



Altered high-density lipoprotein particle structure and antioxidant capacity in preeclampsia

Izmenjena struktura i antioksidativni kapacitet lipoproteinskih čestica visoke gustine u preeklampsiji

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Abstract

Background/Aim. One of the complications that can occur during pregnancy is the development of preeclampsia (PE). The main characteristics of this condition are high blood pressure and very often signs of kidney damage or other organ damage. The condition affects 5–7% of all pregnant women and is one of the main factors of maternal and perinatal morbidity and mortality worldwide. The aim of this study was to investigate the structural and functional modifications of high-density lipoprotein (HDL) particles during high-risk pregnancies (HRP) for PE development. **Methods.** The longitudinal prospective study included a total of 91 pregnant women with a HRP for developing PE. Out of this total number, 71 women did not develop PE until delivery, and this group was designated as the group without PE (WPE). The rest of the 20 HRP women developed PE before delivery and were designated as the PE group. The blood was sampled toward the end of each trimester and before the delivery. The distribution of HDL particles was determined by the vertical 3–31% polyacrylamide gradient gel electrophoresis method. The antioxidative

capacity of HDL particles was measured by the activity of the HDL-associated enzyme – paraoxonase 1 (PON1). PON1 activity was determined by the method of kinetic spectrophotometry from serum samples. **Results.** The results have shown that the proportions of HDL_{2b} particles significantly increased in the 2nd trimester ($p < 0.05$) and remained increased until the end of pregnancy in the WPE group. PON1 activity was significantly higher in the 3rd trimester ($p < 0.05$) of the WPE group. In the PE group, we found that the proportions of HDL_{3a} particles significantly decreased in the 2nd trimester ($p < 0.05$) and remained decreased until the end of pregnancy. PON1 activity has not changed in the PE group during pregnancy. **Conclusion.** Dyslipidemia in pregnancy could be associated with different modifications of HDL particles. The adaptive pregnancy mechanisms expressed as a functional modification of HDL particles in pregnant women who develop PE seem inadequate and, therefore, lose their atheroprotective role.

Key words: antioxidants; lipoproteins, hdl; preeclampsia; pregnancy, complications.

Apstrakt

Uvod/Cilj. Jedna od komplikacija koja se može javiti tokom trudnoće je razvoj preeklampsije (PE). Glavne karakteristike ovog stanja su visok krvni pritisak i vrlo često znači oštećenja bubrega ili drugih organa. Ovo stanje pogađa 5–7% svih trudnica i jedan je od glavnih faktora morbiditeta i mortaliteta trudnica i fetusa ili novorođenčadi. Cilj ovog rada bio je ispitivanje strukturnih i funkcionalnih modifikacija lipoproteinskih čestica visoke gustine (*high-density lipoprotein* – HDL) u trudnoćama sa visokim rizikom (TVR) za razvoj PE. **Metode.** U longitudinalnoj prospektivnoj studiji učestvovala je ukupno 91 trudnica sa TVR od razvoja PE. Od ukupnog broja trudnica, kod njih

71, PE se nije razvila do kraja trudnoće i ova grupa je označena kao grupa bez PE (BPE). Kod preostalih 20 žena sa TVR se razvila PE do porođaja, i one su svrstane u grupu PE. Krv je uzimana za analizu na kraju svakog trimestra i pred porođaj. Raspodela HDL čestica je određivana metodom vertikalne elektroforeze u 3–31% gradijentu poliakrilamidnog gela. Antioksidativni kapacitet HDL čestica je određivan na osnovu aktivnosti enzima paraoksonaze 1 (PON1) vezanog za HDL. Aktivnost PON1 u serumu je određivana metodom kinetičke spektrofotometrije. **Rezultati.** Rezultati su pokazali da se udeo HDL_{2b} čestica značajno povećao u drugom trimestru ($p < 0,05$) i ostao je povećan do kraja trudnoće u grupi BPE. Aktivnost PON1 bila je značajno veća u trećem

trimestru ($p < 0,05$) kod ove grupe trudnica. Udeo HDL_{3a} čestica se značajno smanjio u drugom trimestru u PE grupi trudnica ($p < 0,05$) i ostao je snižen do kraja trudnoće. Aktivnost PON1 enzima u PE gripi se nije menjala u toku trudnoće. **Zaključak.** Dislipidemija u trudnoći može biti posledica različitih modifikacija HDL čestica. Strukturne i funkcionalne modifikacije HDL čestica, kao jedan od

adaptivnih mehanizama, kod trudnica kod kojih se razvila PE, nisu adekvatne i kao takve gube svoju ateroprotektivnu ulogu.

Ključne reči:
antioksidansi; lipoproteini hdl; preeklampsija; trudnoća, komplikacije.

