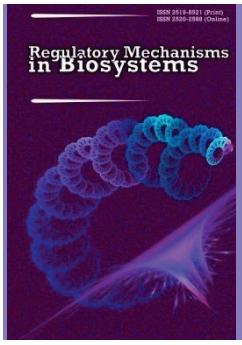

Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst.,
2024, 15(2), 306–314
doi: 10.15421/022444

Biochemical state of brain-liver axis of rats under restraint-induced stress and 2-oxoglutarate impact

O. Dyomshyna, O. Dovban, G. Ushakova

Oles Honchar Dnipro National University, Dnipro, Ukraine

Article info

Received 03.03.2024
Received in revised form 09.04.2024
Accepted 29.04.2024

Oles Honchar Dnipro National University, Naukovy av., 72, Dnipro, 49010, Ukraine.
Tel. +38-067-632-36-13.
E-mail: olga-d2009@ukr.net

Dyomshyna, O., Dovban, O., & Ushakova, G. (2024). Biochemical state of brain-liver axis of rats under restraint-induced stress and 2-oxoglutarate impact. Regulatory Mechanisms in Biosystems, 15(2), 306–314. doi:10.15421/022444

Environmental factors play a significant role in affecting the overall health of organisms, with stress being a notable contributor. The process of urbanization and globalization in modern society introduces additional stressors, exacerbating population health issues. Consequently, there is a need for thorough examination, analysis, and exploration of strategies to mitigate the adverse effects of stress. 2-Oxoglutarate, an essential intracellular metabolite and mediator with metabolite trophic properties, emerges as a promising candidate for intervention. In this study, we aimed to evaluate the combined impact of restraint-induced stress and 2-oxoglutarate on the oxidative-reducing balance, antioxidant system effectiveness, and the functional status of the liver and brain in rats. Restraint-induced stress was found to elevate oxidative stress levels, as evidenced by increased concentrations of malonic dialdehyde and oxidative-modified proteins, particularly in the brain. Additionally, signs of lactic acidosis were observed in the liver, indicating physiological changes in response to stress. Furthermore, restraint-induced stress significantly altered bioenergy components, with decreased superoxide dismutase activity and increased cytochrome C concentration, potentially indicating mitochondrial dysfunction and increased membrane permeability. The incorporation of a 2% solution of 2-oxoglutarate into the diet demonstrated a reduction in malonic dialdehyde and carbonylated protein formation, leading to more effective restoration of oxidative-reducing balance in the brain compared to the liver. Additionally, normalization of the lactate/pyruvate concentration ratio and decreased lactate dehydrogenase activity, alongside elevated alanine aminotransferase levels, suggested a decrease in oxidative stress in the liver. Moreover, exogenous 2-oxoglutarate exhibited a positive effect on superoxide dismutase activity and cytochrome C concentration, indicating a reduction in oxidative tension in the liver and progressive mitochondrial function recovery. Based on these findings, exogenous 2-oxoglutarate emerges as a promising metabolitotrope and adaptogen for managing oxidative stress and improving mitochondrial function.

Keywords: restraint-induced stress; antioxidant system; catalase; superoxide dismutase; cytochrome C; liver; brain; 2-oxoglutarate.

Introduction

Stress serves as a defensive response of the body, triggering adaptive mechanisms to cope with alterations in environmental circumstances. As a result of this occurrence, the activation of individual defense mechanisms enables the biological system to operate effectively. However, modern life, anthropogenic environmental pollution, and new unusual stress factors lead to the tension and exhaustion of this system. In general, the result of this is a violation of the adaptive activity of the organism, the main cause of which is stress. Stress factors can provoke damage to the nervous and cardiovascular system, which causes emotional stress and is accompanied by functional, biochemical, and structural disorders in the central nervous system and peripheral organs (Yaribeygi et al., 2017; Osborne et al., 2020; Hendricks et al., 2023). The modern way of life with the increase in computer technologies and robotics implies a decrease in human motor activity, and sometimes long-term restraint. A certain period of restraint is one of the stress factors for mammals (Gong et al., 2015; Samad et al., 2020). The sedentary human lifestyle, characterized by an increase in time spent on computers, as well as the effects of various frequencies of electromagnetic fields on brain hormones and enzyme activity (Aymen et al., 2017), can be experienced by both children and adults and poses a stressful burden that worsens health. Moreover, COVID-19 caused disruptions in the global social system, government-mandated restraints were imposed on many activities and interactions outside the home were reduced (Okuyama et al., 2021). Stress is a trigger for a complex interplay of nervous, endocrine, and immune mechanisms that involves activation of the sympathetic-adreno-medullar axis, the hypothalamus-pituitary-adrenal axis, and the immune system (Mifsud & Reul, 2018). The main mechanism for stress response is associated with the release of stress hormones (cortisol, adrenaline), which to one degree or another lead to vasospasm, which in turn provokes a state of hypoxia (Majmundar et al., 2010; Shobatake et al., 2022). In turn, hypoxia causes disturbances in the most sensitive organs and tissues due to lack of oxygen: the liver and the brain. However, hypoxia and mitochondrial dysfunction play a decisive role in the pathophysiology of Alzheimer's disease, depression, cardiovascular and other diseases including disturbance of lungs, kidneys, and so on (Panov & Orynbayeva, 2018; Ehtramolsadat et al., 2018; Song et al., 2021). Due to stress hormones, the liver activates the processes of glycogenolysis and glycolysis, disturbs the prooxidant-antioxidant homeostasis, disintegration, and bilirubin-generating functions, and increases the permeability of hepatocyte membranes. The interaction between endoplasmic reticulum stress and oxidative stress plays important roles in nonalcoholic liver disease. Mitochondria-associated membranes act as a structural bridge for the functional clustering of molecules, particularly for lipids and reactive oxygen species exchange (Dyomshyna et al., 2017; Chen et al., 2020).

In a living cell, processes of formation, biotransformation, and elimination of oxidized/carbonylated metabolites are constantly underway, maintaining the stationary level of oxidative processes. Initially, the study of the phenomenon of oxidative/carbonyl stress considered it as a state of enhanced oxidation of carbohydrates and lipids (oxidative stress), or inadequate detoxification or activation of reactive carbonyl compounds for-

med as a result of oxidative chemistry. In modern scientific literature, "carbonyl stress" is defined as the increased acute or chronic elevation of the stationary level of oxidized carbohydrates, lipids, proteins, and nucleic acids, which disrupt the progression of cellular metabolism and consequently lead to damage to cellular components. (Semchyshyn & Lushchak, 2012; Lushchak, 2015; Bhattacharya et al., 2017).

Several natural antioxidants have been well-researched for their potential health benefits: vitamin C (ascorbic acid), vitamin E (tocopherols), beta-carotene: a precursor to vitamin A, flavonoids, zinc, coenzyme Q_{10}, polyphenols, glutathione, omega-3 (Arulselvan et al., 2016; Diniz et al., 2020). It's important to note that the effectiveness of antioxidants can vary, and their benefits are often best realized when obtained through a balanced and varied diet rather than through supplements. Additionally, individual responses to antioxidants can vary, and more research is needed to fully understand their specific health effects in different contexts.

In previous studies, we established positive results in protecting the hippocampus under conditions of ischemic damage with the help of 2-oxoglutarate (Kovalenko et al., 2011; Tkachenko et al., 2018). Other studies have also emphasized the antioxidant properties of this compound, which is an intermediate metabolite of the tricarboxylic acid cycle (Sawa et al., 2017; Zdzisińska et al., 2017; Baulies et al., 2018; Bayliak & Lushchak, 2021).

Given the dependence of the brain and liver on oxygen supply, they are most vulnerable to stress. This work aimed to investigate the key biochemical characteristics of oxidative-reducing balance and the biochemical state of the liver and brain of rats under experimental restraint-induced stress and exposure to 2-oxoglutarate.

Materials and methods

Materials. The experiment was conducted on white Wistar rats weighing between 180 and 230 g, kept under standard animal house conditions. The animals were kept on a standard diet while receiving food and drinking ad libitum under standard sanitary hygiene standards (air temperature: 22 ± 2 °C, light/dark cycle: 12/12 hours). Animal manipulation was carried out according to the rules of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986) and "Regulations on the use of animals in biomedical research". Procedures with rats were carried out following the ethical rules for manipulations with experimental animals (certificate PolLASA N 4109/2016) and were allowed by the local Ethical Committee. The animals were categorized into three experimental groups, each comprising six animals: 1 – control (healthy rats); 2 – rats, which were subjected to restraint-induced stress (RIS) with previous food deprivation for 1 day; 3 – rats subjected to restraint-induced stress with supplementation by 2% 2-oxoglutarate in drinking water for 14 days (RIS+2OG). In the experiment, a modified well-known model of experimental restraint stress was used (Weiner, 1996). Restraint stress is a method used to induce physiological responses in an animal by restricting its free movement.

