ORIGINAL ARTICLE

Cholesterol homeostasis is dysregulated in women with preeclampsia

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KEY WORDS

cholesterol precursors, cholesterol homeostasis, dyslipidemia, phytosterols, preeclampsia

ABSTRACT

INTRODUCTION The link between preeclampsia and dyslipidemia has been established. Even though lipid profile parameters have been intensively investigated in the pathology of preeclampsia, their accurate molecular mechanisms of action have not been fully decoded.

OBJECTIVES We aimed to identify the specifics of cholesterol metabolism in women affected by late-onset preeclampsia and single out potential biomarkers associated with late-onset syndrome.

PATIENTS AND METHODS A total of 90 pregnant women with a priori risk for preeclampsia were monitored at 4 time points during gestation and, based on the outcome of pregnancy, they were classified into the high-risk group (70 women) and the preeclampsia group (20 women). Cholesterol metabolic profiling was done using liquid chromatography-tandem mass spectrometry.

RESULTS The only significant change in the preeclampsia group was an increase in the lathosterol level ($P = 0.001$). The first-trimester lathosterol level was higher in the preeclampsia group compared with the high-risk group ($P = 0.02$). Further, in the preeclampsia group, positive correlations were found between desmosterol and β-sitosterol ($\rho = 0.474$; $P = 0.03$) in the third trimester, desmosterol and campesterol changes between the second and the first ($\rho = 0.546$; $P = 0.02$), and the third and first trimesters ($\rho = 0.754$; $P < 0.001$), as well as between the desmosterol and β-sitosterol differences between the third and first trimesters ($\rho = 0.568$; $P = 0.01$). No similar correlations were found in the high-risk group.

CONCLUSIONS Late-onset preeclampsia could be associated with an altered lipid profile. By studying the quantitative metabolic signatures of cholesterol, we might assume that both cholesterol synthesis and absorption are increased, that is, there is an imbalance in the cholesterol homeostasis regulation in women affected by the disease.

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INTRODUCTION Preeclampsia is new-onset hypertension diagnosed after the twentieth week of gestation with subsequent proteinuria or some sign of end-organ dysfunction.[1] Defective spiral artery remodeling, followed by the formation of pronounced vascular lesions, is the first stage in the onset of this syndrome.[2] Alterations in hemodynamics lead to fibrinoid necrosis of the placental blood vessels and accumulation of foam cells.[3] During the second stage of disease development, maternal systematic inflammatory response and generalized endothelial dysfunction occur in response to placental stress.[4]

While early-onset preeclampsia is supposed to be a fetal disorder associated with placental dysfunction, late-onset preeclampsia was recognized as a maternal disorder, predisposed by specific risk factors such as maternal obesity, diabetes mellitus, or chronic hypertension.[5,6] According to Redman et al,[7] in women with late-onset preeclampsia, restricted intervillous perfusion during placental maturation could lead to placental hypoxia. Placental hypoxia would be responsible for the occurrence of the second stage of the disease, including the maternal clinical signs, common for both early- and late-onset preeclampsia,

WHAT'S NEW?

For the first time, we assessed noncholesterol sterols longitudinally during high-risk pregnancy and preeclampsia. We found that the first-trimester lathosterol level was higher in the preeclampsia group, indicating cholesterol synthesis could be increased from the early beginning in these women, and it could be singled out as a potential biomarker of preeclampsia. Lack of significant change in cholesterol precursors throughout the pregnancy in women with preeclampsia may be related to lower levels of high-density lipoprotein cholesterol. However, the composition of noncholesterol sterols in high-density lipoprotein fraction should be analyzed to confirm the previous assumption. Finally, we found that cholesterol homeostasis, that is, the balance between cholesterol synthesis and absorption, is dysregulated in women with preeclampsia. Thus, cholesterol profiling might detect subtle changes in cholesterol metabolism in preeclampsia.

while maternal risk factors might amplify the vascular response to placental stimuli.[6,7] The clinical manifestations of this disease could be explained through synergistic effects of numerous distinct factors, rather than being a consequence of one specific mediator.[8]

High total cholesterol (TC) and triglyceride levels are included in the pathogenesis of pregnancy-induced hypertension[9] and preeclampsia.[9-11] Moreover, the prevalence of coexisting hypertension and hypercholesterolemia seems to be high even in the general population.[12,13] Increased circulating lipid levels could also be resulting in accelerated accumulation in already damaged endothelium in pregnant women with preeclampsia.

A large meta-analysis[14] reported that maternal serum levels of TC, triglycerides, non–high-density lipoprotein cholesterol (non-HDL-C), and HDL-C during pregnancy are related to the risk of preeclampsia. However, differences in low-density lipoprotein cholesterol (LDL-C) between women with and without preeclampsia were of marginal significance.[14] Also, some investigators hypothesized that an increase in HDL-C concentrations during the second trimester of pregnancy protects the maternal vascular endothelium and possibly has positive effects on the prevention of preeclampsia.[15] Nevertheless, it seems that traditional lipid profile parameters are not the most sensitive and specific preeclampsia biomarkers.[16] On the other hand, Miettinen et al[17] showed that noncholesterol sterols (NCSs) could be more sensitive markers of disbalance in cholesterol homeostasis and highlighted them as interesting markers for further evaluation.

