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Association Between Maternal Folic Acid Supplementation and Congenital Heart Defects in Offspring in Birth Cohorts From Denmark and Norway

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Background—Evidence linking individual-level maternal folic acid supplementation to offspring risk of congenital heart defects is lacking. We investigated whether folic acid supplementation in early pregnancy reduces offspring risk of heart defects in 2 large birth cohort studies.

Methods and Results—Women recruited in early pregnancy within the DNBC (Danish National Birth Cohort), 1996–2003, and MoBa (Norwegian Mother and Child Cohort Study), 2000–2009, were followed until delivery. Information on periconceptional intake of folic acid and other supplements was linked with information on heart defects from national registers. Among 197,123 births, we identified 2247 individuals with heart defects (114/10,000). Periconceptional (4 weeks before through 8 weeks after conception) use of folic acid plus other supplements (54.8%), folic acid only (12.2%), and non–folic acid supplements (5.0%) were compared with no supplement use (28.0%); the adjusted relative risks of heart defects were 0.99 (95% CI, 0.80–1.22), 1.08 (95% CI, 0.93–1.25), and 1.07 (95% CI, 0.97–1.19), respectively. For initiation of folic acid in the preconception period weeks 0 to 4 (33.7%) and the postconception periods 0 to 4 weeks (15.5%), 5 to 8 weeks (17.8%), and 9 to 12 weeks (4.6%), compared with no or late folic acid intake (29.1%), relative risks of heart defect were 1.11 (95% CI, 1.00–1.25), 1.09 (95% CI, 0.95–1.25), 0.98 (95% CI, 0.86–1.12), and 0.97 (95% CI, 0.78–1.20), respectively. Relative risks of severe defects, conotruncal defects, and septal defects showed similar results.

Conclusions—Folic acid was not associated with offspring risk of heart defects, including severe defects, conotruncal defects, or septal defects. (J Am Heart Assoc. 2019;8:e011615. DOI: 10.1161/JAHA.118.011615.)

Key Words: congenital cardiac defect • folate • MoBa (Norwegian Mother and Child Cohort Study) • pregnancy • prospective cohort study

Congenital heart defects are the most common birth defects worldwide, but their etiology is largely unknown.1,2 Gene defects contribute to the occurrence of heart malformations,3 but most heart defects occur in isolation in a family.4 Consequently, a large proportion of heart defects presumably arise in susceptible individuals who carry low-penetrance genes or gene combinations, possibly in interaction with maternal or intrauterine factors. Although it is well established that maternal intake of folic acid around the time of conception reduces the risk of neural tube defect in offspring,5,6 its association with reduced risk of congenital heart defects is only suggestive.7,8

Although the precise role of folic acid supplementation on cardiac morphogenesis remains unclear, folic acid may have a role in the migration of the cardiac neural cells that contribute to the development of the embryonic heart.9,10 It is important...
Clinical Perspective

What Is New?

• In 2 independent prospective birth cohorts of almost 200 000 births, we identified 2247 children with congenital heart defects. Our results do not support the hypothesis that maternal intake of supplements containing folic acid before or after conception reduces the risk of congenital heart defects in offspring.

• Our null association between maternal folic acid supplementation and offspring congenital heart defects was consistent across various definitions of folic acid exposure and types of cardiac defects.

What Are the Clinical Implications?

• Although most likely not harmful, the effect of maternal folic acid supplementation with respect to preventing cardiac birth defects may be questioned, at least in regions with sufficient intake of dietary folate.

• Our finding of no association between individual-level maternal folic acid supplementation and offspring heart defect is in contrast to time trend studies claiming a causal relationship between folic acid and heart defects when comparing trends of birth prevalence of heart defects before and after folic acid fortification of staple food.

We have utilized 2 unique large prospective birth cohorts, the DNBC (Danish National Birth Cohort) and MoBa (Norwegian Mother and Child Cohort Study) to investigate whether periconceptional folic acid supplementation measured as individual-level information of folic acid exposure from up to 4 weeks before through 8 weeks after conception, including the timing of folic acid initiation, reduces the risk of congenital heart defects.

Methods

We can make tabulated data and analytical methods available to other researchers on reasonable request sent to the corresponding author.

Independent Data Access and Analyses

All authors had the ability to query any aspect of the data either directly or through independent analysis. Authors N.Ø., S.B., and J.W. had full access to all data in the study and take responsibility for its integrity and the data analysis.

Birth Cohorts

DNBC, 1996–2003

More than 100 000 Danish women in early pregnancy were recruited to the DNBC. By linkage, all births were updated with information on heart defects in their children, retrieved from the National Patient Register (NPR) and the Causes of Death Register, classified into cardiac phenotypes as published previously. In addition, information on chromosomal aberrations from the Danish Cytogenetic Central Register, University of Aarhus, and familial heart defect cases from the Danish Familial Relational Database were included. The NPR contains information on in-patient diagnoses assigned since 1977 and outpatient diagnoses from 1995 onward. Diagnoses of heart defects in the NPR have been validated against clinical records. The Causes of Death Register contains death certificate information, including underlying cause of death and up to 3 contributing causes of death since 1970, with high validity for infant death causes. The Danish Cytogenetic Central Register was established in 1968 and contains reports on all pre- and postnatal chromosomal analyses performed in Denmark since 1970 and 1960, respectively.

MoBa, 2000–2009

By June 2009, >100 000 pregnant women were included in MoBa. We used the MoBa analytic data file version 7, linked with information from the Medical Birth Registry of Norway. All births in MoBa were updated with information on heart defect
from a national project database on heart defects, Congenital Heart Defects in Norway, Cardiovascular Disease in Norway Project (https://cvdnor.w.uib.no/), in which individuals with heart defects were ascertained from 4 data sources: the Medical Birth Registry of Norway, the hospitals’ patient administrative systems, Oslo University Hospital’s clinical database for children with heart disease, and the Causes of Death Registry.26 Individuals with heart defects had been assigned specific cardiac phenotypes before linkage with MoBa. Information on chromosomal aberration and familial heart defect was ascertained from the Medical Birth Registry.

Dietary Supplement: Assessment of Folic Acid Exposure

In the DNBC, information on folic acid brand names, dosage, and frequency of use was collected at enrollment covering the period from 4 weeks before the last menstrual period until enrollment (around the 12th gestational week). At enrollment the women answered a questionnaire including information on supplement use. This recruitment form had 2 formats during the study period: initially a format with a floating time window and then, halfway through the study period, a format with a fixed time window (each week specified from gestational week 4 to week 14). Information from the first recruitment form was manually set to the same format as the second recruitment form.27 In MoBa, the women answered a questionnaire including information on folic acid supplement use and intake of specific nutrients from 3 months before pregnancy and throughout pregnancy until enrollment (18th gestational week). More detailed information on exposure matrices in both cohorts has been published.28–30 Data have also been used to evaluate health campaigns for the intake of folic acid during pregnancy.29 Self-reported folic acid intake has been validated and found to mirror biological levels of folate in erythrocytes31 and in plasma.32 Therefore, use of folic acid is one of the most well-defined exposures so far characterized collaboratively between the 2 cohorts with the original objective of studying its influence on neural tube defects.

The embryonic heart forms from the second through the eighth weeks after conception.33 Therefore, the presumed window of susceptibility from insufficient maternal folate regarding embryonic heart development was defined as 4 weeks before through 8 weeks after conception. Exposure information of maternal folic acid supplement use was classified by 2 approaches (supplement type and timing), as suggested by Roth and colleagues.34,35 First, we assessed any supplement use 4 weeks before through 8 weeks after conception for 4 mutually exclusive exposure categories: folic acid (0.4 mg) plus other micronutrient supplements, only folic acid, other non–folic acid supplements, and no supplements (as the reference). Next, we evaluated initiation of any folic acid intake (folic acid plus other supplements were combined with folic acid only) for 4 time windows: 4 weeks before conception, 4 weeks after conception, 5 to 8 weeks after conception, and 9 to 12 weeks (9–11 weeks in Denmark) after conception, with no folic acid supplementation (no supplements was combined with non–folic acid supplements) in the period 4 weeks before to 12 weeks (11 weeks in Denmark) after conception (as the reference).

Case Ascertainment and Classification of Congenital Heart Defects

In both birth cohorts, infants with International Classification of Diseases, 10th Revision (ICD-10) codes for heart defects were classified into cardiac phenotypes in a hierarchical system33,26,36: heterotaxia, conotruncal defects, atrioventricular septal defects, anomalous pulmonary venous return, left ventricle outflow tract obstruction, right ventricle outflow tract obstruction, isolated septal defects (ventricular septal defects, atrial septal defects registered after 6 weeks, and ventricular plus atrial septal defects), complex defects, patent ductus arteriosus registered after 6 weeks or with surgical correction in preterm live births (gestational age <37 weeks) or live births at term (gestational age ≥37 weeks), other specified heart defects, unspecified heart defects (Table S1). In tables, we report relative risk estimates for any type of heart defect (not counting patent ductus arteriosus in preterm births), severe heart defects (heterotaxia, conotruncal defects, atrioventricular septal defects, anomalous pulmonary venous return, left or right ventricle outflow tract obstruction, complex defects combined), conotruncal defects, and septal defects (ventricular septal defects, atrial septal defects registered after 6 weeks, and ventricular plus atrial septal defects).

