Published in final edited form as:

Obstet Gynecol. 2012 June ; 119(6): 1243–1250. doi:10.1097/AOG.0b013e318255004e.

Maternal Tryptophan and Kynurenine Pathway Metabolites and Risk of Preeclampsia

Roy M. Nilsen, PhD^a, Anne-Lise Bjørke-Monsen, MD, PhD^b, Øivind Midttun, PhD^c, Ottar Nygård, MD, PhD^{d,e}, Eva R. Pedersen, MD, PhD^d, Arve Ulvik, PhD^c, Per Magnus, MD, PhD^f, Håkon K. Gjessing, PhD^{f,g}, Stein Emil Vollset, MD, Dr. PH^{g,h}, and Per Magne Ueland, MD, PhD^e

^aCentre for Clinical Research, Haukeland University Hospital, Bergen, Norway

^bLaboratory of Clinical Biochemistry, Haukeland University Hospital, Bergen, Norway

^cBevital AS, Bergen, Norway

^dDepartment of Heart Disease, Haukeland University Hospital, Bergen, Norway

^eInstitute of Medicine, University of Bergen, Bergen, Norway

^fDivision of Epidemiology, Norwegian Institute of Public Health, Oslo, Norway

^gDepartment of Public Health and Primary Health Care, University of Bergen, Norway

^hMedical Birth Registry of Norway, Norwegian Institute of Public Health, Bergen, Norway

Abstract

Objective—To estimate the association of maternal plasma concentrations of tryptophan and 6 kynurenine pathway metabolites with the risk of preeclampsia.

Methods—The study was based on a subsample of 2,936 pregnant women who delivered singleton infants in the Norwegian Mother and Child Cohort Study in 2002–2003. Maternal blood plasma was obtained approximately at gestational week 18 and 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 3.2 – 4.7) were subsequently diagnosed with preeclampsia. 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%, $P < 0.001$; adjusted odds ratio 3.6, 95% confidence interval 1.9 – 6.8). This association was significantly stronger in women with prepregnancy body mass index of 25 kg/m² or more (P for interaction = .03; 20.4% compared with 4.2%, $P < 0.001$). 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.

Corresponding Author: Roy Miodini Nilsen, Centre for Clinical Research, Haukeland University Hospital, Paviljongen, 2nd floor, N-5021 Bergen, Norway, Telephone: (+47) 55 97 70 75, Fax number: (+47) 55 97 60 88, roy.miodini.nilsen@helse-bergen.no.

Financial Disclosure: The authors did not report any potential conflicts of interest.

INTRODUCTION

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 pathological 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 (IDO) (1, 2). Kynurenine may be further degraded by vitamins B2 and B6-dependent enzymes to kynurenic acid (KA), anthranilic acid (AA), 3-hydroxykynurenine (HK), xanthurenic acid (XA) and 3-hydroxyanthranilic acid (HAA) (2, 6). During inflammation, however, IDO 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 kynurenines in preeclampsia are scarce. This pregnancy complication has, in retrospective studies, been associated with decreased maternal plasma kynurenine/tryptophan ratio (9) and with increased urinary excretion of XA 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 6 kynurenine pathway metabolites with the subsequent risk of preeclampsia using data from a large pregnancy cohort in Norway. The blood samples were collected around 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 (MoBa), 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 prior to a routine ultrasound examination at their local hospital, usually around 18 weeks of gestation (response rate 43.5%). Informed consent was obtained from each participant prior to the study, and the study has been approved by the Regional Committee for Medical Research Ethics and by the Norwegian Data Inspectorate.

Study population

The present study comprised a quasi-random sample of 3,000 women included in MoBa and who delivered between July 2002 and December 2003 (13). The sub-cohort 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 due to 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 around gestational week 18. By April 2008, 6,723 blood samples had been processed and were ready for retrieval from the MoBa biobank (13).

We selected a simple random sample of 3,000 among women with the available blood samples. Of the 3000 available samples, 8 women had no information on preeclampsia and 3 pregnancies were terminated after prenatal diagnosis. We further excluded 53 twin pregnancies, leaving 2,936 singleton pregnancies for analyses.

