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Original paper

Gene expression of lipid transporters in peripheral blood mononuclear cells of pregnant women: A longitudinal study

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Summary. PLTP, SR-B1, ABCA1 and ABCG1 transporters mediate the uptake of maternal HDL-cholesterol and its transfer to the foetal circulation. Their placental expressions have already been explored, but less is known about changes in their gene expressions in maternal peripheral blood mononuclear cells (PBMC). The aim of this study was to investigate longitudinal changes in gene expression of PLTP, SRB1, ABCA1 and ABCG1 in maternal PBMCs during healthy pregnancy. Changes in PLTP, SR-B1, ABCA1 and ABCG1 gene expressions, in relation to the maternal smoking status before pregnancy, was examined. Quantification of PLTP, SCARB1, ABCA1 and ABCG1 mRNA was performed using quantitative polymerase chain reaction (qPCR) method in total RNA isolated from PBMCs. In the second trimester (T2), PLTP gene expression was upregulated compared to the first trimester (T1), while in the third trimester (T3) it was significantly downregulated compared to T1 and T2. Gene expression of SRB1, ABCA1 and ABCG1 did not change significantly during the course of healthy pregnancy. A significant negative correlation was observed between the concentration of triglycerides and the ABCA1 and ABCG1 genes in the T1 and T2. During whole pregnancy course, a negative correlation of ABCA1 mRNA with total cholesterol was observed. In T2, ABCA1 mRNA correlated negatively with LDL-cholesterol concentrations. A negative correlation of PLTP mRNA with HDL-cholesterol in T2 was observed. SRB1 gene expression was significantly upregulated in pregnant women who had smoked before pregnancy in T1 and T2. Gene expression of ABCA1 and ABCG1 was significantly upregulated in pregnant women who had smoked before pregnancy during T1. PLTP PBMCs gene expression showed changes throughout healthy pregnancy, which is in line with changes in maternal lipid status. Maternal smoking status before pregnancy significantly affected ABCA1, ABCG1 and SR-B1 PBMCs gene expression during healthy pregnancy, which could affect cholesterol homeostasis in mothers.

Keywords: ABCA1, ABCG1, PLTP, pregnancy, SCARB.

INTRODUCTION

Cholesterol is a major nutrient required for fetal growth, synthesis of steroid hormones, and is essential for the development and maturation of fetal organs. During pregnancy, the placenta controls the transport of cholesterol from the mother to the fetus and vice versa (Kallol and Albrecht 2020). Furthermore, healthy pregnancy is accompanied by significant changes in maternal lipid metabolism. During the first two trimesters, lipid metabolism is primarily anabolic, with an increase in lipid synthesis, insulin sensitivity in the first trimester and fat storage in preparation for the exponential increases in fetal energy needs during the late pregnancy (Wild and Feingold 2023). The second phase takes place in the third trimester, and represents a net catabolic phase, associated with insulin resistance and accompanied by enhanced lipolysis of stored triglycerides in adipocytes (Herrera 2002; Wild and Feingold 2023). As pregnancy progresses, there is

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a noticeable increase in all routine lipid parameters, and by the end of the third trimester the levels peak. These changes allow proper supply of nutrients and oxygen to the developing fetus, through numerous receptors and transfer proteins located on the placenta (Kallol and Albrecht 2020; Wild and Feingold 2023).

Changes in lipoprotein levels during pregnancy are similar with the lipoprotein profile in cardiometabolic diseases, while after delivery they rapidly return to normal. The special feature of pregnancy dyslipidemia, which differentiates it from dyslipidemia in cardiometabolic diseases, is an increase in high density lipoprotein cholesterol (HDL-C) concentrations during the second trimester with slight decrease toward the delivery. During pregnancy, low density lipoprotein (LDL) and HDL are enriched in triglycerides (Alvarez et al. 1996). Pregnancy alters HDL particle size and a number of protein clusters on distinct HDLs. It is likely these compositional changes alter the particle function in several metabolic pathways important for maternal and fetal heath (Alvarez et al. 1996; Melchior et al. 2021; Woollett et al. 2022).