Better understanding of cholesterol metabolism in pathologies of pregnancy complications could be of the utmost significance since cholesterol is associated with preeclampsia but without clear insight into its decisive role. We monitored cholesterol synthesis (cholesterol precursors) and absorption markers (plant sterols) longitudinally throughout the gestation in women with a high risk of preeclampsia. We compared cholesterol metabolic pathways between the women who did not and those who did develop preeclampsia, assuming that both the synthesis and absorption pathways would be altered in women who eventually develop the syndrome. We aimed to identify the specifics of cholesterol metabolism in women affected by late-onset preeclampsia and single out new potential biomarkers associated with late-onset preeclampsia.

PATIENTS AND METHODS

Study design In this longitudinal observational study, we followed 90 women with a high-risk singleton pregnancy from the beginning of gestation until term. We recruited women at their first antenatal check-up at the Gynecology and Obstetrics Clinic Narodni Front"(Belgrade, Serbia). The study was approved by the Ethics Committee of the previous clinic (approval number, 24/55–6) and all the participants provided informed written consent. Blood sampling as well as all the experimental procedures were conducted according to the relevant national regulations, institutional policies, and ethical guidelines defined by the Declaration of Helsinki.

Patients Due to the significant risk of developing complications throughout gestation discerned at the first appointment in the maternity care pathway, pregnant women were referred by their gynecologists at primary care units to the Gynecology and Obstetrics Clinic Narodni Front where screening for preeclampsia was carried out. The screening was performed by the end of the first trimester, between 11 weeks 0 days and 13 weeks 6 days of gestation, ensuring that information on which women are at increased risk is obtained exceedingly early during gravidity.

The flow rate through the uterine artery was evaluated by a pulse color Doppler. The mean arterial blood pressure was measured in a standardized way, the height and body weight were measured to assess body mass index (BMI) (calculated as weight in kilograms divided by height in meters), and specific anamnestic data were taken. Pregnant women were recruited based on insufficient flow within the uterine artery or the existing a priori risk of preeclampsia as evaluated by the guidelines of the National Institute for Health and Care Excellence.[18] Namely, all women had to have 1 high-risk or at least 2 moderate-risk factors. Chronic hypertension, hypertensive disease in a previous pregnancy, chronic kidney disease, diabetes mellitus, or presence of autoimmune disease were defined as high-risk factors, while moderate-risk factors included maternal age of more than 40 years, first pregnancy, pregnancy interval of more than 10 years, BMI of 30 kg/m^2 or more at the first visit, and a family history of preeclampsia. Exclusion criteria were: multifetal pregnancy, miscarriage, abortion, infectious disease or exacerbation of existing autoimmune disease at any point during pregnancy, and malignant disease before pregnancy. The primary outcome was preeclampsia. Additionally, we observed

development of the secondary outcomes: hypertensive disorders of pregnancy (pregnancy hypertension, superimposed preeclampsia), intrauterine growth restriction (IUGR), and gestational diabetes mellitus. IUGR was defined as a fetal growth rate that is lower than the expected fetal growth pattern, including infants born with clinical signs of malnutrition and in utero growth retardation regardless of birth weight percentiles.[19] Pregnancy hypertension, preeclampsia, and superimposed preeclampsia were defined according to the relevant guidelines.[1,20,21]

Sample collection and preparation All patients were monitored at 4 time points throughout pregnancy: first (11–14 weeks of gestation), second (22–25 weeks of gestation), third (28–32 weeks of gestation) trimester, and before the delivery. Venous blood samples were obtained after an overnight fast (≥12 h) and collected into serum sample tubes (Becton, Dickinson and Company, New Jersey, United States). Samples were centrifuged at 1500 g for 10 minutes to obtain serum, and aliquots were stored at –80 °C until analyzed.

Methods Fasting serum levels of glucose, urea, creatinine, total proteins, aspartate aminotransferase, and alanine aminotransferase were determined by commercial kits purchased from Beckman Coulter (Brea, California, United States) on an AU480 Chemistry Analyzer (Beckman Coulter). Serum TC, triglyceride, and HDL-C levels were measured by enzymatic methods (Beckman Coulter), while LDL-C levels were calculated according to the Friedewald equation.[22]

Serum cholesterol precursors (desmosterol, 7-dehydrocholesterol, and lathosterol) and plant sterols (campesterol, β-sitosterol) were quantified by liquid chromatography-tandem mass spectrometry, as previously reported.[23]

Statistical analysis Continuous variables were presented as mean (SD) for normally distributed data, or as geometric mean and 95% CI derived from log-normal values. We used a general linear model for repeated measures with Bonferroni CI adjustment to test the difference between the data in the function of time. The Mauchly test of sphericity was performed to check if the data passed the assumption of sphericity. If the presumption of sphericity was violated, the Greenhouse-Geisser correction was used to assess whether the data were statistically significantly different. The independent-samples *t* test was used to compare the geometric means between the 2 study groups: the high-risk group and the preeclampsia group. The Pearson χ^2 test was used for testing relationships between 2 categorical variables. We used the Spearman correlation analysis to analyze the correlation between parameters. The power of the study was calculated for investigated parameters of interest (lipid profile parameters and NCSs) for the repeated measures design using the web-based power and sample size program GLIMMPSE 3.0.0, and it was higher than 0.8.

