Maternal Tryptophan and Kynurenine Pathway Metabolites and Risk of Preeclampsia

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OBJECTIVE: To estimate the association of maternal plasma concentrations of tryptophan and six kynurenine pathway metabolites with the risk of preeclampsia.

METHODS: The study was based on a subsample of 2,936 pregnant women who delivered singleton neonates in the Norwegian Mother and Child Cohort Study in 2002–2003. Maternal blood plasma was obtained at approximately gestational week 18 and was measured for tryptophan, kynurenine, kynurenic acid, anthranilic acid, 3-hydroxykynurenine, xanthurenic acid, and 3-hydroxyanthranilic acid.

RESULTS: Of the 2,936 pregnant women included in this study, 116 (4.0%, 95% confidence interval [CI] 3.2–4.7) had preeclampsia subsequently diagnosed. The prevalence of preeclampsia was significantly higher among women with plasma kynurenic acid concentrations greater than the 95th percentile than among those with concentrations in the 25th–75th percentile (11.0% compared with 3.3%, $P<.001$; adjusted odds ratio 3.6, 95% CI 1.9–6.8). This association was significantly stronger in women with prepregnancy body mass index of 25 or more (P for interaction=.03; 20.4% compared with 4.2%; $P<.001$). No statistically significant associations of preeclampsia with other tryptophan metabolites were found.

CONCLUSION: Elevated maternal plasma kynurenic acid concentrations in early pregnancy are associated with a substantial increased risk of preeclampsia in obese women.

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LEVEL OF EVIDENCE: II

Tryptophan is an essential amino acid that occurs naturally in foods. It is important for the biosynthesis of proteins and is a precursor of serotonin, a neurotransmitter in the central nervous system.[1] The major catabolic route of tryptophan in mammals is the kynurenine pathway leading to the formation of several indole derivatives, collectively called "kynurenines."[2] This pathway has been implicated in various pathologic conditions associated with altered immune response.[3–5]

The first step in the oxidation of tryptophan to kynurenine (Fig. 1) is catalyzed by the hepatic enzyme tryptophan 2,3-dioxygenase or the ubiquitous indoleamine 2,3-dioxygenase.[1,2] Kynurenine may be further degraded by vitamins B2-dependent and B6-dependent enzymes to kynurenic acid, anthranilic acid, 3-hydroxykynurenine, xanthurenic acid, and 3-hydroxyanthranilic acid.[2,6] During inflammation, however, indoleamine 2,3-dioxygenase is induced by several inflammatory mediators, including interferon-γ.[5,7] This may result in decreased blood concentrations of tryptophan and increased blood concentrations of kynurenine.[5,7]

Preeclampsia is considered an inflammatory condition,[8] but data on the role of tryptophan and

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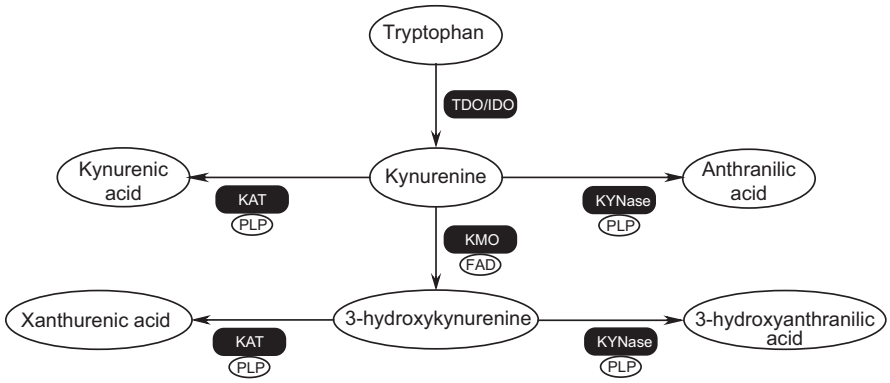

Fig. 1. The kynurenine pathway. TDO, tryptophan 2,3-dioxygenase; IDO, indoleamine 2,3-dioxygenase; KAT, kynurenine aminotransferase; KYNase, kynureninase; KMO, kynurenine 3-monooxygenase; PLP, pyridoxal 5′-phosphate; FAD, flavin adenin dinonucleotide.
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kynurenines in preeclampsia are scarce. This pregnancy complication has, in retrospective studies, been associated with decreased maternal plasma kynurenine-to-tryptophan ratio[9] and with increased urinary excretion of xanthurenic acid after oral tryptophan loading.[10] More recently, a genome-wide transcriptional profiling study of decidua basalis tissue showed that the tryptophan metabolism was the most significant canonical pathway in preeclampsia.[11]

In the present study, we estimated associations of tryptophan and six kynurenine pathway metabolites with the subsequent risk of preeclampsia using data from a large pregnancy cohort in Norway. The blood samples were collected at approximately gestational week 18, long before preeclampsia was clinically recognized.

