Maternal intake of folate during pregnancy and risk of cerebral palsy in the MOBAND-CP cohort

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ABSTRACT

Background: Folate prevents neural tube defects and may play a role in some neurodevelopmental disorders.

Objectives: We investigated whether higher intakes of periconceptional or midpregnancy folate, as recommended, were associated with a reduced risk of offspring cerebral palsy (CP).

Methods: We included participants from the Nordic collaboration cohort consisting of mother-child dyads in the Danish National Birth Cohort and the Norwegian Mother, Father, and Child Cohort Study [combined as MOthers and BAbies in Norway and Denmark (MOBAND-CP)]. A total of 190,989 live-born children surviving the first year of life were included. Missing covariate data were multiply imputed. Our exposures were defined as any or no folic acid supplementation in gestational weeks (GWs) –4 to 8 (periconceptional), 9 to 12, and –4 to 12, and supplemental, dietary, and total folate during midpregnancy (GWs 22–25). CP overall and the unilateral and bilateral spastic subtypes, as well as CP with low or moderate/high gross motor function impairments, were our outcomes of interest.

Results: Periconceptional folic acid supplementation was not associated with CP [adjusted odds ratio (aOR), 1.02; 95% CI: 0.82–1.28]. However, supplementation in GWs 9 to 12 was associated with a reduced risk of CP (aOR, 0.74; 95% CI: 0.57–0.96), and inverse associations were indicated for both the unilateral (aOR, 0.68; 95% CI: 0.46–1.02) and bilateral (aOR, 0.70; 95% CI: 0.49–1.02) spastic subtypes, although the associations were not statistically significant. Supplemental or dietary folate in midpregnancy alone were not associated with CP. Strong inverse associations were observed with low gross motor function impairment (aOR, 0.49; 95% CI: 0.29–0.83), while for unilateral CP the aOR was 0.63 (95% CI: 0.34–1.22) for intakes of ≥500 compared to ≤199 dietary folate equivalents/day during midpregnancy.

Conclusions: Our findings suggest that folate intakes in GWs 9 to 12 and midpregnancy were associated with lower risks of CP, while no association was observed for periconceptional supplementation. *Am J Clin Nutr* 2022;115:397–406.

Keywords: DNBC, MoBa, MOBAND-CP, cerebral palsy, folic acid, nutrition, epidemiology, neurodevelopment, food frequency questionnaire

Introduction

Cerebral palsy (CP) is the most common motor disability in childhood, with a prevalence of approximately 2 per 1000 live births (1, 2). The etiology of CP is multifactorial and the most important CP risk factors operate prenatally (3), yet some of these prenatal risk factors remain to be identified. One plausible prenatal risk factor is insufficient periconceptional folic acid intake. Periconceptional folic acid supplementation lowers the risk of neural tube defects (4–8). Furthermore, intake of folic acid supplements has been associated with lower risks of several neurodevelopmental disorders, including autism spectrum disorder, language delays, and impaired motor function (9–12), although results on autism have been inconsistent (13, 14). To date, only 1 case-control study has examined the association between folic acid supplementation during pregnancy and CP. The findings suggested half the risk of CP in children of supplementing mothers; however, the dietary covariate adjustment was minimal (15). Results from studies with prospectively collected data and adequate confounder controls were therefore warranted.

Our primary aim was to examine whether higher intakes of maternal periconceptional folic acid supplementation, as recommended, are associated with reduced risks of CP. Additionally, we considered the potential role of midpregnancy folate intake.

Methods

Study population

The study population was drawn from the Mothers and Babies in Norway and Denmark (MOBAND-CP) collaboration cohort (16–18), comprising children recruited into 2 large Nordic birth cohorts: the Danish National Birth Cohort (DNBC) (17) and the Norwegian Mother, Father and Child Cohort Study (MoBa), conducted by the Norwegian Institute of Public Health (18). MOBAND-CP includes approximately 210,000 children born in 1996–2009 for whom data on prenatal exposures were collected primarily during pregnancy, as described in more detail elsewhere (16). Due to planned similarities in the timing and content of data collected between the 2 cohorts, it has been possible to create a large number of harmonized variables in MOBAND-CP (16). For our study, we selected live-born offspring with data from the earliest maternal interview/questionnaire. In the DNBC, initial contact with mothers occurred with general practitioners at around gestational weeks (GWs) 6 to 10, when mothers were given an enrolment form that was completed at home and included questions on intake of periconceptional dietary supplementation and medication. Additionally, information on maternal characteristics and exposures was collected in a computer-assisted telephone interview in DNBC at mean GW 16. In MoBa, data on both folic acid supplementation and maternal characteristics were collected through a self-administered, written questionnaire received by mail around GW 15, together with the invitation to participate in the study. Semi-quantitative FFQs were completed in both cohorts, at mean GWs 25 (DNBC) and 22 (MoBa). We excluded infant deaths and observations missing information on folic supplementation or sex, leaving a population for analysis of 190,989 children in our data set (**Supplemental Figure 1**).

