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NITRIC OXIDE CYCLE ACTIVITY IN RAT BICEPS FEMORIS MUSCLE UNDER CONDITIONS OF BACTERIAL LIPOPOLYSACCHARIDE INFLUENCE, EXPERIMENTAL METABOLIC SYNDROME AND THEIR COMBINATION

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There is evidence that long-term organism stimulation with bacterial lipopolysaccharides (LPS), which promotes the secretion of pro-inflammatory cytokines and nitric oxide, may play an important role in metabolic syndrome (MetS) development. Changes in NO production under conditions of MetS have different directions and depend on a specific organ. The purpose of this work was to study the production of nitric oxide and its metabolites in the biceps femoris muscle of rats under conditions of lipopolysaccharide stimulation of the organism, metabolic syndrome and their combination. The study was conducted for 60 days on 24 male Wistar rats divided into control, MetS, LPS and LPS+MetS groups. MetS was reproduced by adding 20% fructose solution to food, LPS stimulation was carried out by intraperitoneal injection of S. typhi LPS. It was demonstrated that stimulation of the rat organism with LPS under conditions of experimental metabolic syndrome increased the production of nitric oxide by L-arginine-dependent pathway, but limited metabolic syndrome-induced increase in nitric oxide production by L-arginine-independent pathway, reduced the concentration of S-nitrosothiols, while increasing the concentration of peroxynitrites and nitrites in the biceps femoris muscle of rats.

K e y w o r d s: metabolic syndrome, bacterial lipopolysaccharide, nitric oxide, reactive nitrogen species, biceps femoris.

Metabolic syndrome (MetS) is one of the most common diseases of civilization that humanity has encountered in the 20-21st century. Approximately 20-25% of the world's adult population suffers from metabolic syndrome, the leaders in the prevalence of MetS are Western Europe (12-26% of the adult population) and the USA (36.9% of the adult population) [1]. MetS can be complicated by the development of type II diabetes, and certain components of the pathogenesis of MetS lead to damage to the heart and liver [2]. The presence of MetS in a person increases the risk of sudden cardiac death by 70%, even in the absence of data on coronary heart disease in the medical history [3].

Nitric oxide (NO) is an important biologically active compound that plays a significant role in the pathogenesis of the development of insulin resistance under MetS conditions [4]. However, changes in NO production under conditions of MetS have different directions and depend on a specific organ or its part (stroma or parenchyma). For example, the production of NO from the endothelial isoform of NO synthase (eNOS) is reduced, which is one of the reasons for impaired physiological insulin signaling [5]. However, NO production from the inducible isoform of NO-synthase (iNOS), on the contrary, increases under conditions of development of MetS and leads to chronic low-intensity inflammation [6].

The main etiological factor leading to the development of MetS is a high-calorie diet and a sedentary lifestyle [7]. Long-term stimulation of the body with bacterial lipopolysaccharides (LPS) can also lead to the development of MetS [8]. Currently, the combined effect of long-term stimulation of the body

with bacterial lipopolysaccharide and a high-calorie diet on the development of metabolic syndrome is insufficiently studied. Also, in the scientific literature, there is a limited amount of data on changes in the production of nitric oxide in skeletal muscles under the conditions of metabolic syndrome.

The purpose of this work is to study the effect of bacterial lipopolysaccharide stimulation of the organism, the influence of metabolic syndrome modeling and their combination on the production of nitric oxide in L-arginine-dependent and L-arginine-independent ways, the concentration of the main metabolites of nitric oxide in the biceps femoris muscle of rats.

Materials and Methods

The study was conducted on 24 mature male Wistar rats weighing 200-260 g. The animals were divided into 4 groups of 6 animals each. The first group was a control group, the animals of this group received manipulations similar to those of the other groups, but instead of the active substances, they received a 0.9% solution of sodium chloride. The second group is the experimental metabolic syndrome group (MetS group). Experimental MetS was reproduced by using a 20% fructose solution as the only source of water for 60 days [9]. The third group is the group of stimulation of the organism with the bacterial LPS of *S. typhi* (LPS stimulation group). LPS stimulation of the organism was carried out according to the following scheme: in the first week, animals were administered LPS at a dose of 0.4 μg/kg intraperitoneally three times a week, then LPS was administered at a dose of 0.4 μg/kg intraperitoneally once a week throughout the experiment (60 days) [10]. The fourth group is the group of the combined effect of stimulation of the LPS organism and reproduction of experimental MetS (LPS+MetS group). Animals of this group received a 20% fructose solution as the only source of water and were administered LPS according to the scheme of group 3.

The animals were kept in the vivarium of the Poltava State Medical University under standard conditions. We worked with laboratory animals according to the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986). The withdrawal of animals from the experiment was carried out under thiopental anesthesia by taking blood from the right ventricle of the heart. All manipulations with laboratory animals were approved by the Bioethics Commission of the Poltava State Medical University (Record No 206 from 24.06.2022).

