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Infant Birth Size Is Not Associated with Maternal Intake and Status of Folate during the Second Trimester in Norwegian Pregnant Women[1,2]

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Abstract

Maternal folate status and smoking are potentially strong risk factors for infant birth size. We assessed the association of several folate indicators and smoking with birth outcomes in a subsample of participants in the Norwegian Mother and Child Cohort Study, consisting of 2934 singleton pregnancies in 2002–2003. Blood plasma folate and cotinine concentrations and self-reported intake of food folate and supplemental folic acid were measured during the second trimester (median 18 wk). Birth outcomes included gestational age, infant birth weight, head circumference, crown-heel length, and small for gestational age (SGA). Mean total dietary folate intake from foods (mean 268.0 μg/d) and supplements (mean 187.7 μg/d) was 455.7 μg/d. Smokers (plasma cotinine $\geq$85 nmol/L) had substantially lower supplemental folic acid intake than nonsmokers, but they did not differ regarding folate intake from food only. Nevertheless, smoking was correlated with plasma folate both before and after adjusting for total dietary folate intake (both $P < 0.001$). We found no significant associations of food folate intake, supplemental folic acid use, total dietary folate intake, or plasma folate with the various birth outcomes after adjustment for potential confounders. Consistent with previous studies, infant birth size was strongly predicted by maternal smoking (adjusted odds ratio for SGA: 2.3; 95% CI: 1.6, 3.3). This study of well-nourished Norwegian pregnant women suggests that dietary folate and plasma folate during the second trimester are not risk factors for infant birth size. J. Nutr. 140: 572–579, 2010.

Introduction

Since randomized trials in the 1990s showed that supplemental folic acid early in pregnancy can lower the risk of neural tube defects in the newborn (1), folic acid has become a well-studied vitamin in perinatal epidemiology. Increased intake of this B-vitamin has also been associated with reduced risk of facial clefts (2), preeclampsia (3), placental abruption (4), spontaneous abortion (5), and gestational hypertension (6).

Some randomized trials suggest that prenatal use of vitamins, including folic acid, is also associated with infant birth size (7,8). In these and several smaller studies (9), the overall difference in birth weight was reported to vary between 40 and 407 g between supplemented and placebo groups. However, results from trials are not consistent, with 3 large trials showing no significant association of folic acid supplementation alone or in combination with other dietary supplements with infant birth size (10–12).

Likewise, several observational studies have examined the relation of prenatal use of folic acid-containing supplements, dietary folate intake, and maternal blood folate status with birth weight or gestational age. While some studies have found a relation (13–19), others have not (20–23). Diverging results are also reported regarding elevated homocysteine (14,20–24), which is a marker of impaired folate status. These conflicting results may be due to methodological issues, such as study design, population size, lack of control for confounding factors, and a variation in population characteristics and nutritional baseline status.

A general limitation of previous studies is the lack of complete information on folic acid supplement use, total intake of folate from food and supplements, as well as maternal blood folate. Examining several folate indicators in relation to the study outcome may be essential to preclude chance findings and to draw valid inference of the results obtained.

In this prospective study, we examined the association of second trimester folic acid supplement use, total dietary folate

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TABLE 1 Sampling scheme of the 3000 mothers in MoBa, 2002–2003

Event	Time window	Subjects
		n
MoBa participants with registered births	July 2002–December 2003	17,588
Donated a blood sample	Median 18 wk of gestation (16–21)[1]	15,644
Returned the baseline questionnaire[2]	Median 18 wk of gestation (16–26)	16,690
Returned the FFQ[2]	Median 18 wk of gestation (16–26)	16,277
Complete data on blood and questionnaires	July 2002–December 2003	14,838
Available for retrieval from the MoBa Biobank[3]	July 2002–December 2003	6723
Random sample used for the present study	July 2002–December 2003	3000

[1] Parentheses indicate the 5th and 95th percentiles.
[2] Until April 2004, both the baseline and FFQ were sent out together with the invitation letter.
[3] By April 2008.

intake, maternal plasma folate, and plasma total homocysteine with birth outcomes in Norwegian women. Because smoking is associated with folate status (25,26) and is probably the strongest environmental predictor of infant birth size (27), we also reported the association of smoking with birth outcomes.

Materials and Methods

This study is based on the Norwegian Mother and Child Cohort Study (MoBa),[9] which is a population-based prospective study of Norwegian pregnant women (28). The cohort includes >100,000 live-born and stillborn infants between 1999 and 2008 and consists of questionnaire data as well as biological specimens from the mother, father, and child. Women were invited to participate through a postal invitation prior to a routine ultrasound examination at their local hospital, usually around 18 wk of gestation (response rate 43%). Hospitals have been included in the cohort on a rolling basis over the course of the study.

Study population. The present study comprised 3000 mothers with babies born during the period July 2002 to December 2003. The mothers were drawn randomly during this period among women who had donated a blood sample at the ultrasound appointment, who were registered in the Medical Birth Registry of Norway and who had returned a baseline questionnaire and a FFQ during the second trimester. Detailed information on the gestational time window of data collection (median 18 wk) and sampling scheme is provided in **Table 1**. Of the 3000 mothers, we excluded 53 multiple pregnancies and 13 pregnancies with no information on either birth weight or gestational age, leaving 2934 singleton births for analyses. The study was approved by the Regional Committee for Medical Research Ethics.