MATERIALS AND METHODS

The study drew on resources from the Norwegian Mother and Child Cohort Study, a long-term prospective study of Norwegian pregnant women and their infants. The cohort includes more than 100,000 pregnancies during the period 1999–2008 and is linked to the Medical Birth Registry of Norway to obtain registered pregnancy outcomes.[12] Women were invited to participate through a postal invitation before a routine ultrasound examination at their local hospital, usually at approximately 18 weeks of gestation (response rate 43.5%). Informed consent was obtained from each participant before the study, and the study has been approved by the Regional Committee for Medical Research Ethics and by the Norwegian Data Inspectorate.

The present study comprised a quasi-random sample of 3,000 women included in the Norwegian Mother and Child Cohort Study and who delivered between July 2002 and December 2003.[13] The subcohort was initially established to examine relations between one-carbon metabolism and pregnancy outcomes,[13,14] and the decision on sample size was made to achieve sufficient statistical power for analyzing low prevalent outcomes. The limited period of sampling was chosen because of logistics related to sample processing. During the selected period, there were initially 17,588 women with registered births. Of these, 14,838 women (84%) had donated a blood sample and had returned a baseline questionnaire at approximately gestational week 18. By April 2008, 6,723 blood samples had been processed and were ready for retrieval from the Norwegian Mother and Child Cohort Study biobank.[13] We selected a simple random sample of 3,000 among women with the available blood samples. Of the 3,000 available samples, eight women had no information on preeclampsia and three pregnancies were terminated after prenatal diagnosis. We further excluded 53 twin pregnancies, leaving 2,936 singleton pregnancies for analyses.

The diagnostic criteria of preeclampsia in Norway are defined as maternal blood pressure higher than 140/90 mm Hg after gestational week 20 combined with proteinuria with more than one dipstick on at least two occasions.[15] The diagnosis is routinely recorded in the Medical Birth Registry of Norway by the attending midwife or obstetrician after birth using a standardized notification form.[16] The birth registry also comprises information on "early" preeclampsia diagnosed before 34 weeks of gestation, eclampsia, and hemolysis, elevated liver enzymes, and low platelet count (HELLP). In this study, we defined preeclampsia to include all these diagnoses.

The main exposures in the present study were plasma tryptophan, kynurenine, kynurenic acid, anthranilic acid, 3-hydroxykynurenine, xanthurenic acid, and 3-hydroxyanthranilic acid. However, because vitamins B2 and B6 are important coenzymes involved in the formation of kynurenic acid, anthranilic acid, xanthurenic acid, and 3-hydroxyanthranilic

acid,[6,17,18] we also included plasma concentrations of these B-vitamins in the analyses. Furthermore, we included plasma neopterin, another marker of immune activation mediated by interferon-γ.[19] Finally, plasma creatinine was included as a marker of renal function[20] to address the question of whether accumulation of kynurenines was related to impaired renal function. Notably, degradation and accumulation kinetics of tryptophan and six kynurenine pathway metabolites in serum and plasma were recently examined.[21] Kynurenine, kynurenic acid, and xanthurenic acid were essentially stable, whereas 3-hydroxykynurenine and 3-hydroxyanthranilic acid decreased and anthranilic acid increased on prolonged storage.

All plasma indices used in this study were analyzed in the laboratory of Bevital AS (www.bevital.no) using nonfasting maternal blood samples. The blood samples were collected into ethylenediaminetetraacetic acid tubes, centrifuged within 30 minutes, and placed in the hospital refrigerator (4°C).[22] They were shipped by mail overnight to the biobank of the Norwegian Mother and Child Cohort Study. On the day of receipt, usually 1–2 days after blood donation, ethylenediaminetetraacetic acid plasma were aliquoted onto polypropylene microtiter plates (300 microliters per well, 96-well format), sealed with heat-sealing foil sheets, and stored at −80°C. Plasma concentrations of tryptophan and kynurenine were analyzed using a gas chromatography–tandem mass spectrometry method,[23] whereas kynurenic acid, anthranilic acid, 3-hydroxykynurenine, xanthurenic acid, 3-hydroxyanthranilic acid, vitamins B2 (riboflavin) and B6 (pyridoxal 5′-phosphate), neopterin, and cotinine were analyzed by liquid chromatography–tandem mass spectrometry.[24] Creatinine was analyzed by including it and its deuterated internal standard (d3-creatinine) in an established liquid chromatography–tandem mass spectrometry method[25] using the ion pairs 114/44.2 and 117/47.2, respectively.

