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Role of p38 activation in changes of nitric oxide production in rat biceps femoris muscle during metabolic syndrome

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Transcriptional changes in organism and muscles especially during development of metabolic syndrome (MetS) is still highly understudied. Role of p38 MAPK activation during MetS is highly debatable.

The aim of this study is to evaluate influence of administration of selective inhibitor of p38 MAPK on production and metabolism of nitric oxide in rat biceps femoris during metabolic syndrome modelling.

Materials and methods. The study was conducted on 24 mature male Wistar rats weighing 200–260 g, which were divided into 4 groups of 6 animals each: I – control group; II – MetS (received 20 % fructose for 60 days); III – SB203580 administration (received SB203580 intraperitoneally in a dose 2 mg/kg once every 3 days for 60 days); IV –SB203580 + MetS modelling. We studied activity of enzymes responsible for NOS-dependent and NOS-independent NO production and content of nitrites, peroxynitrites and nitrosothiols in rat biceps femoris.

Results. MetS modelling increased production of nitric oxide from NO-synthases and nitrtate-nitrite reductive pathway, elevated content of nitrosothiols and peroxynitrite, while SB203580 during MetS modelling attenuated these changes in production of nitric oxide and nitrosothiols and peroxynitrite content.

Conclusions. P38 MAPK activation during MetS modelling increases NOS-dependent and NOS-independent NO production and leads to accumulation of nitrosothiols and peroxynitrite in rat biceps femoris.

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Роль активації p38 у змінах виробництва оксиду азоту в біцепсі стегнового м'яза щурів під час метаболічного синдрому

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Транскрипційні зміни в організмі та м'язах, особливо під час розвитку метаболічного синдрому (МС), до цього часу залишаються маловивченими. Роль активації p38 MAPK під час МС є дуже дискусійною.

Мета роботи – оцінювання впливу введення селективного інгібітора p38 MAPK на продукцію та метаболізм оксиду азоту в двоголовому м'язі стегна щурів під час моделювання метаболічного синдрому.

Матеріали і методи. Дослідження проведено на 24 статевозрілих самцях щурів Вістар вагою 200–260 г, яких розділили на 4 групи по 6 тварин у кожній: I – контрольна група; II – МС (отримували 20 % фруктозу протягом 60 днів); III – введення SB203580 (отримували SB203580 внутрішньочеревно в дозі 2 мг/кг один раз на 3 дні протягом 60 днів); IV – SB203580 + моделювання МС. Досліджували активність ферментів, відповідальних за NOS-залежне та NOS-незалежне утворення NO, а також вміст нітритів, пероксинітритів та нітрозотіолів у двоголовому м'язі стегна щура.

Результати. Моделювання МС збільшило утворення оксиду азоту з NO-синтаз та нітрат-нітритного відновного шляху, підвищило вміст нітрозотіолів та пероксинітриту, тоді як SB203580 під час моделювання МС послабив ці зміни у виробництві оксиду азоту та нітрозотіолів, а також вмісті пероксинітриту.

Висновки. Активація P38 MAPK під час моделювання МС збільшує NOS-залежне та NOS-незалежне утворення NO та призводить до накопичення нітрозотіолів та пероксинітриту у двоголовому м'язі стегна щура.

Сучасні медичні технології. 2025. Т. 17, № 4(67). С. 299-305

Nitric oxide (NO) is an important signalling molecule, which is necessary for upholding physiological functions of the muscle tissue and is often responsible for development of muscle pathologies. NO can influence the thick filament by modulation of calcium influx rate, which changes isometric force development, maximal shortening velocity, and peak power of skeletal muscle [1]. Supplementation with precursors of NO synthesis (L-citrulline and nitrates/nitrites) can augment mechanisms contributing to skeletal muscle performance, hypertrophy, and strength adaptations [2].

