

## Folic Acid Supplementation and Risk for Imperforate Anus in China

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Maternal consumption of folic acid before pregnancy and during early pregnancy is associated with a reduced risk for some birth defects. Whether folic acid can reduce the risk for imperforate anus is unknown. As part of a public health campaign conducted in China from 1993 through 1995, the outcomes of pregnancies of  $\geq 20$  weeks' gestation were evaluated among women using folic acid supplements. The women were asked to take one pill containing 400  $\mu\text{g}$  of folic acid (without other vitamins) every day from the time of their premarital examination until the end of their first trimester of pregnancy. Rates of imperforate anus and risk ratios for imperforate anus among the offspring of these women were calculated according to folic acid use. Among the offspring of women who took folic acid and women who did not take folic acid, 20 and 30 infants with imperforate anus were identified, respectively. The rate of imperforate anus was 3.1 per 10,000 among the offspring of women who did not take folic acid and 1.6 per 10,000 among the offspring of women who took folic acid; adjusted for maternal age, the risk ratio was 0.59 (95% confidence interval: 0.33, 1.07). Daily maternal consumption of 400  $\mu\text{g}$  of folic acid before and during early pregnancy may reduce the risk for imperforate anus. *Am J Epidemiol* 2001;154:1051–6.

abnormalities; anus, imperforate; folic acid; maternal age; pregnancy; primary prevention; risk

Imperforate anus is a birth defect that results from incomplete formation of the hindgut, causing absence or abnormal localization of the anus around the eighth week of development. The rectum may end in a blind pouch or have openings to the urethra, bladder, or vagina (1, 2). Reported estimates of the birth prevalence of imperforate anus have ranged from 2 per 10,000 livebirths to 6.7 per 10,000 livebirths (2, 3). Prenatal thalidomide exposure and maternal diabetes mellitus are the only two recognized teratogenic causes of imperforate anus (2, 3). An association between maternal age and imperforate anus has also been reported (4, 5). No factors are known to prevent or reduce the risk for imperforate anus during pregnancy.

Daily consumption of folic acid with or without multivitamins before pregnancy and during early pregnancy can reduce a woman's risk for having an infant with a neural tube defect (6–8). Recent studies have also suggested that supple-

mentation with multivitamins containing folic acid before and during early pregnancy may reduce the risk for some non-neural-tube birth defects, including cardiovascular defects (9–11), orofacial clefts (12–14), urinary tract defects (10, 14, 15), and limb-reduction defects (9, 16). In these studies, it was not clear whether folic acid or another component of the multivitamins was associated with the reduced risk for these birth defects. None of these studies addressed the effect of the use of folic acid or multivitamins before and during early pregnancy on the risk for imperforate anus.

A public health campaign in China using a folic acid pill without other vitamins to prevent neural tube defects began in October 1993 (8). We used pill-taking and birth-defects surveillance data from this large cohort study to evaluate the effect of maternal daily consumption of 400  $\mu\text{g}$  of folic acid before and during early pregnancy on the risk for imperforate anus.

### MATERIALS AND METHODS

The methods employed in the campaign to prevent neural tube defects have been reported previously (8) and are summarized below.

#### Cohort

The campaign was conducted in one northern province (Hebei) and two southern provinces (Zhejiang and Jiangsu) of China. All pregnant women and women who were preparing for marriage registered with a pregnancy monitoring system that serves as the principal record of prenatal care

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Abbreviations: CI, confidence interval; RR, risk ratio.

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and delivery in all three provinces. Subjects for this evaluation were all women who registered with this monitoring system between October 1, 1993, and September 30, 1995, and had an informative pregnancy (i.e., one in which the presence or absence of a birth defect could be ascertained). The project was approved by the institutional review boards of the US Centers for Disease Control and Prevention and Peking University.