The object of the study was a 10% homogenate of the biceps femoris muscle of rats. Total activity of NO-synthases (NOS) was evaluated by increase in nitrite (NO_2^-) concentration after incubation homogenized tissue samples for 30 min. at temperature 37°C in the incubation solution (3.1 ml) containing: 2.5 ml of 161 mM Tris-buffer (pH 7.4), 0.3 ml of 31 mM L-arginine, 0.1 ml 32 μM NADPH and 0.2 ml of 10% tissue homogenate. Immediately after mixing homogenate with the incubation solution, we took 0.2 ml of the formed mixture to evaluate the initial nitrite concentration. Then the reaction was stopped by adding aliquot 0.02 ml 0.02% of sodium aside. Then 0.2 ml of the mixture was taken to assess the final nitrite concentration. Total NOS activity was calculated as NOS = (A2-A1)×2057÷P (μmol/min per g of protein), where A2 means absorbance of solution taken for final nitrite measurement, A1 stands for absorbance of solution taken for initial nitrite measurement, P is the concentration of protein calculated by Biurette method g/l [10-12].

In order to evaluate the activity of constitutive isoforms of NO-synthase (cNOS) we used the following procedure: 0.2 ml of 10% tissue homogenate was taken for analysis and was incubated for 60 min at t 37°C in incubation solution (3.3 ml) containing: 2.5 ml of 152 mM Tris-buffer (pH 7.4), 0.3 ml of 29 mM L-arginine, 0.2 ml of 545 μM aminoguanidine hydrochloride and 0.1 ml of 30 μM NADPH. Initial nitrite content was evaluated as mentioned before. After 60 min incubation we measured the final nitrite content and calculated cNOS activity by the formula: (A2-A1)×1028.5÷P, (μmol/min per g of protein), where A2 means absorbance of solution taken for final nitrite measurement, A1 stands for absorbance of solution taken for initial nitrite measurement, P is the concentration of protein calculated by Biurette method g/l [11, 12]. The activity of inducible isoform of NO-synthase (iNOS) was calculated by the formula: iNOS = NOS-cNOS (μmol/min per g of protein).

We used Griess-Ilosvay reagent for nitrite estimation (1% sulfanilic acid in 30% acetic acid and 0,1% 1-naphtylamine in the same solvent). To the 0.2 ml of aliquot taken for initial (or final) nitrite assessment we added 1.8 ml of distilled water. Following this, we added 0.2 ml of 1% sulfanilamide

acid and then in 10 min, we added 0.2 ml of 0,1% 1-naphtylamine. The concentration of nitrites was measured by spectrophotometer Ulab-101 (540 nm in cuvette with optical path length of 5 mm) [11].

Total activity of arginases was assessed by difference of L-ornithine concentration before and after incubation of 0.1 ml of 10% tissue homogenate in incubation solution (0.8 ml) containing 0.5 ml of 125 mM phosphate buffer (pH 7.0), 0.2 ml of 6 mM L-arginine. Aliquot 0.1 ml of mixture was immediately taken for estimation of initial L-ornithine concentration. For the final L-ornithine concentration estimation 0.1 ml of mixture was taken for analysis after incubation.

Evaluation of L-ornithine was performed after addition 0.1 ml of modified Chinard's reactive (2.5% ninhydrin on acidic mixture consisting of 2:3 60% orthophosphoric and ice acetic acids mixed at a ratio 6:4 with water) and 1.0 ml of ice acetic acid [12]. The solution was boiled for 40 min to achieve maximal color yield. Then 1 ml of 20% trichloracetic acid was added to precipitate proteins and after centrifugation (1000 g) for 30 min the absorbance of 1 ml of supernatant was measured (10 mm cuvette against water on 515 nm wavelength) [12].

Nitrite reductase (NiR) activity was assessed by a decrease in nitrite content after 60 min at *t* 37°C incubation of 0.2 ml of 10% tissue homogenate in incubation medium (2.3 ml) consisting: 1 ml of 87 mM phosphate buffer (pH 7.0), 1 ml of 4.35 mM sodium nitrite, and 0.1 ml of 61 μM NADH. Nitrites content was measured before and after incubation [11, 12].

Nitrate reductase (NaR) activity was assessed by decrease in nitrate content after 60 min at *t* 37°C incubation of 0.2 ml of 10% tissue homogenate in incubation medium (2.3 ml) consisting: 1 ml of 87 mM phosphate buffer (pH 7.0), 1 ml of 4.35 mM sodium nitrate, and 0.1 ml of 61 μM NADH. We immediately took aliquot 0.2 ml of mixture to measure initial nitrate content [11, 12]. After incubation, we took another aliquot 0.2 ml to estimate final nitrate concentration. In order to measure nitrate concentration, we added to initial and final aliquots 0.1 ml of 0.55% hydrazine sulfate solution and incubated for 10 min at *t* 40˚C to reduce all nitrates to nitrites. Afterwards, we estimated nitrite concentration as mentioned above.

The concentration of peroxynitrites of alkali (Na^+, K^+) and alkali-earth (Ca^{2+}) metals was measured by using its reaction with potassium iodide under pH 7.0 in 0.2 M phosphate buffer with the same pH [12]. For this purpose, we took 0.1 ml of 10% tissue homogenate, which then was solved in 3.9 ml of 156 mM phosphate buffer (pH 7.0) and 1 ml of 6 mM potassium iodide (final volume 5 ml). Test tubes with the mixture were shaken vigorously for 2 min. The following centrifugation (1000 g) lasted 10 min. Then we assessed the absorbance of 1 ml of upper layer against the control (incubation medium without homogenate) in 10 mm cuvette at 355 nm.

