CLINICAL RESEARCH

Protective effect of periconceptional folic acid supplements on the risk of congenital heart defects: a registry-based case–control study in the northern Netherlands

Ingrid M. van Beynum[1]*, Livia Kapusta[1], Marian K. Bakker[5], Martin den Heijer[2,3], Henk J. Blom[4,6], and Hermien E.K. de Walle[5]

[1]Children's Heart Centre, Radboud University Nijmegen Medical Centre, PO Box 9101, Nijmegen 6500 HB, The Netherlands; [2]Department of Endocrinology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; [3]Department of Epidemiology and Biostatistics, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; [4]Laboratory of Paediatrics and Neurology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; [5]EUROCAT Registration Northern Netherlands, Department of Medical Genetics, University Medical Centre Groningen, University of Groningen, Groningen, The Netherlands; and [6]Metabolic Unit, PK 1-X-018, Department of Clinical Chemistry, VU University Medical Centre, Amsterdam, The Netherlands

Aims	To investigate the potentially protective of periconceptional folic acid use on the risk of congenital heart defects (CHDs) relative to other non-folate related malformations.
Methods and results	We analysed data from a large regional register of birth defects (EUROCAT—Northern Netherlands), over a 10 year period (1996–2005) for a case–control study. The cases were mothers who had delivered infants with isolated or complex heart defects, without any related syndrome or genetic abnormality ($n = 611$). We used two control groups; one from the EUROCAT database and another from the general population. The registry controls consisted of mothers of children with a known chromosomal or genetic defect, and with infants with other non-folate related congenital malformations ($n = 2401$). Additional folic acid was taken as a single supplement or as a multivitamin containing folic acid in a dose of ≥ 400 µg daily. Mothers who had used folate antagonists or who had diabetes, and mothers of children with oral clefts, hypospadias, limb reduction- or neural tube defects, were excluded from both groups. Potentially confounding factors of periconceptional folic acid use in relation to CHD were explored, including baby's birth year, maternal body mass index, education, maternal age at delivery of index baby, smoking behaviour, and alcohol use during pregnancy. Periconceptional folic acid use revealed an odds ratio (OR) of 0.82 (95% CI 0.68–0.98) for all types of CHD relative to other malformations. The estimated relative risk for CHDs of additional folic acid use compared with the general population was comparable [OR 0.74 (95%CI 0.62–0.88)]. Subgroup analysis showed an OR of 0.62 (95% CI 0.47–0.82) for isolated septal defects. The proportions of the potential confounders between mothers of case and control infants did not differ significantly.
Conclusion	Our results support the hypothesis that additional periconceptional folic acid use reduces CHD risk in infants. Use of periconceptional folic acid supplements was related to ∼20% reduction in the prevalence of any CHD. Given the relatively high prevalence of CHD worldwide, our findings are important for public health.
Keywords	Periconceptional folic acid use • Prevention • Congenital heart defects • Registries • Epidemiology

* Corresponding author. Tel: +31 24 361 9060, Fax: +31 24 361 9052, Email: i.vanbeynum@cukz.umcn.nl

Introduction

Congenital heart defects (CHDs) are one of the most common birth defects affecting up to 0.6–1.9 in 100 newborns worldwide,[1] with a prevalence of CHDs in the Netherlands of 7–8 per 1000 live births per year,[2,3] accounting for substantial morbidity and mortality among infants.

There is general consensus that folic acid supplements taken during the periconceptional period substantially reduce the risk of neural tube defects (NTD) in infants.[4,5] A few studies have proposed that maternal periconceptional use of folic acid also protects against the occurrence of CHDs, but this has not yet been definitely established. The Hungarian trial with CHD as a secondary outcome measure and two other observational studies identified reductions in CHD risk with the use of multivitamins containing folic acid.[6–8] Two more observational studies found multivitamins containing folic acid had no effect on CHD risk.[9,10] It would be unethical to perform a primary intervention trial to study the potentially beneficial effect of periconceptional folic acid use on CHD risk because of its proven protective effect against NTDs.