Periconceptional folic acid supplementation

We defined our primary exposure as any periconceptional folic acid supplementation in GWs −4 to 8, since we hypothesized that the periconceptional period would be the most sensitive to supplementation. Additionally, we examined supplementation in the subsequent 4-week interval (GWs 9 to 12), as well as a composite measure of the 2 intervals (GWs −4 to 12). The participants in the DNBC were asked to report weekly folic acid and multivitamin supplementation by type and dosage from GWs −4 to 12 on the enrolment form. Two versions of the DNBC enrolment forms were administered, which have been harmonized, so periconceptional supplementation data were available for nearly the entire cohort (13). In MoBa, mothers were asked to report any use of folic acid and multivitamin supplementation (yes compared with no) for 4-week intervals running from GWs −4 to 12. MoBa mothers were not asked to provide data on dosages of periconceptional supplementation.

Since any use included mothers with sporadic use, we also employed an alternative categorization to examine regularity of supplementation in the 2 periods of GWs −4 to 8 and GWs −4 to 12. Data were harmonized so that participants were first classified according to any or no use in the four 4-week intervals from GWs −4 to 12. We then classified exposure for the respective periods as none (no supplementation in any of the 4-week intervals), irregular (any supplementation in only some of the 4-week intervals), or regular (any supplementation in all of the 4-week intervals).

Midpregnancy folate

Dosages of midpregnancy folic acid supplementation were reported in the FFQs and classified as no (0 μg/day), noncompliant (1–399 μg/day) or compliant ($\geq$400 μg/day) supplementation, according to levels of adherence to the Nordic Nutrition Recommendations (NNR) 2012 for folate among women of reproductive age (19). Similarly, dietary folate intakes in μg/day were estimated from the FFQs, using calculations based on standard portion sizes. We classified midpregnancy (GWs 22 to 25) dietary folate intake into 3 groups of low dietary intakes (0–299 μg/day), insufficient dietary intakes (300–399 μg/day), and sufficient dietary intakes ($\geq$400 μg/day), again in accordance with the aforementioned NNR 2012 recommendations (19). To examine the combined effect of folate from supplements and diet, midpregnancy total folate values were calculated as total dietary folate equivalents (DFEs) by multiplying supplemental folic acid intakes by a factor of 1.7, and then aggregating intakes with dietary sources (20). Both supplemental folic acid and dietary folate intakes have been validated in both cohorts (21, 22), and

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Supplemental Figures 1–3, Supplemental Methods, and Supplemental Tables 1–9 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

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Abbreviations used: aOR, adjusted odds ratio; CP, cerebral palsy; DFE, dietary folate equivalent; DNBC, Danish National Birth Cohort; GMFCS, Gross Motor Function Classification System; GW, gestational week; IVF, in vitro fertilization; MoBa, Norwegian Mother, Father and Child Cohort Study; MOBAND-CP, Mothers and Babies in Norway and Denmark; NNR, Nordic Nutrition Recommendations.

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a previous study found that total folate intakes correlated well with plasma folate measured approximately at the same time in a subsample of MoBa participants (23). The FFQs used in DNBC and MoBa are comparable in length and detail and assess intakes of roughly 300 food items and dietary supplements (24). Both have been developed and validated specifically for pregnant women. Further information on the administration and content of the FFQs are available by Olsen et al. (24). The total DFEs were classified into categories of 0–199, 200–299, 300–399, 400–499, and ≥500 DFEs/day.

Total periconceptional folate: only in DNBC

In a subgroup analysis of DNBC participants alone, we categorized periconceptional total DFEs similarly to the aforementioned categories (0–199, 200–399, 400–499, and ≥500 DFEs/day), using midpregnancy dietary folate intake as a proxy for periconceptional intake. Weekly supplemental intakes from GWs −4 to 8 were averaged and converted to DFEs and added to dietary folate intakes.

Measures of CP

Danish children with CP were identified through the Danish National Cerebral Palsy Registry (25), which has full nationwide coverage of children with a neuro-pediatrician-validated diagnosis of CP from age 4 to 6 years. Children with a verified CP diagnosis who die before the age of 4 are also included. A full coverage rate for Norwegian children was obtained by identifying cases from both the Cerebral Palsy Registry of Norway with a neuro-pediatrician-validated diagnosis at age 5 years (26) and additional neuro-pediatrician-validated cases from the Norwegian Patient Registry (27). We examined associations both with CP overall and the 2 major subtypes (unilateral and bilateral spastic CP) as the primary outcomes and with CP according to the Gross Motor Function Classification System (GMFCS), classified as low (GMFCS I) or moderate/high (GMFCS II–V) gross motor function impairment, as the secondary outcomes.