Preeclampsia

The diagnostic criteria of preeclampsia in Norway are defined as maternal blood pressure >140/90 mm Hg after gestational week 20, combined with proteinuria with more than 1 dipstick on at least 2 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, eclampsia, and HELLP (hemolysis, elevated liver enzymes, and low platelet count). In this study, we defined preeclampsia to include all these diagnoses.

Plasma indices

The main exposures in the present study were plasma tryptophan, kynurenine, KA, AA, HK, XA and HAA. However, because vitamins B2 and B6 are important coenzymes involved in the formation of KA, AA, XA, and HAA (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 whether accumulation of kynurenines were related to impaired renal function. Notably, degradation and accumulation kinetics of tryptophan and 6 kynurenine pathway metabolites in serum and plasma was recently examined (21). Kynurenine, KA and XA were essentially stable, whereas HK and HAA decreased and AA increased upon prolonged storage.

Blood sampling and laboratory analyses

All plasma indices used in this study were analyzed in the laboratory of Bevital AS (www.bevital.no) using non-fasting maternal blood samples. The blood samples were collected into ethylenediaminetetraacetic acid (EDTA) tubes, centrifuged within 30 minutes, and placed in the hospitals' refrigerator (4 °C) (22). They were shipped by mail overnight to the biobank of MoBa. On the day of receipt, usually 1–2 days after blood donation, EDTA plasma were aliquoted onto polypropylene micro-titre plates (300 μ L 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 KA, AA, HK, XA, HAA, 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

Covariates for the present analyses were obtained from both the Medical Birth Registry of Norway (collected at the time of hospitalization around birth) and the MoBa baseline questionnaire (collected at median gestational week 18). From the birth registry, we obtained data on maternal age at delivery (years), marital status (single/other, cohabitation, married), parity (0, 1, and 2 previous deliveries), chronic hypertension (no, yes), prepregnancy diabetes (no, yes), gestational diabetes (no, 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 years), prepregnancy body mass index (BMI; kg/m²), and gestational BMI around week 18. A woman was classified as an active smoker if her plasma cotinine concentration was 30 nmol/L. Information was missing for 19 subjects on marital status, 11 subjects on maternal education, 114 subjects on prepregnancy BMI, and 3 subjects on plasma cotinine.

Statistical analyses

Statistical analyses were performed by using SAS (Statistical Analysis System) version 9.2 (SAS Institute, Inc., Cary, North Carolina) and R version 2.13.1 (The R Foundation for Statistical Computing, www.r-project.org) software for Windows. Data were described as percentages or means together with a variability measure such as standard error or confidence interval (CI). The chi-square test was used to test for difference in frequencies, whereas the 2-sample t-test was used to assess difference in means. Spearman's correlation coefficient (*r*) was used to estimate associations between pairs of continuous data.

To explore potential non-linear 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 $3 + 1 = 4$ basis functions 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 below the 1th percentile and above the 99th percentile of the distribution. Statistical significance of effects was determined by likelihood ratio tests. If analyses turned out statistically significant, we went on to examine the relation with preeclampsia according to plasma percentile categories. In these analyses, all data (i.e., 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 (<25, 25 kg/m²) and parity (primiparous and multiparous) 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 listwise deletion.

All *P* values were 2-sided and values < 0.05 were considered statistically significant except for results presented in Table 2 and Fig. 2 where Bonferroni correction for 12 plasma indices was done due to multiple testing (i.e., a significance level of 0.004 was used).

RESULTS

Preeclampsia (including 3 cases of HELLP and 1 case of eclampsia) occurred in 4.0% (95% CI 3.2 – 4.7) of pregnancies (Table 1). The mean $\pm$ 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 (i.e., >24 weeks gestation).

Preeclampsia was significantly more common among younger women, mothers who delivered for the first time, among those with higher prepregnancy BMI, and mothers with chronic hypertension (Table 1). Compared with women without preeclampsia, preeclamptic mothers had lower mean age at delivery (28.4 ± 0.42 years versus 29.8 ± 0.09 years; $P = .001$), higher mean prepregnancy BMI (27.1 ± 0.58 kg/m² versus 24.1 ± 0.08 kg/m²; $P < .001$), and shorter gestations (37.5 ± 0.33 weeks versus 39.5 ± 0.04 weeks; $P < .001$).