Cholesterol homeostasis results from the balance between the *de novo* synthesis of cholesterol, the uptake of cholesterol from lipoproteins, and cholesterol efflux. One of the major determinants of cholesterol homeostasis are lipid transporters (Yu and Tang 2022).

Phospholipid transfer protein (PLTP) is encoded by the human *PLTP* gene. Its main role is to transfer phospholipids from triglyceride-rich lipoproteins to HDL (Tall et al. 1985), and between HDL particles, resulting in the formation of both larger HDL2 and smaller (pre-β) HDL subspecies (Settasatian et al. 2001). It was shown that pregnant females exhibit changes in the composition of HDL subspecies compared to non-pregnant women (Melchior et al. 2021). Therefore, PLTP plays a crucial role in plasma lipoprotein metabolism, in particular in the regulation of HDL remodelling and reverse cholesterol transport (Wolfbauer et al. 1999). PLTP is also able to promote the first step in reverse cholesterol transport – cellular cholesterol and phospholipids efflux mediated by interaction with ATP-binding cassette transporter A1 (ABCA1) (Wolfbauer et al. 1999; Oram et al. 2003). PLTP is also located on the basal membrane of the placenta, functioning as a major transporter of cholesterol to the fetus (Scholler et al. 2012).

ATP-binding transporters (ABC transporters) are ubiquitous transmembrane proteins that play a significant role in the efflux of substrates, such as steroid hormones, cholesterol and inflammatory mediators (cytokines, chemokines, prostaglandins), from the cytosol to the extracellular space. The efflux of cholesterol from cells is mediated predominantly by ABCA1, which is a member of the ABC transporters superfamily (Oram 2002). Cholesterol and phospholipids are transported across cell membranes to apolipoprotein A-I (apoA-I) by ABCA1, which leads to the formation of nascent HDL particles (nHDL). nHDL can accept more free cholesterol from peripheral cells. Free cholesterol is then converted to cholesteryl ester by lecithin:cholesterol acyltransferase to form mature HDL. HDL-bound cholesterol enters the liver for biliary secretion and fecal excretion (Chen et al. 2022).

ABCG1 effluxes excess cholesterol from cells to HDL particles for reverse cholesterol transport, while it also has an important role in intracellular transport of cholesterol. It is ubiquitously expressed in many cell types including immune, placental and endothelial cells (Yu and Tang 2022). ABCA1 and ABCG1 transporters are localized on the placental selective membrane, and modulate the transport of cholesterol and other sterols from the mother to the fetus (Aye et al. 2010).

The scavenger receptor, class B type 1 (SR-B1), is a multiligand membrane receptor protein that functions as a physiologically relevant HDL receptor whose primary role is to mediate selective uptake or influx of HDL-derived cholesteryl esters into cells and tissues. SR-B1 also facilitates the efflux of cholesterol from peripheral tissues, including macrophages, back to liver (Shen et al. 2018). It has a significant role in the synthesis of sex hormones in women, especially during pregnancy (Christianson and Yates 2012). The synthesis of sex hormones in women is facilitated by this receptor, but it has also been demonstrated to have an important role in delivering and providing cholesterol to the fetus (Kallol and Albrecht 2020).

Although changes in serum lipid profile have been extensively documented, changes in the gene expression levels of lipid transporters in maternal PBMCs have not been investigated. Namely, PBMCs include lymphocytes and monocytes which circulate in the body and are exposed to both environmental factors and metabolic tissues. Studies have shown that PBMCs may be used as a surrogate model for liver metabolism, since these cells reflect hepatic regulation of cholesterol metabolism, as well as metabolic and immune responses (Larsen et al. 2018). Therefore, the aim of this study is to examine the longitudinal trajectory of *PLTP*, *SR-B1*, *ABCA1* and *ABCG1* gene expression in PBMCs during healthy pregnancy and their relation to maternal serum lipid profile. In addition, changes in *PLTP*, *SR-B1*, *ABCA1* and *ABCG1* gene expressions in relation to the maternal smoking status before pregnancy was examined.

