

Review

Gender-related features of liver mitochondrial dysfunction in the development of metabolic syndrome and its pharmacological correction

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Abstract

The liver as a central organ of metabolism regulates homeostasis of carbohydrates, proteins and lipids. At the same time, key organelles of any cell are mitochondria including liver cells as well. Mitochondria play a crucial role in the cellular energy metabolism. Their main function is generation of adenosine triphosphate mainly by means of oxidative phosphorylation providing a continuous supply of energy to various vital cellular processes. Therefore, the objective of our research was the study of gender differences in liver mitochondrial dysfunction with metabolic syndrome development and possibility of its pharmacological correction by means of carbacetam. The experiments were carried out on laboratory nonlinear white male and female rats with the body weight of 0.220–0.250 kg. Metabolic syndrome was simulated by keeping the rats on high fat diet and free access to fructose solution (100 g/L). Carbacetam was administered intraperitoneally in a dose of 5 mg/kg during 14 days. Therefore, the results of our research are indicative of the development of mitochondrial dysfunction in the liver cells of rats of different genders under conditions of simulating metabolic syndrome. Male rats appeared to be more susceptible to damage. At the same time, administration of carbacetam as a modulator of GABA-receptors demonstrated positive changes from the point of view of mitochondrial functioning. These changes include a decreased relative rate of mitochondrial swelling and decreased peroxide oxidation of lipids and proteins with increasing activity of antioxidant defense enzymes.

Keywords: metabolic syndrome, carbacetam, liver, protein and lipid peroxide oxidation

Introduction

Nowadays, global overweight and obesity in children and adults is one of the most certain threats to public health, which is often associated with insulin resistance, hypertension, and dyslipidemia. The simultaneous combination of these symptoms is known as metabolic syndrome. In 2023 statistics estimated 1.54 billion adults with this pathology in the world [1]. The data are indicative of an increasing occurrence

associated with age, urbanization and income level, ranging from 7.5% to 45.0% among women and from 6.5% to 59.6% among men in different regions. The results obtained require targeted actions to solve a global burden.

The liver as a central organ of metabolism regulates homeostasis of carbohydrates, proteins and lipids [2]. At the same time, key organelles of any cell are mitochondria including liver cells as well. Mitochondria play a crucial role in the cellular energy metabolism.



Their main function is generation of adenosine triphosphate mainly by means of oxidative phosphorylation providing a continuous supply of energy to various vital cellular processes [3]. The lack of cellular energy supply complicates maintenance of normal physiological processes including those influencing upon metabolic liver function. Moreover, they regulate calcium signaling and its homeostasis, affect the level of reactive oxygen species (ROS) and control it, and stimulate immune response [4, 5]. Therefore, structural integrity of mitochondria are of a crucial value for their functioning, since any disorder can result in impaired energy production and cellular dysfunction.

According to the latest scientific data, GABAergic signaling exists in the non-neuronal cells of the visceral organs [6]. Its physiological and pathophysiological role in the liver cells is studied. Thus, a group of scientists identified GABAergic receptors in the liver cells. Moreover, there is evidence available informing about possible role of these receptors in protection of the liver cells against ethanol cytotoxicity, and pathogenesis of hepatocellular carcinoma [7].

Based on the scientific literature available we have not found any information concerning liver mitochondrial functional state, lipid and protein peroxide oxidation in them, when metabolic syndrome is simulated in rats of different genders and under conditions of carbacetam administration as a GABA-receptor modulator.

Therefore, the objective of our research was the study of gender differences in liver mitochondrial dysfunction with metabolic syndrome development and possibility of its pharmacological correction by means of carbacetam.

Material and methods

The experiments were carried out on laboratory nonlinear albino rats of different genders (males and females), with the body weight of 0.220–0.250 kg. The animals were kept under standard vivarium conditions. All the experimental procedures were carried out according to the main regulations of the Law of Ukraine No. 3447-IV “On the Protection of Animals against Cruel Treatment”, the principles of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (of 18.03.1986), EU Directives 2010/63/EU, the Orders of the Ministry of Health of Ukraine No. 690 of 23.09.2009 and No. 944 of 14.12.2009, and the Order

of the Ministry of Education and Science of Ukraine No. 249 of 01.03.2012.

