Periconceptional nutrient intakes and risks of orofacial clefts in California

Matthew B. Wallenstein¹, Gary M. Shaw¹, Wei Yang¹ and Suzan L. Carmichael¹

BACKGROUND: Evidence indicates that maternal nutrient intake may play a role in the development of birth defects. We investigated the association of maternal periconceptional intake of vitamin supplements and dietary nutrients with risk of developing cleft palate (CP) and cleft lip with or without cleft palate (CLP).

METHODS: Data were from a population-based, case-control study of fetuses and liveborn infants delivered in California in 1999–2003. Analyses included 170 cases with CP, 425 with CLP, and 534 nonmalformed controls. Dietary intake was estimated from a food frequency questionnaire.

RESULTS: Vitamin supplement intake was associated with a modestly decreased risk of clefts, but the confidence intervals (CIs) include 1.0. Among women who did not use vitamin supplements, dietary intake of several micronutrients was associated with risk of clefts. We found at least a twofold elevated risk of CP with low intake of riboflavin, magnesium, calcium, vitamin B12, and zinc; all CIs excluded 1.0. For CLP, we found at least a twofold elevated risk with low intake of niacin, riboflavin, vitamin B12, and calcium, and a decreased risk with high intake of folate and cryptoxanthin; all CIs excluded 1.0.

CONCLUSION: The results suggest that periconceptional nutrient intake may be associated with risk of CP and CLP.

Orofacial clefts are a significant cause of morbidity among children worldwide. The combined prevalence of cleft palate (CP) and cleft lip with or without cleft palate (CLP) is ~1 in 700. The majority of cases are isolated anomalies not associated with an identifiable syndrome. The etiologies of CP and CLP are multifactorial and mostly unknown. Evidence suggests that many cases of CP and CLP may arise from an interaction between susceptible genes and environmental exposures in the prenatal and periconceptional periods (1,2).

There is a growing body of evidence that maternal nutrient intake plays a role in the development of birth defects, beyond the established relationship between neural tube defects and folic acid (1,3–8). Understanding the contribution of nutrient intake to risk of birth defects is important to understanding their etiologies and developing effective prevention strategies. Previous reports have shown reduced risks of orofacial clefting with maternal intake of vitamin supplements (3,9). Previous

reports have also shown that dietary intake of specific nutrients may play a role in CP/CLP, but the extent to which each individual nutrient is associated with CP/CLP still remains unclear (1). In the present study, we investigated the association of periconceptional intake of vitamin supplements and dietary intake of 31 nutrients with the risk of developing CP/CLP, using data from a large population-based, case-control study.

RESULTS

As compared with control mothers, mothers of infants with CP had lower daily energy intake and higher BMI, and mothers of infants with CLP were more likely to be foreign-born Hispanic and have higher BMI (Table 1).

Overall, 63% of control cases reported vitamin intake ($n = 334$), as compared with 56% of CLP cases ($n = 237$) and 62% of CP cases ($n = 105$). Vitamin supplement intake was associated with a modestly decreased risk of clefts, but the confidence intervals (CIs) include 1.0. Adjusted odds ratios (ORs) for any vs. no intake were 0.8 (CI: 0.6, 1.1) for CLP and 0.8 (CI: 0.6, 1.2) for CP.

Dietary intake of many of the nutrients was associated with risk of clefts when comparing intake in the lowest and highest quartiles (“low” or “high” intake) with intake in the middle two quartiles (reference). Among the set of nutrients not typically in vitamin supplements, we observed increased risk of CP with low choline intake (OR: 2, 95% CI: 1.2, 3.2) and low fructose intake (OR: 1.7, 95% CI: 1.1, 2.5), and decreased risk of CLP with low lycopene intake (OR: 0.7, 95% CI: 0.5, 0.9) (Table 2). Other nutrients had associated ORs that tended to be closer to 1.0; several were ≤ 0.7 or ≥ 1.4 , but none had CIs that included 1.0.

For CP, among the set of nutrients that are likely to be included in multivitamin supplements, many of the ORs were ≤ 0.7 or ≥ 1.4 (Table 3). Among women who did not take supplements, the ORs tended to be farther from 1.0 than among women who did take supplements. In particular, among women who did not take supplements, lower dietary intake was associated with at least a twofold increased risk of CP for niacin, riboflavin, thiamin, vitamin B12, vitamin C, vitamin E, zinc, magnesium, and calcium, with many of these CIs excluding 1.0.

¹Division of Neonatal and Developmental Medicine, Department of Pediatrics, Stanford University School of Medicine, Stanford, California. Correspondence: Suzan L. Carmichael (scarmichael@stanford.edu)

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Table 1. Characteristics of mothers of cases and controls, California 1999–2003

