

Vitamin Supplements and the Risk for Congenital Anomalies Other Than Neural Tube Defects

LORENZO D. BOTTO,* RICHARD S. OLNEY, AND J. DAVID ERICKSON

Randomized trials, supported by many observational studies, have shown that periconceptional use of folic acid, alone or in multivitamin supplements, is effective for the primary prevention of neural tube defects (NTDs). Whether this is true also for other congenital anomalies is a complex issue and the focus of this review. It is useful to consider the evidence not only for specific birth defects separately but, importantly, also for all birth defects combined. For the latter, the Hungarian randomized clinical trial indicated, for periconceptional multivitamin use, a reduction in the risk for all birth defects (odds ratio (OR) = 0.53, 95% confidence interval (CI) = 0.35–0.70), even after excluding NTDs (OR = 0.53, 95% CI = 0.38–0.75). The Atlanta population-based case-control study, the only large observational study to date on all major birth defects, also found a significant risk reduction for all birth defects (OR = 0.80, 95% CI = 0.69–0.93) even after excluding NTDs (OR = 0.84, 95% CI = 0.72–0.97). These and other studies also evaluated specific anomalies, including those of the heart, limb, and urinary tract, as well as orofacial clefts, omphalocele, and imperforate anus. For cardiovascular anomalies, two studies were negative, whereas three, including the randomized clinical trial, suggest a possible 25–50% overall risk reduction, more marked for some conotruncal and septal defects. For orofacial clefts, six of seven case-control studies suggest an apparent reduced risk, which could vary by cleft type and perhaps, according to some investigators, by pill dosage. For limb deficiencies, three case-control studies and the randomized trial estimated approximately a 50% reduced risk. For urinary tract defects, three case-control studies and the randomized trial reported reduced risks, as did one study of nonsyndromic omphalocele. All these studies examined multivitamin supplement use. With respect to folic acid alone, a reduced rate of imperforate anus was observed among folic acid users in China. We discuss key gaps in knowledge, possible avenues for future research, and counseling issues for families concerned about occurrence or recurrence of these birth defects. Published 2004 Wiley-Liss, Inc.†

KEY WORDS: congenital heart defects; oral clefts; congenital abnormalities; folic acid; multivitamin supplements; epidemiology; prevention

INTRODUCTION

Decades of studies, including two randomized clinical trials [MRC Vitamin Study Research Group, 1991; Czeizel and Dudas, 1992] have demonstrated the effectiveness of folic acid in preventing

a substantial fraction of neural tube defects. These findings have led to recommendations and actions on the part of medical, public health, and community groups to ensure that such research would translate into the public's benefit.

Whether the risk for birth defects other than neural tube defects is decreased by folic acid alone or multivitamin use is a question of considerable relevance. As a group, congenital anomalies are a major cause of morbidity, disability, and mortality among children

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DOI 10.1002/ajmg.c.30004

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†This article was prepared by a group consisting of both United States Government employees and non-United States Government employees, and as such is subject to 117 U.S.C. Sec. 105.

[Centers for Disease Control and Prevention, 1998; Shibuya and Murray, 1998]. Heart defects, for example, account for a third or more of infant deaths due to congenital anomalies, more than any other congenital anomaly, including neural tube defects [Centers for Disease Control and Prevention, 1998]. Finding simple and inexpensive ways to prevent such congenital anomalies is desirable in any setting and particularly in less affluent countries where 90% of births currently occur and where costly medical and surgical care is not always available.

Because the effect on neural tube defects is established, in this paper we focus on congenital anomalies other than neural tube defects. Animal models are beyond the scope of the review and will not be covered. We first summarize the published evidence, adding some findings from a large case-control study from Atlanta; then we highlight gaps in knowledge; and finally we discuss research and clinical implications.

MATERIALS AND METHODS

We searched Medline using the search terms *vitamin*, *multivitamin*, *folic acid*, *congenital abnormality*, and *birth defects*. We searched for all languages and all available years. We also reviewed reference lists of key articles and reviews for additional articles. We included studies that were published in peer-reviewed journals, were original research articles using epidemiologic data, quantified an association with vitamin use, and had an outcome that was a congenital abnormality in human beings. We also included studies quantifying an association with the common 677C → T variant of the *MTHFR* gene, as a possible indicator of a specific etiologic role of folic acid. We did not include studies on genetic or chromosomal conditions such as Down syndrome.

For the Hungarian randomized clinical trial we used the final data reported in 1998 [Czeizel, 1998]. We reanalyzed data from the Atlanta Birth Defects Case-Control Study, a large population-based study of all major birth

defects [Erickson, 1991], to compute the estimated relative risk for all birth defects combined, with and without neural tube defects, to expand on findings previously reported in abstract form [Mulinare et al., 1995]. In our analysis of this case-control study we compare regular periconceptional multivitamin use (three or more times per week, beginning from before conception) to no use before conception or during first trimester, and adjust via logistic regression model for maternal race, period of birth, and hospital of birth (the study design variables).

From published reports we abstracted features related to study design, patient population, and results. We report the published effect estimates, though we recomputed some for a more uniform presentation. When a finding was unclear to us (e.g., whether a case-control study was population based or not) we left that entry blank.

RESULTS

With respect to supplement use, we selected 17 studies [Li et al., 1995; Shaw et al., 1995a,b, 2000; Tolarova and Harris, 1995; Czeizel et al., 1996; Hayes et al., 1996; Yang et al., 1997; Czeizel, 1998; Scanlon et al., 1998; Werler et al., 1999; Botto et al., 2000, 2002b; Beaty

et al., 2001; Itikala et al., 2001; Loffredo et al., 2001; Myers et al., 2001].

