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THE DEVELOPMENT OF OXIDATIVE STRESS IN THE LIVER AND PANCREAS OF RAT PUPS EXPOSED TO MATERNAL PARTIAL FOOD DEPRIVATION DURING PREGNANCY

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It is known that a deficiency of carbohydrates in pregnant women leads to a delay in intrauterine development in the fetus, causing stress in the mitochondria. The liver is highly susceptible to oxidative stress, taking into account that it is rich in mitochondria. There is a lack of studies on the pancreas of an offspring whose mothers received poor nutrition.

The aim of the study – identification and study of the harmful effects of partial food deprivation in pregnant women on the tissues of the pancreas and liver of the offspring.

Materials and methods. The state of oxidative-antioxidant homeostasis in the tissue of the pancreas and liver was determined in 67 rats, which were divided into 4 groups: control group, newborn rats, 1-month-old rats, 3-month-old rats. The model of partial food deprivation was applied to mother rats of the studied groups during pregnancy.

Results. The study of lipid peroxidation indicators in the pancreatic tissue of newborn rats showed the presence of activation of lipid peroxidation. In the tissue of the pancreas, the activity of superoxide dismutase decreased significantly in the 4th experimental group below the control by 45.2% ($p<0.01$). The same happened with the activity of catalase. The use of partial food deprivation in mothers led to the activation of lipid peroxidation in the hepatocytes of rat pups, in particular, the maximum content of diene conjugates increased reliably by 95.4% ($p<0.01$), and reactive substances of thiobarbituric acid – by 64.4% ($p<0.01$) in the 3rd experimental group compared to the control group. In the 3rd experimental group, there is a sharp decrease in the activity of superoxide dismutase and catalase, and in the 4th group, the indicators balance out and almost reach the target level of the control group.

Conclusions. A high probability in the development of liver pathologies within one month after birth and a complicated course of pancreatic diseases at all stages of the experiment has been proven.

Key words: oxidative stress, pancreas, liver, food nutritional deprivation, pregnancy.

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РОЗВИТОК ОКСИДАТИВНОГО СТРЕСУ У ПЕЧІНЦІ ТА ПІДШЛУНКОВІЙ ЗАЛОЗІ ЩУРЯТ, МАТЕРІ ЯКИХ ПІД ЧАС ВАГІТНОСТІ ЗАЗНАЛИ ЧАСТКОВОЇ ХАРЧОВОЇ ДЕПРИВАЦІЇ

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У статті експериментально доведено шкідливий вплив часткової харчової депривації у вагітних на тканини підшлункової залози та печінки потомства. В одномісячних щурят розвиток оксидативного стресу зі зниженням активності антиоксидантної системи у тканинах печінки сягає свого максимуму, тоді як у тканинах підшлункової залози досліджувані показники зазнають найбільших змін наприкінці 3 місяця від народження. Тому можна говорити про високу ймовірність розвитку патологій печінки у термін один місяць після народження і зростання ризику розвитку ускладненого перебігу захворювань підшлункової залози з народження до 3 місяців спостереження.

Ключові слова: оксидативний стрес, підшлункова залоза, печінка, харчова депривація, вагітність.

Introduction. Throughout pregnancy, the developing fetus relies entirely on the maternal environment for nutrition. The intrauterine setting serves as a pivotal determinant in the intrauterine programming of chronic diseases in adulthood, encapsulated by the concept known

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Стаття поширюється на умовах ліцензії

as the Fetal Origin of Adult Disease (FOAD) [1]. Pregnancy and early postnatal development constitute metabolically intricate periods for both the mother and offspring [2].

Carbohydrate deficiency in pregnant women is recognized for inducing intrauterine growth retardation in the fetus, triggering stress in the mitochondria, a crucial organelle in energy production. Mitochondria, pivotal in aerobic respiration, utilize oxygen as the final electron acceptor; however, the consequence is the hyperproduction of reactive oxygen species (ROS) and ROS byproducts [3, 4]. While low levels of ROS are essential for specific enzymatic reactions and signaling pathways, an excess presence leads to oxidative damage to macromolecules. Oxidative stress ensues when there is an imbalance between ROS production and antioxidant enzymes responsible for converting ROS into less harmful molecules [5]. The mitochondrial electron transport chain, acting as both a source and target of ROS, typically coexists with oxidative stress in cases of mitochondrial dysfunction [6].