Variable	Controls		CP	CLP	
	% (n = 534) ^a	% (n = 170) ^a		% (n = 425) ^a	P value ^b
Race/ethnicity					
Non-Hispanic white	24.3	28.8	0.61	22.8	0.04
US-born Hispanic	23.2	21.2		16.7	
Foreign-born Hispanic	33.0	29.4		39.1	
Other	19.5	20.6		21.4	
Age (years)					
<25	29.6	20.6	0.12	29.9	0.44
25–29	22.1	25.3		26.1	
30–34	28.3	34.1		24.9	
>34	20.0	20.0		19.1	
Education					
<High school	24.5	20.6	0.31	24.2	0.19
High school	24.3	20.0		28.7	
Some college	24.0	28.2		25.4	
4-y College degree or more	27.2	31.2		21.6	
Gravidity					
0	29.6	26.5	0.85	24.2	0.24
1	27.7	28.2		27.3	
2	18.4	20.6		20.9	
>2	24.3	24.7		27.5	
Cigarette use ^c					
None	91.9	92.9	0.67	89.9	0.27
Any	8.1	7.1		10.1	
Alcohol use ^c					
None	82.0	77.6	0.21	79.8	0.38
Any	18.0	22.4		20.2	
	Mean ± SD			Mean ± SD	
BMI (kg/m ²)	24.5 (5.3)	25.3 (6.2)	0.09	25.5 (6.2)	0.007
Daily energy intake (kcal)	2,670.5 (937.9)	2,404.3 (967.0)	0.001	2,630.0 (974.7)	0.51

CLP, cleft lip with or without cleft palate; CP, cleft palate.

^aPercentages may not sum to 100 owing to rounding. ^bP values from χ^2 test for categorical variables and from *F*-test for continuous variables, as compared with controls. ^cDuring the first 2 mo of pregnancy.

For CLP, among vitamin supplement users, intake of micro-nutrients was not associated with substantially increased or decreased risk; only one OR was ≤ 0.7 or ≥ 1.4 (the OR for higher iron was 1.4) (Table 4). Among women who did not take supplements, at least twofold increased risks of CLP were observed for lower intake of niacin, riboflavin, vitamin B12, and calcium, and all of these CIs excluded 1.0. There was a 60% decreased risk of CLP observed for higher intake of folate, and that CI also excluded 1.0.

In a separate analysis, restricted to isolated cases of CP (*n* = 129) and CLP (*n* = 384), our results were similar (data not shown). We again found substantially increased risk of CP and CLP for the lowest quartile intake of several B vitamins, calcium, magnesium, and others. We also examined ORs for CLP using different specifications for BMI (BMI <18.5, 18.5–<25,

25–<30, and ≥ 30 kg/m²), energy intake (<25th percentile, 25th–<75th percentile, and ≥ 75 th percentile, based on the distribution among controls), smoking (none, 10 or fewer cigarettes/day, >10 cigarettes/day), and alcohol (none, nonbinge drinking, binge drinking, i.e., five or more drinks at once). Overall, our inferential paradigm did not change substantially with the inclusion of categorical variables (data not shown). We did not conduct a similar analysis for CP due to small sample size.

Because nutrients co-occur, we created an index of nutrients typically present in supplements (i.e., those in Tables 3 and 4) by scoring each one as 0 (lowest quartile), 1 (middle quartiles), or 2 (highest quartile) and then summing them. After stratification by supplement intake, comparisons of scores in the lowest and highest quartile of the index vs. scores in the middle quartiles yielded one OR for which the 95% CI excluded 1.0:

Table 2. Risks of CP and CLP associated with periconceptional dietary nutrient intakes, California 1999–2003

Nutrient quartiles ^a	Controls (n = 534)	CP (n = 170)	Adjusted OR (95% CI) ^b	CLP (n = 425)	Adjusted OR (95% CI) ^b
Total fat (g)					
<85	134	63	1.3 (0.8, 2.2)	113	1.0 (0.7, 1.5)
85–142.8	268	76	Reference	201	Reference
≥142.9	132	31	1.2 (0.6, 2.3)	111	1.4 (0.9, 2.3)
Linoleic acid (g)					
<14.1	139	57	1.1 (0.7, 1.7)	108	1.0 (0.7, 1.4)
14.1–29.0	272	73	Reference	198	Reference
≥29.1	123	40	1.6 (1.0, 2.7)	119	1.4 (1.0, 1.9)
Oleic acid (g)					
<31.2	131	54	1.0 (0.6, 1.7)	111	1.1 (0.7, 1.6)
31.2–54.8	270	89	Reference	216	Reference
≥54.9	133	27	0.8 (0.4, 1.4)	98	1.0 (0.7, 1.6)
Total carbohydrate (g)					
<227.3	137	65	1.2 (0.7, 2.1)	123	1.2 (0.8, 1.8)
227.3–380.8	268	73	Reference	204	Reference
≥380.9	129	32	1.6 (0.8, 3.2)	98	1.0 (0.6, 1.7)
Fructose (g)					
<22.6	138	72	1.7 (1.1, 2.5)	120	1.2 (0.8, 1.6)
22.6–48.1	272	73	Reference	213	Reference
≥48.2	124	25	0.9 (0.5, 1.6)	92	0.9 (0.6, 1.3)
Glucose (g)					
<19.2	137	68	1.6 (1.0, 2.4)	109	1.0 (0.7, 1.4)
19.2–40.6	272	68	Reference	223	Reference
≥40.7	125	34	1.4 (0.8, 2.3)	93	0.9 (0.6, 1.2)
Galactose (g)					
<0.3	130	45	0.9 (0.6, 1.5)	129	1.4 (1.0, 1.9)
0.3–0.9	274	88	Reference	187	Reference
≥1	130	37	1.1 (0.7, 1.7)	109	1.3 (0.9, 1.9)
Sucrose (g)					
<26.9	136	65	1.5 (1.0, 2.3)	112	1.0 (0.7, 1.5)
26.9–51.6	271	69	Reference	215	Reference
≥51.7	127	36	1.6 (1.0, 2.7)	98	1.0 (0.7, 1.5)
Glycemic index					
<49.4	130	30	0.6 (0.4, 1.0)	101	1.0 (0.7, 1.3)
49.4–53.1	264	89	Reference	208	Reference
≥53.2	140	51	1.0 (0.7, 1.5)	116	1.1 (0.8, 1.5)
Total protein (g)					
<75.0	133	63	1.5 (0.9, 2.5)	114	1.1 (0.7, 1.6)
75.0–131.5	270	78	Reference	212	Reference
≥131.6	131	29	0.9 (0.5, 1.8)	99	1.1 (0.7, 1.7)
Methionine (mg)					
<1,534.1	135	63	1.4 (0.8, 2.3)	119	1.2 (0.8, 1.7)
1,534.1–2,690.5	268	76	Reference	210	Reference
≥2,690.6	131	31	1.1 (0.6, 2.1)	96	0.9 (0.6, 1.5)