It is therefore important to acquire further evidence of the preventive effect of periconceptional folic acid supplements on other birth defects, including CHDs, so that worldwide public health strategies can achieve an increase in periconceptional folic acid intake. This topic is particularly relevant in countries where food fortification with folic acid has not yet been adopted; one of these is the Netherlands, where the government drew up a periconceptional folic acid supplement policy to prevent NTDs in 1993.[11] A study has shown that although the Dutch mass media campaign, in 1995, increased awareness in up to nearly 80% of pregnant women, only ∼50% of women took folic acid supplement daily for the entire advised period, which is from 4 weeks before conception to 8 weeks afterwards.[12]

We aimed to assess the potentially protective effect of periconceptional folic acid use on the risk of CHDs relative to other non-folate related malformations, based on data from the European Registration of Congenital Anomalies and Twins (EUROCAT) Northern Netherlands database.

Methods

Study design and population

The EUROCAT Northern Netherlands database has registered congenital anomalies diagnosed before or after birth since 1981 in the northern provinces of the Netherlands. This population-based register now monitors ∼20 000 births annually. Methods for case ascertainment have not changed over time and are in concordance with EUROCAT Central Registry guidelines (www.eurocat.ulster.ac.uk). The anomalies are reported by midwives, general practitioners, well-baby clinic doctors, and specialists. The major notifier for CHD is the Department of Paediatric Cardiology, University Medical Centre Groningen. Live births, stillbirths and also spontaneous abortions with any CHD, and terminated CHD-affected pregnancies from 1981 have also been registered in the database. To study the potentially protective effect of periconceptional folic acid supplements on CHD risk, we analysed data from children and foetuses born from 1996 to 2005. We chose to start the analysis 1 year after the Dutch mass media campaign which aimed to reduce the occurrence of NTDs. It promoted periconceptional folic acid supplements for all women of child-bearing age.

Written consent was given by all the parents of the children and foetuses before they were registered in EUROCAT.

Data collection

Once an infant with an anomaly has been reported, further information is gathered from the mother. Up until 1996, information about the mothers' conditions was collected through the notifying healthcare provider, but since 1997 the parents have been asked directly to complete a written questionnaire to supply information about the index pregnancy. The questions include information about medical and reproductive history, occupation, demographic characteristics, maternal weight and height, smoking habits and alcohol consumption, and the use of medications and vitamins in the period from 3 months before pregnancy until delivery. A request for written consent providing access to their pharmacy data was also included. After the questionnaire and consent form are returned, the pharmacy is contacted for information on any prescribed medication. Information on whether the medications were actually taken and for which period are verified with the mother in a telephone interview.

Cases and controls: ascertainment and definition

Information about infants' anomalies is collected through midwives, clinical geneticists, physicians, and pathologists. For CHDs, the details regarding each heart defect were also obtained from clinical reports, including those from echocardiography, CT or MRI scans, catheterization, surgery, and/or autopsy. In the EUROCAT database, the CHDs are classified according to a detailed coding system specifying cardiac anatomy based on phenotypic groups. This classification is based on the British Paediatric Association (BPA) Classification of Diseases [a paediatric handbook compatible with the 9th and 10th revisions of the WHO International Classification of Diseases (ICD), 1977]. In diagnoses for which no specific ICD/BPA code was available, a EUROCAT extension code was used. The ICD-9 classification was used for births up until 2001, whereas the ICD-10 coding system has been adopted since 2002.

On the basis of these classifications, each infant with a CHD was given an exclusive diagnosis: (i) *isolated heart defect*, if a heart defect was the only anomaly; (ii) *multiple heart defect*, if a combination of heart defects were present; (iii) *multiple complex*, if the heart defect was associated with other congenital anomalies or a pattern of other birth defects of unknown cause (e.g. VACTERL-association); or (iv) *genetic abnormality*, if the heart defect was known to be related to a genetic aberration (e.g. trisomies, deletions, or single gene defects). Because of the diversity of cardiac phenotypes, each anatomical diagnosis or combination of heart defects was assigned to final subgroups according to two classification systems, based on developmental and epidemiological considerations.[13,14] The cases for the analysis were mothers who had delivered infants with isolated, multiple or complex heart defects, not associated with a known syndrome or genetic abnormality ($n = 611$). Isolated cardiomyopathies, single umbilical artery, and rhythm disorders (i.e. atrioventricular blocks, Wolff–Parkinson–White syndrome) were not considered as CHDs.