MATERIAL AND METHODS

Subjects

Seventy-three healthy women, with healthy, uncomplicated pregnancy participated in this research, whose preg-

nancy was managed from the first trimester until delivery at the Gynecology and Obstetrics Clinic "Narodni Front" in Belgrade. Women with twin pregnancies and chronic systemic diseases were not included in the research. Blood samples for the analysis were taken at three determination points, during regular gynecological examinations in the first (T1), second (T2) and third (T3) trimesters of pregnancy (median values: 13.2 weeks of gestation, 22.3 weeks of gestation and 32.9 weeks of gestation).

Demographic and clinical data were collected, using standardized questionnaires. The entire research is a part of the larger scientific research project HIgh-density lipoprotein MetabolOMe research to improve pregnancy outcome – HI-MOM, financed by the Science Fund of the Republic of Serbia. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. This research was approved by the Ethics Committee of Gynecology and Obstetrics Clinic "Narodni Front" (reference No. 05006-2020-10738); the Ethics Commission of the Faculty of Medicine, University of Belgrade (designation No. 1322/VII-27); and the Ethical Committee for Biomedical Research of the Faculty of Pharmacy, University of Belgrade (reference No. 1156/2).

Laboratory methods

Blood samples were collected after overnight fasting in tubes for the extraction of EDTA plasma and serum. Concentrations of lipid status parameters, total cholesterol (TC), triglycerides (TG) and HDL cholesterol (HDL-C) were determined by using routine automated methods and commercial test reagents (Beckman Coulter, Brea, California, USA). Low-density lipoprotein cholesterol (LDL-C) levels were estimated by the Friedwald equation.

PBMCs were isolated from an EDTA whole blood sample using Ficoll-Paque™ PLUS medium (GE Healthcare, Waukesha, Wisconsin, USA) following the manufacturer's protocol. RNA isolation was performed with TRIzol™ reagent (Ambion, Life technologies, Grand Island, New York), according to the manufacturer instructions, and stored at -80 °C prior to reverse transcription (RT) reactions. RT and real-time PCR experiments were carried out on the 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) and analysed in real-time mode using SDS Version 1.4.0.25 software. RT was performed using High-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA). Real-time PCR was performed using 5'-nuclease TaqMan® gene expression assays (Applied Biosystems, Foster City, CA, USA) for human *PLTP* (Hs01067287_m1), *SR-B1* (Hs00969821_m1), *ABCA1* (Hs01059101_m1) and *ABCG1* (Hs00245154_m1) genes, while beta actin (Hs99999903_m1) was used as the housekeeping gene. Data were expressed as a ratio between the target gene mRNA and the mRNA of the housekeeping gene.

Statistical methods

All data were analysed using IBM® SPSS® Statistics version 22 software. Distribution of data was tested using Kolmogorov-Smirnov test. Continuous variables that followed normal distribution were presented as mean ± standard deviation. Variables with an asymmetric distribution were presented as the median (interquartile range) and analysed using the Mann-Whitney U test, the Friedman test and *post hoc* Wilcoxon signed-rank test. Spearman's correlation analysis was used to examine correlations with the calculation of the correlation coefficient (ρ). Results for P values less than 0.05 were considered statistically significant.

RESULTS

Number of study participants, age, body mass index (BMI) and smoking status before pregnancy are presented in Table 1. During pregnancy, the levels of *PLTP* mRNA have changed significantly. Firstly, in 2nd trimester, PLTP gene expression was upregulated compared to the 1st trimester, while in the 3rd trimester it was significantly downregulated compared both to 1st and 2nd. Gene expression of *SRB1*, *ABCA1* and *ABCG1* did not change significantly during the course of healthy pregnancy (Table 2).

As expected in heathy pregnancy, lipid profile has changed significantly. Total cholesterol, LDL-cholesterol and triglycerides significantly increased troughout pregnancy. HDL-cholesterol levels rose in T2 compared to T1, decreased in T3 compared to T2, but were still significantly higher compared to T1 (Table 3).

