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Daniela Ardalić[1, D], Aleksandra Stefanović[2, A], Jelena Kotur-Stevuljević[2, E], Ana Vujović[2, G], Slavica Spasić[2, F], Vesna Spasojević-Kaliomanvska[2, A], Zorana Jelić-Ivanović[2, C], Vesna Mandić-Marković[1,3, B], Zeljko Miković[1, 3, B], Nikola Cerović[1, B]

The Influence of Maternal Smoking Habits Before Pregnancy and Antioxidative Supplementation During Pregnancy on Oxidative Stress Status in a Non-Complicated Pregnancy*

[1] Narodni Front Clinic of Gynecology and Obstetrics, Belgrade, Serbia
[2] Department of Medical Biochemistry, Faculty of Pharmacy, University of Belgrade, Serbia
[3] Faculty of Medicine, University of Belgrade, Serbia

A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article; **G** – other

Abstract

Background. As a physiological condition closely linked with increased susceptibility to oxidative stress, pregnancy can be further compromised by cigarette smoking. Inadequate nutrition and reduced intake of antioxidants can also disrupt the prooxidant/antioxidant relationship and contribute to oxidative stress. Increased oxidative stress during pregnancy may be involved in several complications of pregnancy, such as preterm labor, fetal growth restriction, preeclampsia and miscarriage.
Objectives. The aim of this study was to investigate the influence of maternal smoking habits before pregnancy on the parameters of oxidative stress and the antioxidative defense system, lipid profile parameters and paraoxonase-1 (PON1) activity during the third trimester of uncomplicated pregnancies.
Material and Methods. Healthy pregnant women (n = 86) were divided into non-smoking and smoking groups, and into groups taking vitamin supplements and not taking them. Oxidative damage was measured through the levels of thiobarbituric acid-reacting substances (TBARS) and plasma antioxidant status was evaluated by measuring total antioxidant capacity (TAC).
Results. TBARS concetration was significantly higher ($p < 0.05$) and PON1 activity was significantly lower ($p < 0.05$) in the smokers' group. No significant differences were found in the investigated parameters in relation to vitamin supplement intake.
Conclusions. Habitual smoking before pregnancy is associated with increased oxidative stress. Vitamin supplementation has no effect on the oxidative stress status of healthy pregnant women (**Adv Clin Exp Med 2014, 23, 4, 575–583**).

Key words: pregnancy, oxidative stress, habitual maternal smoking, antioxidative supplementation.

Pregnancy is a physiological condition closely linked with increased susceptibility to oxidative stress [1]. Oxidative stress is an imbalance between the systemic manifestation of reactive oxygen species (ROS) and the antioxidative system's ability to readily detoxify reactive intermediates [2]. Increased oxidative stress during pregnancy could be involved in several complications of pregnancy, such as preterm labor, fetal growth restriction and preeclampsia [2].

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It is well known that tobacco smoke may invoke oxidative stress [3]. In pregnant women, the nicotine and carbon monoxide components of cigarette smoke may cause damage to both the mother and the fetus, crossing the placental barrier [3]. A number of studies suggest a relationship between maternal smoking and not only intrauterine fetal growth retardation or low birth weight [4], but also with disturbances in postnatal growth and development [5]. As pregnancy is a period of increased metabolic demands, insufficient supplies of essential vitamins and micronutrients can lead to a state of biological competition between the mother and fetus [6]. In such a situation, inadequate nutrition and a reduced intake of antioxidants may also disrupt the prooxidant/antioxidant relationship and contribute to oxidative stress.

In addition, it is well known that lipid metabolism is altered during pregnancy by the effects of estrogen, progesterone, and human placental lactogen [7]. These alterations are characterized by normal concentrations of total cholesterol during early pregnancy, and by hypertriglyceridemia and hypercholesterolemia in late pregnancy [7]. An increase in low-density lipoprotein cholesterol (LDL-C) and its oxidative modification are important steps in the development of atherosclerosis. Higher LDL-C concentrations during late pregnancy followed by intensive oxidative stress could be initial reasons for an increased risk of developing cardiovascular disease in later life.

Paraoxonase-1 (PON1) is part of the enzyme antioxidant potential of the organism, and is related to high-density lipoprotein (HDL) particles. The defensive role of PON1 is the metabolism of oxidative LDL-C particles [8], and lower PON1 activity is connected with cardiovascular disease.