We used two control groups, one from the EUROCAT database and another from the general population. The control group of the database ($n = 2401$) consisted of mothers who had given birth to

infants with a known chromosomal ($n = 782$) or genetic defect, or to infants with other non-folate related congenital malformations ($n = 1619$). Mothers of infants with oral clefts, hypospadias, limb reduction defects, or NTDs were excluded from the case and control groups because of the definite or possibly preventive effect of maternal folic acid supplements on the risk for these defects.[4–6,15,16]

We assumed that the control group, consisting of congenital defects and genetic disorders, is unrelated to exposure status, i.e. periconceptional folic acid use and is representative for the general population. To verify this assumption, we included a control group from the general population. This control group consisted of pregnant women ($n = 3343$) who took part in five previous cross-sectional studies performed in the northern Netherlands in 1996, 1998, 2000, 2003, and 2005. These surveys evaluated the awareness and use of periconceptional folic acid supplements with regards to socio-economic status among women attending antenatal visits, 2 yearly. All studies following the same methodology in the same health professionals' practices. The questionnaire included questions about periconceptional folic acid use similar to those in the EUROCAT registry. The designs of these studies have been described in detail elsewhere.[12,17–20]

Periconceptional folic acid supplements

At the beginning of 1993, the Dutch authorities recommended that women planning a pregnancy should take $\geq 400\ \mu g$ of folic acid supplement daily to help prevent NTDs, starting at least 4 weeks prior to conception and continuing up to 8 weeks thereafter. This period covers the development of the heart in the human embryo, which starts on Day 19, with the basic structures completed at the end of week 9 post-conception.[21] The questionnaire included detailed questions on maternal periconceptional folic acid use.

We defined mothers as users of periconceptional folic acid as those who took folic acid supplements during the entire advised period, those who started taking supplements at least prior to conception and continued for the first 2 months of pregnancy, or those who started after conception and took folic acid regularly in the advised period. Mothers who took folic acid after the advised period or who did not take any folic acid supplement were classified as non-users. If the mothers' use of folic acid was unknown, or the period was unknown, they were excluded from the study. Women who took folic acid supplements had either taken folic acid alone or as multivitamins containing folic acid in a dose of $\geq 400\ \mu g$ daily. The following potentially confounding factors of periconceptional folic acid use in relation to CHD were explored and included: maternal body mass index (BMI, body weight in kg/length in m^2); age at delivery of index baby; year of baby's birth; mother's education level; smoking behaviour and alcohol use during pregnancy. Smoking and alcohol use was considered positive if mothers reported any use, even if they stopped when they became aware they were pregnant. The highest level of education was taken as an indicator of socio-economic status. In total, there were seven levels of education, ranging from elementary school to university.

Taking a folic acid antagonist during pregnancy interferes with folate metabolism and is a known risk factor for CHD[22,23] consequently mothers who reported taking folate antagonists were excluded from the study. Folate antagonists include dihydrofolate reductase inhibitor (methotrexate, sulfasalazine, pyrimethamine, triamterene, trimetroprim) and antiepileptic drugs. Mothers with diabetes mellitus prior to pregnancy or gestational diabetes mellitus were also excluded from further analysis, because CHDs have been associated with maternal diabetes.[24,25]

Statistical analysis

A case–control analysis was performed to assess the possible effect of periconceptional folic acid supplements on CHD risk, using data on all live births and stillbirths with CHD, and on spontaneous abortions and terminated pregnancies with CHD. We used the reported rates, although the true population with CHD, including all conceptions, cannot be fully ascertained.

Potential confounders are those factors that correlate with both the main determinant (i.e. folic acid) and outcome (CHD). The known potentially confounders of periconceptional folic acid use in relation to CHD were explored: maternal age and BMI, smoking, alcohol use, year of baby's birth, and mother's education level. These potential

Table I Distribution of maternal characteristics in the population-based registry in the northern Netherlands 1996–2005

