FULL-LENGTH ORIGINAL RESEARCH

Epilepsia®

Vitamin B status and association with antiseizure medication in pregnant women with epilepsy

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Summary

Objective: Antiseizure medication (ASM) use interacts with vitamin B status in nonpregnant epilepsy populations. We aimed to examine the association between ASM and vitamin B status in pregnant women with epilepsy.

Methods: We performed a cross-sectional study of pregnancies in women with epilepsy enrolled in the Norwegian Mother, Father and Child Cohort Study from 1999 to 2008. Data on ASM and vitamin supplement use were collected from questionnaires. We analyzed maternal plasma concentrations of ASM and metabolites of folate, including unmetabolized folic acid (UMFA), riboflavin (vitamin B2), pyridoxine (vitamin B6), and niacin (vitamin B3) during gestational weeks 17–19.

Results: We included 227 singleton pregnancies exposed to ASM with available plasma samples (median maternal age 29 years, range 18 to 41 years). From the preconception period to gestational week 20, any supplement of folic acid was reported in 208 of pregnancies (94%), riboflavin in 72 (33%), pyridoxine in 77 (35%), and niacin in 45 (20%). High ASM concentrations correlated with high concentrations of UMFA and inactive folate metabolites, and with low concentrations of riboflavin and metabolically active pyridoxine. There was no association between ASM and niacin status.

Significance: ASM concentrations during pregnancy were associated with vitamin B status in pregnant women with epilepsy. Additional studies are needed to determine the clinical impact of these findings, and to define the optimal vitamin doses that should be recommended to improve pregnancy outcomes.

KEYWORDS
anticonvulsants, folic acid, MBRN, MoBa, pyridoxine, riboflavin

1 | INTRODUCTION

Exposure to antiseizure medication (ASM) during pregnancy is associated with an increased risk of congenital malformations and adverse neurodevelopment in the children.[1–4] Several ASMs interact with folate metabolism and reduce folate concentrations,[5–8] adding to the folate-lowering effect of pregnancy itself.[9] Chronic ASM use has been associated with increased folate catabolism.[10] Studies examining the interplay between folate metabolism and ASM use are needed in pregnant women. Women with epilepsy using ASMs are often recommended a high dose of folic acid supplement during pregnancy.[1,3,11] Studies of nonepilepsy populations show that excessive folic acid supplementation results in plasma accumulation of unmetabolized folic acid (UMFA).[12,13] The safety of high supplement doses has been questioned,[12–15] as studies in women without epilepsy have reported negative effects of high UMFA concentrations on neurodevelopment.[14–16]

In nonpregnant epilepsy populations, there is an association between chronic ASM use and low concentrations of non-folate B vitamins such as riboflavin (vitamin B2) and pyridoxine (vitamin B6).[5,7,17,18] Riboflavin and pyridoxine act in close interaction with folate in one-carbon metabolism, representing metabolic pathways fundamental for normal fetal development.[9,19] Niacin (vitamin B3) plays a key role in neuronal development and survival.[20] The association between ASM use and vitamin B status in pregnant women with epilepsy has not been examined in detail. One study reported an association between low folate concentrations and ASM polytherapy, and also with high phenytoin and phenobarbital concentrations.[21] Another study reported low concentrations of active folate metabolite during lamotrigine treatment.[22]

In this study, we aimed to examine the association between various ASM concentrations and vitamin B status during pregnancy in women with epilepsy. Such studies contribute important knowledge to aid decisions on recommendations for vitamin supplements in pregnancy for women with epilepsy using ASM.

2 | MATERIAL AND METHODS

2.1 | Study population

The study population included singleton pregnancies of women with epilepsy using ASM with available plasma samples enrolled in the Norwegian Mother, Father and Child Cohort Study (MoBa). MoBa is a population-based pregnancy cohort study conducted by the Norwegian Institute of Public Health and linked to the compulsory Medical Birth Registry of Norway (MBRN).[23] During the years 1999–2008, pregnant women were invited to participate in gestational weeks 17–19. The participation rate was 41%. Women answered questionnaires in gestational weeks 17–19 and 30 on medication use, vitamin use, social and medical background, and parameters related to current and previous pregnancies.[23] A maternal blood sample was collected during gestational weeks 17–19.[24] The current study is based on version 10 of the quality-assured MoBa data files.

We identified women with epilepsy based on self-reported information in the MoBa questionnaires and from diagnostic data registered by the primary care physician or midwife in the MBRN. The MoBa epilepsy cohort has been described elsewhere.[25–27] We collected information on ASM use from the two pregnancy questionnaires, and from the MBRN.[23] Response rates were 97% for the first questionnaire in gestational weeks 17–19, and 89% for the second in gestational week 30. The epilepsy cohort in MoBa has been validated by a retrospective survey (50% response rate), and in a hospital record examination of a subcohort (n = 40).[25] The validity was high, as 98% of the women who reported a diagnosis of epilepsy in MoBa confirmed this in the retrospective survey.[25] There was 100% agreement between the reported ASM use in MoBa and ASM use registered in the hospital records.[25]

2.2 | Vitamin supplement use

We obtained information on type, timing, and frequency of vitamin supplement use in the questionnaires from gestational weeks 17–19 and 30. The mothers reported on use of folic acid, riboflavin, pyridoxine, and niacin during the following gestational week intervals, with week 0 starting with the first day of the last menstrual period: −4 to 0, 0–4, 5–8, 9–12, 13+ (first questionnaire) and for gestational

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Key point
- Antiseizure medication (ASM) use is associated with low concentrations of folate and other B vitamins in nonpregnant epilepsy populations
- High ASM concentrations correlated with high concentrations of unmetabolized folic acid in pregnant women with epilepsy
- High ASM concentrations correlated with high concentrations of inactive folate metabolites in pregnant women with epilepsy
- High ASM concentrations correlated with both low riboflavin and pyridoxine status in pregnant women with epilepsy

weeks 13–16, 17–20, 21–24, 25–28, and 29+ (second questionnaire). Intake was reported as either daily, 4–6 times per week, or 1–3 times per week. Maternal intake of supplements in the first and second trimester has been associated previously with plasma concentrations in samples collected during gestational weeks 17–19.[28,29] We defined supplement use as any use of a supplement during gestational weeks −4 to 20.