The rats involved in the study were randomized into the following groups: the control group and the group with simulated metabolic syndrome. To reproduce the pattern of metabolic syndrome the rats of different genders were kept during 60 days on high-fat diet with free access to fructose solution (100 g/L). Fat enrichment was provided by adding solid pork fat [8]. The development of metabolic syndrome was confirmed by the determination of glucose concentration in the blood plasma on an empty stomach and by a positive glucose tolerance test. The rats with hyperglycemia lower than 7.0 mmol/L were withdrawn from the experiment. The following stage of the study was carbacetam administration into the peritoneum of rats with metabolic syndrome in a dose of 5 mg/kg during 14 days. The groups of comparison including the control one and that with simulated pathology (7 rats in each group) received 0.9% NaCl solution in a similar mode into the peritoneum.

Euthanasia of rats was performed under light chloroform narcosis. The liver was removed in the cold and thoroughly washed with cooled 0.9% NaCl solution. After that, the liver was minced and homogenized in a 10-fold volume of pH 7.4 buffer (saccharose 250 mM, EDTA 1 mM, Tris-HCl 10 mM) [9].

Mitochondrial fraction was isolated by means of differential centrifugation of homogenate of the structure examined: 700 g during 10 min (4°C), supernatant – at 11 000 g for 20 min (4°C). The sediment obtained was re-suspended in 5 ml of pH 7.4 buffer (without EDTA), and centrifuged again under similar conditions. The mitochondrial fraction (as the sediment obtained) was re-suspended in the same buffer. Then it was used for examinations immediately.

Mitochondrial swelling was registered by their expand-contract ability and optic density changes. Mitochondrial suspension was placed into the incubation pH buffer 7.4 (mmol/L): saccharose – 150, KCl – 50, KH₂PO₄ – 2, succinate – 1, Tris-HCl – 5 (final volume of 3 ml). Optic density level of the mitochondrial suspension was registered at λ 520 nm during 60 min of swelling in the presence of Ca²⁺ inducer (50 μ mol/L). The changes in the level of mitochondrial swelling were determined as the difference between the swelling rate of the organelles on the 5, 10, 15, 20, 30, 40, 60 minutes relative to the initial value. Mitochondrial suspension in the incubation medium in the absence of an inducer was used as a control, followed by recording the optical density for 60 minutes. Protein concentration in the

incubation medium was 0.4 mg/ml. The changes of the E520 values in the incubation medium were used to calculate a relative rate of mitochondrial swelling [9].

Lipid peroxide oxidation (LPO) state in mitochondria was evaluated by the content of 2-thiobarbituric acid reactive substances (TBARS) [10], which characterize lipid peroxide oxidation (LPO); protein peroxide oxidation – by the number of 2,4-dinitrophenylhydrazones derivatives with the formation of carboxylphenylhydrazone (CPH) [11]. The state of the antioxidant defense system in mitochondria was assessed by the enzymatic activity of superoxide dismutase (SOD) and catalase [12]. The protein content in mitochondria was determined by Lowry method [13].

The results of the research were processed statistically by means of the t-Student criterion. A preliminary check of the distribution of values in the samples was carried out to confirm an adequate method of statistical assessment of the average difference between the groups of the study. According to the Shapiro-Wilk criterion, no deviations from the normal distribution was found in the samples ($p > 0.05$). Considering the above, t-Student criterion was thought to be sufficient to obtain valid results. To confirm reliability of the conclusions, nonparametric Mann-Whitney U-criterion was used in parallel, which showed similar results

concerning the calculations according to the t-Student criterion as to p value. Therefore, the differences were considered statistically confident with $p \leq 0.05$.