Data on covariates

The following potential confounders were identified (28) maternal age at birth (<25, 25–29, 30–34, or ≥35 years), in vitro fertilization (IVF) treatment (yes, no), maternal occupational status (employed, unemployed, student, or receiving benefits or pension), prepregnancy BMI (<18.5, 18.5–22.9. 23.0–24.9. 25.0–29.9, or ≥30 kg/m), number of cigarettes smoked per day in the first part of pregnancy (0, 1–9, or ≥10), number of alcoholic beverages per week (0, 0.1–0.5, 0.6–2.5, or >2.5), periconceptional fish oil supplementation (any or none), dietary folate intake reported during midpregnancy, and total dietary and supplemental EPA/DHA intakes reported during midpregnancy. The latter 2 were considered proxies for periconceptional dietary intakes of the specified nutrients, since no data on periconceptional dietary intakes were available. In complete case analyses, these were modeled as restricted cubic splines with 3 knots at the 10th, 50th, and 90th percentiles for dietary folate and 5 knots at the 5th, 27.5th, 50th, 72.5th, and 95th percentiles for total EPA/DHA. In multiple imputation analyses, dietary folate values were included as previously described for exposure classification (low, insufficient, or sufficient dietary intakes) and total EPA/DHA was classified according to cohort deciles.

Most covariate data were self-reported in the earliest interview/questionnaire. In contrast, data on maternal age at birth and IVF were acquired through linkage to the Danish IVF Registry and the Medical Birth Registries of Denmark and Norway.

Statistical analyses

Unless otherwise specified, we identified models for statistical analyses a priori. We estimated ORs and 95% CIs of CP for each exposure, using logistic regression analyses. Robust variance estimators were applied to account for dependency due to siblingship, since mothers could contribute to the cohorts with multiple pregnancies or multiple births. In addition to the univariate models, we fitted multivariate models, including the harmonized covariates. In all analyses of various midpregnancy folate exposures, as well as folic acid exposure in GWs 9 to 12, we additionally controlled for prior folic acid supplementation exposure.

Missing covariate and midpregnancy exposure data for mother-child pairs were multiply imputed using Substantive Model Compatible Fully Conditional Specification, with the number of imputations set at 30 (see **Supplemental Methods**). The continuous covariates of dietary folate and total midpregnancy EPA/DHA were categorized to best account for nonlinear associations, because imputation of fractional polynomials was not feasible.

We examined interactions between periconceptional folic acid supplementation and dietary folate intake sufficiency using a joint effects model, since it has been postulated that the beneficial effects of folic acid supplementation on neurodevelopment may be limited to offspring exposed to insufficient dietary folate intakes (i.e., mothers not compliant with dietary folate recommendations). Likelihood ratio tests were conducted to test for the level of statistical significance. We determined that interaction models included in likelihood ratio tests with P values < 0.2 should be examined further in joint effects models. This cutoff was determined due to the limited power for interaction analyses.

For complete case analyses of total DFEs and GMFCS (post hoc analyses), multinomial logistic regression analyses were conducted, estimating RR ratios and 95% CIs.

Sensitivity analyses

We conducted several sensitivity analyses to check the robustness of our findings. We repeated several multiple imputation analyses after excluding those with prophylactic doses, to see whether confounding by indication may have biased our estimates. We also performed complete case analyses for comparison with multiple imputation analyses.

We conducted several analyses that were feasible only with complete cases. Due to potential residual confounding by healthy dietary patterns, we examined the main effects stratified by cohort affiliation, additionally adjusting for principal component dietary

TABLE 1 Baseline characteristics according to periconceptional folic acid supplementation

	Periconceptional folic acid supplementation (GWs −4 to 8)			
	None		Any	
	n	(%)	*n*	(%)
Total	60,198	100	130,791	100
Offspring sex, female	29,307	48.7	63,833	48.8
IVF, yes	1041	1.7	4917	3.8
Age at birth				
<25	8330	13.8	11,224	8.6
25–29.9	20,223	33.6	47,334	36.2
30–34.9	21,516	35.7	51,167	39.1
≥35	10,129	16.8	21,066	16.1
Socio-occupational position				
Employed	45,172	75.0	104,339	79.8
Unemployed	5674	9.4	8464	6.5
Student	7201	12.0	15,259	11.7
Benefits/pension	1299	2.2	1473	1.1
Missing	852	1.4	1256	1.0
Gestational smoking				
Nonsmokers	49,388	82.0	117,696	90.0
Smokers	10,398	17.3	13,095	10.0
Missing	412	0.7	513	0.4
Gestational alcohol drinking				
Nondrinkers	39,744	66.0	90,572	69.3
Drinkers	16,847	28.0	33,497	25.6
Missing	3607	6.0	6722	5.1
Prepregnancy BMI				
Mean (SD)	24.1	(±4.5)	23.7	(±4.2)
Missing	1791	3.0	2451	1.9
Periconceptional EPA/DHA supplementation, mg/day				
None	55,817	92.7	88,872	68.0
Any	4381	7.3	41,919	32.1
Midpregnancy dietary folate intake, μg/day				
Mean (SD)	312.4	(±118.9)	311.9	(±111.3)
Missing	16,048	26.7	21,949	16.8
Midpregnancy dietary EPA/DHA, mg/day				
Mean (SD)	387.0	(±393.3)	392.5	(±359.7)
Missing	16,048	26.7	21,949	16.8