With respect to *MTHFR* variants we focused on five studies on oral clefts [Shaw et al., 1998; Gaspar et al., 1999; Mills et al., 1999; Martinelli et al., 2001; van Rooij et al., 2003]. For studies on *MTHFR* variants and heart defects, we refer to the discussion in Botto et al. [2003].

For all birth defects (Fig. 1) the risk reduction associated with periconceptional multivitamin use was 47% in the Hungarian randomized trial (odds ratio (OR) = 0.53, 95% confidence interval (CI) = 0.35–0.70) and 20% in the Atlanta case-control study (OR = 0.80, 95% CI = 0.69–0.93). For all birth defects excluding neural tube defects the risk reduction was 47% (OR = 0.53, 95% CI = 0.38–0.75) and 16% (OR = 0.84, 95% CI = 0.72–0.97) in the two studies, respectively.

Specific study elements and findings are here summarized for selected birth defects, including oral clefts (Table I), heart defects (Table II), and other congenital anomalies (Table III). We also summarize findings relative to the 677C → T variant of the *MTHFR* gene and cleft lip and palate, for which more data are available among the anomalies examined here (Table IV).

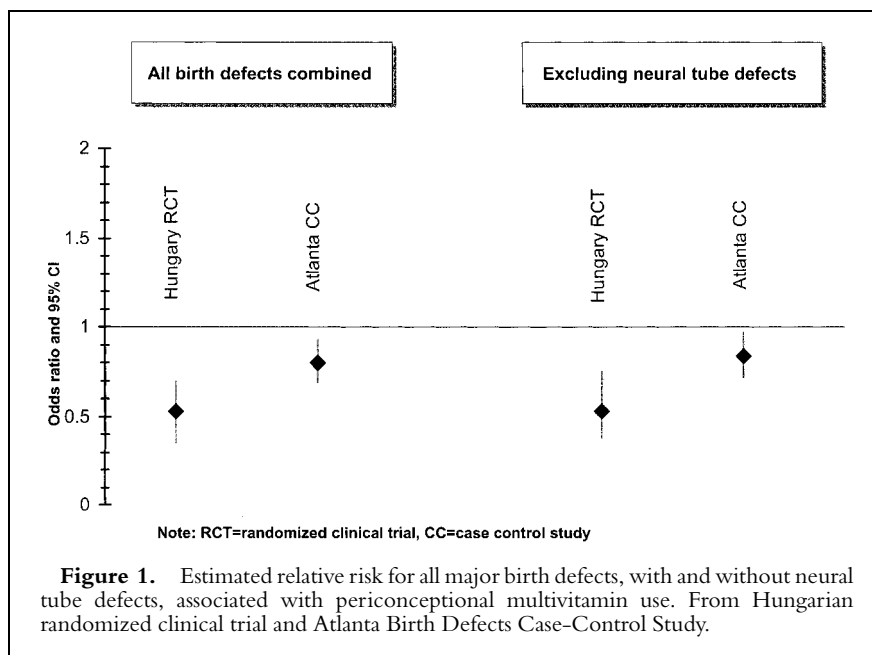


TABLE I. Studies on Multivitamin Supplements/Folic Acid and Orofacial Clefts, 1995–2003

Type of study	Reference	Study years	Location	Study participants ^a	Exposure ^a	Timing of exposure	Orofacial clefts		
							(overall)	CL/P	CP
Randomized controlled trial	Czeizel [1998]	1985–1993	Hungary	2,471 women on MV supplements; 2,391 on trace elements	MV containing 0.8 mg folic acid	Begun at least one month before conception	0.77 (0.22–2.69)	1.29 (0.32–5.22)	0.19 (0.01–4.03)
Nonrandomized recurrence prevention trial	Tolarova and Harris [1995]	1976–1980	Czech Republic	221 women on MV supplements; 1901 not supplemented	MV plus 10.0 mg folic acid	Begun at least two months before conception	–	0.35 (0.09–0.95)	–
Case-control, population-based	Shaw et al. [1995a]	1987–1989	California	489 with clefts ^c ; 734 controls	MV	Any periconceptual use	–	0.50 ^c (0.36–0.68)	0.73 ^c (0.46–1.20)
Case-control, hospital based	Hayes et al. [1996]	1988–1991	Boston, Philadelphia, Toronto	303 with nonsyndromic clefts; 1,167 controls with selected defects	MV or folic acid alone	Regular use in months 1–4, 3, or 4, +/- preconceptional use	1.4 (0.8–1.7)	1.3 (0.8–2.1)	0.9 (0.5–1.6)
Case-control, population-based	Czeizel et al. [1999]	1980–1996	Hungary	473 with clefts ^c ; 525 controls	MV plus 3.0–9.0 mg folic acid	Begun in months 1, 2, 3, or 4 +/- preconceptional use	–	0.87 ^c (0.73–1.03)	0.75 ^c (0.58–0.96)
Case-control, hospital based	Werler et al. [1999]	1993–1996	Boston, Philadelphia, Toronto	160 with nonsyndromic clefts; 521 controls	MV	Regular use in first trimester +/- preconceptional use	–	0.7 (0.4–1.1)	0.4 (0.2–0.9)
Case-control	Loffredo et al. [2001]	1991–1992	Sao Paulo, Brazil	450 with nonsyndromic clefts; 450 controls	MV	Any use in months 1–4	–	0.58 (0.43–0.79)	0.60 (0.37–0.98)
Case-control, population-based	Itikala et al. [2001]	1968–1980	Atlanta	309 with nonsyndromic clefts; 3,029 controls	MV	Regular use in first trimester +/- preconceptional use	0.61 (0.43–0.87)	0.52 (0.34–0.80)	0.81 (0.44–1.52)
Case-control	Beaty et al. [2001]	1992–1998	Maryland	135 with nonsyndromic clefts; 152 controls	MV	Regular use in first trimester +/- preconceptional use	–	0.59 (0.33–1.09)	0.70 (0.31–1.56)

^aMV, multivitamin supplement (folic acid dose likely ≤ 1.0 mg); CL/P, cleft lip with or without cleft palate; CP, cleft palate.