Correlation analysis was used to examine the association between the expression of target genes and the lipid status parameters. A weak, yet significant negative correlation was observed between the concentration of triglycerides and the expression of *ABCA1* and *ABCG1* genes in T1 and T2, while in T3 significant correlation was absent (Tables 4-6).

In all three trimesters, a weak, yet significant negative correlation of *ABCA1* mRNA with total cholesterol was observed (Tables 4-6). In the 2nd trimester, *ABCA1* mRNA correlated negatively with LDL-cholesterol (Table 5). A weak,

Table 1. Characteristics of the study group.

Number of study participants	73
Age, years*	30.6 ± 5.1
BMI before pregnancy, kg/m²*	22.3±3.4
Smoking status before pregnancy, yes/no	23/50

*Data are presented as means with standard deviations

Table 2. Gene expression of lipid transporters during pregnancy.

	1st trimester	2nd trimester	3rd trimester	*P*
PLTP mRNA	0.94 (0.70-1.15)	1.08 (0.73-1.37) [a]	0.69 (0.36-0.99) [a, b]	**<0.001**
SR-B1 mRNA	1.02 (0.75-1.30)	0.96 (0.74-1.14)	1.00 (0.76-1.32)	0.234
ABCA1 mRNA	0.96 (0.64-1.24)	1.01 (0.69-1.31)	0.98 (0.74-1.16)	0.278
ABCG1 mRNA	1.01 (0.76-1.26)	0.89 (0.67-1.31)	1.03 (0.75-1.39)	0.697

Data are presented as medians (interquartile ranges) and compared using the Friedman test and the *post hoc* Wilcoxon signed-rank test.
[a] Significantly different from T1
[b] Significantly different from T2

Table 3. Lipid status parameters during pregnancy.

	1st trimester	2nd trimester	3rd trimester	P
Total cholesterol (mmol/l)	5.24 (4.75-5.84)	6.73 (5.62-7.84) [a]	7.23 (6.02-8.28) [a, b]	**<0.001**
Triglycerides (mmol/l)	1.25 (0.97-1.50)	1.80 (1.39-2.24) [a]	2.30 (1.98-3.02) [a, b]	**<0.001**
HDL- C (mmol/l)	1.69 (1.48-1.91)	1.92 (1.65-2.14) [a]	1.80 (1.63-2.05) [a, b]	**<0.001**
LDL- C (mmol/l)	2.94 (2.53-3.44)	3.98 (3.13-4.93) [a]	4.28 (3.48-5.11) [a, b]	**<0.001**

Data are presented as medians (interquartile ranges) and compared using the Friedman test and the *post hoc* Wilcoxon signed-rank test.
[a] Significantly different from T1
[b] Significantly different from T2

Table 4. Correlation between lipid parameters and the level of gene expression of the examined genes in the 1st trimester.

	PLTP mRNA	*SR-B1* mRNA	*ABCA1* mRNA	*ABCG1* mRNA
Total cholesterol (mmol/l)	-0.063	-0.055	**-0.232***	-0.022
Triglycerides (mmol/l)	0.154	-0.110	**-0.245***	**-0.236***
HDL-C (mmol/l)	-0.144	-0.062	-0.049	0.124
LDL- C (mmol/l)	-0.036	-0.034	-0.186	-0.029

* - $p < 0.05$
** - $p < 0.01$

Table 5. Correlation between lipid parameters and the level of gene expression of the examined genes in the 2nd trimester.

	PLTP mRNA	*SR-B1* mRNA	*ABCA1* mRNA	*ABCG1* mRNA
Total cholesterol (mmol/l)	-0.091	-0.089	**-0.252***	-0.174
Triglycerides (mmol/l)	0.181	-0.134	**-0.310****	**-0.244***
HDL- C (mmol/l)	**-0.254***	-0.106	-0.021	-0.036
LDL- C (mmol/l)	-0.073	-0.073	**-0.239***	-0.175

* - $p < 0.05$
** - $p < 0.01$

Table 6. Correlation between lipid parameters and the level of gene expression of the examined genes in the 3rd trimester.