Introduction

Preeclampsia (PE) is defined as a hypertensive disorder of pregnancy accompanied by proteinuria or some other organ damage, and it is recognized as one of the most severe pregnancy complications¹. With an estimated incidence of 5%, PE is a leading cause of maternal and perinatal morbidity and mortality worldwide². The main pathophysiological feature of PE is the insufficient remodeling of the spiral artery, leading to placental ischemia and creating an imbalance between anti-angiogenic and pro-angiogenic factors, finally inducing maternal endothelial dysfunction³. Despite a lot of research in this field, there are still major doubts regarding the prediction, monitoring, and therapy of this pregnancy complication. PE shares many risk factors with cardiovascular (CV) disease (CVD), such as obesity and hypertension. Thus, the role of the altered lipid profile in the pathogenesis of endothelial dysfunction in PE has become an important area of research during the last several decades⁴. Normal pregnancy is followed by metabolic changes, especially increased levels of plasma lipids, oxidative stress, and inflammation, as well as impaired coagulation processes. However, in PE, all those changes are much more pronounced^{5,6}. The most common lipid abnormalities in PE are hypertriglyceridemia with increased circulating free fatty acids, lower high-density lipoprotein (HDL) cholesterol (HDL-C) concentration, and a higher proportion of small dense (sd) low-density lipoprotein (LDL) particles (sdLDL)⁴.

It has been recognized that PE and CVD share common risk factors, diabetes mellitus (DM), obesity, hypertension, and renal disease. In addition, PE could be associated with an increased risk of CVD development later in life^{4,7}. On the other hand, the role of HDL particle distribution, structure, and function in the CVD risk assessment has been studied extensively during the last few years. Surprisingly, data about this issue in PE is quite limited. HDL particles show diversity in their structure, composition, and functionality, including their different roles in lipid transport, antioxidative capacity, inflammation, and hemostasis⁸. The newest concept in understanding this lipoprotein indicates that HDL composition and functionality, much more than HDL-C concentrations, qualify the multipotent antiatherogenic role of this lipoprotein⁹⁻¹¹. Kontush¹¹ highlighted the importance of dominant HDL diameter and HDL particle distribution profile determination for the improvement of CV risk assessment. It is generally accepted that reduced mean HDL size is associated with an increased risk of CVD development. Namely, it has been shown that elevated cholesterol ester transfer protein (CETP) activity in hypertriglyceridemia in-

duces triglycerides (TG) enrichment of HDL particles, which upon subsequent hydrolysis become smaller and less effective in CV protection^{11,12}. In line with previous research, a higher proportion of small HDL₃ particles in pathological conditions associated with high CV risk is a consequence of structural and functional modifications of HDL₂ particles rather than *de novo* synthesis of small, protein-rich HDL₃ particles¹¹.

The antiatherogenic role of HDL particles is well known, and it is based on several protective mechanisms. The main atheroprotective mechanism of HDL particles is probably reverse cholesterol transport via apolipoprotein A1 (apoA1). However, HDL particles also have antioxidative effects [by the action of HDL-associated antioxidative enzymes paraoxonase 1 (PON1) and platelet-activating factor acetylhydrolase (PAFAH)], and anti-inflammatory and antithrombotic effects⁹. It is known that HDL₃ subclasses are smaller than HDL₂, and in healthy individuals, HDL₃ particles are more protective, but in conditions of hypertriglyceridemia, large particles become smaller and denser, losing their atheroprotective properties. Recently, Sulaiman et al.¹³ hypothesized that the forming of fetoplacental circulation toward the end of the 1st trimester and early 2nd trimester could be associated with the synthesis of new functionally improved HDL particles that protect maternal endothelial function in an uncomplicated pregnancy. These authors also speculate that this adaptive and protective HDL-related mechanism is defective in PE, leading to the development of endothelial dysfunction.

The aim of this study was to investigate the changes in HDL particle distribution and HDL particle antioxidative capacity in pregnant women who remained at risk of PE until delivery and in women who developed PE.

Methods

Study population

For this research, we recruited 91 pregnant women whose median age was 32 years (20–46) and who qualified for this study based on being diagnosed with a high risk of PE development, and who had their regular gynecological check-ups at the Gynecology and Obstetrics Clinic “Narodni Front” in Belgrade, Serbia. The study protocol was in accordance with all relevant national regulations, institutional policies, and ethical guidelines defined by the Declaration of Helsinki. This study was approved by the Ethics Committee of the Obstetrics and Gynecology Clinic “Narodni Front” (approval number: 24/55-6, from June 14, 2016). Informed

consent was obtained from all individual participants included in the study.

According to the recommendations given by the American College of Obstetricians and Gynecologists and the National Institute for Health and Care Excellence, pregnant women were classified as having a high-risk pregnancy (HRP) if one high or two moderate risk factors for PE development were present^{14, 15}. Chronic hypertension, chronic kidney disease, hypertension in previous pregnancy, high uterine artery pulsatility index, DM (type 1 or type 2), autoimmune disease, antiphospholipid syndrome, and history of thrombophilia were defined as high-risk factors, while the maternal age of 40 or older, pregnancy interval > 10 years, body mass index (BMI) > 30 kg/m² before pregnancy, and family history were defined as moderate risk factors^{14, 15}. By the end of pregnancy, the women were divided into two groups for research purposes: 71 women, although being in the HRP group, did not develop PE before delivery and were designated as a group without preeclampsia (WPE group), while 20 HRP women (22%) who did develop PE until the end of pregnancy, were designated as a PE group. Of the 20 pregnant women who developed PE, 4 of them also had pregnancies associated with intrauterine growth restriction (IUGR), and 6 had gestational DM (GDM). PE was defined with *de novo* hypertension ($\geq 140/90$ mmHg) after 20 weeks of gestation, with or without proteinuria (≥ 300 mg/24 hrs), but with clinical signs of edema or organ damage. Only one woman was diagnosed with early-onset PE with delivery before 34 weeks of gestation, while 19 were diagnosed with late-onset PE. Among those who did not develop PE, 13 participants developed gestational hypertension (1 had IUGR, 3 had GDM), 2 developed IUGR and GDM, while 5 developed only IUGR and 4 only GDM until the end of pregnancy. Forty-seven (52%) participants who started their pregnancy with high or moderate risk factors for PE development carried out their pregnancies to term with no manifested complications.

