

# Analysis of the level of free radical lipid peroxidation and antioxidative system activity during different pregnancy weight gain and multifetal pregnancy

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**Objective.** To elucidate the level of lipid peroxidation and changes in oxidative system activity in different degrees of pregnancy weight gain and in multifetal pregnancies.

**Materials and methods.** Eighty-six pregnant women (mean age, 28 years) treated at the Department of Obstetric Pathology, Vilnius City University Hospital were examined in 2003 at the 29th week of pregnancy on average. The patients were divided into three groups according to weight gain: Group I – increase up to 10 kg, Group II – from 10 to 15 kg, and Group III – 15 kg and more. Multifetal pregnancy was diagnosed during ultrasound examination.

Lipid peroxidal status was evaluated according to malonaldehyde concentration in blood serum. The status of the antioxidative system was analyzed by measuring catalase activity in blood serum. Ceruloplasmin concentration was determined by the method of Ravin, modified by Bestuzheva and Kolbe. Supplementary markers of acute phase, sialic acids, were determined by the method of Voren.

**Results.** Lipid peroxidation level in cases of higher weight gain was statistically significantly higher (25%) than in cases of lower weight gain.

CAT activity with weight gain increase did not differ statistically significantly, however, in the group of highest weight gain it was by 15% higher than in the group of lowest, and by 27% higher than in the group of average weight gain. The indices of CER and SA in all groups did not differ. The indices of antioxidant CAT were related to weight gain, blood erythrocyte and Hb levels.

In case of multifetal pregnancy, malonaldehyde concentration increased by 25%, but catalase activity decreased by 19%. Ceruloplasmin concentration was highest in multifetal pregnancies (43%).

Catalase activity was adversely related to blood erythrocyte and thrombocyte counts and lipid peroxidase activity.

**Conclusions.** Lipid peroxidation is significantly more active in cases of a higher pregnancy weight gain. Activity of lipid peroxidation is slightly higher during multifetal pregnancy. The antioxidative system activity is only slightly related to weight gain and the number of foetuses. A dependency between the CER and SA indices and body weight was noted. A significant disbalance of antioxidative system components during multifetal pregnancy was observed. The level of lipid peroxidation was best reflected by the activity of the principal antioxidation enzyme CAT.

**Key words:** lipid peroxidation, antioxidative system, pregnancy, weight gain, multifetal pregnancy

## INTRODUCTION

In the normal physiological status of the organism, one of the elements of cell metabolism, lipid peroxidation, takes place at a certain speed which is strictly regulated

by the antioxidative system, in which such enzymes as catalase, superoxide dismutase, glutathionperoxidase, and glutathionreductase play a key role. Metal-binding proteins, such as ceruloplasmin, albumins, ferritin, transferrin, myoglobin, also exhibit antioxidative properties.

The antioxidative system is one of the adaptive protection systems of the organism. It regulates lipid peroxidation processes, neutralizes harmful effects of lipid peroxidation products (peroxides) both in healthy organism and in case of various diseases (1–4).

Free radicals (FR) are chemical products capable of independent existence with one or more unpaired ions. FR participates in phagocytosis, synthesis of prostaglandins, steroid and thyroid hormones. Reactive particles of oxygen are involved in the processes of cell growth, division and death. Their low concentrations are beneficial for the organism.

It is known that FR participate in the pathogenesis of at least 50 diseases (1, 2, 4). FR especially actively injure the endothelium of blood vessels during oxidation of membrane lipoproteins and polyunsaturated fat acids. In case of the activation of lipid peroxidation and excess FR production, the protective mechanisms of the antioxidative system are activated and protect the organism from the activity of free radicals. Such process is called oxidative stress.

In the opinion of numerous researchers, pregnancy invokes oxidative stress. Lipid peroxidation activity in the blood serum of healthy pregnant women in comparison with non-pregnant is increased. The intensity of stress during different periods of pregnancy varies. With the progression of a normal pregnancy, gradual suppression of lipid peroxidation takes place through the activated production of endogenous antioxidants to protect the fetus from toxic oxygen effects (5). At delivery, due to the rapidly increasing oxygenation during intensive breathing and labor efforts, shifts of the antioxidative system are activated, which in turn are caused by reactive oxygen particles produced in the uterus and placenta tissues (6).