### Use of folic acid supplements

Beginning in October 1993, all women who registered with the pregnancy monitoring system were asked to purchase pills containing 400 µg of folic acid without other vitamins and to take one of these pills every day through the end of the first trimester of pregnancy. Women used one bottle containing 31 folic acid pills for each calendar month. At the end of each month, village health care workers collected the bottles and counted and recorded the number of pills consumed by each woman and the dates on which the women had started and stopped taking folic acid. For each woman, we computed compliance with pill-taking as the percentage of folic acid pills taken compared with the number that could have been taken.

For this study, we used the classification and pattern of pill-taking defined by Berry et al. (8). Women taking any folic acid pills before or during their first trimester of pregnancy were classified as folic acid users. Women with periconceptional use were defined as those who started taking folic acid on or before the date of their last menstrual period prior to conception and who stopped taking folic acid at the end of their first trimester. Women with late use were those who started taking folic acid during their first trimester but after their last menstrual period. Women who discontinued folic acid early were those who started and stopped taking the pills before their last menstrual period prior to conception. Women with missing dates were considered unclassifiable and were not assigned to any group. Women who either were already in their second trimester of pregnancy at registration (i.e., never had the opportunity to start taking folic acid by the end of their first trimester) or did not agree to take folic acid pills at the time of registration were considered not to have taken folic acid.

### Case ascertainment

We identified infants with imperforate anus through a birth defects surveillance system that was established in January 1993. This system collects detailed data about infants and fetuses with external structural birth defects. Included in the surveillance system were liveborn and stillborn infants of at least 20 weeks' gestation who had a birth defect that was diagnosed by 6 weeks of age (17). We also collected information about all pregnancies, even those with gestations of less than 20 weeks, that were electively terminated after the prenatal diagnosis of any birth defect. Three pediatricians who were unaware of the mothers' folic acid status independently reviewed the reports and photographs and assigned diagnostic codes, and a clinical geneticist val-

idated the diagnoses. In this evaluation, the definition of imperforate anus included the absence of an anal opening. Case infants with anal stenosis or anteriorly displaced but patent anus were not included in this study.

### Classification of case infants

We attempted to classify infants into the three groups typically used in epidemiologic studies of birth defects: infants in whom the defect was the only defect or was associated with other, minor anomalies (isolated defect); infants in whom the defect was associated with other, major defects in seemingly unrelated organ systems (multiple defects); and infants in whom the defect was part of a recognized single gene or chromosomal condition (a syndrome). For this study, classification using this method was difficult, because only data on external birth defects were collected by the surveillance system, and imperforate anus is often a component of conditions characterized by anomalous development of caudal body structures, such as cloacal exstrophy and sirenomelia. Thus, we divided the case infants into 1) those with imperforate anus and no other major external birth defects; 2) those with imperforate anus and other major external birth defects only in the caudal region; 3) those with imperforate anus and major external birth defects outside of the caudal region; and 4) those with a recognized syndrome that has imperforate anus as a component. Because imperforate anus with and without other, more severe caudal defects could be part of a spectrum of anomalies varying in severity, we also analyzed infants in the first two groups together.

### Statistical analysis

Any woman registering with the pregnancy monitoring system on or after October 1, 1993, was asked to take folic acid. We expected most women from this cohort to deliver after July 1, 1994. Therefore, our analysis of pregnancy outcomes was limited to the period from July 1, 1994, through December 31, 1996. Each woman contributed only one informative pregnancy to the study. For this cohort, we compared the numbers of women who took folic acid pills and the number who did not in each region (northern and southern) according to maternal age, parity, body mass index (weight (kg)/height (m)<sup>2</sup>), ethnicity, educational level, and occupation. For each region, we calculated the rate of imperforate anus (the number of cases per 10,000 pregnancies of at least 20 weeks' gestation) according to the use of folic acid. We estimated risk ratios by dividing the risk for imperforate anus among the fetuses or infants of all women who took folic acid by the risk among the fetuses or infants of women who did not take folic acid. Logistic regression was used to examine the effects of folic acid use (any; none), maternal education (elementary school or none; junior high school; high school or college), maternal occupation (farmer; factory worker; other), parity (no previous births; one or more previous births), and maternal age at delivery (15–24 years; 25–29 years; ≥30 years) on the risk for imperforate anus. We used SPSS statistical software (SPSS Base 10 for Windows; SPSS, Inc., Chicago, Illinois) for all analyses.