All statistical tests were considered statistically significant at the 0.05 probability level. Statistical analyses were performed with the PASW Statistics 18 (IBM, Armonk, New York, United States).

RESULTS

Study population A total of 114 women with at least 1 high-risk or 2 moderate-risk factors for preeclampsia were selected for the study. However, 24 patients were lost to follow-up; 16 respondents dropped out of the survey, 4 due to miscarriage, and 4 due to fetal anomalies. Finally, 90 women were monitored throughout the pregnancy. By the end of gestation, 20 women (22.2%) had preeclampsia, 4 of which had associated IUGR, while 6 also had gestational diabetes accompanied by preeclampsia. Ten out of these 20 women finished their pregnancy with preeclampsia as the sole complication. Thirteen women developed gestational hypertension without signs of end-organ dysfunction, 2 of which also developed gestational diabetes, while a single patient had IUGR and gestational diabetes following pregnancy hypertension. A total of 12 pregnant women developed IUGR, 5 had IUGR as the only pregnancy complication while 4 had IUGR accompanied by preeclampsia, 2 had gestational diabetes associated with IUGR, and a single patient had pregnancy hypertension and gestational diabetes. Four women developed gestational diabetes as the only pregnancy complication. Six patients had gestational diabetes accompanied by preeclampsia, 2 by pregnancy hypertension, 2 by IUGR, and a single one by IUGR and pregnancy hypertension. Other women (47 patients) delivered without complications despite being at risk. All women were diagnosed with late-onset preeclampsia, with 16 giving birth between the 34 and 37 week of gestation, while 4 of them delivered after week 37.

We additionally classified our respondents based on the prepregnancy BMI and clinical history of prepregnancy diabetes. The first group included pregnant women who had prepregnancy BMI of less than 25 kg/m^2, and the second, patients who were overweight or obese, or had type 1 or 2 diabetes before pregnancy. Twenty pregnant women were overweight before pregnancy, and 15 were obese. A single woman had type 1 diabetes, and 3 had type 2 diabetes before pregnancy, while 2 of them were normally nourished, and 2 were overweight. Thus, a total of 37 women were listed in the first, and 53 in the second group.

Clinical and laboratory characteristics of the study population Basic clinical and laboratory characteristics of both study groups during the follow-up are presented in TABLE 1. Prepregnancy BMI was significantly higher in the preeclampsia group (mean, 26.2; 95% CI, 24.0–28.6) compared with the high-risk group (mean, 23.7; 95% CI, 22.7–24.7; $P = 0.02$). Neonatal birth weights did not differ between the newborns of women

at high-risk and those with preeclampsia (mean [SD], 3313 [457.6] g vs 3178 [516.6] g; $P = 0.29$). There was no difference in the smoking status between the 2 study groups ($P = 0.28$) (data not shown). Changes in lipid profile parameters in 2 study groups throughout the pregnancy are presented in TABLE 2.

Cholesterol metabolic profiling throughout the pregnancy

The increase in the TC levels (TABLE 2) was mostly accompanied by an increase in the concentrations of desmosterol, 7-dehydrocholesterol, and lathosterol in the high-risk group ($P < 0.001$, $P = 0.003$, and $P < 0.001$, respectively) (TABLE 3). On the other hand, despite the increase in TC (TABLE 2), we observed no changes in desmosterol nor in 7-dehydrocholesterol levels in the preeclampsia group ($P = 0.43$ and $P = 0.37$, respectively) (TABLE 3). However, an increase in the lathosterol concentration was noticed from the third trimester compared with the first-trimester concentrations in this study group ($P = 0.001$) (TABLE 3). Moreover, the lathosterol levels in the first trimester were higher in the preeclampsia group compared with the high-risk group ($P = 0.02$). Groups did not differ in any other cholesterol precursor level throughout the pregnancy (P values not shown).

Pregnant women not affected by preeclampsia had significantly lower ratios of cholesterol / desmosterol before delivery compared with those in the first, second, and third trimester ($P < 0.001$ for all), and cholesterol / 7-dehydrocholesterol ratio before delivery compared with the third trimester ($P = 0.04$) (TABLE 4). 7-Dehydrocholesterol / lathosterol ratio was lower in the second ($P = 0.01$) and third trimester ($P = 0.003$), and before delivery compared with the ratio in the first trimester ($P = 0.003$) in the same study group. Similar changes were not observed in the preeclampsia group (TABLE 4). Likewise, the 2 study groups did not differ in any of the ratios throughout the pregnancy (P values not shown).