Blood sampling and biochemical analyses. Blood sample collection and laboratory methods have been described previously (29). Blood samples (nonfasting) used for the preparation of plasma were collected into EDTA tubes, centrifuged within 30 min after collection, and placed in the hospital refrigerator (4°C). They were sent by ordinary post overnight to the Biobank of MoBa at the Norwegian Institute of Public Health. On the day of receipt (usually 1–2 d after blood donation), EDTA plasma samples were aliquoted onto polypropylene microtiter plates (300 μL in each well, 96-well format), sealed with heat-sealing foil sheets, and stored in a freezer at −80°C. The plasma folate concentration was measured by microbiological assay, using a chloramphenicol-resistant strain of *Lactobacillus casei* (30). The assay determines biologically active folate species, predominantly 5-methyl-tetrahydrofolate, and has a CV that corresponds to 4% within-day and 5% between days, at population median. The sample handling did not involve addition of ascorbic acid. Total plasma homocysteine and plasma cotinine concentrations were measured by a MS method (31). A woman was considered an active smoker if her cotinine concentration was ≥85 nmol/L (15 μg/L) (5).

Folate intake. Information on folic acid supplement use and food folate intake was collected using a FFQ (32,33). Upon receiving the questionnaire, the women were asked to recall their diet since the start of pregnancy. Dietary habits were reported by selecting mean frequencies of intake of numerous food items from never to several times monthly, weekly, or daily. Supplement use was reported by textual descriptions or by selecting vitamin supplements from a predefined list of the most commonly used supplements. The frequency of vitamin supplement use ranged from never to 7 times/wk and the quantity was reported by the number of tablets/capsules/spoons. This information was used to calculate the daily mean intake (μg/d) of both food folate and supplemental folic acid from the start of the pregnancy until returning the FFQ. Supplemental folic acid use was further divided into 3 categories: 0, 1–399, and ≥400 μg/d. Food folate represents naturally occurring folate, because foods are not fortified with folic acid in Norway. Using the food questionnaire, we also obtained information on the mean total energy intake per day (kJ/d). Information on dietary and supplemental intake was missing for 4 women and excluded for 38 women with an energy intake <4500 kJ/d or >20,000 kJ/d.

Birth outcomes. Data on birth outcomes was abstracted from the Medical Birth Registry of Norway and included gestational age, infant birth weight, head circumference, crown-heel length, and small for gestational age (SGA) (34). Infant SGA included both preterm and term deliveries. The SGA variable was constructed using national reference weights from gestational wk 20 through 44, defined as infant birth weight below the 10th percentile within strata of infant gender and gestational age (35). To be consistent with the reference population, SGA was obtained by using gestational age based on the last menstrual period date. For all other analyses, we used gestational age based on second trimester ultrasound measurements. Information was missing for 15 participants on gestational age, for 37 participants on head circumference, 93 participants on crown-heel length, and 17 participants on SGA.

Other variables. From the birth registry, we also obtained data on maternal age at delivery (<25, 25–34, ≥35 y), marital status (single, cohabitation, married), and parity (0, 1, 2, ≥3 previous deliveries). From the baseline cohort questionnaire, we obtained data on maternal education and prepregnancy BMI (kg/m^2). The women reported their education by checking 1 of 7 predefined categories (including an undefined category). On the basis of this information, education was divided into 4 categories: primary school (0–9 y), secondary school (10–12 y), university and college (≥13 y), and other education. BMI was based on prepregnancy weight and height and divided into 4 categories: <18.5, 18.5–24.9, 25–29.9, and ≥30 kg/m^2.

Statistical analyses. All statistical analyses were performed using SAS version 9.2 software for Windows (SAS Institute) and R version 2.8.1. All *P*-values were 2 sided and values < 0.05 were considered significant.

[9] Abbreviations used: GAM, generalized additive model; MoBa, Norwegian Mother and Child Cohort Study; OR, odds ratio; SGA, small for gestational age.

Dietary intake and plasma concentrations are described as means ± SD. A test for linear trend or group difference in means was performed by incorporating the group variable as a linear predictor or as a categorized variable in linear regression models. Correlation between pairs of measures was assessed using the Spearman correlation coefficient. The association between exposures and SGA was estimated as odds ratio (OR) with 95% CI using logistic regression models. Folic acid supplement use (0, 1–399, ≥400 μg/d) and smoking (plasma cotinine <85, ≥85 nmol/L) were incorporated in logistic models as categorical variables, with no supplement use and no smoking as the

TABLE 2 Intake of food folate and supplemental folic acid among mothers delivering 2934 singletons in MoBa, 2002–2003, according to maternal characteristics[1]