## RESULTS

## Participants

Among the women who registered with health authorities between October 1, 1993, and September 30, 1995, 223,722 had an informative pregnancy outcome between July 1, 1994, and December 31, 1996. Pregnancies with multiple fetuses were excluded from the analysis, yielding 222,314 singleton pregnancies (28,952 in the North and 193,362 in the South). In both regions, women who took folic acid pills were slightly younger than those who did not and were more likely to be pregnant for the first time (table 1). Women in the northern region were more likely to have continued their education through junior high school and were more likely to be employed in agriculture.

## Imperforate anus

We identified 50 women who had a fetus or infant with imperforate anus: six in the northern region and 44 in the southern region. Overall, male fetuses or infants were more likely to be affected than female fetuses or infants (ratio of males to females, 1.66:1). Sixty percent of imperforate anus cases had no additional external anomalies, 22 percent had additional caudal anomalies such as persistent cloaca, and 16 percent had multiple anomalies; one case was associated with Johanson-Blizzard syndrome. No cases of imperforate anus occurred among women with a medical history of diabetes.

## Use of folic acid supplements

Among women who registered with the pregnancy monitoring system within 6 weeks after their last menstrual period, 88 percent bought and took folic acid pills, and 90 percent of these women continued using the pills until the end of their first trimester. Among all women who did not take folic acid pills, 67 percent did not take folic acid because they registered with the health authorities after the first trimester of pregnancy, and 3 percent refused to take folic acid.

Once women began taking folic acid supplements, compliance with pill-taking remained high (overall mean compliance was more than 90 percent). The most common pattern of use of folic acid pills was periconceptual use in both the northern region (71 percent) and the southern region (54 percent) (table 2).

It was not possible to evaluate rates of imperforate anus by pattern of pill-taking (periconceptual use, late starting, and early stopping), because of the small number of cases in these categories (14 cases, four cases, and two cases, respectively). Instead, we compared rates of imperforate anus between the offspring of women with any folic acid use (periconceptual, late starting, or early stopping) and the offspring of women with no folic acid use. The rate of imperforate anus was lower among offspring of women who took folic acid than among offspring of women who did not take folic acid (risk ratio (RR) = 0.50, 95 percent confidence interval (CI): 0.29, 0.88) (table 3). There was little variation in risk for imperforate anus between regions or

TABLE 1. Characteristics of pregnant women, by region and use of folic acid pills, People's Republic of China, October 1, 1993–September 30, 1995

Characteristic	North (n = 28,952)				South (n = 193,362)			
	Any pill-taking (n = 18,400)		No pill-taking (n = 10,552)		Any pill-taking (n = 108,383)		No pill-taking (n = 84,979)	
	No.	%	No.	%	No.	%	No.	%
Mean age (years)	24.0 (2.5)*		26.4 (4.0)		24.3 (2.4)		25.6 (3.7)	
Parity†								
0	17,636	97	7,061	70	99,627	92	61,808	73
1	596	3	2,988	29	7,756	7	20,892	25
≥2	24	<1	110	1	483	1	1,568	2
Mean body mass index‡	21.4 (2.1)		21.7 (2.0)		20.4 (2.3)		20.8 (2.5)	
Han ethnicity	17,654	96	9,872	94	105,980	98	81,607	96
Education								
High school or college	1,651	9	742	7	12,311	11	8,741	10
Junior high school	13,788	75	7,960	75	67,032	62	45,547	54
Elementary school or none	2,352	13	1,256	12	27,022	25	27,704	33
Unknown	609	3	594	6	2,018	2	2,987	3
Occupation								
Farmer	15,594	85	9,132	87	56,784	52	54,080	64
Factory worker	1,786	10	674	6	43,962	41	23,625	28
Other	464	2	158	1	5,785	5	4,424	5
Unknown	556	3	588	6	1,852	2	2,850	3

\* Numbers in parentheses, standard deviation.