The modified stress model. The modified stress model consists of interchangeable exposure to dry and water-immersion conditions for 3 days. On days 1 and 2, the rats were placed in perforated plastic tubes with a transparent window on top to block their movements. The first 1 hour of the experiment saw the rats subjected to dry immobilization, followed by a period of 4 hours when the tubes were immersed vertically in water (23/27 °C) so that 1/3 of each animal's body was below the water surface. The manipulations took place on the initial day from 8 am to 1 pm and on the subsequent day in the afternoon from 12 pm to 5 pm. On day 3, the rats were subjected to dry immobilization for 1 hour from 8 am to 9 am. Permanent illumination was assured on the first and second days using an artificial light lamp.

After the induction of stress procedures for 3 days, animals were treated for 14 days with 2 % 2-oxoglutarate (SGPlus, Sweden) dissolved in drinking water. The animals drank 62 mL per day (n = 6) after prolonged exposure.

The animals had unrestricted access to food and water before and after manipulations. Upon completion of the experiment, the animals were weighed and subsequently removed from the study under anesthesia (thiopental 60 μg/kg). The liver and brain were removed, washed in saline, and used for further studies. The obtained liver and brain were homogenized with buffer solution: 250 mM saccharose, 1 mM EDTA, 10 mM tris, 2 mM $MgCl_2$, pH 7,4 at 0–3 °C and centrifuged for 5 minutes at 740 g. Water-soluble protein fraction was used for biochemical analyses (Stepchenko et al., 2021).

Enzyme assays. Determinations of the concentration of total protein, lactate dehydrogenase activity (LDH, EC 1.1.1.27), aspartate aminotransferase activity (AST, EC 2.6.1.1), alanine aminotransferase (ALT, EC 2.6.1.2) were measured by commercial kits (Filisit-diagnostics and Reagent, Ukraine, Dnipro) according to (Burtis et al., 2007; Waller-Evans et al., 2013; Pandurangan & Kim, 2015) following the manufacturer's instructions. The catalase activity (CAT, EC 1.11.1.6) was assessed based on the capacity of hydrogen peroxide to generate a stable-colored complex with molybdenum salts (Koroliuk, 1988) and expressed as μcat/mg protein. The superoxide dismutase activity (SOD, EC 1.15.1.1) was determined by its capability to impede quercetin oxidation (Kostjuk, 1990) and quantified in conventional units – c.u./mg protein. One unit of enzyme activity was defined as the amount required to induce a 50% reduction in quercetin oxidation per 1 mg of tissue protein. The concentration of cytochrome C was assessed based on its capacity to regenerate sodium dithionite (Choi & Swanson, 1995). The activity of succinate dehydrogenase (SDH, EC 1.3.99.1) was determined by the ability of the enzyme in the presence of sodium succinate to restore colorless salt in a water-soluble compound of red-color-formazan (Munujos et al., 1993).

Oxidative stress. The concentration of malonic dialdehyde (MDA) was measured by quantifying the formation of a colored complex resulting from the reaction between an acidic medium and thiobarbituric acid (Andreeva, 1988). The MDA content was expressed in micromoles per milligram of protein.

Protein carbonylation. Protein carbonylation was assaying via the level of 2,4-dinitrophenylhydrazone derivatives, which are produced in reactions of oxidized amino acid residues with 2,4-dinitrophenylhydrazine and expressed as nmol of carbonyl protein derivatives per mg of protein (Lushchak et al., 2011).

Pyruvate concentration. The pyruvic acid with 2,4-dinitrophenylhydrazine in an alkaline medium forms 2,4-dinitrophenylhydrazones of pyruvic acid of brown-red color, the intensity of which is proportional to the concentration of pyruvic acid and is determined calorimetrically.

Lactate concentration. Lactic acid is converted into acetic aldehyde due to heating with concentrated sulfuric acid, which forms a brown-red compound in the case of interaction with hydroquinone. The concentration of lactate was determined calorimetrically.

The measurement of the research parameters was carried out on a BS-3000M biochemical analyzer (SINNOWA Medical Science & Technology Co., Ltd, China).

Data analysis and statistics. All data was expressed as mean (x) ± standard deviation (SD). A one-way analysis of variance (ANOVA) test and Tukey post hoc test were applied to assess the differences between experimental groups, considering $P < 0.05$ as statistically significant. The correlation was calculated with Pearson's coefficient.

Results

The impact of concurrent exposure to restraint-induced stress and exogenous 2-oxoglutarate at a concentration of 2 % in drinking water for 14 days on the oxidative-reducing equilibrium, the efficiency of the antioxidant system, and the biochemical profile of the liver and brain of rats was assessed. The induction of experimental restraint-induced stress resulted in an increase in the concentration of malonic dialdehyde (MDA) in both the liver and brain (Fig. 1). The concentration of MDA in the liver of stressed rats was elevated to 2.05 μmoles/mg protein compared to 0.87 μmoles/mg protein of the control animals. The same tendency we have noted for the brain of stressed rats – 9.19 μmoles/mg protein compared to 3.43 μmoles/mg protein of the control animals. To compare the level of MDA concentration in the water-soluble protein fraction of experimental stressed animals treated with 2-oxoglutarate, we have presented data in a percent compared to the control group. We can see a 2.3-fold increased concentration of MDA in the liver and brain of stressed rats compared with the control group of animals.

The elevated concentration of carbonylated proteins was also observed in the liver and brain of experimentally stressed rats also (Fig. 1). However, such changes were indicated much more in the brain, 2.6-fold compared with the control group of animals. The concentration of carbonylated proteins in the liver of stressed rats increased by 1.33-fold compared to the control group. Complement with 2% 2-oxoglutarate in the drinking water in the daily diet for 14 days after stress procedure led back toward the concentration of MDA and carbonylated proteins in the control direction. The concentration of MDA in the liver and brain was decreased almost twice compared to the RIS group. The same situation was noted for the concentration of carbonylated proteins in the brain, in the liver this parameter fell less sharply to 1.26-fold compared to the RIS group.

The status of the antioxidant system, the components of which are catalase and superoxide dismutase, was demonstrated (Fig. 2).

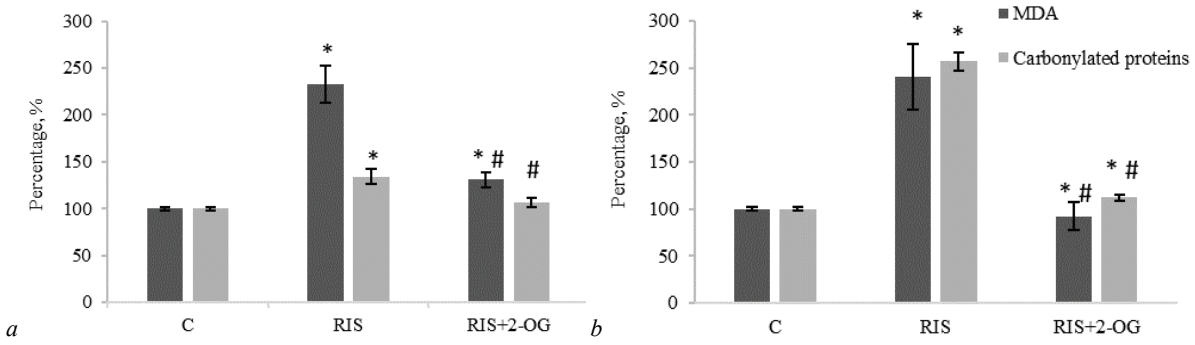

Fig. 1. The level of malonic dialdehyde (MDA) and carbonylated proteins in the liver (*a*) and brain (*b*) of experimental rats compared to the control group (x ± SD, n = 6): C – control group; RIS – animals, which were subjected to the restraint-induced stress with previous food deprivation; RIS+2-OG – animals subjected to restraint-induced stress with supplementation with 2-oxoglutarate 2% in drinking water, * – P < 0.05 regarding control; # – P < 0.05 regarding RIS group

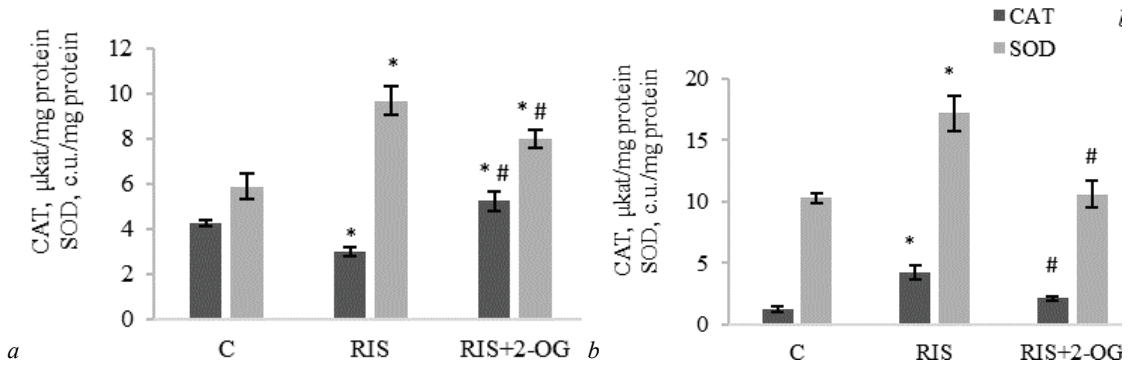

Fig. 2. The activity of catalase (CAT) and superoxide dismutase (SOD) in the liver (*a*) and brain (*b*) of rats (x ± SD, n = 6): see Fig. 1

Underdeveloped stress, a significant increase of SOD activity, was found in the brain and liver of rats (Fig. 2). The activity of SOD in the brains of physiologically healthy rats was 10.27 ± 0.5 c.u./mg protein and increased to 17.19 ± 1.6 c.u./mg protein (P < 0.001) in animals of the RIS group. In the liver of control animals, SOD activity was 5.87 ± 0.5 c.u./mg protein and increased to 9.68 ± 0.6 c.u./mg protein (P < 0.001) in animals of the RIS group. An increase in CAT activity up to 4.25 ± 0.5 μcat/mg protein was also determined in the brains of stressed rats compared to 1.21 ± 0.20 μcat/mg protein of control rats (P < 0.001). In contrast to the brain, a significant decrease in CAT activity was recorded in the liver of stressed animals from 4.28 ± 0.10 μcat/mg protein in control animals to 2.97 ± 0.20 μcat/mg protein in the stressed animals (P < 0.05). The obtained data are consistent with the number of oxidative metabolites of this experimental group (Fig. 1).

