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Dose-dependent Effect of Folic Acid on the Prevention of Orofacial Clefts

Andrew E. Czeizel, MD, DSc; László Timár, MD; and Andrea Sárközi, MD

ABSTRACT. Objective. In 1982, Tolarova found a reduction in the recurrence rate of isolated cleft lip (CL) with or without cleft palate (CP; CL ± CP) after periconceptional supplementation with a multivitamin including a very high dose (10 mg) of folic acid. The Hungarian randomized, double-blind, controlled trial of periconceptional supplementation with a multivitamin including a physiologic dose (8 mg) of folic acid did not show any preventive effect on the first occurrence of isolated CL ± CP and CP. However, the general evaluation of congenital abnormalities in the Hungarian Case-Control Surveillance of Congenital Abnormalities indicated, among others, a reduction of isolated CL ± CP and CP after the use of high doses of folic acid in the critical period for the development of these congenital abnormalities in the 12-year dataset between 1980 and 1991. We hypothesized that the prevention of orofacial clefts by folic acid has a dose-dependent effect, and this hypothesis was tested in 2 recent Hungarian datasets.

Design. In a prospective cohort study, the occurrence of isolated CL ± CP and CP was studied in the newborn infants born to mothers with or without periconceptional folic acid-containing (8 mg) multivitamin supplementation. Supplemented women with confirmed pregnancy were recruited from the participants of the periconceptional service. Unsupplemented women were invited to take part in the study after the first visit between the 8th and 12th week of gestation in the antenatal care. Supplemented and unsupplemented women were matched based on age, socioeconomic status, and residence. In contrast, the occurrence of high-dose (in general daily 6 mg) folic acid supplementation was evaluated in the case-control pairs of CL ± CP and CP, particularly during the critical period of these 2 types of orofacial clefts in the 17 years dataset of the Case-Control Surveillance of Congenital Abnormalities, between 1980 and 1996. Cases were selected from the population-based Hungarian Congenital Abnormality Registry, whereas population controls without congenital abnormality were ascertained from the national birth registry. Two population controls were matched to every case according to sex, week of birth, and district of parental residence. The drug uses, including pregnancy supplements as folic acid, were evaluated based on retrospective self-reported maternal questionnaire and prospective medically documented data of antenatal care logbook.

Results. In the prospective cohort study, of 3019 informative offspring (termination of pregnancies in the second and third trimesters because of fetal defect, stillborn fetuses, and liveborn infants) in the supplemented group, 3 had CL ± CP and 1 was affected with CP, whereas of 3432 informative offspring in the unsupplemented group, 2 had CL ± CP and 1 had CP. The lack of preventive effect was in agreement with the result of the previous Hungarian randomized double-blind controlled trial; thus, these 2 datasets were combined. The preventive effect of a folic acid containing multivitamin used in the periconceptional period for the first occurrence of isolated CL ± CP and CP was estimated by the Mantel-Haenszel test. Of 5488 supplemented women, 6 had CL ± CP, and of 5821 unsupplemented women, 4 had CL ± CP. Of 5489 supplemented women, 1 had CP, and of 5823 unsupplemented pregnant women, 3 had CP. The Hungarian Case-Control Surveillance of Congenital Abnormalities, 1980–1996, included 38 151 population controls (1.8% of the Hungarian births) and 22 865 cases with congenital abnormalities. Within the latter group, 1368 had isolated CL ± CP, and 596 had CP. A significantly more frequent use of high-dose folic acid (in general daily 6 mg) supplementation was found in controls than in cases of 1246 case-control pairs of CL ± CP group and of 537 case-control pairs of CP group, respectively. However, the protective effect for these 2 types of orofacial clefts was seen only after the use of folic acid during the critical period of primary and secondary palate development, ie, during the first and second months of gestation (12.4% in controls vs 9.1% in cases) in the group of CL ± CP and during the first 4 months of pregnancy (39.0% in controls and 32.2% in cases) in the group of CP. There was no difference in the occurrence of folic acid supplementation after the critical period of orofacial clefts between cases and controls.

