DOI: 10.32999/ksu2524-0838/2022-32-7
UDC 57.033: 57.023: 612.66(67): 612.6.05: 612.353

Kurhaluk N.[1], **Tkachenko H.**[1], **Partyka T.**[2]

PHOTOPERIOD-INDUCED ALTERATIONS IN BIOMARKERS OF OXIDATIVE STRESS IN RATS OF DIFFERENT AGES AND INDIVIDUAL PHYSIOLOGICAL REACTIVITY

[1]Institute of Biology and Earth Sciences, Pomeranian University in Słupsk, Poland
e-mail: halyna.tkachenko@apsl.edu.pl
[2]The sector of Agricultural Chemistry, Institute of Agriculture of Carpathian Region National Academy of Agrarian Sciences, Obroshyno, Lviv region, Ukraine
e-mail: tetyana.partyka@gmail.com

This study was performed to investigate the photoperiod-induced changes in biomarkers of oxidative stress in rats of different ages and different physiological reactivity, as assessed by different resistance to hypoxia. The study was conducted on 96 male Wistar rats divided into 16 groups based on resistance to hypoxia (LR, low resistance, HR, high resistance) and age, i.e. 6 and 21 months. The research was conducted at four photoperiod points: winter (January), spring (March), summer (July), and autumn (October). Lower levels of oxidative stress biomarkers ($p < 0.05$) were observed in the younger rats when compared to older rats, as well as in HR rats compared to LR rats. The levels of lipid peroxidation end product, 2-thiobarbituric acid reactive substances (TBARS) as the major indicator of oxidative stress, were found to increase with age, and summer resulted in further elevation compared to other seasons. Also, oxidative stress biomarkers were lower ($p < 0.05$) in winter than in other seasons, especially in the HR rats. TAC level in the hepatic tissue of the 6-months-old rats was significantly higher ($p < 0.05$) when compared to the level value in older rats. A similar higher TAC level was found in the hepatic tissue of HR rats compared to the LR rats. The adult rats with HR maintained TAC with minimal fluctuations throughout the year. It should be noted that the difference in TAC was higher for the groups of the adult animals with HR to hypoxia in winter, spring, and summer, which may indicate effective mechanisms preventing the formation of reactive oxygen species and systems of elimination thereof.

Keywords: *rats, resistance to hypoxia, liver, seasons, lipid hydroperoxides, 2-thiobarbituric acid reactive substances (TBARS), total antioxidant capacity (TAC).*

Кургалюк Н., Ткаченко Г., Партика Т.

ФОТОПЕРІОД-ІНДУКОВАНІ ЗМІНИ БІОМАРКЕРІВ ОКСИДАТИВНОГО СТРЕСУ У ЩУРІВ РІЗНОГО ВІКУ ТА ІНДИВІДУАЛЬНОЇ ФІЗІОЛОГІЧНОЇ РЕАКТИВНОСТІ

Дане дослідження виконано для вивчення індукованих фотоперіодом змін біомаркерів окиснювального стресу у щурів різного віку та різною фізіологічної реактивністі, що оцінюється за різною стійкістю до гіпоксії. Дослідження проведено на 96 щурах-самцях лінії Вістар, розділених на 16 груп залежно від стійкості до гіпоксії (НР, низькорезистентні, ВР, високорезистентні) та віку (6 та 21 міс.). Дослідження проводилися в чотири фотоперіоди: зима (січень), весна (березень), літо (липень) та осінь (жовтень). Нижчі рівні біомаркерів окиснювального стресу ($p < 0,05$) спостерігалося у молодших щурів у порівнянні зі старшими щурів, а також у високорезистентних щурів порівняно з низькорезистентними щурами. Було виявлено, що рівні кінцевого продукту перекисного окиснення ліпідів – ТБК-

продуктів як основних індикаторів окиснювального стресу – збільшуються з віком, а влітку це призводить до подальшого підвищення в порівнянні з іншими сезонами. Крім того, біомаркери окиснювального стресу були нижчими (p < 0,05) взимку, ніж в інші сезони, особливо у високорезистентних щурів. Рівень загальної антиокиснювальної активності (ОАА) у тканині печінки 6-місячних щурів був достовірно вищим (p < 0,05) порівняно зі значеннями у щурів старшого віку. Подібно, більш високий рівень ОАА було виявлено в тканині печінки високорезистентних щурів порівняно з низькорезистентними. У дорослих високорезистентних щурів зберігався високий рівень ОАА із мінімальними змінами протягом року. Слід зазначити, що різниця в ОАА була вищою для груп дорослих тварин з високою резистентністю до гіпоксії взимку, навесні та влітку, що може свідчити про ефективні механізми, що перешкоджають утворенню активних форм кисню, та системи їх елімінації.

Ключові слова: *щурі, стійкість до гіпоксії, печінка, сезони року, гідроперекиси ліпідів, ТБК-продукти, загальна антиоксидантна активність (ОАА).*

INTRODUCTION

The mechanisms of the diurnal and seasonal periodicity of the rhythmic functions of the body are targeted at adaptation to environmental conditions on the one hand and at preservation of the relative constancy of its internal environments on the other hand [7]. For example, the diurnal dynamics of ovulation and oviposition in meat chickens are controlled through endocrine pathways that are modulated by both environmental and internal factors [50]. Environmental factors control seasonal breeding in birds when there are natural photoperiodic cues, i.e. the availability of food for the hatchlings, availability of nesting sites, predation pressure, and climate factors [48]. Special interesting changes in the rhythmic-induced functions of the systems of organisms at the influence of sex, age, season, and individual physiological reactivity [10, 47, 51]. Seasonality refers to season-dependent fluctuations in sleep length, social activity, mood, weight, appetite, and energy level [16]. The underlying mechanism responsible for synchronizing internal biochemical processes with circadian environmental cues in mammals has been comprised of three major components. Firstly, photoreception by the retina and transmission of neural signals along the retinohypothalamic tract; secondly, integration of photoperiodic information with an internal reference circadian pacemaker located in the suprachiasmatic nucleus, and thirdly, dissemination of circadian information to target organs, *via* the autonomic nervous system and through humoral pathways [49].

Effects of hypoxia can be regulated by circadian patterns, and the possible differences in such effects may have biological and clinical implications for the organism [2, 21, 33, 35]. Recent investigations have identified a connection between the circadian rhythm protein Period 2 (PER2) and hypoxia-inducible factor (HIF1A) that may elucidate an evolutionarily conserved cellular network that can be targeted to manipulate metabolic function in stressed conditions like hypoxia or ischemia [3]. The fact that hypoxia modifies the circadian oscillations of variables as important as body temperature and metabolism leads to the expectation that the daily rhythms of many other functions are perturbed by hypoxia, according to their link to the primary variables. It is contemplated that the alterations of the normal circadian oscillations can contribute to many common symptoms at sustained hypoxia, e.g., from sleep fragmentation to malaise and loss of appetite [36]. Circadian clock interaction with HIF1α mediates oxygenic metabolism and anaerobic glycolysis in skeletal muscle [39]. Genetic disruption of the clock activator BMAL1 in skeletal myotubes and fibroblasts increased levels of the hypoxia-inducible factor 1α (HIF1α) under hypoxic conditions. The results of these researchers revealed bidirectional interactions between circadian and HIF pathways that influence metabolic adaptation to hypoxia.