Women who developed preeclampsia had higher mean plasma KA concentrations at gestational week 18 than mothers who did not develop this complication (Table 2; $P < .001$). This difference was larger in mothers who developed preeclampsia before 34 weeks, although the association was not statistically significant when correcting for multiple comparison (12 cases; 25.1 ± 2.69 nmol/L versus 20.8 ± 0.13 nmol/L, $P = .04$). No statistically significant differences in means for other plasma indices were observed (Table 2).

Using generalized additive models, we discovered a distinct non-linear association between log plasma KA and preeclampsia (Fig. 2; $P < .001$). This relation was essentially unchanged after adjustment for potential confounders, as well as after exclusions of smokers, diabetic women, and mothers who suffered from 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 KA (Table 3). The prevalence $\pm$ standard error of preeclampsia within the upper 95th percentile category of plasma KA was $11.0 \pm 2.59\%$ compared with $3.3 \pm 0.46\%$ within the 25th–75th percentile category ($P < 0.001$). Furthermore, women who had plasma KA concentrations greater 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 around week 18), did not change risk estimates (not shown).

Unadjusted logistic regression analyses of KA percentiles further showed that there was a statistically significant effect modification of the plasma KA - preeclampsia association by prepregnancy BMI (P for interaction = .03), with associations substantially stronger among subjects with BMI ≥ 25 kg/m² (Fig. 3). In that group, the OR for preeclampsia was 5.8 (95% CI 2.6 – 13.0) for KA $>$ 95th percentile and 3.0 (95% CI 1.6 – 5.7) for KA between 75th and 95th percentile, relative to those who had concentrations in the 25–75th percentile. The corresponding prevalence of preeclampsia within the upper 95th percentile and the 75th–95th percentile categories of plasma KA were $20.4 \pm 5.53\%$ and $11.8 \pm 2.36\%$ compared with $4.2 \pm 0.95\%$ within the reference group (both $P < 0.001$). The strength of the relation between plasma KA and prepregnancy BMI within each of the two BMI groups (< 25 , ≥ 25 kg/m²) was weak and not statistically significant ($r = 0.04$ and $r = 0.05$, respectively).

When examining the relation between plasma KA percentiles and preeclampsia according to strata of parity (primiparous and multiparous), some deviations in ORs were observed in the upper tail of plasma KA (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 6 kynurenine pathway metabolites with the risk of preeclampsia among 2,936 singleton pregnancies. We found that women who developed preeclampsia had higher plasma KA

concentrations at gestational week 18 than mothers who did not develop this complication. Regression analyses further showed a distinct positive non-linear relation between KA and preeclampsia. Whereas KA was an overall strong risk factor, subgroup analyses revealed that the association was particularly pronounced among subjects with elevated prepregnancy BMI.

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 MoBa was 43.5%, a recent validation study of 8 selected exposure-outcome associations showed that initial self-selection in MoBa did not introduce such bias (28). A weakness of this study is that blood samples were non-fasting. This may have added pre-analytic variability and thereby introduced non-differential 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. We do, however, not suspect that such misclassification differed by plasma concentrations. Finally, the present sub-cohort included biomarkers related to one-carbon metabolism and the kynurenine pathway, but with the primary aim to study 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 is therefore warranted.

Our analyses were adjusted for a number of important covariates, including plasma vitamin B6 and B2, prepregnancy BMI, and smoking. The adjustment procedures, however, had no impact on the statistical results, suggesting little confounding. Results regarding KA 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 KA, 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 KA - preeclampsia association.