Covariates for the present analyses were obtained from both the Medical Birth Registry of Norway (collected at the time of hospitalization at approximately the time of birth) and the Norwegian Mother and Child Cohort Study baseline questionnaire (collected at median gestational week 18). From the birth registry, we obtained data on maternal age at delivery (years), marital status (single or other, cohabitation, married), parity (0, 1, and 2 or more previous deliveries), chronic hypertension (no or yes), prepregnancy diabetes (no or yes), gestational diabetes (no or yes), and gestational age (completed weeks) based on second-trimester ultrasound measurements. From the baseline cohort questionnaire, we obtained data on maternal education (0–9, 10–12, and 13 or more years), prepregnancy body mass index (BMI, calculated as weight (kg)/[height (m)]^2), and gestational BMI at approximately week 18 of gestation. A woman was classified as an active smoker if her plasma cotinine concentration was 30 nmol/L or more. Information was missing for 19 individuals regarding marital status, for 11 individuals regarding maternal education, for 114 individuals regarding prepregnancy BMI, and for 3 individuals regarding plasma cotinine.

Statistical analyses were performed by using SAS 9.2 and R 2.13.1 software for Windows. Data were described as percentages or means together with a variability measure such as standard error or confidence interval (CI). The χ^2 test was used to test for difference in frequencies, whereas the two-sample t test was used to assess difference in means. Spearman correlation coefficient (r) was used to estimate associations between pairs of continuous data.

To explore potential nonlinear associations between preeclampsia and the plasma indices, we used generalized additive logistic regression models (mgcv package in R)[26] with 3 degrees of freedom. Initially, this regression algorithm uses an underlying (thin-plate) spline basis with a $3+1=4$ basis function to estimate 4 regression coefficients for the smooth spline term. However, to ensure identifiability, there is a sum-zero constraint of functions, which reduces the effective number of degrees of freedom to 3. All plasma indices were analyzed on the logarithmic scale, and for each plasma index we excluded outliers defined as concentrations less than the first percentile and more than the 99th percentile of the distribution. Statistical significance of effects was determined by likelihood ratio tests. If analyzes were statistically significant, then we examined the relation with preeclampsia according to plasma percentile categories. In these analyses, all data (ie, no exclusions of outliers) were included in an ordinary logistic regression model.

We estimated both unadjusted and adjusted odds ratios (ORs) with 95% CIs. Adjustment variables were maternal age, parity, prepregnancy BMI, active smoking, chronic hypertension, prepregnancy diabetes, and gestational week at blood sampling. Maternal age, prepregnancy BMI, and gestational week at blood sampling were included as continuous linear terms. Potential effect modifications by prepregnancy BMI (less than 25, 25 or more) and parity (primiparous and multiparous women) were examined by including the product term of the plasma index and each of the categorical variables using likelihood ratio test. Potential confounding factors and relevant effect

modification variables were chosen on the basis of their previous reported roles in preeclampsia.[27] To handle missing values in multiple regression models, we used the method of list-wise deletion.

All *P* values were two-sided, and $P<.05$ was considered statistically significant, except for results presented in Table 1 and Figure 2, in which Bonferroni correction for 12 plasma indices was performed because of multiple testing (ie, a significance level of .004 was used).

RESULTS

Preeclampsia (including three cases of HELLP and one case of eclampsia) occurred in 4.0% (95% CI 3.2–4.7) of pregnancies (Table 2). The mean±standard error gestational age at blood sampling was 18.2±0.16 weeks (range 12–21 weeks) for women with preeclampsia and 18.5±0.04 weeks (range 10–36 weeks) for women without preeclampsia ($P=.16$). Median sampling time was 18 weeks of gestation for both groups. Among those without preeclampsia, only 25 women had donated blood samples in the third trimester (ie, more than 24 weeks of gestation).

Table 1. Concentrations of 12 Plasma Indices in Women With and Without Preeclampsia

Plasma Indices	Women Without Preeclampsia (n=2,820)	Women With Preeclampsia (n=116)	*P**
Tryptophan (micromol/L)	59.0±0.17	59.7±0.88	.40
Kynurenine (micromol/L)	1.11±0.004	1.13±0.02	.44
Kynurenine-to-tryptophan ratio†	19.1±0.08	19.1±0.30	.85
Kynurenic acid (nmol/L)	20.7±0.13	23.3±0.77	<.001
Anthranilic acid (nmol/L)	9.55±0.08	9.64±0.39	.82
3-hydroxykynurenine (nmol/L)	25.6±0.22	24.3±0.93	.22
Xanthurenic acid (nmol/L)	17.8±0.18	19.5±0.96	.07
3-hydroxyanthranilic acid (nmol/L)	41.7±0.29	43.4±1.51	.24
Vitamin B6 (nmol/L)	33.9±0.48	31.9±1.52	.39
Vitamin B2 (nmol/L)	11.8±0.26	10.9±0.97	.47
Creatinine (micromol/L)	49.2±0.12	49.3±0.62	.83
Neopterin (nmol/L)	7.57±0.04	7.49±0.16	.71

Data are mean±standard error unless otherwise specified.
* *P* value for difference in means was obtained by using a two-sample *t* test.
† The kynurenine-to-tryptophan ratio was multiplied by 1,000.