Data are presented as absolute frequencies (*n*) and relative frequencies (%), unless otherwise specified. Abbreviations: GW, gestational week; IVF, in vitro fertilization.

patterns in each cohort (see Supplemental Methods). Briefly, it was not possible to harmonize a variable for dietary pattern, due to the cohort-specific nature of these data. The 2 cohort-specific dietary pattern variables, briefly elaborated on in the Supplemental Methods, described related dietary patterns for each cohort (24).

Our results using the categorical total DFE variable indicated a potential nonlinear association, so we investigated the specific level(s) at which higher midpregnancy total DFEs might be protective in post hoc complete case analyses. We modeled these associations using restricted cubic splines with 3 knots at the 10th, 50th, and 90th percentiles, estimating ORs and 95% CIs for specific intakes of DFEs/day, and visualizing them using 2-way plots. For a crude comparison with the results of the spline regressions in complete cases, we conducted post hoc logistic regressions of tertiles of total DFEs in the multiply imputed population.

Analyses were performed using Stata statistical software, version 16 (StataCorp).

Ethics

Written informed consent was obtained from all participating mothers in the DNBC and MoBa at the time of enrolment. The DNBC has been approved by the Danish Committee on Biomedical Research Ethics [case no. (KF) 01-471/94] and linkage to the Danish National CP Registry and harmonization with MoBa was approved by the Danish Data Protection Agency through the joint notification of the Faculty of Health and Medicine, University of Copenhagen (ref. 514-0214/18-3000). The establishment of MoBa and initial data collection were based on a license from the Norwegian Data protection agency and approval from The Regional Committees for Medical and Health Research Ethics. The MoBa cohort is based on regulations based on the Norwegian Health Registry Act. The Norwegian part of the study, including linkage with the National CP registry of Norway and the Norwegian Patient Register, was further approved by the Regional Committee for Medical Research Ethics (2012/1738).

Results

Of the 190,989 children included in the study, 392 were diagnosed with CP overall, 148 were diagnosed with unilateral spastic CP, and 186 were diagnosed with bilateral spastic CP. The majority of mothers supplemented with folic acid in GWs −4 to 8 (68%). Supplementing mothers were more often employed, between the ages of 25 and 35, nonsmokers, EPA/DHA supplementers, and IVF patients (**Table 1**). In each cohort, women supplementing with folic acid also supplemented with fish oil to a greater degree, although concurrent fish oil supplementation was more prevalent in Norway (**Supplemental Table 1**). Dietary folate intakes differed substantially between the 2 cohorts, with substantially lower mean intakes in Norway (Supplemental Table 1). Supplementation by periconceptional 4-week intervals increased to a greater extent in Norwegian mothers than Danish mothers, although overall levels were fairly similar throughout (**Supplemental Figure 2**).

Periconceptional folate

Our primary analyses showed no associations between any compared to no [adjusted OR (aOR), 1.02; 95% CI: 0.82–1.28; **Table 2**] or regular compared to no folic acid supplementation (aOR, 1.09; 95% CI: 0.84–1.41; **Supplemental Table 2**) in GWs −4 to 8 and CP overall. Periconceptional total intake of DFEs in the DNBC alone was not associated with CP overall (**Supplemental Table 3**). Similar results were observed for CP subtypes. Results from cohort-stratified analyses with adjustment for dietary patterns did not differ substantially (**Supplemental Table 4**). Our analyses indicated that supplementation in GWs 9 to 12 was associated with a reduced risk of CP overall (aOR, 0.74; 95% CI: 0.57–0.96; Table 2), with similar albeit not significant associations for the unilateral (aOR, 0.68; 95% CI: 0.46–1.02; Table 2) and bilateral (aOR, 0.70; 95% CI: 0.49–1.02; Table 2) spastic subtypes. Similar associations for periconceptional and early pregnancy exposures were seen for complete cases as those for multiple imputation analyses (**Supplemental Tables 5–7**).

In the joint effects model examining interactions between low dietary intakes, insufficient dietary intakes, and sufficient dietary intakes and any or no periconceptional supplementation, we observed some evidence of interaction in complete case analyses, where the association between any supplementation and unilateral CP was stronger among those with low dietary intakes (aOR, 0.54; 95% CI: 0.30–0.96; likelihood ratio test, $P = 0.118$; **Supplemental Table 8**). This was, however, not observed in the multiple imputation analyses (**Table 3**).