The aim of this study was to investigate the influence of maternal smoking habits before pregnancy on the parameters of oxidative stress and the antioxidative defense, lipid profile parameters and PON1 activity during the third trimester of uncomplicated pregnancies. The influence of antioxidative vitamin supplementation during pregnancy on oxidative stress status was also examined.

Material and Methods

For the study, 86 healthy pregnant Caucasians were recruited during their regular gynecological check-ups at the Narodni Front Gynecology and Obstetrics Clinic in Belgrade. The median week of pregnancy was 34 (range 31–38), and none of the participants had any identifiable risk factors for the current pregnancy. A full medical history was taken, including noting the presence of any systemic disorders before pregnancy, smoking status, alcohol intake, antioxidant supplementation and family history of cardiovascular disease and diabetes mellitus. The median age of the subjects was 26 years (range 20–35).

Non-smokers were defined as women who had never smoked either before or during pregnancy. Smokers were defined as those who reported that they had smoked habitually before pregnancy and gave up smoking during pregnancy. In the study population 42 women (48.8%) had been active smokers before pregnancy.

All the participants were specifically advised to consume a variety of foods to get all the nutrients they needed. Sparing use of fats and sweets was recommended. Prenatal vitamin and mineral supplements were provided for 42 of the women (48.8%), who were instructed to take them once a day. The supplements included approximately 200–300 mg calcium, 15 mg zinc, 17 mg iron, 400 mcg folic acid, 400 IU vitamin D, 70 mg vitamin C, 3 mg thiamine, 2 mg riboflavin, 20 mg niacin, 6 mcg vitamin B12 and 10 mg vitamin E.

The exclusion criteria for participation in the study were pregnancy-induced hypertension, overweight before pregnancy, a non-singleton pregnancy, gestational diabetes or the presence of any other complication of pregnancy.

All the women underwent a complete clinical and biochemical examination. The study protocol also included height and weight measurement for body-mass index calculation (BMI = weight [kg]/ /height squared [m^2]). Replies to a standard questionnaire were collected in person by trained interviewers. Individuals were considered overweight when their body mass index (BMI) was > 25 kg/m^2 and < 30 kg/m^2 [9].

Ethical Considerations

The study was planned according to the ethical standards detailed in the Declaration of Helsinki (as revised in 1983) and according to local institutional guidelines. The local institutional review committee approved the research proposal and informed consent was obtained from all the individuals involved in the study.

Analytical Methods

Venous blood was drawn from each subject's antecubital vein after nighttime fasting (> 10 h) into one serum sample tube, one EDTA sample tube and one heparin sample tube before immediate centrifugation at 1500 × g for 10 min at 4^0C. Plasma and serum samples were stored at

–80 ^{0}C in aliquots until analysis (one month after the blood sampling). To measure total oxidant status (TOS), plasma from heparinized blood samples was used immediately.

Lipid status parameters (total cholesterol [T-C], LDL-C, HDL-C, triglyceride [TG], apolipoprotein A-1 [apo A-1], apolipoprotein B [apo B]), fasting glucose, uric acid and total protein were measured in serum using an Ilab 300 Plus autoanalyzer employing commercial kits (Bioanalytica, Belgrade, Serbia). The concentration of high sensitive C-reactive protein (hsCRP) was measured in serum by an immunoturbidimetric assay (Dade-Behring, BN II, Marburg, Germany). The Atherogenic Index of Plasma (AIP) was calculated according to the following equation: AIP = log (TG/HDL-c), with units for TG and HDL-c in mmol/L [10]. The concentration of thiobarbituric acid-reactive substances (TBARS) was measured using the molar absorption coefficient of 1.56×10^5 M^{-1} at 535 nm, as described by Girotti [11]. The intra-assay and inter-assay coefficients of variance were 4.8% and 7.2% respectively. Concentrations of lipid hydroperoxides (LOOH) were measured in serum by the ferric-xylenol orange method [12]. The concentration of advanced oxidation protein products (AOPP) was measured as described by Witko-Sarsat [13], employing spectrophotometry at 340 nm. AOPP concentrations were expressed as chloramine-T equivalents. Total oxidative status (TOS) was determined using Erel's method [14], based on the oxidation of ferrous ion to ferric ion in the presence of various oxidant species in serum. Ferric ion was measured using xylenol orange. The assay was calibrated with hydrogen peroxide (H_2O_2) and was measured using the Ilab 300 plus autoanalyzer. The intra-assay and inter-assay coefficients of variance were 5.6% and 9.5% respectively. The results were expressed in terms of micromolar H_2O_2 equivalent per liter (umol H_2O_2 Equiv/L). Total antioxidant capacity (TAC) was determined using an automated method developed by Erel [15], based on the de-coloration of 2.2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid radical cation (ABTS) by antioxidants present in serum. The color change was measured using the Ilab 300 Plus autoanalyzer. The reaction rate was calibrated with Trolox (a water-soluble analogue of vitamin E, 6-hydroxy-2.5.7.8-tetramethylchroman-2-carboxylic acid) and sample TAC values were expressed as mmol Trolox equivalent/L. The intra-assay and inter-assay coefficients of variance were 4.3% and 8.8% respectively.