The concentration of low molecular weight S-nitrosothiols (S-NO) was determined by increase in nitrite concentration after 30 min incubation of 0.2 ml of 10% tissue homogenate in incubation solution (2.6 ml) containing: 2.0 ml of 154 mM phosphate buffer (pH 7.0), 0.1 ml of 923 μM sodium fluoride, and 854 μM mercury chloride. Immediately after addition of tissue homogenate to the incubation solution, we took 0.5 ml of mixture for measurement of initial nitrite concentration. For assessment of final nitrite concentration, we took 0.5 ml of incubation mixture after 30 min. Concentration of S-nitrosothiols was calculated by difference between initial nitrite content (taken before incubation) and final nitrite content (measured after incubation) [11].

The concentration of H_2S was estimated by amount of a color dye formed in reaction of H_2S with specific sulfide coloring reagent (0.4 g of N,N-dimethyl-p-phenylenediamin and 0.6 g of iron (III) chloride ($FeCl_3 \cdot 6H_2O$) dissolved in 100 ml of 6 M HCl). For analysis, we took 0.2 ml of 10% tissue homogenate and added 0.2 ml of sulfide coloring reagent. The mixture was left to stand for 15 min at room temperature. Then 5 ml of 0.1 M HCl was added for the final volume 5.4 ml and absorbance was measured at 667 nm in 10 mm cuvette [11].

In the blood, we studied the concentration of the following metabolic substances: glucose, triglycerides (TG), total cholesterol (TC), cholesterol from low-density lipoproteins (LDL-C), cholesterol from high-density lipoproteins (HDL-C). All the abovementioned substances were evaluated by respective assays produced by "Filisit Diagnistika" (Ukraine). We also calculated body-mass index (BMI) according to recommendations [13].

In order to evaluate the development of insulin resistance, we calculated the following indexes:

Triglyceride glucose-body mass index (TyG-BMI). TyG-BMI = Ln [TG (mg/dl) × Glucose (mg/dl) ÷ 2] × BMI (kg/m^2) [14].

Metabolic score for insulin resistance (METS-IR) index. METS-IR = Ln [(2×Glucose (mg/dl)) +

+ TG (mg/dl)]×BMI (kg/m^2) ÷ Ln [HDL-C (mg/dl)] [14].

The statistical significance of the difference between groups was determined using the non-parametric analysis of variance by Kruskal-Wallis method, followed by pairwise comparisons using the Mann-Whitney U-test. The difference was considered statistically significant at $P < 0.05$.

Results

The development of metabolic syndrome in the group of stimulation of the body with bacterial lipopolysaccharide, the group of simulation of metabolic syndrome using a 20% fructose solution, and in the group of their combination is confirmed by an increase in the level of glucose in the blood, hyperlipidemia, dyslipidemia, and the development of insulin resistance in animals from these groups (Table).

Modeling of metabolic syndrome resulted in a 94.7% increase in total NOS activity in rat biceps femoris muscle compared to control group (Fig. 1, *A*). Under these conditions, cNOS activity decreased by 41.5% (Fig. 2, *B*), and iNOS activity increased by 105.7% when compared with the control group of animals (Fig. 2, *A*). Arginase activity increased by 74.9% compared to the results of the control group (Fig. 1, *B*). After analyzing the changes in the L-arginine-independent pathway of nitric oxide formation, we found that the activity of NaR increased by 54.7% (Fig. 3, *A*), and the activity of NiR increased by 134.3% when compared with the control group (Fig. 3, *B*). At the same time, the concentration of nitrites decreased by 59.2% (Fig. 4, *A*), and the concentration of $ONOO^-$ and S-NO increased by 71.4% (Fig. 5, *A*) and by 24.2% (Fig. 5, *B*), respectively, compared to the control group of animals. H_2S content in the MetS group increased by 65.7% compared to the control group (Fig. 4, *B*). Therefore, the modeling of the metabolic syndrome leads to an increase in the production of nitric oxide in L-arginine-dependent and L-arginine-independent ways. At the same time, the increased activity of arginases against the background of high iNOS activity threatens the development of competition between NOS and arginases for the substrate of the reaction, which can explain the reduced activity of cNOS in this group. It is interesting that according to the results of our research, the formation of peroxynitrites and S-nitrosothiols are the predominant ways of nitric oxide metabolization. The reduced concentration of nitrites can be explained by the increased activity of the nitrate-nitrite reductase pathway of nitric oxide formation.