Midpregnancy folate

Supplementation and dietary sufficiency during midpregnancy (roughly GWs 22–25) were not independently associated with CP overall or CP subtypes. However, when these 2 sources of folate were considered in the aggregated measure, the aOR for unilateral CP was 0.41 (95% CI: 0.14–1.19) for intakes of 400–499 DFEs/day and 0.63 (95% CI: 0.34–1.22) for intakes of ≥500 DFEs/day (**Table 4**). In post hoc analyses, maternal intakes of ≥500 DFEs/day were associated with half the risk of CP with a low gross motor function impairment in offspring,

TABLE 2 Folic acid supplementation in GWs −4 to 8 (periconceptional), 9 to 12, and −4 to 12 and cerebral palsy (full data)

Folic acid supplementation	Pop_{Total} (n)	CP overall			Unilateral CP			Bilateral CP		
		Pop_{CP} (n)	OR (95% CI)		Pop_{CP} (n)	OR (95% CI)		Pop_{CP} (n)	OR (95% CI)	
			Crude model	Adjusted model[1]		Crude model	Adjusted model[1]		Crude model	Adjusted model[1]
GWs −4 to 8 (periconceptional)										
None	35,667	71	1 [Referent]	1 [Referent]	28	1 [Referent]	1 [Referent]	36	1 [Referent]	1 [Referent]
Any	91,986	174	0.99 (0.80–1.23)	1.02 (0.82–1.28)	64	0.88 (0.62–1.23)	0.82 (0.57–1.18)	84	1.02 (0.74–1.39)	1.15 (0.84–1.58)
GWs 9 to 12										
None	42,746	93	1 [Referent]	1 [Referent]	34	1 [Referent]	1 [Referent]	52	1 [Referent]	1 [Referent]
Any	84,907	152	0.84 (0.69–1.03)	0.74[2] (0.57–0.96)	58	0.77 (0.55–1.06)	0.68[2] (0.46–1.02)	68	0.79 (0.59–1.06)	0.70[2] (0.49–1.02)
GWs −4 to 12										
None	29,045	62	1 [Referent]	1 [Referent]	24	1 [Referent]	1 [Referent]	33	1 [Referent]	1 [Referent]
Any	98,608	183	0.90 (0.72–1.12)	0.92 (0.73–1.16)	68	0.76 (0.54–1.07)	0.70 (0.48–1.03)	87	0.89 (0.65–1.22)	1.01 (0.73–1.40)

Absolute frequencies (n) are presented for complete cases; analyses are based on the full multiple imputation data set. Abbreviations: CP, cerebral palsy; GW, gestational week; Pop_{CP}, cases with specified cerebral palsy subtype.

[1]The adjusted model controlled for maternal age at birth, in vitro fertilization treatment, EPA/DHA supplementation, prepregnancy BMI, smoking, alcohol, dietary folate, and total EPA and DHA.

[2]Additionally adjusted for periconceptional folic acid supplementation (GWs −4 to 8)

TABLE 3 Joint effects of dietary and supplemental periconceptional folate on risk of cerebral palsy (full data)

Dietary folate and periconceptional supplementation[1]	Pop Total (*n*)	Pop$_{CP}$ (*n*)	CP overall		Pop$_{CP}$ (*n*)	Unilateral		Pop$_{CP}$ (*n*)	Bilateral	
			Crude	Adjusted[2]		Crude	Adjusted[2]		Crude	Adjusted[2]
Low dietary intakes*No	18,764	41	1 [Referent]	1 [Referent]	[3]	1 [Referent]	1 [Referent]	[3]	1 [Referent]	1 [Referent]
Insufficient dietary intakes*No	10,271	22	1.03 (0.65–1.64)	1.06 (0.66–1.69)	[3]	0.78 (0.39–1.56)	0.82 (0.41–1.64)	[3]	1.25 (0.65–2.42)	1.25 (0.64–2.42)
Sufficient dietary intakes*No	6632	8	0.76 (0.40–1.42)	0.79 (0.42–1.49)	[3]	0.41 (0.11–1.45)	0.43 (0.12–1.56)	[3]	1.00 (0.42–2.38)	0.98 (0.41–2.35)
Low dietary intakes*Any	47,918	101	1.06 (0.78–1.45)	1.09 (0.79–1.50)	[3]	0.73 (0.46–1.18)	0.68 (0.41–1.11)	[3]	1.21 (0.76–1.94)	1.39 (0.86–2.24)
Insufficient dietary intakes*Any	27,601	43	0.80 (0.55–1.15)	0.84 (0.58–1.23)	[3]	0.72 (0.43–1.22)	0.72 (0.42–1.24)	[3]	0.83 (0.47–1.46)	0.92 (0.52–1.64)
Sufficient dietary intakes*Any	16,467	30	0.97 (0.64–1.46)	1.03 (0.68–1.57)	[3]	0.69 (0.37–1.30)	0.71 (0.37–1.35)	[3]	1.23 (0.69–2.21)	1.34 (0.73–2.44)

Absolute frequencies (*n*) are presented for complete cases; analyses are based on the full multiple imputation data set. Abbreviations: CP, cerebral palsy; GW, gestational week; Pop$_{CP}$, cases with specified cerebral palsy subtype; Pop$_{Total}$, total population.