Changes in plant sterols were not as pronounced. We noticed a decrease in β-sitosterol concentrations before delivery in the high-risk group ($P = 0.02$). Significantly lower concentrations of plant sterols were noticed for β-sitosterol in the first and second, and campesterol in the second trimester in the preeclampsia compared with the high-risk group ($P = 0.01$, $P = 0.04$, and $P = 0.03$, respectively) (TABLE 3).

Correlations We observed no correlations between the tested cholesterol precursors and plant sterols in the high-risk group (data not shown). On the other hand, in the preeclampsia group, in the third trimester, desmosterol concentrations correlated positively with β-sitosterol concentrations ($\rho = 0.474$; $P = 0.03$). Moreover, when we observed differences between individual sterol concentrations at later test points relative to the first-trimester concentrations, we found a positive correlation between the change in desmosterol levels between the second and first trimesters and the difference in campesterol concentrations over the same period in the preeclampsia group ($\rho = 0.546$; $P = 0.02$). Likewise, in the same test group, the difference in desmosterol levels between the third and first trimesters positively correlated with the difference in campesterol ($\rho = 0.754$; $P < 0.001$) and β-sitosterol concentrations ($\rho = 0.568$; $P = 0.01$) for the same period. Interestingly, no similar significant correlations were found in the high-risk group (data not shown).

Although individual sterol concentrations did not correlate with neonatal birth weight in both study groups (data not shown), the birth weight of the newborns showed a significant correlation with differences in NCS concentrations between different test points. The change in desmosterol concentrations between the second and first trimesters correlated negatively with the birth weight ($\rho = -0.282$; $P = 0.03$) in the high-risk group. On the other hand, in the preeclampsia group, the difference in lathosterol concentrations between the third and first trimester positively correlated with the newborns' birth weight ($\rho = 0.489$; $P = 0.04$).

Normal weight vs overweight and diabetic patients

Two newly formed groups differed in prepregnancy BMI ($P < 0.001$), as well as in the frequency of women who developed preeclampsia ($P = 0.02$). Although, we observed significant alterations in cholesterol synthesis (desmosterol and 7-dehydrocholesterol), as well as in cholesterol absorption markers (campesterol and β-sitosterol) in the group with BMI <25 kg/m^2 when the changes in the concentrations of NCSs between test points in overweight patients were compared, statistically significant difference was seen only for cholesterol synthesis markers (desmosterol and lathosterol). When we compared the NCSs between these 2 groups, we observed significantly higher lathosterol concentrations in the second group (first trimester, mean, 8.48 vs 14.3 μmol/l; $P = 0.002$; third trimester, mean, 20.1 vs 26.0 μmol/l; $P = 0.03$; and before delivery, mean, 21.0 vs 26.9 μmol/l; $P = 0.05$). On the other hand, campesterol levels were lower in obese and overweight pregnant women (first trimester, mean, 2.75 vs 1.95 μmol/l; $P = 0.003$; and second trimester, mean, 2.77 vs 1.93 μmol/l; $P = 0.002$), and β-sitosterol (first trimester, mean, 6.14 vs 4.72 μmol/l; $P = 0.002$; and second trimester, mean, 6.35 vs 4.41 μmol/l; $P = 0.002$).

DISCUSSION The present study is the first one to monitor changes in the concentration of NCSs longitudinally during the high-risk pregnancy and pregnancy affected by preeclampsia. We proposed that the atherogenic lipid profile and impaired cholesterol metabolism could be contributing factors in preeclampsia. By analyzing cholesterol precursors and plant sterols, we gained a better insight into the changes in the cholesterol homeostasis during pregnancy with preeclampsia.