Conclusions. Folate-folic acid deficiency may have a role in the origin of isolated CL ± CP and CP, and it can be neutralized by the supplementation of folic acid during the critical period of these 2 kinds of orofacial clefts. However, periconceptional daily supplementation with multivitamins including physiologic doses (<1 mg) of folic acid or folic acid alone (and probably folic acid fortified flour/bread) can not reduce the birth prevalence of isolated CL ± CP and CP. Only the high pharmacological doses (eg, 6 mg per day) of folic acid alone in the critical period of the primary and the secondary palate development seem to be effective for the reduction of orofacial clefts. Thus, our hypothesis concerning the dose-dependent effect of folic acid in the prevention of isolated CL ± CP and CP was confirmed, therefore a high dose (eg, 6 mg) of folic acid should be recommended for the reduction of recurrent orofacial clefts during early pregnancy under medical control. The question is the benefit and risk of this kind of primary prevention for the first occurrence of isolated CL ± CP and CP, which have a birth prevalence of 1.5 per 1000 because the upper tolerable level of folic acid is 1 mg for the preventive program because of the possible rare side effects, eg, in women with...
pernicious anemia. It is worth waiting for the specific prevention until the identification of women with high risk for orofacial clefts based on mutant genes. *Pediatrics* 1999;104(6). URL: http://www.pediatrics.org/cgi/content/full/104/6/66; orofacial cleft, cleft lip with or without palate, cleft palate, periconceptual multivitamin/folic acid supplementation, dose-dependent preventive effect of folic acid.

Two major forms of isolated oro-facial clefting are known. Cleft lip (CL) with or without cleft palate (CP; CL ± CP) has a birth prevalence of ~1/1000, whereas ~.4 case with (posterior) CP occurs among 1000 births.1,2 It is worth separating these entities from the so-called multiple or syndromic oro-facial clefts of heterogeneous origin.3 A reduction in the recurrence rate of isolated CL ± CP was found after periconceptual daily supplementation with a multivitamin, including an extremely high dose of folic acid (10 mg), in a nonrandomized study by Tolarova4 in 1982. Two previous Hungarian studies resulted in controversial findings. The randomized double-blind controlled trial of periconceptual multivitamin supplementation (RCT) did not show a reduction in the first occurrence of isolated oro-facial clefts.5,6 The multivitamin used contained .8 mg folic acid, ie, a physiologic dose. However, the possible association between folic acid supplementation and reduction of congenital abnormalities was evaluated in the dataset of the Hungarian Case-Control Surveillance of Congenital Abnormalities (HCCSCA), 1980–1991, and, among others, a significant reduction was found in the first occurrence of oro-facial clefts after a high-dose (3–9 mg) folic acid supplementation in the early post-conceptional period.7

Thus, we hypothesized that the prevention of oro-facial clefts by folic acid has a dose-dependent effect, and this hypothesis was tested in 2 recent independent Hungarian datasets.

### METHODS

**Prospective Cohort Study (PCS)**

The relationship between the use of periconceptual multivitamin supplementation and the incidence of congenital abnormalities, particularly isolated oro-facial clefts, was investigated by following 2 cohorts in the study, 1 of them was exposed (ie, supplemented) and the other was unexposed (ie, unsupplemented).

**Recruitment of Two Cohorts in the PCS**

Women with confirmed pregnancy who were supplemented by a folic acid-containing multivitamin periconceptually were recruited from the participants of the periconceptional service3

### TABLE 1. Distribution of Informative Offspring and Isolated CL ± CP and CP in Supplemented and Unsupplemented Groups

<table>
<thead>
<tr>
<th>Informative Offspring</th>
<th>Supplemented Group (n = 3415)</th>
<th>Unsupplemented Group (n = 3415)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
</tr>
<tr>
<td>Elective termination of pregnancy</td>
<td>22</td>
<td>.07%</td>
</tr>
<tr>
<td>Stillbirth</td>
<td>9</td>
<td>.3%</td>
</tr>
<tr>
<td>Livebirth</td>
<td>2988†</td>
<td>99.0%</td>
</tr>
<tr>
<td>Total</td>
<td>3019</td>
<td>100.0%</td>
</tr>
<tr>
<td>CL ± CP</td>
<td>3</td>
<td>.10%</td>
</tr>
<tr>
<td>CP</td>
<td>1</td>
<td>.03%</td>
</tr>
</tbody>
</table>

* Including 61 twin pairs.
† Including 51 twin pairs.
‡ This control infant was affected with Robin-sequence.
tamin supplementation, they were excluded from the PCS, and they were referred immediately to the regional antenatal care.