Characteristics	Controls (n = 2401) n (%)	Cases (n = 611) n (%)	P-value[a]
Maternal age at delivery (years)			0.13
15–19	20 (0.8)	4 (0.7)	
20–24	170 (7.1)	63 (10.3)	
25–29	791 (32.9)	192 (31.4)	
30–34	962 (40.1)	232 (38.0)	
35–39	377 (15.7)	103 (16.9)	
40–50	81 (3.4)	17 (2.8)	
Body mass index (kg/m^2)			0.39
10–18.5	94 (3.9)	25 (4.1)	
18.5–27	1897 (79.0)	461 (75.5)	
27–32	302 (12.6)	94 (15.4)	
32–35	58 (2.4)	16 (2.8)	
>35	50 (2.1)	25 (2.5)	
Education level			0.31
Low	315 (13.1)	94 (15.4)	
Middle	1411 (58.8)	355 (58.1)	
High	675 (28.1)	162 (26.5)	
Smoking during pregnancy			0.47
Yes	610 (25.4)	164 (26.8)	
No	1791 (74.6)	447 (73.2)	
Alcohol during pregnancy			0.66
Yes	561 (23.4)	148 (24.2)	
No	1840 (76.6)	463 (75.8)	
Year of birth			0.83
1996–1997	492 (20.5)	133 (21.8)	
1998–1999	560 (23.3)	131 (21.4)	
2000–2001	499 (20.8)	133 (21.8)	
2002–2003	471 (19.6)	121 (19.8)	
2004–2005	379 (15.8)	93 (15.2)	

[a]There were no significant differences in proportions between the mothers of case and control infants present (tested two-sided with χ^2 test).

confounders for mothers of case and control infants are given in *Table 1* and proportions for each variable were calculated. For statistical analysis, the reported educational levels were merged into three levels: low (primary school, lower general secondary education, and lower vocational education), middle (higher general secondary education and intermediate vocational education), and high (higher vocational education and university). The maternal age at delivery of the index pregnancy was grouped for each five or ten incremental years: 15–19, 20–24, 25–29, 30–34, 35–39, 40–50 years. Body mass index in kg/m^2 was classified as underweight (10–18.5), normal weight (18.5–27), increased weight (27–32), obese (32–35), severely obese (>35). Differences in proportions between case and control mothers were ascertained using χ^2 test (Pearson, *P*-values tested two-sided).

To estimate the relative risk for CHD, we calculated odds ratios (OR) along with the 95% confidence intervals (CI) using a logistic regression model. In the model, folic acid use was the independent main variable and the presence or absence of a CHD the dependent variable. Furthermore, a multivariate logistic regression model was constructed to explore the contribution of maternal age, BMI, educational level, year of birth, smoking and alcohol use during pregnancy, including them as covariates in the analyses. Folic acid, smoking and alcohol were considered as dichotomous variables, education and birth year as categorical variables, and maternal age and BMI as continuous variables.

The following assumptions of the basic model were verified. We tested whether the variables had normal distributions; no outliers were identified through visual inspections of histograms. Collinearity was also excluded because the tolerance for the variables was larger than 0.1. The order in which the independent variables are entered in the model did not change the prediction of the outcome variable. We tested the linearity assumption between the independent continuous variables and dependent variables with residual plots, which indicated linear relationship.

Two-tailed values of $P < 0.05$ and 95% CI excluding 1.0 were considered to be statistically significant. We used the SPSS software package for Windows (version 16.0, Chicago, IL, USA) for the statistical analyses.

Results

Included participants

From 1996 to 2005, in total 5173 infants with congenital anomalies were registered in the EUROCAT database. Complete data on maternal age, BMI, year of baby's birth, education, smoking and alcohol use during pregnancy were available for 3836 mothers (74.2%). We excluded 19 mothers with pre-existing diabetes mellitus, 33 with documented gestational diabetes, 121 who reported use of folic acid antagonists, and 3 with diabetes and use of folic acid antagonists. Of the remaining 3660 mothers of cases and controls, 648 were excluded because of the presence of a definite or possibly preventive effect of folic acid on the risk of the anomalies in their infants (NTDs $n = 101$, oral clefts $n = 251$, hypospadias $n = 230$, limb reduction defects $n = 66$). In total, we identified mothers of 611 CHD cases and of 2401 controls (782 infants with a chromosomal or genetic aberration and 1619 infants with any other congenital anomaly) for further analysis. The proportions of the maternal characteristics; maternal age, BMI, educational level, year of birth, smoking and alcohol use during pregnancy, between mothers of cases and control infants were

not significantly different (*Table 1*). In the five cross-sectional surveys, performed between 1996 and 2005, 3517 women filled out the questionnaire. Data on periconceptional folic acid use was available for 3343 women.