† Current pregnancy not included.

‡ Weight (kg)/height (m)<sup>2</sup>.

**TABLE 2. Pattern of pill-taking and pill-taking compliance, by region, among all women taking folic acid supplements, People's Republic of China, October 1, 1993–September 30, 1995**

Pill-taking pattern	North ( <i>n</i> = 18,365)*			South ( <i>n</i> = 108,291)*		
	Pregnancies		Pill-taking compliance (mean %)	Pregnancies		Pill-taking compliance (mean %)
	No.	%		No.	%	
Periconceptual	12,965	71	89	58,226	54	94
Late-starting	3,571	19	90	33,093	31	95
Early-stopping	1,829	10	82	16,972	16	90

\* The analysis excluded 35 women in the northern region and 92 in the southern region for whom the pattern of folic acid use could not be classified.

between educational and occupational categories with sufficient numbers of cases. Controlling the results for region, education, and occupation did not change the association between folic acid and imperforate anus. Higher parity and maternal age were associated with an increased risk for imperforate anus. Because parity and maternal age were highly correlated, we could not control for their independent effects. However, controlling for either of these covariates had a small effect on the magnitude of the relative risk estimate. Since maternal age explained the variation in the data

better than parity, and since there was no evidence of interaction between pill-taking and maternal age, we adjusted only for age. The reduction in the risk for imperforate anus among pill-takers was 41 percent when we controlled for maternal age (RR = 0.59, 95 percent CI: 0.33, 1.07).

When we classified cases of imperforate anus into subgroups according to whether the defect was unaccompanied by additional external anomalies, present only with other caudal anomalies, or present with multiple anomalies, a reduced risk for imperforate anus remained among offspring

**TABLE 3. Rates of imperforate anus and unadjusted risk ratios for imperforate anus, according to use of folic acid pills, region, maternal age, parity, occupation, and education, People's Republic of China, October 1, 1993–September 30, 1995**

	No. of pregnancies	Imperforate anus			
		Rate*	No.	Risk ratio	95% confidence interval
Use of folic acid pills					
All non-pill-takers†	95,531	3.1	30		
All pill-takers	126,783	1.6	20	0.50	0.29, 0.88
Region					
South†	193,362	2.3	44		
North	28,952	2.1	6	0.91	0.39, 2.14
Occupation					
Farmert	135,590	2.4	32		
Factory worker	70,047	2.4	17	1.03	0.57, 1.85
Other	10,831	0.9	1	0.39	0.05, 2.86
Education					
Elementary school or none†	58,334	2.4	14		
Junior high school	134,327	2.6	35	1.09	0.58, 2.02
High school or college	23,445	0.4	1	0.18	0.02, 1.35
Maternal age (years)					
15–24†	128,641	1.7	22		
25–29	67,117	2.2	15	1.31	0.68, 2.52
≥30	26,353	4.9	13	2.88	1.45, 5.73
Parity					
0†	186,132	1.9	35		
≥1	36,182	4.1	15	2.20	1.20, 4.04

\* Rate per 10,000 pregnancies of at least 20 weeks' gestation.

† Referent group.

of folic acid users (table 4), but the relative risk estimate was imprecise because of small numbers. The same was true when the first two categories, imperforate anus with no additional external anomalies and imperforate anus with other caudal anomalies, were combined (unadjusted RR = 0.59, 95 percent CI: 0.32, 1.09; after adjustment for maternal age, RR = 0.72, 95 percent CI: 0.38, 1.38).

## DISCUSSION

In our study, the rate of imperforate anus among offspring of women who did not take folic acid (3.1/10,000) was similar to that previously reported (1, 2), while the rate of imperforate anus among offspring of women who took 400 µg of folic acid daily was reduced by one half (1.6/10,000). As did investigators in previous studies (2–5), we found that imperforate anus was more common among males, was commonly associated with other anomalies, and was associated with increasing maternal age. We did not find any association between imperforate anus and maternal history of diabetes, but the prevalence of reported maternal diabetes in our study population was low (0.64/10,000).