We collected information on folic acid dose from 97 of 227 pregnancies (43%) with data from the retrospective validation questionnaire,[25] as this information was not included in the ordinary MoBa questionnaires. The women reported a folic acid dose of 0.4 mg, 1–2 mg, or ≥4 mg during gestational weeks −4 to 24. We grouped the pregnancies according to the highest reported dose during this period: low-dose folic acid (0.4–2 mg) and high-dose folic acid (≥4 mg).

2.3 | Plasma ASM concentrations

We analyzed plasma concentrations of valproate, carbamazepine, lamotrigine, levetiracetam, topiramate, and the oxcarbazepine monohydroxyderivative metabolite.[25] Standardized ASM concentrations were calculated by normalizing the plasma concentrations to the concentration range observed for that drug in the present study according to the formula *100 x (observed concentration − minimum concentration measured for that drug) / concentration range measured for that drug*.[26,30] For ASM polytherapy, the sum of all standardized ASM concentrations was given.

2.4 | Vitamin and metabolite concentrations

We analyzed plasma vitamin and metabolite concentrations at Bevital Laboratory, Bergen (www.bevital.no). We examined folate status by analyzing the biologically active 5-methyltetrahydrofolate (mTHF) metabolite, the mTHF-derived 4-alfa-hydroxy-5-methyltetrahydrofolate (hmTHF) metabolite, and the inactive metabolites para-aminobenzoylglutamate (pABG) and acetamidobenzoyl-glutamate (apABG),[31] as well as unmetabolized folic acid (UMFA).[31] UMFA values below the limit of quantification (LOQ, 0.53 nmol/L)[31] were reported as 0.0 nmol/L. Metabolically active folate concentration was given as the sum of mTHF and hmTHF ("folate").[26,31–33] We calculated the ratio between the active (mTHF plus hmTHF) and inactive (pABG plus apABG) folate metabolites and used a low ratio as a marker of increased folate catabolism. We also calculated the ratio between UMFA and metabolically active folate to better separate the effect of UMFA from the effect of folate.[31,34]

We analyzed plasma riboflavin to examine riboflavin status.[35] We examined pyridoxine status by analyzing metabolically active pyridoxine (pyridoxal-5-phosphate [PLP]) and a functional marker of pyridoxine status, HKr, described in detail elsewhere.[36,37] High HKr indicates low pyridoxine status.[37] We analyzed plasma nicotinamide to examine niacin status.[36]

2.5 | Statistical analysis

We used IBM SPSS Software version 25 for the statistical analyses. We categorized the pregnancies according to ASM monotherapy and ASM polytherapy. Pregnancies where none of the reported ASMs could be detected were categorized into a separate group of suspected low ASM adherence pregnancies. We recorded relevant covariates from the questionnaire in gestational weeks 17–19, and from the MBRN stratified for ASM group[28,29]: maternal age, parity, maternal education, maternal prepregnancy body mass index (BMI), smoking during pregnancy, unplanned pregnancy, epileptic seizures during pregnancy, and tonic-clonic (TC) epileptic seizures during pregnancy. For continuous variables, we reported median values with range. We analyzed vitamin and metabolite concentrations stratified for ASM group and vitamin supplement use, and folate status stratified for ASM group and folic acid dose. Two-sided p-values <0.05 were considered statistically significant. The different ASM groups were compared with the nonparametric Kruskal-Wallis test, due to violation of the assumption of normal distribution and low number of pregnancies in each group. Adjustment for multiple testing was done by multiplying the observed p-value by the number of comparisons made (Dunn-Bonferroni post hoc method). This Bonferroni corrected p-value was considered statistically significant when <0.05. We used Mann-Whitney U test to compare vitamin and metabolite concentrations between supplemented and nonsupplemented pregnancies and between high-dose and low-dose folic acid supplement, stratified for ASM group. We examined the associations between ASM concentrations and vitamin and metabolite concentrations in a nonparametric correlation analysis (Spearman rank correlation). We performed sensitivity analyses by excluding supplement users from the correlation analyses for riboflavin, niacin, and pyridoxine. For folate status, high-dose folic acid supplement users were excluded, because exclusion of nonsupplemented folic acid pregnancies (n = 13) was not meaningful.

2.6 | Standard protocol approvals, registrations, and patient consents

The establishment of MoBa and initial data collection were based on a license from The Norwegian Data Protection Agency and approval from The Regional Committee for Medical Research Ethics. The MoBa cohort is regulated by the Norwegian Health Registry Act. All parents in MoBa have given written consent to participate. The current study was approved by The Regional Committee for Medical Research Ethics (reference 2011/1616).