Potential Confounders and Stratification Variables

We decided a priori on the following potential confounders (variables associated with maternal folic acid supplement use and most likely also with the risk of heart defect in offspring) for the association between maternal folic acid supplementation and offspring heart defect risk: year of birth and birth order, maternal age, prepregnancy body mass index (kg/m²), and epilepsy (or intake of folic acid antagonists for maternal epilepsy). In addition, we also considered maternal socioeconomic status and education, pregestational diabetes mellitus, smoking, and alcohol consumption as possible confounders. We did not, however, include these factors in the final model because they were either associated only with the exposure (maternal education, smoking, alcohol consumption) or only

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with heart defects in the offspring (pregestational diabetes mellitus). We also explored the main effects when restricting the analyses by information on live births, singletons, mother without heart defect, or planned pregnancy. Individuals with missing data for the covariates among all births (10 352 of 197 213, 5.5%) and births with cardiac defect (117 of 2247, 5.2%) were not included in the multivariable regression analyses.

Study Cohort and Statistical Analysis

The 2 study cohorts consisted of live births, and MoBa also included stillbirths and pregnancy termination for fetal abnormalities. We excluded births with chromosomal aberrations (353 in DNBC and 140 births in MoBa). The association between maternal folic acid intake and the risk of heart defect was calculated as risk ratios (RRs) comparing offspring risk of heart defect among those exposed and not exposed to supplementation. The RRs were estimated with 95% CIs in log-linear binomial regression analyses with adjustment for the a priori confounders in the separate analyses of the Danish and Norwegian cohorts, and then with adjustment for interactions between country and the a priori country-specific confounders when combining the 2 cohorts.

Study Size and Power Calculation

A priori, we estimated that we would be able to detect a reduced risk of heart defect (all types combined) and conotruncal defects among infants exposed to folic acid supplementation of at least 16% and 41%, respectively, relative to unexposed infants. Estimates were based on 80% power and a 5% significance level, the Danish prevalence of heart defects without chromosomal aberrations in 93 per 10 000 and in 12 per 10 000 for conotruncal defects,23 and a mean prevalence of folic acid supplementation of 23% (Denmark 13.5%37 and Norway 32.0%28).

Ethics

We have approval from the DNBC’s steering committee (ref. no 2010-05; May 4, 2010), a general approval granted to the Statens Serum Institut from the Danish Data Protection Agency (J.nr.2008-54-0472; September 12, 2008), and an approval from the steering board of the Danish Cytogenetic Central Register. We also have approval from MoBa’s steering committee (PD757; 09/3237; February 5, 2010); the Regional Committee for Medical and Health Research Ethics, South-East Norway (2012/796); and the steering committee of the Cardiovascular Disease in Norway project. Written informed consent was obtained from all participating women.

Results

Denmark: DNBC

In the study cohort from Denmark of 94 228 births without chromosomal aberrations, we present information on mothers’ periconceptional intake of supplements (Table 1). In the period 1996–2003, maternal intake of folic acid plus other supplements not containing folic acid was reported in 59.0% of the births, intake of only folic acid was reported in 4.1%, use of other non–folic acid supplements was reported in 3.7%, and no maternal supplement intake was reported in 33.2%.

Norway: MoBa

The study cohort from Norway consisted of 102 985 births without chromosomal aberrations (Table 2). In the period 1999–2009, the mother reported intake of folic acid plus other supplements in 50.3% of the births, folic acid only in 19.0%, other supplements without folic acid in 6.2%, and no supplement intake in 24.5%.

Maternal use of folic acid or other supplements is shown for maternal and birth characteristics in both cohorts (Tables 1 and 2).

Cardiac Defects and Birth Prevalence in the Danish and Norwegian Birth Cohorts

Country-specific numbers of heart defects and birth prevalence are presented in Table 3. The 2 birth cohorts were combined, and among 197 213 births, there were 2247 infants with any type of heart defect, not counting patent ductus arteriosus among preterm births (birth prevalence 114 per 10 000 births). There were 583 infants with severe heart defect (30 per 10 000), of which 201 had conotruncal defect (10 per 10 000 births), and 1191 infants had septal defect (60 per 10 000).

Folic Acid Supplementation and Risk of Congenital Heart Defects in Denmark and Norway

Excluding 5.2% with missing values, the combined cohort consisted of 186 861 births. In the period from 4 weeks before through 8 weeks after conception, 54.8% of the mothers used folic acid plus other types of non–folic acid supplements, 12.2% took only folic acid supplements, 5.0% used non–folic acid supplements, and 28.0% did not use any supplements (Table 4). The adjusted RRs for any type of heart defect in offspring were 0.99 (95% CI, 0.80–1.22) for maternal intake of folic acid plus other supplements, 1.08 (95% CI, 0.93–1.25) for only folic acid supplements, and 1.07 (95% CI, 0.97–1.19) for non–folic acid supplements, compared with no supplement use. RR estimates were similarly nonsignificant.
### Table 1. Maternal Characteristics by Maternal Use of Supplements From 4 Weeks Before to 8 Weeks After Conception Among 94,228 Births Without Chromosomal Aberrations in the DNBC, Denmark, 1996–2003

| Characteristics                  | Total N=94,228 (100%) | No Supplements n=31,322 (33.2%) | Other Supplements No Folic Acid n=34,53 (3.7%) | Folic Acid Only n=38,89 (4.1%) | Folic Acid Plus Other Supplements n=55,564 (59.0%) | P Value, No Folic Acid vs Folic Acid Use* |
|----------------------------------|------------------------|---------------------------------|-----------------------------------------------|--------------------------------|------------------------------------------------|------------------------------------------|
| **Year of birth**                |                        |                                 |                                               |                                |                                                |                                          |
| 1996                             | 139 (0.15)             | 69 (49.6)                       | 12 (8.6)                                      | 0 (0)                          | 58 (41.7)                                     | <0.0001                                  |
| 1997                             | 750 (0.80)             | 346 (46.1)                      | 43 (5.7)                                      | 9 (1.2)                        | 352 (46.9)                                    |                                          |
| 1998                             | 12 077 (12.8)          | 4442 (36.8)                     | 767 (6.4)                                     | 260 (2.2)                      | 6608 (54.7)                                   |                                          |
| 1999                             | 18 548 (19.7)          | 7188 (38.8)                     | 924 (5.0)                                     | 459 (2.5)                      | 9977 (53.8)                                   |                                          |
| 2000                             | 20 540 (21.8)          | 7230 (35.2)                     | 764 (3.7)                                     | 823 (4.0)                      | 11 723 (57.1)                                 |                                          |
| 2001                             | 20 135 (21.4)          | 6254 (31.1)                     | 555 (2.8)                                     | 933 (4.6)                      | 12 393 (61.6)                                 |                                          |
| 2002                             | 18 084 (19.2)          | 4681 (25.9)                     | 324 (1.8)                                     | 1123 (6.2)                     | 11 956 (61.6)                                 |                                          |
| 2003                             | 3955 (4.2)             | 1112 (28.1)                     | 64 (1.6)                                      | 282 (7.1)                      | 2497 (63.1)                                   |                                          |
| **Maternal age, y**              |                        |                                 |                                               |                                |                                                |                                          |
| ≤24                              | 8996 (9.55)            | 3871 (43.0)                     | 343 (3.8)                                     | 287 (3.2)                      | 4495 (50.0)                                   | <0.0001                                  |
| 25–29                            | 35 891 (38.1)          | 11 515 (32.1)                   | 1187 (3.3)                                    | 1488 (4.2)                     | 21 701 (60.5)                                 |                                          |
| 30–34                            | 35 117 (37.3)          | 11 277 (32.1)                   | 1279 (3.6)                                    | 1479 (4.2)                     | 21 082 (60.0)                                 |                                          |
| ≥35                              | 14 113 (15.0)          | 4628 (32.8)                     | 640 (4.5)                                     | 627 (4.4)                      | 8218 (58.2)                                   |                                          |
| Missing                          | 111 (0.12)             | 31 (27.9)                       | 4 (3.6)                                       | 8 (7.2)                        | 68 (61.3)                                     |                                          |
| **Birth order**                  |                        |                                 |                                               |                                |                                                |                                          |
| First                            | 41 698 (44.3)          | 12 255 (29.4)                   | 1374 (3.3)                                    | 2049 (4.9)                     | 26 020 (62.4)                                 | <0.0001                                  |
| Second                           | 32 425 (34.4)          | 10 829 (33.4)                   | 1226 (3.8)                                    | 1188 (3.7)                     | 19 182 (59.2)                                 |                                          |
| Third                            | 11 435 (12.1)          | 4542 (39.7)                     | 497 (4.4)                                     | 384 (3.4)                      | 6012 (52.6)                                   |                                          |
| Fourth or higher                 | 2649 (2.8)             | 1171 (44.2)                     | 146 (5.5)                                     | 76 (2.9)                       | 1256 (47.4)                                   |                                          |
| Missing                          | 6021 (6.4)             | 2525 (41.9)                     | 210 (3.5)                                     | 192 (3.2)                      | 3094 (51.4)                                   |                                          |
| **Maternal body mass index before pregnancy (kg/m²)** |                        |                                 |                                               |                                |                                                |                                          |
| <20                              | 14 414 (15.3)          | 4516 (31.3)                     | 591 (4.1)                                     | 596 (4.1)                      | 8711 (60.4)                                   | <0.0001                                  |
| 20–24                            | 48 238 (51.2)          | 15 178 (31.5)                   | 1843 (3.8)                                    | 2054 (4.3)                     | 29 163 (60.5)                                 |                                          |
| 25–29                            | 16 866 (17.9)          | 5867 (34.7)                     | 544 (3.2)                                     | 682 (4.0)                      | 9793 (58.0)                                   |                                          |
| 30–34                            | 5255 (5.6)             | 1904 (36.2)                     | 160 (3.0)                                     | 223 (4.2)                      | 2968 (56.5)                                   |                                          |
| ≥35                              | 1993 (2.1)             | 748 (37.5)                      | 41 (2.1)                                      | 94 (4.7)                       | 1110 (56.0)                                   |                                          |
| Missing                          | 7442 (7.9)             | 3109 (41.8)                     | 274 (3.7)                                     | 240 (3.2)                      | 3819 (51.3)                                   |                                          |
| **Maternal heart defect**        |                        |                                 |                                               |                                |                                                |                                          |
| Yes                              | 445 (0.47)             | 136 (30.6)                      | 12 (2.7)                                      | 13 (2.9)                       | 284 (63.8)                                    | 0.14                                     |
| No                               | 93 783 (99.5)          | 31 186 (33.3)                   | 3441 (3.7)                                    | 3876 (4.1)                     | 55 280 (58.9)                                 |                                          |
| **Epilepsy before pregnancy**    |                        |                                 |                                               |                                |                                                |                                          |
| Yes                              | 351 (0.37)             | 82 (23.4)                       | 13 (3.7)                                      | 50 (14.2)                      | 206 (58.7)                                    | <0.0001                                  |
| No                               | 93 877 (99.6)          | 31 240 (33.3)                   | 3440 (3.7)                                    | 3839 (4.1)                     | 55 358 (59.0)                                 |                                          |
| **Pregestational diabetes mellitus** |                        |                                 |                                               |                                |                                                |                                          |
| Yes                              | 285 (0.3)              | 96 (33.7)                       | 12 (4.2)                                      | 9 (3.2)                        | 168 (58.9)                                    | 0.83                                     |
| No                               | 93 943 (99.7)          | 31 226 (33.2)                   | 3441 (3.7)                                    | 3880 (4.1)                     | 55 396 (59.0)                                 |                                          |
| **Maternal smoking in pregnancy** |                        |                                 |                                               |                                |                                                |                                          |
| Nonsmokers                       | 67 769 (71.9)          | 20 869 (30.8)                   | 2440 (3.6)                                    | 3032 (4.5)                     | 41 428 (61.1)                                 | <0.0001                                  |