The liver is highly susceptible to oxidative stress because of its richness in mitochondria and integral role in nutrient metabolism [7]. Various chronic liver diseases, such as nonalcoholic steatohepatitis, exhibit heightened markers of oxidative stress often accompanied by the accumulation of damaged or dysfunctional mitochondria [8]. Studies involving intrauterine growth retardation in offspring reveal evidence of hepatic oxidative stress. For instance, newborn pigs experiencing growth retardation displayed elevated levels of hepatic alpha-1-acid glycoprotein at birth, signifying systemic oxidative stress in hepatocytes [9]. Pro-/antioxidant balance could depend on genetic polymorphism of detoxification liver enzymes. For instance, according to *cytochrome P-4502E1 (CYP2E1)* polymorphism, there is a higher concentration of diene conjugates in the blood and lower plasma catalase activity in the individuals with *"rapid metabolizers"* genotype comparatively with the persons with *"slow metabolizers"* genotypes [10].

Comparatively poor attention has been paid to pancreatic dysfunction despite the fact that it may form the basis for identifying risk groups prone to glandular disease. There is a lack of studies focusing on the functional state and mechanisms of pancreatic dysfunction in children whose intrauterine development occurred under the influence of adverse environmental factors during maternal pregnancy. Among those influential factors, an unbalanced diet in pregnant women, characterized by a deficiency in protein and other essential nutrients plays the main role. The mechanisms of pancreatic cell damage in various pancreatic pathologies often involve oxidative stress resulting from disturbance in oxidative-antioxidant homeostasis (OAH) [11].

Considering the insufficient study of the role of OAH disorders in the pathogenesis of pancreatic and hepatic damage caused by negative exogenous factors taking effect on the mother-fetus system, the significance of experimental research of this scientific problem becomes obvious. Consequently, a better understanding of these mechanisms is crucial, hence the relevance and need for experimental studies to shed light on this complex scientific problem.

The aim of the study is the identification and study of the harmful effects of partial food deprivation in pregnant women on the tissues of the pancreas and liver of the offspring.

Materials and Methods. The research was conducted at the vivarium of I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine. 67 rats were involved for the evaluation of OAH in the pancreatic and liver tissues. The rats were divided into groups: the 1st group (control) consisted of 15 animals with mothers following a balanced diet during pregnancy, the 2nd group, (experimental) consisted of 25 newborn rats from mothers having unbalanced diet, the 3rd group consisted of 1-month-old rats (14 rats), and the 4th group consisting of 3-month-old rats (13 rats).

The model of food deprivation was implemented using a patented method (Ukraine Patent No. 147539 dated 19.05.2021), involving conditions of partial nutritional starvation with a 70% reduction in the ration while maintaining nutrient balance (proteins, fats, carbohydrates) and essential vitamins and minerals [12]. To control starvation and prevent coprophagy, a special grid was placed on the bottom. The rats in the experimental group were individually given a daily ration of 15.0 g of food grain mixture, with unlimited access to water and a 12-hour light/dark regimen. Under these conditions, litter (sawdust) and excrement were excluded from the diet of rats. Partial food deprivation in rats was applied for 15 days from the moment of fixation of pregnancy. Rats in the control group received a daily feed ration of 50 g.

Animals were euthanized by bloodletting under sodium thiopental anesthesia (40 mg/kg of the animal's body weight intraperitoneally), and the pancreas and liver were subsequently removed. The determination of lipid peroxidation (LPO) activity involved assessing diene conjugates (DC) and reactive substances of thiobarbituric acid (TBA-RS), while the antioxidant defence system (AOS) activity was determined by measuring the activity of superoxide dismutase (SOD) and catalase (CAT) using the BioMajesty JCA-BM6010/C biochemical analyzer (manufacturer: DiaSys Diagnostic Systems).

Experimental procedures met the national "General Ethical Principles for Animal Research" (Ukraine, 2001), agreed with the recommendations of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 18.03.86), the Declaration of Helsinki adopted by the General Assembly of the World Medical Association (1964–2000), the Charter of the Ukrainian Bioethics Association, and the norms of Good Laboratory Practice (GLP) (1992). Statistical analysis of the results was performed using STATISTICA-10 software, and Mann-Whitney U-test was used to assess the reliability of differences.