[1]Low dietary intakes are defined as intakes below what is recommended for adults (0–299 μg/day). Insufficient dietary intakes are defined as intakes typically sufficient, but below recommendations for women in the reproductive age (300–399 μg/day). Sufficient dietary intakes are defined as meeting or exceeding the New Nordic Recommendations 2012 for women of reproductive age to consume at minimum 400 dietary folate equivalents. Values are based on midpregnancy dietary folate, as a proxy. Any periconceptional supplementation is defined as any report of supplementation in GWs −4 to 8, while no periconceptional supplementation is defined as no supplementation in the same period.

[2]The adjusted model controlled for maternal age at birth, in vitro fertilization treatment, EPA/DHA supplementation, prepregnancy BMI, smoking, alcohol, dietary folate, and total EPA/DHA.

[3]Clouded due to low number of observations in 1 or more cells

compared to intakes of <200 DFEs/day (aOR, 0.49; 95% CI: 0.29–0.83; **Table 5**). Similar results for the aforementioned midpregnancy exposures were observed for complete cases (Supplemental Tables 6 and **9**). In post hoc analyses with restricted cubic splines, an inverse J-shaped curve was observed for the relationship between total DFEs and unilateral CP, with the lowest association estimates between 800 and 900 DFEs/day (**Supplemental Figure 3**). No association was observed for bilateral CP (Supplemental Figure 3). Similar effect estimates were observed when including the covariates for dietary patterns in cohort-specific spline regressions, albeit with broader CIs (data not shown).

Discussion

We found no evidence that periconceptional folic acid supplementation in GWs −4 to 8 was associated with a decreased risk of CP. However, several of our findings suggest reduced risks with higher folate intakes later in the first trimester, as any supplementation in GWs 9 to 12 was associated with lower risks of both unilateral and bilateral CP by nearly a third. Moreover, higher intakes of total DFEs during midpregnancy (roughly GWs 22–25) were associated with a decreased risk of CP with a low gross motor function impairment, in a nonlinear dose-response manner, indicating that the risk of less-impaired CP phenotypes may be influenced by maternal folate status. It is difficult to make comparisons with previous research, since (to our knowledge) only 1 study has examined associations between prenatal folate and CP (15).

We initially hypothesized that folate might be of importance in the periconceptional window. Although we found no effect in the earliest (pre)pregnancy period, our results suggest that the later-pregnancy maternal folate status may have an effect on CP. The latter finding is compatible with evidence of the importance of mid- to late-pregnancy folate to fetal neurodevelopment (29). Potential explanations for the lack of association in the earlier period are lacking.

A potential mechanism explaining our findings might be the prevention of in utero fetal growth restriction through folic acid supplementation (30). For this to hold, we would expect to see greater effects of folate on bilateral CP than unilateral CP, as bilateral CP is much more common among preterm births and children born preterm are more likely to be growth restricted. Given that this was not the case, we suggest that any true effect of maternal folate is not likely to be primarily mediated by promoting normal fetal growth.

Nevertheless, it is not entirely surprising that we found an indication of a lower risk of unilateral CP with high folate intake, since increasing evidence suggests prenatal exposures play a greater role in the etiology of CP among cases born to term; that is, most unilateral CP cases and cases with a low gross motor function impairment (3). Animal studies indicate that maternal folate statuses both before and in pregnancy impact different biological processes in the developing brain, such as DNA synthesis, regulation of gene expression, synthesis of neurotransmitters, and phospholipids, among other things (31). However, the specific underlying mechanism for CP and why this would not apply to the early periconceptional period for developing CP must be further explored.

TABLE 4 Midpregnancy total, supplemental, and dietary folate and cerebral palsy (full data)