There are two primary pathways of nitric oxide synthesis in muscles: "oxidative" pathway and "reductive" pathway. "Oxidative" pathway is performed by set of enzymes called Nitric Oxide Synthases (NOS, EC 1.14.13.39) and is usually called NOS-dependent pathway. These set of enzymes consists from three isoforms inducible NO-synthase, endothelial NO-synthase and neuronal NO-synthase, the last two being calmodulin-dependent and are often called constitutive NO-synthases [3]. All three isoforms catalyse single reaction of L-arginine oxidation by oxygen with $NADPH_2$ as electron donor, which results in NO and L-citrulline formation. "Reductive" pathway of NO synthesis in muscles involves enzyme-dependent and enzyme-independent reduction of nitrates (two electron reduction) and nitrites (one electron reduction) to NO [4]. This pathway is often called NOS-independent.

However, excessive formation of NO in muscles during inflammatory or hypoxic conditions may reveal adverse effects of NO and its metabolites on muscle function. During inflammation caused by muscle injury increased production of NO is often observed. Main NO producing enzyme during inflammation is inducible NO-Synthase [5]. At the same time, a decrease in endothelial NO-synthase activity is often associated with oxidative stress development in skeletal muscles [6]. Therefore, not only the amount of NO produced from NOS-dependent pathway is important, but also the exact enzyme responsible for its production. Disruption of the balance between activities of different NOS isoforms plays a significant role in pathological processes. Skeletal muscles can be considered a reservoir for nitrate and nitrite in our body and have strong ability to activate NOS-independent pathway of NO production due abundance of xanthine oxidoreductase (XOR, EC 1.17.3.2) in muscles [7]. However, excessive activation of xanthine oxidoreductase leads to oxidative damage to the muscles [8].

It should be noted, that not only NO can cause damage to the skeletal muscles, but its metabolites may also have a pathogenetic role. For instance, during abundance of NO in tissue and subsequent increased production of reactive oxygen species (ROS) formation of peroxynitrite ($ONOO^-$) is often observed. Excessive peroxynitrite formation causes protein nitration and leads to nitrosative stress, which, in turn, may cause muscle waning [9]. NO in large quantities or formed from specific enzyme (endothelial NO-synthase) may cause nitrosylation of thiol groups of proteins leading to increased leukocyte adhesion to cells containing these S-nitrosothiols and inflammation enhancement [10]. Hydrogen sulfide (H_2S) acts as another important signalling molecule often interacting with NO signalling [11].

Metabolic syndrome (MetS) is a systemic condition which is often accompanied with generalized inflammatory response in different organs and tissues (metaflammation). MetS systemically activates several transcriptional factors like (NF-κB, Nrf-2, STAT-3, AP-1, etc.), which control reactive oxygen (ROS) and nitrogen species (RNS) production [12]. However, one of important for muscle tissue transcriptional factors, namely, p38-MAPK axis, is usually omitted in studies dedicated to influence of MetS on skeletal muscles or treated as part of NF-κB cascade, despite its ability to independently influence ROS production and glucose metabolism [13,14]. The exact role of p38-MAPK activation in metabolic changes in skeletal muscles during metabolic syndrome remains controversial and demands further studies.

Aim

The aim of this study is to evaluate influence of administration of selective inhibitor of p38 MAPK (SB203580) on production and metabolism of nitric oxide in rat biceps femoris muscle during metabolic syndrome modelling.

Materials and methods

The study was performed using 24 adult male Wistar rats (200–260 g), obtained from the accredited vivarium of Poltava State Medical University. Animals were housed under standard laboratory conditions. The chow received by animals was standard, as described by Y. Frenkel et al. (2023), and did not contain external sources of nitrates and nitrites [15]. All experimental procedures adhered to the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes. Euthanasia was conducted under thiopental anesthesia via cardiac puncture of the right ventricle for blood collection. All animal protocols received prior approval from the Bioethics Committee of Poltava State Medical University (Protocol No. 206, dated 24.06.2022). During the preparation of this scientific article, the Bioethics Committee of Poltava State Medical University re-examined the materials contained therein, and no violations were found. (Protocol No. 240, dated 20.08.2025).