Characteristics	Study participants[2]	Food folate	Supplemental folic acid	Intake of folate + folic acid
	n		*μg/d*	
All women	2934	268.0 ± 91.9	187.7 ± 230.3	455.7 ± 250.3
Maternal age, *y*				
<25	373	269.1 ± 96.5	163.9 ± 210.8	433.0 ± 242.0
25–34	2117	266.0 ± 90.4	191.8 ± 234.5	457.8 ± 251.9
≥35	444	276.5 ± 94.3	187.7 ± 224.8	464.2 ± 248.7
P-trend[3]		0.20	0.19	0.09
Marital status				
Single	84	267.9 ± 86.3	98.4 ± 167.1	366.4 ± 201.3
Cohabitation	1315	264.8 ± 91.5	184.0 ± 222.3	448.9 ± 242.2
Married	1517	270.6 ± 92.6	195.8 ± 239.2	466.4 ± 258.4
P for difference[3]		0.25	<0.001	<0.001
Maternal education, *y*				
0–9	85	275.7 ± 96.8	143.8 ± 219.3	419.5 ± 258.6
10–12	1126	268.0 ± 102.8	152.6 ± 207.1	420.6 ± 230.0
≥13	1649	268.0 ± 83.6	212.2 ± 234.7	480.1 ± 252.9
Other	63	260.9 ± 90.5	231.5 ± 389.5	492.4 ± 404.3
P for difference[3]		0.81	<0.001	<0.001
Parity, *n*				
0	1269	271.2 ± 92.4	219.8 ± 236.9	491.0 ± 255.4
1	1101	259.9 ± 83.8	178.5 ± 230.6	438.3 ± 251.0
2	431	273.3 ± 98.7	146.3 ± 210.4	419.6 ± 229.0
≥3	133	287.4 ± 118.2	95.4 ± 167.4	382.8 ± 213.2
P-trend[3]		0.41	<0.001	<0.001
Prepregnancy BMI, *kg/m²*				
<18.5	79	267.7 ± 99.2	192.5 ± 217.4	460.2 ± 251.4
18.5–24.9	1840	270.7 ± 90.6	196.5 ± 231.6	467.1 ± 250.6
25.0–29.9	593	264.4 ± 87.9	184.3 ± 219.6	448.8 ± 236.2
≥30.0	306	260.4 ± 104.5	162.7 ± 258.0	423.1 ± 284.1
P-trend[3]		0.05	0.02	0.004
Plasma cotinine, *nmol/L*				
<85	2572	268.5 ± 90.5	194.1 ± 231.4	462.6 ± 250.8
85–499	198	271.4 ± 109.4	146.0 ± 217.5	417.4 ± 240.2
≥500	161	256.9 ± 90.7	133.5 ± 217.6	390.3 ± 242.2
P-trend[3]		0.25	<0.001	<0.001
Plasma homocysteine quartiles, *μmol/L*				
<4.3	720	277.5 ± 87.9	268.1 ± 241.6	545.6 ± 255.0
4.3–5.0	739	269.4 ± 91.2	213.3 ± 230.4	482.7 ± 251.1
5.1–5.7	731	262.9 ± 90.6	167.5 ± 240.9	430.4 ± 256.6
≥5.8	738	262.2 ± 97.1	103.4 ± 169.2	365.6 ± 199.5
P-trend[3]		<0.001	<0.001	<0.001
Plasma folate quartiles, *nmol/L*				
<5.9	730	259.9 ± 90.3	70.8 ± 149.5	330.7 ± 177.3
5.9–8.7	731	265.9 ± 91.2	135.2 ± 208.2	401.2 ± 226.4
8.8–14.7	731	271.8 ± 92.3	211.8 ± 215.6	483.6 ± 238.1
≥14.8	731	273.3 ± 88.7	332.2 ± 249.4	605.6 ± 262.8
P-trend[3]		0.002	<0.001	<0.001

[1] Values are presented as mean ± SD. Information on dietary and supplemental intake was missing for 4 participants and excluded for 38 participants with an energy intake < 4500 kJ/d or >20,000 kJ/d.
[2] Information was missing for 18 participants on marital status, 11 participants on maternal education, 116 participants on prepregnancy BMI, 3 participants on plasma cotinine, 6 participants on plasma homocysteine, and 11 participants on plasma folate.
[3] *P*-trend or group difference was calculated by incorporating the group variable as a linear predictor or as a categorized variable in linear regression models (chi-square test).

reference categories. Dietary folate intake, plasma folate, and homocysteine were incorporated in logistic models as categorical variables (<25th, 25th–74th, ≥75th percentile) with the middle category as reference (cut points based on all available study subjects). To explore the potential dose-response relation between continuous exposures and dichotomous outcome, we used generalized additive logistic regression models (GAM; MGCV package of R). Exposure data were right-skewed and thus log-transformed to increase readability of GAM curves.

All regression analyses were performed unadjusted and with adjustment for maternal age, marital status, maternal education, parity, prepregnancy BMI, and smoking. In the analyses of dietary folate intake, additional adjustment was made for total energy intake (in quartiles) and gestational age at the time the FFQ was returned (in 4-wk intervals). In the analyses of plasma folate and homocysteine, additional adjustment was made for gestational age at blood collection (in 4-wk intervals).

Results

The mean maternal age of the 2934 study participants was 29.8 y ± 4.6 (range: 15–43 y). Nearly 97% of the mothers were married or cohabiting, 56% had a university or college education, and 43% delivered for the first time (**Table 2**). The mean maternal prepregnancy BMI was 24.2 ± 4.4 kg/m^2. About

TABLE 3 Mean birth outcome values among 2934 singletons in MoBa, 2002–2003, according to maternal dietary and plasma folate indicators, and plasma cotinine[1]

Folate indicator	Study participants	Gestational age	Infant birth weight	Head circumference	Crown-heel length
	n	*d*	*g*	*cm*	
All women	2934	278.7 ± 14.5	3619 ± 611	35.4 ± 1.8	50.4 ± 2.7
Food folate,[2] *μg/d*					
<204	723	279.1 ± 12.9	3616 ± 575	35.4 ± 1.6	50.4 ± 2.3
205–314	1446	278.4 ± 15.4	3609 ± 615	35.3 ± 1.9	50.4 ± 2.8
≥315	723	279.0 ± 14.6	3644 ± 641	35.4 ± 1.8	50.4 ± 2.8
P-trend[3]		0.92	0.39	0.63	0.88
Adjusted *P*-trend[4]		0.77	0.99	0.27	0.77
Supplemental folic acid, *μg/d*					
0	1196	279.0 ± 13.6	3639 ± 611	35.4 ± 1.8	50.5 ± 2.6
1–399	849	278.8 ± 14.3	3622 ± 617	35.4 ± 1.8	50.4 ± 2.8
≥400	847	278.3 ± 16.0	3589 ± 608	35.3 ± 1.7	50.3 ± 2.7
P-trend[3]		0.29	0.07	0.10	0.21
Adjusted *P*-trend[4]		0.22	0.44	0.44	0.46
Intake of folate + folic acid[2], *μg/d*					
<258	723	278.4 ± 14.4	3618 ± 623	35.4 ± 1.8	50.4 ± 2.7
259–632	1446	279.0 ± 13.9	3632 ± 601	35.4 ± 1.8	50.5 ± 2.6
≥633	723	278.4 ± 15.8	3596 ± 623	35.3 ± 1.8	50.3 ± 2.8
P-trend[3]		0.92	0.48	0.17	0.46
Adjusted *P*-trend[4]		0.90	1.00	0.42	0.73
Plasma folate[2], *nmol/L*					
<5.9	730	278.7 ± 15.5	3618 ± 660	35.3 ± 1.9	50.4 ± 2.9
5.9–14.7	1462	279.1 ± 13.8	3643 ± 592	35.4 ± 1.7	50.5 ± 2.7
≥14.8	731	278.0 ± 14.8	3577 ± 598	35.3 ± 1.8	50.2 ± 2.5
P-trend[3]		0.36	0.20	0.75	0.12
Adjusted *P*-trend[4]		0.15	0.54	0.53	0.09
Plasma homocysteine[2], *μmol/L*					
<4.3	720	278.7 ± 14.0	3631 ± 587	35.4 ± 1.7	50.4 ± 2.5
4.3–5.7	1470	278.9 ± 14.8	3626 ± 619	35.4 ± 1.8	50.5 ± 2.8
≥5.8	738	278.2 ± 14.4	3593 ± 620	35.3 ± 1.9	50.2 ± 2.8
P-trend[3]		0.43	0.24	0.17	0.22
Adjusted *P*-trend[4]		0.84	0.99	0.48	0.93
Plasma cotinine, *nmol/L*					
<85	2572	278.7 ± 14.6	3641 ± 611	35.4 ± 1.8	50.5 ± 2.7
85–499	198	280.9 ± 11.0	3570 ± 497	35.3 ± 1.5	50.2 ± 2.2
≥500	161	276.7 ± 16.8	3336 ± 670	34.8 ± 2.0	49.2 ± 3.0
P-trend[3]		0.55	<0.001	<0.001	<0.001
Adjusted *P*-trend[4]		0.58	<0.001	<0.001	<0.001