Enhanced tryptophan catabolism along the kynurenine pathway caused by interferon- γ mediated activation of IDO is observed during inflammation, and an increased plasma kynurenine/tryptophan ratio is often seen in cancer and autoimmune diseases as well as normal pregnancy (5, 7, 19, 30). The pathway per se, however, mediates immunosurveillance. IDO 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/tryptophan ratio was decreased instead of increased in preeclamptic women, and also the activity of IDO 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 IDO expression, but 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/tryptophan ratio or neopterin with the risk of preeclampsia. The inconsistent findings between studies could be due to the timing of blood sampling. While 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 KA which may link KA to preeclampsia. Notably, KA is a glutamate receptor antagonist, which 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 KA is a potent inhibitor of the

synthesis of fatty acids (34, 35). These effects of KA 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 elevated KA is a compensatory mechanism that counteracts key pathogenic features in preeclampsia, including components of the metabolic syndrome. Such mechanisms could possibly involve reduced enzyme activity of kynurenine 3-monooxygenase (38, 39), thereby shunting the metabolism of kynurenine to the end-stage metabolite KA (39). In addition, the high risk estimates of preeclampsia may partly reflect the unique stability of KA 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 was also observed in the present study (see Table 1) and has previously been hypothesized to be due to increased maternal immunologic tolerance to the fetus mediated through earlier pregnancies (40). Our study, however, found no evidence of an interaction between plasma KA and parity. Thus, the strong association of KA 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 6 kynurenine pathway metabolites with the risk of preeclampsia. Our study suggests that elevated plasma concentrations of the tryptophan catabolite KA in early pregnancy are strongly associated with increased risk of preeclampsia in women with elevated prepregnancy BMI. Potential pathogenic roles of KA in preeclampsia, as well as in other obesity-associated diseases, should be further explored.

Acknowledgments

Funding/Support:

The Norwegian Mother and Child Cohort Study was supported by the Norwegian Ministry of Health and the Ministry of Education and Research, NIH/NIEHS (contract no. NO-ES-75558), NIH/NINDS (grant no. 1 UO1 NS 047537-01), and the Norwegian Research Council/FUGE (grant no. 151918/S10). The present study was also supported by the Foundation to Promote Research into Functional Vitamin B12-Deficiency.

References

1. Le Floch N, Otten W, Merlot E. Tryptophan metabolism, from nutrition to potential therapeutic applications. *Amino Acids*. 2011; 41:1195–1205. [PubMed: 20872026]
2. Stone TW, Darlington LG. Endogenous kynurenines as targets for drug discovery and development. *Nature reviews 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. [PubMed: 20190767]
4. Mellor AL, Munn DH. IDO expression by dendritic cells: tolerance and tryptophan catabolism. *Nat Rev Immunol*. 2004; 4:762–74. [PubMed: 15459668]
5. Schrocksnadel K, Wirleitner B, Winkler C, Fuchs D. Monitoring tryptophan metabolism in chronic immune activation. *Clin Chim Acta*. 2006; 364:82–90. [PubMed: 16139256]
6. Stevens CO, Henderson LM. Riboflavin and hepatic kynurenine hydroxylase. *J Biol Chem*. 1959; 234:1191–4. [PubMed: 13654345]
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. [PubMed: 12814390]
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. [PubMed: 17433431]

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. [PubMed: 12634647]
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. [PubMed: 14846824]
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.e1–27.10.1016/j.ajog.2010.08.043 [PubMed: 20934677]
12. 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. [PubMed: 16926217]
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. [PubMed: 20089778]
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; 127:262–4.10.1016/j.jaci.2010.10.004 [PubMed: 21094522]
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. [PubMed: 19451820]
16. Irgens LM. The Medical Birth Registry of Norway. Epidemiological research and surveillance throughout 30 years. *Acta Obstet Gynecol Scand.* 2000; 79:435–9. [PubMed: 10857866]
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; 141:611–17. [PubMed: 21310866]
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. [PubMed: 16695856]
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. [PubMed: 12479977]
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. [PubMed: 1394976]
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. [PubMed: 22194632]
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. [PubMed: 17031521]
23. Ueland PM, Midttun O, Windelberg A, Svoldal 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. [PubMed: 17963453]
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. [PubMed: 19337982]
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. [PubMed: 12560353]
26. Hastie, TJ.; Tibshirani, RJ. Generalized Additive Models. New York: Chapman and Hall; 1990.
27. Sibai B, Dekker G, Kupferminc M. Pre-eclampsia. *Lancet.* 2005; 365:785–99. [PubMed: 15733721]