3 | RESULTS

We identified 227 singleton pregnancies in 203 mothers with epilepsy who had available plasma samples from gestational weeks 17–19 (Figure 1 and Table 1). The mothers used ASM monotherapy in 183 pregnancies and ASM polytherapy in 44 pregnancies (Table S1). The reported ASM used during pregnancy was detected in 199 pregnancies (88%) (Table S1). We studied eight ASM groups: six monotherapy groups with the reported ASM detected in plasma for valproate (n = 24), lamotrigine (n = 65), carbamazepine (n = 48), levetiracetam (n = 11), topiramate (n = 8), and oxcarbazepine (n = 5); one polytherapy group with at least one of the reported ASMs detected in plasma (n = 40); and one low-adherence group with none of the reported ASMs detected in plasma (n = 26) (Table 1). Most women in the latter group admitted low adherence, because only 25% reported regular ASM intake in gestational week 13+ in the first questionnaire, compared to 78%-90% in the other ASM groups.

In 221 pregnancies with available supplement data, the women reported any folic acid supplement use in 208 (94%), riboflavin supplement in 72 (33%), niacin in 45 (20%), and pyridoxine in 77 pregnancies (35%) (Table 1). Intake was reported as ≥4–6 times per week or daily in ≥90% of the pregnancies for all supplements.

Among the included pregnancies from the retrospective validation survey, 76 (33%) had precise information on folic acid dose from gestational weeks −4 to 24. High-dose folic acid (≥4 mg) was reported in 39 pregnancies, and low-dose (0.4–2 mg) was reported in 37 pregnancies.

3.1 | Folate status and association with ASM concentrations

High ASM concentrations correlated with high concentrations of UMFA and inactive folate metabolites, and with a low ratio between active and inactive folate metabolites (Figure 2 and Table S2).
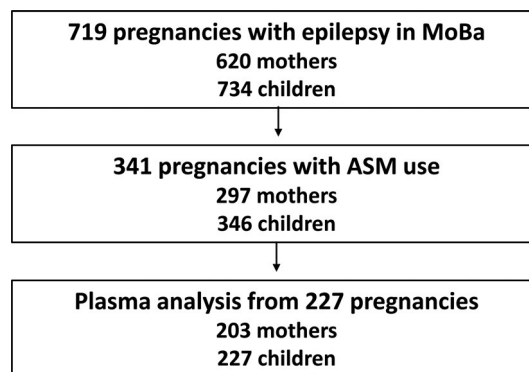

FIGURE 1 Flow chart of excluded and included cases. The flow chart shows excluded and included cases. MoBa, The Norwegian Mother, Father and Child Cohort Study; ASM, antiseizure medication.

The low-adherence group had the lowest folate concentrations; otherwise there were few differences in folate between the different ASM (Table 2). The UMFA concentrations were higher in mothers using valproate, lamotrigine, carbamazepine, or ASM polytherapy, respectively, compared to the low-adherence group (Table 2). The concentrations of the inactive folate metabolites and ratio between active and inactive folate metabolites differed between individual ASMs (Table 2). The concentrations of inactive folate metabolites were higher in ASM polytherapy users compared to lamotrigine users, levetiracetam users, and the low-adherence group, and in valproate users compared to the low-adherence group and levetiracetam users (Table 2). Mothers using levetiracetam had the highest ratio between active and inactive folate metabolites (Table 2). Women using carbamazepine monotherapy or ASM polytherapy had a lower ratio between active and inactive folate metabolites compared to mothers using lamotrigine (Table 2).

In particular, high valproate concentrations correlated with high concentrations of inactive folate (Figure 3 and Table S2). The correlation strength remained unchanged after removing high-dose folic acid users (Table S2). High topiramate concentrations correlated with high UMFA concentrations, but the correlation strength was reduced after removal of high-dose supplement users (Table S2).

High-dose folic acid supplement users had higher folate and UMFA concentrations compared to low-dose users (Figure S1). High folate concentrations correlated with high UMFA concentrations (Figure S1). High-dose users did not differ in ASM concentrations compared to low-dose users (data not shown). After stratification for ASM group, the concentrations of the different folate metabolites in high-dose users compared to low-dose users were essentially the same across ASM groups (Figure S2).

TABLE 1 Clinical characteristics stratified for antiseizure medication (ASM) treatment.

	Valproate n = 24	Lamotrigine n = 65	Carbamazepine n = 48	Levetiracetam n = 11	Topiramate n = 8	Oxcarbazepine n = 5	Polytherapy n = 40	Low-adherence group[1] n = 26
Maternal age, y; median (range)	27.0 (19.0)	29.0 (21.0)	30.0 (23.0)	30.0 (14.0)	28.5 (12.0)	29.0 (5.0)	28.0 (20.0)	29.0 (19.0)
Parity[2]; median (range)	1.0 (2.0)	0.0 (4.0)	1.0 (4.0)	0.0 (3.0)	0.5 (2.0)	0.0 (1.0)	1.0 (3.0)	0.5 (4.0)
Maternal prepregnancy BMI; median (range)	23.3 (13.4)	22.9 (20.2)	24.7 (18.0)	24.4 (18.4)	20.4 (6.1)	23.9 (13.5)	24.1 (18.8)	24.5 (22.8)
Maternal low education[3]; n (%)	0 (0)	0 (0)	2 (4)	0 (0)	0 (0)	0 (0)	2 (6)	2 (8)
Smoking during pregnancy; n (%)	8 (33)	9 (14)	7 (15)	1 (9)	1 (13)	2 (40)	7 (18)	3 (12)
Unplanned pregnancy; n (%)	3 (14)	16 (25)	11 (23)	0 (0)	0 (0)	2 (40)	10 (26)	4 (17)
≥1 epileptic seizure during pregnancy; n (%)	3 (30)	6 (18)	3 (11)	4 (44)	1 (17)	0 (0)	10 (50)	0 (0)
TC seizure(s) during pregnancy; n (%)	2 (20)	4 (12)	2 (7)	1 (11)	0 (0)	0 (0)	5 (25)	0 (0)
Plasma ASM concentration (μmol/L)[4]; median (range)	314.0 (409.0)	7.6 (26.2)	29.5 (39.0)	57.0 (146.0)	12.0 (17.0)	35.4 (56.3)	NA	0.0 (0.0)[1]
Folic acid supplement use[5]; n (%)	21 (96)	62 (97)	45 (94)	11 (100)	7 (100)	4 (80)	38 (95)	20 (83)
Riboflavin supplement use[5]; n (%)	3 (14)	30 (47)	10 (21)	3 (27)	4 (57)	2 (40)	14 (35)	6 (25)
Pyridoxine supplement use[5]; n (%)	3 (14)	32 (50)	11 (23)	3 (27)	4 (57)	2 (40)	15 (38)	7 (29)
Niacin supplement use[5]; n (%)	2 (9)	17 (27)	6 (13)	2 (18)	3 (43)	2 (40)	9 (23)	4 (17)