	PLTP mRNA	*SR-B1* mRNA	*ABCA1* mRNA	*ABCG1* mRNA
Total cholesterol (mmol/l)	0.064	-0.108	**-0.244***	-0.177
Triglycerides (mmol/l)	0.116	-0.115	-0.134	-0.177
HDL- C (mmol/l)	-0.011	0.204	-0.064	0.019
LDL- C (mmol/l)	0.068	-0.081	-0.153	-0.094

* - $p < 0.05$
** - $p < 0.01$

yet significant negative correlation of PLTP mRNA with HDL-cholesterol levels in the 2nd trimester was observed (Table 6).

Gene expression of SR-B1 was significantly upregulated in pregnant women who had smoked before pregnancy compared to non-smokers in T1 and T2. Gene expression of *ABCA1* and *ABCG1* was significantly upregulated in pregnant women who had smoked before pregnancy compared to non-smokers during the 1st trimester. Smoking status before pregnancy did not affect *PLTP* gene expression during pregnancy. Lipid status parameters were not significantly affected by smoking status before pregnancy (data not shown).

DISCUSSION

The aim of this study was to examine changes in the expression of *PLTP*, *SRB1*, *ABCA1* and *ABCG1*, during three points in pregnancy. Previous studies examined changes in the expression of these genes in the placenta and in the fetus (Kallol and Albrecht 2020). To the best of our knowledge, this is the first study to examine changes in gene expression of these lipid transporters in PBMCs of healthy pregnant women in all three trimesters.

During pregnancy, the levels of *PLTP* mRNA have changed significantly. *PLTP* gene expression was upregulated in T2 compared to T1, while in the T3 it was significantly downregulated compared to both T1 and T2 (Table 2). *PLTP* is upregulated by insulin, since fetal insulin itself can also act on the placenta and is thought to increase the expression of *PLTP* (Scholler et al. 2012). T1 and T2 trimesters are characterised by increased insulin sensitivity in mothers, which could explain the rise in mother's PBMCs' PLTP gene expression levels through T1 and T2, and the decrease in T3, since the last trimester is characterised by insulin resistance (Herrera 2002; Wild and Feingold 2023).

Changes in *PLTP* expression levels were accompanied with the changes in HDL-cholesterol levels, which rose in T2 comparing to T1, decreased in T3 compared to T2, but were still significantly higher compared to T1 (Table 3). Also, a negative correlation of *PLTP* mRNA level with HDL-C levels in T2 was observed (Table 6). As already stated, PLTP plays a central role in HDL remodelling and reverse cholesterol transport (Wolfbauer et al. 1999). Namely, HDL apolipoproteins remove both cholesterol and phospholipids from cells by an active process induced by cholesterol loading of cells, but only HDL apolipoproteins that contain little or no lipids are eligible for this process. PLTP mediates the conversion of HDL apolipoproteins to lipid-poor particles that are active acceptors of cellular cholesterol and in that way enhances the removal of cellular cholesterol and phospholipids by HDL apolipoproteins. In addition, PLTP activity is a significant positive predictor of the capacity of human plasma to promote cholesterol efflux from cultured cells, since lipid-poor apo A-I, generated from plasma HDL by PLTP, mobilizes lipids from cells (Wolfbauer et al. 1999).

Although levels of *PLTP* expression accompanied the rise in HDL cholesterol, the two parameters showed negative corelation in T2. This is in line with the fact that adenovirus-mediated overexpression of *PLTP* by mouse livers markedly reduced plasma HDL levels which was associated with increased metabolism and hepatic clearance of HDL lipids (Föger et al.1997; Wolfbauer et al. 1999).

As expected in heathy pregnancy (Herrera 2002; Wild and Feingold 2023), lipid profile has changed significantly (Table 3). Even though there was no significant change in expression of *SR-B1*, *ABCA1* and *ABCG1* in mother's PBMCs during pregnancy, a statistically significant, yet very weak negative correlation was observed between the concentration of triglycerides and the expression of *ABCA1* and *ABCG1* in the first and second trimesters. In all three trimesters, a negative correlation of *ABCA1* mRNA with total cholesterol was observed (Tables 4-6). In the 2nd trimester, *ABCA1* mRNA correlated negatively with LDL-cholesterol (Table 5).