According to data of some authors, antioxidant (antioxidative enzymes such as ceruloplasmin, superoxide dismutase) concentration increase at the beginning of pregnancy helps the organism to protect itself against an increasing intensity of the oxidative stress (5). The first trimester miscarriage is considered to be related to a decreased activity of the antioxidant superoxide dismutase (7).

Numerous experimental studies have demonstrated that oxidative stress is induced in the placental tissues, trophoblasts, the endothelium of maternal blood vessels (5, 8). The endothelium damaged by FR locally releases vasoconstrictive mediators (endothelin, thromboxan) and produces pathological processes such as blood vessel inflammation, vasoconstriction, thrombosis, ischemia (9). Active mediators, tissue cytokines evoke degeneration on the periphery of the placenta, apoptosis and participate in the pathogenesis of miscarriage, pre-eclampsia and pre-term delivery (10). Placental FR, getting into general circulation, invoke the inflammatory status of the maternal organism, blood vessel spasm, induce pla-

cental hypoxia (8). A marked increase of stress activity is noted at the second and third trimesters of pregnancy and for a certain period after delivery, in case of chronic hypertension and pre-eclampsia, gestational diabetes (11–14). Activated free radical lipid peroxidation can influence an increased duration of delivery (6).

In the literature, there are numerous data on the oxidative stress during pregnancy and delivery, in cases of various pregnancy and extragenital pathologies. However, there is not much information on oxidative stress in multifetal pregnancies, on the influence of weight increase on lipid peroxidation degree and antioxidative system activity fluctuations.

**The objective of the current study** was to elucidate lipid peroxidation degree and antioxidative system activity changes in various degrees of pregnancy weight gain and in multifetal pregnancies.

## MATERIALS AND METHODS

Eighty-six pregnant women that were treated at the Department of Obstetric Pathology, Vilnius City University Hospital, were studied. The average age of the participants was 28 years. The pregnant women were examined on the 29th week of pregnancy on average.

Only women without acute inflammatory diseases, diabetes, kidney and liver diseases, pre-eclampsia and eclampsia were included into the study sample. The patients were under treatment usually due to threatening miscarriage, vomiting due to pregnancy, colpitis and fetal hypotrophia.

All women were clinically examined, and anthropometrics (stature, weight measurements, body mass index (BMI) calculation according to the formula [weight kg/ (stature m)<sup>2</sup>], weight gain during pregnancy), blood, biochemical, urine, cytological, instrumental (ultrasound examination of internal organs of mother and fetus) examinations were performed. Blood samples for oxidative stress indices determination were taken before treatment, after admission to hospital.

Results of analysis were evaluated taking into account weight gain during pregnancy and the number of fetuses *in utero*.

Weight gain was measured as part of the standard procedure. Routinely a woman gains about 14 kg of weight during pregnancy (15). Pregnant women were divided into three groups according to the degree of weight gain: group I – weight increase up to 10 kg, group II – from 10 to 15 kg, and group III group – 15 kg and more. Multifetal pregnancy was determined on the basis of ultrasound examination.

The quantity of lipid peroxide metabolites produced during free-radical reactions was determined according to malondialdehyde (MDA) concentration in blood serum. MDA is found in human blood plasma and is considered to be a marker of oxidative stress,

as it shows the degree of membrane lipid peroxidation. Malondialdehyde, the final product of fat acid peroxidation, reacts with thiobarbituric acid, making a colored complex defined by absorption maximum at a wavelength of 532 nm (16).

The status of the antioxidative system was analyzed by measuring the catalase (CAT) activity and concentrations of ceruloplasmin (CER) and sialic acids in blood serum. CAT, as glutathione peroxidase and superoxide dismutase, belongs to the enzymes that indicate intracellular antioxidative activity. Catalase activity is defined by a decrease of hydrogenium peroxide quantity during a certain length of time. Hydrogenium peroxide makes a stable colored complex with ammonium molybdate at a wavelength of 410 nm (17). Activity of the enzyme is expressed in nmol/ml.

Ceruloplasmin concentration was determined according to the method of Ravin, modified by Beszuzheva and Kolbe. Activity of the enzyme is expressed in mg/l (18).

An additional marker of the acute phase of inflammation, sialic acids (SA) (acetylated compounds of neuraminic acid), was determined by the method of Woren, using the reaction of periodate-thiobarbituric acid. Reactive FR can damage tissues during the oxidative stress and produce inflammation. SA activity is expressed in mmol/l (19).