Antenatal care is mandatory in Hungary for pregnant women. All pregnant women after the first visit between the 8th and 12th week of gestation were informed about the purpose of the PCS and were invited to take part in the PCS in the region where cases were recruited. Eligibility of controls was based on 3 criteria: First, no mother (or folic acid) supplementation was taken in the periconceptional period. Data concerning their previous and present vitamin supplements and diet, particularly the consumption of citrus fruit and leafy dark green vegetables (eg, spinach) were obtained by printed questionnaire. Second, eligibility was based on appropriate matching of age (±1 year), socioeconomic status, and residence based on the personal card of supplemented pregnant women. Each supplemented pregnant woman received 2 matched unsupplemented pregnant women, the goal of the second reserve woman was to reduce drop-out. Third, all participation was voluntary. If unsupplemented pregnant women were eligible for the PCS and they agreed to participate, they were asked to sign an informed consent form. (The informed consent authorized us to visit both supplemented and unsupplemented pregnant women at home if appropriate information about their pregnancy outcome after 1 month of expected date of delivery was not available.) The mean number of visits of unsupplemented pregnant women was ~8 in the antenatal care. Unsupplemented pregnant women also were asked to deliver personally or to mail their completed pregnancy certificate to the regional antenatal care after the ending of pregnancy.

Endpoints of the PCS

The endpoints of the PCS were congenital abnormalities in informative offspring, ie, 1) malformed fetuses diagnosed attributable to antenatal examinations in the second trimester usually followed by termination of pregnancy, 2) stillborn fetuses, and 3) liveborn infants. In Hungary, all deliveries and terminations of pregnancy occur in inpatient obstetric clinics and the birth attendants are obstetricians. In most inpatient obstetric clinics, pediatricians also examine neonates. Autopsy was obligatory in infant deaths and usually was performed in stillborn fetuses. The pregnancy certificates of supplemented and unsupplemented women were completed by mothers after the end of pregnancy and were countersigned by their physicians. The certificate included the date of pregnancy outcome, the type of births (singleton or multiple), sex, birth weight, gestational age, and particularly any developmental defects.

The evaluation of congenital abnormalities was based on 2 approaches. If congenital abnormalities were diagnosed in the fetus or newborn infants, an attempt was made to collect all available medical documents (ultrasound films, discharge summaries, autopsy report, etc). Infants with particular or questionably defects were referred to our department for a detailed medical examination. In contrast, an attempt was made to examine all infants without reported congenital abnormalities by the pediatrician of the PCS (L.T.) until the 12 months of life. The pediatrician was not informed whether infants examined belonged to the supplemented or unsupplemented cohorts. If families did not take part in this examination of infants, the invitation was repeated. If there was no response, we contacted the regional or family pediatricians and obtained case histories with particular reference to congenital abnormalities. Congenital abnormalities were evaluated blindly and classified by 1 of us (A.E.C.), eg, isolated and multiple (syndromic) cases of CL ± CP and CP were differentiated.

Considerable efforts were made to evaluate pregnancy outcomes in all supplemented and unsupplemented participants. If the completed pregnancy certificate was not sent back within 1 month of the expected date of delivery, participants were contacted by telephone or mail and asked to send the completed certificate and/or discharge summary. If there was no response, the study coordinators visited these families at home. A complete failure of contact was considered to be a dropout from the PCS.