Table 2 Continued on next page

Table 2. Continued

Nutrient quartiles ^a	Controls (n = 534)	CP (n = 170)	Adjusted OR (95% CI) ^b	CLP (n = 425)	Adjusted OR (95% CI) ^b
Choline (mg)					
<293.0	133	69	2.0 (1.2, 3.2)	127	1.5 (1.0, 2.1)
293.0–505.7	269	75	Reference	213	Reference
≥505.8	132	26	0.7 (0.4, 1.4)	85	0.7 (0.5, 1.1)
Betaine (mg)					
<88.7	128	50	1.0 (0.6, 1.6)	110	1.0 (0.7, 1.4)
88.7–193.5	270	89	Reference	222	Reference
≥193.6	136	31	0.8 (0.5, 1.4)	93	0.9 (0.6, 1.3)
Lutein (μg)					
<1,231.3	127	43	0.8 (0.5, 1.3)	122	1.2 (0.9, 1.7)
1,231.3–5,211.2	269	88	Reference	204	Reference
≥5,211.3	138	39	0.8 (0.5, 1.3)	99	0.9 (0.7, 1.3)
Lycopene (μg)					
<2,398.6	135	48	0.9 (0.6, 1.4)	93	0.7 (0.5, 0.9)
2,398.6–7,064.4	267	87	Reference	229	Reference
≥7,064.5	132	35	1.0 (0.6, 1.7)	103	1.0 (0.7, 1.5)
Cryptoxanthin (μg)					
<114.9	135	56	1.1 (0.7, 1.8)	119	1.2 (0.9, 1.6)
114.9–312.7	276	84	Reference	223	Reference
≥312.8	123	30	1.0 (0.6, 1.7)	83	0.8 (0.5, 1.1)
α-Carotene (μg)					
<267.3	134	40	0.8 (0.5, 1.2)	110	1.0 (0.7, 1.3)
267.3–870.9	270	92	Reference	223	Reference
≥871.0	130	38	1.2 (0.7, 2.0)	92	0.8 (0.6, 1.2)

CI, confidence interval; CLP, cleft lip with or without cleft palate; CP, cleft palate.

^aQuartile cut-offs (lowest quartile and highest quartile, as compared with middle two quartiles) determined from intake levels among all control mothers with nutrient data. ^bOdds ratio (OR) adjusted for maternal race/ethnicity, age, education, BMI, gravidity, smoking, alcohol use, vitamin/mineral supplement use, and energy intake.

the OR for a lowest-quartile score was 2.2 (95% CI: 1.2, 4.3) for CLP, among women who did not take supplements. The OR was similar for CP, but the CI included 1.0 (OR: 2, 95% CI: 0.8, 4.7). The other ORs were closer to 1.0 (Tables 3 and 4).

DISCUSSION

This study found that lower dietary intakes of several micronutrients were associated with an elevated risk of CP or CLP. Analytic consideration of several potential confounders did not reveal alternative interpretations of the results.

Our analysis specifically showed at least a twofold increased risk of CP for lower intake of niacin; riboflavin; thiamin; vitamins B12, C, and E; zinc; magnesium; calcium; and choline; at least a twofold elevated risk of CLP for lower intake of niacin, riboflavin, vitamin B12, and calcium; and a 60% reduction in risk of CLP associated with higher intake of folate. These associations are noteworthy in that they replicate, as well as provide new clues beyond, previously observed associations with folic acid-containing supplements. The observation of multiple nutrient associations with CP as well as with CLP is also noteworthy, given that most previous studies have not examined these two phenotypes separately. In addition, we demonstrated

that these associations tended to be observed only among women who did not take vitamin supplements during early pregnancy, which makes sense given that supplement use may compensate for lower dietary intake of many nutrients.

Most previous literature on micronutrients and clefting has focused on folic acid and multivitamin intake. Previous studies indicate reduced occurrence of CP and CLP with intake of vitamin supplements with or without folic acid (9), although more recent studies have not shown an association with CP (10,11) or CLP (10). Our results showed a modest reduced risk of clefts for multivitamin intake, although the 95% CIs included 1.0.