Periconceptional folic acid supplementation

The distribution of periconceptional folic acid use in mothers of cases and controls is shown in *Table 2*, with 41.6% of the case mothers and 37.1% of the control mothers reporting no use of folic acid during the advised period (4 weeks before conception to 8 weeks after). Periconceptional folic acid use during the entire or part of the advised period (which we defined as adequate use) was reported by 56.0% of case mothers and 61.1% of control mothers.

The estimated relative risks for different types of CHD (without a known syndrome or genetic defect) associated with maternal periconceptional folic acid supplements are presented in *Table 3*. Overall, mothers who had used periconceptional folic acid had an 18% lower risk of delivering an infant with any type of CHD [OR 0.82 (95% CI 0.68–0.98)]. In the case of septal heart defects, the most frequent specific diagnosis, the risk was reduced by 38% [OR 0.62 (95% CI 0.47–0.82)]. Among the isolated ventricular septal defects (VSDs), the perimembranous defects appeared to account for the largest proportion of risk reduction [OR 0.58 (95% CI 0.31–1.07)]. Adequate periconceptional folic acid use was also associated with a reduced risk for isolated type II atrial septal defects [OR 0.54 (95% CI 0.31–0.94)]. Subgroup analysis revealed no significant reduced risk for conotruncal heart defects, atrioventricular septal defects, left sided- and right sided-obstruction defects or for the group of complex heart defects. All estimates of relative risk did not change after adjustment for maternal age, BMI, educational level, year of birth, smoking and alcohol use during pregnancy. After applying all these variables as covariates in the logistic regression model, the OR was 0.82 (95% CI 0.68–0.997) for any CHD.

Table 2 Distribution of periconceptional folic acid use by mothers of case and control infants in the population-based registry in the northern Netherlands 1996–2005

Maternal use of folic acid	Case mothers ($n = 611$)	Control mothers ($n = 2401$)
(1) Adequate periconceptional use[a]	342 (56.0%)	1468 (61.1%)
(2) No use or use after advised period	254 (41.6%)	891 (37.1%)
(3) Use unknown or unknown for which period[b]	15 (2.4%)	42 (1.8%)

[a]Adequate use of periconceptional folic acid includes: (1) use during the entire advised period, 4 weeks before conception to 8 weeks after; (2) use started at least before conception and continued during the first 8 weeks of pregnancy; (3) folic acid supplements taken regularly during the officially advised period, but started after conception.
[b]Mothers in this category were excluded from the study.

Table 3 Relative risk for congenital heart defects, without known syndrome or genetic defect, associated with maternal periconceptional folic acid use relative to non-folate related malformations as controls, in the population-based registry in the northern Netherlands 1996–2005

Diagnoses	Non-users[a]	Users[b]	OR (95% CI)
Controls[c]	891	1468	Reference
Any congenital heart defect	254	342	0.82 (0.68–0.98)
Isolated septal heart defects	108	111	0.62 (0.47–0.82)
Isolated VSD	72	82	0.69 (0.50–0.96)
Perimembranous VSD	21	20	0.58 (0.31–1.07)
Muscular VSD	32	39	0.74 (0.46–1.19)
VSD combined/not otherwise specified	19	23	0.74 (0.40–1.36)
Isolated ASDII	27	24	0.54 (0.31–0.94)
VSD + ASDII	9	5	0.34 (0.11–1.01)
Conotruncal heart defects	45	57	0.77 (0.52–1.15)
Tetralogy of Fallot	17	26	0.93 (0.50–1.72)
Transposition great arteries	17	22	0.79 (0.42–1.49)
Double outlet right ventricle	4	3	0.46 (0.10–2.04)
Interrupted aortic arch	5	1	0.12 (0.01–1.04)
Truncus arteriosus	2	5	1.52 (0.29–7.84)
Atrioventricular septal defects	3	7	1.42 (0.37–5.49)
Left ventricle outflow obstruction	36	73	1.23 (0.82–1.85)
Hypoplastic left heart syndrome	12	19	0.96 (0.46–1.99)
Other aortic arch anomalies	3	2	0.41 (0.07–2.43)
Aortic valve stenosis	3	16	3.24 (0.94–11.14)
Coarctation aortae	9	19	1.28 (0.58–2.84)
CoA + VSD	8	9	0.68 (0.26–1.78)
Mitral valve stenosis	1	2	1.21 (0.11–13.41)
Biscupid aortic valve	0	6	—
Right ventricle outflow obstruction	33	58	1.07 (0.69–1.65)
Tricuspid atresia/stenosis	1	6	3.64 (0.44–30.30)
Ebstein anomaly	3	4	0.81 (0.69–1.65)
Pulmonary valve stenosis	19	28	0.89 (0.50–1.61)
PS + ASDII and/or VSD	8	13	0.99 (0.41–2.39)
Pulmonary atresia + intact ventricular septum	1	6	3.64 (0.44–30.30)
Peripheral pulmonary stenosis + VSD	1	1	0.61 (0.04–9.72)
Complex heart defects	14	19	0.82 (0.41–1.65)
Heterotaxy + other defects	1	2	1.20 (0.11–13.41)
Single ventricle	4	5	0.76 (0.20–2.83)
Multiple complex heart anomalies	9	12	0.81 (0.34–1.93)
Other cardiac defects	15	17	0.69 (0.34–1.38)
Isolated persistent ductus arteriosus	7	7	0.61 (0.21–1.74)
Abnormal pulmonary venous connection	3	5	1.01 (0.24–4.24)
Aberrant coronary arteries	2	2	0.61 (0.09–4.32)
Remaining cardiac defects	3	3	0.61 (0.12–3.01)