There are many studies showing that individuals within each animal species and humans differ significantly in their reactions to oxygen deficiency [19, 20, 27, 28]. Analyses of animal reactions to oxygen deficiency have demonstrated that, in any group of intact animals (in particular,

white rats) of the same age and sex reared in the same conditions and placed on a critical altitude (11,000-12,000 m), it is possible to identify individuals that remain viable at the 95% death rate in experimental animals [8]. Such naturally hypoxia-resistant individuals can survive for a long time in gas environments containing only 2-3% of O_2. These animals maintain rhythmic breathing movements in acute hypoxic conditions (equivalent to the height of 12,000 m above sea level) for 5-6 minutes, and some even more than 10 minutes, while most animals display agonal breathing and convulsions that end in death after 5-60 seconds. Animals with high resistance (HR) to acute hypobaric hypoxia were identified among laboratory mice, rats, rabbits, and guinea pigs [38, 43, 44]. Intra-species differences in the reaction of individuals to acute hypoxia also vary significantly, and they are related to both genotypic and phenotypic characteristics of individuals [9, 13].

Aging is one of the main risk factors for various diseases. On the other hand, oxidative stress is a key element responsible for the development of age-related pathologies [1]. In addition, the alteration of circadian rhythms also contributes to cardiovascular pathology [3]. Aging modifies the temporal organization of antioxidant defenses and blood pressure, probably, as a consequence of a disruption in the circadian rhythm of the clock's transcriptional regulator, BMAL1, in the heart [1]. The loss of temporal organization of the activity of the antioxidant enzymes, the oxidative status, and the cellular clock machinery could result in a temporally altered antioxidant defense system also in the aging brain [26]. Rhythmic changes in oxidative damage of protein and lipid molecules have been also reported [15, 41].

The present study was focused on photoperiod- and age-related variability between the activity of oxidative stress biomarkers in rats with different physiological reactivity estimated by different resistance to hypoxia. Thus, the aim of our study was the assessment of levels of lipid hydroperoxides, 2-thiobarbituric acid reactive substances (TBARS), and total antioxidant capacity (TAC) in the hepatic tissue of male rats of different ages (adult – 3 months old, old – 21 months old) and physiological reactivity (LR, low resistance to hypoxia and HR, high resistance to hypoxia) in different photoperiods (winter, spring, summer, autumn). This study was carried out during the Scholarship Program supported by The Visegrad Fund in the Department of Zoology and Animal Physiology, Institute of Biology and Earth Sciences, Pomeranian University in Słupsk (Poland), and we are grateful to The Visegrad Fund the supporting our study.

MATERIALS AND METHODS

Animals and experimental design. The study was carried out on 96 male Wistar rats divided into 16 groups based on resistance to hypoxia (LR, low resistance, HR, high resistance) and age, i.e. 6 and 21 months. The rats were randomly assigned to sixteen groups. There were six animals in each group. The studies were conducted at four photoperiod points: winter (January), spring (March), summer (July), and autumn (October). The day/night ratio in the different photoperiod points was as follows: winter – 8:16, spring – 12:12, summer – 16:8, and autumn – 10:14.

Group I and group II – adult 6-month-old males with low resistance (n = 6) and high resistance to hypoxia (n = 6) studied in winter; Group III and group IV – old males (21 months old) with low resistance (n = 6) and high resistance to hypoxia (n = 6) studied in winter; Group V and Group VI – adult 6-month-old males with low resistance (n = 6) and high resistance to hypoxia (n = 6) studied in spring; Group VII and group VIII – old males (21 months old) with low resistance (n = 6) and high resistance to hypoxia (n = 6) studied in spring; Group IX and Group X – adult 6-month-old males with low resistance (n = 6) and high resistance to hypoxia (n = 6) studied in summer; Group XI and group XII – old males (21 months old) with low resistance (n = 6) and high resistance to hypoxia (n = 6) studied in summer; Group XIII and Group XIV – adult 6-month-old males with low resistance (n = 6) and high resistance to hypoxia (n = 6) studied in autumn; Group XV and group XVI – old males (21 months old) with low resistance (n = 6) and high resistance to hypoxia (n = 6) studied in autumn.

Prior to the experiments, the animals were divided into 2 groups: LR and HR. The hypoxia resistance of the rats was evaluated as survival time (min) in an altitude chamber 11,000 m above sea level. The survival time was measured after achieving the «altitude». Cessation of breathing served as a criterion for hypoxia resistance [9, 23, 28]. Animals with a maximum survival time after the second agonistic breath were classified as high-resistance animals, and those with a minimal survival time were regarded as low-resistance animals. After the survival assessment, the animals were housed for at least 3 weeks in vivarium conditions to adapt.

The male rats were housed at a constant temperature of 20 ± 2 °C. The animals had free access to feed and water throughout the experiments. During the study, the animals were kept on a standard diet and temperature conditions under natural lighting. The influence of artificial light sources was prevented. Blood was sampled at the peak secretion of melatonin, i.e. from 2.00 to 4.00 AM.

Tissue isolation. Tissues were removed from rats after decapitation. One rat was used for each homogenate sample. Briefly, the liver was excised, weighed, and washed in an ice-cold buffer. The minced tissue was rinsed clear of blood with cold isolation buffer and homogenized in a glass Potter-Elvehjem homogenizing vessel with a motor-driven Teflon pestle on ice. The isolation buffer consisted of 120 mM KCl, 2 mM K_2CO_3, 10 mM HEPES, and 1 mM EDTA; pH was adjusted to 7.2 with KOH. The hepatic homogenate was used for the determination of the levels of lipid hydroperoxide (LHPO), 2-thiobarbituric acid reactive substances (TBARS), and total antioxidant capacity (TAC). The Bradford method [6] with bovine serum albumin as a standard was used for the quantification of proteins. Absorbance was recorded at 595 nm.

Assay of the lipid hydroperoxide (LHPO) level. The acyl hydroperoxide level was assessed in the tissue samples with the method proposed by Kamyshnikov [18]. 4 mL of a «heptane-isopropanol» mixture was added to 0.2 mL of homogenate and vortexed vigorously. Then, 1 mL of HCl (pH 2.0) and 2 mL of heptane reagent were added, vortexed, and centrifuged at 3,000 rpm for 5 min. The lipid hydroperoxide level was read spectrophotometrically at 233 nm and expressed as nmol per mg of protein. A mixture of distilled water was used in the blank samples.

Assay of 2-Thiobarbituric acid reactive substances (TBARS). The level of lipid peroxidation was determined by quantifying the concentration of 2-thiobarbituric acid reacting substances (TBARS) with the method developed by Kamyshnikov [18]. This method is based on the reaction of the degradation of the lipid peroxidation product, i.e. malonic dialdehyde (MDA), with 2-thiobarbituric acid (TBA) at high temperature and acidity to generate a colored adduct that can be measured spectrophotometrically. The nmol of MDA per mg protein was calculated using $1.56 \cdot 105$ mM^{-1} cm^{-1} as the extinction coefficient.