Study procedures

All participants were advised to take standard vitamin and antioxidative supplements. In order to preserve their unique lipid profile, no participants were treated with lipid-lowering therapy. An exclusion criterion for participation in the study was multiple gestations.

The study was designed as a longitudinal prospective study. Blood samples were drawn after night-time fasting, towards the end of each trimester, and before the delivery [1st trimester (11–13 weeks of gestation), 2nd trimester (20–23 weeks of gestation), 3rd trimester (28–32 weeks of gestation) and before the delivery (37–38 weeks of gestation)]. Samples were collected into one serum sample tube and one EDTA sample tube, which were centrifuged at 1500xg for 10 min at 4°C. Plasma and serum samples were aliquoted and stored at -80°C until analysis.

Height and weight measurements were done for BMI calculation [BMI = weight (kg)/squared height (m²)] and weight gain (%) assessment. Pregnancy weight gain was calculated as the difference between the last recorded maternal

weight at the time when blood was sampled and self-reported maternal weight before conception. Lipid profile parameters [TG, total cholesterol (TC), HDL-C, and apoA1] were measured on Beckman auto-analyzer AU 480 employing commercial kits (Beckman, USA) in serum samples. Low-density lipoprotein cholesterol (LDL-C) concentration was calculated by the Friedewald equation¹⁶. The atherogenic index of plasma (AIP) was calculated according to the following equation: AIP = log (TG/HDL-C)¹⁷. Dominant LDL and HDL diameter, as well as HDL particle distribution, were determined by the vertical 3–31% polyacrylamide gradient gel electrophoresis method, standardized in our laboratory¹⁸. After electrophoretic separation, lipoprotein subclasses were assessed using Image Scanner (Amersham Pharmacia Biotech, Vienna, Austria) with Image Quant software (version 5.2; 1999; Molecular Dynamics). We determined LDL and HDL particle sizes by estimating the diameters of the most dominant peaks in the corresponding LDL and HDL regions of each scan. The relative content of each HDL subclass was approximated by determining the areas under the peaks of densitometric scans according to previously defined regions: HDL_{2b} (9.70–12.00 nm), HDL_{2a} (8.80–9.69 nm), HDL_{3a} (8.20–8.79 nm), HDL_{3b} (7.80–8.19 nm), and HDL_{3c} (7.20–7.79 nm)¹⁹. PON1 activity was determined by the method of kinetic spectrophotometry in serum, previously described by Richter and Furlong²⁰. The concentration of paraoxon was 1.2 mmol/L, and it was purchased from Chem Service (West Chester, PA, USA). The method was modified and optimized in our laboratory²¹.

Statistical analysis

The data are shown as median and interquartile range. Longitudinal changes of variables through pregnancy were measured by the Friedman test with post hoc Wilcoxon's test using Bonferroni correction for the number of mutual comparisons. Differences between two continuous variables were analyzed by the Mann-Whitney *U* test. Statistical analyses were performed using the Medcalc software (MedCalc Software, Ostend, Belgium) and PASW Statistics 18 (IBM, Armonk, New York, USA). All statistical tests were considered significant at the $p = 0.05$ probability level.

Results

The general characteristics of the study groups – the WPE group (71 participants) and the PE group (20 participants) – are presented in Table 1. Samples were taken toward the end of each trimester with numbers expressed as median (min-max): 1st trimester [12.7 (12.1–13.3) weeks of gestation], 2nd trimester [23.4 (22.7–23.8) weeks of gestation], 3rd trimester [29.6 (28.4–30.7) weeks of gestation], and before the delivery [36.7 (36.3–37.6) weeks of gestation]. In both groups, BMI significantly increased during pregnancy. As expected, systolic and diastolic blood pressure were significantly higher in the PE compared with the WPE group in almost all stages of pregnancy. We have further focused our research on lipid profile parameters in those two groups. In

the WPE group, TG, TC, and LDL-C concentrations significantly increased during the pregnancy (all $p < 0.001$), HDL-C concentration in this study group significantly increased in the 2nd trimester ($p < 0.001$) and remained increased by the end of pregnancy ($p < 0.001$), as well as apoA1 ($p < 0.001$) (Table 2). There was a significant increase in AIP values in all stages of pregnancy ($p < 0.001$), while the dominant LDL diameter significantly decreased in the 2nd trimester, and these changes persisted until the end of pregnancy in the WPE group. In the PE group, TG, TC, and LDL-C concentrations also significantly increased alongside the course of pregnancy. TG concentrations were significantly higher in the PE compared with the WPE group in all the stages of pregnancy ($p < 0.05$). We did not notice significant changes

in HDL-C concentration during pregnancy in the PE group, while apoA1 significantly increased in the 2nd trimester and remained increased in all following stages of pregnancy. In the PE group, AIP significantly increased in the 2nd trimester compared with the 1st trimester ($p < 0.05$) and stayed increased by the end of pregnancy. AIP was significantly higher in the PE group compared with HRP from 2nd trimester until the delivery (Table 2).