VSD, ventricular septal defects; ASDII, atrial septal defects type II; CoA, Coarctation Aortae; PS, pulmonary valve stenosis.
[a]Non-users were defined as not having taken any periconceptional folic acid supplements or only after the advised period (4 weeks before conception to 8 weeks after).
[b]Users were defined as having taken periconceptional folic acid supplements during all or part of the advised period.
[c]Controls consisted of infants with chromosomal or genetic aberrations and of infants with any other non-folic acid associated congenital malformation.

Selected control group of genetic defects

The group of control infants with any other congenital anomaly ($n = 1619$) may have also included congenital malformations which might be associated with a preventive working of periconceptional folic acid use. We therefore compared mothers of infants with CHD to mothers whose infants had a chromosomal or other genetic aberration only ($n = 782$). In this selected group of control mothers, 61.9% reported taking folic acid supplements periconceptionally, a figure similar to the whole control group (61.1%, $n = 2401$). In this group, the risk reduction for having a child with a CHD associated with periconceptional folic acid use remained virtually unchanged for any type of heart defect [0.83 (0.67−1.03)] and also for the specific subgroups of anatomic heart defects (data not shown).

Reference group from the general population

Furthermore, we included pregnant women representative for the general population in the northern Netherlands as a reference group. In this group, 35.4% ($n = 1183$) of the women reported no use of folic acid and 64.6% ($n = 2160$) used additional folic acid at some time during the advised period. *Table 4* summarizes the estimates of relative risk for different types of CHD among periconceptional folic acid users compared with the non-users with controls from general population as reference group. The point estimates of relative risk for CHDs of additional folic acid use using the control group from the general population were slightly lower to those found when using the non-folate related malformed controls. Overall, maternal periconceptional folic acid use was associated with a 26% reduction in risk for any type of CHD [OR 0.74 (95%CI 0.62−0.88)].

Discussion

In this relatively large population-based case−control study, we found a preventive effect from periconceptional folic acid use on the risk for CHDs relative to other non-folate related malformations. Women who reported using folic acid in the advised period had an ∼20% lower risk of having infants with any type of heart defect. The apparently protective effect varies and appears to be strongest for septal heart defects, including isolated VSDs and secundum type atrial septal defects.

In principle, randomized placebo controlled trials would be required to establish the effect of periconceptional folic acid use on various pregnancy outcomes, including CHD risk. However, since women are advised to take periconceptional folic acid supplements to reduce the occurrence risk of NTDs, it would be unethical to perform intervention studies. There was a national campaign in the Netherlands to encourage the use of periconceptional folic acid in 1995. This, together with detailed information on periconceptional folic acid use and on potential confounders registered in EUROCAT (for the northern Netherlands region) made it possible to explore the potentially preventive effect of periconceptional folic acid use on CHD risk.