Measurement of total antioxidant capacity (TAC). The TAC level in the samples was estimated by measuring the 2-thiobarbituric acid reactive substances (TBARS) level after Tween 80 oxidation. This level was determined spectrophotometrically at 532 nm [12]. Sample inhibits the Fe^{2+}/ascorbate-induced oxidation of Tween 80, resulting in a decrease in the TBARS level. The absorbance of the obtained solution was measured at 532 nm. The absorbance of the blank was defined as 100%. The level of TAC in the sample (%) was calculated with respect to the absorbance of the blank samples.

Statistical analysis. The results were expressed as mean ± S.D. Before the analysis, all variables were tested for normal distribution using the Kolmogorov-Smirnov test ($p > 0.05$), and homogeneity of variance was assessed using Levene's test. The significance of differences in the level of lipid peroxidation processes, amino acid carbonyl derivatives, total antioxidant capacity, antioxidant enzyme activity, and energy metabolism biomarkers between all examined groups was determined using one-way analysis of variance (ANOVA) and multifactorial analysis of variance (MANOVA) according to Zar [52]. Differences were considered significant at $p < 0.05$. Also, the relationships between data of all individuals were evaluated using Pearson's correlation analysis. All statistical calculations were performed on separate data from each individual with STATISTICA

13.3 software (StatSoft Inc., Poland). We used Bonferroni's post-test for the analysis of inequality. The statistical analysis was carried out in a triple way: the levels of biomarkers of oxidative stress and aerobic and anaerobic pathways in the groups were compared in relation to age, resistance to hypoxia, and photoperiods. The combined effects of age, individual physiological reactivity, and photoperiods and their significance (main effects) were compared with the biomarkers of lipid peroxidation and total antioxidant capacity, separately. The correlation and regression analysis comprised the correlation coefficient (r), regression equation, and significance of these dependencies (p). We used the coefficients of multiple correlation analysis (R), the coefficient of determination (R^2), and its corrected form reduced by random errors (R^2 adjusted) in the data analysis for the description of the full statistical model.

RESULTS AND DISCUSSION

Lipid hydroperoxides are formed in lipid systems in the process of lipid peroxidation, a complex multi-stage chain process of oxygen oxidation in lipid substrates (mainly polyunsaturated fatty acids), including stages with the participation and formation of free radicals [34]. Lipid hydroperoxides are formed as a result of enzymatic or non-enzymatic reactions involving chemically activated products, such as reactive oxygen species (ROS), possessing a toxic effect on the body and causing various tissue damage. In addition, ROS include oxidized forms of lipids or peroxide radicals, singlet oxygen, and peroxynitrites, which are formed from nitrogen oxides (NO). These groups of atoms behave as a whole and are known as free radicals. These chemical forms contain one or more unpaired electrons and are capable of independent existence. They are formed either by loss or by adding one electron to a non-radical and can easily be formed when a covalent bond is broken as a result of a homolytic cleavage [11].

The level of lipid hydroperoxides in the hepatic tissue of male rats of different ages and physiological reactivity in different photoperiods (winter, spring, summer, autumn) is presented in Fig. 1.

Figures 1 and 2 show the biomarkers of lipid peroxidation at the initial stage (lipid hydroperoxides, LHPO) associated with the initiation of this process, and the content of TBARS, i.e. the end product of lipid peroxidation, in the hepatic tissue of male rats of different ages and resistance to hypoxia in the different photoperiods of the year. Significant differences depending on age, resistance to hypoxia, and photoperiods were observed in three groups of animals. Adult animals, as well as individuals with HR, were mainly less exposed to lipid peroxidation at the initial (Fig. 1) and final stages of this process (Fig. 2). However, the temporal photoperiodical activity of these processes is as follows. In the group of the adult rats with LR, the minimum level of LHPO was noted in spring, while the maximum value was recorded in autumn, respectively. A similar trend in LHPO was also observed in the group of adult rats with HR. In the group of adult rats with LR, the minimum level of TBARS was observed in winter, while the maximum level was noted in summer. The trend in these changes was similar in the group of the adult animals with HR, i.e. the minimum TBARS level was observed in winter, but there were no statistically significant changes during the other photoperiods.

Lipid peroxidation (LPO) is the process of oxidative destruction (oxidative degradation) of lipids at the action of free radicals. It is known that under normal conditions of cell activity, a certain level of lipid peroxidation is constantly present, induced by the formation of ROS [40]. Lipid peroxidation in the cell is maintained at a constant level due to the multilevel antioxidant defense system. Thus, the balance between both parts of this system, i.e. peroxidation on the one hand and antioxidant activity on the other, is a necessity for maintaining normal cell activity. Given the need to maintain the prooxidant-antioxidant balance in a stationary regime, it can be assumed that its shift is one of the first nonspecific links in the development of stress response and can serve as a biologically important change in the internal environment of the cell that triggers other defense mechanisms [14, 37, 40].