Results and discussion

Under physiological conditions, mitochondrial pore opening is known to be initiated by the combined action of the following factors: free radical and peroxide compounds, such as reactive oxygen and nitrogen species, respiratory inhibitors and depolarizing agents. Acting in coordination, these agents promote mitochondrial pore opening in pathological cell conditions. To study the induction of mitochondrial pore opening under the action of the excessive cytotoxic forms in the mitochondria of the liver cells of rats we used the fact that formation of a mitochondrial pore is characterized by a decreased light absorption in the suspension. The process is caused by swelling of the mitochondria and Ca^{2+} release into the extra-mitochondrial space after Ca^{2+} -overload of the organelle.

Based on the studied performed, the dynamics of changes in the intensity of mitochondrial swelling in the liver cells of rats of different genders was found (Figure 1A and Figure 1B). Thus, in the control group of

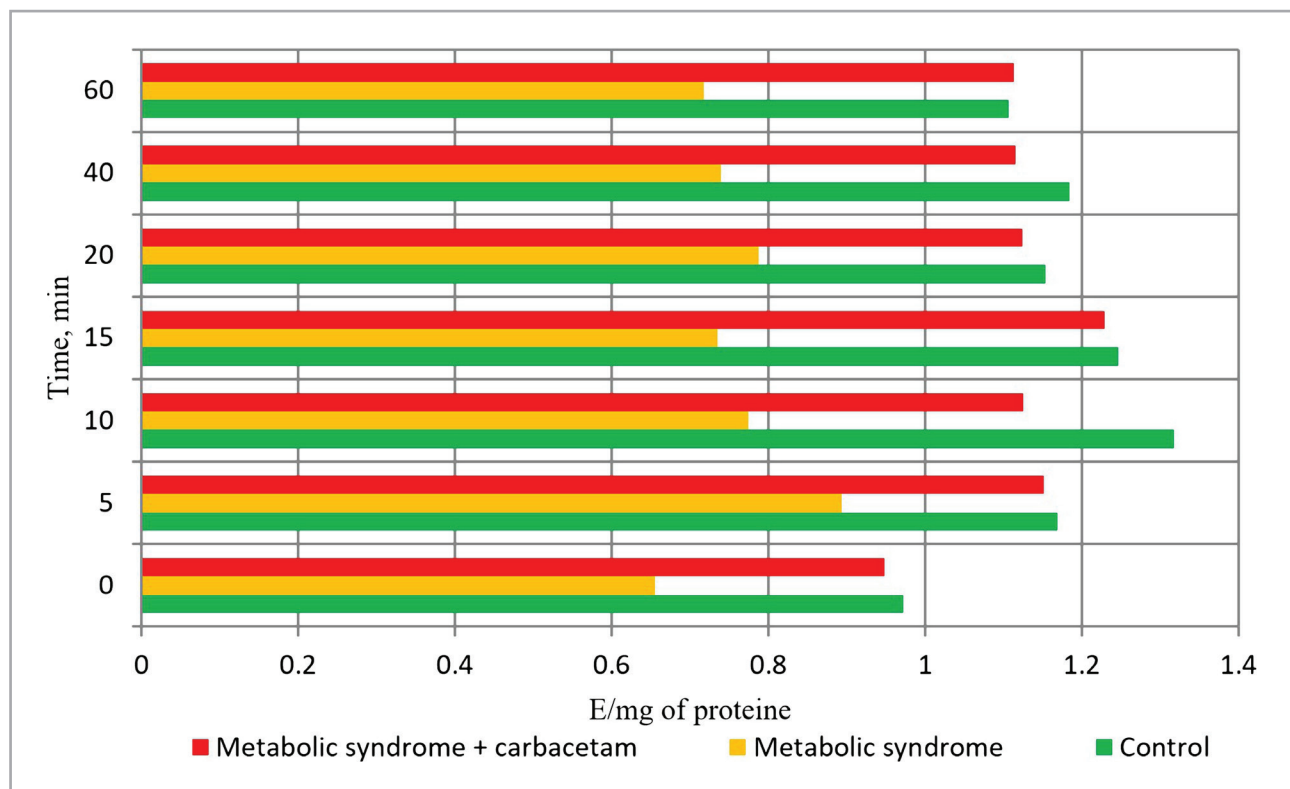


Figure 1A: Intensity of mitochondrial swelling of hepatocytes of male rats with metabolic syndrome after 14 days of administration of carbacetam at a dose of 5 mg/kg ($M \pm m$, $n=7$).