Abbreviations: ASM, antiseizure medication; BMI, body mass index; maternal low education, total missing n = 14; SD, standard deviation; seizures during pregnancy, total missing n = 115 (due to validation study data, see Methods); smoking during pregnancy, total missing n = 1; TC seizure(s), tonic-clonic seizure(s); unplanned pregnancy, total missing n = 11; vitamin supplement use, total missing n = 6.

[1] Consist of pregnancies where the mother reported ASM use, but no ASM was detected in plasma samples.

[2] Number of all previous pregnancies >12 weeks of gestation, values from 0 (nulliparous) to 4, where 4 means 4 or more. When the median parity value is between 0 and 1, the median is given as 0.5.

[3] 9 or fewer years of schooling.

[4] Maternal plasma concentration in gestational weeks 17–19 for each monotherapy group.

[5] Any supplement use during gestational weeks −4 to 20.
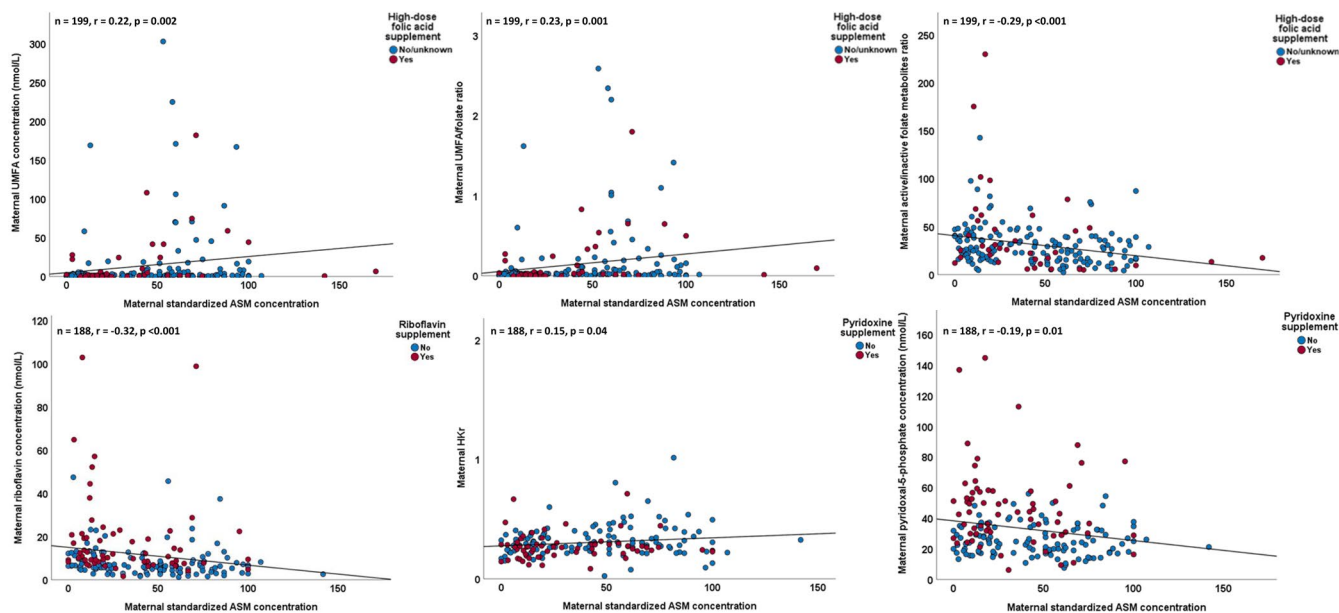
FIGURE 2 Correlation between maternal standardized antiseizure medication (ASM) concentrations and concentrations of vitamin B metabolites. The figure shows the correlation between standardized ASM and vitamin B metabolite concentrations. Blue dots represent no supplement or no or unknown folic acid supplement dose; red dots represent supplemented pregnancies or high-dose folic acid supplement. ASM, antiseizure medication. UMFA, unmetabolized folic acid; HKr, marker of pyridoxine deficiency; r, Spearman's rho; p, *p*-value.

3.2 | Riboflavin status and association with ASM concentrations

High ASM and high lamotrigine concentrations correlated with low concentrations of riboflavin (Figure 2 and Table S2). We observed minor changes in the correlation coefficients after removal of supplement users (Table S2). The riboflavin concentration did not differ between individual ASMs (Table 2 and Table 3).