Stimulation of the organism with bacterial LPS led to an increase in total NOS activity by 39.4% in the biceps femoris muscle of rats when compared with the control group. Under these conditions, cNOS activity decreased by 45.3%, and iNOS activity increased by 45.9% when compared with the control group of animals. Arginase activity decreased by 57.7% compared to the results of the control group. NaR activity decreased by 25.7% and NiR activity decreased by 52.6% when compared to the control group. At the same time, the concentration of nitrites and S-NO decreased by 29.9 and 60.6%, respectively, and the concentration of $ONOO^-$ in-

Table. Metabolic changes in rat blood and insulin resistance indexes under conditions of metabolic syndrome and stimulation of the organism with bacterial lipopolysaccharide (M ± m, n = 6)

Parameters	Groups			
	Control	MetS	LPS stimulation	LPS + MetS
Glucose, mmol/l	3.89 ± 0.06	8.24 ± 0.11*	5.85 ± 0.09 */#	9.79 ± 0.03 */#/^
TG, mmol/l	0.90 ± 0.06	2.75 ± 0.06*	1.65 ± 0.05 */#	3.34 ± 0.03 */#/^
TC, mmol/l	1.18 ± 0.01	1.77 ± 0.02*	1.42 ± 0.01 */#	2.09 ± 0.01 */#/^
LDL-C, mmol/l	0.17 ± 0.005	0.28 ± 0.01*	0.21 ± 0.003 */#	0.37 ± 0.01 */#/^
HDL-C, mmol/l	0.55 ± 0.02	0.38 ± 0.01*	0.50 ± 0.01 */#	0.33 ± 0.01 */#/^
TyG-BMI	40.91 ± 1.93	64.65 ± 0.88*	54.62 ± 0.57 */#	68.38 ± 0.79 */#/^
METS-IR	5.61 ± 0.05	6.79 ± 0.02*	6.24 ± 0.02 */#	7.03 ± 0.01 */#/^

Note: *The data are statistically significantly different from the control group ($P < 0.05$). #The data are statistically significantly different from the experimental metabolic syndrome group ($P < 0.05$). ^The data are statistically significantly different from the group of stimulation of the organism by bacterial lipopolysaccharide ($P < 0.05$)

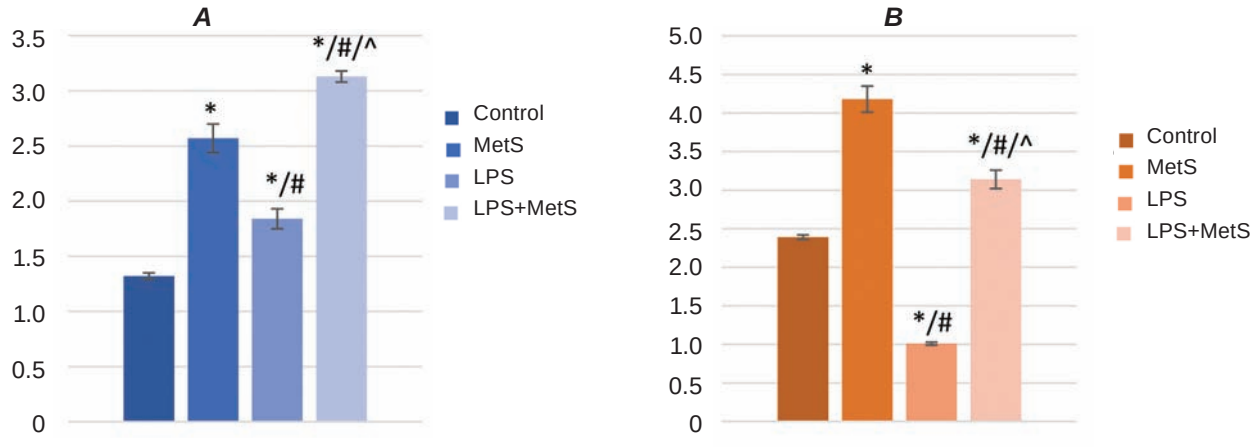

Fig. 1. ***A*** *– Total activity of NOS in rat biceps femoris, μmol/min per g of protein;* ***B*** *– Activity of arginase in rat biceps femoris, μmol/min per g of protein. *The data are statistically significantly different from the control group (P < 0.05); #the data are statistically significantly different from the experimental metabolic syndrome group (P < 0.05); ^the data are statistically significantly different from the group of stimulation of the organism by bacterial lipopolysaccharide (P < 0.05)*