ABCA1 and ABCG1 participate in the reverse cholesterol transport and the formation of HDL particle (Oram 2002; Chen et al. 2022). Negative correlation of *ABCA1* and *ABCG1* with triglycerides, total cholesterol and LDL-cholesterol is in line with the function of these transporters in the formation of protective HDL particles, and maintaining a good balance between pro- and antiatherogenic lipid particles. This implies an active protective role of ABCA1 and ABCG1 transporters during healthy pregnancy (Antonić et al. 2021).

Lipid status parameters were not significantly affected by the maternal smoking status before pregnancy (data not shown). However, expression of *SR-B1* during 1st and 2nd trimester, as well as of *ABCA1* and *ABCG1* during 1st trimester, was significantly upregulated in smokers, while *PLTP* expression was not affected by maternal smoking status before pregnancy (Fig. 1).

Cigarette smoke contains thousands of chemical components, and its toxic effect is mainly due to the presence of free radical compounds (reactive oxygen species, ROS) and volatile electrophilic compounds such as α,β-unsaturated aldehydes. Tobacco smoking induces changes in true DNA methylation, hydroxymethylation and gene expression in bronchoalveolar lavage cells (Ringh et al. 2019). Continuous active exposure to tobacco smoke leads to demethylation, and subsequent increased activity of inflammatory processes in the lungs (Ringh et al. 2019).

Pathway analysis of transcriptional changes in tobacco smoking revealed that the most enriched canonical pathway is related to liver X receptor-retinoid X receptor (LXR/RXR)