To assess the type of maintenance of general metabolism, the level of key elements of aerobic and anaerobic oxidation of glucose – lactate and pyruvate – was determined as energetically important components. The balance of lactate/pyruvate was 10:1 within the norm. Changes in this index lead to the development of lactic acidosis and, consequently, changes in the redox balance in the cell. The obtained data indicate an increase in the concentration of both pyruvate and lactate in the liver and brain as well (Fig. 3). In the liver, the increase in the concentration of these intermediate metabolites was significantly higher (2.2–2.5-fold, P < 0.05) than in the brain (1.1–1.5 fold, P < 0.05). Administration of 2-oxoglutarate resulted in a notable rise in the concentration of pyruvate and a slight decrease in the amount of lactate in the liver compared to the group of stressed animals. In the brain, no major changes in the amount of pyruvate and lactate were detected among animals of the RIS and RIS+2-OG groups.

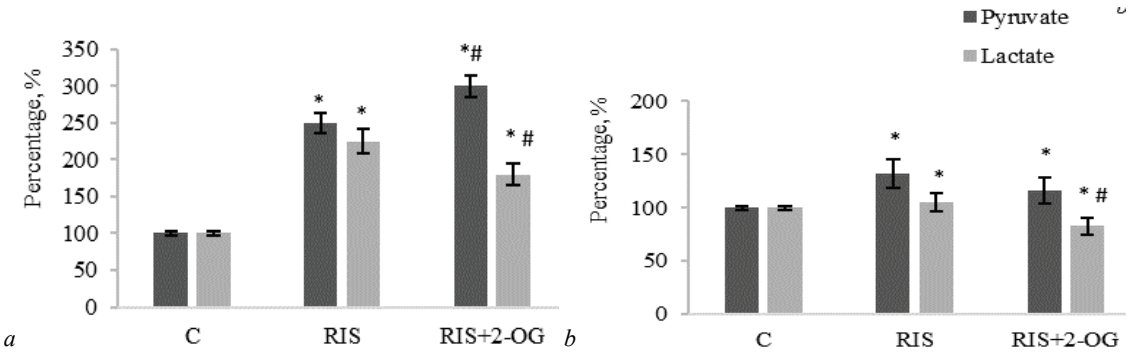

Fig. 3. The level of pyruvate and lactate in the liver and brain of experimental rats compared to the control group (x ± SD, n = 6): see Fig. 1

An enzyme that determines the direction of the use of pyruvate/lactate is lactate dehydrogenase (LDH). Hence, the activity of lactate dehydrogenase (LDH) in the liver of rats subjected to restraint-induced stress surged threefold (226 ± 46 U/kg of tissue) in comparison to the control group (76 ± 7 U/kg of tissue). In the group of animals treated with 2-oxoglutarate, LDH activity was nearly equivalent to that of the control group (88 ± 8 U/kg of tissue).

Alanine aminotransferase (ALT) is the enzyme that reversibly converts alanine into pyruvate with the participation of 2-oxoglutarate. The activity of ALT in the liver was elevated to 60.57 ± 4.27 U/kg tissue of RIS animals compared to 39.17 ± 1.47 U/kg tissue of control rats (Fig. 4). At the same time, the activity of aspartate aminotransferase (AST) in the liver of stressed animals decreased to 13.62 ± 0.87 U/kg tissue compared to 22.91 ± 1.68 U/kg in the tissue of control rats. 2-Oxoglutarate treatment for 14 days of stressed animals didn't normalize the ALT activity, it was still higher than the control level. However, the AST activity in the liver was restored with 2-oxoglutarate supplementation.

Important parts of energy-supplying processes in the cell are succinate dehydrogenase (SDH) and cytochrome C. In the liver, a moderate decrease in succinate dehydrogenase activity due to stress development was observed, with a 1.6-fold reduction, while in the brain, the reduction was more pronounced, reaching 2.25-fold compared to the control group (Fig. 5). Additionally, a notable increase in the level of cytochrome C was detected in the brain of stressed animals, showing a 2.36-fold increase, whereas in the liver, there was a 0.64-fold increase.

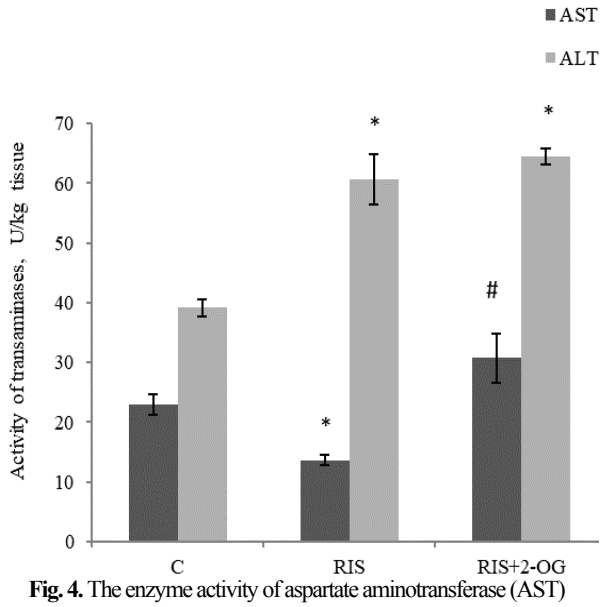

Fig. 4. The enzyme activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the liver of rats (x ± SD, n = 6): see Fig. 1

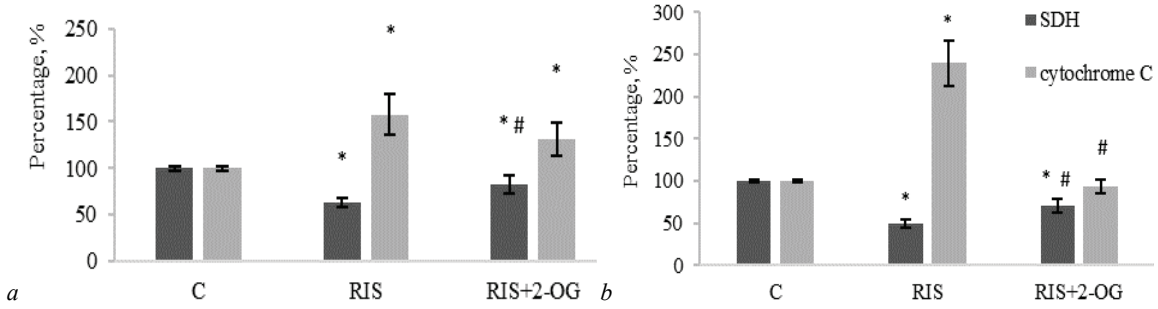

Fig. 5. The activity of succinate dehydrogenase (SDH) and quantity of cytochrome C in the liver and brain of experimental rats compared to the control group (x ± SD, n = 6): see Fig. 1

Supplementation of 2-oxoglutarate resulted in a gradual increase in SDH activity to the level of the control group, both in the liver and in the brain. The level of cytochrome C was decreased significantly in the brain to 2.7-fold relative to the control group. In the liver, no significant differences in the concentration of cytochrome C in the cytosolic fraction between the RIS and RIS+2-OG groups were detected.