^bRR/OR, relative risk or odds ratio; 95%CI, 95% confidence interval.

^cResults for isolated clefts only shown here.

TABLE II. Studies on Multivitamin Supplements and Congenital Heart Defects, 1992–2000

Type of study	Reference	Study years	Population	Location	Study participants ^a	Exposure ^a	Timing of exposure	RR/OR (95% CI) ^b		
								Heart defects (overall)	Outflow tract defects	Ventricular septal defect
Randomized clinical trial	Czetz et al. [1998]	1985–1993	1985–1993	Hungary	2,471 women on MV; 2,391 on trace elements	MV with 0.8 mg folic acid	Begun at least one month before conception	0.42 (0.19–0.98)	0.48 (0.04–5.34)	0.24 (0.05–1.14)
Case-control, population based	Shaw et al. [1995b]	1987–1988	1987–1988	California	207 with OTD, 481 controls	MV	Any periconceptional use	–	0.70 (0.46–1.1)	–
Case-control, population based	Scanlon et al. [1998]	1986–1989	1981–1989	Baltimore–Washington	126 with OTD, 679 controls	MV with folic acid	Average use in year before conception	–	0.97 (0.6–1.6)	–
Case-control, population based	Botto et al. [2000]	1968–1980	1968–1980	Atlanta	958 with heart defects, 3,029 controls	MV	Regular periconceptional use	0.76 (0.60–0.97)	0.46 (0.24–0.86)	0.61 (0.38–0.99)
Case-control, hospital based	Werler et al. [1999]	1993–1996	1993–1996	Boston, Philadelphia, Toronto	157 with OTD, 186 with VSD, 521 controls	MV	Regular use in first trimester +/- periconceptional	–	1.00 (0.70–1.50)	1.20 (0.80–1.80)

^aMV, multivitamin supplement.

^bRR/OR, relative risk or odds ratio; 95%CI, 95% confidence interval.

To provide an evaluation of potential benefits as a preventable fraction (rather than a relative risk), we computed the number of cases potentially prevented by periconceptional multivitamin use in a hypothetical cohort of 370,000 newborns (roughly the estimated number of births worldwide daily). This calculation assumes that the relative risk from the literature (Tables I–III) reflect a causal relation. We considered only those anomalies with at least two published relative risk estimates, all of which were in same direction. We computed a range of preventable cases using three parameters: the highest and lowest published point estimates and an estimate of defect occurrence rates (per 1,000 births). These parameters were as follows: all birth defects (OR = 0.80 and 0.53, rate = 30/1,000); heart defects (OR = 0.76 and 0.42, rate = 8/1,000); conotruncal defects (OR = 1.00 and 0.46, rate = 0.8/1,000); limb defects (OR = 0.64 and 0.19, rate = 0.5/1,000); and urinary tract defects (OR = 0.60 and 0.17, rate = 4/1,000). The findings, rounded to the closest 50 units, are summarized in Figure 2.

DISCUSSION

The interpretation of these data is ultimately founded on a reasoned balance between methodology, data, biology, and context. Because these may vary by type of birth defect and can hardly be conveyed adequately in this short summary, we shall focus mainly on issues that are shared across types of birth defects. For more comprehensive, defect-specific discussion refer to recent reviews [Munger, 2002; Botto et al., 2003].

It is useful to defer the discussion of specific defects and examine first the question of whether supplements reduce the overall risk for birth defects. This question is key to preventive clinical practice and public health action and, because of sheer numbers, could be answered more easily than questions on specific, rarer anomalies. However, few studies have examined systematically the overall association with birth defects. The Hungarian randomized trial reported a 47% decrease for all birth defects

TABLE III. Use of Multivitamin Supplements/Folic Acid and Risk for Selected Birth Defects, 1995–2003