Folic acid has garnered a great deal of attention in the medical literature, but our observations contribute to the growing body of evidence that dietary intake of other nutrients may also contribute to the risk of developing clefts (10,12,13). In particular, some studies of nutrients (other than folic acid) involved in one-carbon metabolism have found strong associations between risk of clefts and lower intake or serum levels of nutrients (e.g., vitamin B12, vitamin B6, inositol, and methionine) (10,13–16), although findings for each nutrient are not entirely consistent across studies (10,13). Our findings are

Table 3. Risks of CP associated with periconceptual dietary nutrient intakes stratified by vitamin/mineral supplement use, California 1999–2003

Nutrient quartiles ^a	Vitamin/mineral supplement users			Vitamin/mineral supplement nonusers		
	Cases (n = 105)	Controls (n = 334)	Adjusted OR (95% CI) ^b	Cases (n = 65)	Controls (n = 200)	Adjusted OR (95% CI) ^b
Folate (μg)						
<293.8	40	85	1.5 (0.8, 2.8)	25	51	1.4 (0.6, 3.2)
293.8–546.3	51	167	Reference	30	101	Reference
≥546.4	14	82	0.7 (0.3, 1.4)	10	48	0.5 (0.2, 1.4)
Niacin (mg)						
<18.5	37	88	1.2 (0.6, 2.3)	27	46	2.4 (1.0, 5.7)
18.5–31.5	53	163	Reference	22	107	Reference
≥31.6	15	83	0.7 (0.3, 1.7)	16	47	1.9 (0.7, 5.0)
Riboflavin (mg)						
<1.8	39	87	1.4 (0.7, 2.6)	31	45	4.1 (1.7, 9.9)
1.8–3.2	48	167	Reference	25	102	Reference
≥3.3	18	80	1.1 (0.5, 2.4)	9	53	0.4 (0.1, 1.1)
Thiamin (mg)						
<1.4	39	78	1.8 (0.9, 3.5)	29	53	2.1 (0.9, 5.1)
1.4–2.3	49	168	Reference	24	105	Reference
≥2.4	17	88	0.8 (0.4, 1.9)	12	42	1.1 (0.4, 3.2)
Vitamin B6 (mg)						
<1.8	38	88	1.2 (0.6, 2.2)	22	47	1.1 (0.5, 2.4)
1.8–3.1	50	166	Reference	29	105	Reference
≥3.2	17	80	1.1 (0.5, 2.4)	14	48	1.6 (0.6, 4.0)
Vitamin B12 (μg)						
<3.6	37	91	1.1 (0.6, 2.0)	26	39	2.9 (1.3, 6.5)
3.6–7	50	162	Reference	28	110	Reference
≥7.1	18	81	1.1 (0.6, 2.3)	11	51	0.7 (0.3, 1.8)
Vitamin C (mg)						
<138.9	39	88	1.1 (0.7, 2.0)	25	47	2.0 (1.0, 4.2)
138.9–289.8	51	171	Reference	27	101	Reference
≥289.9	15	75	0.9 (0.4, 1.8)	13	52	1.1 (0.5, 2.5)
Vitamin E (mg)						
<11.7	40	99	1.0 (0.6, 1.7)	23	39	2.1 (1.0, 4.6)
11.7–20.9	50	157	Reference	30	107	Reference
≥21.0	15	78	0.8 (0.4, 1.6)	12	54	0.7 (0.3, 1.6)
Vitamin A (RE)						
<1,040.1	30	86	0.8 (0.4, 1.4)	20	48	1.1 (0.5, 2.3)
1,040.1–2,134.2	54	169	Reference	36	105	Reference
≥2,134.3	21	79	1.4 (0.7, 2.7)	9	47	0.6 (0.2, 1.6)
β-Carotene						
<2,844.5	33	80	1.1 (0.6, 2.1)	17	50	0.7 (0.3, 1.6)
2,844.5–7,345.2	47	172	Reference	37	102	Reference
≥7,345.3	25	82	1.4 (0.7, 2.5)	11	48	0.7 (0.3, 1.5)
Zinc (mg)						
<10.3	39	89	1.2 (0.7, 2.3)	26	44	2.7 (1.1, 6.5)
10.3–18.5	47	163	Reference	28	109	Reference
≥18.6	19	82	1.3 (0.6, 2.7)	11	47	0.7 (0.2, 1.9)

Table 3 Continued on next page

Table 3. Continued

Nutrient quartiles ^a	Vitamin/mineral supplement users			Vitamin/mineral supplement nonusers		
	Cases (n = 105)	Controls (n = 334)	Adjusted OR (95% CI) ^b	Cases (n = 65)	Controls (n = 200)	Adjusted OR (95% CI) ^b
Iron (mg)						
<13.0	39	82	1.6 (0.8, 3.2)	26	51	1.5 (0.6, 3.7)
13.0–22.5	49	165	Reference	25	108	Reference
≥22.6	17	87	0.9 (0.4, 2.0)	14	41	1.7 (0.6, 5.0)
Magnesium (mg)						
<272.2	36	88	1.1 (0.6, 2.1)	29	48	3.0 (1.3, 7.0)
272.2–504.6	50	158	Reference	26	115	Reference
≥504.7	19	88	0.8 (0.4, 1.7)	10	37	1.0 (0.3, 3.0)
Calcium (mg)						
<818.5	39	92	1.3 (0.7, 2.3)	28	43	3.3 (1.4, 7.6)
818.5–1,593.2	48	165	Reference	28	106	Reference
≥1,593.3	18	77	1.1 (0.5, 2.2)	9	51	0.5 (0.2, 1.3)
Nutrient index ^c						
<8	37	81	1.4 (0.7, 2.8)	25	44	2.0 (0.8, 4.7)
8–19	53	168	Reference	27	109	Reference
≥20	15	85	0.7 (0.3, 1.8)	13	47	1.0 (0.3, 2.9)

CI, confidence interval; CP, cleft palate; RE, retinol equivalent.