[1] Values are mean ± SD. Information was missing for 15 participants on gestational age, 37 participants on head circumference, and 93 participants on crown-heel length.
[2] Categories were constructed corresponding to <25th, 25th–75th, and ≥75th percentiles, based on all available study subjects.
[3] *P*-trend was calculated by incorporating the group variable as a linear predictor in linear regression models (chi-square test).
[4] Adjustment for maternal age, marital status, maternal education, parity, prepregnancy BMI, and smoking; for dietary analyses, additional adjustment for supplemental folic acid intake, energy intake, and gestational age when the FFQ was returned; for plasma analyses, additional adjustment for gestational age at blood collection.

10% of the women were in the highest prepregnancy BMI group (≥30 kg/m^2) and 12% smoked at the time of blood collection (plasma cotinine ≥85 nmol/L). The mean total energy intake was 9819 ± 2542 kJ/d.

The mean intake of food folate and supplemental folic acid from the start of the pregnancy until returning the FFQ (median 18 wk) was 268.0 and 187.7 μg/d, respectively (Table 2). Mean supplemental folic acid intake was higher among women who were married or cohabiting, had a higher education and lower parity, had lower prepregnancy BMI, and did not smoke (Table 2). Mean folate intake from food only did not differ significantly according to these variables, including smoking.

The frequency of folic acid supplement use ≥ 400 μg/d since the beginning of pregnancy was 28.9%. However, ~53% of all women had a total dietary folate intake of ≥400 μg/d. Four women had a total folate intake ≤ 100 μg/d and 342 (11.7%) women had a total folate intake ≤200 μg/d.

Mean total dietary folate intake from food and supplements increased by 274.9 μg/d over plasma folate quartiles and decreased by 180.0 μg/d over plasma homocysteine quartiles (both $P < 0.001$; Table 2). These trends were strongly attributed to supplemental folic acid use. Overall, plasma folate was correlated (Spearman) with food folate alone ($r = 0.07$), total dietary folate intake ($r = 0.44$), plasma homocysteine ($r = -0.48$), and plasma cotinine ($r = -0.15$) ($P < 0.001$). The correlation with cotinine was -0.12 ($P < 0.001$) after adjusting for total dietary folate intake.

Mean infant birth weight was 3619 g (**Table 3**). Regression analyses showed no linear trends in mean values of infant birth weight, gestational age, head circumference, or crown-heel length over the percentile categories of the various folate indicators. Reductions in mean birth outcome values over smoking categories, however, were observed for all outcomes (all $P < 0.001$), except for gestational age ($P = 0.58$).

We also examined folate consumption and plasma folate in relation to infant SGA. As a first analysis, we compared SGA prevalence according to food folate quartiles and categories of supplemental folic acid intake (**Fig. 1**). The crude comparison of groups suggested a potential dose-response relation, ranging from 11.2 to 5.3% of SGA, with higher percentages at lower levels of folate intake. However, we observed no significant trends in the risk of SGA over food folate quartiles or supplemental categories in separate analyses after adjustment of each other, total energy intake, and other covariates (**Table 4**). Furthermore, we found no significant SGA risk trends over the percentile categories when total folate intake from both foods and supplements was examined. Similarly, plasma folate and plasma homocysteine appeared not to be associated with SGA.

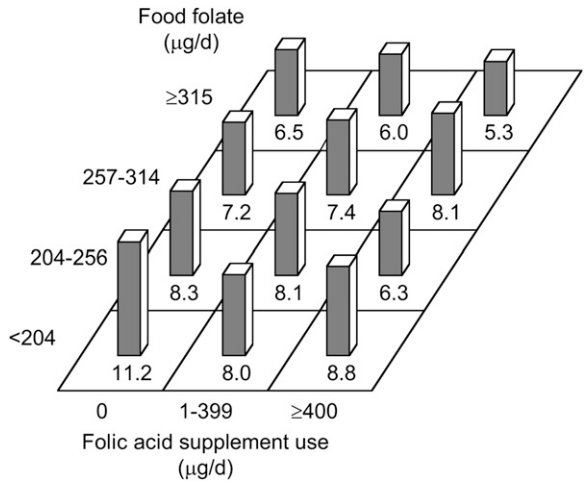

FIGURE 1 Infant SGA prevalence (%) among mothers delivering 2934 singletons in MoBa, 2002–2003, according to quartiles of maternal intake of food folate and categories of supplemental folic acid. Prevalence of SGA is shown in each cell together with a vertical bar.