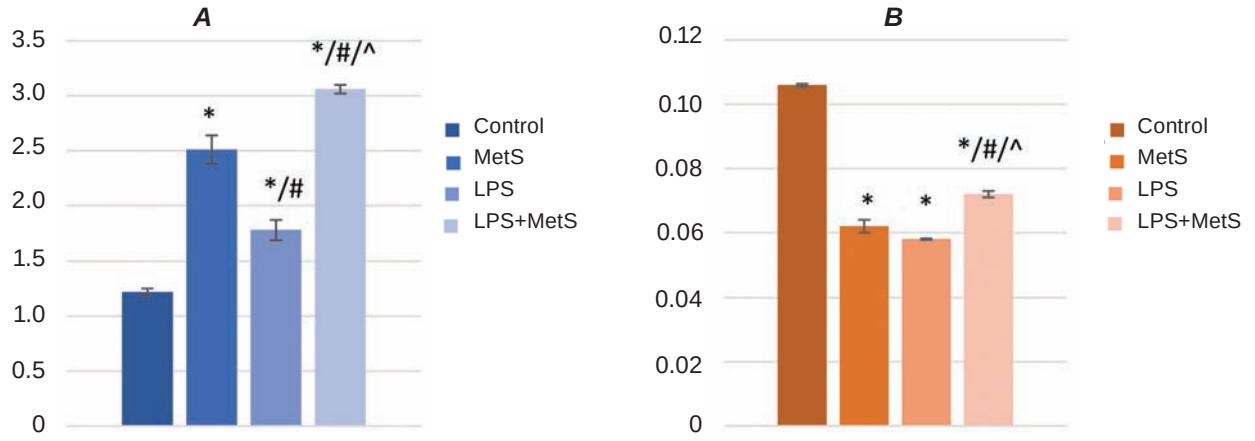

Fig. 2. ***A*** *– Activity of iNOS in rat biceps femoris, μmol/min per g of protein;* ***B*** *– Activity of cNOS in rat biceps femoris, μmol/min per g of protein. *The data are statistically significantly different from the control group (P < 0.05); #the data are statistically significantly different from the experimental metabolic syndrome group P < 0.05); ^the data are statistically significantly different from the group of stimulation of the organism by bacterial lipopolysaccharide (P < 0.05)*

creased by 335.2% compared to the control group of animals. The content of H_2S in the group of stimulation of the body with bacterial LPS decreased by 22.0% compared to the control group. Therefore, LPS stimulation of the organism leads to an increase in the production of nitric oxide in the biceps femoris due to increased activity of the L-arginine-dependent pathway of its formation. At the same time, the predominant way of metabolizing nitric oxide is the formation of peroxynitrites.

When comparing the data of LPS stimulation group and MetS group, we found that LPS stimulation reduced the total activity of NOS in the biceps femoris muscle by 28.4% compared to MetS group. There was no statistically significant difference between cNOS activities in these groups. iNOS activity in the group of organism stimulation with bacterial LPS was 29.1% lower when compared with the MetS group. The activities of arginases, NaR, and NiR in the LPS stimulation group were by 75.8, 52.0 and 79.8% lower, respectively, compared to the MetS

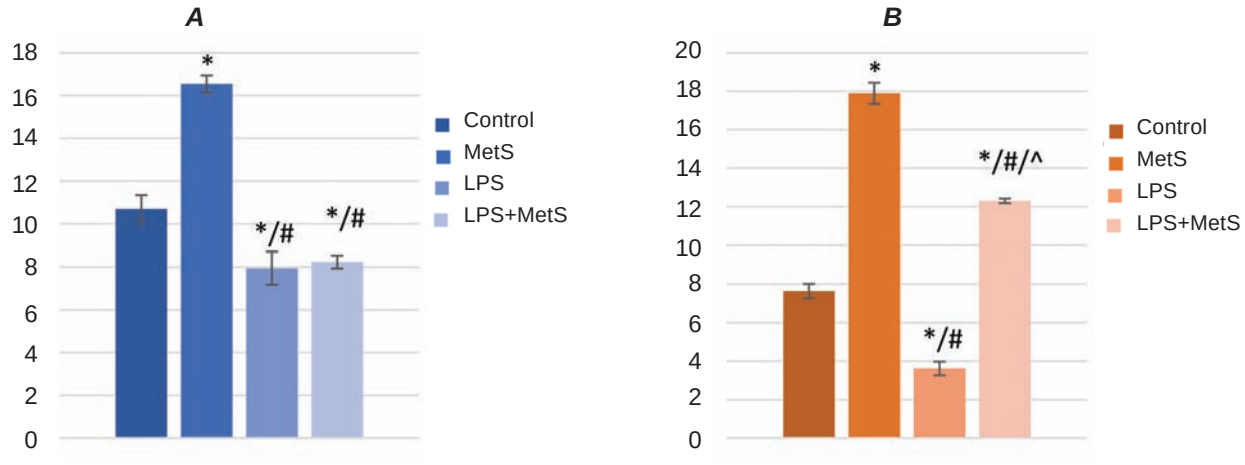

Fig. 3. **A** *– Activity of NaR in rat biceps femoris, μmol/min per g of protein;* **B** *– Activity of NiR in rat biceps femoris, μmol/min per g of protein. *The data are statistically significantly different from the control group (P < 0.05); #the data are statistically significantly different from the experimental metabolic syndrome group (P < 0.05); ^the data are statistically significantly different from the group of stimulation of the organism by bacterial lipopolysaccharide (P < 0.05)*

