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ORIGINAL PAPER

Chromium picolinate modulates nitric oxide pathways but enhances myocardial peroxynitrite formation in a rat heart during metabolic syndrome modeling

Oleh Akimov [1], Andrii Mykytenko [2], Vitalii Kostenko [1]

[1] Department of Pathophysiology, Poltava State Medical University, Poltava, Ukraine
[2] Department of Biological and Bioorganic Chemistry, Poltava State Medical University, Poltava, Ukraine

ABSTRACT

Introduction and aim. Metabolic syndrome (MetS) is a global non-communicable health burden. Chromium picolinate (CRPIC) as modulator of p38 MAPK cascade may have a potential therapeutic effect on MetS.

The objective of the present study is to evaluate the effects of CRPIC administration on nitric oxide generation and myocardial levels of nitric oxide metabolites in rats under conditions of metabolic syndrome.

Material and methods. The experiment was performed on 24 Wistar rats, which were randomly allocated into four groups (n=6 per group): Group I, the control group; Group II, the metabolic syndrome (MetS) group, in which MetS was induced by providing a 20% fructose solution as the sole source of drinking water for 60 days; Group III, the CRPIC-treated group, receiving CRPIC at a dose of 80 μg/kg; and Group IV, the CRPIC+MetS group, in which CRPIC administration was carried out under conditions of experimentally induced metabolic syndrome. The following biochemical parameters were evaluated: total nitric oxide synthase (NOS) activity, inducible NOS (iNOS) and constitutive NOS (cNOS) activities, arginase activity, nitrite reductase and nitrate reductase activities, as well as the concentrations of nitrites, peroxynitrites, nitrosothiols, and hydrogen sulfide.

Results. Administration of CRPIC under MetS conditions resulted in a 38.2% reduction in NOS activity and a 40.2% decrease in iNOS activity, accompanied by a 48.9% increase in cNOS activity compared with the MetS group. CRPIC treatment also reduced arginase activity by 13.2%. While the activity of nitrate reductase remained unchanged, nitrite reductase activity decreased by 37.0%. Furthermore, CRPIC increased nitrite levels by 95.2% and peroxynitrite concentrations by 35.2%, while the content of nitrosothiols was reduced by 49.1%. H_2S levels also decreased by 16.8%.

Conclusion. Administration of CRPIC on the background of metabolic syndrome modeling alleviates enhanced nitric oxide production from the L-arginine-dependent and L-arginine-independent pathways, but increases peroxynitrite compared to the metabolic syndrome group.

Keywords. chromium picolinate, heart, metabolic syndrome, nitric oxide, p38-MAPK

Introduction

According to the State Committee of Statistics of Ukraine, in the year 2020, 39.7 % of adults over 18 years were considered overweight and 16% obese, with 0.7 % classified as extremely obese.[1] Obesity often leads to metabolic syndrome (MetS) and type II diabetes development.[2] MetS is often accompanied by cardiovascular diseases, which develop due to oxidative-nitrosative stress in heart tissues caused by altered metabolism.[3]

Corresponding author: Oleh Akimov, e-mail: o.akimov@pdmu.edu.ua

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Nitrosative component of oxidative-nitrosative stress is usually caused by excessive production of nitric oxide (NO) and peroxynitrite (ONOO-) formation. Most of existing therapeutic strategies targeting NO production in rat heart during MetS influence transcriptional factor NF-κB activity, which controls inducible NO-synthase (iNOS) activity.[4] However, prolonged transcriptional factor NF-κB activity inhibition may have adverse effects on other organs and tissues, which may be one of underlying mechanisms of metformin hepatotoxicity.[5]

Chromium picolinate (CRPIC) has a potential to improve lipid metabolism during MetS, which is one of etiological factors of oxidative-nitrosative stress development.[6] Therefore, it can be considered a therapeutic tool for treatment of heart damage caused by MetS. However, its influence on sources of NO production and its metabolism in heart during MetS remain insufficiently studied.

Current therapeutic approaches largely focus on inhibiting NF-κB–dependent iNOS expression; however, prolonged NF-κB suppression may induce adverse systemic effects. Therefore, there is a pressing need for alternative strategies capable of modulating nitrosative signaling without direct interference in NF-κB activity. CRPIC, a nutritional supplement known to improve lipid and carbohydrate metabolism, represents a promising candidate in this regard. Despite evidence supporting its beneficial effects on metabolic homeostasis, the potential of CRPIC to influence cardiac NO metabolism and mitigate nitrosative stress during MetS remains largely unexplored. The present study addresses this gap by investigating the impact of CRPIC on NO production and its metabolism in the myocardium under MetS conditions, thus providing a novel mechanistic perspective on its cardioprotective potential.