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. [PubMed: 19840297]
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. [PubMed: 19155537]
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. [PubMed: 1722946]
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. [PubMed: 15063632]
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. [PubMed: 9712583]
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. [PubMed: 20065145]
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. [PubMed: 3966788]
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. [PubMed: 4684700]
36. Zavalza-Gomez AB. Obesity and oxidative stress: a direct link to preeclampsia? *Arch Gynecol Obstet.* 2011; 283:415–22. [PubMed: 21076925]
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. [PubMed: 8890098]
38. Oxenkrug GF. Metabolic syndrome, age-associated neuroendocrine disorders, and dysregulation of tryptophan-kynurenine metabolism. *Ann N Y Acad Sci.* 2010; 1199:1–14.10.1111/j.1749-6632.2009.05356.x [PubMed: 20633104]
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. [PubMed: 21693093]
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. [PubMed: 11821338]

Fig. 1.

The kynurenine pathway. Abbreviations: 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.

Fig. 2.

Associations between plasma kynurenic acid concentrations and preeclampsia. Odds ratios were estimated by using generalized additive logistic models. Unadjusted odds ratio with 95% confidence interval is shown as a solid black line with gray shaded area. Adjusted odds ratio is shown as a solid blue line, whereas adjusted odds ratio after exclusions of smokers, diabetic women, and women with chronic hypertension is shown as a solid red line (confidence intervals not shown). The odds ratio 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 confidence interval represent the 5th, 25th, 75th, and 95th percentile of plasma kynurenic acid.

Fig. 3.

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

Table 1

Prevalence of Preeclampsia According to Maternal Characteristics

Characteristics	Total Number of Women *	Women With Preeclampsia		<i>P</i> [†]
		n	% ± SE	
All women	2936	116	4.0 ± 0.36	
Maternal age (years)				.03
Younger than 25	373	23	6.2 ± 1.25	
25–29	1002	45	4.5 ± 0.65	
30–34	1115	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	1315	55	4.2 ± 0.55	
Married	1519	58	3.8 ± 0.49	
Maternal education (years)				.96
0–9	85	4	4.7 ± 2.30	
10–12	1125	43	3.8 ± 0.57	
13 or more	1652	66	4.0 ± 0.48	
Other	63	2	3.2 ± 2.21	
Parity				< .001
0	1271	76	6.0 ± 0.67	
1	1101	25	2.3 ± 0.45	
2 or more	564	15	2.7 ± 0.68	
Prepregnancy BMI (kg/m ²)				< .001
Less than 18.5	80	4	5.0 ± 2.44	
18.5–24.9	1843	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	2540	106	4.2 ± 0.40	
Yes	393	10	2.5 ± 0.79	
Chronic hypertension				< .001
No	2916	110	3.8 ± 0.35	
Yes	20	6	30.0 ± 10.3	
Prepregnancy diabetes				.14
No	2917	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 on marital status, 11 women on maternal education, 114 women on prepregnancy BMI, and three women on plasma cotinine.

[†] *P* value for difference in proportions was calculated by chi-square tests.

[‡]Plasma cotinine concentration 30 nmol/L.

Table 2

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*
	Mean ± SE	Mean ± SE	
Tryptophan (μmol/L)	59.0 ± 0.17	59.7 ± 0.88	.40
Kynurenine (μmol/L)	1.11 ± 0.004	1.13 ± 0.02	.44
Kynurenine/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 (μmol/L)	49.2 ± 0.12	49.3 ± 0.62	.83
Neopterin (nmol/L)	7.57 ± 0.04	7.49 ± 0.16	.71

SE, standard error.

* P value for difference in means was obtained by using a 2-sample t-test.

[†]The kynurenine/tryptophan ratio was multiplied by 1,000

Table 3

Association Between Plasma Kynurenic Acid and Preeclampsia

Women With Preeclampsia					
Kynurenic Acid Percentiles	Total Number of Women [*]	n	% ± SE	Odds Ratio [†] (95% CI)	Adjusted Odds Ratio [‡] (95% CI)
5th or lower	147	5	3.4 ± 1.50	1.0 (0.4 – 2.7)	1.3 (0.5 – 3.5)
5–25th	587	17	2.9 ± 0.69	0.9 (0.5 – 1.5)	1.0 (0.5 – 1.8)
25–75th [§]	1467	48	3.3 ± 0.46	1	1
75–95th	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)
<i>P</i> 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.