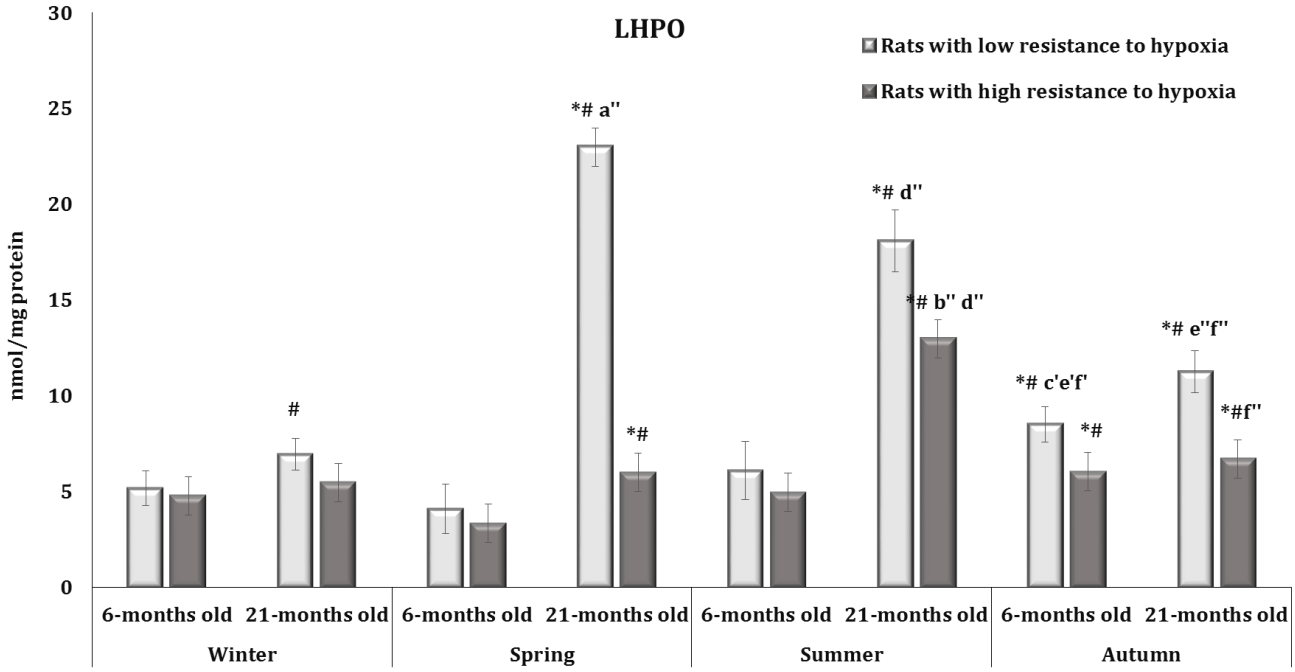

Fig. 1. Level of lipid hydroperoxides (LHPO, nmol/mg protein) in the hepatic tissue of male rats of different ages (Adult – 3 months old, Old – 21 months old) and physiological reactivity (LR – low resistance to hypoxia and HR – high resistance to hypoxia) in different photoperiods (winter, spring, summer, autumn).

The results are expressed as mean ± S.D. The differences between the experimental groups (n = 6) were analyzed using one-way ANOVA and Bonferroni post-hoc test. The differences were considered statistically significant at $p<0.05$.

Significant differences between groups are designated as follows:

*– Low resistant group vs. High resistant group in one photoperiod; # – Adult group vs. Old group in one photoperiod.

In the Adult groups: a'– Winter group vs. Spring group; b' – Winter group vs. Summer group; c' – Winter group vs. Autumn group; d' – Spring group vs. Summer group; e' – Spring group vs. Autumn group; f' – Summer group vs. Autumn group.

Into the Old groups: a'' – Winter group *vs.* Spring group; b'' – Winter group *vs.* Summer group; c'' – Winter group *vs.* Autumn group; d'' – Spring group *vs*. Summer group; e'' – Spring group *vs.* Autumn group; f'' – Summer group *vs.* Autumn group.

The level of TBARS as biomarkers of lipid peroxidation in the hepatic tissue of male rats of different ages and physiological reactivity in different photoperiods (winter, spring, summer, autumn) is presented in Fig. 2.

In the groups of the old rats with LR, the minimum level of LHPO was noted in winter, while the maximum values were recorded in spring. The trends in these processes in the groups of the old animals with HR were similar, i.e. the minimum and maximum TBARS levels were observed in winter and summer, respectively. Aging in the rats with LR was accompanied by the intensification of lipid peroxidation and formation of highly toxic products, e.g. malonic dialdehyde, compared to both the adult animals and the individuals with HR. The minimum level of TBARS in the hepatic tissue of this group was observed in winter, and the maximum value was recorded in summer. Aging in the animals with HR was accompanied by a less pronounced level of TBARS as end products of lipid peroxidation, i.e. the lowest level was shown in summer, and the highest value was noted in autumn.

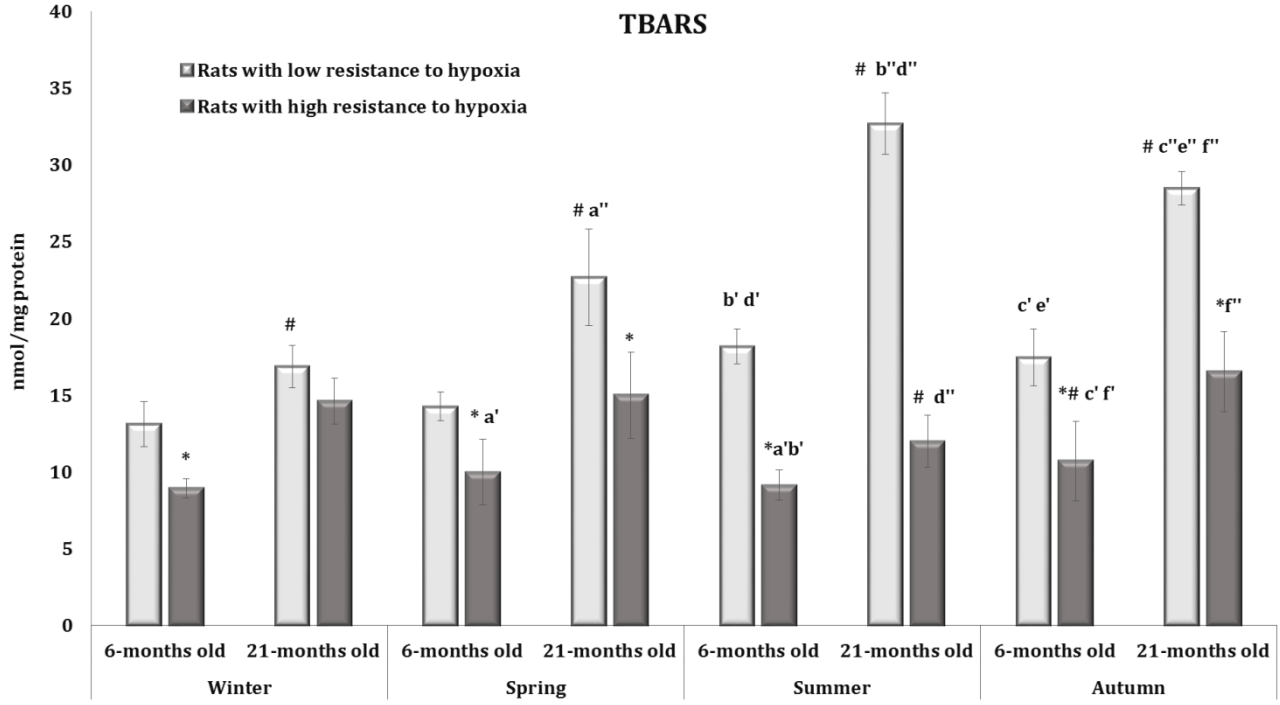

Fig. 2. TBARS level (nmol/mg protein) in the hepatic tissue of male rats of different ages (Adult – 3 months old, Old – 21 months old) and physiological reactivity (LR – low resistance to hypoxia and HR – high resistance to hypoxia) in different photoperiods (winter, spring, summer, autumn).

The results are expressed as mean ± S.D. The differences between the experimental groups (n = 6) were analyzed using one-way ANOVA and Bonferroni post-hoc test. The differences were considered statistically significant at $p<0.05$.

Significant differences between groups are designated as follows:

*– Low resistant group vs. High resistant group in one photoperiod; # – Adult group vs. Old group in one photoperiod.

In the Adult groups: a'– Winter group vs. Spring group; b' – Winter group vs. Summer group; c' – Winter group vs. Autumn group; d' – Spring group vs. Summer group; e' – Spring group vs. Autumn group; f' – Summer group vs. Autumn group.

Into the Old groups: a'' – Winter group *vs.* Spring group; b'' – Winter group *vs.* Summer group; c'' – Winter group *vs.* Autumn group; d'' – Spring group *vs.* Summer group; e'' – Spring group *vs.* Autumn group; f'' – Summer group *vs.* Autumn group.