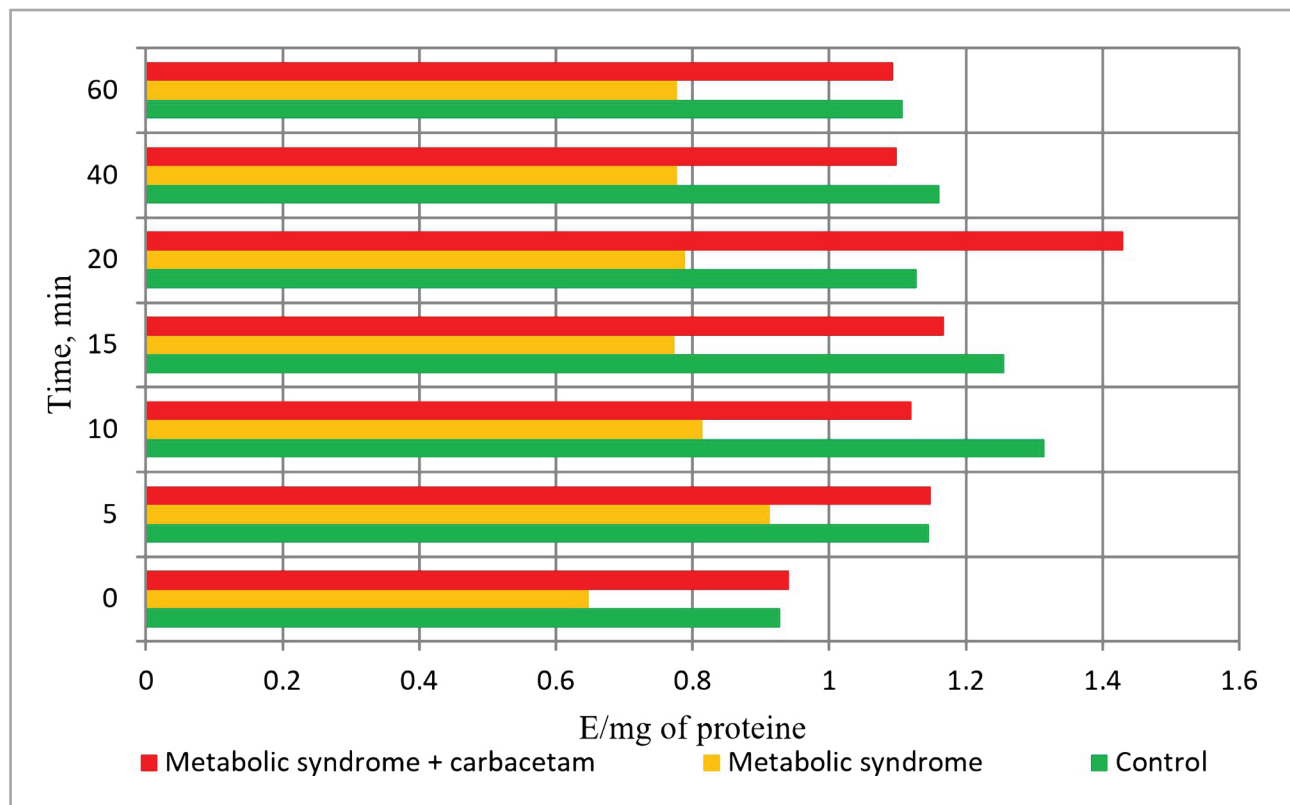


Figure 1B: Intensity of mitochondrial swelling of hepatocytes of female rats with metabolic syndrome after 14 days of administration of carbacetam at a dose of 5 mg/kg ($M \pm m$, $n=7$)

rats after 60-minute incubation of the mitochondrial suspension of the liver cells taken from males and females light scattering level 9.8 and 8.4% decreased respectively.