Statistical Evaluation of Data

At the evaluation of informative offspring with orofacial cleft, adjusted odds ratios (OR) with 95% confidence intervals (95% CI) for potential confounders were calculated using a conditional logistic regression model by the STATA statistical software package.
The proportion of fully and partially supplemented subgroups was 95% and 5%. Pregnancy outcomes could not be clarified in 24 (0.7%) supplemented and 64 (1.9%) unsupplemented women attributable to changed or untraceable addresses, moving abroad, or noncooperation. However, the final dataset included 3415 pregnant women both in supplemented and unsupplemented groups because the unsupplemented group was complemented with 40 second reserve matched pregnant women to replace the higher dropout rate.

Matched variables were similar in the 2 study groups (mean age in years: 27.6 ± 4.0 in supplemented and 27.8 ± 4.2 in unsupplemented pregnant women, proportion of different school grades: ≤8 = 3%, 9–12 = 48%, and ≥13 = 49%; distribution by employment status: professional = 48%, managerial = 26%, skilled worker = 18%, semiskilled/unskilled worker = 2%, and others = 6%). The birth order was lower in the supplemented group (1.2 ± .6) because of the larger proportion of women planning first pregnancies compared with the unsupplemented group (1.4 ± .7) \(t = 12.7; P < .001\). However, the rate of fetal deaths (27.3% in supplemented and 13.9% in unsupplemented pregnant women) and infant deaths (5.6% in supplemented and 9% in unsupplemented groups), in addition to the occurrence of congenital abnormalities (6.1% in supplemented and 6% in unsupplemented informative offspring) in previous pregnancies were much higher in supplemented than in unsupplemented women. Of 153 malformed offspring in the previous pregnancies of supplemented women, 2 had CL + CP and 1 was affected with CP, whereas of 21 previously malformed offspring in the unsupplemented group, 1 had CL + CP. The consumption of citrus fruit and dark green vegetables in unsupplemented women corresponded to the diet of participants in the periconceptional care.15

The distribution of informative offspring is shown in Table 1. The lower number of liveborn infants in the supplemented group was explained by the higher number of first trimester pregnancy terminations (11 vs 0), ectopic pregnancies (17 vs 0) and miscarriages (429 vs 34) attributable to the earlier recruitment of women. (Twin births increased the number of informative offspring; therefore, the latter exceeded the number of pregnant women in the unsupplemented group.) Sex, birth weight, and gestational age of liveborn infants did not show significant differences between the 2 cohort groups.

All orofacial clefts occurred in liveborn infants (Table 1). The occurrence of CL ± CP (OR: 1.71 with 95% Cl: .34–8.66) and CP (OR: 1.14 with 95% Cl: .12–10.94) did not show difference between the supplemented and unsupplemented groups. The manifestation of CL ± CP was the following: CL alone 0:2 (both left) while CL + CP 3:0 (2 left, 1 bilateral CL) in the supplemented and unsupplemented groups, respectively. The supplemented asthmatic mother of 1 infant with CL + CP missed multivitamin tablets several times for 3 to 4 days. There was 1 recurrent CL + CP in the infant of a mother with full supplementation. In the unsupplemented group, 1 twin infant had Robin-sequence, but in general this abnormality entity is included in the group of CP; the other twin was healthy.

The combination of results of the RCT and PCS offers an opportunity to estimate the efficacy of periconceptional folic acid-containing multivitamin in the reduction of the first occurrence of isolated oral clefts (Table 2). In the supplemented group, 1 infant in the RCT and 1 supplemented infant in the PCS had recurrent CL + CP; therefore, they were excluded from the analysis. In addition, the newborn infants of 1 supplemented and 1 unsupplemented woman with previous CL + CP child and 1 supplemented woman with a previous CP infant in the PCS also were excluded from the denominators attributable to the 50-fold higher recurrence risk.3 There were 6 and 4 CL ± CP infants in the supplemented and unsupplemented groups, respectively. In addition, there were 1 and 3 children with CP in the supplemented and unsupplemented groups, respectively. Adjusted OR with wide range of CI for founders (parity and previous pregnancy outcomes) did not show difference between the 2 cohort groups. In addition, there was no significant difference in the first occurrence of CL ± CP \((P = .47)\) and CP \((P = .35)\) between the supplemented and unsupplemented groups according to the Mantel-Haenszel test.