Animals were randomized into four experimental groups (n = 6 per group) as follows:

Group I – Control: Rats received identical handling as experimental groups but were administered 0.9 % sodium chloride solution in place of active compounds.

Group II – Metabolic Syndrome (MetS): MetS was experimentally induced by providing a 20 % fructose solution as the sole drinking fluid for 60 days [16].

Group III – SB203580 Treatment: Rats received intraperitoneal injections of SB203580 (Analytical grade, Sigma-Aldrich) at a dose of 2 mg/kg every third day over 60 days [17].

Group IV – MetS + SB203580: Animals were simultaneously administered SB203580 intraperitoneally (2 mg/kg every third day) and provided 20 % fructose solution as the sole source of water for 60 days.

In order to asses metabolic syndrome development we measured body-mass index, glucose content and triglycerides content in rat blood. These parameters were used to calculate insulin resistance index TyG-BMI according to Y. Zhang et al. (2022) [18]. Glucose content and triglycerides content in rat blood

were measured by kits produced by OOO NPP "Filisit-Diagnostics' (HP009.02 for glucose and HP022.04 for triglycerides).

Biochemical assays were conducted using 10 % homogenates of the biceps femoris muscle (cytosolic fraction). Total nitric oxide synthase (tNOS) activity was assessed by measuring the increase in nitrite (NO_2^-) concentration following 30-minute incubation at 37 °C in an incubation medium (3.1 mL) comprising: 2.5 mL of 161 mM Tris buffer (pH 7.4), 0.3 mL of 31 mM L-arginine, 0.1 mL of 32 μM NADPH, and 0.2 mL of 10 % tissue homogenate [19].

Constitutive NOS (cNOS) activity was determined using a modified incubation mixture (3.3 mL): 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, with 0.2 mL of 10 % tissue homogenate incubated at 37 °C for 60 minutes. Inducible NOS (iNOS) activity was calculated as the difference between total NOS and cNOS activities, expressed as μmol/min/g protein [19].

Nitrite concentration was quantified using the Griess-Ilosvay method with 1 % sulfanilic acid in 30 % acetic acid and 0.1 % 1-naphthylamine. A 0.2 mL aliquot of the sample was diluted with 1.8 mL distilled water, followed by addition of 0.2 mL sulfanilic acid and, after 10 minutes, 0.2 mL of 1-naphthylamine. Absorbance was measured spectrophotometrically at 540 nm using a Ulab-101 spectrophotometer with a 5 mm path length cuvette [19].

Arginase (Agn) activity was determined by measuring the increase in L-ornithine concentration before and after incubation of 0.1 mL of 10 % tissue homogenate in 0.8 mL of incubation buffer containing 0.5 mL of 125 mM phosphate buffer (pH 7.0) and 0.2 mL of 6 mM L-arginine.

Nitrite reductase (NiR) activity was assessed by measuring the reduction in nitrite concentration following 60-minute incubation at 37 °C of 0.2 mL of 10 % homogenate in 2.3 mL of incubation medium containing 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 [19,20].

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 [19,20]. 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 aliquote 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.

Peroxynitrite ($ONOO^-$) concentrations of alkali (Na^+, K^+) and alkaline earth (Ca^{2+}) metal derivatives were measured based on their reaction with potassium iodide in 0.2 M phosphate buffer at pH 7.0 [20].

Low-molecular-weight S-nitrosothiol (S-NO) concentrations were quantified by the nitrite increase following 30-minute incubation of 0.2 mL of 10 % homogenate in 2.6 mL of incubation medium comprising 2.0 mL of 154 mM phosphate buffer (pH 7.0), 0.1 mL of 923 μM sodium fluoride, and 0.1 mL of 854 μM mercury chloride. Nitrite content was measured immediately and after 30 minutes. The difference between initial and final values represented the S-NO concentration [19].

Hydrogen sulfide (H_2S) levels were determined spectrophotometrically based on the formation of a chromogenic complex with a specific sulfide reagent (0.4 g N,N-dimethyl-p-phenylenediamine and 0.6 g $FeCl_3 \cdot 6H_2O$ dissolved in 100 mL of 6 M HCl) [20].