Rats with metabolic syndrome demonstrated decreased light scattering of the mitochondrial suspension after 60-minute incubation by 27.1% in males and 22.4% – in females. After 14-day carbacetam administration, the rats with simulated pathology presented gradual decreased light scattering both in males and females – by 8.5 and 8.3% respectively.

According to the results of our research (Figure 2), a relative rate of mitochondrial swelling in rats with metabolic syndrome 53.3 and 28.3% increased in comparison with the control group of males and females. It should be admitted that after carbacetam administration, compared to the data obtained in the rats with simulated pathology, a relative rate of mitochondrial swelling in the groups of rats of both genders decreased: by 31.9% – in males and 18.6% – in females.

Based on scientific data that the triggering factor for the development of oxidative stress is an imbalance between the production of reactive oxygen species and the systems of antioxidant cell defense, the following stage of our research was to study the state of the pro-

and antioxidant systems of the liver mitochondria. Based on the results of our research (Figure 3) we have determined that in rats with metabolic syndrome of both genders the content of TBARS 74.1% increased in males and 100.9% – in females as compared to the control group data. Carboxylphenylhydrazine (CPH) content increased in rats of both genders as well: by 45.3% – in males and 40.8% – in females. The data obtained were indicative of intensified processes of free radical oxidation of lipids and proteins. At the same time, males tend to have more severe damage to proteins than lipids that might be caused by physiological features of their organisms.

Further analysis of the results obtained determined functional disorders of the enzymatic systems of antioxidant defense due to the indices of SOD and catalase activity. Thus (Table 1), SOD activity in males 24.2% decreased, and 22.9% decreased in females. Catalase activity decreased in both groups of the research: in male rats – by 19.5%, and in female rats – by 25.3%. The changes observed are indicative of more pronounced decrease of antioxidant defense in females than in males.

When analyzing the results obtained, we have noticed that in the group of rats of both genders with