Plasma superoxide dismutase (SOD) activity was measured according to the method described by Misra and Fridovich [16]. SOD-mediated inhibition of adrenalin auto-oxidation to adrenochrome was monitored. One unit of SOD activity was defined as the activity that inhibits the auto-oxidation of adrenalin by 50%. The intra-assay and inter-assay coefficients of variance were 6.3% and 9.2% respectively. The pro-oxidant/antioxidant balance (PAB) was measured using a method described by Alamdari et al. [17], in which 3,3',5,5'-tetramethyl benzidine and its cation are used as redox indicators participating in two simultaneous reactions. PAB values were expressed in arbitrary HK units, which correspond to the percentage of H_2O_2 in the standard solution. The concentration of sulfhydryl (SH) groups in plasma was determined using 0.2 mmol/L 5,5'--dithiobis (2-nitrobenzoic acid) (DTNB) as described by Ellmann [18]. DTNB reacts with aliphatic thiols (at pH 9.0), producing 1 mole of p-nitrophenol per mole of thiol. p-Nitrophenol was measured by spectrophotometry at 412 nm. Paraoxonase-1 (PON1) activity was measured as the rate of diazoxone hydrolysis and was measured spectophotometrcally in serum using a continuous spectrophotometer (Pharmacia LKB, Cambridge, UK) as described by Richter and Furlong [19].

Statistical Analysis

Data are shown as mean ± standard deviation for normally-distributed variables and as relative or absolute frequencies for categorical variables. Comparisons of continuous variables were performed using Student's *t*-test. The analyses of categorical variables used the chi-square test for contingency tables. A logarithmic transformation of TG levels, hsCRP and POase activity were performed because of the skewed distribution in the analysis using Student's *t*-test [20]. Spearman's nonparametric correlation analysis was employed to determine the correlations between the parameter of oxidative stress (TBARS) and lipid profile, and the oxidative stress status parameters. A multiple regression analysis was used to estimate the independent contribution of predictors to the variance in TBARS levels. Spearman's rho correlation test was used to screen the independent variables. If the P values were < 0.10, the variables were included in a further regression analysis. Weight gain, triglyceride, uric acid and hsCRP concentrations, as well as TAC (a representative parameter of the antioxidative defense status) and TOS (representative of oxidative stress) were included in the primary regression model. As smoking habits and vitamin intake could influence the TBARS concentration, those variables were also included in the regression model. The tolerance option was used to prevent multicollinearity among the independent variables [21].

All the statistical analyses were performed using Medcalc software (version 13.2). Statistical comparisons were considered significant at the 0.05 probability level.

Results

Table 1 shows the clinical and laboratory parameters of lipid status and oxidative stress status parameters in pregnant women grouped according to their smoking habits before pregnancy.