Protein content in 10 % muscle tissue homogenate was measured by Biuret method.

Statistical analyses were performed using the Kruskal–Wallis non-parametric ANOVA, followed by pairwise comparisons using the Mann–Whitney U-test. Differences were considered statistically significant at $p < 0.05$. Data are presented as mean (M) ± standard error of the mean (SEM).

Results

Addition of 20 % fructose solution to rat daily ration in order to stimulate MetS development led to increase in blood glucose levels by 111.8 % compared to control (*Table 1*). Triglycerides content elevated by 206.5 %. BMI increased by 26.9 %. These changes were accompanied by increase of TyG-BMI index by 58.0 %. Therefore, we can assume, that rats developed changes typical for metabolic syndrome: hyperglycemia, insulin resistance, increased body weight and lipid content in blood.

Administration of SB203580 to animals on which we modelled MetS led to decrease in blood glucose levels by 17.2 % compared to MetS group. Triglyceride content under these conditions did not change compared to MetS group. BMI decreased by 7.6 %. and TyG-BMI index dropped by 10.1 % compared to MetS group.

Modelling of MetS led to increased total NO-synthase activity of in rat biceps femoris muscle by 94.7 % compared to control group. Administration of p38 inhibitor SB203580 to healthy animals did not change the total NO-synthase activity compared to control group, however, total NO-synthase activity was by 49.0 % lower compared to MetS group. Administration of p38 inhibitor SB203580 on the background of MetS modelling decreased total NO-synthase activity by 26.5 % compared to MetS group, however, compared to control group and SB203580 administration group total NO-synthase activity was increased by 43.2 % and 44.3 %, respectively (*Table 2*).

Analysis of constitutive NO-synthases activity revealed that MetS modelling decreased constitutive NO-synthases activity by 41.5 % compared to control group. Administration of p38 inhibitor SB203580 to healthy animals reduced constitutive NO-synthase activity compared to control group by 34.9 %, however, it was 11.3 % higher compared to MetS group. Combination of MetS modelling and SB203580 administration had no influence on constitutive NO-synthases activity in rat biceps femoris muscle compared to MetS group.

Activity of inducible NO-synthase showed the same tendency as total NO-synthase activity. It increased by 105.7 % compared to control group. Administration of SB203580 had no effect on inducible NO-synthase activity in rat biceps femoris muscle compared to control group. Combination of MetS modelling and SB203580 administration decreased inducible NO-synthase

Table 1. Glucose and triglyceride content in rat blood, and BMI under conditions of metabolic syndrome and p38 activation inhibition by SB203580 (M ± SEM)

Parameter, units of measurement	Groups			
	Control, n = 6	MetS group, n = 6	SB203580 administration group, n = 6	MetS + SB203580 administration group, n = 6
Blood glucose content, mg/dL	70.02 ± 1.10	148.30 ± 1.99*	71.89 ± 1.33#	122.72 ± 0.42*,#,^
Blood triglyceride content, mg/dL	79.30 ± 5.06	243.09 ± 4.96*	87.45 ± 9.30#	226.79 ± 6.50*,^
BMI, g/cm^2	0.52 ± 0.02	0.66 ± 0.01*	0.58 ± 0.005*,#	0.61 ± 0.004*,#,^
TyG-BMI index	40.91 ± 1.93	64.65 ± 0.88*	46.68 ± 0.90*,#	58.12 ± 0.42*,#,^

***:** 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 SB203580 administration group ($p < 0.05$).

Table 2. Parameters of NO production and metabolism of in rat biceps femoris muscle under conditions of metabolic syndrome and p38 activation inhibition by SB203580 (M ± SEM)