Continued
for conotruncal defects, severe heart defects, and septal defects for any combination of intake of folic acid and non–folic acid supplementation.

We next analyzed initiation of any folic acid supplementation within the period from 4 weeks before to 12 weeks after conception (Table 4). Among the 186,861 births, 33.7% of the mothers started folic acid supplementation during weeks 0 to 4, 15.3% started during weeks 5 to 8, and 4.6% started during weeks 9 to 12, whereas 29.1% did not take folic acid supplementation or started to take folic acid supplementation after week 12. The adjusted RRs of any heart defect in offspring were 1.11 (95% CI, 1.00–1.25), 1.09 (95% CI, 0.95–1.25), 0.98 (95% CI, 0.86–1.12), and 0.97 (95% CI, 0.78–1.20) for maternal folic acid intake in weeks 0 to 4, 5 to 8, and 9 to 12, respectively, compared with the reference group with no or late folic acid intake. RR estimates were similarly nonsignificant for severe heart defects, conotruncal defects, and septal defects for any timing of folic acid use compared with no folic acid use.

Numbers and relative risks of offspring heart defect for maternal intake of folic acid and other non–folic acid supplements were estimated separately for Denmark and Norway (Tables S2 and S3). In each country, the relative risk of heart defects in offspring was nonsignificant when comparing folic acid with no folic acid in early pregnancy.

In Table S4, adjusted RRs in the combined cohort showed similar null findings for the association between folic acid and any heart defect or conotruncal defects using the final model, model 2 with further adjustment for maternal education, or model 3 with...
Table 2. Maternal Characteristics by Maternal Use of Supplements From 4 Weeks Before to 8 Weeks After Conception Among 102,985 Births Without Chromosomal Aberrations in MoBa, Norway, 1999–2009

| Characteristics                              | Total N=102 985 (100%) | No Supplements n=25 229 (24.5%) | Other Supplements n=6 431 (6.24%) | Folic Acid Only n=19 555 (19.0%) | Folic Acid Plus Other Supplements n=51 770 (50.3%) | P Value, No Folic Acid vs Folic Acid use* |
|-----------------------------------------------|------------------------|---------------------------------|----------------------------------|---------------------------------|-----------------------------------------------|------------------------------------------|
| **Year of birth†**                            |                        |                                 |                                  |                                 |                                               |                                          |
| 1999                                          | 46 (0.04)              | 30 (65.2)                       | 4 (8.7)                          | 6 (13.0)                        | 6 (13.0)                                      | <0.0001                                  |
| 2000                                          | 2010 (2.0)             | 1047 (52.1)                     | 223 (11.1)                       | 316 (15.7)                      | 424 (21.1)                                    |                                          |
| 2001                                          | 3931 (3.8)             | 1890 (48.8)                     | 443 (11.3)                       | 609 (15.5)                      | 989 (25.2)                                    |                                          |
| 2002                                          | 8293 (8.1)             | 3960 (47.8)                     | 846 (10.2)                       | 1321 (15.9)                     | 2166 (26.1)                                   |                                          |
| 2003                                          | 12 142 (11.8)          | 4615 (38.0)                     | 1133 (9.3)                       | 2208 (18.2)                     | 4186 (34.5)                                   |                                          |
| 2004                                          | 13 073 (12.7)          | 3282 (25.1)                     | 845 (6.5)                        | 2541 (19.4)                     | 6405 (49.0)                                   |                                          |
| 2005                                          | 15 117 (14.7)          | 3181 (21.0)                     | 785 (5.2)                        | 3094 (20.5)                     | 8057 (53.3)                                   |                                          |
| 2006                                          | 16 775 (16.3)          | 2872 (17.1)                     | 867 (5.2)                        | 3177 (18.9)                     | 9859 (58.8)                                   |                                          |
| 2007                                          | 15 474 (15.0)          | 2265 (14.6)                     | 661 (4.3)                        | 3028 (19.6)                     | 9520 (61.5)                                   |                                          |
| 2008                                          | 12 904 (12.5)          | 1695 (13.1)                     | 514 (4.0)                        | 2620 (20.3)                     | 8075 (62.6)                                   |                                          |
| 2009                                          | 3220 (3.1)             | 392 (12.2)                      | 110 (3.4)                        | 635 (19.7)                      | 2083 (64.7)                                   |                                          |
| **Maternal age, y‡**                          |                        |                                 |                                  |                                 |                                               |                                          |
| ≤24                                           | 11 301 (11.0)          | 3999 (35.4)                     | 883 (7.8)                        | 2011 (17.8)                     | 4408 (39.0)                                   | <0.0001                                  |
| 25–29                                         | 33 730 (32.8)          | 7920 (23.5)                     | 1846 (5.5)                       | 6739 (20.0)                     | 17 225 (51.1)                                 |                                          |
| 30–34                                         | 39 916 (38.8)          | 8881 (22.3)                     | 2378 (6.0)                       | 7772 (19.5)                     | 20 885 (52.3)                                 |                                          |
| ≥35                                           | 18 038 (17.5)          | 4429 (24.6)                     | 1324 (7.3)                       | 3033 (16.8)                     | 9252 (51.3)                                   |                                          |
| **Birth order†**                              |                        |                                 |                                  |                                 |                                               |                                          |
| First                                         | 46 367 (45.0)          | 9566 (20.6)                     | 2723 (5.9)                       | 8282 (17.9)                     | 25 796 (55.6)                                 | <0.0001                                  |
| Second                                        | 36 576 (35.5)          | 8936 (24.4)                     | 2105 (5.8)                       | 7616 (20.8)                     | 17 919 (49.0)                                 |                                          |
| Third or higher                               | 20 042 (19.5)          | 6727 (33.6)                     | 1603 (8.0)                       | 3657 (18.3)                     | 8055 (40.2)                                   |                                          |
| **Maternal body mass index before pregnancy (kg/m²)†**                   |                        |                                 |                                  |                                 |                                               |                                          |
| <20                                           | 12 700 (12.3)          | 2953 (23.3)                     | 875 (6.9)                        | 2104 (16.6)                     | 6768 (53.3)                                   | <0.0001                                  |
| 20–24                                         | 56 079 (54.5)          | 12 656 (22.6)                   | 3592 (6.4)                       | 10 450 (18.6)                   | 29 381 (52.4)                                 |                                          |
| 25–29                                         | 21 735 (21.1)          | 5671 (26.1)                     | 1273 (5.9)                       | 4505 (20.7)                     | 10 286 (47.3)                                 |                                          |
| 30–24                                         | 7067 (6.9)             | 2154 (30.5)                     | 366 (5.2)                        | 1442 (20.4)                     | 3105 (43.9)                                   |                                          |
| ≥35                                           | 2608 (2.5)             | 784 (30.1)                      | 132 (5.1)                        | 553 (21.2)                      | 1139 (43.7)                                   |                                          |
| Missing data                                  | 2796 (2.7)             | 1011 (36.2)                     | 193 (6.9)                        | 501 (17.9)                      | 1091 (39.0)                                   |                                          |
| **Maternal heart defect**                     |                        |                                 |                                  |                                 |                                               |                                          |
| Yes                                           | 640 (0.62)             | 160 (25.0)                      | 48 (7.5)                         | 103 (16.1)                      | 329 (51.4)                                    | 0.20                                     |
| No                                            | 102 345 (99.4)         | 25 069 (24.5)                   | 6383 (6.2)                       | 19 452 (19.0)                   | 51 441 (50.3)                                 |                                          |
| **Epilepsy before pregnancy**                 |                        |                                 |                                  |                                 |                                               |                                          |
| Yes                                           | 687 (0.67)             | 160 (23.3)                      | 28 (4.1)                         | 140 (20.4)                      | 359 (52.3)                                    | 0.08                                     |
| No                                            | 102 288 (99.3)         | 25 069 (24.5)                   | 6403 (6.3)                       | 19 415 (19.0)                   | 51 407 (50.3)                                 |                                          |
| **Prepregnancy diabetes mellitus§**           |                        |                                 |                                  |                                 |                                               |                                          |
| Yes                                           | 698 (0.68)             | 172 (24.6)                      | 40 (5.7)                         | 123 (17.6)                      | 363 (52.0)                                    | 0.70                                     |
| No                                            | 102 287 (99.3)         | 25 057 (24.5)                   | 6391 (6.25)                      | 19 432 (19.0)                   | 51 407 (50.2)                                 |                                          |
| **Smoking before pregnancy**                  |                        |                                 |                                  |                                 |                                               |                                          |
| Daily                                         | 18 598 (18.1)          | 6574 (35.4)                     | 1318 (7.1)                       | 3438 (18.5)                     | 7268 (39.1)                                   | <0.0001                                  |