Table 3 shows changes in HDL particle sizes and subclasses, as well as PON1 activity through pregnancy. In the WPE group, the proportions of HDL_{2b} particles significantly increased in the 2nd trimester ($p < 0.001$) and remained increased until the end of pregnancy, while proportions of HDL_{2a} ($p < 0.001$) and HDL_{3a} ($p < 0.001$) particles

Table 1

Clinical parameters in high-risk pregnancy

Group	1 st trimester	2 nd trimester	3 rd trimester	37 th WG	<i>p</i> -value
WPE					
BMI (kg/m ²)	23.6 (21.0–27.4)	25.2 ^{a**} (23.5–29.9)	26.7 ^{a**,b**} (24.6–31.1)	28.1 ^{a**,b**,c**} (25.5–32.2)	< 0.001
Weight gain (%)	4.0 (2.0–6.0)	7.5 ^{a**} (5.0–10.0)	5.0 ^{b**} (3.0–6.0)	5.0 ^{b**} (3.0–6.0)	< 0.001
SBP (mmHg)	114.2 (102.0–123.0)	109.2 (101.1–117.6)	108.0 (102.6–121.5)	114.2 ^{b*} (104.0–122.6)	0.003
DBP (mmHg)	72.0 (63.0–80.2)	69.2 (62.5–76.0) ^{a*}	71.0 (65.0–76.0)	75.5 ^{b**,c**} (69.5–81.0)	< 0.001
PE					
BMI (kg/m ²)	27.5 ^{d#} (24.2–31.1)	28.4 ^{a**,d#} (26.4–32.7)	30.0 ^{a**,b**,d#} (27.1–34.4)	31.2 ^{a**,b**,c**,d#} (28.6–36.1)	< 0.001
Weight gain (%)	4.0 (2.3–5.9)	5.5 (4.0–8.7)	5.0 (2.0–6.7)	4.6(1.2–7.0)	0.109
SBP (mmHg)	120.7 (110.6–130.2)	120.0 (105.6–126.2)	122.0 ^{d#} (112.6–136.5)	127.5 ^{d#} (117.5–139.1)	0.011
DBP (mmHg)	77.7 ^{d#} (73.0–83.4)	75.2 ^{d#} (71.5–85.0)	80.0 ^{d#} (72.6–88.6)	84.0 ^{b*,d#} (78.9–90.1)	0.166

WPE – group without the development of preeclampsia; PE – group with development of preeclampsia; BMI – body mass index; SBP – systolic blood pressure; DBP – diastolic blood pressure; WG – week of gestation.

Data are expressed as median and interquartile range. Bolded values are statistically significant.

Mean significantly different from: ^a the 1st trimester; ^b the 2nd trimester; ^c the 3rd trimester; ^d the same trimester of WPE.

* $p < 0.05$; ** $p < 0.001$ (pairwise comparison: Friedman test with Wilcoxon *post hoc* test; Bonferroni corrected).

[#] $p < 0.05$; ^{##} $p < 0.001$ (Mann-Whitney *U* test).

Table 2

Lipid profile parameters in high-risk pregnancy

Group	1 st trimester	2 nd trimester	3 rd trimester	37 th WG	<i>p</i> -value
WPE					
TG (mmol/L)	1.29 (1.06–1.55)	1.88 ^{a**} (1.54–2.27)	2.31 ^{a**,b**} (1.80–2.95)	2.97 ^{a**,b**,c**} (2.39–3.65)	< 0.001
TC (mmol/L)	5.2 (4.7–6.2)	6.9 ^{a**} (5.8–7.6)	7.3 ^{a**,b**} (6.0–8.6)	7.3 ^{a**,b**} (6.2–8.9)	< 0.001
LDL-C (mmol/L)	2.9 (2.3–3.5)	3.9 ^{a**} (3.0–4.7)	4.1 ^{a**,b**} (3.2–5.2)	3.9 ^{a**} (3.2–5.1)	< 0.001
HDL-C (mmol/L)	1.78 (1.53–2.02)	2.11 ^{a**} (1.85–2.45)	2.00 ^{a**,b**} (1.74–2.35)	1.90 ^{a**,b**} (1.70–2.27)	< 0.001
ApoA1 (g/L)	1.94 (1.67–2.20)	2.31 ^{a**} (2.06–2.58)	2.31 ^{a**} (1.98–2.54)	2.25 ^{a**} (1.95–2.47)	< 0.001
AIP	-0.15 (-0.24–-0.048)	-0.068 ^{**} (-0.18–0.045)	0.045 ^{a**,b**} (-0.051–0.19)	0.16 ^{a**,b**,c**} (0.068–0.27)	< 0.001
Dominant LDL diameter (nm)	26.3 (25.5–27.1)	25.8 ^{a**} (24.9–26.6)	25.2 ^{a**,b**} (24.7–26.0)	25.2 ^{a**,b**} (24.3–26.0)	< 0.001
PE					
TG (mmol/L)	1.60 ^{d#} (1.19–1.97)	2.38 ^{a**,d#} (1.75–2.92)	2.75 ^{a**,b*,d#} (2.21–3.66)	3.62 ^{a**,b**,c*,d#} (2.78–4.61)	< 0.001
TC (mmol/L)	5.3 (5.1–5.8)	6.5 ^{a**} (5.5–7.4)	6.7 ^{a**} (6.0–8.1)	7.4 ^{a**,b*} (5.9–8.2)	< 0.001
LDL-C (mmol/L)	2.7 (2.3–3.3)	3.2 ^{a**} (2.9–4.4)	3.1 ^{a*} (2.6–4.9)	3.1 (2.3–4.8)	0.012
HDL-C (mmol/L)	1.75 (1.45–1.97)	1.83 ^{d#} (1.66–2.10)	1.85 (1.70–2.12)	1.84 (1.73–2.16)	0.108
ApoA1 (g/L)	1.84 (1.65–2.39)	2.27 ^{a*} (1.95–2.68)	2.26 ^{a*} (2.01–2.53)	2.30 ^{a*} (2.02–2.67)	< 0.001
AIP	-0.033 (-0.17–0.097)	0.13 (-0.035–0.19) ^{a*,d#}	0.18 ^{a**,d#} (0.085–0.35)	0.32 ^{a**,b*,d#} (0.12–0.38)	< 0.001
Dominant LDL diameter (nm)	26.7 (25.5–27.3)	25.7 ^{a*} (24.5–26.6)	24.8 ^{a**} (23.8–26.1)	24.8 ^{a**} (24.4–26.3)	< 0.001