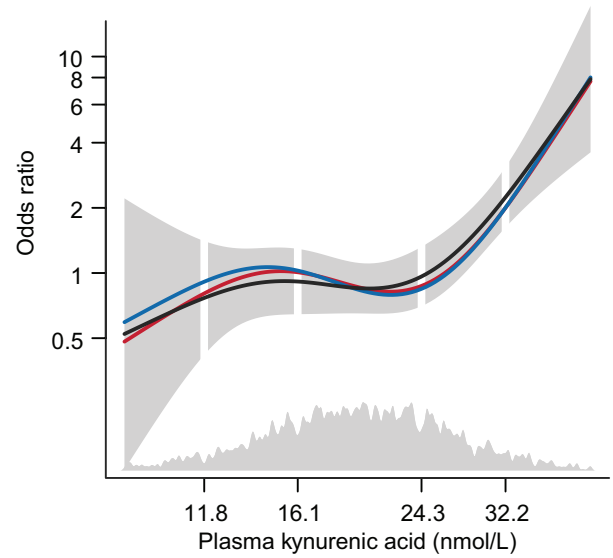

Fig. 2. Associations between plasma kynurenic acid concentrations and preeclampsia. Odds ratios (ORs) were estimated by using generalized additive logistic models. Unadjusted OR with 95% confidence intervals (CIs) are shown as a *solid black line* with *gray shaded area*. Adjusted OR is shown as a *solid blue line*, and adjusted OR after exclusions of women who smoke, diabetic women, and women with chronic hypertension is shown as a *solid red line* (CIs not shown). The OR scale is centered and set to 1 on the average estimated population risk (4.0%) in the unadjusted model. The distribution of log plasma kynurenic acid is shown on the X axis. The *vertical white lines* intersecting the CI represent the 5th, 25th, 75th, and 95th percentiles of plasma kynurenic acid.

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Preeclampsia was significantly more common among younger women, mothers who delivered for the first time, those with higher prepregnancy BMI, and mothers with chronic hypertension (Table 2). Compared with women without preeclampsia, preeclamptic mothers had lower mean age at delivery (28.4±0.42 years compared with 29.8±0.09 years; $P=.001$), higher mean prepregnancy BMI (27.1±0.58 compared with 24.1±0.08; $P<.001$), and shorter gestations (37.5±0.33 weeks compared with 39.5±0.04 weeks; $P<.001$).

Women who had development of preeclampsia had higher mean plasma kynurenic acid concentrations at gestational week 18 than mothers who did not have development of this complication (Table 1; $P<.001$). This difference was larger in mothers who had development of preeclampsia before 34 weeks, although the association was not statistically significant when correcting for multiple comparison (12 cases; 25.1±2.69 nmol/L compared with 20.8±0.13

Table 2. Prevalence of Preeclampsia According to Maternal Characteristics

Characteristic	Total No. of Women*	Women With Preeclampsia		P†
		n	%±SE	
All women	2,936	116	4.0±0.36	
Maternal age (y)				.03
Younger than 25	373	23	6.2±1.25	
25–29	1,002	45	4.5±0.65	
30–34	1,115	36	3.2±0.53	
35 or older	446	12	2.7±0.77	
Marital status				.87
Single or other	83	3	3.6±2.05	
Cohabitation	1,315	55	4.2±0.55	
Married	1,519	58	3.8±0.49	
Maternal education (y)				.96
0–9	85	4	4.7±2.30	
10–12	1,125	43	3.8±0.57	
13 or more	1,652	66	4.0±0.48	
Other	63	2	3.2±2.21	
Parity				<.001
0	1,271	76	6.0±0.67	
1	1,101	25	2.3±0.45	
2 or more	564	15	2.7±0.68	
Prepregnancy BMI (kg/m²)				<.001
Less than 18.5	80	4	5.0±2.44	
18.5–24.9	1,843	41	2.2±0.34	
25.0–29.9	594	34	5.7±0.95	
30.0 or higher	305	29	9.5±1.68	
Active smoking‡				.12
No	2,540	106	4.2±0.40	
Yes	393	10	2.5±0.79	
Chronic hypertension				<.001
No	2,916	110	3.8±0.35	
Yes	20	6	30.0±10.3	
Prepregnancy diabetes				.14
No	2,917	114	3.9±0.36	
Yes	19	2	10.5±7.03	

SE, standard error; BMI, body mass index.
* Information was missing for 19 women regarding marital status, 11 women regarding maternal education, 114 women regarding prepregnancy BMI, and 3 women regarding plasma cotinine.
† *P* value for difference in proportions was calculated by χ^2 tests.
‡ Plasma cotinine concentration ≥30 nmol/L.

nmol/L; *P*=.04). No statistically significant differences in means for other plasma indices were observed (Table 1).