^aQuartile cut-offs (lowest quartile and highest quartile, as compared with middle two quartiles) determined from intake levels among all control mothers with nutrient data. ^bOdds ratio (OR) adjusted for maternal race/ethnicity, age, education, BMI, gravidity, smoking, alcohol use, and energy intake. ^cSee Results for definition.

generally consistent with these results, such as our relatively large adjusted ORs for several B vitamins. The biologic plausibility of one-carbon-donor deficiency contributing to the etiology of orofacial clefting is persuasive. One-carbon donors are involved in the synthesis of numerous cellular structures and enzymes that play a role in fetal organogenesis.

Antioxidants are another set of nutrients that we examined. In theory, an excess of reactive oxygen species during fetal growth may interfere with the fidelity of cellular division and the synthesis of proteins involved in organ development, including oromaxillofacial development. In the present study, we examined several antioxidants (e.g., vitamins C and E, zinc, vitamin A, and several carotenoids). Existing literature regarding these nutrients is sparse. One report found an increased risk of clefts with lower intake of vitamins C and E and β -carotene (12). Two studies found an association between maternal serum zinc and clefting (14,17), although a third study did not (18). Another study, from Norway, found an association between increased vitamin A intake and reduced risk of CP only (19). In general, previous findings are consistent with our findings of increased risk of CP associated with lower intakes of zinc and vitamins C and E.

One other large study of CP and CLP investigated multiple dietary nutrients after folic acid fortification, using data from a large, multicenter, case-control study (10). Shaw *et al.* found some evidence for decreased risk of CLP with higher intakes of several one-carbon donors (e.g., choline, methionine, thiamin, and riboflavin) (10). However, in the current study, we found significantly larger adjusted ORs for a wider range of micro- and macronutrients. The differences may be attributable to

the present study utilizing a more extensive food frequency questionnaire, which may have improved accuracy of nutrient intake estimates. The strengths of our study include its population-based ascertainment of cases and controls, its relatively short period for maternal recall between delivery and interview, its relatively high participation by study subjects, and its separate analysis of CP and CLP, which are likely to be unique etiologic entities (1). Our study had several limitations that should be noted. Inherent in our study design is a potential for measurement error. Our study relied on a food frequency questionnaire to assess nutrient intake. Limitations of this type of instrument have been described (20–22). Although the instrument we used was not internally validated, validation studies have revealed that it provides reasonable estimates of usual dietary intake of nutrients even for diets in the distant past (20,22). Error in recall of food intake is unlikely to be systematic based on case-control status; as such, the error would bias risk estimates toward the null. Another limitation of our study is that bioavailability and placental exchange of nutrients vary greatly among individuals. As a result, our analysis of dietary intake may not accurately represent actual fetal exposure to each nutrient. A strength of this study was the adjustment for several potential confounders, including variables related to socioeconomic status and behaviors that might affect risks of birth defects. However, as noted above, results were similar before and after adjustment. We also acknowledge that residual confounding remains a possibility, although we are unsure what those confounders would be, given the limitations of our understanding of what causes orofacial clefts. Given the large number of nutrients, we believed that a single nutrient analytic

Table 4. Risks of CLP associated with periconceptional nutrient intakes, stratified by vitamin/mineral supplement use, California 1999–2003