Type of study	Reference	Birth defect	Period	Location	Study participants ^a	Exposure ^a	Timing of exposure	RR/OR ^b (95% CI)
Randomized controlled trial	Czeizel et al. [1998]	Limb deficiency	1985–1993	Hungary	2,471 women on MV supplements; 2,391 on trace elements	MV containing 0.8 mg folic acid	Begun at least one month before conception	0.19 (0.03–1.18)
Case-control, population-based	Shaw et al. [1995b]	Limb deficiency	1987–1988	California	178 with nonsyndromic limb deficiencies; 481 controls	MV	Any periconceptual use	0.64 (0.41–1.0)
Case-control, population-based	Yang et al. [1997]	Limb deficiency	1968–1980	Atlanta	117 with nonsyndromic limb deficiencies; 3,029 controls	MV	Regular periconceptual use	0.47 (0.23–0.97)
Case-control, hospital based	Werler et al. [1999]	Limb deficiency	1993–1996	Boston, Philadelphia, Toronto	31 with nonsyndromic limb deficiencies; 521 controls	MV	Regular use 1st trimester ± preconceptional use	0.5 (0.2–1.1)
Evaluation of public health campaign	Myers et al. [2001]	Imperforate anus	1993–1995	China	126,783 women on folic acid supplements, 95,531 women on no supplements	0.4 mg folic acid only (pill)	Any periconceptual use	0.50 (0.29–0.88)
Randomized controlled trial	Czeizel et al. [1998]	Urinary tract defects	1985–1993	Hungary	2,471 women on MV supplements; 2,391 on trace elements	MV containing 0.8 mg folic acid	Begun at least one month before conception	0.21 (0.05–0.99)
Case-control	Li et al. [1995]	Urinary tract defects	1990–1991	Western Washington	117 with defects of kidney/ureter/bladder/urethra; 385 controls	MV	Any periconceptual use	0.17 (0.06–0.48)
Case-control, hospital based	Werler et al. [1999]	Urinary tract defects	1993–1996	Boston, Philadelphia, Toronto	184 with defects of kidney/ureter/bladder/urethra; 521 controls	MV	Regular use in first trimester ± preconceptional use	0.6 (0.4–0.9)
Case-control, population-based	Botto et al. [2002b]	Omphalocele	1968–1980	Atlanta	72 cases of nonsyndromic omphalocele (40 isolated, 32 multiples), 3,029 controls	MV	Regular periconceptual use	0.4 (0.2–1.0)
Case-control, population-based	Shaw et al. [2000]	Multiple congenital anomalies	1993–1996	California	112 with multiple congenital anomalies; 195 controls	MV	Any periconceptual use	2.6 (1.1–6.2)
Case-control	Czeizel et al. [1996]	Multiple congenital anomalies		Hungary	1,133 with multiple congenital anomalies; 1,133 matched controls	MV	Any use during pregnancy	0.97 (0.81–1.16)

^aMV, multivitamin supplement.

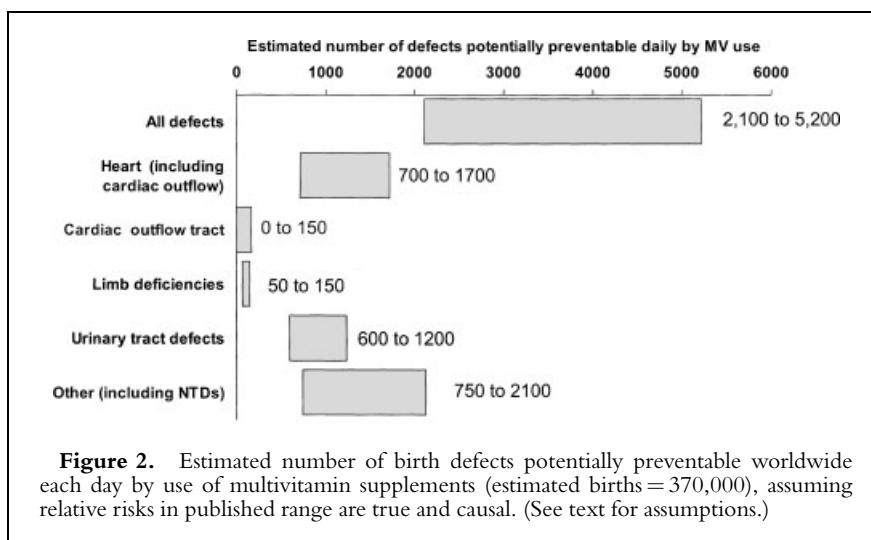
TABLE IV. 677C->T Variant of MTHFR and Risk of Cleft Lip With or Without Cleft Palate

Location	Reference	Cases (%)				Controls (%)				TT vs. CC		CT vs. CC	
		All	TT	CT	CC	All	TT	CT	CC	OR (95% CI)	OR (95% CI)		
California ^a	Shaw et al. [1995a]	310	40 (12.9)	127 (41.0)	143 (46.1)	382	49 (12.8)	178 (46.6)	155 (40.6)	0.84 (0.52–1.35)	0.7 (0.53–1.02)		
Netherlands	van Rooij et al. [2003]	105	6 (5.7)	45 (42.9)	54 (51.4)	128	4 (3.1)	54 (42.2)	70 (54.7)	1.9 (0.5–7.2)	1.1 (0.6–1.8)		
Brazil	Gaspar et al. [1999]	77	8 (10.4)	39 (50.6)	30 (39.0)	113	15 (13.3)	49 (43.4)	49 (43.4)	0.87 (0.33–2.30)	1.3 (0.70–2.41)		
Italy	Martinelli et al. [2001]	64	12 (18.8)	30 (46.9)	22 (34.4)	106	17 (16.0)	43 (40.6)	46 (43.4)	1.47 (0.60–3.60)	1.5 (0.73–2.90)		
Ireland	Mills et al. [1999]	66	10 (15.2)	nr ^b	nr	848	83 (9.8)	nr	nr	TT vs. (CT plus CC) 1.65 (0.81–3.35)			

^aPopulation-based study.^bnr, genotype not reported.

Because more than 10,000 children are estimated to be born daily with congenital anomalies worldwide . . . , even a 15–20% decrease in the overall risk for birth defects, if true, would be important.