Parameter, units of measurement	Groups			
	Control, n = 6	MetS group, n = 6	SB203580 administration group, n = 6	MetS + SB203580 administration group, n = 6
Total NO-synthase activity, μmol/min per g of protein	1.32 ± 0.03	2.57 ± 0.13*	1.31 ± 0.03#	1.89 ± 0.07*,#,^
Constitutive NO-synthase activity, μmol/min per g of protein	0.106 ± 0.0004	0.062 ± 0.002*	0.069 ± 0.0004*,#	0.063 ± 0.002*,^
Inducible NO-synthase activity, μmol/min per g of protein	1.22 ± 0.03	2.51 ± 0.13*	1.24 ± 0.03#	1.83 ± 0.07*,#,^
Arginase activity, μmol/min per g of protein	2.39 ± 0.03	4.18 ± 0.17*	1.80 ± 0.05*,#	1.49 ± 0.09*,#,^
Nitrate reductase activity, μmol/min per g of protein	10.69 ± 0.65	16.54 ± 0.40*	12.11 ± 0.32#	12.93 ± 0.30*,#
Nitrite reductase activity, μmol/min per g of protein	7.64 ± 0.37	17.90 ± 0.55*	9.95 ± 0.21*,#	11.51 ± 0.29*,#,^
Nitrite concentration, nmol/l	9.68 ± 0.27	3.95 ± 0.11*	5.83 ± 0.15*,#	7.65 ± 0.15 *,#,^
Peroxynitrite concentration, μmol/g	0.91 ± 0.01	1.56 ± 0.03*	0.66 ± 0.02*,#	0.83 ± 0.07#,^
Concentration of nitrosothiols, μmol/g	0.33 ± 0.01	0.41 ± 0.01*	0.225 ± 0.002*,#	0.18 ± 0.01*,#,^
Concentration of H_2S, μmol/g	8.54 ± 0.07	14.15 ± 0.15*	8.29 ± 0.23#	9.60 ± 0.59#

***:** 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 SB203580 administration group ($p < 0.05$).

activity by 27.1 % compared to MetS group. Therefore, we can assume that changes in total NO-synthase activity may be attributed primarily to changes in activity of inducible NO-synthase.

Arginase activity in rat biceps femoris during MetS modelling increased by 74.9 % compared to control group. SB203580 decreased arginase activity compared to control and MetS groups by 24.7 % and by 56.9 %, respectively. SB203580 administration against the background of MetS modelling decreased arginase activity by 37.7 % compared to control, by 64.4 % compared to MetS group and by 17.2 % compared to SB203580 administration group.

Nitrate reductase activity during MetS modelling increased by 54.7 % compared to control group. Administration of SB203580 had no influence on nitrate reduction compared to control group, however, it was decreased compared to MetS group by 26.8 %. Combination of MetS modelling and SB203580 administration decreased nitrate reductase activity by 21.8 % compared to MetS group, however, it increased by 21.0 % compared to control group.

Modelling of MetS led to increased nitrite reductase activity in rat biceps femoris by 134.3 % compared to control group. SB203580 increased nitrite reductase activity by 30.2 % compared to control group but decreased nitrite reductase activity by 44.4 % compared to MetS group. SB203580 administration against the background of MetS modelling increased nitrite reductase activity by 50.7 % compared to control, and by 15.7 % compared to SB203580 administration group, but decreased nitrite reductase activity by 35.7 % compared to MetS group.

Nitrite content in rat biceps femoris during MetS modelling decreased by 59.2 % compared to control group. SB203580 administration decreased nitrite content by 39.8 % compared to control group, but increased nitrite content compared to MetS group by 47.6 %. Combination of MetS modelling and SB203580

administration decreased nitrite content by 21.0 % compared to control group, but elevated nitrite content by 93.7 % compared to MetS group and by 31.2 % compared to SB203580 group.

Content of peroxynitrites of alkali and alkali-base metals in rat biceps femoris during MetS modelling increased by 71.4 % compared to control group. SB203580 decreased peroxynitrite content by 27.5 % compared to control group and by 57.7 % compared to MetS group. SB203580 administration against the background of MetS modelling did not change peroxynitrite content compared to control, increased it by 25.8 % compared to SB203580 administration group, but decreased it by 46.8 % compared to MetS group.