Correlation analysis made it possible to establish connections between the studied indicators of metabolism in the liver (Fig. 6) and brain (Fig. 7) in the stressed animals under the influence of 2-oxoglutarate. In the liver a strong direct relationship with a high degree of significance was established between CAT activity and cytochrome C concentration ($r = 0.899$, $P < 0.001$) under stress development (Fig. 6b); as for CAT activity and concentration of carbonylated proteins ($r = 0.911$, $P < 0.001$) (Fig. 6b); ALT and LDH ($r = 0.898$, $P < 0.001$) (Fig. 6e); MDA concentration and CAT activity ($r = 0.870$, $P < 0.01$) (Fig. 6a); ALT activity and concentration pyruvate ($r = 0.829$, $P < 0.01$) (Fig. 6e); a moderate direct relationship between the concentration of MDA and the concentration of cytochrome C ($r = 0.730$, $P < 0.05$) (Fig. 6a); MDA concentration and carbonylated proteins ($r = 0.695$, $P < 0.05$) (Fig. 6a); concentrations of cytochrome C and carbonylated proteins ($r = 0.754$, $P < 0.05$) (Fig. 6d); AST activity and SDH concentration ($r = 0.707$, $P < 0.05$) (Fig. 6f). A moderate inverse relationship between AST and SDH concentrations is likely ($r = – 0.789$, $P < 0.05$) (Fig. 6f); lactate concentration and activity AST ($r = – 0.840$, $P < 0.01$) (Fig. 6f). For RIS+2-OG group a strong direct relationship with a high degree of significance was established between the amount of MDA and CAT activity ($r = 0.832$, $P < 0.01$) (Fig. 6a); concentrations of MDA and cytochrome C ($r = 0.800$, $P < 0.01$) (Fig. 6a); a moderate direct relationship between SOD activity and AST (ALT) ($r = 0.752$, $P < 0.05$) (Fig. 6c); lactate concentration and SDH activity ($r = 0.747$, $P < 0.05$) (Fig. 6h); AST activity and ALT ($r = 0.595$, $P < 0.05$) (Fig. 6f). A moderate inverse relationship between activity SOD and cytochrome C concentration ($r = – 0.900$, $P < 0.001$) (Fig. 6c); cytochrome C concentration and ALT activity ($r = – 0.872$, $P < 0.01$) (Fig. 6d); cytochrome C concentration and AST activity ($r = – 0.835$, $P < 0.01$) were noted (Fig. 6d).

In the brain developed stress modulates a strong direct relationship with a high degree of significance between cytochrome C concentration and carbonylated proteins ($r = 0.959$, $P < 0.001$) (Fig. 7d); SOD activity and MDA concentration ($r = 0.932$, $P < 0.001$) (Fig. 7a); concentrations of cytochrome C and MDA ($r = 0.889$, $P < 0.01$) (Fig. 7a); concentrations of carbonylated proteins and MDA ($r = 0.841$, $P < 0.01$) (Fig. 7a); moderate direct relationship between SDH activity and lactate concentration ($r = 0.783$, $P < 0.05$) (Fig. 7e); CAT activity and cytochrome C concentration ($r = 0.754$, $P < 0.05$) (Fig. 7c); CAT activity and pyruvate concentration ($r = 0.708$, $P < 0.05$) (Fig. 7c); SOD activity and cytochrome C concentration ($r = 0.678$, $P < 0.05$) (Fig. 7b); CAT activity and carbonylated proteins concentration ($r = 0.649$, $P < 0.05$) (Fig. 7c); SOD activity and lactate concentration ($r = 0.630$, $P < 0.05$) (Fig. 7b); SOD activity and carbonylated proteins concentration ($r = 0.603$, $P < 0.05$) (Fig. 7b). A moderate inverse relationship between lactate and pyruvate concentrations is likely ($r = – 0.774$, $P < 0.05$) (Fig. 7f). Supplementation with 2-oxoglutarate induced a strong direct relationship with a high degree of significance between CAT activity and cytochrome C concentration ($r = 0.812$, $P < 0.01$) (Fig. 7c); SOD activity and cytochrome C concentration ($r = 0.733$, $P < 0.05$) (Fig. 7b); concentrations of cytochrome C and MDA ($r = 0.694$, $P < 0.05$) (Fig. 7a); CAT activity and MDA concentration ($r = 0.650$, $P < 0.05$) (Fig. 7a); a moderate inverse relationship between concentrations of lactate and pyruvate ($r = – 0.913$, $P < 0.001$) (Fig. 7f); SDH activity and lactate concentration ($r = – 0.687$, $P < 0.05$) (Fig. 7e); SDH activity and MDA concentration ($r = – 0.669$, $P < 0.05$) (Fig. 7a).

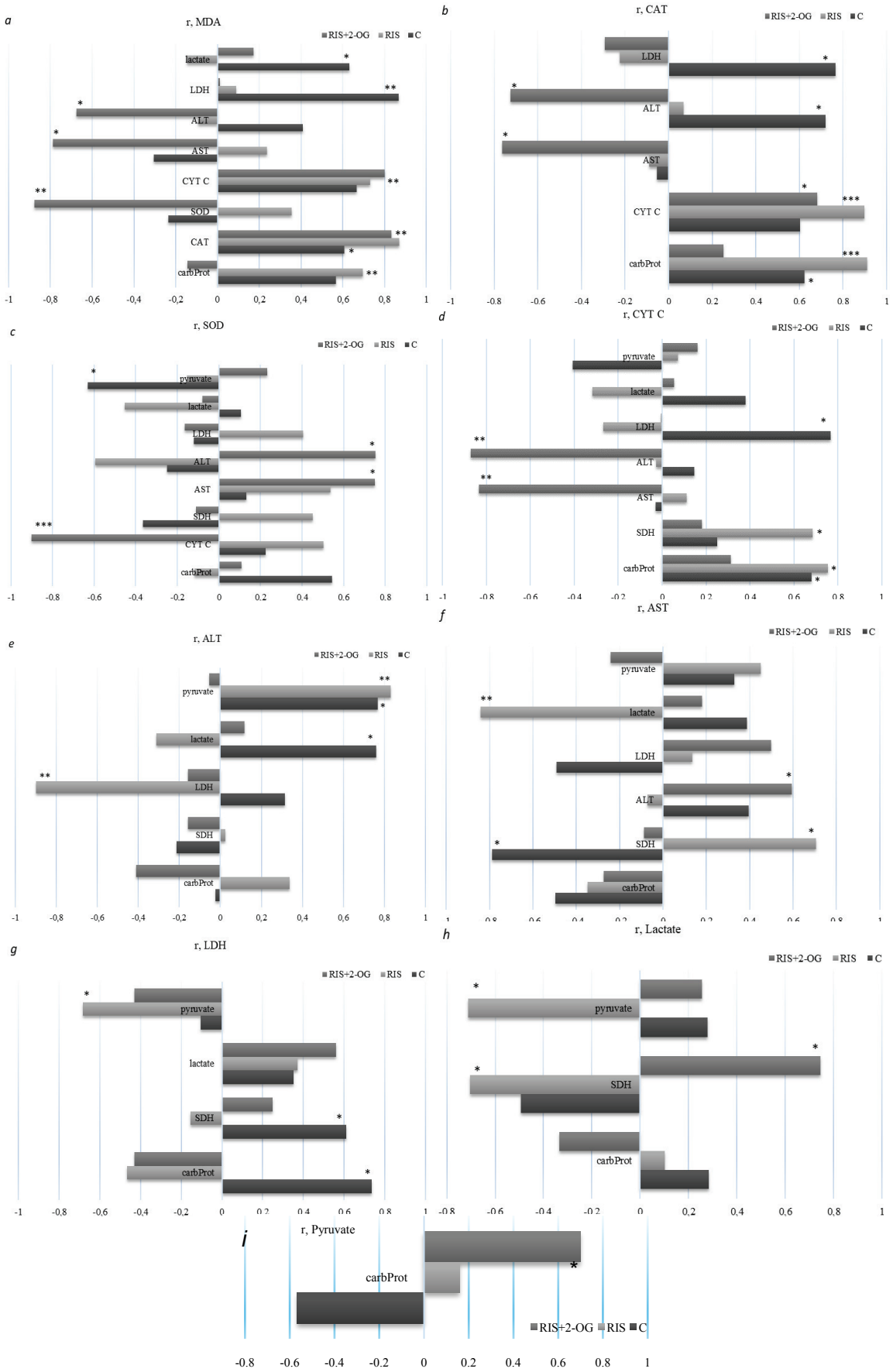

Fig. 6. The Pearson correlation coefficient (r) between experimental parameters of rat liver:
C – control group; RIS – animals, which were subjected to the restraint-induced stress with previous food deprivation; RIS+2-OG – animals subjected to restraint-induced stress with supplementation with 2-oxoglutarate 2% in drinking water, * – $P < 0.05$, ** – $P < 0.01$, *** – $P < 0.001$; MDA – malonic dialdehyde; CAT – catalase; SOD – superoxide dismutase; CYT C – cytochrome C; ALT – alanine aminotransferase; AST – aspartate aminotransferase