Moving to the question of specific birth defects, the two studies just cited,



combined (the same even after excluding neural tube defects). In the Atlanta case-control study the overall reduction was approximately 20% (16% excluding neural tube defects). The difference between these two estimates (20% and 47%) can be due to many factors, including bias and confounding (e.g., differential and nondifferential reporting bias in the case-control study), as well as the relative size of the two studies. One should also note that the randomized trial could estimate risks with limited precision given its size, and that the Atlanta study was observational and retrospective. However, because more than 10,000 children are estimated to be born daily with congenital anomalies worldwide (3% of the estimated 135 million births that occur yearly), even a 15–20% decrease in the overall risk for birth defects, if true, would be important.

which survey many types of anomalies, already underscore the challenges of identifying which anomalies are related to multivitamin use and which are not. For example, for some anomalies (e.g., cardiac, limb) there is evidence of a risk reduction in both studies, whereas for others (e.g., orofacial clefts) such evidence is present in one but not the other study. Adding the data from other focused studies (summarized in Tables I–III) provides a complex picture with conflicting findings.

In part, the variation in the findings could reflect the heterogeneity of the studies. In the studies on oral clefts (Table I), for example, the assessment of multivitamin supplement use varied considerably, with respect to pattern (e.g., any use vs. regular use), timing (first trimester vs. from before conception), and folic acid content (from unknown to 10.0 mg). These studies often used different study populations (e.g., hospitals/clinics or population-based setting), comparison groups (no supplement use vs. irregular use), and endpoints (e.g., all cases vs. isolated cases). Some geographic locations also had considerable ethnic heterogeneity. This diversity might have reduced the power of these studies to show an effect, particularly if a particular polymorphism was involved, due to genetic heterogeneity among multiple ethnic groups.

The studies on heart defects highlight further difficulties in interpretation and in drawing inferences that are

engendered by heterogeneity, including defect classification (which could influence the analysis and possibly the effect estimates) and screening for 22q11 (which if undiagnosed could inflate etiologic heterogeneity and bias the effects, likely toward null results). Such sources of heterogeneity, well appreciated in such exposure studies [Werler, 2002], remain a considerable challenge in interpreting the current body of literature. To what extent such heterogeneity accounts for the variation of the effect estimates is unclear. Nevertheless, to disregard such heterogeneity is probably unwise, at this stage of knowledge, as doing so implicitly assumes, without proof, that the factors that vary across studies have no effect on the association.

It may be helpful to conclude these methodologic considerations with a comparative look at neural tube defects, for which the effect of supplements and folic acid is well established [Botto et al., 1999; Moore, 2001]. For neural tube defects, the data are considerable and include robust randomized clinical trials as well as several recurrence and occurrence studies. Both folic acid alone and multivitamin supplements have been studied. Findings are remarkably consistent across different study designs, geographic areas, and ethnic backgrounds.

Genetic data on the 677C → T variant of the *MTHFR* gene pooled from several studies, though variable, are globally convincing. Ease of diagnosis and limited morphologic variation further simplify the study of neural tube defects.

These considerations also highlight the need for large, well-designed studies (Table V). This approach can leverage multicenter collaboration to achieve statistical power, but requires shared epidemiologic and clinical protocols to ensure comparability and validity of the findings. Such studies could provide critical knowledge on key aspects of the relation between supplement use and birth defects, which we will now briefly examine.

Effect Direction and Size

We already noted the marked variability of the published findings. For example, the risk reduction for conotruncal heart defects estimated in different studies varied from 0–50% (Table II). For oral clefts, the point estimate varied even in the same study framework during different study periods, from 1.3 (30% increased risk) [Hayes et al., 1996] to 0.7 (30% decreased risk) [Werler et al., 1999]. For limb deficiencies, a similar risk reduction in two studies [Shaw et al., 1995b; Yang et al., 1997] was driven by

apparently different limb deficiency subgroups.

These findings further underscore the critical role for medical geneticists to ensure valid and consistent clinical classification, in particular of defect subgroups, in such epidemiologic studies.

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In addition to validity, precision of the effect estimate is a desired objective, which requires large study populations achievable only through multicenter collaboration. For example, finding a 25% decrease in risk for conditions such as hypoplastic left heart, with occurrence rates of 1 in 5,000 births, even in reasonable and favorable study settings [Botto et al., 2003] could require an underlying birth cohort the size of all annual births in the United States.

TABLE V. Understanding Whether Vitamins Prevent Birth Defects: Challenges, Gaps, and Strategies

Challenges and gaps	Strategies
Establish effect with reasonable precision	Randomized trials with sufficient power Collaborative well-designed case-control studies (e.g., National Birth Defects Prevention Study - NBDPS)
Etiologic heterogeneity, subgroup classification	Participation of clinical geneticists in studies Evaluate for known teratogens, genetic causes
Identify and quantify dose-effect relation	Randomized trials with treatment arms with varying vitamin dose Observational studies stratified by vitamin content
Evaluate effect in occurrence vs. recurrence setting	Recurrence prevention trials Observational studies with family history components
Ethical issues related to randomized trials of folic acid supplementation	All treatment arms include 400 mcg of folic acid Higher doses can be tested
Background of flour fortification and baseline multivitamin use in countries where studies are conducted	Incorporation of biologic sample collection with biochemical analyses into epidemiologic studies
Assess effect by genotype	Incorporation of biologic sample collection with molecular testing into epidemiologic studies

Role of Specific Micronutrients

Because nearly all studies examined multivitamin supplements, little is known on the role of specific micronutrients. Folic acid, for example, has been studied nearly exclusively as part of such multivitamins (Tables I–III). Because of this, indirect data can be quite useful, including those on folic acid antagonists [Hernandez-Diaz et al., 2000] or folate genes (e.g., Table IV). For example, by searching for distortions from the expected Mendelian randomization of polymorphic variants related to micronutrient metabolism, such genetic studies can suggest an etiologic role of specific micronutrients [Clayton and McKeigue, 2001; Davey Smith and Ebrahim, 2003]. Understanding the role of specific micronutrients has important practical implications, as it can help develop better prevention strategies, whether by fortification, supplementation, improving diet, or a combination of these.