3.3 | Pyridoxine status and association with ASM concentrations

High concentrations of ASM correlated with low concentrations of metabolically active pyridoxine, and with a high value for the marker of low pyridoxine status (HKr) (Figure 2 and Table S2). Removal of pyridoxine supplement users only slightly reduced the strength of the correlations (Table S2). The metabolically active pyridoxine concentration did not differ between individual ASMs (Table 2), but between supplement users and nonusers (Table 3). However, for individual ASMs, high valproate concentrations correlated with high HKr (Figure 3 and Table S2). In this group, the HKr was higher than in the other ASM groups (Table 2), particularly in those without pyridoxine supplement use (Table 3).

3.4 | Niacin status and association with ASM concentrations

We found no correlation between concentrations of ASM and nicotinamide (Table S2). The nicotinamide concentrations did not differ between different ASM groups (Table 2 and Table 3).

4 | DISCUSSION

In this cohort of pregnant women with epilepsy using ASMs, we found an association between plasma ASM concentrations and folate, riboflavin, and pyridoxine status, all of them part of one-carbon metabolism. High ASM concentrations were associated with high concentrations of inactive folate metabolites and UMFA, and with low concentrations of riboflavin and metabolically active pyridoxine. The associations were present even though many women used various types of vitamin B supplements during pregnancy.

The median folate concentration was lowest in the group with low adherence to ASM therapy and probably also to folic acid supplement use. Low folate concentrations during valproate, carbamazepine, oxcarbazepine, topiramate,[6,7] and lamotrigine treatment[8] have been reported in nonpregnant epilepsy populations compared to controls without ASM use. Folic acid supplement use in

TABLE 2 Maternal plasma vitamin B metabolite concentrations in gestational weeks 17–19 stratified for antiseizure medication (ASM) treatment

	Valproate n = 24	Lamotrigine n = 65	Carbamazepine n = 48	Levetiracetam n = 11	Topiramate n = 8	Oxcarbazepine n = 5	Polytherapy n = 40	Low-adherence group[1] n = 26
Plasma folate status								
Folate[2] (nmol/L); median (range)	**80.0 (84.7)**	65.9 (119.0)	65.5 (103.8)[b]	73.9 (107.1)	66.9 (86.4)	67.1 (68.4)	**74.2 (112.1)**[b]	**38.2 (96.7)**[a]
pABG (nmol/L); median (range)	**1.6 (19.5)**	1.1 (20.7)[b]	**1.3 (29.5)**	0.9 (17.4)	1.2 (9.2)	1.0 (5.3)	**1.5 (24.8)**[b]	**0.8 (9.7)**[a]
apABG (nmol/L); median (range)	**0.9 (2.4)**	0.8 (6.7)[b]	**0.9 (2.6)**	**0.6 (1.1)**[a]	1.2 (1.3)	0.6 (2.0)	**1.1 (2.4)**[b,c]	0.8 (2.2)[c]
Ratio active/inactive metabolites[3]; median (range)	25.5 (76.6)	27.5 (225.6)[b,c]	22.7 (76.2)[b]	41.3 (168.7)[a]	31.4 (58.1)	39.2 (32.7)	19.4 (94.2)[c]	23.8 (180.4)
UMFA (nmol/L); median (range)	**2.7 (303.0)**	**1.1 (169.0)**	**1.5 (182.0)**	0.8 (108.0)	1.9 (41.6)	0.8 (8.4)	**1.6 (167.0)**	**0.6 (63.8)**[a]
Ratio UMFA/folate[4]; median (range)	**0.03 (2.6)**	0.02 (1.6)	**0.03 (2.2)**	0.01 (0.8)	0.02 (0.3)	0.01 (0.1)	**0.02 (1.4)**	**0.009 (0.8)**[a]
Plasma riboflavin status								
Riboflavin (nmol/L); median (range)	5.9 (22.8)	10.0 (100.3)	6.6 (97.2)	9.6 (62.4)	7.4 (19.6)	6.4 (17.6)	6.9 (46.2)	7.6 (53.0)
Plasma pyridoxine status								
PLP (nmol/L); median (range)	24.7 (45.1)	32.7 (135.3)	23.1 (78.4)	24.9 (121.5)	31.1 (49.9)	27.8 (60.9)	28.9 (105.4)	27.2 (138.9)
HKr[5]; median (range)	**0.4 (0.9)**[a]	**0.3 (0.6)**	**0.3 (0.5)**	**0.3 (0.2)**	**0.3 (0.3)**	**0.2 (0.2)**	**0.3 (0.7)**	**0.3 (0.4)**
Plasma niacin status								
Nicotinamide (nmol/L); median (range)	485.3 (1058.5)	337.1 (1108.5)	370.2 (1652.3)	499.6 (501.2)	266.6 (526.7)	277.7 (1063.6)	412.9 (1099.0)	434.5 (1514.7)

All ASM groups are compared with each other by using Kruskal-Wallis test. Groups that differ significantly (p-value < 0.05) from the group marked with an [a] are marked with bold text, additional groups that significantly differ from each other are both marked with [b] and than with [c]. Significant differences after Bonferroni correction for multiple tests are underlined.

Abbreviations: ASM, 3-hydroxyanthranilic acid; XA, 3-hydroxykynurenine; pABG, 4-alfa-hydroxy-5-methyltetrahydrofolate; hmTHF, 5-methyltetrahydrofolate; UMFA, acetamidobenzoylglutamate; HAA, anthranilic acid; antiseizure medication, N may vary slightly due to missing data for plasma riboflavin status, plasma niacin status and plasma pyridoxine status in the following ASM treatment groups: lamotrigine (n = 1), carbamazepine (n = 1), levetiracetam (n = 2), polytherapy (n = 7); kynurenic acid, AA; mTHF; para-aminobenzoylglutamate, apABG; pyridoxal-5-phosphate, PLP; pyridoxal-5-phosphate, HK; unmetabolized folic acid, PLP; xanthurenic acid, KA.