Aim

The objective of the present study is to evaluate the effects of CRPIC administration on nitric oxide generation and myocardial levels of nitric oxide metabolites in rats under conditions of metabolic syndrome.

Material and methods

The study was carried out on 24 mature male Wistar rats weighing 200-260 g that were obtained from accredited animal facility of Poltava State Medical University. The sample size was determined a priori for one-way analysis of variance with four groups. An effect size (f = 0.4), p = 0.05 and power (1–β)=0.80 were assumed based on the previous literature and pilot data. The calculation yielded a minimum of 24 animals (6 per group). The animals were housed under standard vivarium conditions. All experimental procedures involving animals were conducted in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes. Animals were withdrawn from the experiment under thiopental anesthesia by cardiac puncture with blood collection from the right ventricle. All manipulations were approved by the Bioethics Commission of Poltava State Medical University (Record No. 206 from 24.06.2022).

Experimental design

The animals were distributed into IV experimental groups (6 animals per group):

I – Control group: animals in this group underwent the same manipulations as those in the experimental groups but received 0.9% sodium chloride solution instead of the active substances.

II – MetS group. Animals on which MetS was modelled. Induction of MetS was achieved by exposure to a 20% fructose solution as the only drinking fluid for 60 days.[7]

III – group of chromium picolinate administration (CRPIC group). Animals from this group received chromium picolinate (Sigma Aldrich, Cas Number: 14639-25-9) intragastrically daily at a dose 80 μg/kg for 60 days.[8] The dose of CRPIC was chosen according to Sahin K. et al (2013) as dose showing anti-diabetic effects.[8] Duration of CRPIC was chosen according to the time necessary to induce MetS in our chosen model.[7]

IV – group of simultaneous chromium picolinate administration and metabolic syndrome modelling (CRPIC+MetS group). Animals in this group received chromium picolinate intragastrically daily at a dose of 80 μg/kg for 60 days and had a 20% fructose solution as the only source of water for 60 days.

Rats were housed in cages containing six animals each. The cages were used as a randomization unit.

Biochemical analysis of rat heart homogenate

Determination of L-arginine-dependent NO production

The study object was a 10% homogenate of rat heart tissue. The total activity of nitric oxide synthase (gNOS) was assessed based on the increase in the concentration of nitrite (NO_2^-) concentration.[9]

To assess the activity of constitutive nitric oxide synthase isoforms (cNOS), aminoguanidine hydrochloride was employed as a selective inhibitor of inducible NO-synthase (iNOS). The activity of the inducible isoform was subsequently calculated using the formula: iNOS = NOS – cNOS (μmol/min per g of protein).[9]

Nitrite levels were determined using the Griess–Ilosvay reagent, consisting of 1% sulfanilic acid in 30% acetic acid and 0.1% 1-naphthylamine in the same solvent. The concentration of nitrites was measured spectrophotometrically using a Ulab-101 spectrophotometer (λ=540 nm in cuvette with optical path length of 5 mm, ULAB, Nanjing, China).[10]

Evaluation of arginase activity
Total arginase activity was determined by the change in L-ornithine concentration before and after incubation of 0.1 mL of a 10% tissue homogenate in 0.8 mL of incubation medium containing 0.5 mL of 125 mM phosphate buffer (pH 7.0) and 0.2 mL of 6 mM L-arginine.

Determination of L-arginine-independent NO production
Nitrite reductase (NiR) activity was evaluated based on the reduction in nitrite concentration following incubation of 0.2 mL of a 10% tissue homogenate for 60 minutes at 37°C. Nitrite levels used for the calculation of NiR activity were determined both before and after the incubation period.[9-10] The activity of nitrate reductase (NaR) was evaluated by measuring the decrease in nitrate concentration after incubating 0.2 mL of a 10% tissue homogenate for 60 min at 37°C.[9-10] Nitrite concentration, used for the calculation of NaR activity, was determined spectrophotometrically using a Ulab-101 at a wavelength of 540 nm in a cuvette with an optical path length of 5 mm.

Estimation of peroxynitrite content
The concentration of peroxynitrite derivatives of alkali (Na^+, K^+) and alkaline earth (Ca^{2+}) metals was determined based on their reaction with potassium iodide under neutral conditions (pH 7.0) in 0.2 M phosphate buffer.[10] Quantification was performed spectrophotometrically using a Ulab-101 at a wavelength of 355 nm with a cuvette optical path length of 10 mm.

Evaluation of S-nitrosothiols concentration
The concentration of low-molecular-weight S-nitrosothiols (S-NO) was assessed indirectly by quantifying the increase in nitrite levels following a 30-min incubation of 0.2 mL of a 10% tissue homogenate.[9] S-nitrosothiol content was calculated as the difference between baseline nitrite concentration measured prior to incubation and the nitrite concentration determined after incubation. Nitrite levels were quantified spectrophotometrically using a Ulab-101 at 540 nm with a cuvette optical path length of 5 mm.