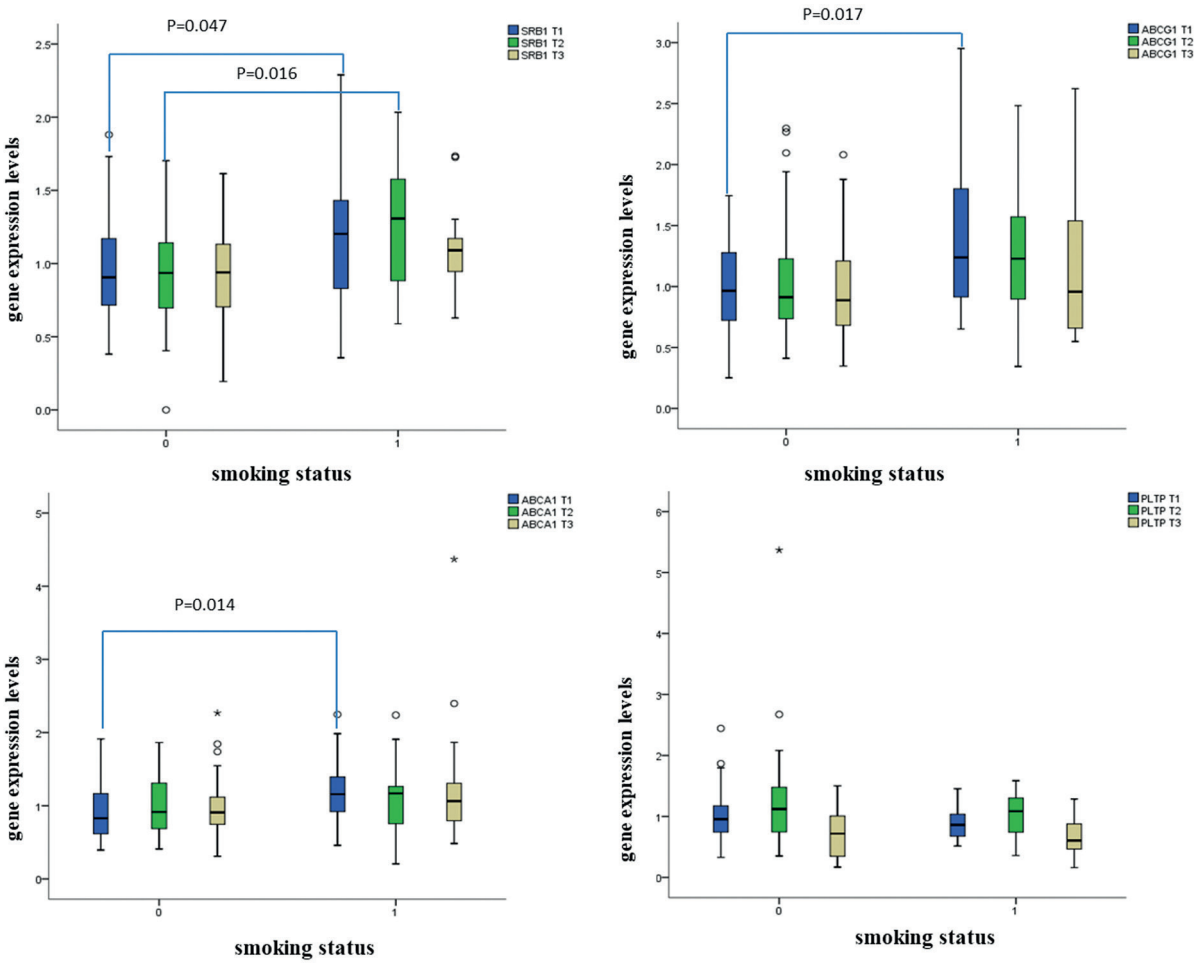

Fig. 1. Changes in gene expression of lipid transporters during pregnancy according to smoking status before pregnancy analysed by the Mann-Whitney U test.

activation, with the involvement in lipid metabolism and inflammation (Nelson et al. 2017).

Namely, the sterol-responsive nuclear receptors – liver X receptors α (LXRα, NR1H3) and β (LXRβ, NR1H2), are key determinants of cellular cholesterol homeostasis. LXRs are activated under conditions of high cellular sterol load and induce expression of cholesterol efflux transporters ABCA1 and ABCG1 to promote efflux of excess cellular cholesterol (Zelcer and Tontonoz 2006; Nelson et al. 2017). It was shown that the activation of LXR and subsequent upregulation of newly identified LXR-target gene *EEPD1*, leads to EEPD1 recruitment to stall replication forks, induced by DNA damage in smoking, as a part of response to the replication fork stress. In parallel, it was shown that EEPD1 promotes cellular cholesterol efflux in macrophages, by controlling cellular levels and the activity of ABCA1 (Nelson et al. 2017).

Furthermore, novel aspects of LXR signalling in innate immunity revealed that LXR activation induces a proinflammatory trained immunity phenotype in human monocytes through epigenetic and metabolic reprogramming (Sohrabi et al. 2020).

Therefore, smoking can extensively influence regulation of gene expression, leading to extensive changes in lipid metabolism by inducing lipid transporter genes, as well as inducing inflammation, trough LXR partway (Zelcer and Tontonoz 2006; Nelson et al. 2017; Ringh et al. 2019; Sohrabi et al. 2020).

In line with our results, *ABCA1* expression in human keratinocytes was increased (both mRNA and protein levels) after cigarette smoke exposure, and the effect was mediated through LXR (Sticozzi et al. 2010).

SR-B1 regulation of gene expression is subjected to many transcriptional factors, including LXR. In both human and rodent preadipocytes and liver cells, LXRα/RXR and LXRβ/RXR were shown to bind to the *SR-B1* promoter and induce its transcription (Malerød et al. 2002). In contrast to our results, it was shown that acute exposure to cigarette smoke reduced lung *SR-B1* expression (protein and mRNA)

in mice and in human alveolar epithelial cells (Valacchi et al. 2011). However, we should bear in mind that in humans, smokers have chronic exposure to tobacco smoke, and that our study is based on subjects' PBMCs.

In conclusion, *PLTP* gene expression in PBMCs shows significant changes through healthy pregnancy, which is in line with changes in maternal lipid status. Maternal smoking status before pregnancy significantly affects *ABCA1*, *ABCG1* and *SR-B1* expression in PBMCs during healthy pregnancy, which could influence cholesterol homeostasis in mothers.

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