Of interest, we also examined the association between folate indicators and preterm birth (<37 wk of gestation; $n = 164$). We found no evidence that low dietary folate intake, low plasma folate or high plasma homocysteine concentration increased preterm birth risk (data not shown).

Since the analyses of quartiles and linear trends may not adequately reveal difference in the upper and lower tails of the exposure distributions, we therefore explored dose-response relations between continuous folate exposure and SGA using GAM curves (**Fig. 2**). The OR for SGA increased with lower values of food folate, total dietary folate intake, and plasma folate concentrations but results were not significant due to wide CI (as indicated by the gray-shaded areas in Fig. 2).

There was no significant evidence for effect modification of the folate-SGA associations by smoking (data not shown). On the other hand, active maternal smoking (plasma cotinine ≥ 85 nmol/L) was a strong determinant for SGA (adjusted OR: 2.3; 95% CI: 1.6, 3.3). Additional adjustment for plasma folate and homocysteine quartiles did not alter this association (adjusted OR: 2.2; 95% CI: 1.5, 3.3). Specifically, women with a plasma cotinine concentration ≥500 nmol/L had a 3.5-fold greater risk of having a SGA infant than those who did not smoke (Table 4).

Discussion

We found that dietary folate intake, supplemental folic acid use, and maternal plasma folate concentrations, measured in the second trimester, were not associated with gestational age, infant birth weight, head circumference, or crown-heel length. There was, however, a tendency for increased SGA risk at lower folate values (Table 4), but analyses yielded nonsignificant associations, possibly due to the low number of individuals with low folate status. As expected, we found a strong effect of second trimester smoking on the risk of birth outcomes, except gestational age.

We analyzed data from a relatively homogenous population. Compared with the total Norwegian pregnant population, MoBa includes a higher portion of primiparous and older women and a substantially higher portion of nonsmokers and vitamin users (36). A homogenous population may have advantages, because it may provide better control for confounding factors that vary in the general population. In this study, adjustment for socioeconomic data, maternal anthropometric data, maternal smoking, and total energy intake had little impact on the observed results, suggesting that important unmeasured confounding by other variables is also unlikely, unless they are strongly related to both the exposures and outcomes.

We do not suspect selection bias to have influenced exposure-outcome associations. Maternal characteristics of the subsample in our study are essentially the same as those observed in the full cohort of MoBa (36). Furthermore, a recent validation of 8 exposure-outcome associations in MoBa against the Medical Birth Registry of Norway showed essentially no bias due to initial self-selection in the cohort study, even though exposures such as smoking and vitamin use were markedly under- or overrepresented (36).

TABLE 4 Infant SGA risk among mothers delivering 2934 singletons in MoBa, 2002–2003, according to maternal dietary and plasma folate indicators, and plasma cotinine[1]

Folate indicator	Participants, *n*	SGA, *n (%)*	Unadjusted OR (95% CI)	Adjusted OR (95% CI)[2]
All women	2934	225 (7.7)		
Food folate,[3] *μg/d*				
<204	723	70 (9.7)	1.3 (0.9, 1.8)	1.1 (0.8, 1.6)
205–314	1446	110 (7.6)	1.0 (ref)	1.0 (ref)
≥315	723	43 (6.0)	0.8 (0.5, 1.1)	0.8 (0.5, 1.1)
P-trend[4]			0.009	0.16
Supplemental folic acid, *μg/d*				
0	1196	100 (8.4)	1.0 (ref)	1.0 (ref)
1–399	849	63 (7.5)	0.9 (0.6, 1.2)	0.9 (0.6, 1.3)
≥400	847	60 (7.1)	0.8 (0.6, 1.2)	0.8 (0.6, 1.2)
P-trend[4]			0.26	0.27
Intake of folate + folic acid,[3] *μg/d*				
<258	723	66 (9.2)	1.3 (0.9, 1.8)	1.3 (0.9, 1.8)
259–632	1446	103 (7.2)	1.0 (ref)	1.0 (ref)
≥633	723	54 (7.5)	1.1 (0.7, 1.5)	1.1 (0.7, 1.5)
P-trend[4]			0.25	0.42
Plasma folate,[3] *nmol/L*				
<5.9	730	65 (9.0)	1.3 (1.0, 1.8)	1.3 (0.9, 1.8)
5.9–14.7	1462	101 (6.9)	1.0 (ref)	1.0 (ref)
≥14.8	731	58 (8.0)	1.2 (0.8, 1.6)	1.1 (0.8, 1.6)
P-trend[4]			0.47	0.49
Plasma homocysteine,[3] *μmol/L*				
<4.3	720	44 (6.1)	0.7 (0.5, 1.0)	0.7 (0.5, 1.1)
4.3–5.7	1470	125 (8.6)	1.0 (ref)	1.0 (ref)
≥5.8	738	56 (7.6)	0.9 (0.6, 1.2)	0.8 (0.6, 1.2)
P-trend[4]			0.29	0.69
Plasma cotinine, *nmol/L*				
<85	2572	176 (6.9)	1.0 (ref)	1.0 (ref)
85–499	198	18 (9.1)	1.4 (0.8, 2.2)	1.5 (0.9, 2.5)
≥500	161	31 (19.3)	3.2 (2.1, 4.9)	3.5 (2.2, 5.6)
P-trend[4]			<0.001	<0.001

[1] Information on SGA was missing for 17 participants.
[2] Adjustment for maternal age, marital status, maternal education, parity, prepregnancy BMI, and smoking; for dietary analyses, additional adjustment for supplemental folic acid intake, energy intake, and gestational age when the FFQ was returned; for plasma analyses, additional adjustment for gestational age at blood collection.
[3] Categories were constructed corresponding to <25th, 25th–74th, and ≥75th percentiles, based on all available study subjects.
[4] *P*-trend was calculated by incorporating the group variable as a linear predictor in logistic regression models (chi-square test).