Using generalized additive models, we discovered a distinct nonlinear association between log plasma kynurenic acid and preeclampsia (Fig. 2; *P*<.001). This relation was essentially unchanged after adjustment for potential confounders, as well as after exclusions of women who were smokers, diabetic women, and mothers who had chronic hypertension (Fig. 2). No statistically significant associations of preeclampsia with other metabolites were found (not shown).

We also estimated the overall prevalence of preeclampsia according to percentile categories of plasma kynurenic acid (Table 3). The prevalence± standard error of preeclampsia within the upper 95th percentile category of plasma kynurenic acid was 11.0%±2.59% compared with 3.3%±0.46% within the 25th–75th percentile category (*P*<.001). Furthermore, women who had plasma kynurenic acid concentrations more than the 95th percentile had an adjusted OR of 3.6 (95% CI 1.9–6.8) for preeclampsia, compared with those who had concentrations in the 25th–75th percentile. This association was slightly stronger after additional adjustment for plasma creatinine (OR 4.1, 95% CI 2.1–8.0), whereas additional adjustment for plasma B6 and B2, gestational diabetes, or gestational BMI (reported at approximately week 18 of gestation) did not change risk estimates (not shown).

Unadjusted logistic regression analyzes of kynurenic acid percentiles further showed that there was a statistically significant effect modification of the plasma kynurenic acid and preeclampsia association by prepregnancy BMI (*P* for interaction=.03), with associations substantially stronger among individuals with BMI 25 or more (Fig. 3). In that group, the OR for preeclampsia was 5.8 (95% CI 2.6–13.0) for kynurenic acid more than the 95th percentile and 3.0 (95% CI 1.6–5.7) for kynurenic acid between the 75th and 95th percentile, relative to those who had concentrations in the 25th–75th percentile. The corresponding prevalence of preeclampsia within the upper 95th percentile and the 75th–95th percentile categories of plasma kynurenic acid were 20.4%± 5.53% and 11.8%±2.36% compared with 4.2%±0.95% within the reference group (both *P*<.001). The strength of the relation between plasma kynurenic acid and prepregnancy BMI within each of the two BMI groups (less than 25, 25 or more) was weak and not statistically significant (r=0.04 and r=0.05, respectively).

When examining the relation between plasma kynurenic acid percentiles and preeclampsia according to strata of parity (primiparous and multiparous), some deviations in ORs were observed in the upper tail of plasma kynurenic acid (excess risk for multiparous women), although 95% CIs largely overlapped (*P* for interaction=.47).

DISCUSSION

This study investigated the association of maternal plasma concentrations of tryptophan and six kynure-

Table 3. Association Between Plasma Kynurenic Acid and Preeclampsia

Kynurenic Acid Percentiles	Total No. of Women*	Women With Preeclampsia		Odds Ratio† (95% CI)	Adjusted Odds Ratio‡ (95% CI)
		n	%±SE		
5th or lower	147	5	3.4±1.50	1.0 (0.4–2.7)	1.3 (0.5–3.5)
5th–25th	587	17	2.9±0.69	0.9 (0.5–1.5)	1.0 (0.5–1.8)
25th–75th§	1,467	48	3.3±0.46	1	1
75th–95th	586	30	5.1±0.91	1.6 (1.0–2.5)	1.4 (0.8–2.3)
Greater than 95th	146	16	11.0±2.59	3.6 (2.0–6.6)	3.6 (1.9–6.8)
P for trend‖				<.001	.004

SE, standard error; CI, confidence interval.
* Information on plasma kynurenic acid was missing for three women.
† Odds ratios were estimated by using ordinary logistic regression models.
‡ Adjusted for maternal age, parity, prepregnancy body mass index, active smoking, chronic hypertension, prepregnancy diabetes, and gestational week at blood sampling.
§ Reference category.
‖ *P* for trend was obtained by including the categorical variable as a continuous term in ordinary logistic regression models.

nine pathway metabolites with the risk of preeclampsia among 2,936 singleton pregnancies. We found that women who had development of preeclampsia had higher plasma kynurenic acid concentrations at gestational week 18 than mothers who did not have development of this complication. Regression analyzes further showed a distinct positive nonlinear relation between kynurenic acid and preeclampsia. Whereas kynurenic acid was an overall strong risk factor, subgroup analyses revealed that the association was particularly pronounced among individuals with elevated prepregnancy BMI.