The next step in our research consisted of the determination of the total antioxidant capacity (TAC) in the liver (Fig. 3) for effective antioxidative protection at the level of both enzymes and other cell components. Currently, to assess the functional state of the antioxidant defense system, along with the determination of the content of individual antioxidants in samples, an indicator is designated as total antioxidant capacity (TAC). TAC is an integral indicator that reflects its ability to counteract the development of free radical reactions in any model system. The main components of such model systems are a radical generation system and a substrate (or target molecule) that undergoes free radical oxidation [4].

The level of TAC in the hepatic tissue of male rats of different ages and physiological reactivity in different photoperiods (winter, spring, summer, autumn) is presented in Fig. 3.

It was shown that the adult animals with LR had a minimum level of TAC in spring and a maximum value in autumn. The adult rats with HR maintained TAC with minimal fluctuations throughout the year. It should be noted that the difference in TAC was higher for the groups of the adult animals with HR in winter, spring, and summer, which may indicate effective mechanisms preventing the formation of reactive oxygen species and systems of elimination thereof.

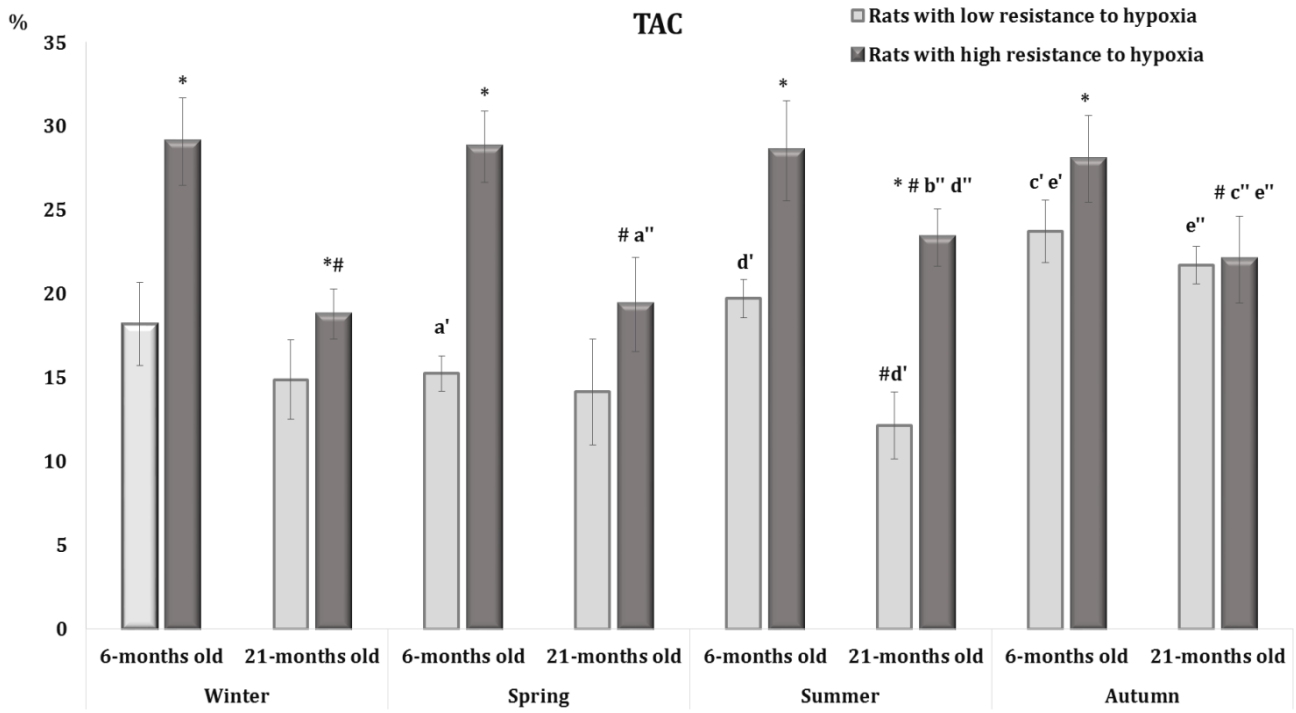

Fig. 3. TAC level (%)in the hepatic tissue of male rats of different ages (Adult – 3 months old, Old – 21 months old) and physiological reactivity (LR – low resistance to hypoxia and HR – high resistance to hypoxia) in different photoperiods (winter, spring, summer, autumn).

The results are expressed as mean ± S.D. The differences between the experimental groups (n = 6) were analyzed using one-way ANOVA and Bonferroni post-hoc test. The differences were considered statistically significant at $p<0.05$.

Significant differences between groups are designated as follows:

*– Low resistant group vs. High resistant group in one photoperiod; # – Adult group vs. Old group in one photoperiod.

In the Adult groups: a'– Winter group vs. Spring group; b' – Winter group vs. Summer group; c' – Winter group vs. Autumn group; d' – Spring group vs. Summer group; e' – Spring group vs. Autumn group; f' – Summer group vs. Autumn group.

Into the Old groups: a'' – Winter group *vs.* Spring group; b'' – Winter group *vs.* Summer group; c'' – Winter group *vs.* Autumn group; d'' – Spring group *vs.* Summer group; e'' – Spring group *vs.* Autumn group; f'' – Summer group *vs.* Autumn group.

Our earlier investigations demonstrated that the ability of HR animals to endure physical training better than LR rats depends on the initial physiological state of the organism. It is determined by the predominance of the cholinergic (in animals with high resistance to hypoxia) or adrenergic (in animals with a low resistance to hypoxia) regulatory mechanisms of physiological functions [24]. Some extreme influences are accompanied by the modulation of the NO-ergic mode of physiological regulation. Thus, treatment with L-arginine, i.e. the precursor of NO production, before swimming to exhaustion, as shown in our data, can be an important factor for correction of stress-induced reactions and increasing the animal's resistance [24]. It is important that NO is regarded as a regulating signaling factor in hypoxia states [19]. Recent data have shown that resistance to hypoxia is also associated with antioxidant defenses [21, 46]. In the heart of highly-resistant Sprague Dowley rats, the level of such enzymes as superoxide dismutase and catalase that protect cells from oxidative stress is elevated [17].

Lukyanova and Kirova [30] studied the effect of single hypoxic preconditioning exposure (hypobaric hypoxia, 5000 m, 60 min) on free radical processes, glutathione system, and antioxidant defense enzymes in tissues of rats with different resistance to acute hypoxia. The intensity of free

radical processes was shown to increase or decrease on day 1 after hypoxic preconditioning. These changes were tissue-specific and opposite in animals with genetically determined differences in the resistance to hypoxia. Hypoxic preconditioning contributes to the immediate resistance. The effect was more pronounced in low resistant animals, who did not exhibit signs of oxidative stress in tissues during the early post-hypoxic period. By contrast, hypoxic preconditioning was followed by activation of free radical processes in tissues of highly resistant animals. These rats were characterized by low ability for the development of immediate resistance. Activation of free radical processes in the early period of adaptation (first hours after hypoxic preconditioning) does not play a role in the induction of immediate adaptive mechanisms for hypoxia [30].