WPE – group without the development of preeclampsia; PE – group with development of preeclampsia; WG – week of gestation; TG – triglycerides; TC – total cholesterol; LDL-C – low-density lipoprotein cholesterol; HDL-C – high-density lipoprotein cholesterol; ApoA1 – apolipoprotein A1; AIP – atherogenic index of plasma.

Data are expressed as median and interquartile range. Bolded values are statistically significant.

Mean significantly different from: ^a the 1st trimester; ^b the 2nd trimester; ^c the 3rd trimester; ^d the same trimester of WPE.

* $p < 0.05$; ** $p < 0.001$ (pairwise comparison: Friedman test with Wilcoxon *post hoc* test; Bonferroni corrected).

[#] $p < 0.05$; ^{##} $p < 0.001$ (Mann-Whitney *U* test).

Table 3

High-density lipoprotein particle size, subclass distribution, and paraoxonase 1 activity through high-risk pregnancy

Group	1 st trimester	2 nd trimester	3 rd trimester	37 th WG	<i>p</i> -value
WPE					
Dominant HDL diameter (nm)	10.81 (10.38–11.11)	10.89 (10.46–11.07)	10.75 (10.41–11.12)	10.80 (10.47–11.11)	0.594
HDL _{2b} (%)	50.91 (44.77–56.18)	54.18 (50.05–59.06) ^{***}	53.09 ^{a*} (47.93–59.28)	52.15 ^{a*} (47.34–58.16)	< 0.001
HDL _{2a} (%)	20.10 (18.65–21.76)	18.98 ^{a**} (17.09–20.62)	18.67 ^{a**} (17.20–20.96)	19.57 ^{a*} (17.70–20.61)	< 0.001
HDL _{3a} (%)	13.67 (12.05–15.17)	12.38 ^{a**} (11.05–14.28)	12.44 ^{a**} (10.61–13.78)	12.36 ^{a**} (11.04–13.97)	< 0.001
HDL _{3b} (%)	7.90 (6.73–9.53)	7.76 (6.45–9.31)	8.09 (6.74–9.35)	8.37 (6.46–9.77)	0.389
HDL _{3c} (%)	5.58 (4.30–9.01)	5.94 (4.81–8.24)	6.79 (4.65–10.23)	6.78 (4.61–9.67)	0.249
PON1 (U/L)	284.5 (190.0–603.2)	346.5 ^{a**} (254.5–785.2)	358.5 ^{a**} (262.5–772.0)	325.0 ^{b*} (219.5–681.2)	< 0.001
PE					
Dominant HDL diameter (nm)	10.88 (10.30–11.08)	10.62 (10.50–10.88)	10.67 (10.59–10.96)	10.83 (10.34–11.12)	0.569
HDL _{2b} (%)	52.16 (44.38–56.32)	57.50 (46.99–60.88)	55.06 ^{a**} , ^{b*} (50.63–62.43)	57.40 (45.57–61.60)	0.001
HDL _{2a} (%)	19.78 (16.84–22.12)	19.17 ^{a*} (17.47–20.18)	17.86 ^{a**} (15.46–18.73)	16.86 ^{d#} (15.88–18.96)	0.009
HDL _{3a} (%)	13.33 (12.26–15.67)	12.02 ^{a*} (11.25–14.60)	12.19 (10.98–13.79)	11.88 (10.92–14.17)	0.001
HDL _{3b} (%)	7.83 (6.16–9.90)	7.47 (5.82–10.06)	7.55 (6.39–9.69)	7.53 (6.22–10.06)	0.363
HDL _{3c} (%)	6.15 (5.69–8.67)	6.53 (4.31–8.06)	6.18 (4.20–7.59)	5.87 (4.70–9.30)	0.643
PON1 (U/L)	698.0 ^{d#} (395.5–968.0)	697.5 (379.0–1024.7)	803.5 ^{d#} (431.2–1305.0)	771.0 ^{d#} (357.7–1162.0)	0.109

WPE – group without the development of preeclampsia; PE – group with development of preeclampsia; WG – week of gestation; PON1 – paraoxonase 1; HDL – high-density lipoprotein.

Data are expressed as median and interquartile range. Bolded values are statistically significant.