At the evaluation of the HCCSCA dataset, the study period covered 2,146,574 total births in Hungary. Hence the 38,151 population controls represented 1.8% of the Hungarian births. Of 22,865 cases with congenital abnormalities, 1368 had CL ± CP and 596 had CP. Finally 1246 and 537 case–control pairs were evaluated in the group of CL ± CP and CP, respectively.

Maternal age (in years: 25.3 ± 5.2 vs 25.4 ± 4.9) did not show any difference between case and control subjects \((t = 1.29; P = .20)\), but birth order \((1.79 ± 1.1\)

\[\text{TABLE 2. The First Occurrence of Isolated CL ± CP and CP in the Previous Hungarian RCT and the PCS of Periconceptional Folic Acid-containing (.8 mg) Multivitamin Supplementation} \]

<table>
<thead>
<tr>
<th>Study</th>
<th>Isolated Orofacial Clefts</th>
<th>Supplemented Group</th>
<th>Unsupplemented Group</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>Number</td>
<td>Per 1000</td>
<td>N</td>
</tr>
<tr>
<td>RCT</td>
<td>CL ± CP</td>
<td>2471</td>
<td>4</td>
<td>1.62</td>
<td>2390</td>
</tr>
<tr>
<td>PCS</td>
<td></td>
<td>3017</td>
<td>2</td>
<td>.66</td>
<td>3431</td>
</tr>
<tr>
<td>PCS</td>
<td>CL ± CP</td>
<td>5488</td>
<td>6</td>
<td>1.09</td>
<td>5821</td>
</tr>
<tr>
<td>PCS</td>
<td>CP</td>
<td>2471</td>
<td>0</td>
<td>0.00</td>
<td>2391</td>
</tr>
<tr>
<td>PCS</td>
<td></td>
<td>3018</td>
<td>1</td>
<td>.33</td>
<td>3432</td>
</tr>
<tr>
<td>PCS</td>
<td></td>
<td>5489</td>
<td>0</td>
<td>.18</td>
<td>5823</td>
</tr>
</tbody>
</table>

\[N\] indicates number of informative offspring evaluated.
vs 1.70 ± .9) was higher in the case group (t = 7.85; P < .001). In general, the occurrence of pregnancy complications, maternal disorders, and other drug uses were similar between case and control pregnant women. However, common cold, influenza, acetylsalicylic acid, and aminophylline use were more frequent in cases with CL ± CP, whereas edema in CP cases compared with that of matched controls.

As Table 3 shows that pregnant women rarely used (12.5–14.3%) folic acid alone. Therefore, folic acid alone and with other drugs were evaluated together, because most other drugs including pregnancy supplements (other vitamins, irons, and calcium derivatives) have similar use in cases and controls. The mothers of newborn infants with CL ± CP had a significantly lower use of folic acid (50.0%) than did the mothers of matched population controls (54.8%). It was the case in the group of CP (47.3%) as well compared with their matched controls (54.2%). The proportion of medically documented folic acid supplementation was similar in the combined control (65.6%) and case (64.2%) groups (χ² = 5.1; P = .25).

It is worth evaluating the start of folic acid supplementation according to the gestational months (Table 3). Folic acid was used only in a small proportion of women in the first month of gestation, which corresponded to the preconceptional supplementation. The increase of folic acid use in the second month of gestation may reflect the early visits to the antenatal care or voluntary folic acid supplementation after the recognition of pregnancy. The maximum rate was seen in the third month of gestation, at the usual time of the first visit to the antenatal care. The later start of folic acid supplementation can be explained by a postponed visit in the antenatal care or a delay in folic acid use. The major point is the use of folic acid at the development of primary and secondary palate. Thus, the cumulative number of pregnant women with high-dose folic acid supplementation was calculated during the critical period of CL ± CP and of CP. However, the critical period of the primary palate development (CL ± CP) includes the last week of the second and the first week of the third gestational months, whereas the secondary palate development (ie, CP forming period) overlaps the last 2 weeks of the third and first 2 weeks of the fourth gestational months. This explains why there were 2 estimates for the protective effect of folic acid. At the evaluation of the primary palate development, there was a significantly higher rate of supplementation with a high dose (in general 6 mg/day) of folic acid during the first and second months of gestation in the control group (12.4% vs 9.1% in the case group), which may indicate a protective effect for CL ± CP. This was the case at the evaluation of folic acid supplementation in the first 4 months of gestation (39.0% in controls and 32.2% in cases) in the group of CP. There was no difference in the occurrence of folic acid supplementation after the critical period of these 2 kinds of orofacial clefts between cases and controls, respectively.