A significantly higher percentage of women who received (antioxidative) vitamin supplementation

Table 1. Clinical and laboratory parameters, oxidative stress/antioxidative defense parameters, PON1 activities and PON1 phenotypes distribution in the two study groups according maternal smoking habits before pregnancy

Parameter	Non-smokers (n = 44)	Smokers (n = 42)	P[1]
Age – years	29.9 ± 3.8	30.1 ± 4.6	0.862
Vitamins intake – n (%)	n (36%)	n (62%)	< 0.05
BMI – kg/m^2	26.3 ± 4.1	26.5 ± 2.8	0.869
Weight gain (%)	20.4 ± 6.6	26.5 ± 7.00	< 0.001
Week of gestation	34.4 ± 2.8	33.2 ± 1.7	0.912
SBP – mm Hg	107 ± 8.4	108 ± 7.0	0.852
DBP – mm Hg	69 ± 6.0	69 ± 7.0	0.902
Glucose – mmol/L	4.3 ± 0.5	4.4 ± 0.44	0.539
TC – mmol/L	7.0 ± 1.1	6.6 ± 1.1	0.057
LDL-C – mmol/L	3.3 ± 1.0	3.9 ± 1.	< 0.05
HDL-C – mmol/L	1.9 ± 0.5	2.0 ± 0.5	0.742
TG – mmol/L#	3.01 (2.377–3.825)	2.76 (2.53–3.027)	< 0.05
Apo A-I – g/L	1.6 ± 0.3	1.7 ± 0.2	0.090
Apo B – g/L	1.06 ± 0.18	1.03 ± 0.27	0.452
AIP	0.10 (–0.01–0.15)	0.18 (0.12–0.24)	0.183
Uric Acid – μmol/L	233.5 ± 45.4	247.1 ± 45.34	0.170
hsCRP – mg/L#	2.38 (1.71–3.31)	3.01 (2.38–3.82)	0.240
TBARS – μmol/L	1.7 ± 0.8	2.3 ± 1.0	< 0.05
LOOH – μmol/L	9.3 ± 2.7	3.4 ± 2.3	0.846
AOPP – μmol/L	21.6 ± 7.45	21.6 ± 7.0	0.971
PAB – HK units	159.4 ± 34.6	167.7 ± 29.2	0.250
TOS – μmol/L	15.9 ± 5.4	18.9 ± 5.4	< 0.05
TAC – mmol/L	1.28 ± 0.15	1.30 ± 0.18	0.571
SOD – kU/L	158.0 ± 11.16	150.3 ± 34.0	0.362
SH-groups – g/L	0.49 ± 0.06	0.48 ± 0.08	0.849
POase – U/L#	342.7 (231.5–507.3)	253.01 (201.0–318.5)	< 0.05
DZOase – U/L	5219 ± 2944	5107 ± 3577	0.877
PON1 phenotype (%)			
QQ	4.5%	3%	0.062
QR	34%	17%	
RR	61.5%	80%	

Data are expressed as mean ± SD; # – means and 95th confidence intervals derived from log-normal values.

[1] Continuous variables were compared using Student's *t*-test and categorical variables by the χ^2 test.

was present in the smokers' group (Table 1). Also, weight gain was significantly higher in that group compared with the non-smokers ($p < 0.001$). LDL-c concentrations were significantly higher and triglyceride concentrations significantly lower in the smokers' group. No other significant differences in lipid profile parameters were present between the two groups, but those parameter concentrations were close to high risk levels.

In this study only non-complicated pregnancies were investigated, so it is not surprising that no significant differences in glucose levels, uric acid levels and hsCRP concentrations were found between the two groups.

TBARS and TOS concentrations were significantly higher in the smokers' group, while PON1 activity was significantly lower (Table 1). The other oxidative stress status parameters were similar in both groups.

Table 2. Clinical and laboratory parameters, oxidative stress/antioxidative defense parameters, PON1 activities and PON1 phenotype distribution in the two study groups according to maternal vitamin supplementation during pregnancy