[1] Consists of pregnancies where the mother reported ASM use, but no ASM was detected in plasma samples.

[2] Based on sum of mTHF and hmTHF.

[3] Ratio between active and inactive folate metabolites; mTHF + hmTHF: pABG + apABG.

[4] Ratio between UMFA and mTHF + hmTHF.

[5] Ratio between HK: (KA + XA + AA + HAA). This is a functional marker of vitamin B6 status; a high ratio shows an inverse association to the concentration of PLP.
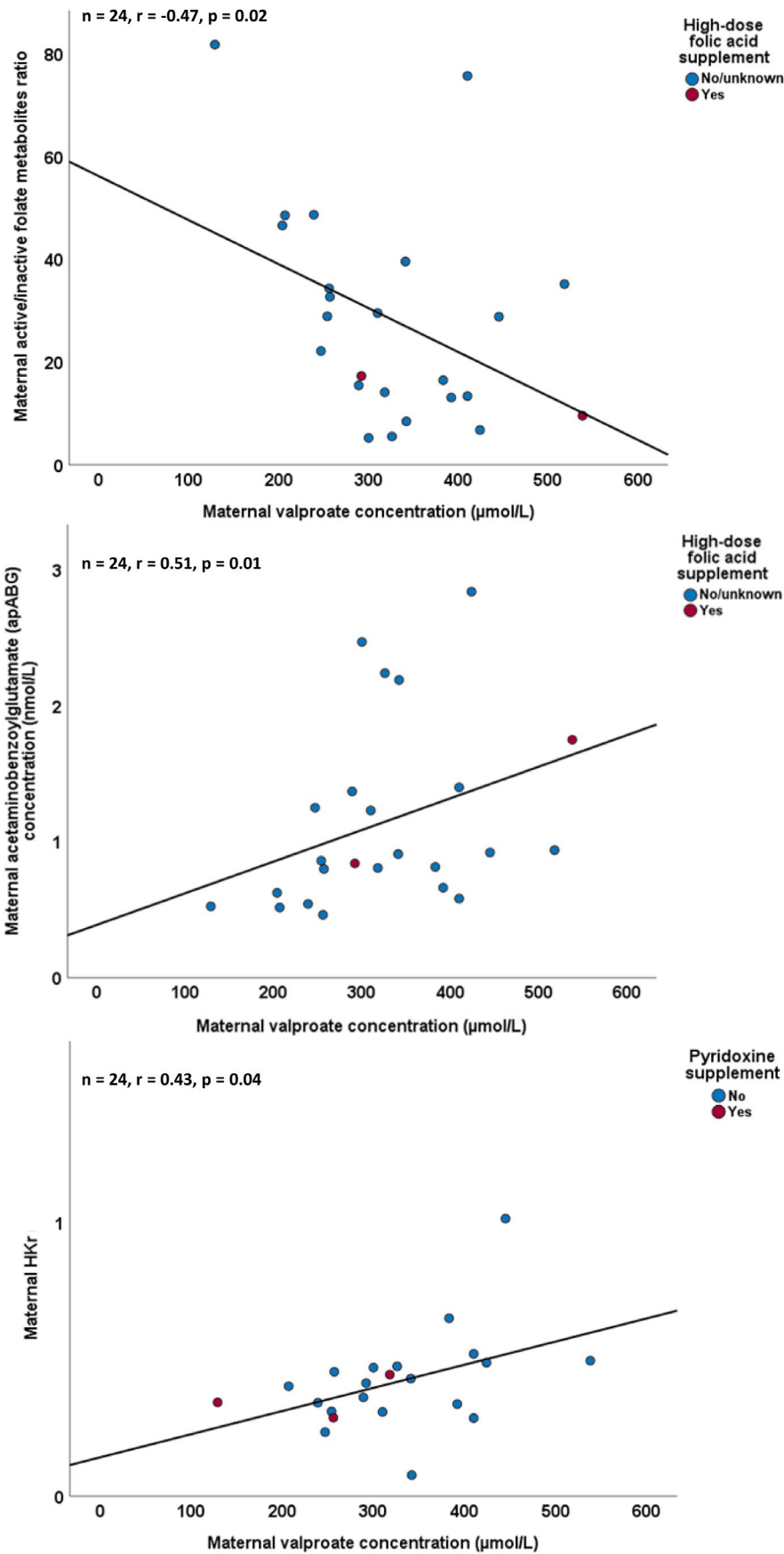
FIGURE 3 Correlation between maternal valproate concentrations and concentrations of vitamin B metabolites. The figure shows the correlation between maternal valproate concentrations in monotherapy users and vitamin B metabolites. Blue dots represent no supplement or no or unknown folic acid supplement dose; red dots represent supplemented pregnancies or high-dose folic acid supplement; HKr, marker of pyridoxine deficiency; r, Spearman's rho; p, p-value.