Our findings of no association between various folate indicators and birth outcomes may not be generalizable to all pregnant women. The study population included relatively well-nourished women, with 53% of the women having a total dietary folate intake of ≥400 μg/d. Furthermore, based on the Norwegian guidelines for nutritional intake (37), our population included only 4 women with a daily total folate intake below the lower limit (100 μg/d) and only 342 (11.7%) women below the limit of estimated average requirements (200 μg/d). In addition, the total folate intake from both foods and supplements in our study was higher than that seen in other populations of pregnant women (22,38).

Our study showed a weak, nonsignificant tendency for increased risk of SGA at lower folate values (Table 4). Recently, a study from the UK reported a higher incidence of SGA (<10th percentile) at low folate intake during late pregnancy in an adolescent population (38). These authors also showed a greater risk of SGA with lower concentrations of serum and RBC folate during the 3rd trimester. Furthermore, a recent large study from The Netherlands demonstrated a 60% reduced risk of SGA (<5th percentile) with periconceptional folic acid supplement use (39). However, because our study differs from others with respect to population characteristics, nutritional baseline status, and the timing and methods used to collect exposures and outcomes data, a direct comparison with previous studies is difficult. A strength of the present study is that we had information on dietary folate, supplemental folic acid, and plasma folate in a relatively large population of 2934 singleton pregnancies.

The findings of a strong association of smoking with SGA and no association with gestational age agree with previous studies (27,40). We also found that plasma cotinine was inversely correlated with plasma folate ($r = -0.15$; $P < 0.001$). A reduction in blood folate and other vitamins by smoking has been reported by others and may be attributed to increased oxidative stress as well as decreased folate intake in smokers (25,26). Adjustment for total folate intake from food and supplements only modestly reduced the correlation between

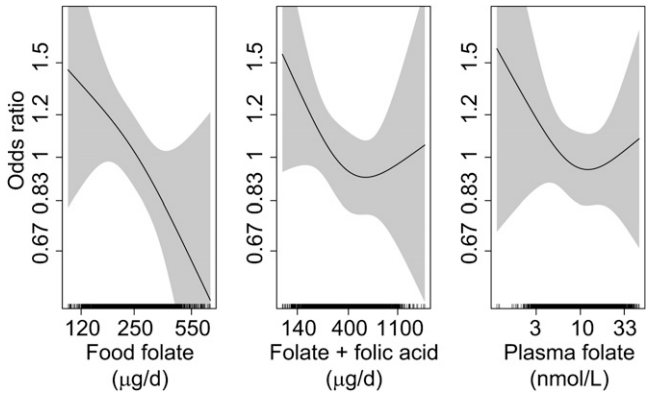

FIGURE 2 Infant SGA risk among mothers delivering 2934 singletons in MoBa, 2002–2003, according to log-transformed maternal values of food folate, total dietary folate, and plasma folate. OR were predicted from GAM adjusted for variables similar to those in the analyses in Table 4. The OR scale is centered and set to 1 on the mean of log values.

plasma folate and cotinine ($r = -0.12$; $P < 0.001$), supporting that smoking alone affects blood folate status.

A general limitation of this study is that information on folate exposures and smoking was collected only once during pregnancy. Particularly, we did not have information on plasma folate and folate consumption during late pregnancy. This is a potential problem, because changes during pregnancy may lead to some exposure misclassification (41), which again may lead to some underestimation of possible effects of these folate indicators in the third trimester on birth outcomes. To avoid this problem, future studies should include longitudinal data on exposures. However, longitudinal measurements in large-scale epidemiological studies are a resource-demanding task, which may explain why such investigations on folate indicators and birth outcomes have not been undertaken.

The FFQ used for this study assessed food folate and supplemental folic acid intake over the first 4–5 mo of pregnancy (median 18 wk). It is likely that many women do not accurately recall their diets at the start of pregnancy through the mid-second trimester. Furthermore, during early pregnancy, many women experience nausea or vomiting and changes in appetite and eating patterns. Nevertheless, a validation study showed that the FFQ produces a realistic estimate of the habitual intake and is a valid tool for ranking pregnant women according to high and low intakes of nutrients (42). We further showed that the total folate intake was strongly correlated with blood plasma folate concentrations ($r = 0.44$; $P < 0.001$) and that this was largely attributed to folic acid supplement use (Table 2). Because naturally occurring folate is unstable and has less bioavailability than synthetic folic acid pills (43), the observed weaker correlation with food folate only ($r = 0.07$; $P < 0.001$) was not unexpected.

Due to the large population size (>100,000 pregnancies) and the complex recruitment procedures of MoBa, the logistics did not allow fasting blood samples. Consequently, measures of plasma folate in our study may have been influenced by recent dietary intakes. Although this may add preanalytical variability, the overall plasma results were supported by data on folate intake and folic acid supplementation, suggesting that valid results were obtained from nonfasting plasma folate.

Another concern is that the folate concentration in EDTA plasma is likely to decrease at room temperature for the first 1–2 d, especially in the absence of a stabilizer like ascorbic acid (44). Such folate loss may result from an conversion of biologically active forms of folate to other forms that cannot be detected with standard microbiological assay methods (45). However, there are indications that the remaining biologically active folate still reflects overall folate status. We found that plasma folate was strongly inversely correlated with plasma total homocysteine ($r = -0.48$; $P < 0.001$), which is stable at room temperature (46). Thus, plasma folate may have degraded at a constant rate over the distribution of folate concentrations and the relative difference in folate concentration between women may have been upheld.