Sympathetic regulation is predominant in LR rats while parasympathetic tone predominates in HR animals [25]. It is believed that LR animals have a weak type of nervous system, less developed internal inhibition, increased excitability, and emotional reactivity. They respond to hypoxia with excitement and high locomotor activity. LR animals are more prone to the development of diseases such as diabetes, obesity, thyrotoxicosis, atherosclerosis, etc. [27, 29]. On the contrary, in HR animals, excitability and anxiety are reduced, and moderate aggressiveness, more pronounced internal inhibition, low sensitivity to any provoking factors, and a tendency to social domination are manifested, and they are more resistant to anesthesia. They react to acute hypoxia, cerebral ischemia, and carbon monoxide poisoning with an inhibitory reaction [27, 29].

To investigate the correlation between oxidative stress and aging, Tian and co-workers [45] have determined the levels of oxidative protein damage and lipid peroxidation in the brain and liver, and activities of antioxidant enzymes in the brain, liver, heart, kidney, and serum from the Fisher rats at ages of 1, 6, 12, 18, and 24 months. The results showed that the level of oxidative protein damage (measured as carbonyl content) in the brain and liver was significantly higher in older animals than in young animals. No statistical difference was observed in the lipid peroxidation of the liver and brain between young and old animals. The activities of antioxidant enzymes in most tissues displayed an age-dependent decline. Superoxide dismutases in the heart, kidney, and serum, glutathione peroxidase activities in the serum and kidney, and catalase activities in the brain, liver, and kidney, significantly decreased during aging. Cytochrome c oxidase, an enzyme involved in electron transport in mitochondria, initially increased, but subsequently decreased in the aged brain, whereas no significant alteration was observed in the liver mitochondrial antioxidant enzymes. The studies of Tian and co-workers [48] suggest that the accumulation of oxidized proteins during aging is most likely to be linked with an age-related decline of antioxidant enzyme activities, whereas lipid peroxidation is less sensitive to predicting the aging process.

The liver is one of the most complex organs in the body and is involved in a variety of functions. Liver regeneration is the body's protection mechanism against loss of functional liver tissue. Biondo-Simões Mde and co-workers [5] have evaluated the effect of aging on liver regeneration in rats and revealed that age is related to delay in liver regeneration in rats. Aging in male Wistar rats is associated with changes in intestinal microbiota, gut structure, and cholecystokinin-mediated gut-brain axis function [42]. Several mechanisms have been proposed by Rubio and co-workers [42] such as the presence of low-grade chronic inflammation in different tissues, as well as leptin and insulin resistance, but the primary alteration is not fully elucidated. The gut microbiota has recently emerged as a key player in a variety of metabolic and neurological disorders. The gut-brain axis refers to alterations in the gut that mediate effects in the central nervous system, including those related to the control of energy balance [42].

Mármol and co-workers [32] have studied the response of several parameters related to oxidative stress in the liver of aging rats. Male Wistar rats aged 1.5, 3, 18, and 24 months were used. Livers showed an increase in superoxide anion concentration at 1.5 and 18 months of age compared to the 3-month-old group; a decrease in superoxide dismutase (SOD) was seen at 1.5 months and catalase concentrations remained unaltered throughout the aging process. Nitric oxide (NO) progressively declined with age; a significant decrease was particularly apparent at 18 and 24 months of age. TBARS level was decreased significantly at 1.5 months, whereas it increased at 18

and 24 months of age. Concentrations of prostaglandin E2, adenine nucleotides, and their metabolites, remained unchanged throughout the aging process. Although the mitochondrial damage caused by oxidative stress can result in reduced ATP production and compromised cell function, results obtained by Mármol and co-workers [32] on adenosine nucleotides and their metabolites support the notion that the integrity of mitochondria and enzymatic activity remain mostly unchanged with aging. These researchers have observed a significant decrease in the levels of NO in the older groups of rats and hence in its antioxidant activity. This could explain the observed increase in lipid peroxides which suggests an important role for NO in oxidative stress in the liver of older rats [32]. Also, Mármol and co-workers [31] have evaluated the presence of oxidative stress and alterations in the levels of two cytoprotective agents, prostaglandin E2 and nitric oxide, in the gastrointestinal tract of aging rats. The absence of macroscopic gastric injury throughout the gastrointestinal tract indicates that the oxidative stress in the stomach and the significant decrease of nitric oxide in the duodenum in the old rats are not sufficient to disrupt the mucosal defense network. The results support the notion that the disruption of the mucosal network is essentially regulated by the cytoprotective agent's prostaglandin E2 and nitric oxide, and that injury appears only when both substances are concurrently reduced [31].

Heat-induced liver injury in old rats is associated with exaggerated oxidative stress and altered transcription factor activation [22, 53]. Older organisms showed extensive hepatic damage, along with increased morbidity and mortality, after environmental heating. After a heat-stress protocol, time-course changes in reactive oxygen species (ROS) levels, oxidative damage markers, glutathione (GSH)/glutathione disulfide (GSSG) ratios, and activation of stress-response transcription factors (AP-1 and NF-kappaB) were measured in young and old rats. A small, transient increase in hepatic oxidative damage, with minimal injury, was observed in young rats. However, old rats showed widespread hepatic injury that was manifested over a 24 h period after heating. This pathology was preceded by elevated steady-state levels of ROS, along with large increases in lipid peroxidation products, prolonged hepatic DNA oxidation damage, aberrant GSH/GSSG profiles, and altered activation patterns for AP-1. These data indicate that young animals have an effective oxidation-reduction buffering system in the liver that provides protection from oxidative damage to intracellular macromolecules under stress conditions. In sharp contrast, an environmental challenge in older animals produces exaggerated oxidative stress and alterations in signal transduction pathways, which can contribute to cellular dysfunction and age-related reductions in stress tolerance [53].

CONCLUSIONS

Lower levels of oxidative stress biomarkers ($p < 0.05$) were observed in the younger rats when compared to older rats, as well as in HR rats compared to LR rats. The levels of lipid peroxidation end product, TBARS as the major indicator of oxidative stress, were found to increase with age, and summer resulted in further elevation compared to other seasons. Also, oxidative stress biomarkers were lower ($p < 0.05$) in winter than in other seasons, especially in the HR rats. TAC level in the hepatic tissue of the 6 months aged rats was significantly higher ($p < 0.05$) elevated when compared to older rats. A similar higher TAC level was in the hepatic tissue of HR rats compared to the LR rats. It was shown that the adult animals with LR had a minimum level of TAC in spring and a maximum value in autumn. The adult rats with HR maintained TAC with minimal fluctuations throughout the year. It should be noted that the difference in TAC was higher for the groups of the adult animals with HR in winter, spring, and summer, which may indicate effective mechanisms preventing the formation of reactive oxygen species and systems of elimination thereof.

ACKNOWLEDGMENT

This study was carried out during the Scholarship Program supported by The Visegrad Fund in the Department of Zoology and Animal Physiology, Institute of Biology and Earth Sciences, Pomeranian University in Słupsk (Poland), and we are grateful to The Visegrad Fund the supporting our study.