Nutrient quartiles ^a	Vitamin/mineral supplement users			Vitamin/mineral supplement nonusers		
	Cases (n = 237)	Controls (n = 334)	Adjusted OR (95% CI) ^b	Cases (n = 188)	Controls (n = 200)	Adjusted OR (95% CI) ^b
Folate (μg)						
<293.8	71	85	1.2 (0.7, 1.9)	56	51	1.6 (0.9, 2.8)
293.8–546.3	107	167	Reference	101	101	Reference
≥546.4	59	82	1.2 (0.7, 2.0)	31	48	0.4 (0.2, 0.9)
Niacin (mg)						
<18.5	62	88	0.9 (0.5, 1.4)	60	46	2.1 (1.2, 3.9)
18.5–31.5	117	163	Reference	88	107	Reference
≥31.6	58	83	1.2 (0.7, 2.1)	40	47	0.8 (0.4, 1.6)
Riboflavin (mg)						
<1.8	69	87	1.0 (0.6, 1.7)	64	45	2.8 (1.5, 5.3)
1.8–3.2	113	167	Reference	83	102	Reference
≥3.3	55	80	1.1 (0.7, 1.9)	41	53	0.6 (0.3, 1.2)
Thiamin (mg)						
<1.4	67	78	1.3 (0.8, 2.1)	58	53	1.5 (0.8, 2.8)
1.4–2.3	110	168	Reference	90	105	Reference
≥2.4	60	88	1.1 (0.6, 1.9)	40	42	1.0 (0.5, 2.1)
Vitamin B6 (mg)						
<1.8	62	88	0.8 (0.5, 1.4)	54	47	1.5 (0.8, 2.7)
1.8–3.1	116	166	Reference	91	105	Reference
≥3.2	59	80	1.3 (0.8, 2.2)	43	48	0.9 (0.5, 1.7)
Vitamin B12 (μg)						
<3.6	66	91	1.0 (0.6, 1.6)	58	39	2.5 (1.4, 4.4)
3.6–7.0	115	162	Reference	91	110	Reference
≥7.1	56	81	1.1 (0.6, 1.7)	39	51	0.7 (0.4, 1.4)
Vitamin C (mg)						
<138.9	66	88	1.0 (0.6, 1.5)	57	47	1.4 (0.8, 2.5)
138.9–289.8	121	171	Reference	96	101	Reference
≥289.9	50	75	1.0 (0.6, 1.6)	35	52	0.6 (0.3, 1.0)
Vitamin E (mg)						
<11.7	70	99	0.9 (0.6, 1.4)	42	39	1.4 (0.8, 2.5)
11.7–20.9	107	157	Reference	87	107	Reference
≥21.0	60	78	1.2 (0.7, 2.0)	59	54	1.2 (0.7, 2.1)
Vitamin A (RE)						
<1,040.1	61	86	0.9 (0.6, 1.4)	61	48	1.9 (1.1, 3.3)
1,040.1–2,134.2	127	169	Reference	92	105	Reference
≥2,134.3	49	79	0.8 (0.5, 1.3)	35	47	0.7 (0.4, 1.2)
β-Carotene						
<2,844.5	59	80	1.0 (0.6, 1.6)	54	50	1.2 (0.7, 2.1)
2,844.5–7,345.2	121	172	Reference	102	102	Reference
≥7,345.3	57	82	1.0 (0.7, 1.6)	32	48	0.6 (0.4, 1.1)
Zinc (mg)						
<10.3	64	89	0.9 (0.5, 1.4)	51	44	1.6 (0.8, 3.0)
10.3–18.5	114	163	Reference	96	109	Reference
≥18.6	59	82	1.3 (0.7, 2.2)	41	47	0.9 (0.5, 1.8)

Table 4 Continued on next page

Table 4. Continued

Nutrient quartiles ^a	Vitamin/mineral supplement users			Vitamin/mineral supplement nonusers		
	Cases (n = 237)	Controls (n = 334)	Adjusted OR (95% CI) ^b	Cases (n = 188)	Controls (n = 200)	Adjusted OR (95% CI) ^b
Iron (mg)						
<13.0	59	82	0.8 (0.5, 1.4)	53	51	1.3 (0.7, 2.4)
13.0–22.5	115	165	Reference	96	108	Reference
≥22.6	63	87	1.4 (0.8, 2.5)	39	41	1.0 (0.5, 2.1)
Magnesium (mg)						
<272.2	68	88	1.0 (0.6, 1.6)	61	48	1.9 (1.0, 3.6)
272.2–504.6	109	158	Reference	95	115	Reference
≥504.7	60	88	1.1 (0.7, 1.8)	32	37	0.9 (0.5, 1.9)
Calcium (mg)						
<818.5	67	92	0.9 (0.5, 1.4)	65	43	3.1 (1.7, 5.6)
818.5–1,593.2	117	165	Reference	85	106	Reference
≥1,593.3	53	77	1.1 (0.7, 1.8)	38	51	0.7 (0.4, 1.4)
Nutrient index ^c						
<8	64	81	1.0 (0.6, 1.7)	56	44	2.2 (1.2, 4.3)
8–19	113	168	Reference	94	109	Reference
≥20	60	85	1.3 (0.7, 2.3)	38	47	0.6 (0.3, 1.3)

CI, confidence interval; CLP, cleft lip with or without cleft palate; RE, retinol equivalent.

^aQuartile cut-offs (lowest quartile and highest quartile, as compared with middle two quartiles) determined from intake levels among all control mothers with nutrient data. ^bOdds ratio (OR) adjusted for maternal race/ethnicity, age, education, BMI, gravidity, smoking, alcohol use, and energy intake. ^cSee Results for definition.

approach was more appropriate than an integrated analytic model. In addition, sample sizes for many comparisons were small and the article includes multiple comparisons. For these reasons, our results should be interpreted with caution.

Finally, we evaluated each nutrient individually without analysis of nutrient interactions. In a recent article, we found that overall maternal diet quality was associated with a reduced risk of CP and CLP, implying that multiple nutrients and nutrient interactions are probably involved in the etiology of clefting (8). Two other studies have also found a relationship between clefting and overall diet quality (23,24). Nutrition is complex, and understanding both the role of individual nutrients and the impact of overall dietary intake is potentially important in understanding the etiologies of clefts and other birth defects. It is important that multiple approaches to assessment and analysis continue to be explored.

The literature on nutrient intake and risk of CP and CLP highlights the importance of maternal diet in reducing birth defects. The present study has helped to elucidate the role of individual micronutrients and the extent of their impact on the development of CP/CLP.