		CP overall			Unilateral CP			Bilateral CP		
	Pop_{Total} (*n*)	Pop_{CP} (*n*)	OR (95% CI)		Pop_{CP} (*n*)	OR (95% CI)		Pop_{CP} (*n*)	OR (95% CI)	
			Crude model	Adjusted model[1]		Crude model	Adjusted model[1]		Crude model	Adjusted model[1]
Total DFEs/day										
0–199	7328	22	1 [Referent]	1 [Referent]	10	1 [Referent]	1 [Referent]	9	1 [Referent]	1 [Referent]
200–299	16,966	33	0.76 (0.46–1.25)	0.77 (0.46–1.29)	13	0.61 (0.28–1.34)	0.62 (0.28–1.37)	13	0.81 (0.37–1.79)	0.83 (0.38–1.81)
300–399	13,396	35	0.87 (0.54–1.41)	0.91 (0.56–1.48)	[2]	0.86 (0.40–1.83)	0.91 (0.42–1.94)	[2]	0.94 (0.44–2.03)	0.96 (0.45–2.05)
400–499	9866	17	0.72 (0.40–1.30)	0.75 (0.42–1.35)	[2]	0.38 (0.13–1.10)	0.41 (0.14–1.19)	[2]	1.13 (0.49–2.57)	1.11 (0.49–2.55)
≥500	80,097	138	0.69 (0.46–1.04)	0.71 (0.47–1.10)	49	0.58 (0.31–1.09)	0.63 (0.34–1.22)	72	0.82 (0.43–1.57)	0.80 (0.42–1.58)
Supplemental folic acid, μg/day										
0	37,922	88	1 [Referent]	1 [Referent]	33	1 [Referent]	1 [Referent]	41	1 [Referent]	1 [Referent]
1–399	50,633	89	0.82 (0.63–1.07)	0.84[3] (0.64–1.11)	36	0.87 (0.57–1.35)	0.93[3] (0.60–1.45)	43	0.82 (0.56–1.21)	0.83[3] (0.55–1.24)
≥400	39,098	68	0.83 (0.62–1.10)	0.84[3] (0.62–1.13)	23	0.86 (0.55–1.35)	0.94[3] (0.59–1.51)	36	0.81 (0.54–1.23)	0.77[3] (0.49–1.21)
Dietary folate, μg/day										
0–299	66,682	142	1 [Referent]	1 [Referent]	52	1 [Referent]	1 [Referent]	66	1 [Referent]	1 [Referent]
300–399	37,872	65	0.83 (0.65–1.08)	0.88[4] (0.67–1.15)	27	0.91 (0.61–1.34)	0.97[4] (0.65–1.45)	32	0.84 (0.57–1.23)	0.85[4] (0.56–1.27)
≥400	23,099	38	0.87 (0.63–1.19)	0.93[4] (0.66–1.30)	13	0.74 (0.43–1.27)	0.81[4] (0.46–1.43)	22	1.02 (0.66–1.56)	1.01[4] (0.64–1.60)

Absolute frequencies (*n*) are presented for complete cases; analyses are based on the full multiple imputation data set. Abbreviations: CP, cerebral palsy; DFE, dietary folate equivalent; GW, gestational week; Pop_{CP}, cases with specified cerebral palsy subtype; Pop_{Total}, total population.

[1]The adjusted model was controlled for maternal age at birth, in vitro fertilization treatment, EPA/DHA supplementation, prepregnancy BMI, smoking status, alcohol intake, dietary folate intake, dietary EPA/DHA, periconceptional folic acid supplementation, and folic acid supplementation in GWs 9 to 12.

[2]Clouded due to a low number of observations in 1 or more cells or the potential deduction thereof.

[3]Additionally adjusted for dietary folate.

[4]Additionally adjusted for supplemental folic acid.

TABLE 5 Early and midpregnancy folate and cerebral palsy with low or high gross motor function impairment (full data)

		GMFCS I (low impairment)			GMFCS II–IV (moderate/high impairment)		
			OR (95% CI)			OR (95% CI)	
	Pop_{Total} (n)	Pop_{CP} (n)	Crude model	Adjusted model[1]	Pop_{CP} (n)	Crude model	Adjusted model[1]
Early pregnancy							
Folic acid supplementation							
GWs −4 to 8 (periconceptional)							
None	35,666	[2]	1 [Referent]	1 [Referent]	[2]	1 [Referent]	1 [Referent]
Any	91,983	[2]	0.91 (0.67–1.25)	0.92 (0.66–1.27)	[2]	1.15 (0.85–1.56)	1.23 (0.89–1.68)
GWs 9 to 12							
None	28,983	[2]	1 [Referent]	1 [Referent]	[2]	1 [Referent]	1 [Referent]
Any	98,425	[2]	0.81 (0.60–1.09)	0.76 (0.51–1.12)	[2]	0.90 (0.68–1.19)	0.73 (0.51–1.04)
GWs −4 to 12							
None	42,653	[2]	1 [Referent]	1 [Referent]	[2]	1 [Referent]	1 [Referent]
Any	84,755	[2]	0.84 (0.61–1.16)	0.84 (0.60–1.18)	[2]	1.00 (0.73–1.37)	1.06 (0.76–1.48)
Midpregnancy							
Total DFEs/day							
0–199	7328	[2]	1 [Referent]	1 [Referent]	[2]	1 [Referent]	1 [Referent]
200–299	16,966	[2]	0.57 (0.30–1.09)	0.573 (0.30–1.09)	[2]	1.07 (0.45–2.53)	1.12[3] (0.47–2.64)
300–399	13,396	22	0.74 (0.38–1.43)	0.743 (0.38–1.45)	13	1.22 (0.51–2.91)	1.30[3] (0.54–3.09)
400–499	9866	11	0.51 (0.23–1.12)	0.51[3] (0.23–1.12)	7	1.28 (0.50–3.27)	1.37[3] (0.51–3.49)
≥500	80,097	80	0.49 (0.29–0.82)	0.49[3] (0.29–0.83)	55	1.17 (0.55–2.47)	1.24[3] (0.58–2.65)

Absolute frequencies (n) are presented for complete cases; analyses are based on the full multiple imputation data set. Abbreviations: DFE, dietary folate equivalent; GMFCS, Gross Motor Function Classification System; GW, gestational week; Pop_{CP}, cases with specified cerebral palsy subtype; Pop_{Total}, total population.