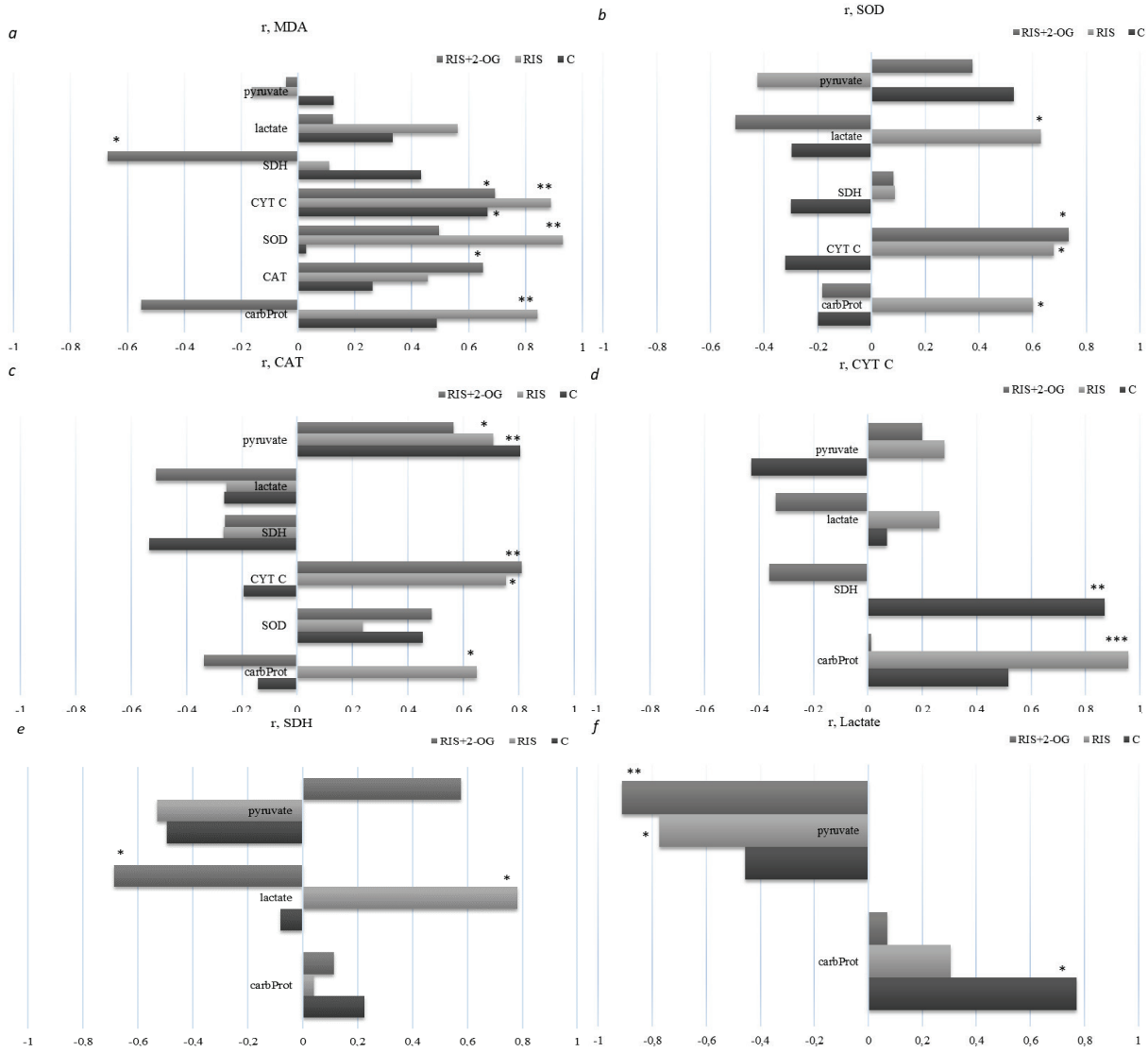

Fig. 7. The Pearson correlation coefficient (r) between biochemical parameters of rat brain: see Fig. 6

The correlation analysis between the studied indicators of the liver and brain metabolism showed the presence of correlations under stress (Fig. 8). Thus, a strong direct relationship with a high degree of significance was established for the concentration of MDA ($r = 0.809$, $P < 0.01$), cytochrome C concentration ($r = 0.763$, $P < 0.05$) and CAT ($r = 0.619$, $P < 0.05$) and a strong inverse relationship of high significance with lactate concentration is likely ($r = -0.912$, $P < 0.001$). Supplementation with 2-oxoglutarate induced a direct relationship with a high degree of significance for lactate concentration ($r = 0.633$, $P < 0.05$) and a strong inverse relationship of significance for pyruvate concentration is likely ($r = -0.606$, $P < 0.05$).

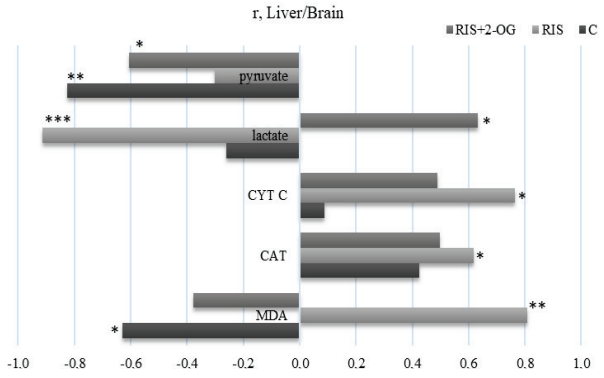

Fig. 8. The Pearson correlation coefficient (r) between experimental parameters of liver/brain: see Fig. 6

Discussion

Over the past 10 years, research into the interdependent metabolic processes of the complex axis connecting the intestinal, hepatic, and central nervous systems has significantly increased (Giuffrè & Moretti, 2023). About 50 articles were located in the PubMed search engine using the keywords "brain liver axis" in 2013, and already 210 in 2023. On the one hand, one of the key aspects of this interaction is the regulatory influence of the brain on the functions of the intestines and liver, on the other hand, the intestinal tract and liver significantly affect brain trophic and general mental health (Ding et al., 2020; Nguyen & Swain, 2023).

Most research on the gut-liver-brain axis has focused on functional gastrointestinal disorders (such as irritable bowel syndrome), considering neuro-emotional stress as a trigger for such diseases. Abnormalities in the liver-brain system are also associated with several neurological diseases, including hepatic encephalopathy, autism, and depression. However, the exact mechanisms behind this relationship remain largely unexplored (D'Mello & Swain, 2011).

The physiological activity of the brain must first be stored due to the complete supply of living substances and, first, due to the presence of oxygen, because the nerve tissue must be stored in the ATP pool from oxide phosphorylation. The brain utilizes 20 % of the basic oxygen supply to sustain ATP-demanding cognitive functions. So, the brain can be adjusted to continuously control the balance between the demand for oxygen and the risk of accumulation of oxide products.

The initial response of the brain to acute stress typically involves the activation of the hypothalamic-pituitary-adrenal (HPA) axis, which releas-

es the adrenocorticotropic hormone and stress hormones like cortisol and adrenaline into the bloodstream. These hormones help the body respond to stress by increasing heart rate, blood pressure, and energy levels, preparing the individual to face the perceived threat or challenge. During stress, the body's demand for energy increases, which can result in higher metabolic activity and, consequently, increased ROS generation as byproducts. Additionally, stress can impair the function of antioxidant defense mechanisms in the brain, making it more vulnerable to oxidative damage.

Oxidative stress, both in the brain and liver under stress, arises from an imbalance between the production of reactive oxygen species (ROS) and the body's capacity to detoxify or neutralize these detrimental molecules. Stress, whether psychological, environmental, or physiological, can trigger an elevation in ROS production within cells. The brain, with its elevated metabolic rate, substantial oxygen consumption, and comparatively lower levels of antioxidant enzymes relative to other organs, is especially vulnerable to oxidative stress. Oxidative stress in the brain can lead to damage to lipids, proteins, and DNA, which can contribute to neurodegenerative diseases, cognitive decline, and other neurological disorders (Wang & Michaelis, 2010).

Our data showed that restraint-induced stress led to an 2.3-fold increase in concentration of MDA in the liver and brain compared with the control group of animals (Fig. 1), which was confirmed by previous data (Li et al., 2020). The Pearson correlation coefficient ($r = 0.87$, $P < 0.01$, Fig. 8) indicates that the relationship between the concentration of MDA in the brain and the liver is linear with a high degree of significance. Thus, it illustrates a corresponding rise in the concentration of MDA in the liver with the increase observed in the brain due to the impact of restraint-induced stress (RIS). The changes in the concentration of carbonylated proteins in both organs have another trend. In the liver a significant increase was not observed, within the limit of 0.33-fold. The analysis of the calculation of Pearson's correlation coefficient confirmed the existence of a direct relationship between the increased concentration of the liver`s carbonylated proteins as a result of increasing the concentration of MDA ($r = 0.696$, $P < 0.05$, Fig. 6a) and concentrations of cytochrome C ($r = 0.754$, $P < 0.05$, Fig. 6d), decreased CAT activity ($r = 0.911$, $P < 0.001$,(Fig. 6b). In the brain there was a 2.6-fold increase compared with the control group of animals. Correlation analysis revealed a direct linear relationship with a high degree of probability ($r = 0.841$, $P < 0.01$, Fig. 7a) between the increase in carbonylated proteins concentration and the rise in cytochrome C concentration ($r = 0.958$, $P < 0.001$, Fig. 7d), CAT activation (CAT $r = 0.649$, $P < 0.05$, Fig. 7c) and SOD ($r = 0.603$, $P < 0.05$, Fig. 7b).