Results and Discussion. The study was conducted to determine the adverse effects of maternal partial nutritional deficiency during pregnancy on the pancreatic and liver tissues of the offspring.

Prooxidant/antioxidant balance in the pancreas. The examination of LPO indicators in the pancreatic tissue of newborn rats (Table 1) revealed a notable activation of lipid

peroxidation: a significant increase in the DC and TBA-RS levels compared to the control group. In 1-month-old baby rats, the studied parameters probably exceed the control levels observed both in new-borns and in the same age group. Although the activation of POL is comparatively milder than in neonates, accumulation of both primary and intermediate POL products is observed. This trend persists in 3-month-old rats, indicating a sustained activation of peroxidation processes, a factor contributing to membrane destabilisation. Exposure of hydroxyl radicals to diene conjugates of fatty acids leads to the formation of lipid hydroperoxides causing conformational disturbances in cell membranes [13].

The enzymatic link of antioxidant defence is represented by SOD and catalase. The activity of AOS indicators (SOD and CAT) was significantly lower than in animals of the control group. The study revealed a statistically significant decrease of SOD activity in the pancreas tissue of the 2nd experimental group – by 29.8%. It is noteworthy that this decrease reached its apogee in the 4th experimental group, registering a decrease of 45.2% compared to the control. A similar situation was observed with catalase activity. The stability of AOS indices observed from the first to the third month of life of baby rats is intriguing: SOD and catalase activity indices decreased, but insignificantly (Table 2). This may indicate a partial activation of the antioxidant defence system to preserve the functioning of pancreatic cells [14]. The question about subsequent changes in AOS activity in baby rats after one month remains unanswered. Overall, the assessment of LPO and AOS indicators shows a disruption of OAH in the pancreas of newborn rats subjected to hypocaloric maternal nutrition during pregnancy. This disruption manifests through the activation of LPO coupled with a reduction in AOS activity, leading to the onset of oxidative stress. Oxidative stress, which is known to cause damage to cell membranes and intracellular structures [15], may serve as a critical factor in the pathogenesis of prenatal damage to the pancreas. Such damage may cause not only functional disorders but also organic pathology in the postnatal period, at least up to 3 months of the offspring's life.

Prooxidant/antioxidant balance in the liver. It was determined that subjecting mothers to partial food deprivation resulted in the heightened activation of lipid peroxidation in rat hepatocytes. Specifically, the 3rd experimental group exhibited a significant increase, with 95.4% rise in DC and 64.4% elevation in TBA-RS, compared to the control group. Notably, both DC and TBA-RS indicators exhibited a substantial decrease in the 4th experimental group when compared to 1-month-old rats (Table 3). Considering that increased lipid peroxidation combined with decreased antioxidant defence serves as a trigger of organ failure [16], it can be assumed that

Table 1

Indices of lipid peroxidation in pancreatic tissue of rat pups subjected to partial maternal nutritional deprivation during pregnancy

Indicators	Groups of rat pups			
	1st group	2nd group	3rd group	4th group
Diene Conjugates (μmol/g protein)	1.67±0.16 (1.39; 2.23)	3.68±0.22* (3.06; 4.01)	4.21±0.13* (3.82; 4.81) p_1<0,01	5.09±0.09* (4.88; 5.23) p_2<0,01 p_3<0,01
Reactive Substances of Thiobarbituric Acid (μmol/g protein)	3.04±0.30 (2.87; 3.33)	5.70±0.50* (5.34; 6.11)	6.01±0.11* (5.89; 6.48) p_1<0,01	7.02±0.18 (6.92; 7.39) p_2<0.01 p_3<0.01

Note for tables 1–4. * – the difference is significant compared to control animals ($p<0.05$); p_1 – the difference is significant between 2nd and 3rd groups, p_2 – the difference is significant between 2nd and 4th groups, p_3 – the difference is significant between 3rd and 4th groups (here and in the following tables).