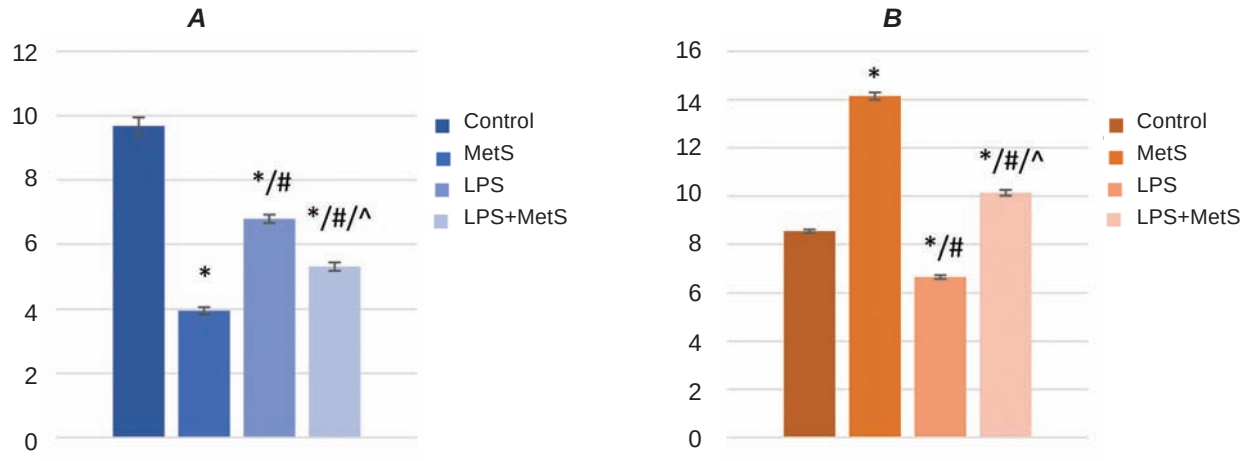

Fig. 4. **A** *– Nitrite concentration in rat biceps femoris, nmol/l;* **B** *– H_2S concentration in rat biceps femoris, μmol/g of tissue. *The data are statistically significantly different from the control group (P < 0.05); #the data are statistically significantly different from the experimental metabolic syndrome group (P < 0.05); ^the data are statistically significantly different from the group of stimulation of the organism by bacterial lipopolysaccharide (P < 0.05)*

group. The concentration of nitrites was higher by 71.9%, and peroxynitrites by 153.8%. The content of S-nitrosothiols and H_2S was lower by 68.3 and 52.9%, respectively.

The combination of MetS and LPS stimulation increased the total activity of NOS by 137.1% in the biceps femoris muscle compared to control group. Activity of cNOS decreased by 32.1%, and iNOS activity increased by 150.8% compared to the control group. The activity of arginase under these conditions increased by 31.4% compared to the control group. NaR activity decreased by 23.0%, NiR activity increased by 61.0% compared to the control group. The concentration of nitrites decreased by 45.0% and the concentration of S-nitrosothiols decreased by 36.4%, while the concentration of peroxynitrites increased by 268.1% compared to the control group. The H_2S content increased by 18.6% compared to the control group. Therefore, the combination of metabolic syndrome and LPS stimula-

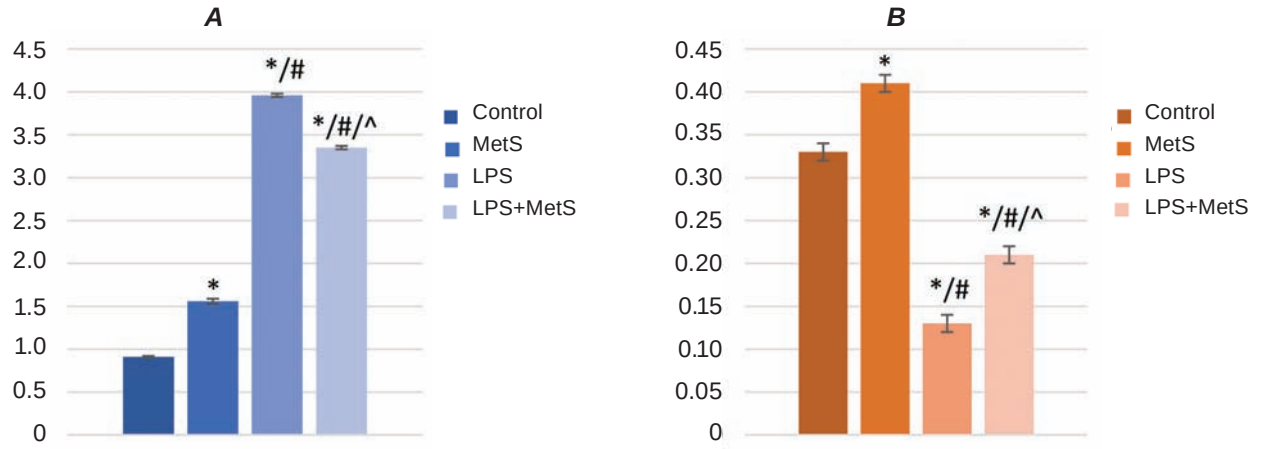

Fig. 5. ***A*** *– Concentration of $ONOO^-$ in rat biceps femoris, μmol/g of tissue;* ***B*** *– Concentration of S-nitrosothiols in rat biceps femoris, μmol/g of tissue. *The data are statistically significantly different from the control group (P < 0.05); #the data are statistically significantly different from the experimental metabolic syndrome group (P < 0.05); ^the data are statistically significantly different from the group of stimulation of the organism by bacterial lipopolysaccharide (P < 0.05)*

tion of the organism, by analogy with the group of isolated modeling of metabolic syndrome, leads to increased production of nitric oxide in both L-arginine-dependent and L-arginine-independent ways. However, most of the nitric oxide is metabolized into peroxynitrite, by analogy with isolated LPS stimulation of the body.