Continued
the following covariates added to model 1: maternal diabetes mellitus, maternal smoking before conception, and maternal alcohol consumption 3 months before conception.

In sensitivity analyses, we restricted the combined cohort to live births, singleton births, births to mothers who did not have a heart defect, or births of a planned pregnancy, and we estimated sets of RRs for offspring risk of any heart defect (Table S5). The RRs showed no association between folic acid and any heart defect regardless of various restrictions of the birth cohort.

**Discussion**

In 2 independent national birth cohorts in Denmark and Norway of almost 200,000 pregnancies with prospectively collected information on folic acid supplement intake with follow-up of all births, we identified 2247 births with congenital heart defects in the offspring. We did not find support for the hypothesis that maternal periconceptional supplementation containing folic acid would reduce the risk of congenital heart defects. The findings were consistent using 2 exposure approaches, any intake of folic acid supplementation during the period from 4 weeks before and 8 weeks after conception, and timing of such intake in the periconceptional period. Specifically the risks of conotruncal defects, severe heart defects, or septal defects were not reduced by such supplementation.

**Previous Findings From Studies of the Association Between Folic Acid and Congenital Heart Defects**

There are studies comparing birth prevalence trends before and after staple food folic acid fortification\(^3\),\(^3\) claiming a causal relationship between folic acid and heart defect.\(^3\) However, a prerequisite for discussion of causal inference are studies

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**Table 2. Continued**

| Characteristics                      | Total N=102 985 (100%) | No Supplements n=25 229 (24.5%) | Other Supplements n=6431 (6.24%) | Folic Acid Only n=19 555 (19.0%) | Folic Acid Plus Other Supplements n=51 770 (50.3%) | P Value, No Folic Acid vs Folic Acid use* |
|--------------------------------------|------------------------|----------------------------------|----------------------------------|----------------------------------|-----------------------------------------------|------------------------------------------|
| Sometimes                            | 10 309 (10.0)          | 2388 (23.2)                      | 628 (6.1)                       | 1917 (18.6)                      | 5376 (52.2)                                   |                                          |
| No                                   | 61 352 (59.6)          | 11 346 (18.5)                    | 3315 (5.4)                      | 11 929 (19.4)                    | 34 762 (56.7)                                 |                                          |
| Missing                              | 12 726 (12.4)          | 4921 (38.7)                      | 1170 (9.2)                      | 2271 (17.9)                      | 4364 (34.3)                                   |                                          |
| Prepregnancy alcohol intake*         |                        |                                  |                                  |                                  |                                               |                                          |
| Yes                                  | 88 509 (85.9)          | 16 824 (20.4)                    | 4635 (5.61)                     | 16 027 (19.4)                    | 45 149 (54.6)                                 | <0.0001                                  |
| No                                   | 7167 (6.96)            | 1459 (34.0)                      | 363 (8.46)                      | 785 (18.3)                       | 1686 (39.3)                                   |                                          |
| Missing                              | 7309 (7.10)            | 1878 (44.7)                      | 419 (9.97)                      | 702 (16.7)                       | 1205 (28.7)                                   |                                          |
| Maternal education, y                |                        |                                  |                                  |                                  |                                               |                                          |
| <12                                  | 20 535 (19.9)          | 7987 (38.9)                      | 1648 (8.03)                     | 3661 (17.8)                      | 7239 (35.3)                                   | <0.0001                                  |
| 12                                   | 14 458 (14.0)          | 4393 (30.4)                      | 1081 (7.48)                     | 2717 (18.8)                      | 6267 (43.4)                                   |                                          |
| 13–16                                | 39 916 (38.8)          | 8021 (20.1)                      | 2229 (5.58)                     | 8071 (20.2)                      | 21 595 (54.1)                                 |                                          |
| ≥17                                  | 22 816 (22.2)          | 3337 (14.6)                      | 1116 (4.89)                     | 4166 (18.3)                      | 14 197 (62.2)                                 |                                          |
| Missing data                         | 5260 (5.11)            | 1491 (28.4)                      | 357 (6.79)                      | 940 (17.9)                       | 2472 (47.0)                                   |                                          |
| Planned pregnancy                    |                        |                                  |                                  |                                  |                                               |                                          |
| Yes                                  | 82 078 (79.7)          | 18 144 (22.1)                    | 4664 (5.68)                     | 16 377 (20.0)                    | 42 893 (52.3)                                 | <0.0001                                  |
| No                                   | 19 673 (19.1)          | 6858 (33.8)                      | 1672 (8.50)                     | 2997 (15.2)                      | 8346 (42.4)                                   |                                          |
| Missing data                         | 1234 (1.20)            | 427 (34.6)                       | 95 (7.70)                       | 181 (14.7)                       | 531 (43.0)                                    |                                          |
| Plurality†                           |                        |                                  |                                  |                                  |                                               |                                          |
| Single                               | 99 320 (96.4)          | 24 450 (24.6)                    | 6226 (6.3)                      | 18 800 (18.9)                    | 49 844 (50.2)                                 | <0.0001                                  |
| Twins/triplets                       | 3665 (3.6)             | 779 (21.3)                       | 205 (5.6)                       | 755 (20.6)                       | 1926 (52.6)                                   |                                          |

Data are shown as n (%). MBRN indicates Medical Birth Registry of Norway; MoBa, Norwegian Mother and Child Cohort Study.

*No folic acid use (no/other supplements, no folic acid) vs folic acid use (folic acid only/folic acid plus other supplements) 4 weeks before to 8 weeks after gestation (combining –4 to 0, 0–4, and 5–8 weeks).

†Numbers from MBRN.

Diabetes mellitus type 1 or type 2 before pregnancy, registered in MoBa and/or MBRN.

*Alcohol intake 3 months before pregnancy.
Interpretation of the Null Association Between Periconceptional Folic Acid and Cardiac Defects

Table 3. Numbers and Birth Prevalence of Congenital Heart Defects Among 94 228 Births in the DNBC, 1996–2003, and Among 102 985 Births in MoBa, Norway, 2000–2009

|                              | Denmark (n=94 228) | Norway (n=102 985) |
|------------------------------|--------------------|--------------------|
|                              | n                  | n/10 000           | n                  | n/10 000 |
| Any heart defect             | 1077               | 114                | 1434               | 139     |
| Any heart defect without preterm PDA | 995  | 106                | 1252               | 122     |
| Heterotaxia                  | 16                 | 1.7                | 13                 | 1.3     |
| Conotruncal defect           | 105                | 11                 | 96                 | 9.3     |
| AVSD                         | 34                 | 3.6                | 27                 | 2.6     |
| APVR                         | 8                  | 0.8                | 12                 | 1.2     |
| LVOTO                        | 96                 | 10                 | 84                 | 8.2     |
| RVOTO                        | 43                 | 4.6                | 48                 | 4.7     |
| Septal defect, isolated      | 445                | 47                 | 746                | 72      |
| VSD                          | 235                | 25                 | 586                | 60      |
| ASD                          | 141                | 15                 | 138                | 13      |
| VSD+ASD                      | 34                 | 3.6                | 12                 | 1.2     |
| Unspecified septal defect    | 35                 | 3.7                | 10                 | 1.0     |
| Other complex defects        | 1                  | 0.1                | 0                  |         |
| Isolated PDA*                | 155                | 16                 | 279                | 27      |
| At-term gestation            | 73                 | 7.7                | 97                 | 9.4     |
| Preterm gestation            | 82                 | 8.7                | 182                | 18      |
| Other specified defects      | 87                 | 9.2                | 91                 | 8.8     |
| Unspecified cardiac defects  | 87                 | 9.2                | 38                 | 3.7     |

APVR indicates anomalous pulmonary venous return; ASD, atrial septal defect; AVSD, atrioventricular septal defect; DNBC, Danish National Birth Cohort; LVOTO, left ventricle outflow tract obstruction; MoBa, Norwegian Mother and Child Cohort Study; PDA, patent ductus arteriosus; RVOTO, right ventricle outflow tract obstruction; VSD, ventricular septal defect.