The cell system, which first interacts with oxidative metabolites, is an antioxidant system, the components of which are catalase and superoxide dismutase (Li et al., 2020). The activity of the antioxidant system increased SOD activity 1.67-fold and CAT activity 3.5-fold compared with the control group in the brain under RIS (Fig. 2). The obtained data are consistent with the number of oxidative metabolites in the stressed animals (Fig. 1) and confirmed by the correlation of the direct linear relationship of a high degree of probability ($r = 0.932$, $P < 0.001$, Fig. 7a) between the SOD activity and the MDA concentration. Other trends were observed in the liver. The CAT activity decreased by 1.44-fold and the SOD activity increased by 1.65-fold compared to the control group (Fig. 2). Similar results were obtained by Rostami et al. (2022). The results of the correlation analysis indicate the presence of a direct linear relationship with a high degree of probability between the CAT activity and: the concentration of MDA ($r = 0.870$, $P < 0.01$, Fig. 6a), the concentration of cytochrome C ($r = 0.899$, $P < 0.001$, Fig. 6b), the concentration of carbonylated proteins ($r = 0.911$, $P < 0.001$, Fig. 6b).

Pyruvate is the main product of the decomposition of the main nutriaents: carbohydrates, fatty acids, and proteins. In the norm, one way to utilize excess pyruvate with intense metabolism is to form lactate. Lactate is delivered to the liver, which is included in gluconeogenesis (Selen et al., 2022). The concentration of lactate/pyruvate is 10:1 for the physiological state. Changes in this index lead to the development of lactic acidosis and, consequently, changes in the redox balance in the cell. Therefore, studies on the concentration of lactate and pyruvate are important indicators of energy metabolism and the state of prooxidant/antioxidant equilibrium.

Our findings indicated a consistent 2.5-fold increase in the concentration of pyruvate and a 2.2-fold increase in lactate compared to the control group in the livers of rats under restraint-induced stress (RIS, Fig. 3). This increase of lactate concentration in the liver of rats indicates the development of lactic acidosis (Bakker, 2013). Also, a simultaneous increase in the concentration of pyruvate and lactate serves as a marker of mitochondrial damage and, therefore, a violation of energy processes in the liver cells. The results of the correlation analysis indicate the presence of an inverse linear relationship with a high degree of probability ($r = -0.710$, $P < 0.05$, Fig. 6h) between the concentration of lactate and pyruvate. In the brain, RIS caused a 1.3-fold increase in pyruvate concentration, while the value of lactate concentration fluctuated within the limits of the control group (Fig. 3). The results of the correlation analysis indicate the presence of an inverse linear relationship with a high degree of probability ($r = -0.774$, $P < 0.05$, Fig. 7f) between the concentration of lactate and pyruvate. A similar trend occurs in the liver. Also, correlations were established of the direct linear relationship with a high degree of probability ($r = 0.783$, $P < 0.05$, Fig. 7e) between lactate concentration and SDH activity; ($r = 0.630$, $P < 0.05$) between lactate concentration and SOD activity (Fig. 7b); ($r = 0.708$, $P < 0.05$) between pyruvate concentration and CAT activity (Fig. 7c). It is noteworthy that the correlations established in the brain are not present in the liver of the animals within this research group. The restraint-induced stress may provoke the destruction of the mitochondrial membrane and the formation of mitochondrial dysfunction, which coheres with data about an almost 1.6-fold increase in concentration of cytochrome C in the rat liver under the conditions of RIS (Fig. 5). The correlation analysis results indicate a direct linear relationship ($r = 0.685$, $P < 0.05$) between the concentration of cytochrome C and SDH activity (Fig. 6d) and ($r = 0.730$, $P < 0.05$) between of the concentration of cytochrome C and MDA (Fig. 6a) This assumption was confirmed by a simultaneously elevated content of pyruvate and lactate in the liver, which is a marker of mitochondrial damage. In the brain under the conditions of RIS, the concentration of cytochrome C increased by 2.4 times, which is confirmed by correlations of the direct linear dependence of a high degree of significance on the concentration of MDA ($r = 0.889$, $P < 0.01$, Fig. 7a), the concentration of carbonylated proteins ($r = 0.959$, $P < 0.001$, Fig. 7d), CAT activity ($r = 0.754$, $P < 0.05$, Fig. 7c), SOD activity ($r = 0.678$, $P < 0.05$, Fig. 7b).

Stress caused by water immobilization with previous food deprivation leads to changes in the homeostasis of animals. The brain is the most sensitive organ, and the blood is the tissue sensitive to the effects of stress (Grimm et al., 2014; Sawa et al., 2017; Usende et al., 2018). Changes in the blood lead to the development of hypoxia (Dyomshina et al., 2018). Hypoxia, in turn, is the cause of hypoxic phenomena occurring both in the brain and in other organs with intensive blood circulation. One of these organs is the liver (Dyomshina et al., 2018; Stepchenko et al., 2021). One of the ways to overcome the effects of stress and restore the normal functioning of the body is the use of substances of the main metabolic pathways. Of particular importance are substances that are involved in providing energy to cells. 2-Oxoglutarate (also known as alpha-ketoglutarate) is indeed an intermediate metabolite in the tricarboxylic acid (TCA) cycle, which is central to cellular metabolism. While traditionally known for its role in energy production, emerging research suggests that 2-oxoglutarate also possesses antioxidant properties. Studies have demonstrated that 2-oxoglutarate can scavenge ROS, thus reducing oxidative stress in cells (Satpute et al, 2014). This dicarboxylic acid is a multifunctional substance (Ushakova et al., 2010; Kovalenko et al., 2011; Tkachenko et al., 2018). In our experiment, we investigated the combined effects of restraint-induced stress on animals and a 2% solution of 2-oxoglutarate in drinking water administered for 14 days after the stress. We evaluated the oxidative-reducing balance, the efficiency of the antioxidant system, and the functional status of the liver and brain in rats under these conditions. The use of 2-oxoglutarate during the RIS (Fig. 1) decreased the formation of MDA and the carbonylated proteins (within the limit of the control group) compared with the group of animals, which were subjected to RIS with previous food deprivation. However, in the liver concentration of MDA was elevated by 0.31-fold in the control group. Thus, the use of 2-oxoglutarate led to the restoration of oxidative-reducing balance in the brain more effectively than the liver under the conditions of the physical stress factor. The 2-OG contributed to restoring the CAT activity and gradually decreased SOD activity in the liver of experimental animals. 2-Oxoglutarate ser-

ves as a substrate for enzymes known as dioxygenases, which regulate the expression of genes involved in antioxidant defense mechanisms. By modulating gene expression, 2-oxoglutarate can enhance the cellular antioxidant capacity (Zeng et al., 2015). 2-oxoglutarate has been shown to interact with signaling pathways involved in stress response and longevity, such as the mTOR pathway and the AMP-activated protein kinase (AMPK) pathway. These interactions can modulate cellular responses to oxidative stress and promote cell survival too (Su et al., 2019).

The introduction of a 2% solution of 2-oxoglutarate into the diet resulted in a 1.25-fold decrease in lactate concentration in the liver. Nevertheless, this concentration was higher compared to the control group of animals, suggesting a gradual attenuation of hypoxic effects. In the brain, the concentration of lactate decreased by 1.3 times compared to both the group of animals with RIS and the control group. Correlation analysis showed a strengthening of an inverse linear relationship with a high degree of probability ($r = -0.913$, $P < 0.001$) between the concentration of lactate and pyruvate in the brain (Fig. 7f). This fact indicates a decrease in oxidative stress in the liver and brain by speeding the incorporation of the three-carbon molecule into the total metabolism.

At the same time, high concentrations of pyruvate remain. An enzyme that determines the direction of the use of pyruvate/lactate is lactate dehydrogenase (LDH). The RIS provoked a 3-fold increase in the activity of LDH compared with the control group. The search for correlations showed the presence of an inverse linear relationship ($r = -0.684$, $P < 0.05$) between pyruvate concentration and LDH activity (Fig. 6g). This increase is the result of a redirection of metabolic processes in the liver to preserve important glucose precursors under conditions of limited oxygen supply induced by RIS. The conclusion was confirmed by the increase in the concentration of pyruvate and lactate (Fig. 3). In the group of animals with the addition of 2-oxoglutarate, LDH activity was recorded almost at the level of the control group. Thus, the simultaneous decrease in lactate concentration and LDH activity and the persistence of high pyruvate concentrations under the action of 2-OG testifies to the activation of other metabolic pathways for the exchange of pyruvate.