Mean significantly different from: ^a the 1st trimester; ^b the 2nd trimester; ^c the 3rd trimester; ^d the same trimester of WPE.

****p* < 0.05; ***p* < 0.001 (pairwise comparison: Friedman test with Wilcoxon *post hoc* test; Bonferroni corrected).**

[#]*p* < 0.05; ^{##}*p* < 0.001 (Mann-Whitney *U* test).

significantly decreased in the 2nd trimester compared to the 1st trimester. In the WPE group, PON1 activity was significantly higher in the 2nd and 3rd trimesters compared with the 1st trimester. On the other hand, in the PE group, the proportions of HDL_{2a} (*p* = 0.009) and HDL_{3a} (*p* = 0.001) particles significantly decreased in the 2nd trimester. Moreover, there was a significantly lower proportion of HDL_{2a} particles in the PE group compared with the WPE group (*p* < 0.05). The PON1 activity in the PE group did not change during the pregnancy (*p* = 0.109) but was significantly higher in the PE group compared with the WPE group in the 1st and 3rd trimesters (Table 3).

Discussion

This study followed changes in the distribution and function of HDL particles in pregnant women at risk of developing PE. The lipid profile changes in uncomplicated pregnancy are similar to the ones in atherogenic pregnancy, but with appropriate compensatory and adaptive mechanisms, HDL particles manage to regulate the changes in order to avoid the development of complications in pregnancy. By monitoring pregnant women at risk of developing PE, we concluded that these adaptive changes in HDL particle function do not occur, i.e., endothelial protection is insufficient and, consequently, it is associated with clinical manifestations of PE.

A healthy pregnancy is associated with complex and intensive changes in lipid metabolism, which increase plasma lipid concentrations. These metabolic changes are necessary for the physiological course of pregnancy and fetal development²². In the early anabolic phase of pregnancy, increased

lipid synthesis results in enlarged fat storage, while in the catabolic phase during the 3rd trimester, fat deposits have been degraded as a consequence of enhanced adipose tissue lipolytic activity²³. These intensive metabolic changes arise as a result of a sudden and dramatic increase of estrogen, progesterone, and other reproductive hormones, as well as due to insulin resistance development, which is a physiological adaptation of pregnant women aimed to ensure adequate nutrient supply for the fetus development²³. The main characteristics of an altered lipid profile during uncomplicated pregnancy are hypertriglyceridemia and an increase in TC and LDL-C concentrations, alongside an increase in HDL-C concentration and apoA1⁴. In the current study, we showed that WPE is followed by changes in the size of LDL particles. The results of previous studies indicated that as a consequence of the TG concentration increase during pregnancy, the activity of CETP also increases and leads to the formation of sdLDL particles²⁴. Furthermore, recently published studies have suggested that maternal predisposition for PE could be partially explained by altered lipid profile in the early course of pregnancy⁵. The results of our study showed that only TG concentrations were significantly higher in the PE group compared with the WPE during the whole course of pregnancy. That was partially the expected result because, even if it is generally accepted that PE is associated with more prominent alterations toward proatherogenic lipid profile in comparison with an uncomplicated pregnancy, in our study, all participants experienced HRP and the differences in lipid parameters between the WPE and PE groups were not so obvious. In addition, we did not notice significant changes in HDL-C concentrations through pregnancy in the PE group. HDL-C concentration is found to increase toward

the end of the 1st and the beginning of the 2nd trimester of uncomplicated pregnancy^{4,13}. We also found this pattern in the WPE group, and this increase indicates the potential protective role of HDL particles. Lipid alterations found in the PE group, an increase in TG concentration, and a lack of significant increase in HDL-C concentration could be the factors responsible for endothelial dysfunction, which is the base of PE development. Moreover, these findings highlighted the importance of AIP determination during pregnancy. Our previous results indicated that AIP, a relatively inexpensive test to perform, could be used routinely as a possible marker of pregnancy complications, especially PE²⁵.

This study did not notice a significant change in the dominant HDL diameter in the WPE group. We found that during HRP, the relative proportion of large HDL_{2b} particles significantly increases in the 2nd trimester and stays increased until the delivery. This increase in large HDL_{2b} particles is associated with an increase in TC concentration and an increase in PON1 activity in the 2nd and 3rd trimesters. As we have pointed out before, the role of distinct HDL particles through HRP has been poorly investigated, and the data are inconsistent¹³. The results of our previous study indicated that during normal pregnancy, proportions of smaller, denser HDL_{3b} and HDL_{3c} particles significantly increase, and this is followed by a decrease in the proportion of HDL_{2a} subclasses²⁶. Similarly, Alvarez et al.²⁷ reported a decrease in HDL_{2a} and an increase in HDL_{3c} subclasses in the 3rd trimester, while Sulaiman et al.²⁸ found that the final phase of a normal pregnancy is associated with an increase in large HDL₂ particles. The remodeling of HDL particles during pregnancy was explained as a subclass shift toward smaller, denser, TG-rich, and less potent particles in terms of atheroprotection^{26,27}. Recently, Sulaiman et al.¹³ presented an intriguing hypothesis according to which enhanced synthesis of new HDL particles occurs as a compensatory mechanism aimed to protect vascular endothelial function in normal pregnancy. In light of this observation, we could speculate that the observed increase in small HDL₃ particles in studies of uncomplicated pregnancy could be explained as the triggering of new, protein-rich, atheroprotective, and antioxidative potent HDL particle synthesis by the placental circulation. In HRP, this adaptive mechanism was not observed, and this could also explain the fact that almost half of these pregnancies are characterized by the development of complications.