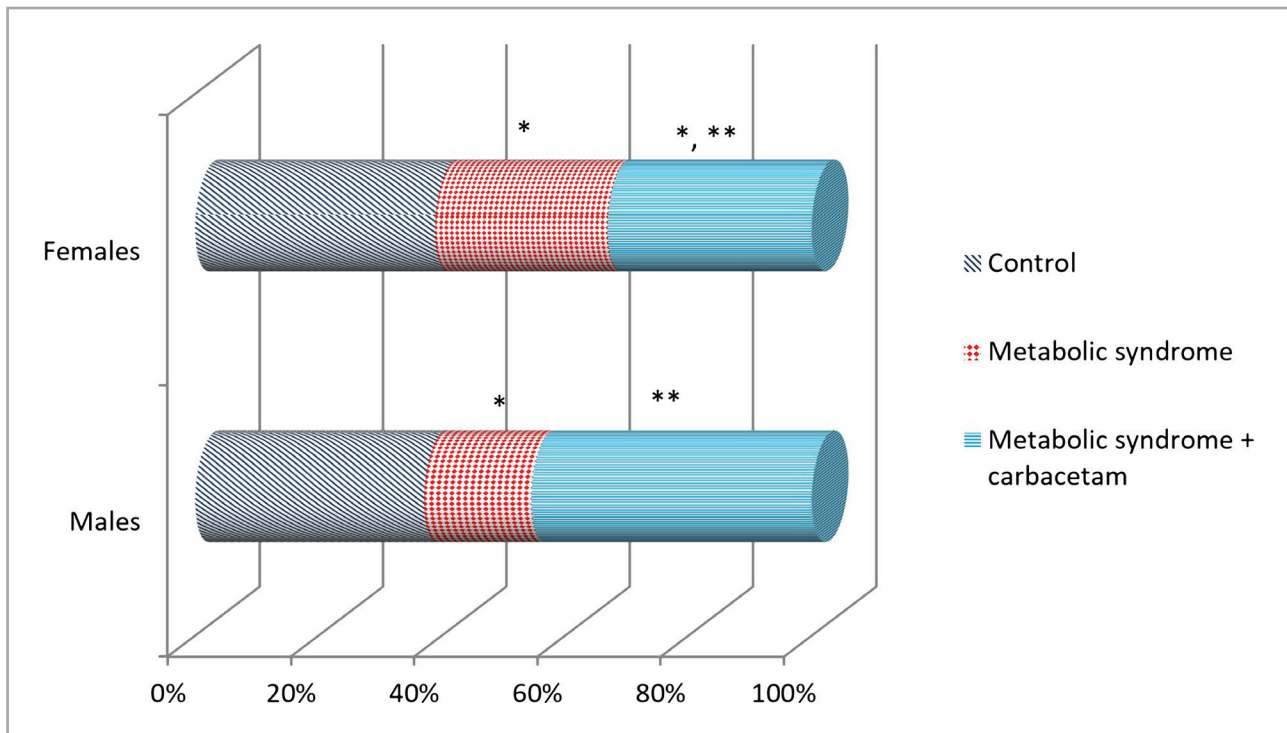


Figure 2: Relative rate of mitochondrial swelling of hepatocytes of rats of different breeds with metabolic syndrome after 14 days of carbacetam administration at a dose of 5 mg/kg ($M \pm m$, $n=7$). * – reliability of differences compared with the control group of rats; ** – reliability of differences compared with the group of rats with metabolic syndrome.