Table 2

Antioxidant defence system indicators in the pancreatic tissue of rat pups subjected to maternal partial food deprivation during pregnancy

Indicators	Groups of rat pups			
	1st group	2nd group	3rd group	4th group
Superoxide Dismutase (conditional units/mg protein)	22.41±0.22 (21.40; 23.62)	15.73±0.21* (14.70; 17.21)	13.88±0.18* (12.32; 15.41) p_1<0.01	12.28±0.11* (11.91; 13.23) p_2<0.01 p_3<0.01
Catalase (μmol/min per 1 mg of protein)	6.66±0.16 (5.8; 7.4)	5.70±0.50* (5.34; 6.11)	4.04±0.08* (3.19; 4.98) p_1<0.01	4.01±0.11* (3.02; 4.33) p_2<0.01

Table 3

Indices of lipid peroxidation in liver tissues of baby rats subjected to partial maternal nutritional deprivation during pregnancy

Indicators	Groups of rat pups			
	1st group	2nd group	3rd group	4th group
Diene Conjugates (μmol/g protein)	2.40±0.09 (2.01; 2.93)	2.66±0.06 (2.26; 2.98)	4.69±0.16* (3.61; 5.24) p_1<0.01	3.41±0.07* (2.01; 2.93) p_2<0.01 p_3<0.01
Reactive Substances of Thiobarbituric Acid (μmol/g protein)	4.50±0.09 (3.87; 4.93)	4.69±1.34 (4.34; 5.11)	7.40±0.25* (5.83; 8.48) p_1<0.01	6.03±0.22* (5.04; 6.53) p_2<0.01 p_3<0.01

activation of lipid peroxidation in the liver may contribute to the development of hepatobiliary dysfunction [17]. These data emphasise that the greatest accumulation of H_2O_2, known for its toxicity to liver cells, occurs particularly in rats belonging to study group 3.

AOS activity in the liver tissues of newborn rats was slightly increased compared to the control group, mainly due to an increase in SOD activity by 11.7%. Simultaneously, catalase activity is lower by 14.3%, indicating an insufficient capacity of catalase to neutralize the produced hydrogen peroxide (H_2O_2) resulting from the catalytic action of SOD in the dismutation reaction of superoxide anion radicals (O_2^-) [18]. In the 3rd experimental group, a pronounced decrease in both SOD and catalase activities was noted, correlating with an elevation in LPO indicators within this group. While one might consider the depletion of the antioxidant system of hepatocytes, the 4th group demonstrates a restoration of balance, with indicators almost reaching the target levels observed in the control group (Table 4). It is noteworthy that heightened

Table 4

Indices of antioxidant defence system in liver tissues of rat pups subjected to partial maternal nutritional deprivation during pregnancy

Indicators	Groups of rap pups			
	1st group	2nd group	3rd group	4th group
Superoxide Dismutase (conditional units/mg protein)	17.03±0.19 (16.20; 17.90)	19.03±0.13* (18.17;20.13)	13.14±0.10* (12.21; 14.10) p_1<0.01	16.42±0.16 (15.68; 17.02) p_2<0.01 p_3<0.01
Catalase (μmol/min per 1 mg of protein)	5.67±0.29 (4.20; 7.10)	4.82±0.23* (3.84; 6.11)	2.01±0.11* (1.99; 4.08) p_1<0.01	4.52±0.16* (3.92; 5.33) p_3<0.01

immune responses reacting to oxidative stress at a young age contribute to the activation of the AOS [19].

Conclusions. In 1-month-old rats, whose mothers underwent partial food deprivation during pregnancy, the development of oxidative stress in liver tissues reaches its maximum (significantly higher values of DC by 76.32% and TBA-RS by 57.78% vs data of newborn rats), whereas in the pancreatic tissue, lipid peroxidation rates are highest at the end of the 3rd month after birth (p<0.01). At the same time, the activity of the indicators of the antioxidant system in the tissues of the pancreas significantly decreases during the observation period, and in the tissues of the liver it decreases to the maximum in 1-month-old rats (the activity of SOD by 44.82% and catalase by 139.80% vs data of newborn rats) with the following increase in the activity of the studied enzymes in 3-month-old rats (p<0.01). This suggests a high probability in the development of liver pathologies within the first month after birth and an increase in the risk of pancreatic diseases during 3 months of observation.

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