Results of our study also confirmed an increase in PON1 activity in WPE in the 2nd and 3rd trimesters. PON1 is an HDL-associated esterase that metabolizes oxidized lipids within LDL particles, and lower PON1 activities are associated with a higher risk of CVD development²⁹. Additionally, PON-1 determines the capacity of HDL to stimulate nitric oxide production and protect the endothelial function³⁰. Results regarding PON1 activities in pregnancy are controversial, but it is generally accepted that PON1 activity increases through pregnancy as an additional adaptive mechanism of HDL particles in the condition of increased oxidative species formation characteristic of pregnancy³¹⁻³³.

Another important finding of our study is that alterations in HDL particles in women with overt PE are different

than in those with a high risk of PE development. We did not find significant changes in the dominant HDL diameter in this group. There were no changes in HDL-C concentrations, and this result is in agreement with previous studies^{4,25}. A deeper analysis of the distribution of HDL particles through pregnancy complicated with PE showed a decrease in the relative portion of small HDL_{3a} particles in the 2nd trimester. This result brings us back to Sulaiman et al.¹³ hypotheses that uncomplicated pregnancy is associated with *de novo* synthesis of HDL particles (increase in the relative proportion of small HDL₃ particles), and in PE, this adaptive mechanism is lost, which might be further associated with endothelial dysfunction development. As already mentioned, PON1 activity in the WPE group increased as a result of the adaptive mechanism, while in the PE group, there were no changes in PON1 enzyme activity. However, PON1 activity is higher in the PE group in the 1st and 3rd trimesters and before delivery compared to the WPE group, which indicates that pregnant women in the PE group needed greater antioxidant protection. PON1 was initially higher in the PE group, but this increase was insufficient to protect the pregnant women from complications³⁴.

Our study is limited due to the small sample size and should be confirmed in larger studies. Another limitation is the absence of a control group, which could affect the sensitivity and specificity of biomarkers as assessed in a previously selected high-risk population.

Conclusion

Overall, our results indicate that HDL particles go through structural and functional changes during HRP. The structural and functional modifications of HDL particles that go on through HRP without the development of PE are different from those that take place in HRP with the development of PE. Dyslipidaemia in pregnancy is characterized by hypertriglyceridemia and an increase in HDL-C concentration, and this specific lipid profile constellation could be associated with adverse modifications of HDL particles, which are comparable to atherogenic dyslipidemia. Adaptive pregnancy mechanisms expressed as a functional modification of HDL particles seem to lack in PE. A better understanding of the HDL particle's role in pregnancy is necessary for further investigation of pregnancy complications treatment, especially for preventing PE development.

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Conflict of interest

The authors declare no conflict of interest.