*Isolated PDA in preterm infants (gestational age <37 weeks) was not included in the regression analyses.

designed with individual-level information of maternal folic acid supplement use and infant risk of heart defect. Although several studies have individual-level information of folic acid intake, these studies have conflicting findings most likely related to different design, bias, or confounding, they could also be explained by regional differences in dietary vitamin insufficiency. A detailed discussion of previous reports in the context of regions—for example, the United States,7,15,16,40–42 Hungary,11,43 Western Australia,14 the Netherlands,8,13 China,12 and Norway17—can be found in Data S1.

To summarize previous findings from studies of the association between folic acid and congenital heart defects, we found 1 randomized controlled trial reporting a nonsignificant protective effect of high-dose folic acid supplements,11 whereas 1 cohort study17 and most other case–control studies7,13–16,40,42 except one of the Dutch studies8 do not support the hypothesis that folic acid supplementation prevents congenital heart defects. The overall lack of a protective effect from folic acid supplementation for heart defect risk in previous studies supports the null findings in the present study and is in stark contrast to inference drawn from trend studies of birth defects, including heart defects, before-and-after folic acid fortification, or folic acid recommendation to fertile women.38,44

In support of the null findings in the present birth cohort study from Norway and Denmark, in particular for conotruncal defects, is a report using a Mendelian randomization approach that genotyped >3000 individuals with conotruncal defects for the variants in the MTHFR (methylene tetrahydrofolate reductase) gene.45 The variant 677TT has previously been found to be associated with reduced folate levels in blood and could be associated with offspring risk of heart defects. The multicenter study found no association between the variant 677TT and the risk of conotruncal defects compared with healthy controls. The authors also performed a meta-analysis of 16 published studies on the relationship between conotruncal heart defects and the variant 677TT and found a weak positive association, likely explained by publication bias: namely, that the studies with nonsignificant or null associations are less frequently published.
### Table 4. Relative Risk of Congenital Heart Defect* (Overall), Severe Heart Defect†, Conotruncal Defect‡, and Septal Defect§ by Maternal Intake of Folic Acid Supplements in the Periconceptional Period, Combining 94,228 Births in the DNBC, Denmark, 1996–2003, and 102,985 Births in MoBa, Norway 1999–2009

| Supplement use (4 wk before to 8 wk after conception) | Total Births, N=186,861 | Any Congenital Heart Defect, n=2,213 | Severe Heart Defect, n=551 | Conotruncal Heart Defect, n=187 | Septal Defect, n=1,139 |
|---------------------------------------------------------|--------------------------|--------------------------------------|----------------------------|---------------------------|-----------------------|
| None (**4 wk before to 12 wk after conception**)       | 52,401 (28.0)            | 569 (1 Reference)                    | 157 (1 Reference)          | 55 (1 Reference)          | 296 (1 Reference)     |
| Other supplements, no folic acid                       | 9,414 (5.0)              | 104 (0.99 0.80–1.22)                | 25 (0.92 0.61–1.41)        | 8 (0.84 0.40–1.77)        | 61 (1.06 0.80–1.39)   |
| Folic acid only                                        | 22,695 (12.2)            | 279 (1.08 0.93–1.25)                | 68 (1.10 0.82–1.48)        | 22 (0.98 0.59–1.64)       | 155 (1.03 0.84–1.26)  |
| Folic acid plus other supplementation                  | 102,351 (54.8)           | 1,178 (1.07 0.97–1.19)              | 301 (1.02 0.84–1.25)       | 102 (0.93 0.66–1.31)      | 627 (1.06 0.92–1.22)  |

Initiation of folic acid† (4 wk before to 12 wk after conception)

| initiation of folic acid† | Total Births, N=142 (28.4) | Any Congenital Heart Defect, n=96 | Severe Heart Defect, n=57 | Conotruncal Heart Defect, n=14 | Septal Defect, n=52 |
|---------------------------|-----------------------------|-----------------------------------|---------------------------|-----------------------------|---------------------|
| None (**4 wk before to 12 wk after conception**)       | 53,142 (57.7)              | 1,111 (1.10 1.00–1.25)            | 192 (1.08 0.87–1.34)      | 65 (0.95 0.66–1.38)        | 397 (1.08 0.92–1.26) |
| Weeks –4 to –1            | 62,924 (33.7)              | 753 (1.11 1.00–1.25)              | 192 (1.08 0.87–1.34)      | 65 (0.95 0.66–1.38)        | 397 (1.08 0.92–1.26)   |
| Weeks 0–4                 | 28,961 (15.5)              | 348 (1.09 0.95–1.25)              | 82 (1.01 0.77–1.33)       | 29 (0.93 0.59–1.48)        | 199 (1.11 0.92–1.33)   |
| Weeks 5–8                 | 33,161 (17.8)              | 356 (0.98 0.86–1.12)              | 95 (1.00 0.77–1.30)       | 30 (0.85 0.54–1.33)        | 186 (0.93 0.77–1.11)   |
| Weeks 9–12**              | 86,732 (4.6)               | 96 (0.97 0.78–1.20)               | 24 (0.97 0.63–1.50)       | 6 (0.68 0.29–1.58)         | 56 (1.00 0.75–1.32)    |

12 weeks after conception all showed a null association with offspring risk of cardiac malformation. Fifth, it could be that folic acid supplements prevent neural tube defects5,6 but not cardiac malformations. Maternal folate insufficiency may act differently during different time windows in embryonic organ development; folate insufficiency probably disturbs neural tube closure corresponding to an early period in embryo formation but not formation of the later embryonic heart development corresponding to a somewhat later period. Sixth, there could be nondifferential misclassification or measurement error of folic acid exposure and timing, which may bias the estimate to the null value. Interestingly, we found similar nonsignificant country-specific relative risks despite differences in collection and classification of folic acid intake.

Although most likely not harmful,46,47 the effect of maternal folic acid supplementation with respect to preventing cardiac birth defects may be questioned, at least in regions with sufficient intake of dietary folate.

### Study Strengths and Limitations

The use of prospectively collected information on individual-level folic acid intake in early pregnancy precluded recall bias of exposure. We have complete follow-up of all births (except for 204 mothers who gave birth abroad) through the nationwide Medical Birth Registers. Before the analyses, we used an established method for classifying folic acid exposure by type (folic acid plus other supplements, only folic acid, non-folic supplements, no supplements) in the period from 4 weeks before through 8 weeks after conception and by timing (initiation of supplement use) in the period from 4 weeks before through 11 to 12 weeks after conception,34,35

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corresponding to fetal cardiac development (third to eighth weeks after conception). Folic acid is a well-characterized exposure in the 2 cohorts\textsuperscript{27,28,48} and is found to mirror blood plasma folate.\textsuperscript{49} We did not have information on perigestational dietary folate, but from previous studies, supplemental folic acid outweighs dietary folate, as women reporting folic acid intake had significantly higher plasma folate levels than nonusers regardless of dietary intake.\textsuperscript{32}

Low ascertainment of severe heart defects, including conotruncal defects, is unlikely because these defects almost always come to medical attention, either because of the need for surgery or at death. In Denmark there is good agreement between severe heart defect diagnoses in the NPR compared with hospital records,\textsuperscript{24} and in Norway, nationwide health registries and databases, as well as a clinical register, were combined to ensure virtually complete ascertainment of all heart defects among live births, stillbirths, and terminated pregnancies, including information on chromosomal aberrations and familial heart defect cases. All cases from the clinical register at Oslo University Hospital have been coded by senior pediatric cardiologists.

By combining country-specific findings in 2 national birth cohorts, our study cohort was of sufficient size to produce narrow CIs, although we could not estimate RRs for the very rare outcomes of severe defects. A large set of potential confounders were available for evaluation, for adjustment or restriction of the study population for sensitivity analyses. Finally, although exposure data were collected somewhat differently in the 2 cohorts, we decided not to harmonize exposure among the 2 birth cohorts.\textsuperscript{50} The combined country-specific estimates with corresponding country-specific confounders illustrate that despite the variation in exposure collection and country-specific birth prevalence of heart defect, we found no significant association between maternal folic acid supplementation and heart defect risk in either birth cohort.

In conclusion, maternal periconceptional use of folic acid supplements was not associated with offspring risk of congenital heart defects, including conotruncal defects or other severe defects, as well as septal defects, after combining 2 national birth cohorts with prospectively collected information of periconceptional supplement intake. The possible effect of maternal folic acid supplementation with respect to preventing cardiac birth defects may be questioned, at least in regions with sufficient intake of dietary folate.

