amount of folic acid usually found in unit doses of multivitamins. Analyses involving cereal consumption were limited to women who did not use vitamin supplements containing folic acid in the 4-month period.

	Vitamin use*	
	Crude odds ratio	95% confidence interval
Controls		
CL±P, isolated	0·50	0·36–0·68
CL±P, multiple	0·61	0·35–1·1
CP, isolated	0·73	0·46–1·2
CP, multiple	0·64	0·35–1·2
Cleft, known aetiology	0·68	0·35–1·3

*Women who began use in the third month post-conception were excluded from analyses.

Table 2: **Orofacial cleft risk and any maternal use of multivitamins containing folic acid from 1 month before through 2 months after conception**

Results

We interviewed 731 (84·7%) of the 863 eligible case mothers and 734 (78·2%) of the 939 eligible control mothers. Information was unavailable from 2·3% of case and 3·5% of control mothers who refused to be interviewed, and from 13·0% of case and 18·4% of control mothers who could not be located. The 731 cases consisted of 348 infants with isolated CL±P, 100 with multiple CL±P, 141 with isolated CP, 74 with multiple CP, and 68 clefts whose aetiology is known.

Most case (57·2%) and control (69·1%) mothers used multivitamins containing folic acid from 1 month before conception to 2 months afterwards (table 1). Approximately 90% of users reported daily use. Control mothers were more likely to report vitamin use in the month before conception compared with case mothers (18·9% *vs* 14·3%).

Women who reported any use of multivitamins containing folic acid in the period 1 month before, through the first 2 months after conception were compared with women who did not take vitamin pills containing folic acid. For this comparison, the odds ratio was below 1·0 for each of the phenotypic cleft groups, indicating that case mothers were less likely to have taken multivitamins with folic acid periconceptionally (table 2). The greatest risk reduction, 50%, was observed for isolated CL±P, the only group whose risk estimate had an associated confidence interval that did not include 1·0. The risk estimates for 3 levels of average daily intake, <0·4, 0·4–0·9, and ≥1·0 mg, of folic acid from multivitamins were similar to the odds ratios for any use (table 3). Data were too sparse in the highest intake group to estimate risk adequately.

We controlled our analyses for maternal race, ethnicity (white, black, hispanic, asian, other), education (below high school, graduate, high school graduate, some college, college graduate), age (below 20, 20–24, 25–29, 30–34, 35–39, above 39 years), gravidity (0, 1, 2, 3, 4+ previous pregnancies), smoking (0, 1–19, over 19 cigarettes per day), and alcohol use. For isolated CL±P, the multivitamin association was modified by alcohol use. The odds ratio for multivitamin use was 0·61 (0·40–0·91) for women who did not use alcohol in the 4-month period, 0·16 (0·05–0·55) for women who used alcohol at least once a week, and 0·48 (0·26–0·89) for women who used alcohol less often. Simultaneous adjustment for the other covariates, with data stratified by the 3 alcohol use groups, did not substantially alter the risk estimates for multivitamin use. For the other cleft phenotypes, there was no effect modification from alcohol use on the association with multivitamin use. Single variable adjustment revealed risk estimates that were generally similar to the crude estimates.

The reduced risk from multivitamin use was not substantially influenced by maternal use of oral contraceptives, by other folate-antagonist medications, or by excluding from analyses cases and controls who had a family history (parent or older sibling) of orofacial clefts, or whose mothers had diabetes or epilepsy or seizures.

Among 332 women who did not use multivitamins containing folic acid in the 4-month time frame, we found

	Average amount (mg) used/day*	No of cases	Odds ratio	95% CI
CL±P, isolated	0	102	Reference	—
	<0·4	99	0·47	0·33–0·67
	0·4–0·9	81	0·52	0·36–0·76
	≥1·0	4	0·77	0·18–3·0
CL±P, multiple	0	24	Reference	—
	<0·4	29	0·58	0·32–1·1
	0·4–0·9	25	0·68	0·36–1·3
	≥1·0	1	0·82	0·04–7·2
CP, isolated	0	33	Reference	—
	<0·4	55	0·81	0·49–1·3
	0·4–0·9	34	0·68	0·39–1·2
	≥1·0	0	0	—
CP, multiple	0	20	Reference	—
	<0·4	26	0·63	0·33–1·2
	0·4–0·9	20	0·66	0·32–1·3
	≥1·0	0	0	—
Cleft, known aetiology	0	16	Reference	—
	<0·4	24	0·73	0·36–1·5
	0·4–0·9	14	0·57	0·25–1·3
	≥1·0	0	0	—

*Among control mothers: no use, n=137; <0·4 mg/day, n=283; 0·4–0·9 mg/day, n=209; and ≥1·0 mg/day, n=7. Also, women who began use in the third month postconception were excluded from analyses.

Table 3: **Orofacial cleft risk and estimated average daily amount (mg) of folic acid from multivitamins taken 1 month before through 2 months after conception**

that maternal daily cereal consumption (32 control and 22 case mothers) was associated with a reduction in cleft risk. However, only the risk estimate for isolated CL±P had an associated confidence interval that did not include 1·0. The odds ratios were: 0·41 (0·17–0·98), 0·21 (0·03–1·1), 0·26 (0·03–1·5), 0·26 (0·03–1·5), and 1·0 (0·15–7·1) for isolated CL±P, multiple CL±P, isolated CP, multiple CP, and known aetiology clefts, respectively. Less than daily cereal consumption gave risk estimates generally closer to 1·0.