REFERENCES

1. Altamirano FG, Castro-Pascual IC, Ferramola ML, Tula ML, Delgado SM, Anzulovich AC, Lacoste MG. Aging disrupts the temporal organization of antioxidant defenses in the heart of male rats and phase shifts circadian rhythms of systolic blood pressure. Biogerontology. 2021;22(6):603-621. doi: 10.1007/s10522-021-09938-7
2. Bartke A, Amador AG, Chandrashekar V, Klemcke HG. Seasonal differences in testicular receptors and steroidogenesis. J Steroid Biochem. 1987;27(1-3):581-7. doi: 10.1016/0022-4731(87)90357-8
3. Bartman CM, Eckle T. Circadian-Hypoxia Link and its Potential for Treatment of Cardiovascular Disease. Curr Pharm Des. 2019;25(10):1075-1090. doi: 10.2174/1381612825666190516081612
4. Bartosz G. Total antioxidant capacity. Adv Clin Chem. 2003;37:219-92. doi: 10.1016/s0065-2423(03)37010-6
5. Biondo-Simões Mde L, Matias JE, Montibeller GR, Siqueira LC, Nunes Eda S, Grassi CA. Effect of aging on liver regeneration in rats. Acta Cir Bras. 2006;21(4):197-202. doi: 10.1590/s0102-86502006000400002
6. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72:248-54. doi: 10.1006/abio.1976.9999
7. Buijs RM, Scheer FA, Kreier F, Yi C, Bos N, Goncharuk VD, Kalsbeek A. Organization of circadian functions: interaction with the body. Prog Brain Res. 2006;153:341-60. doi: 10.1016/S0079-6123(06)53020-1
8. Dzhalilova D, Makarova O. Differences in Tolerance to Hypoxia: Physiological, Biochemical, and Molecular-Biological Characteristics. Biomedicines. 2020;8(10):428. doi: 10.3390/biomedicines8100428
9. Dzhalilova DS, Diatroptov ME, Tsvetkov IS, Makarova OV, Kuznetsov SL. Expression of Hif-1α, Nf-κb, and Vegf Genes in the Liver and Blood Serum Levels of HIF-1α, Erythropoietin, VEGF, TGF-β, 8-Isoprostane, and Corticosterone in Wistar Rats with High and Low Resistance to Hypoxia. Bull Exp Biol Med. 2018;165(6):781-785. doi: 10.1007/s10517-018-4264-x
10. Emens JS, Burgess HJ. Effect of Light and Melatonin and Other Melatonin Receptor Agonists on Human Circadian Physiology. Sleep Med Clin. 2015;10(4):435-53. doi: 10.1016/j.jsmc.2015.08.001
11. Fang YZ, Yang S, Wu G. Free radicals, antioxidants, and nutrition. Nutrition. 2002;18(10):872-9. doi: 10.1016/s0899-9007(02)00916-4
12. Galaktionova LP, Molchanov AV, El'chaninova SA, Varshavskiĭ BIa. Sostoianie perekisnogo okisleniia u bol'nykh s iazvennoĭ bolezn'iu zheludka i dvenadtsatiperstnoĭ kishki [Lipid peroxidation in patients with gastric and duodenal peptic ulcers]. Klin. Lab. Diagn., 1998;(6):10-14.
13. Grek OR, Pupyshev AB, Tikhonova EV. Effect of transitory ischemia on liver lysosomal apparatus in rats with different resistance to hypoxia. Bull Exp Biol Med. 2003;136(1):11-3. doi: 10.1023/a:1026016224694

14. Halliwell B, Chirico S. Lipid peroxidation: its mechanism, measurement, and significance. Am J Clin Nutr. 1993;57(5 Suppl):715S-724S; discussion 724S-725S. doi: 10.1093/ajcn/57.5.715S
15. Hardeland R, Coto-Montes A, Poeggeler B. Circadian rhythms, oxidative stress, and antioxidative defense mechanisms. Chronobiol Int. 2003;20(6):921-62. doi: 10.1081/cbi-120025245
16. Höller Y, Gudjónsdottir BE, Valgeirsdóttir SK, Heimisson GT. The effect of age and chronotype on seasonality, sleep problems, and mood. Psychiatry Res. 2021;297:113722. doi: 10.1016/j.psychres.2021.113722
17. Jain K, Suryakumar G, Prasad R, Ganju L. Differential activation of myocardial ER stress response: a possible role in hypoxic tolerance. Int J Cardiol. 2013;168(5):4667-77. doi: 10.1016/j.ijcard.2013.07.180
18. Kamyshnikov, V. 2004. A reference book on the clinic and biochemical researches and laboratory diagnostics. MEDpress-inform, Moscow. (In Russian).
19. Kurgaliuk, N.N. Oksid azota kak faktor adaptatsionnoĭ zashchity pri gipoksii [Nitric oxide as an adaptive protection factor in hypoxia]. Usp. Fiziol. Nauk, 2002;33(4), 65-79. [In Russian].
20. Kurhaluk N, Lukash O, Nosar V, Portnychenko A, Portnichenko V, Wszedybyl-Winklewska M, Winklewski PJ. Liver mitochondrial respiratory plasticity and oxygen uptake evoked by cobalt chloride in rats with low and high resistance to extreme hypobaric hypoxia. Can J Physiol Pharmacol. 2019;97(5):392-399. doi: 10.1139/cjpp-2018-0642
21. Kurhaluk N, Tkachenko H, Lukash O. Melatonin modulates oxidative phosphorylation, hepatic and kidney autophagy-caused subclinical endotoxemia and acute ethanol-induced oxidative stress. Chronobiol Int. 2020;37(12):1709-1724. doi: 10.1080/07420528.2020.1830792
22. Kurhaluk N, Tkachenko H. Melatonin and alcohol-related disorders. Chronobiol Int. 2020;37(6):781-803. doi: 10.1080/07420528.2020.1761372.
23. Kurhaluk N, Zaitseva OV, Sliuta A, Kyriienko S, Winklewski PJ. Melatonin diminishes oxidative stress in plasma, retains erythrocyte resistance and restores white blood cell count after low dose lipopolysaccharide exposure in mice. Gen Physiol Biophys. 2018;37(5):571-580. doi: 10.4149/gpb_2018010
24. Kurhalyuk N, Tkachenko H. L-arginine modulates mitochondrial function in rat liver during physical training. J Vet Res. 2007;51:641–647.
25. Kurhalyuk NM, Serebrovskaya TV, Kolesnikova EE. Role of cholino- and adrenoreceptors in regulation of rat antioxidant defense system and lipid peroxidation during adaptation to intermittent hypoxia. *Probl Eco.* Med Genet Cell Immunol. 2001;7:126-137. [In Ukrainian].
26. Lacoste MG, Ponce IT, Golini RL, Delgado SM, Anzulovich AC. Aging modifies daily variation of antioxidant enzymes and oxidative status in the hippocampus. Exp Gerontol. 2017;88:42-50. doi: 10.1016/j.exger.2016.12.002
27. Luk'ianova LD Molekuliarnye mekhanizmy tkanevoĭ gipoksii i adaptatsii organizma [Molecular mechanisms of tissue hypoxia and organism adaptation]. Fiziol Zh, 2003;49(3):17-35. (In Russian).
28. Lukyanova LD, Kirova YI, Germanova EL. The Role of Succinate in Regulation of Immediate HIF-1α Expression in Hypoxia. Bull Exp Biol Med. 2018;164(3):298-303. doi: 10.1007/s10517-018-3976-2
29. Lukyanova LD, Kirova YI. Mitochondria-controlled signaling mechanisms of brain protection in hypoxia. Front Neurosci. 2015;9:320. doi: 10.3389/fnins.2015.00320
30. Lukyanova LD, Kirova YI. Effect of hypoxic preconditioning on free radical processes in tissues of rats with different resistance to hypoxia. Bull Exp Biol Med. 2011;151(3):292-6. English, Russian. doi: 10.1007/s10517-011-1312-1