The alanine aminotransferase (ALT) enzyme reversibly converts alanine into pyruvate with the participation of 2-oxoglutarate. So, the activity of ALT still rose 2-fold in the group of animals subjected to RIS. The results of the correlation analysis indicate the presence of an inverse linear relationship with a high degree of probability ($r = -0.899$, $P < 0.001$) between the ALT activity and LDH activity (Fig. 6e) that indicated the involvement of the enzymes in the exchange of the same substrates in the liver. Also, the direct linear relationship with a high degree of probability ($r = 0.829$, $P < 0.01$) between pyruvate concentration and ALT activity (Fig. 6e) indicated speeding the exchange of the pyruvate by hypoxia. The activity of AST decreased 2-fold compared with the group of control animals. A direct linear correlation ($r = 0.707$, $P < 0.05$) was established between the activity of AST and SDH (Fig. 6f), which are involved in the exchange of dicarboxylic acids. Also, an inverse linear correlation ($r = -0.840$, $P < 0.01$) was found between AST activity and lactate concentration (Fig. 6f), which indicates inhibition of metabolic processes involving AST in lactic acidosis conditions. Use of 2-oxoglutarate can lead to recovery of AST activity and preserve a high level of ALT. The combination of factors for increasing the activity of ALT and LDH, the concentration of pyruvate and lactate may indicate a shift in pH, reducing the ratio NAD+/NADH. Application of 2-oxoglutarate under RIS resulted in the preservation of high values of pyruvate concentration and ALT activity (Fig. 3, 4), while the remaining indices decreased. This was noted in the involvement of ALT in the metabolism of pyruvate under the conditions of action endogenous 2-oxoglutarate.

Important parts of energy-supplying processes in the cell are succinate dehydrogenase (SDH) and cytochrome C. Succinate dehydrogenase is the II complex of the respiratory chain and, simultaneously, the enzyme of the Krebs cycle of mitochondria. Cytochrome C fulfills the function of the electron carrier of the respiratory chain between the III and IV complexes (Hüttemann et al., 2011). Thus, both indicators indicate the integrity of mitochondrial membranes, the effectiveness of energy, oxidation-reduction processes, and the intensity of synthetic processes in the cytosol. The correlation analysis results indicate a direct linear relationship ($r = 0.640$, $P < 0.05$) between the SDH activity and concentration of MDA (Fig. 6a) that confirms the destruction of the membrane and release of the integral protein to the cytosol. In the brain a 2-fold decrease in SDH activity was shown. Also, a direct linear correlation ($r = 0.783$, $P < 0.05$) between SDH activity and lactate concentration was indicated (Fig. 7e). The application of 2-oxoglutarate to rats under RIS led to the renewal of enzyme activity at the level of the control group. This fact may indicate the integrity of the inner mitochondrial membrane and the work of the II complex of the respiratory chain in the norm. Also, this assumption is confirmed by the presence of a direct linear correlation ($r = 0.748$, $P < 0.05$) between SDH activity and lactate concentration in the liver (Fig. 6h). Also, most likely, this is due to the deactivation/activation of synthesis SDH processes by 2-oxoglutarate. Thus, our assumption is supported by the studies of Zhao (2021), which showed the regulatory activity of intracellular 2-oxoglutarate of liver stellate cells. A similar regulatory function of 2-oxoglutarate was proven by Marino et al. (2014), who showed inhibition of cardiomyocyte fibrogenesis. Within the brain, the activity of SDH steadily rises while the concentration of MDA and lactate decreases, all due to the influence of 2-oxoglutarate, as validated by an inverse linear correlation ($r = -0.669$, $P < 0.05$, Fig. 7a) and ($r = -0.687$, $P < 0.05$, Fig. 7e), respectively.

As a key intermediate in the TCA cycle, 2-oxoglutarate plays a critical role in mitochondrial function. By supporting mitochondrial health and metabolism, 2-oxoglutarate indirectly contributes to the maintenance of cellular redox balance and antioxidant defense mechanisms.

Conclusion

The restraint-induced stress with previous food deprivation caused a significant shift in the oxidative-reducing balance in the liver and brain of rats. The effects of 2-oxoglutarate on animals under restraint-induced stress are likely to involve a combination of antioxidant, signaling, and metabolic mechanisms. Further research is needed to fully elucidate the impact of 2-oxoglutarate supplementation on stress resilience and its potential therapeutic applications in stress-related disorders. Managing stress levels and ensuring adequate antioxidant support through a balanced diet, and stress-reducing activities can help mitigate oxidative stress and maintain brain health.

References

Andreeva, L. Y., Kozhemjakyn, L. A., & Kyshkun, A. A. (1988). Modification of the method for the determination of lipid peroxides in the test with thiobarbituric acid. Laboratornoe Delo, 2, 41–43 (in Russian).

Arulselvan, P., Fard, M. T., Tan, W. S., Gothai, S., Fakurazi, S., Norhaizan, M. E., & Kumar, S. S. (2016). Role of antioxidants and natural products in inflammation. Oxidative Medicine and Cellular Longevity, 2016, 5276130.

Baulies, A., Montero, J., Matías, N., Insausti, N., Terrones, O., Basañez, G., Vallejo, C., Conde de La Rosa, L., Martinez, L., Robles, D., Morales, A., Abian, J., Carrascal, M., Machida, K., Kumar, D. B. U., Tsukamoto, H., Kaplowitz, N., Garcia-Ruiz, C., & Fernández-Checa, J. C. (2018). The 2-oxoglutarate carrier promotes liver cancer by sustaining mitochondrial GSH despite cholesterol loading. Redox Biology, 14, 164–177.

Bayliak, M. M., & Lushchak, V. I. (2021). Pleiotropic effects of alpha-ketoglutarate as a potential anti-ageing agent. Ageing Research Reviews, 66, 101237.

Bhattacharya, R., Gujar, N. L., Kumar, D., & John, J. J. (2017). Protective efficacy of various carbonyl compounds and their metabolites, and nutrients against acute toxicity of some cyanogens in rats: Biochemical and physiological studies. Interdisciplinary Toxicology, 10(1), 1–10.

Burtis, C. A., Ashwood, E. R., & Bruns, D. E. (Eds.). (2007). Tietz fundamentals of clinical chemistry. 6th ed. Saunders Elsevier, St. Louis.

Chen, Z., Tian, R., She, Z., Cai, J., & Li, H. (2020). Role of oxidative stress in the pathogenesis of nonalcoholic fatty liver disease. Free Radical Biology and Medicine, 152, 116–141.

Choi, S., & Swanson, J. M. (1995). Interaction of cytochrome *c* with cardiolipin: An infrared spectroscopic study. Biophysical Chemistry, 54, 271–278.

Ding, J. H., Jin, Z., Yang, X. X., Lou, J., Shan, W. X., Hu, Y. X., Du, Q., Liao, Q. S., Xie, R., & Xu, J. Y. (2020). Role of gut microbiota via the gut-liver-brain axis in digestive diseases. World Journal Gastroenterology, 26, 6141–6162.

Diniz, L. R. L., Bezerra Filho, C. D. S. M., Fielding, B. C., & de Sousa, D. P. (2020). Natural antioxidants: A review of studies on human and animal coronavirus. Oxidative Medicine and Cellular Longevity, 2020, 3173281.

D'Mello, C., & Swain, M. G. (2011). Liver-brain inflammation axis. American Journal of Physiology-Gastrointestinal and Liver Physiology, 301(5), 749–761.