The combination of MetS and LPS stimulation increased the total NOS activity by 21.8% in the biceps femoris muscle of rats compared to MetS group. Activity of cNOS increased by 16.1%, and iNOS activity by 21.9% compared to the indicators of the MetS group. Under these conditions, the activity of arginase decreased by 24.9% compared to the MetS group of animals. NaR activity decreased by 50.2%, NiR activity by 31.3% compared to the MetS group. The concentration of nitrites increased by 34.7%, and the concentration of S-nitrosothiols decreased by 48.8%, while the concentration of peroxynitrites increased by 114.7% compared to the MetS group. The content of H_2S decreased by 28.4% compared to the MetS group.

The combination of MetS and LPS stimulation increased total NOS activity by 70.1% in the biceps femoris muscle of rats compared to LPS stimulation group. The activity of cNOS increased by 24.1%, and the activity of iNOS by 71.9% compared to the indicators of the LPS stimulation group. Under these conditions, the activity of arginase increased by 210.9% compared to the LPS stimulation group of animals. The activity of NiR increased by 239.8% compared to the LPS stimulation group. Nitrite concentration decreased by 21.7% and S-nitrosothiols concentration increased by 61.5%, while peroxynitrite concentration decreased by 15.4% compared to the LPS stimulation group. The H_2S content increased by 52.1% compared to the LPS stimulation group.

Discussion

The increase in iNOS activity under the conditions of modeling the metabolic syndrome by using a 20% fructose solution as the only source of drinking water can be explained by the excessive activation of the nuclear factor kappa-amplifier of light chains of activated B cells (NF-κB), under the transcriptional control of which the iNOS genes are located [15]. The development of MetS and its complications of type II diabetes are often accompanied by endothelial dysfunction and uncoupling of eNOS from its substrate [16-18]. One of the reasons for the development of endothelial dysfunction during MetS is the increased activity of arginases, which are competitors with NO-synthases for the substrate. Increased arginase activity is seen as a result of the development of hyperglycemia during MetS [19]. Constitutive isoforms of NOS, under conditions of competition with arginases and iNOS, find themselves in conditions of substrate deficiency, which explains the reduced activity of cNOS in the metabolic syndrome group.

The increased activity of NiR and NaR under conditions of metabolic syndrome explains the reduced concentration of nitrites in this group of rats, and may be an adaptive reaction, since according to M. Peleli et al. introduction of exogenous stimulators of the L-arginine-independent way of formation of nitric oxide (nitrates) improves the course of MetS [20]. Increased reduction of nitrates and nitrites under conditions of MetS may be associated with increased activity of xanthine oxidoreductase (XOR, EC 1.17.1.4 and EC 1.17.3.2), which participates both in the synthesis of uric acid and the formation of active forms of oxygen (superoxide anion radical, hydrogen peroxide), and through its reductase domain under certain conditions (decrease in pH, excess of electron donors) can act as a reducing agent for nitrates and nitrites [21].

The increased concentration of peroxynitrites and S-nitrosothiols under MetS conditions is a consequence of the increased production of nitric oxide. According to our research, the predominant way of nitric oxide metabolism in this group is the formation of peroxynitrites, since their concentration increases to a greater extent when compared to S-nitrosothiols, and the formation of nitrites and nitrates is complicated by the increased activity of NiR and NaR. The predominance of peroxynitrite formation under the conditions of MetS can be explained by the increase in the production of reactive oxygen species and the development of oxidative stress under these conditions, which was shown in our previous work [22].

An increase in the concentration of sulfides in the MetS group can be considered an adaptive response to the development of insulin resistance in this group since, in the scientific literature, there are data on the increase in glucose uptake by cells under the influence of insulin due to the exogenous introduction of H_2S and polysulfides [23]. According to A. Berenyiova et al., enriching the diet of rats with fructose can increase the expression of cystathionine β-synthase genes, which can also explain the increase in the pool of endogenous H_2S observed in our study [24].

Increased iNOS activity in the LPS body stimulation group is associated with the effect of LPS on Toll-like receptors and NF-κB activation [25]. Activation of the nuclear transcription factor κB under physiological conditions prevents the development of oxidative-nitrosative stress, however, under the conditions of the addition of a pathological stimulus or excessive tissue damage, it can stimulate the formation of reactive forms of oxygen and nitrogen [26]. Reduced arginase activity in this group is associated with a change in LPS-induced polarization of tissue macrophages to a pro-inflammatory (M1) phenotype characterized by increased iNOS expression and blockade of arginase expression [25]. The decrease in cNOS activity under the influence of LPS may be related to the ability of LPS to affect the activity of the eNOS gene promoter, which leads to a decrease in the expression of eNOS, and, as a result, leads to a decrease in its activity [27].