TABLE 1 Changes in basic clinical and biochemical parameters in study groups

Parameter	First trimester		Second trimester		Third trimester		Before delivery		P_1 value[a]	P_2 value[b]
	High-risk group	Preeclampsia group	High-risk group	Preeclampsia group	High-risk group	Preeclampsia group	High-risk group	Preeclampsia group		
WG, mean (SD)	12.8 (0.8)	12.7 (0.7)	23.3 (0.8)	23.4 (1.0)	29.6 (1.3)	29.8 (1.4)	36.9 (0.9)	36.6 (1.0)	–	–
BMI, kg/m^2	24.5 (23.4–25.5)	27.4 (25.5–29.5)[h]	26.3 (25.3–27.4)[cf]	29.1 (27.3–31.0)[cf,h]	27.6 (26.6–28.7)[cf,df]	30.4 (28.7–32.3)[cf,df,h]	28.9 (27.9–30.0)[cf,df,ef]	31.8 (30.0–33.7)[cf,df,ef,h]	<0.001	<0.001
Weight gain, kg	2.2 (1.6–2.9)	2.6 (1.2–4.0)	5.5 (5.0–6.0)[cf]	5.0 (3.4–6.5)	3.4 (2.9–3.9)[cg,df]	3.4 (2.3–4.5)	3.6 (3.0–4.2)[cg,df]	4.0 (2.7–5.4)	<0.001	0.03
Weight gain, %	3.30 (2.35–4.26)	3.92 (2.28–5.58)	7.40 (6.66–8.14)[cf]	6.39 (4.30–8.52)	4.45 (3.85–5.05)[df]	4.21 (2.92–5.54)	4.57 (3.92–5.24)[df]	4.50 (2.87–6.15)	<0.001	0.07
Glucose, mmol/l	4.7 (4.6–4.9)	4.9 (4.5–5.3)	4.5 (4.4–4.7)	5.0 (4.6–5.5)	4.8 (4.5–5.0)	5.0 (4.7–5.4)	4.4 (4.3–4.6)[eg]	4.6 (4.3–5.0)	0.02	0.14
Urea, mmol/l	2.73 (2.57–2.90)	2.90 (2.65–3.17)	2.65 (2.50–2.80)	2.46 (2.16–2.81)	2.50 (2.34–2.67)[cg]	2.71 (2.38–3.08)	2.55 (2.40–2.72)	2.93 (2.47–3.46)[dg]	0.07	0.02
Creatinine, µmol/l	55.8 (53.9–57.8)	58.1 (54.7–61.7)	54.7 (53.0–56.5)	55.3 (51.6–59.3)	54.5 (53.0–56.1)	55.9 (52.5–59.5)	57.9 (56.0–59.8)[dg,eg]	58.3 (54.5–62.4)	0.003	0.37
Total protein, g/l	66.6 (65.6–67.7)	66.8 (65.1–68.7)	63.2 (62.2–64.2)[cf]	62.9 (60.9–65.0)[cg]	62.5 (61.6–63.4)[cf]	61.9 (60.0–63.8)[cf]	61.7 (60.8–62.6)[cf,dg]	60.6 (59.0–62.2)[cf]	<0.001	<0.001
AST, U/l	16.6 (15.7–17.5)	17.3 (15.4–19.5)	17.3 (15.8–18.8)	16.9 (14.6–19.6)	17.0 (15.8–18.3)	17.2 (15.5–19.0)	17.8 (16.8–18.9)	17.7 (15.8–19.9)	0.36	0.90
ALT, U/l	14.5 (13.1–16.1)	17.3 (14.4–20.9)	15.7 (13.7–18.1)	16.2 (13.1–20.1)	14.7 (13.1–16.5)	16.2 (13.6–19.2)	13.4 (12.1–14.8)	14.2 (12.3–16.4)	0.12	0.26

Data are shown as geometric mean (95% CI) unless indicated otherwise.

a ANOVA repeated measures for high-risk group

b ANOVA repeated measures for preeclampsia group

c Pairwise comparison: mean difference significantly different from the first trimester

d Pairwise comparison: mean difference significantly different from the second trimester

e Pairwise comparison: mean difference significantly different from the third trimester

f P <0.001 (Bonferroni corrected)

g P <0.05 (Bonferroni corrected)

h Significantly different from the high-risk group, P <0.05

Abbreviations: ANOVA, analysis of variance; AST, aspartate aminotransferase; ALT, alanine aminotransferase; BMI, body mass index; WG, week of gestation

TABLE 2 Changes in lipid profile parameters in high-risk and preeclampsia group

Parameter	First trimester		Second trimester		Third trimester		Before delivery		P_1 value[a]	P_2 value[b]
	High-risk group	Preeclampsia group	High-risk group	Preeclampsia group	High-risk group	Preeclampsia group	High-risk group	Preeclampsia group		
WG, mean (SD)	12.8 (0.8)	12.7 (0.7)	23.3 (0.8)	23.4 (1.0)	29.6 (1.3)	29.8 (1.4)	36.9 (0.9)	36.6 (1.0)	–	–
TC, mmol/l	5.22 (4.97–5.48)	5.41 (5.07–5.77)	6.68 (6.35–7.02)[cf]	6.49 (5.93–7.11)[cf]	7.17 (6.82–7.54)[cf,df]	6.84 (6.22–7.53)[cf]	7.31 (6.94–7.71)[cf,df]	7.01 (6.32–7.79)[cf,dg]	<0.001	<0.001
HDL-C, mmol/l	1.73 (1.65–1.82)	1.84 (1.63–2.07)	2.08 (1.99–2.18)[cf]	1.84 (1.67–2.04)[h]	1.97 (1.87–2.08)[cf]	1.95 (1.71–2.21)	1.90 (1.78–2.03)[cg]	1.89 (1.74–2.05)	<0.001	0.83
LDL-C, mmol/l	2.82 (2.63–3.02)	2.74 (2.48–3.04)	3.65 (3.39–3.93)[cf]	3.46 (2.99–3.99)[cg]	4.03 (3.75–4.34)[cf,df]	3.38 (2.83–4.04)[cf,h]	3.96 (3.67–4.28)[cf]	3.11 (2.37–4.08)	<0.001	0.007
TG, mmol/l	1.27 (1.18–1.38)	1.53 (1.27–1.85)[h]	1.85 (1.72–1.99)[cf]	2.33 (2.00–2.71)[cf,h]	2.33 (2.16–2.51)[cf,df]	2.86 (2.52–3.25)[cf,dg,h]	2.93 (2.72–3.16)[cf,df,ef]	3.52 (3.07–4.04)[cf,df,eg,h]	<0.001	<0.001