TABLE 3 Nonfolate vitamin B status stratified for antiseizure medication (ASM) treatment and supplement use in gestational weeks −4 to 20

Supplement	Valproate	Lamotrigine	Carbamazepine	Levetiracetam	Topiramate	Oxcarbazepine	Polytherapy	Low-adherence group[5]
n riboflavin status[1]	n = 3/19	n = 30/33	n = 9/38	n = 3/6	n = 4/3	n = 2/3	n = 12/21	n = 6/18
Riboflavin, nmol/L *median (range)*								
Riboflavin	22.6 (5.5)[f]	12.7 (97.6)[f]	8.8 (94.7)[f]	12.1 (56.3)	7.6 (19.6)	13.6 (17.6)	9.3 (14.6)[f]	9.7 (8.8)
No riboflavin	5.5 (18.1)[f]	6.9 (20.6)[f]	6.0 (44.0)[f]	8.2 (14.6)	7.9 (7.3)	6.4 (14.4)	5.7 (46.2)[f]	6.9 (53.0)
n pyridoxine status[1]	n = 3/19	n = 32/31	n = 10/37	n = 3/6	n = 4/3	n = 2/3	n = 13/20	n = 7/17
PLP, nmol/L HKr[4] *median (range)*								
Pyridoxine	51.1 (12.3)[f] 0.3 (0.2)	46.8 (129.3)[f] 0.3 (0.6)[f]	33.3 (76.9)[f] 0.3 (0.1)	57.7 (87.6)[f] 0.2 (0.1)	42.7 (45.0) 0.3 (0.3)	46.8 (60.9) 0.2 (0.0)	33.0 (103.6)[f] 0.3 (0.6)	43.8 (51.1) 0.3 (0.3)
No pyridoxine	24.5 (36.8)[f] 0.4 (0.9)[a]	24.1 (33.6)[f] 0.3 (0.5)[f]	20.9 (45.0)[f] 0.3 (0.5)	22.4 (18.6)[f] 0.3 (0.2)	26.0 (39.4) 0.3 (0.2)	27.8 (5.3) 0.2 (0.2)	23.5 (40.7)[f] 0.3 (0.7)	25.1 (138.9) 0.3 (0.4)
n niacin status[1]	n = 2/20	n = 17/46	n = 5/42	n = 2/7	n = 3/4	n = 2/3	n = 7/26	n = 4/20
Nicotinamide, nmol/L *median (range)*								
Nicotinamide	706.0 (11.2)	342.7 (1092.7)	185.7 (460.1)	529.4 (127.6)	416.6 (474.5)	215.2 (125.1)	432.9 (1099.0)	309.1 (442.4)
No nicotinamide	420.7 (1058.5)	337.7 (882.3)	377.7 (1652.3)	499.6 (501.2)	219.1 (114.8)	694.3 (1063.6)	376.5 (795.1)	434.5 (1498.1)

Note: .: All ASM groups are compared with each other by using Kruskal-Wallis test stratified for supplement use. Groups that differ significantly (*p*-value <0.05) from the group marked with an [a] are marked with bold text, additional groups that significantly differ from each other are both marked with [b c d], [e], and [e]. Significant differences after Bonferroni correction for multiple tests are underlined. Statistically significant (two-sided *p*-values <0.05) differences in concentrations between the two supplement groups stratified for ASM group by using Mann-Whitney *U* test are marked with [f].

Abbreviations: 3-hydroxyanthranilic acid, N may vary slightly due to missing data: supplemented pregnancies, n = 3 (carbamazepine n = 1, polytherapy n = 2); anthranilic acid, HAA; antiseizure medication, PLP; ASM; kynurenic acid, AA; non-supplemented pregnancies, n = 8 (lamotrigine n = 1, levetiracetam n = 2, polytherapy n = 5). Six pregnancies are not included in the table due to missing supplement data; pyridoxal-5-phosphate, HK;3-hydroxykynurenine, XA; xanthurenic acid, KA.

[1]Number of pregnancies for each supplement category (supplement/no supplement).

[2]Based on sum of mTHF and hmTHF.

[3]Ratio between active and inactive folate metabolites; mTHF + hmTHF: pABG + apABG.

[4]Ratio between HK: (KA + XA + AA + HAA). This is a functional marker of vitamin B6 status; a high ratio shows low functional PLP status.

[5]Consists of pregnancies where the mother reported ASM use, but no ASM was detected in plasma samples.

pregnancy has been associated with a higher IQ in children of mothers taking lamotrigine or carbamazepine treatment particularly.[38,39] In a study of pregnant women with epilepsy and levetiracetam or lamotrigine treatment, low concentrations of biologically active folate (mTHF) were found with lamotrigine, but folic acid supplement status was not reported.[22] Lamotrigine use was furthermore associated with changes in one-carbon metabolism, with altered pathways involving folate, purine, and sulfur amino acid metabolism.[22] Associations between ASM polytherapy and low folate concentrations, and high phenytoin and phenobarbital concentrations and low folate concentrations have been reported in another study in pregnant women with epilepsy.[21] Possibly, lamotrigine and carbamazepine particularly influence one-carbon metabolism in pregnancy, but concentrations of folate were not clearly different between the ASM groups in our study. Longitudinal studies with multiple sampling before and during pregnancy, and with different folic acid substitution regimens, are needed to fully understand the interplay between ASM, plasma folate status, and supplement use during pregnancy.