Parameter	No vitamin supplementation (n = 44)	Vitamin supplementation (n = 42)	P[1]
Age – years	30.1 ± 3.2	29.8 ± 4.1	0.906
Smoking – n (%)	n (36%)	n (62%)	< 0.05
BMI – kg/m^2	26.6 ± 3.6	26.3 ± 3.4	0.711
Weight gain (%)	23.2 ± 6.4	23.6 ± 8.4	0.803
Week of gestation	34.4 ± 2.9	34.5 ± 2.9	0.891
SBP – mm Hg	107 ± 10.1	108 ± 8.3	0.762
DBP – mm Hg	69 ± 7.1	68 ± 6.9	0.915
Glucose – mmol/L	4.4 ± 0.6	4.3 ± 0.4	0.727
TC – mmol/L	6.7 ± 1.0	6.9 ± 1.1	0.447
LDL-C – mmol/L	3.6 ± 1.0	3.7 ± 1.1	0.958
HDL-C – mmol/L	1.9 ± 0.4	2.0 ± 0.5	0.690
TG – mmol/L#	2.4 (2.18–2.65)	2.7 (2.49–2.94)	0.112
Apo A-I – g/L	1.6 ± 0.3	1.7 ± 0.3	0.788
Apo B – g/L	1.04 ± 0.18	1.07 ± 0.17	0.443
AIP	0.11 (0.021–0.140)	0.17 (0.100–0.218)	0.284
Uric Acid – μmol/L	235.9 ± 17.6	244.9 ± 13.4	0.368
hsCRP – mg/L#	2.9 (2.26–3.75)	2.5 (1.79–3.39)	0.790
TBARS – μmol/L	1.9 ± 0.3	1.9 ± 0.1	0.979
LOOH – μmol/L	9.1 ± 2.6	9.5 ± 2.3	0.343
AOPP – μmol/L	22.4 ± 7.5	20.7 ± 6.8	0.270
PAB – HK units	163.5 ± 31.8	163.4 ± 32.9	0.970
TOS – μmol/L	16.9 ± 5.9	17.6 ± 5.2	0.591
TAC – mmol/L	1.31 ± 0.16	1.28 ± 0.16	0.497
SOD – kU/L	147.9 ± 17.8	160.8 ± 17.9	0.128
SH-groups – g/L	0.49 ± 0.07	0.48 ± 0.08	0.529
POase – U/L#	257.0 (198.4–332.9)	334.0 (231.5–484.2)	0.239
DZOase – U/L	5145 ± 3040	5186 ± 3479	0.955

Data are expressed as mean ± SD; # – means and 95th confidence intervals derived from log-normal values.

[1] Continuous variables were compared using Student's *t*-test and categorical variables by the χ^2 test.

Table 2 shows the same parameters for women with or without (antioxidative) vitamin supplementation. No significant differences were found in any parameter between the two vitamin-supplementation groups (Table 2).

Spearman's correlation analyses were performed to test for associations between oxidative stress parameters and other parameters. (Table 3). TBARS was significantly positively correlated with BMI, weight gain, triglyceride, TOS, PAB concentrations and SOD activity, and significantly negatively correlated with TAC (Table 3).

Table 4 summarizes the multiple regression analysis to examine the influence of weight gain, smoking, TG, vitamin intake, uric acid, hsCRP, TAC and TOS as independent variables on TBARS. Weight gain, TAC and TOS concentrations were independently associated with the concentration of TBARS. Unexpectedly, habitual maternal smoking before pregnancy was not independently associated with the concentration of TBARS.

Table 3. Spearman's non-parametric correlations between TBARS and biochemical and oxidative status parameters

Parameter	**ρ**	**p**
BMI	0.710	< 0.001
Weight gain (%)	0.846	< 0.001
Glucose – mmol/L	0.146	0.278
T-C – mmol/L	0.082	0.546
LDL-C – mmol/L	0.125	0.353
HDL-C – mmol/L	0.058	0.671
TG – mmol/L#	0.415	< 0.001
Apo A1 – g/L	0.204	0.127
Apo B – g/L	0.171	0.205
AIP	0.206	0.125
Uric acid – μmol/L	0.257	0.056
hsCRP – mg/L	0.238	0.075
LOOH – μmol/L	–0.056	0.678
AOPP – μmol/L	0.120	0.375
PAB – HK units	0.417	< 0.001
TOS – μmol/L	0.739	< 0.001
TAC – mmol/L	–0.468	< 0.001
SOD – kU/L	0.314	< 0.05
SH-groups – g/L	–0.122	0.365
POase activity – U/L	–0.125	0.428
DZOase activity	0.171	0.212

Table 4. Multiple regression analysis for the association of various parameters with TBARS – Model 1