Effect on Risk from Other Exposures

A relevant question is: What are the components of the birth defects' risk reduction by supplements? For example, it would be useful to know whether the risk associated with exposures such as febrile illnesses [Botto et al., 2002a; Shaw et al., 2002], maternal diabetes [Correa et al., 2003], and other common factors [Shaw et al., 2002] are specifically reduced by supplements.

Dose

For preventing spina bifida, 400 μg (0.4 mg) of folic acid/day is effective [Berry et al., 1999], and even lower intakes, for example, from fortification alone [Persad et al., 2002], probably have some preventive effect. The effective dose for other birth defects (if indeed folic acid alone prevents some) is unknown but could be different. For example, some findings relative to orofacial clefts suggest that high-dose folic acid might be more effective than low-dose folic acid [Tolarova and Harris,

1995; Czeizel et al., 1999]. These studies were not randomized.

The question of dosage is relevant not only to fortification but also to clinical counseling for recurrence. Only one clinical trial [Tolarova and Harris, 1995] provides data directly addressing recurrence risk, and because this study was nonrandomized, included only one birth defect, and used a very high dose of folic acid (10 mg), one is left with insufficient data on which to base public health recommendations. In general, data from several observational studies outlined in Tables I–III suggest low doses of folic acid with multivitamins reduce occurrence risks also. More data on dosage effects are desirable but not yet available.

Interaction and Mechanism

Quantifying the effect of gene–gene and gene–environment interactions requires large, carefully conducted studies, but could help improve prevention efforts among groups who might be at higher risk of disease because of their genotype. Elucidating the mechanism of action of micronutrients will not only help establish causality, but can provide important clues on the basic pathogenesis of birth defects.

Research Options: Observational Studies, Epidemiologic Monitoring, Clinical Trials

Finding valid and precise answers to these questions requires new, well-designed studies. These may include population-based case–control studies, case–trios, randomized clinical trials, and birth defects monitoring, each providing complementary evidence. Larger, if fewer, studies are preferable to ensure sufficient sample size for an analysis of specific case groups in relation to micronutrient intake from diet and supplements and possibly by genotype. One such promising approach (Table V) is the National Birth Defects Prevention Study (NBDPS) [Yoon et al., 2001], a multicenter, multistate study that incorporates clinical classification [Rasmussen et al., 2003], detailed exposure and dietary history, and biological data collection [Rasmussen et al., 2002].

Birth defect monitoring in areas where widespread flour fortification is in place can provide considerable complementary information. For example, a decrease of birth defects rates after flour fortification would suggest that folic acid is effective alone and in low doses. Negative results could have more complex interpretations, and in general inferences based on monitoring of necessity must consider complicating factors such as the etiologic heterogeneity of many birth defects, the background trends in occurrence, and the influence of selective pregnancy terminations.

Randomized clinical trials can provide powerful evidence. However, the logistic and financial challenges are numerous, and one must realistically consider what goals are achievable within the constraints of resources. What might be doable, with considerable effort, is one or two studies to look at a few specific vitamins, doses, or defects. Alternatively, the trial could focus on assessing the overall reduction of the risk for congenital anomalies other than neural tube defects. This approach would sidestep many interesting issues of specific causal associations with individual birth defects, but could be doable and provide important evidence for population-wide prevention. Another issue is the ethical necessity for all trial participants to be provided with at least the daily recommended dose of folic acid. Depending on the specifics of the doses used, and the particular results, it could be very difficult to understand the effect of folic acid per se. For example, if a study compared 2 doses of folic acid and found equal rates in both dose groups it would be difficult to know if this was because both doses were equally protective or equally ineffective; making an inference in such a circumstance would require reference to data from outside the study.

CONCLUSIONS AND CLINICAL IMPLICATIONS

Overall, the findings on supplement use from the Hungarian randomized clinical trial, supported by the Atlanta

Birth Defects Case-Control Study, are consistent with the notion that periconceptional multivitamin use reduces the overall occurrence of birth defects, in addition to the demonstrated effect on neural tube defects

Overall, the findings . . . are consistent with the notion that periconceptional multivitamin use reduces the overall occurrence of birth defects, in addition to the demonstrated effect on neural tube defects.

(Fig. 1). The extent of risk reduction differs somewhat in the two studies, but even the more conservative estimate (15%, in addition to that on neural tube defects), if true, would be important. These and other observational studies suggest specific reductions for certain common and severe birth defects. In our view, these findings deserve to be followed up quickly by further and well-designed studies, given the potential clinical and public health benefits associated with preventing these congenital anomalies (Fig. 2).

What kind of advice should providers now give in a clinical setting? With respect to genotyping for folate-related genes such as *MTHFR*, such testing, while important in research studies, does not appear currently to be useful as a basis for clinical or public health practice.

Genotyping for folate-related genes such as MTHFR, such testing, while important in research studies, does not appear currently to be useful as a basis for clinical or public health practice.

The absolute risk associated with particular alleles would be small, and genotyping would not be an efficient screening tool in clinical practice [Davey Smith and Ebrahim, 2003].

Existing recommendations regarding supplementation with 0.4 mg of folic acid to prevent neural tube defects remain the most practical immediate advice for prevention of occurrence or recurrence of other birth defects. Because nearly all studies of birth defects that reported a reduced risk for birth defects other than neural tube defects examined multivitamin supplements, not folic acid alone, one could consider using a multivitamin supplement with the recommended daily dose of folic acid [Oakley and Erickson, 1995] as an alternative to folic acid alone.