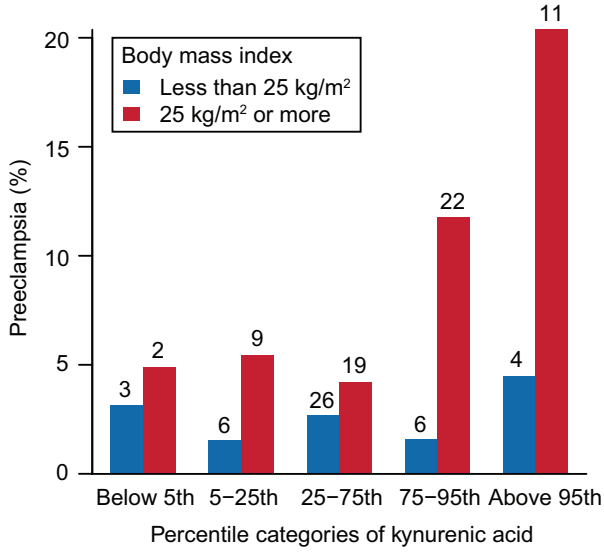

Fig. 3. Prevalence of preeclampsia according to plasma kynurenic acid and prepregnancy body mass index (BMI). Numbers above bars refer to the number of individuals with preeclampsia. Unadjusted ordinary logistic regression model showed a statistically significant effect modification by prepregnancy BMI (*P* for interaction=.03). Information on prepregnancy BMI was missing for 114 participants, and information on plasma kynurenic acid was missing for three participants.

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Strengths of this study include the large sample size, the standardized collection and laboratory analyses of blood samples, and the information on additional plasma indices, like vitamins B2 and B6, neopterin, creatinine, and cotinine. We do not suspect that selection bias has affected our results. Although the attendance rate in Norwegian Mother and Child Cohort Study was 43.5%, a recent validation study of eight selected exposure–outcome associations showed that initial self-selection in Norwegian Mother and Child Cohort Study did not introduce such bias.[28] A weakness of this study is that blood samples were nonfasting. This may have added preanalytic variability, thereby introducing nondifferential information bias to results. Furthermore, we have not validated preeclampsia against hospital medical records. Therefore, we do not know whether the report of preeclampsia was subject to some misclassification. However, we do not suspect that such misclassification differed by plasma concentrations. Finally, the present subcohort included biomarkers related to one-carbon metabolism and the kynurenine pathway, but with the primary aim of studying the relations between one-carbon metabolism and pregnancy outcomes.[13,14] As such, the associations presented in this study could be spurious and the need for an independent validation therefore is warranted.

Our analyses were adjusted for a number of important covariates, including plasma vitamin B6 and B2, prepregnancy BMI, and smoking. The adjust-

ment procedures, however, had no effect on the statistical results, suggesting little confounding. Results regarding kynurenic acid also remained unchanged after exclusions of women with chronic hypertension and prepregnancy diabetes, risk factors that are strongly associated with preeclampsia.[27] Because serum concentrations of kynurenines, including kynurenic acid, increase with severity of chronic kidney disease,[29] we also adjusted for plasma creatinine. We found no supportive evidence that creatinine was important for the plasma kynurenic acid–preeclampsia association.

Enhanced tryptophan catabolism along the kynurenine pathway caused by interferon-γ–mediated activation of indoleamine 2,3-dioxygenase is observed during inflammation, and an increased plasma kynurenine-to-tryptophan ratio is often seen in cancer and autoimmune diseases as well as in normal pregnancy.[5,7,19,30] The pathway per se, however, mediates immunosurveillance. Indoleamine 2,3-dioxygenase is normally highly expressed within placental tissues and confers maternal tolerance to fetal tissues.[31,32] In a previous retrospective case-control study,[9] maternal kynurenine-to-tryptophan ratio was decreased instead of increased in preeclamptic women, and also the activity of indoleamine 2,3-dioxygenase in placentas was found to be lower compared with that of normal pregnancy, suggesting impaired immunologic tolerance. A recent genome-wide transcriptional profiling study of decidua basalis tissue, however, found no preeclampsia-associated changes in indoleamine 2,3-dioxygenase expression, but it reported significant changes in several genes encoding for other enzymes involved in the kynurenine pathway.[11] Further, in our study, there was no statistically significant association of maternal plasma concentrations of tryptophan, kynurenine, kynurenine-to-tryptophan ratio, or neopterin with the risk of preeclampsia. The inconsistent findings between studies could be attributable to the timing of blood sampling. Although ours were drawn long before preeclampsia was clinically recognized, the previous case-control study was performed in women who were overtly preeclamptic.

Animal studies have revealed diverse metabolic effects of kynurenic acid, which may link kynurenic acid to preeclampsia. Notably, kynurenic acid is a glutamate receptor antagonist that can block the release of insulin from pancreatic β-cells,[2] it reduces the lumbar sympathetic nerve activity and arterial blood pressure in hyperinsulinemic rats,[33] and kynurenic acid is a potent inhibitor of the synthesis of fatty acids.[34,35] These effects of kynurenic acid are essentially in the opposite direction of the symptoms observed in patients with preeclampsia, which is characterized by increased insulin resistance and hyperlipidemia,[36] high arterial blood pressure, and increased sympathetic nerve activity.[37] Thus, one may speculate if increased kynurenic acid is a compensatory mechanism that counteracts key pathogenic features in preeclampsia, including components of the metabolic syndrome. Such mechanisms possibly could involve reduced enzyme activity of kynurenine 3-monooxygenase,[38,39] thereby shunting the metabolism of kynurenine to the end-stage metabolite kynurenic acid.[39] In addition, the high risk estimates of preeclampsia may partly reflect the unique stability of kynurenic acid in blood samples,[21] reducing potential for measurement errors.