Statistical data analysis was performed using the SPSS package v.10. Relations among the indices were tested with the help of correlation analysis (Pearson's correlation coefficient  $r$ ). Mean data values are

Table 1. Clinical characteristics of examined pregnant women

Patients (n = 86)	Average	95% CI
Age (years)	27.7	26.32–29.10
Pregnancy weeks	28.6	26.5–30.6
BMI	23.5	21.6–25.4
Body weight increase (kg)	9.8	8.3–11.2
Hemoglobin (g/l)	113	110.4–115.6
Erythrocytes ( $10^{12}/l$ )	3.7	3.68–3.86
Thrombocytes ( $10^9/l$ )	208.6	196.9–220.3

Table 2. Indices of lipid peroxidation and antioxidative system activity (means and 95% CI) in three groups of weight increase during pregnancy

Weight increase (kg) group /number of women	MDA	CAT (nmol/ml)	CER (mg/l)	SA (mmol/l)
Group I: up to 10 kg (n = 36)	5.51 (4.9–6.0)	31.08 (27.8–34.4)	573.6 (525.5–621.8)	2.15 (2.02–2.27)
Group II: 10–14.9 kg (n = 22)	6.33 (5.13–7.52)	28.05 (23.7–32.4)	560.6 (494.5–626.8)	2.15 (2.03–2.27)
Group III: 15 and more kg (n = 18)	6.86* (5.39–8.3)	35.77 (22.4–49.1)	596.7 (489.1–704.7)	2.12 (1.89–2.34)

\*  $p < 0.05$  when Group I and III are compared.

presented with 95% confidence interval (CI). Differences among the groups were considered statistically significant at the probability of error  $p < 0.05$ .

## RESULTS

Characteristics of 86 pregnant women examined are presented in Table 1. Their gestational age, body weight increase, BMI, mean blood indices were corresponding to the recommended values (norms). Thirty-six pregnant women gained up to 10 kg, 22 up to 15 kg, and 18 gained 15 kg and more. Six women were expecting twins. Besides, 28 confessed that they were smoking before and during pregnancy.

The lipid peroxidation and antioxidative system activity indices were measured in the groups of women with low, moderate and high weight increase (Table 2).

Table 2 shows that the level of lipid peroxidation (reflected by the MDA index) was highest in the blood of the pregnant women that had the highest weight gain. In them, as compared to Group I, it was higher by 25% ( $p < 0.05$ ).

CAT activity with weight gain increase did not differ statistically significantly, however, in women of Group III it was by 15% higher than in Group I and by 27% than in Group II. The indices of CER and SA in all groups showed no notable differences.

Correlation analysis in the group of the lowest weight gain revealed that activity of antioxidative markers CER and SA was directly correlated with the level of weight increase ( $r = 0.36$  and  $0.41$ , respectively;  $p < 0.05$ ).

In Group III, CER activity directly correlated with MDA ( $r = 0.58$ ;  $p < 0.025$ ), but negatively with CAT ( $r = -0.49$ ;  $p < 0.05$ ). The indices of red blood exerted a direct influence on the antioxidative system: with increasing erythrocyte count and Hb concentration, CAT activity was increasing ( $r = 0.56$ ;  $p < 0.05$ ) and ( $r = 0.6$ ;  $p < 0.025$ ).

Lipid peroxidation level and antioxidative system activity were compared for women with single fetus and multifetal pregnancies (Table 3).

In our study, the lipid peroxidation level in multifetal pregnancies was higher by 25% (however, sta-

Table 3. Indices of lipid peroxidation and antioxidative system (means and 95% CI) in single fetus and multifetal pregnancies

Pregnancy / number of women	MDA (nmol/ml)	CAT (nmol/ml)	CER (mg/l)	SA (mmol/l)
Group I: single fetus (n = 80)	6.08 (5.57–6.59)	30.8 (28.5–33.02)	554.6 (522.2–587.1)	2.16 (2.07–2.24)
Group II: multifetal (n = 6)	7.6 (3.1–12.1)	24.8 (19.8–29.8)	792.97* (548.6–1037.4)	2.08 (1.97–2.2)

\*  $p < 0.001$  when group I and II are compared.

tistically insignificantly), and antioxidant CAT activity was lower by 19%. Relative difference between MDA and CAT activity was 0.3 (in the case of normal single fetus pregnancies this ratio was 0.19). CER concentration indicating activity in the blood of Group II patients was higher by 43% ( $p < 0.001$ ) as well. SR indices statistically did not differ.