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Disclosures
None.

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SUPPLEMENTAL MATERIAL
Data S1.

A detailed discussion on previous findings from studies of the association between folic acid and congenital heart defects

From the United States, there are six case-control studies worth mentioning. From California, 1987-1998, mothers of 207 children with conotruncal heart defects and 481 randomly selected infants without malformations were telephone interviewed. There was a reduced risk of conotruncal heart defects in children of mothers who had taken multivitamins or folic acid fortified cereals (OR 0.70 95% CI 0.46-1.1), but with a non-significant estimate. In a later study from California, 1999-2004, no preventive effect of multivitamins with folic acid on conotruncal defects was found, when comparing 186 infants with transposition of the great arteries or Tetralogy of Fallot (ToF) to 426 live controls. In the Slone Epidemiology Unit Birth Defects Study, 1993-1996, 101 infants with conotruncal defects, 86 ventricular septal defect (VSD), and 521 controls were recruited from hospitals in Boston, Philadelphia, and Toronto, Canada. No significant association were found between prenatal vitamins containing folic acid, and the types of heart defect. In the Baltimore-Washington Infant Study, 1987-1989, 126 non-syndromic outflow tract defects (53 transposition of the great arteries (TGA) and 73 other, e.g. ToF, double outlet right ventricle (DORV), truncus arteriosus (TA), supracristal VSD) were compared with 679 controls; the authors found no preventive effect of folic acid supplementations before pregnancy, a finding also supported by null findings for folic acid intake in the 5 weeks pregestational period, and for the lowest quartile of total folate intake. In a study from the US National Birth Defects Prevention Study, 1997-2002, with the intention to investigate the association between maternal smoking and offspring heart defect, the authors also reported the potential confounder maternal folic acid intake in the periconceptional period one month later.
before and 2 months after conception. By our calculation of the authors’ numbers (2284 exposed heart
defect out of 3,067 all heart defects; 2,935 non-exposed controls out of 3947 controls), the unadjusted OR
was 1.01 (95% CI 0.90-1.12), i.e. the association between folic acid and heart defect risk was null. In
another report from the National Birth Defects Prevention Study, 1997-2004 (which might overlap with the
study above), from Arkansas, California, Georgia (Atlanta), Iowa, Massachusetts, New Jersey (through
2002), New York, North Carolina (beginning 2003), Texas, Utah (from 2003 onwards) on the joint effect of
maternal diabetes and folic acid containing multivitamins on the risk of different types of heart defect in a
multi-center study,\textsuperscript{6} we calculated an unadjusted OR of 0.95 (95% CI 0.85-1.06) of any heart defect for
maternal use of multivitamins with folic acid (5,205 exposed with heart defects out of 5,979 individuals
with heart defects; 4,737 exposed controls out of 5,408 controls).

The Hungarian randomized controlled trial of neural tube defects,\textsuperscript{7} containing other defects as well, were
extended with 8 months follow-up,\textsuperscript{8} and reported a reduced risk of heart defect, but the difference was
non-significant comparing the group receiving multivitamin supplements containing high dose of 0.8 mg
folic acid with the group receiving trace element supplements other than folic acid. The numbers were
small, with only 10 exposed and 17 unexposed heart defect cases. In a later follow-up, but in case-control
design, 598 children with heart defect born in Hungary 1980-1996 were compared to 902 matched
controls;\textsuperscript{9} The authors reported a reduced risk of conotruncal heart defect in the group exposed to a very
high dose of folic acid, on average 5.6 mg/d.

In Western Australia, 1997-1998, with no folate fortification of staple food, there was no association
between low dose folic acid (0.2 mg or more) and offspring heart defect risk comparing several birth defect
outcomes, including 151 heart defects, to 578 live controls without birth defects, syndromes or
chromosomal aberrations.\textsuperscript{10}
Two recent Dutch case-control studies report from different areas. From the Northern Netherlands, 1996-2005, including 611 children with heart defect identified within a birth defect registry, and two control groups; 2,401 supposedly non-folate related birth defects, and 3,343 births of women participating in previous cross-sectional studies, reported an adjusted ORs of 0.82 (95% CI 0.68-0.98) and 0.74 (95% CI 0.62-0.88) of heart defect among offspring of women using periconceptional supplements with 0.4 mg/d folic acid comparing birth defect controls and live controls, respectively. However, from the Western Netherlands, 2003 onwards, the authors report a non-significant reduction of heart defect for folic acid intake of 0.4 mg or more (p=0.16), by our calculation, OR 0.79 (95% CI 0.57-1.10) when comparing 283 heart defect (ToF, TGA, atrioventricular septal defect (AVSD), Coarctation of aorta, Aortic valve stenosis, pulmonary valve stenosis, Hypoplastic left heart syndrome) to 308 controls.

From China, a hospital-based case-control study with 358 prenatally identified heart defect and 422 controls recruited consecutively among women receiving prenatal investigation in the provinces of Guangdong, Hubei, Fujian, and Shanxi, folic acid supplements reduced heart defect risk by 65%. However, there may be limitations of the study, not discussed by the authors, such as design, selection into the study, and classification of exposure.

In the recent study of around 520,000 births registered in the Medical Birth Registry of Norway, 1999-2009, with pre-birth registered use of folic acid and multivitamin supplements before pregnancy and during pregnancy, the authors identified 1,153 births with severe type of heart defect (heterotaxia, conotruncal defects, AVSD, anomalous pulmonary venous return (APVR), left or right ventricle outlet tract obstructions (LVOTO/RVOTO), other specified defects) and 3,280 with isolated septal defects (VSD, atrial septal defect (ASD), VSD+ASD). There was no significant association between intake of folic acid supplements (containing 0.4 mg folic acid) and severe heart defect, but a surprising 20% increase of septal defects.
Table S1. The classification system for congenital heart defect phenotypes.

| Main groups                                    | Detailed cardiac phenotypes                                                                 |
|------------------------------------------------|---------------------------------------------------------------------------------------------|
| Heterotaxia                                    | Situs inversus                                                                             |
|                                                | Isomerism                                                                                 |
|                                                | Dextrocardia or levocardia with other heart defect                                        |
| Conotruncal defects                            | Truncus arteriosus                                                                         |
|                                                | Transposition of the great arteries (TGA)                                                  |
|                                                | Tetralogy of Fallot (ToF)                                                                  |
|                                                | Pulmonary atresia with ventricular septal defect (ToF type)                                |
|                                                | Double outlet right ventricle (DORV)                                                       |
|                                                | Conoventricular septal defect                                                             |
|                                                | Interrupted aortic arch type B or C                                                        |
|                                                | Supravalvular aortic stenosis                                                             |
|                                                | Aortopulmonary window                                                                     |
| Atrioventricular septal defects                | Atrioventricular septal defects                                                           |
| Anomalous pulmonary venous return (APVR)       | Total anomalous pulmonary venous return                                                    |
|                                                | Partial anomalous pulmonary venous return                                                  |
| Left Ventricular Outflow Tract Obstructions (LVOTO) | Hypoplastic left heart syndrome (HLHS)                                                    |
|                                                | Mitral valve stenosis                                                                     |
|                                                | Coarctation of the aorta (CoA)                                                             |
|                                                | Interrupted aortic arch type A                                                             |
|                                                | Valvular aortic stenosis                                                                  |
| Right Ventricular Outflow Tract Obstructions (RVOTO) | Tricuspid atresia / stenosis                                                               |
|                                                | Hypoplastic right heart syndrome (HRHS)                                                    |
|                                                | Ebstein anomaly                                                                            |
|                                                | Valvular pulmonary atresia (not ToF anatomy)                                               |
|                                                | Arterial pulmonary atresia                                                                |
|                                                | Valvular pulmonary stenosis                                                                |
| Septal defects                                 | Atrial septal defects (ASD)                                                                 |
|                                                | Ventricular septal defects (VSD)                                                           |
|                                                | ASD + VSD                                                                                 |
|                                                | Otherwise specified or not specified septal defects                                        |
| Other complex cardiac defects                  | Congenitally corrected transposition of the great arteries (ccTGA)                         |
|                                                | Single ventricle (non-HLHS, non-HRHS)                                                      |
|                                                | Double inlet left ventricle (DOLV)                                                         |
|                                                | Absent PV                                                                                 |
| Other cardiac defects                          | Infundibular pulmonary stenosis                                                            |
|                                                | Pulmonary insufficiency                                                                    |
|                                                | Subaortic stenosis                                                                         |
|                                                | Aortic insufficiency                                                                       |
|                                                | Mitral insufficiency                                                                       |
|                                                | Pulmonary arterial stenosis                                                                |
|                                                | Cor triatriatum                                                                           |
|                                                | Coronary malformations                                                                     |
|                                                | Other specified malformation of the heart                                                  |
|                                                | Unspecified malformations of the heart, great arteries, great veins                        |
| Isolated Patent ductus arteriosus (PDA)        | Isolated patent ductus arteriosus (PDA)                                                    |
Severe heart defects: Heterotaxia, Conotruncal defects, Atrioventricular septal defect, APVR, LVOTO, RVOTO (except valvular pulmonary stenosis), Other complex defects.
Table S2. Relative risk (RR) of congenital heart defect* (overall), severe heart defect†, conotruncal defect‡, and septal defect§ by maternal intake of folic acid supplements in the periconceptional period among 94,228 births The Danish National Birth Cohort (DNBC), Denmark, 1996-2003.