Nitrosothiols content in rat biceps femoris during MetS modelling increased by 24.2 % compared to control group. SB203580 administration decreased nitrosothiols content by 31.8 % compared to control group and by 45.1 % compared to MetS group. Combination of MetS modelling and SB203580 administration decreased nitrosothiols content by 45.5 % compared to control group, by 56.1 % compared to MetS group and by 20.0 % compared to SB203580 group.

Content of hydrogen sulfide (H_2S) in MetS group increased by 65.7 % compared to control group. Neither administration of SB203580 alone, nor its administration during MetS modelling did not influence hydrogen sulfide content in rat biceps femoris muscle compared to control group. However, compared to MetS group, SB203580 administration decreased hydrogen sulfide content by 41.4 %, while combination of MetS modelling and SB203580 administration decreased hydrogen sulfide content by 32.2 %.

Discussion

Our research results demonstrated elevated production of NO from NOS-dependent pathway of its formation in rat biceps femoris muscle during MetS modelling, as evidenced by increased total and inducible NO-synthase activities (*Table 2*). Constitutive isoforms of NO-synthase showed different tendency contributing to decrease of NO synthesis in endothelium and neurons. Such results are comparable to data obtained by other scientists and may be the evidence of metabolic changes in muscle tissue occurred due to introduction of our model of MetS [21,22]. Administration of SB203580 to healthy animals showed no disruption of NO synthesis from inducible isoform of NO-synthase, however, we discovered a reduction in its synthesis from constitutive isoforms of NO-synthase (*Table 1*). Our study can be supplemented by data obtained by Xu D. et al. (2021), who showed that p38 MAPK inhibition decreases inducible NO-synthase activity in astrocytes during inflammation [23]. According to data obtained by Xu D. et al. (2021) p38 MAPK activation under influence of Orexin-A does not fall below control levels, which corresponds to our data showing that SB203580 cannot lower p38 MAPK activation below control levels. This indicates the presence of a possible baseline of p38 MAPK activation. However, combination of MetS modelling and SB203580 administration revealed ability of SB203580 to decrease inducible NO-synthase activity due to p38 MAPK inhibition. Decrease in constitutive NO-synthase activity observed in group of SB203580 administration to healthy animals corresponds to the results obtained by Huang S. et al. (2023), who showed that p38 MAPK activation stimulates endothelial NO-synthase activity [24].

Arginases (EC 3.5.3.1) are group of two isoenzymes, which control NO production by scavenging (“stealing”) the substrate of NO-synthases. Increased arginase activity may lead to development of endothelial dysfunction and is a suitable target for treatment of endothelial dysfunction during MetS [25]. According to data obtained by S. Mazrouei et al. (2023) increased arginase activity always leads to decreased endothelial NO-synthase activity in endothelial cells [26]. Administration of SB203580 during MetS modelling led to decrease in arginase activity, thus lowering the possibility of endothelial dysfunction development. Li X. et al. (2023) showed that SB203580 can decrease arginase activity due to its ability to inhibit p38 MAPK activation, which can explain results observed in our study [27].

Besides their synthetizing activities inducible NO-synthase and arginase are marker enzymes of macrophage polarization, where prevalence of inducible NO-synthase in macrophage indicates M1 (pro-inflammatory) polarization, while prevalence of arginase in macrophage indicates M2 (anti-inflammatory) polarization [28]. Therefore, we can state, that modelling of MetS leads to chronic inflammation in muscles in which processes of alteration controlled by M1 macrophages are countered by processes of regeneration controlled by M2 macrophages, eventually resulting in unresolvable inflammatory state. Administration of SB203580 during MetS modelling lowers both inducible NO-synthase and arginase activities leading to an almost balanced state, where inducible NO-synthase activity slightly dominates, which may indicate presence of chronic inflammation of lower grade compared to MetS group. Such low-grade chronic inflammation will not obstruct muscle contractility, but may initiate metabolic changes in muscle, which demands further investigation.