We found higher concentrations of inactive folate metabolites and a lower ratio between active and inactive folate metabolites in women using valproate, carbamazepine, and ASM polytherapy, compared to several of the other ASM groups. Chronic high-dose folic acid supplement use could partly explain higher concentrations of inactive folate metabolites, probably inducing an increase in folate catabolism. However, both high-dose use and any use of folic acid supplement were widespread across the ASM groups. Furthermore, there were no differences in ASM concentrations among high-dose users compared to low-dose users. Supplement use could therefore not fully explain the correlation between high ASM concentrations and high inactive folate metabolites. It is possible that valproate, carbamazepine, and ASM polytherapy use increase folate catabolism to a larger degree than other ASMs. In addition to ASMs, also pregnancy itself and chronic folic acid supplementation may influence folate catabolism.[10]

High-dose folic acid supplement users had high concentrations of UMFA. However, studies in both nonpregnant[40] and pregnant[34,41] women from the general population found that the UMFA concentration does not depend solely on the intake of folic acid, and suggested that there are mechanisms by which the body adapts to high supplement intake, thus limiting high plasma concentrations of UMFA. High ASM concentrations correlated with high UMFA concentrations in our study, and this indicates that these mechanisms may be influenced by ASM use. The optimal folic acid dose for women with epilepsy is not known,[4,42] and the safety of high supplement doses has been questioned.[12–15] Animal studies have

reported adverse effects of high UMFA concentrations on genetic programming and neuronal development.[14,15] One study found an association between higher concentrations of cord blood UMFA and increased risk of autism spectrum disorder in some population groups in the United States.[16] The large range and high maximum UMFA concentrations in this study may point to a closer monitoring of folate status during pregnancy in women with epilepsy on ASMs in order to avoid unnecessary high folic acid doses.

We found an association between high ASM concentrations and low riboflavin and pyridoxine status. The findings persisted after excluding women with supplement use of these vitamins in a sensitivity analysis. Low riboflavin concentrations have been reported in patients using carbamazepine, phenobarbital, phenytoin, and primidone in nonpregnant epilepsy populations.[18] Low pyridoxine concentrations during ASM use have also been reported, but less consistently.[5,18,43] Few studies have examined the association between ASM and nonfolate B vitamins in pregnant women with epilepsy. Adequate riboflavin and pyridoxine status is important for optimal folate metabolic function in one-carbon metabolism, and for normal fetal brain development during pregnancy.[19] We found no association between ASM and niacin status, the only vitamin in our study not participating in one-carbon metabolism.[19] Our findings show an association between ASM and riboflavin and pyridoxine status. The benefit of multivitamin B supplements in women with epilepsy planning and undergoing pregnancy should be further investigated.

Strengths of our study include a validated epilepsy diagnosis, prospectively collected data on supplement use, and analyses of plasma ASMs and vitamin and metabolite concentrations in a large sample of pregnancies. Women with epilepsy in MoBa are representative of women with epilepsy in Norway.[25] Multivitamin and folic acid supplement users are overrepresented in MoBa, whereas smokers are underrepresented.[44] Hence, we assume that women included in this study had a healthier lifestyle than women refusing inclusion, and a better vitamin B status. This would bias our results towards the null. Limitations of the study include plasma concentrations being measured only once during the pregnancy in the second trimester, a limited numbers of pregnancies in the ASM groups with the exception of valproate, lamotrigine, and carbamazepine, and self-reported information on vitamin supplement use. Genetic factors influence vitamin concentrations, but we did not have access to genetic information. We did not adjust for multiple pregnancies in the same mother. Only 22 mothers contributed with more than one pregnancy. The variance in vitamin and metabolite concentrations explained by ASM concentrations was low in some of our analyses, illustrating that factors other

than ASM treatment also influence the vitamin status during pregnancy. We presented standardized maternal ASM concentrations in addition to the individual ASM concentrations. By using relative plasma concentrations, we adjusted for differences in pharmacokinetics between the ASMs. Even though different ASMs have different pharmacological mechanisms, the majority of the ASMs have been associated with low folate status and their direct mechanism of action related to vitamin B status could share common pathways.[5–8] The folic acid dose data were collected retrospectively and were not available for the entire cohort.

In conclusion, we found important associations between plasma ASM concentrations and maternal folate status in pregnant women with epilepsy. Interactions between ASM and folate metabolic pathways, the pregnancy itself, and use of high folic acid doses may explain these findings. Furthermore, we found an association between ASM concentrations and low riboflavin and pyridoxine status. Optimal concentrations of both these vitamins are required for normal folate metabolic functioning and are thus essential for normal fetal development. Our findings provide new information regarding the association between ASM and vitamin B status during pregnancy. Additional studies are needed to determine the clinical impact of these findings, and to define the optimal vitamin doses in pregnancy.

CONFLICTS OF INTEREST
E. S. N. Husebye reports no disclosures relevant to the manuscript. B. Riedel reports no disclosures relevant to the manuscript. A.-L. Bjørke-Monsen reports no disclosures relevant to the manuscript. O. Spigset reports no disclosures relevant to the manuscript. A. K. Daltveit has received project funding from Pfizer Inc. N. E. Gilhus reports no disclosures relevant to the manuscript. Marte Helene Bjørk has received speaking and/or consultant honoraria from Novartis, Teva, Eisai, and Lilly, and project funding from Novartis unrelated to the present work.

AUTHOR CONTRIBUTIONS
Elisabeth Synnøve and Nilsen Husebye: designed and conceptualized study, analyzed the data, interpreted the data, drafted and revised the manuscript, and assisted with funding. Bettina Riedel: designed and conceptualized study, provided statistical advice, interpreted the data, and provided critical revision of manuscript. Anne-Lise Bjørke-Monsen: designed and conceptualized study, provided statistical advice, interpreted the data, and provided critical revision of manuscript. Olav Spigset: had a major role in the acquisition of data, interpreted the data, and provided critical revision of manuscript. Anne Kjersti Daltveit: provided methodological advice and critical revision of manuscript. Nils Erik Gilhus: had a major role in the acquisition of data, interpreted the data, provided critical revision of manuscript, and assisted with funding. Marte Helene Bjørk: had a major role in the acquisition of data, designed and conceptualized study, provided statistical advice, interpretation of data, and provided critical revision of manuscript and study supervision, and assisted with funding.

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