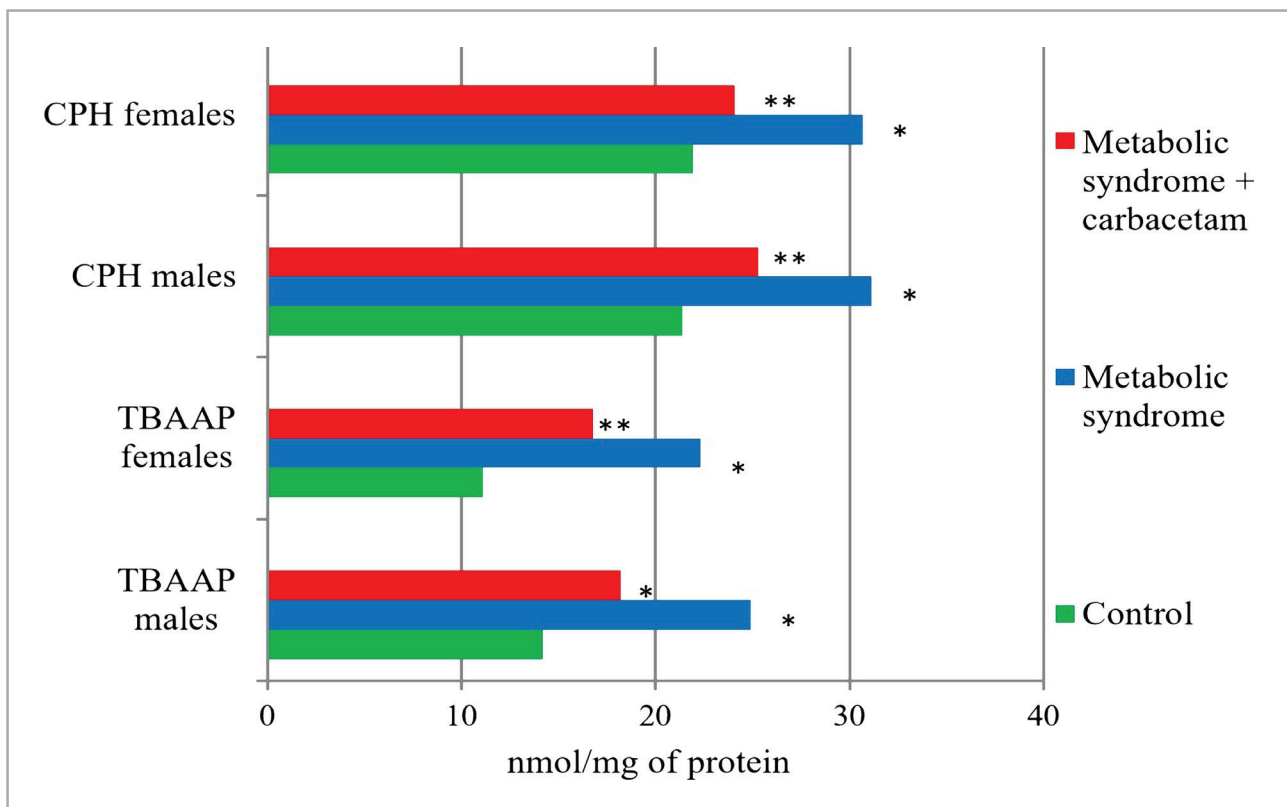


Figure 3: Effect of carbacetam on free radical oxidation of lipids and proteins in liver mitochondria of rats of different strains with experimental metabolic syndrome and after carbacetam administration ($M \pm m$, $n=7$). * – reliability of differences compared with the control group of rats; ** – reliability of differences compared with the group of rats with metabolic syndrome.

Table 1: Effect of carbacetam on antioxidant defense indices in liver mitochondria of rats of different strains with experimental metabolic syndrome and after carbacetam administration (M+m, n=7).

Indices	Sex	Control	Metabolic syndrome	Metabolic syndrome + carbacetam
SOD units/mg of protein	Males	0.393±0.03	0.298±0.01 *	0.375±0.01 **
	Females	0.472±0.03	0.364±0.02 *	0.446±0.02 **
Catalase mcmol H ₂ O ₂ /min. mg of protein	Males	168.874±8.11	135.838±2.61 *	163.174±5.98 **
	Females	187.484±6.69	140.054±5.06 *	169.909±6.07 **

Note: * – reliability of differences compared with the control group of rats; ** – reliability of differences compared with the group of rats with metabolic syndrome.

metabolic syndrome administered with carbacetam, the contents of TBARS and CPH decrease. Thus, TBARS content on an average 25.8% decreased, and CPH – 20.2% decreased in rats of both genders. At the same time, enzymatic activity of antioxidant defense increased. Thus, SOD activity 25% increased in male rats and 22.5% – in females. Catalase activity 20.2% increased in male rats and 21.3% – in female rats.

Therefore, the results of our research are indicative of the development of mitochondrial dysfunction in the liver cells of rats of different genders under conditions of simulating metabolic syndrome. Male rats appeared to be more susceptible to damage. At the same time, administration of carbacetam as a modulator of GABA-receptors demonstrated positive changes from the point of view of mitochondrial functioning. These changes include a decreased relative rate of mitochondrial swelling and decreased peroxide oxidation of lipids and proteins with increasing activity of antioxidant defense enzymes.

One of the probable mechanisms of carbacetam action is that activation of GABA-signaling protects liver function against its damage with D-galactosamine, decreasing toxic damage of the liver cells and decreasing proliferation of epithelial cells lining the intrahepatic bile ducts, forming the “biliary tree” [14, 15]. At the same time, these processes in our opinion, promote the decrease of mitochondrial swelling rate, intensity of lipid and protein peroxidation (decrease of the TBARS and CPH contents) and increase activity of antioxidant defense enzymes such as SOD and catalase. Thus, an important fact is that our results demonstrated a protective role of modulation of GABA-receptors in the liver depending on gender. Nevertheless, a therapeutic value of this mechanism activation in prevention and treatment of liver damage requires further studies.

Conclusions

Under conditions of experimentally induced metabolic syndrome in rats of both sexes, light scattering decreased, while the relative rate of mitochondrial swelling and free radical oxidation of lipids and proteins increased, with more pronounced alterations observed in male rats. Administration of carbacetam, a modulator of GABA receptors, reduced the relative rate of mitochondrial swelling and the levels of thiobarbituric acid-reactive substances and protein oxidative modification products, while increasing catalase and superoxide dismutase activities in both sexes, with more pronounced effects in male rats. These findings suggest that carbacetam exerts mitochondrial protective and antioxidant effects in rats with metabolic syndrome, supporting its potential neuroprotective properties, with the effects being more marked in males.

Conflict of interest

The authors declare no conflict of interest.

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