31. Mármol F, Sánchez J, López D, Martínez N, Mitjavila MT, Puig-Parellada P. Oxidative stress, nitric oxide and prostaglandin E2 levels in the gastrointestinal tract of aging rats. J Pharm Pharmacol. 2009;61(2):201-6. doi: 10.1211/jpp/61.02.0009
32. Mármol F, Sánchez J, López D, Martínez N, Xaus C, Peralta C, Roselló-Catafau J, Mitjavila MT, Puig-Parellada P. Role of oxidative stress and adenosine nucleotides in the liver of aging rats. Physiol Res. 2010;59(4):553-560. doi: 10.33549/physiolres.931768
33. McClung CA. Circadian rhythms and mood regulation: insights from pre-clinical models. Eur Neuropsychopharmacol. 2011;21 Suppl 4(Suppl 4):S683-93. doi: 10.1016/j.euroneuro.2011.07.008
34. Miyazawa T. Lipid hydroperoxides in nutrition, health, and diseases. Proc Jpn Acad Ser B Phys Biol Sci. 2021;97(4):161-196. doi: 10.2183/pjab.97.010
35. Mortola JP. Gender and the circadian pattern of body temperature in normoxia and hypoxia. Respir Physiol Neurobiol. 2017;245:4-12. doi: 10.1016/j.resp.2016.11.002
36. Mortola JP. Hypoxia and circadian patterns. Respir Physiol Neurobiol. 2007;158(2-3):274-9. doi: 10.1016/j.resp.2007.02.005
37. Niki E. Lipid peroxidation products as oxidative stress biomarkers. Biofactors. 2008;34(2):171-80. doi: 10.1002/biof.5520340208
38. Padhy G, Sethy NK, Ganju L, Bhargava K. Abundance of plasma antioxidant proteins confers tolerance to acute hypobaric hypoxia exposure. High Alt Med Biol. 2013;14(3):289-97. doi: 10.1089/ham.2012.1095
39. Peek CB, Levine DC, Cedernaes J, Taguchi A, Kobayashi Y, Tsai SJ, Bonar NA, McNulty MR, Ramsey KM, Bass J. Circadian Clock Interaction with HIF1α Mediates Oxygenic Metabolism and Anaerobic Glycolysis in Skeletal Muscle. Cell Metab. 2017;25(1):86-92. doi: 10.1016/j.cmet.2016.09.010
40. Pré J. La lipopéroxydation [Lipid peroxidation]. Pathol Biol (Paris), 1991;39(7):716-736. [In French].
41. Reiter RJ, Tan DX, Terron MP, Flores LJ, Czarnocki Z. Melatonin and its metabolites: new findings regarding their production and their radical scavenging actions. Acta Biochim Pol. 2007;54(1):1-9. Epub 2007 Mar 9. PMID: 17351668
42. Rubio C, Lizárraga E, Álvarez-Cilleros D, Pérez-Pardo P, Sanmartín-Salinas P, Toledo-Lobo MV, Alvarez C, Escrivá F, Fernández-Lobato M, Guijarro LG, Valverde AM, Carrascosa JM. Aging in Male Wistar Rats Associates With Changes in Intestinal Microbiota, Gut Structure, and Cholecystokinin-Mediated Gut-Brain Axis Function. J Gerontol A Biol Sci Med Sci. 2021;76(11):1915-1921. doi: 10.1093/gerona/glaa313
43. Serebrovskaya TV, Xi L. (2012). Individualized intermittent hypoxia training: principles and practices. In: Intermittent Hypoxia and Human Diseases, L. Xi and T. Serebrovskaya, Eds., Springer: London, UK.
44. Strauss E, Waliszewski K, Oszkinis G, Staniszewski R. Polymorphisms of genes involved in the hypoxia signaling pathway and the development of abdominal aortic aneurysms or large-artery atherosclerosis. J Vasc Surg. 2015;61(5):1105-13.e3. doi: 10.1016/j.jvs.2014.02.007
45. Tian L, Cai Q, Wei H. Alterations of antioxidant enzymes and oxidative damage to macromolecules in different organs of rats during aging. Free Radic Biol Med. 1998;24(9):1477-84. doi: 10.1016/s0891-5849(98)00025-2
46. Tkachenko H, Kurhalyuk N, Khabrovska L, Kamiński P. Effect of L-arginine on lead induced oxidative stress in the blood of rats with different resistance to hypoxia. Pol J Food Nutr Sci. 2007;57(3):387-394.
47. Travaglio M, Ebling FJP. Role of hypothalamic tanycytes in nutrient sensing and energy balance. Proc Nutr Soc. 2019;78(3):272-278. doi: 10.1017/S0029665118002665

48. Ubuka T, Bentle GE. (2011). Neuroendocrine control of reproduction in birds. In: D.O. Norris, K.H. Lopez (Eds.), Hormones and Reproduction of Vertebrates. Academic Press, London, p. 1-25.
49. Urbanski HF, Sorwell KG. Age-related changes in neuroendocrine rhythmic function in the rhesus macaque. Age (Dordr). 2012;34(5):1111-21. doi: 10.1007/s11357-011-9352-z
50. van der Klein SAS, Zuidhof MJ, Bédécarrats GY. Diurnal and seasonal dynamics affecting egg production in meat chickens: A review of mechanisms associated with reproductive dysregulation. Anim Reprod Sci. 2020;213:106257. doi: 10.1016/j.anireprosci.2019.106257
51. Yan L, Lonstein JS, Nunez AA. Light as a modulator of emotion and cognition: Lessons learned from studying a diurnal rodent. Horm Behav. 2019;111:78-86. doi: 10.1016/j.yhbeh.2018.09.003
52. Zar, J.H. (1999). Biostatistical Analysis. 4th ed., Prentice-Hall Inc., Englewood Cliffs, New Jersey.
53. Zhang HJ, Xu L, Drake VJ, Xie L, Oberley LW, Kregel KC. Heat-induced liver injury in old rats is associated with exaggerated oxidative stress and altered transcription factor activation. FASEB J. 2003;17(15):2293-5. doi: 10.1096/fj.03-0139fje

Стаття надійшла до редакції 21.05.2022.
The article was received 21 May 2022.