| Supplement use (4 weeks before to 8 weeks after conception) | Total no. of births (N=995) | Any congenital heart defect* N=303 | Severe heart defect† N=105 | Conotruncal heart defect‡ N=445 | Septal defect§ N=445 |
|---------------------------------------------------------------|-----------------------------|----------------------------------|---------------------------|-------------------------------|---------------------|
| Supplement use (4 weeks before to 8 weeks after conception) | 94,228                      |                                  |                           |                               |                     |
| None                                                          | 31,322 (33.2)               | 311 99 1 1                       | 100 32 1 1               | 36 11 1 1                     | 134 43 1 1         |
| Other supplements, no folic acid                              | 3,453 (3.7)                 | 43 125 1.25 0.91-1.72            | 9 26 0.82 0.41-1.62      | 2 6 0.50 0.12-2.09            | 26 75 1.76 1.16-2.67|
| Folic acid only                                               | 3,889 (4.1)                 | 36 93 0.93 0.66-1.31            | 8 21 0.65 0.31-1.32      | 4 10 0.90 0.32-2.52           | 15 39 0.90 0.53-1.54|
| Folic acid plus other supplementation                         | 55,564 (59.0)              | 605 109 1.10 0.96-1.26          | 186 33 1.05 0.82-1.34    | 63 11 0.99 0.66-1.49          | 270 49 1.14 0.92-1.40|
| Initiation of folic acid** (4 weeks before to 11 weeks after conception) | 32,252 (34.9)               | 323 100 1 1                      | 101 31 1 1               | 38 12 1 1                     | 142 44 1 1         |
| Week 4 to 1                                                   | 32,917 (34.2)               | 360 109 1.09 0.94-1.27          | 106 32 1.03 0.78-1.35    | 38 12 0.98 0.63-1.54          | 161 49 1.11 0.89-1.39|
| Week 5 to 8                                                   | 10,785 (11.5)               | 107 99 0.99 0.80-1.23           | 31 29 0.92 0.61-1.37    | 10 9 0.79 0.39-1.58           | 47 44 0.99 0.71-1.38|
| Week 5 to 8                                                   | 15,751 (16.7)               | 174 110 1.10 0.92-1.32          | 57 36 1.16 0.84-1.60    | 19 12 1.02 0.59-1.78          | 77 49 1.11 0.84-1.47|
| Week 9 to 11                                                  | 2,523 (2.7)                 | 31 123 1.23 0.85-1.77           | 8 32 1.01 0.50-2.15      | 0                              | 18 71 1.62 0.99-2.64|

Note: Relative risk (RR) and 95% confidence interval (95% CI) calculated for each group compared to the reference group (no folic acid).
*†§ Congenital heart defects: see definitions in Table 4.

|| The number of events includes the entire cohort, whereas in the adjusted analyses, individuals with missing values for covariates were excluded (total births n=7,556; any heart defect n=85; severe defect n=22, conotruncal defects n=10; septal defects n=36).

# Relative risk (RR) with 95% confidence interval (CI) comparing supplement use with no use (reference) 4 weeks before to 8 weeks after conception (upper panel) or comparing initiation of folic acid supplements with no use/non-folic acid supplements (reference) 4 weeks before to 12 weeks after conception (lower panel). RRs adjusted for year of birth (1996-97, 1998, 2000, 2001, 2002-03), maternal age (≤24, 25-29, 30-34, ≥35 years), birth order (1, 2, 3+), maternal body mass index (<20, 20-24, 25-29, 30-34, ≥35), maternal heart defect (yes/no), maternal epilepsy before pregnancy (yes/no). Categories for adjustment variables were combined for severe and conotruncal defects.

** The four exposure categories in supplement use collapsed into no/yes; “no” is no folic acid use (no supplements and other supplements, no folic acid), and “yes” is folic acid use (folic acid only and folic acid plus other supplementation).
Table S3. Relative risk (RR) of congenital heart defect* (overall), severe heart defect†, conotruncal defect‡, and septal defect§ by maternal intake of folic acid supplements in the periconceptional period among 102,985 births in the Norwegian Mother and Child Cohort Study (MoBa), Norway, 1999-2009.

| Supplement use (4 weeks before to 8 weeks after conception) | Any congenital heart defect* N=1,252 | Severe heart defect† N=280 | Conotruncal heart defect‡ N=96 | Septal defect§ N=746 |
|-------------------------------------------------------------|-------------------------------------|-----------------------------|-------------------------------|---------------------|
| Total no. of births (%) || Crude RR 95% CI | Adjusted RR# 95% CI | Crude RR 95% CI | Adjusted RR# 95% CI | N | No. per 10,000 | Crude RR 95% CI | Adjusted RR# 95% CI | N | No. per 10,000 | Crude RR 95% CI | Adjusted RR# 95% CI | N | No. per 10,000 | Crude RR 95% CI | Adjusted RR# 95% CI |
| None | 25,229 (24.5) | 312 124 1 1 | 70 28 1 1 | 23 9 1 1 | 184 73 1 1 |
| Other supplements, no folic acid | 6,431 (6.2) | 65 101 0.82 0.63-1.07 0.83 0.64-1.09 | 16 25 0.90 0.52-1.54 0.97 0.56-1.67 | 6 9 1.02 0.42-2.51 1.08 0.43-2.67 | 36 56 0.77 0.54-1.10 0.77 0.54-1.11 |
| Folic acid only | 19,555 (19.0) | 248 127 1.03 0.87-1.21 1.07 0.90-1.27 | 62 32 1.14 0.81-1.61 1.25 0.87-1.79 | 19 10 1.07 0.58-1.96 1.07 0.56-2.03 | 142 73 1.00 0.80-1.24 1.00 0.80-1.25 |
| Folic acid plus other supplementation | 51,770 (50.3) | 627 121 0.98 0.86-1.12 1.02 0.88-1.18 | 132 25 0.92 0.69-1.23 1.03 0.75-1.42 | 48 9 1.02 0.62-1.67 1.00 0.58-1.73 | 384 74 1.02 0.85-1.21 1.00 0.83-1.21 |
| Initiation of folic acid** (4 weeks before to 12 weeks after conception) | | | | | |
| None | 25,084 (24.3) | 304 121 1 1 | 66 26 1 1 | 23 10 1 1 | 178 71 1 1 |
| Week -4 to -1 | 32,827 (31.9) | 417 127 1.05 0.91-1.21 1.09 0.93-1.28 | 95 29 1.10 0.80-1.50 1.25 0.88-1.76 | 32 10 1.06 0.62-1.82 1.07 0.59-1.93 | 248 76 1.06 0.88-1.29 1.04 0.85-1.28 |
| Week 0 to 5 | 19,471 (18.9) | 254 130 1.08 0.91-1.27 1.13 0.95-1.35 | 54 28 1.05 0.74-1.51 1.19 0.82-1.73 | 21 11 1.18 0.65-2.13 1.21 0.65-2.27 | 157 81 1.14 0.92-1.41 1.13 0.91-1.42 |
| Week 5 to 8 | 19,027 (18.5) | 204 107 0.89 0.74-1.06 0.91 0.76-1.09 | 45 24 0.90 0.62-1.31 0.91 0.61-1.36 | 14 7 0.80 0.41-1.56 0.75 0.37-1.51 | 121 64 0.90 0.71-1.13 0.88 0.70-1.12 |
| Week 9 to 12 | 6,576 (6.4) | 73 111 0.92 0.71-1.18 0.90 0.70-1.17 | 20 30 1.15 0.70-1.90 1.08 0.64-1.83 | 6 9 0.99 0.40-2.44 1.04 0.42-2.59 | 42 64 0.90 0.64-1.26 0.88 0.63-1.24 |
*†§ Congenital heart defects: see definitions in Table 4

The number of events includes the entire cohort, whereas in the adjusted analyses, individuals with missing values for covariates were excluded (total births n=2,796; any heart defect n=32; severe defect n=10, conotruncal defects n=4; septal defects n=16).

# Relative risk (RR) with 95% confidence interval (CI) comparing supplement use with no use (reference) 4 weeks before to 8 weeks after conception (upper panel) or comparing initiation of folic acid supplements with no use/non-folic acid supplements (reference) 4 weeks before to 12 weeks after conception (lower panel). RRs adjusted for year of birth (1999-2000, 2001, 2002, 2003, 2004, 2005, 2006, 2007, 2008-09), maternal age (years ≤24, 25-29, 30-34, ≥35), birth order (1, 2, 3+), maternal body mass index (<20, 20-24, 25-29, 30-34, ≥35), maternal heart defect (yes/no), maternal epilepsy before pregnancy (yes/no). Categories for adjustment variables combined for severe and conotruncal defects.