**DISCUSSION**

Two different Hungarian datasets are presented and compared in this paper. There were 3415 evaluated pregnant women both in the supplemented and unsupplemented cohorts during the 3 years of the study period in the PCS. The birth order was higher in the unsupplemented group, because primiparous females preferred participation in the periconceptional service and periconceptional multivitamin supplementation is part of the periconceptional care. However, the higher number of women with previous unsuccessful pregnancy outcomes was a more obvious trend in the supplemented group because of the expected higher medical standard of the periconceptional service. Thus, the higher potential recurrence risk for congenital abnormalities including orofacial clefts has to be considered at the comparison of the supplemented and unsupplemented groups.

The major finding of the PCS confirmed that

<table>
<thead>
<tr>
<th>Gestational Month</th>
<th>CL ± CP (n = 1246)</th>
<th>OR (95% CI)</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>46</td>
<td>3.7</td>
<td>65</td>
<td>5.2</td>
</tr>
<tr>
<td>II</td>
<td>68</td>
<td>5.5</td>
<td>89</td>
<td>7.1</td>
</tr>
<tr>
<td>III</td>
<td>224</td>
<td>18.0</td>
<td>220</td>
<td>17.7</td>
</tr>
<tr>
<td>IV</td>
<td>93</td>
<td>7.5</td>
<td>107</td>
<td>8.6</td>
</tr>
<tr>
<td>V</td>
<td>91</td>
<td>7.3</td>
<td>91</td>
<td>7.3</td>
</tr>
<tr>
<td>VI</td>
<td>38</td>
<td>3.0</td>
<td>57</td>
<td>4.6</td>
</tr>
<tr>
<td>VII</td>
<td>35</td>
<td>2.8</td>
<td>35</td>
<td>2.8</td>
</tr>
<tr>
<td>VIII</td>
<td>23</td>
<td>1.8</td>
<td>15</td>
<td>1.2</td>
</tr>
<tr>
<td>IX</td>
<td>5</td>
<td>0.4</td>
<td>4</td>
<td>0.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gestational Month</th>
<th>CP (n = 537)</th>
<th>OR (95% CI)</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>10</td>
<td>1.9</td>
<td>13</td>
<td>2.4</td>
</tr>
<tr>
<td>II</td>
<td>25</td>
<td>4.7</td>
<td>45</td>
<td>8.4</td>
</tr>
<tr>
<td>III</td>
<td>100</td>
<td>18.6</td>
<td>93</td>
<td>17.3</td>
</tr>
<tr>
<td>IV</td>
<td>38</td>
<td>7.1</td>
<td>58</td>
<td>10.8</td>
</tr>
<tr>
<td>V</td>
<td>32</td>
<td>6.0</td>
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<td>7.6</td>
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<tr>
<td>VI</td>
<td>19</td>
<td>3.5</td>
<td>22</td>
<td>4.1</td>
</tr>
<tr>
<td>VII</td>
<td>24</td>
<td>4.5</td>
<td>12</td>
<td>2.2</td>
</tr>
<tr>
<td>VIII</td>
<td>5</td>
<td>1.9</td>
<td>6</td>
<td>1.1</td>
</tr>
<tr>
<td>IX</td>
<td>1</td>
<td>1.2</td>
<td>1</td>
<td>0.2</td>
</tr>
</tbody>
</table>

| Total             | 623          | 50.0        | 683   | 54.8     |
|                   | 254          | 47.3        | 291*  | 54.2     |
| Folic acid alone  | 163          | 13.1        | 178   | 14.3     |