Discussion

This study shows that women who take multivitamins containing folic acid periconceptionally have a 25–50% decreased risk of having children with orofacial clefts. These data, however, cannot adequately disentangle whether the reduced risk is attritutable to folic acid, to another vitamin or mineral constituent in multivitamin supplements, or to healthy behaviour correlated with folic acid, multivitamin, or cereal use. Too few mothers reported use of a vitamin preparation that contained only folic acid (n=2), or reported use of a vitamin supplement without folic acid (n=27), to assess whether the reduced risk was solely due to folic acid. Nevertheless, the reduction is consistent with previous findings.

Some studies have shown a reduced prevalence of subsequent offspring (recurrence risk) with orofacial clefts among women who consumed vitamins including folic acid (0·5–5 mg) in early pregnancy.[17–21] In general, these studies were small and women were not randomised to receive vitamin supplements. In a nonrandomised trial design, Tolarova[12,13] also found decreased (relative risk=0·17) recurrent risk of clefts among offspring of mothers who took multivitamin supplements and 10 mg of folic acid daily compared with women who did not take supplements.

Few studies have provided data on the possible reduction in occurrence risk for clefting in babies from the maternal use of vitamins.[10,22–24] In their case-control study of Atlanta births, Khoury et al[22] provided sufficient data to show a 30% reduced risk for CL±P among children born to women who used multivitamins. By contrast, Czeizel[10] did not find a difference in prevalence of orofacial clefts in a Hungarian trial in which one group of women received a daily multivitamin suppement containing 0·8 mg of folic acid and another group of women received a supplement containing only trace minerals. However, because the trace mineral group had only 5 children with an orofacial cleft, the trial lacked statistical power to detect prevalence differences in the order of 50%. In a case-control study, Hill et al[23] also could not find a reduced orofacial clefting risk among UK women who used multivitamin supplements or folic acid by itself. In that study, information about vitamin use may have been incomplete (only 5% of mothers were users), because it came from medical records and only referred to prescribed vitamin use. Saxen[24] in a case-control study of Finnish women did not find reduced risk associated with "iron and/or vitamin" use. Inclusion of women who started use during the first trimester but after lip and palate formation and closure, might have biased estimates towards no association. We do not know whether folic acid was a vitamin constituent when women were taking these supplements in 1966–1971. Other evidence that supports a relation between folate and clefts comes from the association of maternal anticonvulsant use and clefting.[23,24] Some anticonvulsants are known folate antagonists,[25] and this has been proposed as one potential underlying teratogenic mechanism of anticonvulsant drugs.[26] Maternal cigarette smoking has been associated with an increased risk of clefting and other evidence suggests that smoking may lower a pregnant woman's serum folate.[22] Further evidence shows that clefts have been seen in the offspring of maternal animals who were exposed to a folic acid deficient diet,[28] or treated with folate anti-metabolites[29] such as methotrexate.

Despite the available evidence, chance or bias might explain our findings. Some of the computed risks had associated confidence intervals of 1·0, indicating limited precision. Our findings need to be considered in respect of the response among those eligible for study. If the proportion of women who used vitamins in early pregnancy differed among the 15% (cases) and 22% (controls) who were not interviewed, then the observed risks might be slightly higher or lower. It is unlikely that the risk reduction was because nonresponding control mothers had a substantially smaller proportion of vitamin users given that the percentage of control mothers who reported use in the first trimester (81%) compared well with other studies.[8,9] In addition, it is unlikely that the risk reduction was because vitamin users were more common among nonresponding case mothers. With respect to recall bias, case mothers may have under-reported their use of vitamins and control mothers over-reported their use of vitamins. This would result in a spurious reduced risk. Two previous studies did not find recall bias of maternal vitamin use as a likely explanation for an association between neural-tube defects and maternal vitamin use.[8,9]

We can estimate the magnitude of reporting error necessary to create our results. For the odds ratio of 0·50 observed for isolated CL±P to have arisen solely as a result of biased reporting, 18% of vitamin-using control mothers would have falsified their response as to using vitamins when they had not, or 39% of nonvitamin-using case mothers would have incorrectly reported that they

did not use vitamins whereas, in fact, they had. Further evidence that supports our argument is the association with maternal cereal consumption. Most women were probably unaware of why they were asked for details about their cereal intake.

Orofacial clefts are common congenital anomalies. In addition to the anguish associated with these anomalies, babies born with these conditions have difficulty feeding as infants, have more frequent speech problems and ear infections, and need a series of corrective surgical operations. The estimated lifetime medical cost for children with orofacial clefts born each year in California is $86 352 000.[30] Our results, and findings from other studies, indicate a substantial risk reduction for orofacial clefts among pregnant women who used multivitamins containing folic acid periconceptionally.[16,17] If this association proves causal, many of these anomalies will be preventable.

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