Many of the strengths and limitations of the data obtained from this public health campaign have been previously discussed (8). Strengths include the population-based nature of the study, with nearly complete ascertainment of outcomes among large numbers of women whose pregnancies lasted at least 20 weeks, and the prospective monthly recording of folic acid use before the outcome of pregnancy was known. Other strengths are the use of a system of prospective surveillance for birth defects, which was established before our evaluation began, and the establishment of diagnoses on the basis of photographs taken at birth and reviews of reports and photographs by several clinicians.

One limitation of the study was that folic acid use was not randomized, and the women who took folic acid pills may have differed systematically from those who did not in terms of factors that could influence the frequency of imperforate

anus. Controlling for region, education, and occupation did not change our findings. Because parity and maternal age were correlated, we could not control for their independent effects. However, controlling for either of these covariates had a small effect on the magnitude of the relative risk estimate. We did not collect information on maternal smoking, alcohol drinking, or use of other nutritional supplements. However, smoking and alcohol drinking are uncommon among women in China. At the time of this study, prenatal vitamins were not part of routine prenatal care in China, and multivitamin supplements were not available for purchase. Although we do not have data on other potential confounders, the population studied was relatively homogeneous. The general lifestyle among women in rural China, including local housing, access to health care, and health-seeking behavior, tends to be uniform.

Imperforate anus is a birth defect that requires surgical correction and is associated with significant infant morbidity. Prognosis is most favorable for low lesions and is variable for intermediate and high lesions (1). It has been postulated that imperforate anus and other caudal birth defects result from abnormal development of the umbilical arteries in the form of a single umbilical artery of vitelline rather than allantoic origin (1). However, the etiology of imperforate anus, like that of most birth defects, has remained ill-defined. Recent observations that maternal prenatal consumption of folic acid may reduce the risk for neural tube defects both with and without other major birth defects (18) suggest that the pathways through which folic acid works to reduce birth defect risk may be nonspecific and that folic acid may also have a protective effect against other birth defects. Our finding that maternal folic acid consumption may reduce the risk for imperforate anus with and without other anomalies is consistent with this idea.

The US Public Health Service has recommended that all women who might become pregnant take 400 µg of folic acid daily to reduce the risk for neural tube defects (19). Other countries have adopted this recommendation (20).

**TABLE 4. Rates of imperforate anus with no additional external anomalies and imperforate anus associated with other anomalies, and risk ratio for any use of folic acid pills, People's Republic of China, October 1, 1993–September 30, 1995**

Pill-taking group	No. of pregnancies	All regions (n = 222,314)							
		IA* with no additional external anomalies		IA with other caudal anomalies		IA with multiple anomalies‡		All cases of IA	
		Rate†	No.	Rate	No.	Rate	No.	Rate	No.
All non-pill-takers	95,531	1.9	18	0.5	5	0.6	6	3.1	30
All pill-takers	126,783	0.9	12	0.5	6	0.2	2	1.6	20
Risk ratio		0.50		0.90		0.25		0.50	
95% confidence interval		0.24, 1.04		0.28, 2.96		0.05, 1.24		0.29, 0.88	
Risk ratio, adjusted for maternal age		0.66		0.90		0.27		0.59	
95% confidence interval		0.31, 1.42		0.27, 3.06		0.05, 1.38		0.33, 1.07	

\* IA, imperforate anus.

† Rate per 10,000 pregnancies of at least 20 weeks' gestation.

‡ Excludes one infant with Johanson-Blizzard syndrome.

Our results suggest that the implementation of such recommendations may also help reduce the risk for other, non-neural-tube birth defects. The preventive effect of folic acid use before and during early pregnancy on non-neural-tube birth defects requires further exploration.

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