METHODS

This case-control study included liveborn, stillborn (fetal deaths at >20 wk gestation), and prenatally diagnosed, electively terminated cases that occurred in mothers residing in Los Angeles, San Francisco, and Santa Clara counties. The study included data on deliveries that had estimated due dates from July 1999 to June 2003. Case information was abstracted from multiple hospital reports and medical records, and reviewed by a clinical geneticist. Infants with diagnoses of single-gene disorders or chromosomal aneuploidy (based on information gathered from chart reviews) were ineligible.

Orofacial cleft cases were those infants or fetuses born with CP or with CLP as confirmed by clinical description or surgical or autopsy report. Infants with clefts believed to be secondary to other defects (e.g., amniotic band sequence or holoprosencephaly) were excluded. Cases were classified as isolated if there was no concurrent major malformation and as nonisolated if there was at least one accompanying major malformation. Nonmalformed, liveborn controls were selected randomly from birth hospitals, to represent the population from which the cases were derived. Specifically, controls were randomly selected from area hospitals in proportion to their contribution to the total population of liveborn infants (i.e., the number of eligible control infants from each hospital was in proportion to that hospital's contribution to the most recent birth cohort for which vital statistics data were available).

Mothers were eligible for interview if they were the biologic mother and carried the pregnancy of the selected study subject, they were not incarcerated, and their primary language was English or Spanish. Maternal interviews were conducted using a standardized, computer-based questionnaire, primarily by telephone, in English or Spanish, and no earlier than 6 wk after the infant's estimated date of delivery. Informed consent was obtained before each interview. A variety of exposures were assessed, focusing on the periconceptional time period, which was defined as 2 mo before through 2 mo after conception.

The interview also included a modified version of the National Cancer Institute Health Habits and History Questionnaire, a well-known, semi-quantitative food frequency questionnaire with demonstrated reliability and validity (22,25). The food frequency questionnaire was modified to include ethnic foods appropriate to a diverse study population. This questionnaire was used to derive estimates of dietary intake of nutrients. The questionnaire has been adjusted over time to update the nutrient values of the food items, primarily to account for folic acid fortification.

Overall, 84% of eligible case mothers (199 CP and 502 CLP) and 80% of control mothers (626) were interviewed. Nine percent of eligible case mothers and 11% of control mothers were not locatable, and the remainder of nonparticipants declined interview. Median time between estimated date of delivery and interview completion

was 11 mo for cases and 8 mo for controls. We excluded from analyses eight cases and one control with mothers who had type 1 or 2 diabetes.

Analyses were performed for CP and CLP separately. We estimated risks using ORs and 95% CIs that were derived from logistic regression models (SAS 9.3; SAS Institute, Cary, NC). We first estimated CP and CLP risks associated with any vs. no maternal periconceptional use of multivitamin/mineral supplements (most women consumed prenatal or nonprenatal formulations of multivitamin/mineral supplements, and most took supplements daily; most supplements contained 400–800 mcg of folic acid). Models were then constructed to assess associations with dietary nutrients. Specifically, we categorized nutrient intakes as <25th percentile, 25th–74th percentile (reference), and ≥75th percentile, based on the distributions among all controls with nutrient data. Analyses involving certain nutrients (total fat, linoleic acid, oleic acid, total carbohydrates, fructose, glucose, galactose, sucrose, glycemic index, total protein, methionine, choline, betaine, lutein, lycopene, cryptoxanthin, and α-carotene) included all subjects. Analyses involving other nutrients (folate, niacin, riboflavin, thiamin, vitamin B6, vitamin B12, vitamin C, vitamin E, vitamin A, β-carotene, zinc, iron, magnesium, and calcium) were stratified based on whether women did or did not use vitamin/mineral supplements in the periconceptional period because these nutrients were likely contained in prenatal multivitamin/mineral supplements.

All nutrient analyses were adjusted for energy intake (kcal), and further adjusted for maternal race/ethnicity (non-Hispanic white, foreign-born Hispanic, US-born Hispanic, other), age (<25, 25–29, 30–34, and >34 y), education (<high school, high school graduate, some college, college graduate, or more), prepregnancy BMI (kg/m²), gravidity (0, 1, 2, and >2), any (vs. none) periconceptional cigarette smoking, alcohol intake during the first 2 mo of pregnancy, or intake of vitamin/mineral supplements during 2 mo before and the first 2 mo of pregnancy (for analyses not stratified on this variable). Covariates were selected *a priori* based on known associations with orofacial clefts (1). We present only results adjusted for all covariates because results were similar before and after adjustment. Final analyses included 170 cases with CP, 425 with CLP, and 534 nonmalformed controls with complete data on nutrient intake and covariates. Seven cases were stillbirths and five were elective terminations. This study was approved by the institutional review board at Stanford University.