Dyomshyna, O. O., Ushakova, G. A., & Stepchenko, L. M. (2017). The effect of biologically active feed additives of humilid substances on the antioxidant system in liver mitochondria of gerbils. Regulatory Mechanisms in Biosystems, 8(2), 185–190.
Ehtramolsadat, S., Maghsoud Peeri, M., Hosseini, M.-J., & Azarbyjani, M. A. (2018). Cardiac oxidative stress following maternal separation stress was mitigated following adolescent voluntary exercise in adult male rats. Physiology and Behavior, 183, 39–45.
Giuffrè, M., & Moretti, R. (2023). The gut-liver-brain axis: From the head to the feet. International Journal of Molecular Sciences, 24(21), 15662.
Gong, S., Miao, Y. L., Jiao, G. Z., Sun, M. J., Li, H., Lin, J., Luo, M. J., & Tan, J. H. (2015). Dynamics and correlation of serum cortisol and corticosterone under different physiological or stressful conditions in mice. PLoS One, 10(2), e0117503.
Hendricks, B., Quinn, T. D., Price, B. S., Dotson, T., Claydon, E. A., & Miller, R. (2023). Impact of stress and stress mindset on the prevalence of cardiovascular disease risk factors among first responders. BMC Public Health, 23(1), 1929.
Koroliuk, M. A., Ivanova, L. I., Mayorova, I. G., & Tokarev, V. E. (1988). A method of determining catalase activity. Laboratornoe Delo, 1, 16–19 (in Russian).
Kostjuk, V. A., Potapovych, A. Y., & Kovaleva, Z. V. (1990). Prostoy i chuvstvitel'nyy metod opredeleniya aktivnosti superoksiddismutazy, osnovannyy na reaktsii okisleniya kvertsetina [A simple and sensitive method for determining the activity of superoxide dismutase, based on the oxidation reaction of quercetin]. Questions of Medical Chemistry, 36(2), 88–91 (in Russian).
Kovalenko, T. N., Ushakova, G. A., Osadchenko, I., Skibo, G. G., & Pierzynowski, S. G. (2011). The neuroprotective effect of 2-oxoglutarate in the experimental ischemia of hippocampus. Journal Physiology Pharmacology, 62(2), 239–246.
Li, S., Li, H., Xu, X., Saw, P. E., & Zhang, L. (2020). Nanocarrier-mediated antioxidant delivery for liver diseases. Theranostics, 10(3), 1262–1280.
Lushchak, V. I. (2014). Free radicals, reactive oxygen species, oxidative stress and its classification. Chemico-Biological Interactions, 224, 164–175.
Lushchak, V. I. (2015). Free radicals, reactive oxygen species, oxidative stresses and their classifications. Ukrainian Biochemical Journal, 87(6), 11–18.
Lushchak, V. I., Semchyshyn, H. M., & Lushchak, O. V. (2011). The classic methods to measure oxidative damage: Lipid peroxides, thiobarbituric acid reactive substances, and protein carbonyls. In: Abele, D., Vázquez-Medina, J.P., & Zenteno-Savín, T. (Eds.). Oxidative stress in aquatic ecosystems. John Wiley & Sons, Inc., New Jersey. Pp. 420–431.
Majmundar, A. J., Wong, W. J., & Simon, M. C. (2010). Hypoxia-inducible factors and the response to hypoxic stress. Molecular Cell, 40(2), 294–309.
Mariño, G., Pietrocola, F., Eisenberg, T., Kong, Y., Malik, S. A., Andryushkova, A., Schroeder, S., Pendl, T., Harger, A., Niso-Santano, M., Zamzami, N., Scoazec, M., Durand, S., Enot, D. P., Fernández, Á. F., Martins, I., Kepp, O., Senovilla, L., Bauvy, C., Morselli, E., & Kroemer, G. (2014). Regulation of autophagy by cytosolic acetyl-coenzyme A. Molecular Cell, 53(5), 710–725.
Mifsud, K. R., & Reul, J. M. H. M. (2018). Mineralocorticoid and glucocorticoid receptor-mediated control of genomic responses to stress in the brain. Stress, 21(5), 389–402.
Munujos, P., Coll-Cantí, J., González-Sastre, F., & Gella, F. J. (1993). Assay of succinate dehydrogenase activity by a colorimetric-continuous method using iodonitrotetrazolium chloride as electron acceptor. Analytical Biochemistry, 212(2), 506–509.
Nguyen, H. H., & Swain, M. G. (2023). Avenues within the gut-liver-brain axis linking chronic liver disease and symptoms. Frontiers in Neuroscience, 17, 1171253.
Okuyama, J., Seto, S., Fukuda, Y., Funakoshi, S., Amae, S., Onobe, J., Izumi, S., Ito, K., & Imamura, F. (2021). Mental health and physical activity among children and adolescents during the covid-19 pandemic. The Tohoku Journal of Experimental Medicine, 253(3), 203–215.
Osborne, M. T., Shin, L. M., Mehta, N. N., Pitman, R. K., Fayad, Z. A., & Tawakol, A. (2020). Disentangling the links between psychosocial stress and cardiovascular disease. Circulation: Cardiovascular Imaging, 13(8), e010931.
Pandurangan, M., & Kim, D. H. (2015). ZnO nanoparticles augment ALT, AST, ALP and LDH expressions in C2C12 cells. Saudi Journal Biological Science, 22(6), 679–684.
Panov, A., & Orynbayeva, Z. (2018). Determination of mitochondrial metabolic phenotype through investigation of the intrinsic inhibition of succinate dehydrogenase. Analytical Biochemistry, 552, 30–37.
Rostami, R., Eslamifar, Z., Nazemi, S., Hosseini, S. Z., Behvandi, M. M., & Jafaripour, L. (2022). The effect of thyme essential oil on liver injuries caused by renal ischemia-reperfusion in rats. BioMed Research International, 2022, 2988334.
Samad, N., Ali, A., Yasmin, F., Ullah, R., & Bari, A. (2020). Behavioral and biochemical effects of *Makia madrespatana* following single immobilization stress on rats. Medicina (Kaunas), 56(7), 350.
Satpute, R., Lomash, V., Hariharakrishnan, J., Rao, P., Singh, P., Gujar, N., & Bhattacharya, R. (2014). Oxidative stress and tissue pathology caused by subacute exposure to ammonium acetate in rats and their response to treatments with alpha-ketoglutarate and N-acetyl cysteine. Toxicology and Industrial Health, 30(1), 12–24.
Sawa, K., Uematsu, T., Korenaga, Y., Hirasawa, R., Kikuchi, M., Murata, K., Zhang, J., Gai, X., Sakamoto, K., Koyama, T., & Satoh, T. (2017). Krebs cycle intermediates protective against oxidative stress by modulating the level of reactive oxygen species in neuronal ht22 cells. Antioxidants, 6(1), 21.
Selen, E. S., Rodriguez, S., Cavagnini, K. S., Kim, H. B., Na, C. H., & Wolfgang, M. J. (2022). Requirement of hepatic pyruvate carboxylase during fasting, high fat, and ketogenic diet. The Journal of Biological Chemistry, 298(12), 102648.
Semchyshyn, H. M., & Lushchak, V. I. (2012). Interplay between oxidative and carbonyl stresses: Molecular mechanisms, biological effects and therapeutic strategies of protection. In: Lushchak, V. I., & Semchyshyn, H. M. (Eds.). Oxidative stress – molecular mechanisms and biological effects. IntechOpen Limited, London. Pp. 15–46.
Shobatake, R., Ota, H., Takahashi, N., Ueno, S., Sugie, K., & Takasawa, S. (2022). The impact of intermittent hypoxia on metabolism and cognition. International Journal of Molecular Science, 23(21), 12957.
Song, T., Song, X., Zhu, C., Patrick, R., Skurla, M., Santangelo, I., Green, M., Harper, D., Ren, B., Forester, B. P., Öngür, D., & Du, F. (2021). Mitochondrial dysfunction, oxidative stress, neuroinflammation, and metabolic alterations in the progression of Alzheimer's disease: A meta-analysis of *in vivo* magnetic resonance spectroscopy studies. Ageing Research Reviews, 72, 101503.
Stepchenko, L., Dyomshyna, O., & Ushakova, G. (2021). The impact of the humate nature feed additives on the antioxidative status of erythrocytes, liver, and muscle in chickens, hens, and gerbils. Biointerface Research in Applied Chemistry, 11(5), 13202–13213.
Su, Y., Wang, T., Wu, N., Li, D., Fan, X., Xu, Z., Mishra, S. K., & Yang, M. (2019). Alpha-ketoglutarate extends *Drosophila* lifespan by inhibiting mTOR and activating AMPK. Aging, 11(12), 4183–4197.
Tkachenko, V., Kovalchuk, Y., Bondarenko, N., Bondarenko, O., Ushakova, G., & Shevtsova, A. (2018). The cardio- and neuroprotective effects of corvitin and 2-oxoglutarate in rats with pituitrin-isoproterenol-induced myocardial damage. Biochemistry Research International, 2018, 9302414.
Waller-Evans, H., Hue, C., Fearnside, J., Rothwell, A. R., Lockstone, H. E., Caldérari, S., Wilder, S. P., Cazier, J. B., Scott, J., & Gauguier, D. (2013). Nutrigenomics of high-fat diet-induced obesity in mice suggests relationships between susceptibility to fatty liver disease and the proteasome. PLoS One, 8, e82825.
Wang, X., & Michaelis, E. K. (2010). Selective neuronal vulnerability to oxidative stress in the brain. Frontiers in Aging Neuroscience, 2, 12.
Warillea, A. A., Altunc, G., Elamina, A. A., Kaplanc, A. A., Mohameda, H., Yurtc, K. K., & Elhaj, A. E. (2017). Skeptical approaches concerning the effect of exposure to electromagnetic fields on brain hormones and enzyme activities. Journal of Microscopy and Ultrastructure, 5, 177–184.
Weiner, H. (1996). Use of animal models in peptic ulcer disease. Psychosomatic Medicine, 58(6), 524–545.
Yaribeygi, H., Panahi, Y., Sahraei, H., Johnston, T. P., & Sahebkar, A. (2017). The impact of stress on body function: A review. EXCLI Journal, 16, 1057–1072.
Zdzisińska, B., Żurek, A., & Kandefer-Szerszeń, M. (2017). Alpha-ketoglutarate as a molecule with pleiotropic activity: Well-known and novel possibilities of therapeutic use. Archivum Immunologiae et Therapiae Experimentalis, 65(1), 21–36.
Zeng, X., Wu, J., Wu, Q., & Zhang, J. (2015). L-malate enhances the gene expression of carried proteins and antioxidant enzymes in the liver of aged rats. Physiological Research, 64(1), 71–78.
Zhao, J., Jiang, Y., Sun, X., Liu, X., Liu, F., Song, M., & Zhang, L. (2020). The mechanism and role of intracellular α-ketoglutarate reduction in hepatic stellate cell activation. Bioscience Reports, 40(3), BSR20193385.