** The four exposure categories in supplement use collapsed into no/yes; “no” is no folic acid use (no supplements and other supplements, no folic acid), and “yes” is folic acid use (folic acid only and folic acid plus other supplementation).
Table S4. Relative risks (RR) of congenital heart defect* (overall), severe heart defect†, conotruncal defect‡, and septal defect§ by maternal intake of folic acid supplements, adjusting for covariates using three models, combining 94,228 births|| in The Danish National Birth Cohort (DNBC), Denmark, 1996-2003, and 102,985 births|| in The Norwegian Mother and Child Cohort Study (MoBa), Norway, 1999-2009.

| Supplement use (4 weeks before to 8 weeks after conception) | Any congenital heart defect* | Severe heart defect† | Conotruncal defect‡ | Septal defect§ |
|---------------------------------------------------------------|-----------------------------|---------------------|-------------------|--------------|
| Model 1 n=2,130 | Model 2 n=2,037 | Model 3 n=1,916 | Model 1 n=551 | Model 2 n=524 | Model 3 n=497 | Model 1 n=187 | Model 2 n=180 | Model 3 n=170 | Model 1 n=1,139 | Model 2 n=1,090 | Model 3 n=1,025 |
| None | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Other supplements, no folic acid | 0.99 (0.80,1.22) | 1.01 (0.97,1.10) | 1.10 (0.97,1.15) | 0.92 (0.61,1.41) | 0.89 (0.57,1.38) | 1.01 (0.64,1.58) | 0.84 (0.40,1.77) | 0.79 (0.36,1.75) | 0.76 (0.32,1.78) | 1.06 (0.80,1.39) | 1.07 (0.81,1.42) | 1.20 (0.89,1.60) |
| Folic acid only | 1.08 (0.93,1.25) | 1.12 (0.97,1.10) | 1.15 (0.98,1.15) | 1.10 (0.81,1.48) | 0.98 (0.64,1.56) | 1.21 (0.88,1.66) | 1.03 (0.84,1.26) | 1.00 (0.86,1.29) | 1.13 (0.66,1.94) | 1.07 (1.03,1.05) |
| Folic acid plus other supplementation | 1.07 (0.97,1.19) | 1.06 (0.96,1.18) | 1.10 (0.99,1.23) | 1.02 (0.81,1.22) | 0.93 (0.66,1.31) | 1.02 (0.83,1.26) | 1.06 (0.92,1.22) | 0.93 (0.93,1.24) | 1.11 (0.95,1.29) |
| Initiation of folic acid** (4 weeks before to 12 weeks after conception) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| None | 1.11 (1.00,1.25) | 1.13 (1.01,1.27) | 1.15 (1.02,1.30) | 1.08 (0.87,1.34) | 1.06 (0.85,1.32) | 1.11 (0.88,1.40) | 0.95 (0.66,1.38) | 0.93 (0.64,1.37) | 0.99 (0.67,1.46) | 1.08 (0.92,1.26) | 1.10 (0.94,1.30) | 1.13 (0.96,1.33) |
| Week -4 to -1 | 1.09 (0.95,1.25) | 1.08 (0.94,1.25) | 1.08 (0.93,1.25) | 1.01 (0.77,1.33) | 0.97 (0.73,1.32) | 0.98 (0.59,1.48) | 0.93 (0.59,1.64) | 0.82 (0.59,1.69) | 0.81 (0.66,1.94) | 1.03 (0.84,1.26) | 1.05 (0.86,1.33) |
| Week 0 to 4 | 0.98 (0.86,1.12) | 0.98 (0.85,1.13) | 1.00 (0.86,1.15) | 1.00 (0.77,1.30) | 0.99 (0.76,1.29) | 1.00 (0.76,1.32) | 0.85 (0.54,1.33) | 0.82 (0.51,1.31) | 0.82 (0.56,1.46) | 0.93 (0.77,1.11) | 0.93 (0.77,1.13) | 0.93 (0.78,1.16) |
| Week 5 to 8 | 0.97 (0.78,1.20) | 0.97 (0.78,1.21) | 1.00 (0.79,1.27) | 0.97 (0.63,1.50) | 0.93 (0.60,1.46) | 1.02 (0.64,1.64) | 0.68 (0.29,1.58) | 0.62 (0.25,1.56) | 0.72 (0.28,1.82) | 1.00 (0.75,1.32) | 0.99 (0.74,1.33) | 1.02 (0.75,1.40) |
| Week 9 to 12†† | 0.97 (0.78,1.21) | 1.00 (0.79,1.27) | 0.97 (0.63,1.50) | 0.93 (0.60,1.46) | 1.02 (0.64,1.64) | 0.68 (0.29,1.58) | 0.62 (0.25,1.56) | 0.72 (0.28,1.82) | 1.00 (0.75,1.32) | 0.99 (0.74,1.33) | 1.02 (0.75,1.40) |

*†‡§ Congenital heart defects: see definitions in Table 4.
|| Individuals with missing values of covariates were excluded in the adjusted analyses, see numbers in Table 4.
# Relative risk (RR) with 95% confidence interval (CI) comparing supplement use with no use (reference) 4 weeks before to 8 weeks after conception (upper panel) or comparing initiation of folic acid supplements with no use/non-folic acid supplements (reference) 4 weeks before to 12 weeks after conception (lower panel). Model 1: RRs adjusted for country, year of birth, maternal age (years <=24, 25-29, 30-34, >=35), birth order (1, 2, 3+), maternal epilepsy before conception (yes/no), maternal body mass index (BMI) (<20, 20-24, 25-29, 30-34, =<35), maternal heart defect (yes/no). Model 2: RRs adjusted for covariates as in Model 1, in addition, maternal socioeconomic status (Denmark: level 1, 2, 3, 5, 6, 7) and maternal education (Norway: years <12, 12, 13-16, >=17). Model 3: RRs adjusted for covariates as in Model 1, in addition, maternal pregestational diabetes (yes/no), maternal smoking before conception (daily, no), maternal alcohol consumption 3 months before conception (yes/no).

** The four exposure categories in supplement use collapsed into no/yes; “no” is no folic acid use (no supplements and other supplements, no folic acid), and “yes” is folic acid use (folic acid only and folic acid plus other supplementation).

†† In DNBC, including week 11.
Table S5. Relative risk (RR) of congenital heart defects* (overall), by maternal intake of folic acid supplements in the periconceptional period, in sensitivity analyses with restrictions to live births, singleton births, mother without heart defect, or planned pregnancies, combining 94,228 births in The Danish National Birth Cohort (DNBC), Denmark, 1996-2003, and 102,985 births in The Norwegian Mother and Child Cohort Study (MoBa), Norway, 1999-2009.

| Supplement use (4 weeks before to 8 weeks after conception) | Live births N=186,257||2,092 births with heart defect | Singletons N=179,514||1,957 infants with heart defect | Mother without heart defect N=185,818||2,104 infant with heart defect | Planned pregnancy N=157,382||1,797 infants with heart defect |
|-------------------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| None                                                       | 563 1 Reference                                 | 520 1 Reference                                 | 654 1 Reference                                 | 445 1 Reference                                 |
| Other supplements, no folic acid                            | 103 1.00 0.81,1.23                              | 95 0.99 0.80,1.23                               | 104 1.00 0.81,1.23                              | 86 1.06 0.85,1.34                              |
| Folic acid only                                            | 274 1.09 0.94,1.27                              | 255 1.09 0.94,1.28                              | 276 1.08 0.93,1.28                              | 243 1.11 0.94,1.30                              |
| Folic acid plus other supplementation                      | 1,152 1.06 0.96,1.18                            | 1,087 1.09 0.98,1.22                            | 1,160 1.06 0.96,1.18                            | 1,023 1.07 0.96,1.21                            |
| Initiation of folic acid** (4 weeks before to 12 weeks after conception) | | | | |
| None                                                       | 572 1 Reference                                 | 523 1 Reference                                 | 574 1 Reference                                 | 460 1 Reference                                 |
| Week -4 to -1                                              | 738 1.11 0.99,1.24                              | 682 1.13 1.01,1.27                              | 740 1.10 0.98,1.23                              | 706 1.10 0.98,1.24                              |
| Week 0 to 4                                                | 339 1.08 0.94,1.24                              | 324 1.13 0.98,1.30                              | 344 1.08 0.95,1.24                              | 296 1.07 0.92,1.24                              |
| Week 5 to 8                                                | 349 0.97 0.85,1.11                              | 336 1.01 0.88,1.16                              | 352 0.97 0.85,1.11                              | 264 0.95 0.82,1.11                              |
| Week 9 to 12††                                             | 94 0.97 0.73,1.11                               | 92 1.02 0.82,1.27                               | 94 0.95 0.77,1.18                               | 71 0.92 0.72,1.18                               |

*Any congenital heart defect: see definitions in Table 4.

|| Individuals with missing values of covariates were excluded in the adjusted analyses (10,339 among 196,596 live births; 9,986 among 189,500 singletons; 10,310 among 196,128 births of mothers without heart defect; 2,939 among 160,321 planned births).
Relative risk (RR) with 95% confidence interval (CI) comparing supplement use with no use (reference) 4 weeks before to 8 weeks after conception (upper panel) or comparing initiation of folic acid supplements with no use/non-folic acid supplements (reference) 4 weeks before to 12 weeks after conception (lower panel). RRs adjusted for country, year of birth, maternal age, birth order, maternal epilepsy, maternal BMI, maternal heart defect.

** The four exposure categories in supplement use collapsed into no/yes; “no” is no folic acid use (no supplements and other supplements, no folic acid), and “yes” is folic acid use (folic acid only and folic acid plus other supplementation).

†† In DNBC, including week 11.
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