The suggestions that high-dose folic acid (several milligrams) might be better than a daily multivitamin with 0.4–0.8 mg of folic acid to prevent the occurrence and recurrence of some birth defects (other than neural tube defects) are intriguing but not conclusive. Randomized trials that compare the standard dose of folic acid with higher doses or vitamin combinations would be both ethical and useful.

For the time being, women should consider the daily use of a folic acid-containing multivitamin supplement. This, along with a healthy diet, is a rational approach to preventing birth defects that is simple, inexpensive, and consistent with (if not proven by) current data.

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REFERENCES

- Beatty TH, Wang H, Hetmanski JB, Fan YT, Zeiger JS, Liang KY, Chiu YF, Vanderkolk CA, Seifert KC, Wulfsberg EA, Raymond G, Panny SR, McIntosh I. 2001. A case-control study of nonsyndromic oral clefts in Maryland. *Ann Epidemiol* 11:434–442.
- Berry RJ, Li Z, Erickson JD, Li S, Moore CA, Wang H, Mulinare J, Zhao P, Wong LY, Gindler J, Hong SX, Correa A. 1999. Prevention of neural-tube defects with folic acid in China. China-U.S. Collaborative Project for Neural Tube Defect Prevention. *N Engl J Med* 341:1485–1490.
- Botto LD, Moore CA, Khoury MJ, Erickson JD. 1999. Neural-tube defects. *N Engl J Med* 341:1509–1519.
- Botto LD, Mulinare J, Erickson JD. 2000. Occurrence of congenital heart defects in relation to maternal multivitamin use. *Am J Epidemiol* 151:878–884.
- Botto LD, Erickson JD, Mulinare J, Lynberg MC, Liu Y. 2002a. Maternal fever, multivitamin use, and selected birth defects: evidence of interaction? *Epidemiology* 13:485–488.
- Botto LD, Mulinare J, Erickson JD. 2002b. Occurrence of omphalocele in relation to maternal multivitamin use: a population-based study. *Pediatrics* 109:904–908.
- Botto LD, Mulinare J, Erickson JD. 2003. Do multivitamin or folic acid supplements reduce the risk for congenital heart defects? Evidence and gaps. *Am J Med Genet* 121A:95–101.
- Centers for Disease Control and Prevention. 1998. Trends in infant mortality attributable to birth defects—United States, 1980–1995. *MMWR Morb Mortal Wkly Rep* 47:773–778.
- Clayton D, McKeigue PM. 2001. Epidemiological methods for studying genes and environmental factors in complex diseases. *Lancet* 358:1356–1360.
- Correa A, Botto L, Liu Y, Mulinare J, Erickson JD. 2003. Do multivitamin supplements attenuate the risk for diabetes-associated birth defects? *Pediatrics* 111:1146–1151.
- Czeizel AE. 1998. Periconceptional folic acid containing multivitamin supplementation. *Eur J Obstet Gynecol Reprod Biol* 78:151–161.
- Czeizel AE, Dudas I. 1992. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. *N Engl J Med* 327:1832–1835.
- Czeizel AE, Toth M, Rockenbauer M. 1996. Population-based case control study of folic acid supplementation during pregnancy. *Teratology* 53:345–351.
- Czeizel AE, Timar L, Sarkozi A. 1999. Dose-dependent effect of folic acid on the prevention of orofacial clefts. *Pediatrics* 104:e66.
- Davey Smith G, Ebrahim S. 2003. ‘Mendelian randomization’: can genetic epidemiology contribute to understanding environmental determinants of disease? *Int J Epidemiol* 32:1–22.
- Erickson JD. 1991. Risk factors for birth defects: data from the Atlanta Birth Defects Case-Control Study. *Teratology* 43:41–51.
- Gaspar DA, Pavanello RC, Zatz M, Passos-Bueno MR, Andre M, Steman S, Wyszynski DE, Mاتيولي SR. 1999. Role of the C677T