Several studies have shown that the prevalence of preeclampsia is at least twice as high during first pregnancies as during second or later pregnancies.[27] This also was observed in the present study (Table 2) and has previously been hypothesized to be attributable to increased maternal immunologic tolerance to the fetus mediated through earlier pregnancies.[40] Our study, however, found no evidence of an interaction between plasma kynurenic acid and parity. Thus, the strong association of kynurenic acid with preeclampsia does not seem to be linked to increased maternal immunologic tolerance in subsequent pregnancies.

In a large cohort, we examined the association of tryptophan and six kynurenine pathway metabolites with the risk of preeclampsia. Our study suggests that elevated plasma concentrations of the tryptophan catabolite kynurenic acid in early pregnancy are strongly associated with increased risk of preeclampsia in women with increased prepregnancy BMI. Potential pathogenic roles of kynurenic acid in preeclampsia, as well as in other obesity-associated diseases, should be further explored.

REFERENCES

1. Le Floc'h N, Otten W, Merlot E. Tryptophan metabolism, from nutrition to potential therapeutic applications. Amino Acids 2011;41:1195–205.
2. Stone TW, Darlington LG. Endogenous kynurenines as targets for drug discovery and development. Drug Discovery 2002;1:609–20.
3. Wang Y, Liu H, McKenzie G, Witting PK, Stasch JP, Hahn M, et al. Kynurenine is an endothelium-derived relaxing factor produced during inflammation. Nat Med 2010;16:279–85.
4. Mellor AL, Munn DH. IDO expression by dendritic cells: tolerance and tryptophan catabolism. Nat Rev Immunol 2004;4:762–74.
5. Schrocksnadel K, Wirleitner B, Winkler C, Fuchs D. Monitoring tryptophan metabolism in chronic immune activation. Clin Chim Acta 2006;364:82–90.