Folate degradation is likely unrelated to the outcome under study, but it can still introduce nondifferential misclassification and thus attenuated risk estimates if a fixed dichotomous cutoff of plasma concentration is used (47). In this study, we analyzed relative cutoffs instead of fixed cutoffs by dividing the distribution of plasma folate into quartile exposures. In addition, we used GAM to examine dose-response effects of continuous plasma folate concentration on birth outcomes.

In conclusion, in this prospective cohort of Norwegian women, we found no evidence that low dietary folate intake or low plasma folate concentration during the second trimester is associated with infant birth size. As expected, however, we found that infant birth size was strongly associated with second trimester smoking. The absence of significant associations with dietary and plasma folate might be explained by an adequate intake of folate from diet and supplements in most women in our study population.

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Literature Cited

1. Botto LD, Moore CA, Khoury MJ, Erickson JD. Neural-tube defects. N Engl J Med. 1999;341:1509–19.
2. Wilcox AJ, Lie RT, Solvoll K, Taylor J, McConnaughey DR, Abyholm F, Vindenes H, Vollset SE, Drevon CA. Folic acid supplements and risk of facial clefts: national population based case-control study. BMJ. 2007;334:464–7.
3. Wen SW, Chen XK, Rodger M, White RR, Yang Q, Smith GN, Sigal RJ, Perkins SL, Walker MC. Folic acid supplementation in early second trimester and the risk of preeclampsia. Am J Obstet Gynecol. 2008;198:45.e1–7.
4. Nilsen RM, Vollset SE, Rasmussen SA, Ueland PM, Daltveit AK. Folic acid and multivitamin supplement use and risk of placental abruption: a population-based registry study. Am J Epidemiol. 2008;167:867–74.
5. George L, Mills JL, Johansson AL, Nordmark A, Olander B, Granath F, Cnattingius S. Plasma folate levels and risk of spontaneous abortion. JAMA. 2002;288:1867–73.
6. Hernandez-Diaz S, Werler MM, Louik C, Mitchell AA. Risk of gestational hypertension in relation to folic acid supplementation during pregnancy. Am J Epidemiol. 2002;156:806–12.
7. Fawzi WW, Msamanga GI, Urassa W, Hertzmark E, Petraro P, Willett WC, Spiegelman D. Vitamins and perinatal outcomes among HIV-negative women in Tanzania. N Engl J Med. 2007;356:1423–31.
8. Rolschau J, Kristoffersen K, Ulrich M, Grinsted P, Schaumburg E, Foged N. The influence of folic acid supplement on the outcome of pregnancies in the county of Funen in Denmark. Part I. Eur J Obstet Gynecol Reprod Biol. 1999;87:105–10, discussion 103–4.
9. Tamura T, Picciano MF. Folate and human reproduction. Am J Clin Nutr. 2006;83:993–1016.