[1]The adjusted model controlled for maternal age at birth, in vitro fertilization treatment, EPA/DHA supplementation, prepregnancy BMI, smoking status, alcohol intake, dietary folate intake, dietary EPA/DHA, and periconceptional folic acid supplementation (for GWs 9 to 12).

[2]Clouded due to a low number of observations in 1 or more cells or the potential deduction thereof.

[3]Additionally adjusted for periconceptional folic acid supplementation (GWs −4 to 8) and folic acid supplementation in GWs 9 to 12.

Strengths and limitations

Certain limitations must be considered when interpreting our results, the most compelling of which is potential residual dietary confounding. We controlled for other known potential confounders and found similar results in cohort-stratified analyses of periconceptional supplementation controlling for dietary quality. The assertion that residual dietary confounding might explain our findings—including the nonlinear dose-response association we observed—would require that another dietary factor highly correlated with folate has a similar dose-response relationship as that observed. We also did not observe drastic changes between crude and adjusted models. That inclusion of the considered dietary and demographic confounders did not substantially impact our results may increase confidence that these results are less likely to be influenced by residual confounding factors.

Although we were able to control for dietary folate and EPA/DHA, only intakes in midpregnancy were available. These served as proxies for earlier dietary intakes. Expectant mothers change consumption patterns in pregnancy, generally to more healthy choices. In MoBa, this includes an increased intake of some folate-containing foods (32). We were not able to assess whether these changes differ by the baseline dietary quality. Changes in dietary intake may be suspected to be differential, as supplement use is an expression of baseline health consciousness, which likely influences dietary choices in expectant mothers. Nonetheless, we expect a certain level of consistency between early pregnancy and midpregnancy intakes (33).

Another limitation is that a substantial portion of the cohort were missing data on midpregnancy folate and EPA/DHA. We therefore imputed. To get an idea of how much our results were affected by bias due to assumptions in the imputation process or missing data in the complete case analyses, we compared and provided results from both. Generally, our complete case analyses were similar to the multiple imputation analyses, with a few exceptions. Another consideration worth bearing in mind is that despite the large study sample, we did not expect to have sufficient power to examine CP subtypes for all exposures, even in analyses with multiply imputed data (34). In the potentially underpowered sample of complete cases, more efficient estimation was, however, obtained by modeling total folate as a continuous measure in restricted cubic splines. Several analyses with small strata, such as those for the joint effects model, require cautious interpretation.

A final consideration worth bearing in mind is that we dichotomized periconceptional folic acid supplementation, because folic acid doses were not available for the full cohort. This exposure classification is likely to be too inclusive and may have biased these results towards the null.

Despite these potential limitations, our results are especially relevant since randomized controlled trials comparing insufficient with sufficient intakes of folate would be unethical, given the convincing evidence available on the prevention of neural tube defects due to folic acid supplementation. The present study also has several notable strengths worth highlighting. To date, this is the only cohort study examining the association between

folate and CP. Especially noteworthy is the large population with validated CP diagnoses and detailed, prospectively collected exposure and covariate data. Finally, in analyses stratified by cohort affiliation, we could examine the consistency of findings across cohorts and thereby country, as well as potential confounding by dietary patterns.

Conclusion

Folic acid supplementation in the periconceptional period is likely not associated with a reduced risk of CP overall. Supplementation in GWs 9 to 12 is associated with moderately decreased risks of both spastic CP subtypes. Adequate maternal total folate intake in midpregnancy was associated with CP with a low gross motor function impairment in an exposure-dependent manner. However, the latter findings were not hypothesized and should be interpreted with greater caution. If confirmed in other studies, these findings would suggest that continued adherence to recommendations for maternal folate intake past the periconceptional period benefits fetal neurodevelopment.

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The authors' responsibilities were as follows—JG: is the guarantor of the study and had primary responsibility for the data management, analytical strategy, analyses, interpretation, and drafting of the manuscript; TGP, PS, KS-L, CG, and SFO: were involved in data acquisition and harmonization and the overall study design; TGP and KS-L: were primarily responsible for the data harmonization process; JG, TGP, TM, SFO, and KS-L: were involved in the statistical approach; and all authors: were involved in the design and/or analytical approach and manuscript revisions and read and approved the final manuscript. The authors report no conflicts of interest.

Data Availability

Data described in the manuscript, code book, and analytic code will not be made available because of data protection regulations in the countries and cohorts involved.

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