Previous studies on the preventive effect of periconceptional folic acid and CHD risk in human offspring have shown both positive and negative associations.[6−10,26] Some of the ambiguity in the results may be due to differences in study design or the types of heart defects included. The Hungarian trial was primarily designed to study the effect of periconceptional multivitamin use on the reduction of the first occurrence of NTDs, with CHDs as a secondary outcome measure. Apart from this trial, our study specifically focusing on periconceptional folic acid supplements in the prevention of CHD is the first such study performed in Europe. The four others were observational studies performed in the USA before the introduction of food fortification with folic acid in 1998. Czeizel[6] was the first to observe that CHDs (mainly VSDs) were reduced by 58% [OR 0.42 (95% CI 0.19−0.98)] in the women receiving multivitamins containing 800 µg folic acid compared with the women receiving trace elements. This randomized trial was inconclusive regarding a potentially preventive effect on other types of heart defects. The observational studies, except for one, analysed only limited types of heart defects (mainly conotruncal).[7−10,26]

Shaw *et al.*[8] studied only conotruncal heart defects and found a reduced risk associated with maternal use of any multivitamin supplement containing folic acid compared with no use [adjusted OR

Table 4 Relative risk for congenital heart defects, without known syndrome or genetic defect, associated with maternal periconceptional folic acid use with pregnant women from general population as controls, in the northern Netherlands 1996−2005

Diagnoses	Non-users	Users	OR (95% CI)
Controls	1183	2160	Reference
Any congenital heart defect	254	342	0.74 (0.62−0.88)
Isolated septal heart defects	108	111	0.56 (0.43−0.74)
Conotruncal heart defects	45	57	0.69 (0.47−1.03)
Atrioventricular septal defects	3	7	1.28 (0.33−4.95)
Left ventricle outflow obstruction	36	73	1.11 (0.74−1.67)
Right ventricle outflow obstruction	33	58	0.96 (0.62−1.48)
Complex heart defects	14	19	0.74 (0.37−1.49)
Other cardiac defects	15	17	0.62 (0.31−1.25)

0.53 (95% CI 0.34−0.85)]. In the Atlanta population-based case−control study, the periconceptional use of multivitamin supplements containing folic acid was associated with a reduced risk of conotruncal CHD [OR 0.57 (95% CI 0.33−1.00)].[26] A second publication based on the same Atlanta birth registry included all types of CHDs, including also these conotruncal CHDs.[7] Periconceptional multivitamin use was associated with a reduced risk for any type of cardiac defect in the offspring [OR 0.76 (95% CI 0.60−0.97)], mainly for outflow tract defects [OR 0.46 (95% CI 0.24−0.86)], and for VSDs [OR 0.61 (95% CI 0.38−0.99)]. However, they gave no information about the folic acid component or dosage.[7] In these two studies, based on Atlanta Birth Defects Case−Control Study, the information was gathered in 1982−1983 from infants born between 1968 and 1980. The data were obtained through a questionnaire, but details up to 14 years old are difficult to recall accurately and should be interpreted with caution.[7,26] Scanlon et al.[9] found no preventive effect from multivitamins containing folic acid taken before pregnancy on the risk of outflow tract defects [OR 0.97 (95% CI 0.6−1.6)]. Werler et al.[10] performed a hospital-based case−control study and found no effect on the risk of conotruncal [OR 1.0 (95% CI 0.7−1.5)] or VSDs [n = 186; OR 1.2 (95% CI 0.8−1.8)] associated with periconceptional use of multivitamins containing folic acid. The finding of a preventive effect of folic acid on CHD risk in our study is consistent with the risk reduction observed in the large positive studies.[6−8] The point estimates for risk reduction of CHD are essentially similar and the strongest association was found for VSDs and conotruncal heart defects. However, we found no significant protective effect of folic acid on risk for conotruncal heart defects. The absence of a potential preventive effect on other CHD with higher complexity is either true but more likely due to relatively small numbers. To establish a potentially preventive effect of folic acid on specific heart defects with a low occurrence rate, very large study populations are required and these can only be achieved through multicentre collaborations.