* The start of folic acid supplementation was unknown is 1 control.
periconceptional supplementation with multivitamins including .8 mg of folic acid does not reduce the incidence of orofacial clefts. In the previous Hungarian RCT,6,8,9 the unsupplemented group had 5 children with an orofacial cleft (1 was a recurrent CL + CP), giving a statistical power which could detect incidence differences only on the order of 50%. Thus, it is worth combining the 2 datasets of the RCT and PCS to obtain more accurate estimates for the effect of periconceptional multivitamin supplementation for the reduction of the first occurrence of isolated orofacial clefts (Table 2). The data of about 5500 supplemented and 5800 unsupplemented pregnant women do not indicate a protective effect of multivitamins including physiologic dose (<1 mg) of folic acid for isolated orofacial clefts.

One observational study using a multivitamin including 4–8 mg of folic acid in the periconceptional period showed a reduction in the occurrence of CL ± CP,16 whereas another case–control observational study did not indicate a reduction either in CL ± CP or in CP after the intake of the usual folic acid-containing multivitamins or folic acid alone.17

The strength and weakness of the HCCSCA have been discussed previously.13 However, we note that this is the largest case–control dataset of its type in the world and population-based sampling makes risk–benefit assessment possible. A significant reduction was found in the rate of isolated CL ± CP and CP attributable to the pharmacological doses (in general, 6 mg daily) of folic acid supplementation during pregnancy. This protective effect was found only after the use of folic acid before and during the critical period of orofacial clefts, but not after the development of primary and secondary palate. This finding is in agreement with the previously mentioned results of the study of Tolarova.4 In addition, Nelson et al18 were able to induce CL in >90% of the rat offspring subjected to a transitory folic acid deficiency attributable to an antimetabolite during the critical period of this congenital abnormality.

In conclusion, folic acid deficiency may have a role in the origin of orofacial clefts and it can be neutralized by the supplementation of folic acid during the critical period of the primary and secondary palate because their developmental disturbances cause CL ± CP and CP. However, the protective effect of folic acid seems to be dose-dependent because only the high-dose folic acid supplementation was found effective on the reduction of isolated orofacial clefts.

Three points need additional discussion. First, the origin of neural tube defects and oral clefts may have some similarities (neural crest cells, schisis-midline defects, etc).19,20 However, there are also differences, for example, studies showed an association between homozygosity for a variant form of the C677T genotype in the methylene-tetrahydrofolate reductase gene and risk for neural tube defects.21 However, the study of Shaw et al22 did not indicate increased risk for CL ± CP among infants homozygous for the C677T genotype, nor do they indicate an interaction between infant C677T genotype and maternal multivitamin use on the occurrence of CL ± CP. Thus, a multivitamin including a physiologic dose of folic acid is appropriate23 and flour–bread fortification with folic acid24 may be effective for the reduction of neural tube defects but not for orofacial clefts. A similar prevention of orofacial clefts requires a high dose of folic acid. The second point is whether the reduction of isolated orofacial clefts is caused by the folic acid supplementation (ie, causal effect) or may be connected with other indirect effects (eg, lifestyle). A causal effect can be supposed if the folic acid deficiency or the preventive effect of high-dose folic acid exists only during the critical period of orofacial clefts. However, if the protective effect of high folic acid use can be observed independent of the critical period of orofacial clefts, it may indicate indirect effects. Our analysis showed a protective effect of high-dose folic acid supplementation only during the critical period of orofacial clefts. The third point is related to practical use of our knowledge. A higher dose (~6 mg) of folic acid should be recommended for the reduction of recurrent orofacial clefts during early postconceptional period under medical control. The question is whether it is worth recommending the use of a high-dose folic acid supplementation after the early diagnosis of pregnancy in all women to reduce the first occurrence of isolated CL ± CP and CP, which together have a birth prevalence of about 1.5/1000. The pharmacological dose of folic acid, however, may have some side effects, eg, in women with pernicious anaemia. The optimal solution would be a specific prevention only in women at high genetic risk for orofacial clefts based on the identification of mutant genes.25–27

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