Data are shown as geometric mean (95% CI) unless indicated otherwise.

a ANOVA repeated measures for high-risk group

b ANOVA repeated measures for preeclampsia group

c Pairwise comparison: mean difference significantly different from the first trimester

d Pairwise comparison: mean difference significantly different from the second trimester

e Pairwise comparison: mean difference significantly different from the third trimester

f P <0.001 (Bonferroni corrected)

g P <0.05 (Bonferroni corrected)

h Significantly different from the high-risk group, P <0.05

Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides; others, see **TABLE 1**

TABLE 3 Changes in noncholesterol sterols' concentrations in high-risk and preeclampsia group

Parameter	First trimester		Second trimester		Third trimester		Before delivery		P_1 value[a]	P_2 value[b]
	High-risk group	Preeclampsia group	High-risk group	Preeclampsia group	High-risk group	Preeclampsia group	High-risk group	Preeclampsia group		
WG, mean (SD)	12.8 (0.8)	12.7 (0.7)	23.3 (0.8)	23.4 (1.0)	29.6 (1.3)	29.8 (1.4)	36.9 (0.9)	36.6 (1.0)	–	–
Desmosterol, μmol/l	1.56 (1.43–1.69)	1.92 (1.42–2.60)	2.15 (1.90–2.43)[cg]	2.07 (1.39–3.08)	2.56 (2.22–2.96)[cg]	2.64 (1.77–3.93)	3.17 (2.78–3.61)[cf,df]	2.68 (2.00–3.60)	<0.001	0.43
7-DHC, μmol/l	1.47 (1.31–1.65)	1.64 (1.27–2.12)	1.93 (1.62–2.29)	1.75 (1.19–2.57)	2.05 (1.72–2.45)[cg]	1.98 (1.40–2.81)	2.26 (1.93–2.64)[cg]	2.09 (1.48–2.95)	0.003	0.37
Lathosterol, μmol/l	9.49 (7.87–11.46)	15.0 (10.8–20.8)[h]	17.9 (15.6–20.5)[cf]	20.2 (15.9–25.7)	21.9 (19.0–25.2)[cf,dg]	24.3 (19.1–30.7)[cg]	22.9 (19.8–26.5)[cf,df]	24.7 (19.8–30.9)[cg]	<0.001	0.001
Campesterol, μmol/l	2.47 (2.19–2.78)	2.08 (1.46–2.98)	2.56 (2.25–2.90)	1.87 (1.40–2.49)[h]	2.54 (2.21–2.93)	2.21 (1.71–2.87)	2.12 (1.81–2.48)[eg]	1.87 (1.44–2.43)	0.09	0.36
β-Sitosterol, μmol/l	5.83 (5.37–6.32)	4.53 (3.50–5.86)[h]	5.84 (5.09–6.70)	4.34 (3.47–5.41)[h]	5.34 (4.82–5.91)	4.69 (3.90–5.65)	4.97 (4.50–5.48)[cg,dg]	4.04 (3.22–5.08)	0.01	0.42

Data are shown as geometric mean (95% CI) unless indicated otherwise.

a ANOVA repeated measures for high-risk group

b ANOVA repeated measures for preeclampsia group

c Pairwise comparison: mean difference significantly different from the first trimester

d Pairwise comparison: mean difference significantly different from the second trimester

e Pairwise comparison: mean difference significantly different from the third trimester

f P <0.001 (Bonferroni corrected)

g P <0.05 (Bonferroni corrected)

h Significantly different from the high-risk group, P <0.05

Abbreviations: 7-DHC, 7-dehydrocholesterol; others, see **TABLE 1**

TABLE 4 Metabolic indices in high-risk and preeclampsia group

Parameter	First trimester		Second trimester		Third trimester		Before delivery		P_1 value[a]	P_2 value[b]
	High-risk group	Preeclampsia group	High-risk group	Preeclampsia group	High-risk group	Preeclampsia group	High-risk group	Preeclampsia group		
WG, mean (SD)	12.8 (0.8)	12.7 (0.7)	23.3 (0.8)	23.4 (1.0)	29.6 (1.3)	29.8 (1.4)	36.9 (0.9)	36.6 (1.0)	–	–
TC/desmosterol, mmol/μmol	3.35 (3.09–3.63)	2.82 (2.09–3.81)	3.11 (2.75–3.51)	3.14 (2.07–4.76)	2.80 (2.42–3.23)	2.59 (1.70–3.95)	2.31 (2.02–2.54)[cf,df,ef]	2.61 (1.90–3.59)	<0.001	0.25
TC/7-DHC, mmol/μmol	3.55 (3.14–4.03)	3.30 (2.56–4.26)	3.46 (2.90–4.14)	3.72 (2.50–5.52)	3.50 (2.91–4.20)	3.45 (2.42–4.92)	3.24 (2.76–3.80)[eg]	3.35 (2.32–4.85)	0.21	0.12
7-DHC/lathosterol	0.15 (0.13–0.19)	0.11 (0.08–0.15)	0.11 (0.09–0.13)[cg]	0.09 (0.06–0.12)	0.09 (0.08–0.11)[cg]	0.08 (0.06–0.10)	0.10 (0.09–0.11)[cg]	0.08 (0.07–0.11)	<0.001	0.22
HDL-C/desmosterol, mmol/μmol	1.11 (1.02–1.22)	0.96 (0.69–1.32)	0.97 (0.85–1.09)	0.89 (0.59–1.35)	0.77 (0.67–0.89)[cf,df,ef]	0.74 (0.47–1.14)	0.59 (0.52–0.67)[cf,df,ef]	0.69 (0.50–0.96)	<0.001	0.08
HDL-C/7-DHC, mmol/μmol	1.18 (1.05–1.33)	1.12 (0.86–1.46)	1.08 (0.90–1.29)	1.06 (0.70–1.59)	0.96 (0.79–0.87)	0.98 (0.68–1.42)	0.82 (0.70–0.96)[cf,dg,ef]	0.87 (0.59–1.29)	<0.001	0.06