Potent ability of muscles to convert nitrates and nitrites to NO is owned to the presence of large quantities of XOR in muscle tissues. In group, where we modelled MetS we observed increased activities of nitrate reductases and nitrite reductases. As evidenced by other scientists XOR activity is often elevated in various tissues and organs during metabolic syndrome [29,30]. Administration of SB203580 during MetS modelling lowers both activities of nitrate reductases and nitrite reductases, which may be associated with inhibition of XOR activity. Despite absence of direct control of p38 MAPK over XOR activity, it can decrease XOR activity by inhibiting STAT3 activation [31].

Summarizing state of NO production in rat biceps femoris muscle during MetS modelling we can conclude, that there is increased NO production from both NOS-dependent and NOS-independent pathways. Introduction of specific inhibitor of p38 MAPK, namely SB203580, during MetS modelling attenuated increased NO production from both NOS-dependent and NOS-independent pathways. Therefore, we can assume, that p38 MAPK activation during MetS modelling leads to NO overproduction in rat biceps femoris muscle.

Analysis of content of NO metabolites revealed, that nitrites are decreased in all study groups except control group. In

case of MetS group sharp decrease in nitrite content can be explained by radical increase in activities of nitrate reductases and nitrite reductases. Decreased nitrite content and increased nitrite reductase activity in SB203580 group can be seen as compensatory response to lowered constitutive NO-synthase activity in this group. In group of administration of SB203580 on the background of MetS modelling we can observe higher content of nitrites that in MetS and SB203580 groups, which can be explained by increased nitrate reductase and inducible NO-synthase activities, which do not compensate NO usage for formation of other NO metabolites.

Peroxynitrite, a substance most responsible for damaging effects of excessive NO production, is increased only in MetS group, which evidences SB203580 effectiveness in nitrosative stress reduction caused by MetS modelling. Decreased peroxynitrite in groups treated with SB203580 can be connected with its ability to lower ROS production [32].

Nitrosothiols may be considered the most stable and safe form of NO in the cell. Whenever necessary (lack of NO synthesis, hypoxia) nitrosothiols can release NO molecule or they can store it in for of S-NO bond (in case of abundance of NO). Nitrosothiols content is increased only in MetS group, while in groups treated with SB203580 it falls below control values, which may be indicative of insufficient amount of biologically available NO. Lack of biologically available NO naturally creates conditions where storage of nitric oxide is hampered.

Hydrogen sulfide (H_2S) is an important gaseous transmitter, which plays role in NO metabolism. In case of abundant production of NO and formation of excessive amounts of peroxynitrite, hydrogen sulfide can act as scavenger of nitrosating molecules and attenuate negative effects of excessive NO production [33]. Therefore, we can consider elevation of hydrogen sulfide in MetS group as adaptive/compensatory response to increase of NO production. Despite absence of scientific evidences about direct positive connection of p38 MAPK activation and activity of H_2S-producing enzymes our study suggests, that H_2S production depends on p38 MAPK activation, because introduction of SB203580 lowers H_2S content to control levels. Possible mechanism of such effects may lie in p38 MAPK with other redox-sensitive transcriptional cascades [34,35].

Conclusions

1. Activation of p38 MAPK during metabolic syndrome modelling increases total NOS-dependent nitric oxide production in rat biceps femoris muscle primarily due to enhancement of inducible NO-synthase activity.

2. In addition, p38 MAPK increases NOS-independent nitric oxide production and leads to accumulation of nitrosothiols and peroxynitrite in rat biceps femoris muscle during metabolic syndrome modelling.

3. Adaptive increase in hydrogen sulfide content in rat biceps femoris muscle during metabolic syndrome modelling depends on activation of p38 MAPK.

Perspectives of further scientific research: a viable approach to further investigation of possible usage of specific p38 MAPK inhibitors for treatment of metabolic syndrome is to study influence of these specific inhibitors on production and metabolism of reactive oxygen species, antioxidant defence and degradation of connective tissue components not only in skeletal muscles, but in other organs and tissues.

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