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REFERENCES

- Dixon MJ, Marazita ML, Beaty TH, Murray JC. Cleft lip and palate: understanding genetic and environmental influences. *Nat Rev Genet* 2011;12:167–78.
- Wu T, Liang KY, Hetmanski JB, et al. Evidence of gene-environment interaction for the IRF6 gene and maternal multivitamin supplementation in controlling the risk of cleft lip with/without cleft palate. *Hum Genet* 2010;128:401–10.
- Munger R. Maternal nutrition and oral clefts. In: Wyszynski D, ed. *Cleft Lip and Palate: From Origin to Treatment*. New York: Oxford University Press, 2002:170–92.
- Kapusta L, Haagmans ML, Steegers EA, Cuypers MH, Blom HJ, Eskes TK. Congenital heart defects and maternal derangement of homocysteine metabolism. *J Pediatr* 1999;135:773–4.
- Shaw GM, Velie EM, Schaffer DM. Is dietary intake of methionine associated with a reduction in risk for neural tube defect-affected pregnancies? *Teratology* 1997;56:295–9.
- Shaw GM, Carmichael SL, Laurent C, Siega-Riz AM. Periconceptional glycaemic load and intake of sugars and their association with neural tube defects in offspring. *Paediatr Perinat Epidemiol* 2008;22:514–9.
- Velie EM, Block G, Shaw GM, Samuels SJ, Schaffer DM, Kulldorff M. Maternal supplemental and dietary zinc intake and the occurrence of neural tube defects in California. *Am J Epidemiol* 1999;150:605–16.
- Carmichael SL, Yang W, Feldkamp ML, et al.; National Birth Defects Prevention Study. Reduced risks of neural tube defects and orofacial clefts with higher diet quality. *Arch Pediatr Adolesc Med* 2012;166:121–6.
- Goh YI, Bollano E, Einarson TR, Koren G. Prenatal multivitamin supplementation and rates of congenital anomalies: a meta-analysis. *J Obstet Gynaecol Can* 2006;28:680–9.
- Shaw GM, Carmichael SL, Laurent C, Rasmussen SA. Maternal nutrient intakes and risk of orofacial clefts. *Epidemiology* 2006;17:285–91.
- Wilcox AJ, Lie RT, Solvoll K, et al. Folic acid supplements and risk of facial clefts: national population based case-control study. *BMJ* 2007;334:464.
- Krapels IP, van Rooij IA, Ocké MC, West CE, van der Horst CM, Steegers-Theunissen RP. Maternal nutritional status and the risk for orofacial cleft offspring in humans. *J Nutr* 2004;134:3106–13.
- Krapels IP, van Rooij IA, Ocké MC, van Cleef BA, Kuijpers-Jagtman AM, Steegers-Theunissen RP. Maternal dietary B vitamin intake, other than folate, and the association with orofacial cleft in the offspring. *Eur J Nutr* 2004;43:7–14.
- Krapels IP, Rooij IA, Wevers RA, et al. Myo-inositol, glucose and zinc status as risk factors for non-syndromic cleft lip with or without cleft palate in offspring: a case-control study. *BJOG* 2004;111:661–8.
- van Rooij IA, Swinkels DW, Blom HJ, Merkus HM, Steegers-Theunissen RP. Vitamin and homocysteine status of mothers and infants and the risk of nonsyndromic orofacial clefts. *Am J Obstet Gynecol* 2003;189:1155–60.
- Tamura T, Munger RG, Nepomuceno B, Corcoran C, Cembrano J, Solon F. Maternal plasma pyridoxal-5'-phosphate concentrations and risk of isolated oral clefts in the Philippines. *Birth Defects Res Part A Clin Mol Teratol* 2007;79:276–80.
- Tamura T, Munger RG, Corcoran C, Bacayao JY, Nepomuceno B, Solon F. Plasma zinc concentrations of mothers and the risk of nonsyndromic oral clefts in their children: a case-control study in the Philippines. *Birth Defects Res Part A Clin Mol Teratol* 2005;73:612–6.
- Munger RG, Tamura T, Johnston KE, Feldkamp ML, Pfister R, Carey JC. Plasma zinc concentrations of mothers and the risk of oral clefts in their children in Utah. *Birth Defects Res Part A Clin Mol Teratol* 2009;85:151–5.
- Johansen AM, Lie RT, Wilcox AJ, Andersen LF, Drevon CA. Maternal dietary intake of vitamin A and risk of orofacial clefts: a population-based case-control study in Norway. *Am J Epidemiol* 2008;167:1164–70.
- Willett W. *Nutritional Epidemiology*, 2nd edn. New York: Oxford University Press, 1998.
- Block G. A review of validations of dietary assessment methods. *Am J Epidemiol* 1982;115:492–505.
- Willett WC, Sampson L, Stampfer MJ, et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. *Am J Epidemiol* 1985;122:51–65.
- Vujkovic M, Ocké MC, van der Spek PJ, Yazdanpanah N, Steegers EA, Steegers-Theunissen RP. Maternal Western dietary patterns and the risk of developing a cleft lip with or without a cleft palate. *Obstet Gynecol* 2007;110(2 Pt 1):378–84.
- Vujkovic M, Steegers EA, Looman CW, Ocké MC, van der Spek PJ, Steegers-Theunissen RP. The maternal Mediterranean dietary pattern is associated with a reduced risk of spina bifida in the offspring. *BJOG* 2009;116:408–15.
- Willett WC, Reynolds RD, Cottrell-Hoehner S, Sampson L, Browne ML. Validation of a semi-quantitative food frequency questionnaire: comparison with a 1-year diet record. *J Am Diet Assoc* 1987;87:43–7.