Data are shown as geometric mean (95% CI) unless indicated otherwise.

a ANOVA repeated measures for high-risk group

b ANOVA repeated measures for preeclampsia group

c Pairwise comparison: mean difference significantly different from the first trimester

d Pairwise comparison: mean difference significantly different from the second trimester

e Pairwise comparison: mean difference significantly different from the third trimester

f P <0.001 (Bonferroni corrected)

g P <0.05 (Bonferroni corrected)

h Significantly different from the high-risk group, P <0.05

Abbreviations: see **TABLES 1**, **2**, and **3**

According to our findings, women who developed preeclampsia by the end of their pregnancies had higher BMI even before pregnancy. Significant differences in BMI persisted throughout the observation period (TABLE 1). It is worth noting that overweight and obesity could be the key risk factors for preeclampsia and that BMI over 30 kg/m^2 is coupled to an almost tripled risk for preeclampsia.[24,25]

Moreover, higher body weight in patients with preeclampsia was accompanied by a more atherogenic lipid profile (TABLE 2). Women with preeclampsia in this study had a significantly higher triglyceride concentration throughout the whole observational period compared with the high-risk group. Alterations in cholesterol levels were not as apparent (TABLE 2). Although there was an increase in the concentrations of TC and LDL-C both during high-risk pregnancy and preeclampsia (TABLE 2), the differences in these concentrations between the high-risk and preeclampsia group were either slight or not of statistical and clinical significance. When we look at cholesterol in the context of pregnancy, the changes in HDL-C are the most thought-provoking. Though an increase in HDL-C levels is commonly observed in healthy pregnant women,[15] in preeclampsia, an expected increase in HDL-C during the first part of pregnancy does not usually happen.[26] Similarly, women with preeclampsia in our study lacked a protective increase in HDL-C levels in the second trimester (TABLE 2). Additionally, second trimester HDL-C levels were lower in the preeclampsia group juxtaposed to the high-risk group. While the progressive elevation of lipid concentrations in uncomplicated pregnancies is considered physiological and nonatherogenic, changes in the lipid profile in women with preeclampsia are rather referred to as dyslipidemia and potentially atherogenic.[15,27]

The insight into the concentrations of cholesterol synthesis and absorption markers, as well as their interrelationships, would be a step further in understanding the imbalance in cholesterol regulation. In the high-risk group, in parallel with the increase in the concentration of TC, LDL-C, and HDL-C (TABLE 2), an increase in cholesterol precursors, without significant change in plant sterols was spotted (TABLE 3). Elevated cholesterol levels are probably the result of the increased synthesis of this physiologically irreplaceable biomolecule. Interestingly, in the preeclampsia group, despite the increase in TC and LDL-C (TABLE 2), there was no parallel increase in the desmosterol and 7-dehydrocholesterol concentrations (TABLE 3). We could hypothesize that the lack of change in cholesterol precursors might be associated with lower HDL particle synthesis and lower HDL-C blood levels in women with preeclampsia. Additional studies examining HDL particle production and cholesterol incorporation, which are beyond the scope of this study, are necessary to confirm the previous hypothesis. We should not neglect the increase in lathosterol concentrations in the same study group. However, this increase became significant only approaching the end of pregnancy (TABLE 3), when we would expect cholesterol levels to stop rising, as the fetus in the second part of pregnancy relies primarily on its cholesterol production.[28] Furthermore, lathosterol concentrations in the first trimester were higher in preeclampsia juxtaposed to the high-risk group. The assumption is that the cholesterol synthesis in preeclampsia could be higher compared with women with risk for preeclampsia already from the beginning of pregnancy, which seems to be visible only by comprehensive analysis of cholesterol precursors.