	TBARS $R^2 = 0.814$ **adjusted** $R^2 = 0.813$		
	β	**SE (β)**	***P***
Weight gain (%)	0.525	0.011	< 0.001
Smoking	0.110	0.124	0.116
Vitamin supplementation	–0.064	0.112	0.306
TG – mmol/L	0.014	0.087	0.848
Uric acid – μmol/L	–0.131	0.002	0.145
hsCRP – mg/L	0.081	0.015	0.195
TOS – μmol/L	0.367	0.016	< 0.001
TAC – mmol/L	–0.342	0.715	< 0.001

Table 5. Multiple regression analysis for the association of various parameters with TBARS – Model 2

	TBARS $R^2 = 0.788$ **adjusted** $R^2 = 0.762$		
	β	**SE (β)**	***P***
Weight gain (%)	0.847	0.009	< 0.001
Smoking	0.084	0.145	0.280
Vitamin supplementation	–0.059	0.129	0.394
TG – mmol/L	0.124	0.086	0.086
Uric acid – μmol/L	0.010	0.001	0.880
hsCRP – mg/L	0.122	0.017	0.073

To prevent multicolinearity, TAC and TOS concentrations as parameters of oxidative stress status were excluded from Model 2 (Table 5). This showed that weight gain was independently associated with the concentration of TBARS.

Discussion

Pregnancy is a physiological condition with changed lipid profile parameters [1] and increased susceptibility to oxidative stress [2]. During pregnancy, increased production of ROS exceeding the mother's antioxidant potential leads to oxidative stress, which can negatively affect the health of both the mother and the fetus, leading to complications in pregnancy, as well as adverse pregnancy outcomes [2]. It is well known that smoking is followed by intense oxidative stress [3]. Pregnancy

complications can arise due to the increased free radical damage caused by smoking [4]. Cigarette smoking leads to increases in the concentrations of serum TC, LDL-c and TG, and decreases in the level of anti-atherogenic HDL-c [22]. It is presumed that, as Jeeyar et al. wrote, "nicotine stimulates the sympathetic adrenal system leading to increased secretion of catecholamines resulting in increased lipolysis and increased concentration of plasma-free fatty acids, which results in increased synthesis of hepatic TG, along with VLDL-c in the blood stream" [22].

High levels of LDL-c, VLDL-c, and TG are strongly associated with the development of coronary artery disease [22].

Tobacco smoke contains a large number of free radicals that are capable of initiating or promoting oxidative injury [5]. Smokers have higher concentrations of oxidatively modified lipid products than non-smokers [22]. The current study showed that pregnant women who smoked had significantly higher LDL-c ($p < 0.05$) than pregnant non-smokers (Table 1). LDL-c from smokers was more susceptible to peroxidative modification when compared with that from non-smokers [23], and oxidation of LDL-c might be an important mechanism whereby cigarette smoking can accelerate atherogenesis [22].

PON1 (aryldialkylphosphatase, EC 3.1.8.1) is an esterase binding to HDL that metabolizes oxidative LDL-c particles and has strong antioxidant potential [8]. Min et al. reported that low levels of PON1 in preeclamptic women lead to lipid peroxidation and increased susceptibility to the oxidation of LDL-cholesterol [24]. Low PON1 activities could be associated with an increased risk of developing CVD [24]. Significant reduction in PON1 activity during uncomplicated pregnancies has been described by Ferre et al. [25]. In the present study, pregnant women who smoked had significantly lower PON1 activity ($p < 0.05$) than pregnant non-smokers. Although PON1 activity differed significantly between smokers and non-smokers, no difference was found in the distribution of PON1 phenotypes between those groups. The PON1 polymorphism phenotype appears to be involved in the pathogenesis of some diseases, especially CVD and diabetes mellitus type 2 [8].

Pregnancy has been described in numerous studies [1, 2, 6] as a physiological state with a pro-atherogenic effect, an altered lipid profile and increased oxidative stress. The results of the present study show that pregnant smokers are at a greater risk of developing atherosclerosis than pregnant non-smokers, as they have significantly higher levels of LDL-c and TBARS, as well as lower PON1 enzyme activity. Pregnant women who smoked before pregnancy had higher TOS values ($p < 0.05$) compared with women who were non-smokers (Table 1). Also, their concentration of TBARS was significantly higher, while PON1 activity was significantly lower compared with non-smokers. These results indicate that pregnant smokers are in a state of increased oxidative stress. This is compatible with the findings of Aycicek et al. [26], who reported that TOS was significantly higher in the active and passive smoker groups than in the non-smoking controls. TBARS includes MDA and related aldehydes, which are by-products of lipid peroxidation and which are responsible for some of the damaging effects of free radicals on DNA and on cell membranes [27]. According to Devasagayam et al., the damage to some cellular structures caused by lipid peroxidation "is highly detrimental to the functioning of the cell and its survival" [27].