6. Stevens CO, Henderson LM. Riboflavin and hepatic kynurenine hydroxylase. J Biol Chem 1959;234:1191–4.
7. Wirleitner B, Rudzite V, Neurauter G, Murr C, Kalnins U, Erglis A, et al. Immune activation and degradation of tryptophan in coronary heart disease. Eur J Clin Invest 2003;33:550–4.
8. Saito S, Shiozaki A, Nakashima A, Sakai M, Sasaki Y. The role of the immune system in preeclampsia. Mol Aspects Med 2007;28:192–209.
9. Kudo Y, Boyd CA, Sargent IL, Redman CW. Decreased tryptophan catabolism by placental indoleamine 2,3-dioxygenase in preeclampsia. Am J Obstet Gynecol 2003;188:719–26.
10. Sprince H, Lowy RS, Folsome CE, Behrman JS. Studies on the urinary excretion of "xanthurenic acid" during normal and abnormal pregnancy: a survey of the excretion of "xanthurenic acid" in normal nonpregnant, normal pregnant, pre-eclamptic, and eclamptic women. Am J Obstet Gynecol 1951;62:84–92.
11. Loset M, Mundal SB, Johnson MP, Fenstad MH, Freed KA, Lian IA, et al. A transcriptional profile of the decidua in preeclampsia. Am J Obstet Gynecol 2011;204:84.e1–27.
12. Magnus P, Irgens LM, Haug K, Nystad W, Skjaerven R, Stoltenberg C, et al. Cohort profile: the Norwegian Mother and Child Cohort Study (MoBa). Int J Epidemiol 2006;35: 1146–50.
13. Nilsen RM, Vollset SE, Monsen AL, Ulvik A, Haugen M, Meltzer HM, et al. Infant birth size is not associated with maternal intake and status of folate during the second trimester in Norwegian pregnant women. J Nutr 2010;140:572–9.
14. Haberg SE, London SJ, Nafstad P, Nilsen RM, Ueland PM, Vollset SE, et al. Maternal folate levels in pregnancy and asthma in children at age 3 years. J Allergy Clin Immunol 2011;127:262–4.
15. Haugen M, Brantsaeter AL, Trogstad L, Alexander J, Roth C, Magnus P, et al. Vitamin D supplementation and reduced risk of preeclampsia in nulliparous women. Epidemiology 2009;20: 720–6.
16. Irgens LM. The Medical Birth Registry of Norway. Epidemiological research and surveillance throughout 30 years. Acta Obstet Gynecol Scand 2000;79:435–9.
17. Midttun O, Ulvik A, Ringdal Pedersen E, Ebbing M, Bleie O, Schartum-Hansen H, et al. Low plasma vitamin B-6 status affects metabolism through the kynurenine pathway in cardiovascular patients with systemic inflammation. J Nutr 2011;141: 611–7.
18. Brown RR, Thornton MJ, Price JM. The effect of vitamin supplementation on the urinary excretion of tryptophan metabolites by pregnant women. J Clin Invest 1961;40: 617–23.
19. Schrocksnadel K, Widner B, Bergant A, Neurauter G, Schennach H, Schrocksnadel H, et al. Longitudinal study of tryptophan degradation during and after pregnancy. Life Sci 2003;72:785–93.
20. Perrone RD, Madias NE, Levey AS. Serum creatinine as an index of renal function: new insights into old concepts. Clin Chem 1992;38:1933–53.
21. Hustad S, Eussen S, Midttun O, Ulvik A, van de Kant PM, Morkrid L, et al. Kinetic modeling of storage effects on biomarkers related to B vitamin status and one-carbon metabolism. Clin Chem 2012;58:402–10.
22. Ronningen KS, Paltiel L, Meltzer HM, Nordhagen R, Lie KK, Hovengen R, 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.
23. 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.
24. Midttun O, Hustad S, Ueland PM. Quantitative profiling of biomarkers related to B-vitamin status, tryptophan metabolism and inflammation in human plasma by liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom 2009;23:1371–9.
25. Holm PI, Ueland PM, Kvalheim G, Lien EA. Determination of choline, betaine, and dimethylglycine in plasma by a high-throughput method based on normal-phase chromatography-tandem mass spectrometry. Clin Chem 2003;49:286–94.
26. Hastie TJ, Tibshirani RJ. Generalized additive models. New York (NY): Chapman and Hall; 1990.
27. Sibai B, Dekker G, Kupferminc M. Pre-eclampsia. Lancet 2005;365:785–99.
28. Nilsen RM, Vollset SE, Gjessing HK, Skjaerven R, Melve KK, Schreuder P, et al. Self-selection and bias in a large prospective pregnancy cohort in Norway. Paediatr Perinat Epidemiol 2009;23:597–608.
29. Schefold JC, Zeden JP, Fotopoulou C, von Haehling S, Pschowski R, Hasper D, et al. Increased indoleamine 2,3-dioxygenase (IDO) activity and elevated serum levels of tryptophan catabolites in patients with chronic kidney disease: a possible link between chronic inflammation and uraemic symptoms. Nephrol Dial Transplant 2009;24:1901–8.
30. Brown RR, Ozaki Y, Datta SP, Borden EC, Sondel PM, Malone DG. Implications of interferon-induced tryptophan catabolism in cancer, auto-immune diseases and AIDS. Adv Exp Med Biol 1991;294:425–35.
31. Kudo Y, Boyd CA, Spyropoulou I, Redman CW, Takikawa O, Katsuki T, et al. Indoleamine 2,3-dioxygenase: distribution and function in the developing human placenta. J Reprod Immunol 2004;61:87–98.
32. Munn DH, Zhou M, Attwood JT, Bondarev I, Conway SJ, Marshall B, et al. Prevention of allogeneic fetal rejection by tryptophan catabolism. Science 1998;281:1191–3.
33. Bardgett ME, McCarthy JJ, Stocker SD. Glutamatergic receptor activation in the rostral ventrolateral medulla mediates the sympathoexcitatory response to hyperinsulinemia. Hypertension 2010;55:284–90.
34. Fabregat I, Vitorica J, Satrustegui J, Machado A. The pentose phosphate cycle is regulated by NADPH/NADP ratio in rat liver. Arch Biochem Biophys 1985;236:110–8.
35. Barth CA, Hackenschmidt HJ, Weis EE, Decker KF. Influence of kynuremate on cholesterol and fatty acid synthesis in isolated perfused rat liver. J Biol Chem 1973;248:738–9.
36. Zavalza-Gomez AB. Obesity and oxidative stress: a direct link to preeclampsia? Arch Gynecol Obstet 2011;283:415–22.
37. Schobel HP, Fischer T, Heuszer K, Geiger H, Schmieder RE. Preeclampsia – a state of sympathetic overactivity. N Engl J Med 1996;335:1480–5.
38. Oxenkrug GF. Metabolic syndrome, age-associated neuroendocrine disorders, and dysregulation of tryptophan-kynurenine metabolism. Ann N Y Acad Sci 2010;1199:1–14.
39. Holtze M, Saetre P, Engberg G, Schwieler L, Werge T, Andreassen OA, et al. Kynurenine 3-monooxygenase polymorphisms: relevance for kynurenic acid synthesis in patients with schizophrenia and healthy controls. J Psychiatry Neurosci 2012;37:53–7.
40. Trogstad LI, Eskild A, Magnus P, Samuelsen SO, Nesheim BI. Changing paternity and time since last pregnancy; the impact on pre-eclampsia risk. A study of 547 238 women with and without previous pre-eclampsia. Int J Epidemiol 2001;30:1317–22.