Cholesterol / desmosterol and cholesterol / 7-dehydrocholesterol ratios were assessed as surrogate markers of cholesterol synthesis, representing 24- and 7-reductase enzyme activity, while 7-dehydrocholesterol / lathosterol ratio was an indicator of sterol-C5-desaturase-like enzyme activity.[16] Hence, lower activities of 24- and 7-reductase were seen before delivery compared with the first 3 test points in the high-risk group. The activity of the sterol-C5-desaturase-like enzyme appeared to decline already from the second trimester in the same group. Vice versa, in women affected by preeclampsia, there was no change in the enzyme activities (TABLE 4), implying that a high degree of cholesterol synthesis persisted even until childbirth. Likewise, Lee et al[16] found that the patients with preeclampsia had higher ratios of cholesterol / desmosterol and cholesterol / 7-dehydrocholesterol in maternal serum than women without preeclampsia. We observed no difference in these ratios between women from the high-risk and preeclampsia group. Lack of significant difference between our study groups could be ascribed to the fact that all women included in the study were at substantial risk of preeclampsia, that is, they had some underlying risk factors, such as high BMI or hypertension, at the time of inclusion.

It is expected in healthy individuals that increased synthesis will be accompanied by decreased absorption and vice versa to maintain the equilibrium. However, altered patterns of cholesterol synthesis and absorption are commonly spotted in different metabolic conditions.[29] Therefore, it should come as no surprise that in the preeclampsia group in the third trimester, concentrations of desmosterol correlated positively with β-sitosterol concentrations, suggesting that cholesterol homeostasis was partially lost in the preeclampsia group at this point. Furthermore, a greater change in the concentration of desmosterol, cholesterol precursor, between the first and second trimester in these women was accompanied by the greater change in campesterol, a plant sterol, over the same period. Likewise, an observed increase in desmosterol between the third and first trimester in the preeclampsia group was followed by an increase in the plant sterols in the same period. Our findings suggest that in women affected by preeclampsia, increased

cholesterol synthesis was not accompanied by decreased absorption. The same changes were not observed in the second study group, which further confirms our suspicions that women with preeclampsia experience a substantial dysregulation of cholesterol homeostasis.

Previous studies have shown variable associations between maternal serum NCSs and neonatal weight.[30,31] While there is no consensus on this issue, to the best of our knowledge, there are no data about the same correlation in offspring of mothers affected by preeclampsia. Though it seems that individual NCS levels in maternal blood do not affect neonatal weight, the change in cholesterol precursors among the trimesters could have a conceivable impact. According to our results, the difference in lathosterol concentrations between the third and first trimester was found to positively correlate with the newborns' birth weight in women affected by preeclampsia. On the other hand, the impact might be different in individuals unaffected by preeclampsia, as we found that the greater change in desmosterol levels between the second and first trimester correlated with the lower birth weight in the high-risk group. The significance of these results remains to be clarified, especially because of the fact that we did not notice significant changes in neonatal birth weight between investigated groups. Either way, one thing is certain—the fetus depends on the maternal cholesterol metabolism up to 19 weeks of pregnancy, when fetal endogenous cholesterol synthesis takes the lead.[28]

The results of our study indicated no significant differences in TC and LDL-C between the study groups (TABLE 2). On the other hand, by analyzing the NCS blood levels, it became clear that cholesterol synthesis is increased, as well as that the process of regulation of cholesterol homeostasis is disturbed in the patients with preeclampsia. These partially inconsistent results might be explained in 2 ways. First, we included only women with elevated risk for preeclampsia in the study, whereby those who finished their pregnancies without preeclampsia were controls for the preeclampsia group. The absence of a control group consisting of women without risk factors could bias the results, as the differences were assessed in a selected high-risk population. Certain risk factors, based on which women with high-risk pregnancies are selected from the general population of pregnant women, are already implemented in clinical practice. However, it remains unclear what are the decisive factors that determine which woman at high risk will and which will not develop preeclampsia. Hence, one of the challenges that still await the scientific answer is how to distinguish between these 2 groups. Besides, the absence of significant differences could be attributed to the relatively small number of respondents, which is another limitation of this study. It is possible that metabolic profiling of cholesterol provides insight into subtle changes in cholesterol homeostasis, which are not visible only by observing the blood levels of traditional lipid profile parameters.[16] Only by comprehensive analysis of longitudinal changes in lipid and NCS levels in maternal blood one might get a clear and complete picture of definite changes in cholesterol metabolism in such a complex disease as preeclampsia.

To conclude, we suggest that late-onset preeclampsia is associated with a significantly altered metabolic and lipid profile, whose fundamental characteristic is its proatherogenic nature. By studying the quantitative metabolic signatures of cholesterol, we might assume that both cholesterol synthesis and absorption are increased, that is, there is an imbalance in the regulation of cholesterol homeostasis in women affected by the disease. We could single out a cholesterol precursor, such as lathosterol, as a potential marker of preeclampsia, emphasizing that its specificity and sensitivity, as well as clinical significance, need to be clarified in further, broader prospective surveys.

ARTICLE INFORMATION

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CONTRIBUTION STATEMENT TA drafted the manuscript. AS, ZM, and DA conceived the concept and designed the study; GB, DA, PC, and NKO were responsible for data acquisition; TA, SV, and GB performed the laboratory analysis. TA and AS were responsible for statistical analysis and interpretation of data; ZM, VSK, AS, and AZ critically revised the manuscript for important intellectual content. AS was responsible for study supervision. All authors have approved the final version of article.

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