10. Charles DH, Ness AR, Campbell D, Smith GD, Whitley E, Hall MH. Folic acid supplements in pregnancy and birth outcome: re-analysis of a large randomised controlled trial and update of Cochrane review. Paediatr Perinat Epidemiol. 2005;19:112–24.
11. Christian P, Khatry SK, Katz J, Pradhan EK, LeClerq SC, Shrestha SR, Adhikari RK, Sommer A, West KP Jr. Effects of alternative maternal micronutrient supplements on low birth weight in rural Nepal: double blind randomised community trial. BMJ. 2003;326:571.
12. Czeizel AE, Dudas I, Metneki J. Pregnancy outcomes in a randomised controlled trial of periconceptional multivitamin supplementation. Final report. Arch Gynecol Obstet. 1994;255:131–9.
13. Catov JM, Bodnar LM, Ness RB, Markovic N, Roberts JM. Association of periconceptional multivitamin use and risk of preterm or small-for-gestational-age births. Am J Epidemiol. 2007;166:296–303.
14. Lindblad B, Zaman S, Malik A, Martin H, Ekstrom AM, Amu S, Holmgren A, Norman M. Folate, vitamin B12, and homocysteine levels in South Asian women with growth-retarded fetuses. Acta Obstet Gynecol Scand. 2005;84:1055–61.
15. Neggers YH, Goldenberg RL, Tamura T, Cliver SP, Hoffman HJ. The relationship between maternal dietary intake and infant birthweight. Acta Obstet Gynecol Scand Suppl. 1997;165:71–5.
16. Relton CL, Pearce MS, Parker L. The influence of erythrocyte folate and serum vitamin B12 status on birth weight. Br J Nutr. 2005;93:593–9.
17. Scholl TO, Hediger ML, Bendich A, Schall JI, Smith WK, Krueger PM. Use of multivitamin/mineral prenatal supplements: influence on the outcome of pregnancy. Am J Epidemiol. 1997;146:134–41.
18. Scholl TO, Hediger ML, Schall JI, Khoo CS, Fischer RL. Dietary and serum folate: their influence on the outcome of pregnancy. Am J Clin Nutr. 1996;63:520–5.
19. Siega-Riz AM, Savitz DA, Zeisel SH, Thorp JM, Herring A. Second trimester folate status and preterm birth. Am J Obstet Gynecol. 2004;191:1851–7.
20. Hogg BB, Tamura T, Johnston KE, Dubard MB, Goldenberg RL. Second-trimester plasma homocysteine levels and pregnancy-induced hypertension, preeclampsia, and intrauterine growth restriction. Am J Obstet Gynecol. 2000;183:805–9.
21. Ronnenberg AG, Goldman MB, Chen D, Aitken IW, Willett WC, Selhub J, Xu X. Preconception homocysteine and B vitamin status and birth outcomes in Chinese women. Am J Clin Nutr. 2002;76:1385–91.
22. Takimoto H, Mito N, Umegaki K, Ishiwaki A, Kusama K, Abe S, Yamawaki M, Fukuoka H, Ohta C, et al. Relationship between dietary folate intakes, maternal plasma total homocysteine and B-vitamins during pregnancy and fetal growth in Japan. Eur J Nutr. 2007;46:300–6.
23. Watanabe H, Fukuoka H, Sugiyama T, Nagai Y, Ogasawara K, Yoshiike N. Dietary folate intake during pregnancy and birth weight in Japan. Eur J Nutr. 2008;47:341–7.
24. Vollset SE, Refsum H, Irgens LM, Emblem BM, Tverdal A, Gjessing HK, Monsen AL, Ueland PM. Plasma total homocysteine, pregnancy complications, and adverse pregnancy outcomes: the Hordaland Homocysteine study. Am J Clin Nutr. 2000;71:962–8.
25. Pagan K, Hou J, Goldenberg RL, Cliver SP, Tamura T. Effect of smoking on serum concentrations of total homocysteine and B vitamins in mid-pregnancy. Clin Chim Acta. 2001;306:103–9.
26. Piyathilake CJ, Macaluso M, Hine RJ, Richards EW, Krumdieck CL. Local and systemic effects of cigarette smoking on folate and vitamin B-12. Am J Clin Nutr. 1994;60:559–66.
27. Savitz DA, Dole N, Terry JW Jr, Zhou H, Thorp JM Jr. Smoking and pregnancy outcome among African-American and white women in central North Carolina. Epidemiology. 2001;12:636–42.
28. Magnus P, Irgens LM, Haug K, Nystad W, Skjaerven R, Stoltenberg C. Cohort profile: the Norwegian Mother and Child Cohort Study (MoBa). Int J Epidemiol. 2006;35:1146–50.
29. Ronningen KS, Paltiel L, Meltzer HM, Nordhagen R, Lie KK, Hovengen R, Haugen M, Nystad W, Magnus P, et al. The biobank of the Norwegian Mother and Child Cohort Study: a resource for the next 100 years. Eur J Epidemiol. 2006;21:619–25.
30. O'Broin S, Kelleher B. Microbiological assay on microtitre plates of folate in serum and red cells. J Clin Pathol. 1992;45:344–7.
31. Ueland PM, Midttun O, Windelberg A, Svardal A, Skalevik R, Hustad S. Quantitative profiling of folate and one-carbon metabolism in large-scale epidemiological studies by mass spectrometry. Clin Chem Lab Med. 2007;45:1737–45.
32. Brantsaeter AL, Haugen M, Alexander J, Meltzer HM. Validity of a new food frequency questionnaire for pregnant women in the Norwegian Mother and Child Cohort Study (MoBa). Matern Child Nutr. 2008;4:28–43.
33. Brantsaeter AL, Haugen M, Hagve TA, Aksnes L, Rasmussen SE, Julshamn K, Alexander J, Meltzer HM. Self-reported dietary supplement use is confirmed by biological markers in the Norwegian Mother and Child Cohort Study (MoBa). Ann Nutr Metab. 2007;51:146–54.
34. Irgens LM. The Medical Birth Registry of Norway. Epidemiological research and surveillance throughout 30 years. Acta Obstet Gynecol Scand. 2000;79:435–9.
35. Skjaerven R, Gjessing HK, Bakketeig LS. Birthweight by gestational age in Norway. Acta Obstet Gynecol Scand. 2000;79:440–9.
36. Nilsen RM, Vollset SE, Gjessing HK, Skjaerven R, Melve KK, Schreuder P, Alsaker ER, Haug K, Daltveit AK, et al. Self-selection and bias in a large prospective pregnancy cohort in Norway. Paediatr Perinat Epidemiol. 2009;23:597–608.
37. NNR Project Group. Nordic Nutrition Recommendations. 4th ed. Copenhagen: Nordic Council of Ministers; 2004.
38. Baker PN, Wheeler SJ, Sanders TA, Thomas JE, Hutchinson CJ, Clarke K, Berry JL, Jones RL, Seed PT, et al. A prospective study of micronutrient status in adolescent pregnancy. Am J Clin Nutr. 2009;89:1114–24.
39. Timmermans S, Jaddoe VW, Hofman A, Steegers-Theunissen RP, Steegers EA. Periconception folic acid supplementation, fetal growth and the risks of low birth weight and preterm birth: the Generation R Study. Br J Nutr. 2009;102:777–85.
40. Horta BL, Victora CG, Menezes AM, Halpern R, Barros FC. Low birthweight, preterm births and intrauterine growth retardation in relation to maternal smoking. Paediatr Perinat Epidemiol. 1997;11:140–51.
41. Hertz-Picciotto I, Pastore LM, Beaumont JJ. Timing and patterns of exposures during pregnancy and their implications for study methods. Am J Epidemiol. 1996;143:597–607.
42. Meltzer HM, Brantsaeter AL, Ydersbond TA, Alexander J, Haugen M. Methodological challenges when monitoring the diet of pregnant women in a large study: experiences from the Norwegian Mother and Child Cohort Study (MoBa). Matern Child Nutr. 2008;4:14–27.
43. McNulty H, Cuskelly GJ, Ward M. Response of red blood cell folate to intervention: implications for folate recommendations for the prevention of neural tube defects. Am J Clin Nutr. 2000;71:S1308–11.
44. O'Broin JD, Temperley IJ, Scott JM. Erythrocyte, plasma, and serum folate: specimen stability before microbiological assay. Clin Chem. 1980;26:522–4.
45. Hannisdal R, Ueland PM, Eussen SJ, Svardal A, Hustad S. Analytical recovery of folate degradation products formed in human serum and plasma at room temperature. J Nutr. 2009;139:1415–8.
46. Fiskerstrand T, Refsum H, Kvalheim G, Ueland PM. Homocysteine and other thiols in plasma and urine: automated determination and sample stability. Clin Chem. 1993;39:263–71.
47. Rothman KJ, Greenland S, Lash TL. Modern epidemiology. Philadelphia: Lippincott Williams & Wilkins; 2008. p. 137–46.