The decrease in the activities of NiR and NaR may be related to the effect of the bacterial LPS administered in this group on the functional state of XOR. LPS can enhance the activity of XOR, which is an enzyme that ensures LPS-induced polarization of macrophages to the M1 phenotype [28]. However, this effect is not unidirectional in relation to the two functional forms of this enzyme: xanthine dehydrogenase (EC 1.17.1.4) and xanthine oxidase (EC 1.17.3.2). LPS is able to suppress the activity of xanthine dehydrogenase, which leads to a decrease in NiR and NaR activities, but increases the activity of xanthine oxidase, which increases the formation of reactive oxygen species and ensures the development of LPS-induced oxidative damage to various organs [28]. The literature provides data on the ability of exogenous nitrates, which are substrate inducers of xanthine dehydrogenase, to prevent the development of LPS-induced inflammation [29].

A decrease in the concentration of nitrites and S-nitrosothiols in the muscles of rats from the group of body stimulation with bacterial LPS against the background of an increase in the concentration of peroxynitrites indicates the predominance of the peroxynitrite pathway of nitric oxide metabolism, which threatens the development of nitrosative stress in these animals.

A decrease in the concentration of endogenous H_2S may be associated with the inhibitory effect of LPS on the activity of cystathionine β-synthase [30]. This can be considered a negative phenomenon, since the increase in the activity of this enzyme under the conditions of exposure to LPS can suppress the intensity of the inflammatory process [31].

The introduction of bacterial LPS against the background of MetS simulation shows a synergistic effect on iNOS activity. MetS and LPS stimulation of the organism has an antagonistic effect on the activity of arginases. Under the conditions of the combined influence of MetS and LPS, the stimulating effect of MetS on the activity of arginases is

more pronounced. The inhibitory effect of LPS on NaR dominates in the conditions of the combined effect of LPS and MetS, but the activity of NiR is more affected by the activating effect of MetS. Under the conditions of a combination of LPS and MetS, the main pathway of metabolic transformations of NO is the formation of peroxynitrites, which corresponds to the LPS-dependent trend. The activating effect of an excess of fructose in the diet of rats in the group of the combined effect of LPS and MetS on the activity of cystathionine β-synthase dominates over the inhibitory effect of the LPS component on the activity of this enzyme, as seen from increase in H_2S concentration compared to LPS stimulation group.

Conclusions. Modeling of metabolic syndrome by the introduction of a fructose-rich diet increases the production of nitric oxide both in L-arginine-dependent and L-arginine-independent pathways, reduces the concentration nitrites, while increasing the concentration of peroxynitrites and S-nitrosothiols in the biceps femoris muscle of rats.

Stimulation of the rat organism with bacterial lipopolysaccharide increases the production of nitric oxide in L-arginine-dependent pathway, but lowers its production by L-arginine-independent pathway, reduces the concentration of S-nitrosothiols and nitrites, while increasing the concentration of peroxynitrites in the biceps femoris muscle of rats.

Stimulation of the rat organism with bacterial lipopolysaccharide under conditions of experimental metabolic syndrome increases the production of nitric oxide in L-arginine-dependent pathway, but limits metabolic syndrome-induced increase in nitric oxide production by L-arginine-independent pathway, reduces the concentration of S-nitrosothiols, while increasing the concentration of peroxynitrites and nitrites in the biceps femoris muscle of rats.

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АКТИВНІСТЬ ЦИКЛУ ОКСИДУ АЗОТУ В ДВОГОЛОВОМУ М’ЯЗІ СТЕГНА ЩУРІВ ЗА УМОВ ВПЛИВУ БАКТЕРІАЛЬНОГО ЛІПОПОЛІСАХАРИДУ, ЕКСПЕРИМЕНТАЛЬНОГО МЕТАБОЛІЧНОГО СИНДРОМУ ТА У РАЗІ ЇХ ПОЄДНАННЯ

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Існують докази, що тривала стимуляція організму бактеріальним ліпополісахаридом (ЛПС), яка стимулює секрецію прозапальних цитокінів та оксиду азоту, може грати значну роль у розвитку метаболічного синдрому (МетС). Зміни у продукції оксиду азоту за умов МетС мають різну спрямованість та залежать від конкретного органу. Метою даної роботи було вивчення продукції оксиду азоту та його метаболітів у двоголовому м’язі стегна щурів за умов стимуляції організму ліпополісахаридом, метаболічного синдрому та у разі їх поєднання. Дослідження проводили протягом 60 днів на 24 щурах-самцях лінії Wistar, які були поділені на контрольну, МетС, ЛПС та МетС+ЛПС групи. МетС було відтворено шляхом додавання 20% розчину фруктози до раціону, ЛПС стимуляція була проведена за допомогою внутрішньоочеревинної ін’єкції ЛПС *S. typhi*. Продемонстровано, що стимуляція організму щурів ЛПС за умов експериментального метаболічного синдрому збільшує продукцію оксиду азоту L-аргінін-залежним шляхом, проте обмежує збільшення продукції оксиду азоту L-аргінін-незалежним шляхом, яке обумовлене метаболічним синдромом, знижує концентрацію S-нітрозотіолів, збільшуючи при цьому концентрацію пероксинітритів та нітритів у двоголовому м’язі стегна щурів.

Ключові слова: метаболічний синдром, бактеріальний ліпополісахарид, оксид азоту, активні форми азоту, двоголовий м’яз стегна.

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