- polymorphism at the MTHFR gene on risk to nonsyndromic cleft lip with/without cleft palate: results from a case-control study in Brazil. *Am J Med Genet* 87:197–199.
- Hayes C, Werler MM, Willett WC, Mitchell AA. 1996. Case-control study of periconceptional folic acid supplementation and oral clefts. *Am J Epidemiol* 143:1229–1234.
- Hernandez-Diaz S, Werler MM, Walker AM, Mitchell AA. 2000. Folic acid antagonists during pregnancy and the risk of birth defects. *N Engl J Med* 343:1608–1614.
- Itikala PR, Watkins ML, Mulinare J, Moore CA, Liu Y. 2001. Maternal multivitamin use and orofacial clefts in offspring. *Teratology* 63:79–86.
- Junker R, Kotthoff S, Vielhaber H, Halimeh S, Kosch A, Koch HG, Kassenbohmer R, Heineking B, Nowak-Gottl U. 2001. Infant methylenetetrahydrofolate reductase 677T genotype is a risk factor for congenital heart disease. *Cardiovasc Res* 51:251–254.
- Li DK, Daling JR, Mueller BA, Hickok DE, Fantel AG, Weiss NS. 1995. Periconceptional multivitamin use in relation to the risk of congenital urinary tract anomalies. *Epidemiology* 6:212–218.
- Loffredo LC, Souza JM, Freitas JA, Mossey PA. 2001. Oral clefts and vitamin supplementation. *Cleft Palate Craniofac J* 38:76–83.
- Martinelli M, Scapoli L, Pezzetti F, Carinci F, Carinci P, Stabellini G, Bisceglia L, Gombos F, Tognon M. 2001. C677T variant form at the MTHFR gene and CL/P: a risk factor for mothers? *Am J Med Genet* 98:357–360.
- Mills JL, Kirke PN, Molloy AM, Burke H, Conley MR, Lee YJ, Mayne PD, Weir DG, Scott JM. 1999. Methylenetetrahydrofolate reductase thermolabile variant and oral clefts. *Am J Med Genet* 86:71–74.
- Moore LL. 2001. Is the jury still out on folic acid and congenital anomalies? *Epidemiology* 12:141–144.
- MRC Vitamin Study Research Group. 1991. Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. *Lancet* 338:131–137.
- Mulinare J, Erickson JD, James LM, Berry RJ. 1995. Does periconceptional use of multivitamins reduce the occurrence of birth defects? *Am J Epidemiol* 141:S3.
- Munger RG. 2002. Maternal nutrition and oral clefts. In: Wyszynski DF, editor. *Cleft lip and palate*. New York: Oxford University Press. p 159–169.
- Myers MF, Li S, Correa-Villasenor A, Li Z, Moore CA, Hong SX, Berry RJ. 2001. Folic acid supplementation and risk for imperforate anus in China. *Am J Epidemiol* 154:1051–1056.
- Oakley GP Jr, Erickson JD. 1995. Vitamin A and birth defects. Continuing caution is needed. *N Engl J Med* 333:1414–1415.
- Persad VL, Van den Hof MC, Dube JM, Zimmer P. 2002. Incidence of open neural tube defects in Nova Scotia after folic acid fortification. *Canadian Medical Association Journal* 167:241–245.
- Rasmussen SA, Lammer EJ, Shaw GM, Finnell RH, McGehee RE Jr, Gallagher M, Romitti PA, Murray JC. 2002. Integration of DNA sample collection into a multi-site birth defects case-control study. *Teratology* 66:177–184.
- Rasmussen SA, Olney RS, Holmes LB, Lin AE, Keppler-Noreuil KM, Moore CA. 2003. Guidelines for case classification for the National Birth Defects Prevention Study. *Birth Defects Res Part A Clin Mol Teratol* 67:193–201.
- Scanlon KS, Ferencz C, Loffredo CA, Wilson PD, Correa-Villasenor A, Khoury MJ, Willett WC. 1998. Preconceptional folate intake and malformations of the cardiac outflow tract. *Baltimore-Washington Infant Study Group. Epidemiology* 9:95–98.
- Shaw GM, Lammer EJ, Wasserman CR, O'Malley CD, Tolarova MM. 1995a. Risks of orofacial clefts in children born to women using multivitamins containing folic acid periconceptionally. *Lancet* 346:393–396.
- Shaw GM, O'Malley CD, Wasserman CR, Tolarova MM, Lammer EJ. 1995b. Maternal periconceptional use of multivitamins and reduced risk for conotruncal heart defects and limb deficiencies among offspring. *Am J Med Genet* 59:536–545.
- Shaw GM, Rozen R, Finnell RH, Todoroff K, Lammer EJ. 1998. Infant C677T mutation in MTHFR, maternal periconceptional vitamin use, and cleft lip. *Am J Med Genet* 80:196–198.
- Shaw GM, Croen LA, Todoroff K, Tolarova MM. 2000. Periconceptional intake of vitamin supplements and risk of multiple congenital anomalies. *Am J Med Genet* 93:188–193.
- Shaw GM, Nelson V, Carmichael SL, Lammer EJ, Finnell RH, Rosenquist TH. 2002. Maternal periconceptional vitamins: interactions with selected factors and congenital anomalies? *Epidemiology* 13:625–630.
- Shibuya K, Murray CJL. 1998. Congenital anomalies. In: Murray CJL, Lopez AD, editors. *Health dimensions of sex and reproduction, Volume III*. Boston: Harvard University Press. p 455–512.
- Tolarova M, Harris J. 1995. Reduced recurrence of orofacial clefts after periconceptional supplementation with high-dose folic acid and multivitamins. *Teratology* 51:71–78.
- van Rooij IA, Vermeij-Keers C, Kluijtmans LA, Ocke MC, Zielhuis GA, Goorhuis-Brouwer SM, van der Biezen JJ, Kuijpers-Jagtman AM, Steegers-Theunissen RP. 2003. Does the interaction between maternal folate intake and the methylenetetrahydrofolate reductase polymorphisms affect the risk of cleft lip with or without cleft palate? *Am J Epidemiol* 157:583–591.
- Werler MM. 2002. Exposure assessment in studies of oral clefts. In: Wyszynski DF, editor. *Cleft lip and palate*. New York: Oxford University Press. p 108–116.
- Werler MM, Hayes C, Louik C, Shapiro S, Mitchell AA. 1999. Multivitamin supplementation and risk of birth defects. *Am J Epidemiol* 150:675–682.
- Yang Q, Khoury MJ, Olney RS, Mulinare J. 1997. Does periconceptional multivitamin use reduce the risk for limb deficiency in offspring? *Epidemiology* 8:157–161.
- Yoon PW, Rasmussen SA, Lynberg MC, Moore CA, Anderka M, Carmichael SL, Costa P, Druschel C, Hobbs CA, Romitti PA, Langlois PH, Edmonds LD. 2001. The National Birth Defects Prevention Study. *Public Health Rep* 116(Suppl 1):32–40.