Higher concentrations of hydroperoxides have been found in pregnant smokers than in non-smokers [5]. Chelchovska et al. also found a positive correlation between MDA and cotinine concentrations (the major metabolite of nicotine in body fluids), both in smoking mothers and in their children [5].

Regarding the effects of smoking on antioxidative markers, a linear relationship has been found between smoking duration, daily cigarette consumption, lipid peroxidation, and nitric oxide formation both in plasma and erythrocytes [28]. In that study, when smokers stopped smoking for six months, plasma levels of vitamin C and E, beta-carotene, erythrocyte superoxide dismutase, catalase and glutathione peroxidase were higher than in those who continued to smoke, but remained significantly lower than those of a matched non-smoking group [28].

As a further step, the authors of the current study wanted to establish whether smoking was the primary reason for increased concentrations of TBARS, the main indicator of oxidative stress. For this purpose, multiple regression analysis was used as a test model in which smoking and other parameters that showed a significant correlation with TBARS were included. The results unexpectedly showed that weight gain during pregnancy, but not smoking, was an independent positive predictor of an increased concentration of TBARS (Table 5).

Among the participants in the current study, pregnant smokers had a significantly higher weight gain during pregnancy ($p < 0.001$) than non-smokers (Table 1). Since cigarette smoking decreases appetite [29], it seems likely that the gains in weight were a consequence of stopping cigarette smoking during pregnancy.

Weight gain during pregnancy is also associated with altered lipid parameters that have a pro-atherogenic character [30]. Although smoking and

diabetes have been associated with increased oxidative stress in a number of studies, the finding that obesity, as measured by BMI, is independently associated with oxidative stress is relatively new [30]. In view of the results of the present study it can be said that pregnant smokers who have a significantly higher weight gain than pregnant non-smokers undergo greater oxidative stress. Although all the pregnant women who smoked in the present study finished their pregnancies without complications, the results suggest that weight gain is an independent risk factor for the development of oxidative stress. However, the pregnant women who had smoked before pregnancy had higher TACs than pregnant non-smokers, suggesting that these women preserved their antioxidant potential, and this may explain why they brought their pregnancies to term without complications.

Pregnant women who took vitamin supplements showed no significant differences in the levels of antioxidant defense parameters in comparison with women who did not use supplements (Table 2). This may be explained by the fact that all the pregnant women were advised to include a lot of fruits and vegetables in their diets and thus they could have had a sufficient intake of antioxidants.

In the present study there was a significantly higher percent of smokers in the group of women who received antioxidative supplementation (62%). Cigarette smoke decreases antioxidant potential, which may explain the lack of any demonstrable effect of supplementation on the parameters of antioxidant defense. All the women were healthy with an intact antioxidant potential that was not compromised by any complications of pregnancy.

Studies have shown lower α-tocopherol retinol, and β-carotene levels in maternal plasma and cord plasma in smokers [5], though another study showed no differences between smokers and non-smokers in antioxidant vitamin levels in maternal plasma and cord plasma [3].

Some authors have shown that taking vitamins, especially vitamin C and vitamin E, may have a positive effect on pregnancy and birth outcome [6]. On the other hand, a healthy diet rich in fruits and vegetables can also provide enough vitamins and minerals [7] for a normal pregnancy.

The present study confirmed that smoking habits before pregnancy were associated with increased oxidative stress, lipid changes expressed through the creation of increased LDL-c, and decreased PON1 activity. A multiple regression analysis showed that weight gain during pregnancy, not smoking, increases TBARS. In addition, the results indicate that vitamin supplementation has no effect on the parameters of oxidative stress status in healthy pregnant women.

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Address for correspondence:

Aleksandra Stefanović
Department of Medical Biochemistry
University of Belgrade
Vojvode Stepe 450
11000 Belgrade
Serbia
E-mail: alex@pharmacy.bg.ac.rs

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