In Group II, the index of lipid peroxidation level positively correlated with CER and SR ( $r = 0.89$  and  $0.91$ , respectively;  $p < 0.025$ ). CAT activity was inversely related with erythrocyte ( $r = -0.98$ ;  $p < 0.001$ ), thrombocyte ( $r = -0.85$ ;  $p < 0.025$ ) counts and MDA activity ( $r = -0.84$ ;  $p < 0.05$ ).

## DISCUSSION

Causes of oxidative stress activity changes are intensively investigated during experimental modeling of the processes that take place in the uterus and the placenta.

Our analysis of relations between oxidative system and weight gain during pregnancy revealed that lipid peroxidation level in case of higher weight gain was statistically significantly higher (25%) as compared to pregnant women with a low weight gain. Differences of antioxidative system markers were insignificant. However, these markers are related to weight gain, erythrocyte and Hb levels in blood.

Weight increase during pregnancy most probably caused blood lipidemia and increased lipid autooxidation (11–13). Increasing dislipidemia in the process of weight gain at pregnancy can intensify the oxidation processes, as spontaneous lipid autooxidation into peroxides is taking place. The intensifying FR production „exhausts“ the protective potential of the antioxidative system. Experimental animal studies have shown that lipid peroxidation is induced in the placenta and tissues of the uterus at the second half of pregnancy. Simultaneously, antioxidative defense in the myometrium, endometrium and liver is intensified (20). Oxidative stress in the placenta induces apoptosis and cytokine secretion, and in this way participates in the pathogenesis of miscarriages, pre-eclampsia, preterm labor (8, 9).

Our study revealed that activity of the antioxidative system in the case of multifetal pregnancy becomes insufficient or inadequate. Probably the antioxi-

dants participating in this process cannot neutralize completely the reactive radicals of oxygen or their expenditure becomes excessive. Defensive activity of the antioxidant enzyme CAT was adversely related to thrombocyte and erythrocyte levels and MDA activity.

However, activity of CER, which is also characterized by antioxidant properties, was statistically significantly higher (by 43%), and this most probably determined the activated lipid peroxidation, increased production of pro-inflammatory cytokines in the placenta at the conditions of oxidative stress. It is known that cytokines in systemic bloodstream induce inflammatory reactions, activate leukocytosis, and evoke activation of endothelium cells (8). Probably CER plays a rather significant role in the equilibrium system of oxidative stress, acting both as an FR suppressor and regulator of inflammation homeostasis, Fe metabolism, especially under conditions of relative hypoxia during multifetal pregnancy (21, 22).

It is established that free Cu and Fe ions are powerful catalyzers. CER, metalloprotease, while binding Cu protects from oxidative effects of Cu ions. As the principal ferroxidase of plasma, CER takes part in oxidation processes of Fe ions, binds these ions with transferrin and transports them to liver and other tissues. It is known that lipid peroxides produced during oxidative reactions destroy cells. Intracellular Fe and Cu ions are released into surrounding tissues, thus triggering peroxidation reactions. In this way CER protects from participation of Fe ions in FR production. CER is considered to be a destroyer of cellular superoxides and other FR. Besides, CER has a unique property to mobilize Fe from liver and macrophages under hypoxic conditions and enhances Fe penetration into cells, Fe circulation between tissues and cells (22, 23). CER concentration was found to increase during pregnancy and during various hypoxic conditions of the organism. It is important to underline that CER, a marker of acute inflammation phase, which in the antioxidative defense chain becomes actual due to its complex role, can be beneficial for the evaluation of oxidative stress status at pregnancy.

Our study has confirmed that free radical oxidation during pregnancy becomes activated. The inten-

sity of the oxidative stress adjusts to the dynamic physiology of pregnancy, mother's body weight and changes of blood lipid concentration. The antioxidative system is heavily loaded in case of multifetal pregnancy.