Thanks to the detailed information on heart defects in our EUROCAT registry, we were enabled to differentiate between several types of heart defects. This may contribute to explaining how the pathophysiological mechanisms of CHD are associated with folic acid, but the precise mechanism underlying folic acid's protective effect on CHD remains to be elucidated. In several animal experiments, severe folate deficiency induced in knockout models has been shown to cause CHDs, with VSDs and conotruncal heart defects in particular being frequently observed.[27,28] In recent years, the methylation hypothesis has been developed and suggests that folic acid prevents congenital defects, in particular NTDs, by stimulating cellular methylation reactions.[29]

In general, CHDs have great psycho-emotional implications and are a burden for society and the healthcare system. Primary prevention is the ideal strategy to lower the prevalence and CHD-related morbidity and mortality.[30] The protective effect of periconceptional folic acid use on CHD risk, in particular for septal heart defects, is important for public health given the high prevalence of these defects worldwide. In the Netherlands, a relatively small country, ~1500 children are born with a CHD each year. Ventricular septal defects account for a substantial proportion of all CHDs and made up 25% in our study population.

The incidence of VSDs has also been demonstrated to be more frequent.[31,32] The tiny muscular defects and small perimembranous defects can be treated conservatively and need follow-up, but approximately one-fifth of the VSDs are haemodynamically important and require surgical intervention.[33] Secundum type of atrial septal defect is also a frequent diagnosis. In one-third of the cases, there is an indication for closure of the defect. Long life follow-up is required because of an increased risk for arrhythmias in later life, notably in those >50 years of age.[33] On the basis of our findings, ~20−40% of septal heart defects, and possibly a proportion of conotruncal defects, could possibly be prevented with periconceptional folic acid use. Our results provide further evidence that folic acid plays a role in the prevention of CHD and may have implications for public health strategies in achieving optimal periconceptional intake of folic acid.

Our observation of a preventive effect of folic acid on CHD risk should be discussed in the light of the strengths and limitations of this study. The main strengths of our study were a relatively large number of infants with all types of CHD drawn from a well-defined and homogeneous population, including all type of births irrespective of gestational age. In previous publications, analysis was often limited to a subgroup of heart defects.[8−10,26]

Information on periconceptional folic acid use was obtained from a retrospective questionnaire; some of this information is thus likely to be inaccurate. The possible differences in maternal recall of folic acid use and in information on confounders between malformed and non-malformed cases are minimized by choosing affected controls. The risk of confounding is, in general, a threat to observational studies. One important potential confounder is if the mother was using a folic acid antagonist. The earlier observational studies did not exclude or adjust for use of a folic acid antagonist. We were well informed about periconceptional use of medications, including folic acid antagonists, and excluded these mothers from our analysis.

Another relevant question is whether malformed controls are representative for the general population. With the careful selection of the affected controls, we assumed this control group representative for the general population. This assumption was confirmed by the reasonably similar estimates of relative risk when the folic acid use was compared with the reference group from the general population. The slightly higher percentages of risk reduction of CHD even might suggest that the affected controls had included birth defects that could possibly be prevented by periconceptional folic acid supplements.

In this study, we considered the folate hypothesis, which proposes that the folic acid component in supplements is responsible for reducing the CHD risk. From the database, we were not able to determine precisely whether the use of folic acid alone or of multivitamins containing folic acid was better in preventing CHD. However, in general, 88% of the women in the northern Netherlands used folic acid in the advised period as a single supplement. Indirect support for the importance of folate in reducing CHD risk was provided by Hernandez-Diaz et al. Their study showed that periconceptional intake of medications acting as a folic acid antagonist doubled the risk of CHDs [OR 2.2 (95% CI 1.4−3.5)], including conotruncal defects, VSDs, and others. Moreover, they observed that the folic acid component

in multivitamins lessened the CHD risk associated with folic acid antagonists.[23]

The optimal dose of periconceptional folic acid supplements to prevent CHD cannot be deduced from our study or the previous studies because most women had taken supplements with at least 400 μg. Whether a lower or higher dose would be more effective is difficult to explore since 400 μg is the level that has been advised for preventing NTDs. There is even growing evidence that with mothers becoming heavier (increasing BMI), the daily dose of folic acid will need to be higher to maintain a similar preventive effect on.[34,35]

In conclusion, our results support the hypothesis that folic acid supplements taken periconceptionally help prevent CHDs. Periconceptional use of folic acid supplements appears to reduce the prevalence of CHD by ~20%. Given the relatively high prevalence of CHDs worldwide, our findings are important for public health and for developing national policies to achieve the highest percentage of periconceptional folic acid intake by women in their fertile period. Women who want to become pregnant should take folic acid supplements around the time of conception, not only to prevent NTDs but also to reduce the risk of CHDs.

Funding

Conflict of interest: none declared.

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