R E F E R E N C E S

- Tranquilli AL, Dekker G, Magee L, Roberts J, Sibai BM, Steyn W, et al. The classification, diagnosis and management of the hypertensive disorders of pregnancy: a revised statement from the ISSHP. *Pregnancy Hypertens* 2014; 4(2): 97–104.
- Abalos E, Cuesta C, Grosso AL, Chou D, Say L. Global and regional estimates of preeclampsia and eclampsia: a systematic review. *Eur J Obstet Gynecol Reprod Biol* 2013; 170(1): 1–7.
- Romero R, Chairworapongsa T. Preeclampsia: a link between trophoblast dysregulation and an antiangiogenic state. *J Clin Invest* 2013; 123(7): 2775–7.
- Kaaja R. Lipid abnormalities in pre-eclampsia: implications for vascular health. *Clin Lipidol* 2011; 6(1): 71–8.
- Spracklen CN, Smith CJ, Safflas AF, Robinson JG, Ryckman KK. Maternal hyperlipidemia and the risk of preeclampsia: a meta-analysis. *Am J Epidemiol* 2014; 180(4): 346–58.
- Konrad E, Güralp O, Shaalan W, Elzarkaa AA, Moftah R, Alemam D, et al. Correlation of elevated levels of lipoprotein(a), high-density lipoprotein and low-density lipoprotein with severity of preeclampsia: a prospective longitudinal study. *J Obstet Gynaecol* 2020; 40(1): 53–8.
- Sandsäter HL, Horn J, Rich-Edwards JW, Haugdahl HS. Preeclampsia, gestational diabetes and later risk of cardiovascular disease: Women's experiences and motivation for lifestyle changes explored in focus group interviews. *BMC Pregnancy Childbirth* 2019; 19: 448.
- Shah AS, Tan L, Long JL, Davidson WS. Proteomic diversity of high-density lipoproteins: our emerging understanding of its importance in lipid transport and beyond. *J Lipid Res* 2013; 54(10): 2575–85.
- Ben-Aicha S, Badimon L, Vilabur G. Advances in HDL: Much More than Lipid Transporters. *Int J Mol Sci* 2020; 21(3): 732.
- Stadler JT, Wadsack C, Marsche G. Fetal High-Density Lipoproteins: Current Knowledge on Particle Metabolism, Composition and Function in Health and Disease. *Biomedicines* 2021; 9(4): 349.
- Kontush A. HDL particle number and size as predictors of cardiovascular disease. *Front Pharmacol* 2015; 6: 218.
- Kontush A, Chapman MJ. Antiatherogenic function of HDL particle subpopulations: focus on antioxidative activities. *Curr Opin Lipidol* 2010; 21(4): 312–8.
- Sulaiman WNW, Caslake MJ, Delles C, Karlsson H, Mudler MT, Graham D et al. Does high-density lipoprotein protect vascular function in healthy pregnancy? *Clin Sci (Lond)* 2016; 130(7): 491–7.
- Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. *Obstet Gynecol* 2013; 122(5): 1122–31.
- National Institute for Health and Care Excellence. Hypertension in pregnancy: diagnosis and management. NICE guideline. Available from: www.nice.org.uk/guidance/ng133 (2019 June 25).
- Friedwald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972; 18(6): 499–502.
- Dobiášová M. Atherogenic index of plasma [log(triglycerides/HDL-cholesterol)]: theoretical and practical implications. *Clin Chem* 2004; 50(7): 1113–5.
- Vekic J, Topic A, Zeljkovic A, Jelic-Ivanovic Z, Spasojevic-Kalimanovska V. LDL and HDL subclasses and their relationship with Framingham risk score in middle-aged Serbian population. *Clin Biochem* 2007; 40(5): 310–6.
- Blanche PJ, Gong EL, Forte TM, Nichols AV. Characterization of human high-density lipoproteins by gradient gel electrophoresis. *Biochim Biophys Acta* 1981; 665(3): 408–19.
- Richter RJ, Furlong CE. Determination of paraoxonase (PON1) status requires more than genotyping. *Pharmacogenetics* 1999; 9(6): 745–53.
- Kotur-Stevuljeric J, Spasic S, Stefanovic A, Zeljkovic A, Bogarac-Stanojevic N, Kalimanovska-Oštrić D, et al. Paraoxonase-1 (PON1) activity, but not PON1(Q192R) phenotype, is a predictor of coronary artery disease in a middle-aged Serbian population. *Clin Chem Lab Med* 2006; 44(10): 1206–13.
- Wild R, Weedon EA, Wilson D. Dyslipidemia in Pregnancy. *Cardiol Clin* 2015; 33(2): 209–15.
- Zeng Z, Liu F, Li S. Metabolic Adaptations in Pregnancy: A Review. *Ann Nutr Metab* 2017; 70(1): 59–65.
- Alabakoon TI, Medbury HJ, Williams H, Lee VW. Lipid profiling in maternal and fetal circulations in preeclampsia and fetal growth restriction—a prospective case control observational study. *BMC Pregnancy Childbirth* 2020; 20(1): 61.
- Ardalić D, Stefanović A, Banjac G, Cabunac P, Miljković M, Mandić-Marković V, et al. Lipid profile and lipid oxidative modification parameters in the first trimester of high-risk pregnancies - possibilities for preeclampsia prediction. *Clin Biochem* 2020; 81: 34–40.
- Zeljkovic A, Vekic J, Spasic S, Jelic-Ivanovic Z, Spasojevic-Kalimanovska V, Gojkovic T, et al. Changes in LDL and HDL subclasses in normal pregnancy and associations with birth weight, birth length and head circumference. *Matern Child Health J* 2013; 17(3): 556–65.
- Alvarez JJ, Montelongo A, Iglesias A, Lasunción MA, Herrera E. Longitudinal study on lipoprotein profile, high density lipoprotein subclass, and postheparin lipases during gestation in women. *J Lipid Res* 1996; 37(2): 299–308.
- Silliman K, Shore V, Forte TM. Hypertriglyceridemia during late pregnancy is associated with the formation of small dense low-density lipoproteins and the presence of large buoyant high-density lipoproteins. *Metabolism* 1994; 43(8): 1035–41.
- Kim DS, Marsillach J, Furlong CE, Jarvik GP. Pharmacogenetics of paraoxonase activity: elucidating the role of high-density lipoprotein in disease. *Pharmacogenomics* 2013; 14(12): 1495–515.
- Besler C, Heinrich K, Rohrer L, Doerries C, Rivanto M, Shis DM, et al. Mechanisms underlying adverse effects of HDL on eNOS-activating pathways in patients with coronary artery disease. *J Clin Invest* 2011; 121(7): 2693–708.
- Roy AC, Loke DF, Saba N, Viegas OA, Tay JS, Ratnam SS. Interrelationships of serum paraoxonase, serum lipids and apolipoproteins in normal pregnancy. A longitudinal study. *Gynecol Obstet Invest* 1994; 38(1): 10–33.
- León-Reyes G, Maída-Claros RF, Urrutia-Medina AX, Jorge-Galarza E, Guzmán-Grenfell AM, Fuentes-García S, et al. Oxidative profiles of LDL and HDL isolated from women with preeclampsia. *Lipids Health Dis* 2017; 16(1): 90.
- Stefanovic A, Ardalic D, Kotur-Stevuljeric J, Vujovic A, Spasic S, Spasojevic-Kalimanovska V, et al. Longitudinal changes in PON1 activities, PON1 phenotype distribution and oxidative status throughout normal pregnancy. *Reprod Toxicol* 2012; 33(1): 20–6.
- Uzun H, Benian A, Madazli R, Topcuoglu MA, Aydin S, Albayrak M. Circulating oxidized low-density lipoprotein and paraoxonase activity preeclampsia. *Gynecol Obstet Invest* 2005; 60(4): 195–200.

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