## CONCLUSIONS

1. Lipid peroxidation was significantly more active in case of higher weight gain during pregnancy.

2. Activity of lipid peroxidation was slightly higher in case of multifetal pregnancy.

3. Activity of antioxidative system is only slightly related to the weight gain and the number of fetuses. A relation between the CER and SA indices and body weight of pregnant women was noted.

4. In case of multifetal pregnancy, a notable antioxidation system component disbalance was observed. The level of lipid peroxidation is best reflected by activity of the antioxidation enzyme CAT.

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## LAISVARADIKALINĖS LIPIDŲ PEROKSIDACIJOS LAIPSNIO IR ANTIOKSIDACINĖS SISTEMOS AKTYVUMO IŠTYRIMAS ESANT AVAIRIAM NĖDĖIŲŲ PRIEAUGIUI IR DAUGIAVAISIAM NĖDTUMUI

Santrauka

**Tikslas.** Darbo tikslas buvo išsiaiškinti lipidų peroksidacijos laipsną ir antioksidacinės sistemos aktyvumo pokyčius esant avairiam nėdėiųjų prieaugiui ir daugiavaisiam nėdtumui.

**Medžiaga ir metodai.** Tirtos 86 nėdėios moterys, kurios gydėsi VMUL Akušerijos patologijos skyriuje 2003 metais. Tyrimo dalyvavusių nėdėiųjų vidutinis amžius – 28 metai.

Moterys buvo tiriamos vidutiniškai 29 nėštumo savaitę. Nėštiosios pagal svorio prieaugį suskirstytos į tris grupes: I grupė – prieaugis iki 10 kg, II grupė – nuo 10 iki 15 kg ir III grupė – 15 kg ir daugiau. Daugiavaisis nėštumas buvo nustatytas ultragarsiniu tyrimu.

Tiriamųjų lipidų peroksidinė būklė buvo vertinama pagal malondialdehido koncentraciją kraujo serume. Antioksidacinės sistemos būklė buvo analizuojama matuojant katalazės aktyvumą kraujo serume. Ceruloplazmino koncentracija buvo nustatoma pagal Ravino metodiką, modifikuotą Bestuļevos ir Kolbe. Papildomas ūmios fazės įymuo – siauro rūgštys – buvo nustatomos pagal Voreno metodiką.

**Rezultatai.** Lipidų peroksidacijos laipsnis, esant didesniam svorio prieaugiui, buvo statistiškai reikšmingai didesnis (25%), lyginant su mažu prieaugiu turinėjusiose nėštiosiose.

KAT aktyvumas didėjant prieaugiui visose grupėse ryškiai nesiskyrė, tačiau, turinėjus didžiausią prieaugį, buvo 15% didesnis nei nėštiosiose, turinėjus mažiausią prieaugį, bei 27% didesnis nei nėštiosiose, turinėjus vidutiną svorio prieaugį CER ir SR rodmenys visose grupėse praktiškai nesiskyrė. Antiok-

sidatoriaus KAT rodmenys buvo susiję su svorio prieaugiu bei eritrocitų ir Hb kiekiais kraujyje.

Esant daugiavaisiam nėštumui padidėjo malondialdehido koncentracija 25%, sumažėjo katalazės aktyvumas 19%, tačiau ceruloplazmino koncentracija buvo didžiausia – 43%.

Katalazės aktyvumas atvirkštiniais ryšiais buvo susijęs su eritrocitų, trombocitų kiekiu kraujyje bei su lipidų peroksidacijos aktyvumu.

**Išvados.** Lipidų peroksidacija gerokai aktyvesnė esant didesniam nėštiosiose prieaugiui. Lipidų peroksidacijos aktyvumo laipsnis didesnis daugiavaisio nėštumo atveju. Nustatyta nedidelė antioksidacinės sistemos aktyvumo priklausomybė nuo svorio prieaugio ir nuo vaisių skaičiaus. Pastebėta priklausomybė tarp CER bei SR rodmenų ir nėštiosiose svorio. Esant daugiavaisiam nėštumui stebimas ryškus antioksidacinės sistemos komponentų disbalansas. Lipidų peroksidacijos laipsnis geriausiai atspindėjo pagrindinio antioksidatoriaus fermento KAT aktyvumas.

**Rakta požymiai:** lipidų peroksidacija, antioksidacinė sistema, nėštumas, svorio prieaugis, daugiavaisis nėštumas