Determination of hydrogen sulfide content
H_2S concentration was determined colorimetrically based on the formation of a chromogenic complex resulting from the reaction of H_2S with a specific sulfide-detecting reagent composed of N,N-dimethyl-p-phenylenediamine (0.4 g) and iron(III) chloride hexahydrate (0.6 g $FeCl_3 \cdot 6H_2O$) dissolved in 100 mL of 6 M HCl.[10] The resulting sulfide concentration was quantified spectrophotometrically using a Ulab-101 at a wavelength of 667 nm with a cuvette optical path length of 10 mm.

Biochemical analysis of rat blood
In the blood concentration of following metabolic substances were studied: glucose (REF# HP009.02; Calibrator solution contains glucose 10.0±0.5 mmol/L), triglycerides (TG, REF# HP022.04; Calibrator solution contains triglycerides 2.26±0.1 mmol/L), total cholesterol (TC, REF# HP026.07, Calibrator solution contains cholesterol 5.17±0.1 mmol/L), cholesterol from low-density lipoproteins (LDL-C, REF# HP026.05, Calibrator solution contains cholesterol 5.17±0.1 mmol/L), cholesterol from high-density lipoproteins (HDL-C, REF# HP026.03, Calibrator solution contains cholesterol 5.17±0.1 mmol/L). All abovementioned substances were evaluated by respective assays produced by "Filisit Diagnistika" (Ukraine) using spectrophotometer Ulab-101. Body mass index (BMI) was additionally calculated in accordance with established methodological recommendations.[11]

Determination of insulin-resistance indexes
In order to evaluate development of insulin resistance following indexes were calculated:
Triglyceride glucose index (TyG). TyG=Ln [TG (mg/dL)×FPG (mg/dL)÷2]
Triglyceride/high-density lipoproteins index (TG/HDL-C). TG/HDL-C=TG (mg/dL)÷HDL-C (mg/dL)
Triglyceride glucose body mass index (TyG-BMI). TyG-BMI=Ln [TG (mg/dL) × Glucose (mg/dL) ÷ 2] × BMI (kg/m^2).[12]
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)].[12]

Statistical analysis
Statistical differences between groups were assessed using nonparametric analysis of variance according to the Kruskal-Wallis test, followed by pairwise post hoc comparisons using the Mann-Whitney U test. Differences were considered statistically significant at $p<0.05$. Data are presented as median (M) with the interquartile range (IQR). To control for type I error associated with multiple comparisons, a Bonferroni correction was applied. All statistical analyzes were performed using Microsoft Office Excel with the Real Statistics 2019 add-in (Charles Zaiontz).

Results

Changes in blood metabolic parameters.
Analysis of rat blood revealed that MetS modeling leads to increase in blood glucose level by 110.9% (Table 1). Under these conditions, the triglycerides content increased by 194.5%, the total cholesterol content increased by 51.5%, the LDL-C content increased by 60.0%, while HDL-C content decreased by 31.0%. Rat weight and BMI increased by 14.5% and 20.0%, respec-

tively. Analysis of insulin resistance indexes revealed that all studied indexes increased. TyG, TG/HDL-C, TyG-BMI and METS-IR increased by 22.5%, 310.3%, 50.8% and 21.4%, respectively.

All abovementioned changes correspond to typical symptoms of metabolic syndrome: hyperglycemia, hyperlipemia, dyslipidemia and insulin resistance.

Table 1. Metabolic changes in rat blood and insulin resistance indexes under conditions of metabolic syndrome and CRPIC administration (M(IQR))[a]

Parameters	Groups			
	Control, n=6	MetS group, n=6	CRPIC administration group, n=6	MetS+CRPIC administration group, n=6
Glucose, mg/dL	70.6 (68.1–71.9)	148.9 (145.0–151.0) *	68.9 (67.0–71.2) #	85.6 (82.3–87.6) */#/^
Triglycerides, mg/dL	82.3 (70.0–87.8)	242.4 (236.8–247.9) *	80.0 (75.6–91.2) #	108.9 (103.4–111.2) */#/^
Total cholesterol, mg/dL	45.6 (45.0–46.4)	69.1 (67.4–69.9) *	41.5 (40.4–45.8) #	53.3 (52.8–53.9) */#/^
LDL–C, mg/dL	6.5 (6.2–6.5)	10.4 (9.6–11.8) *	4.8 (4.4–5.4) */#	7.1 (6.6–7.7) #/^
HDL–C, mg/dL	21.6 (20.8–22.0)	14.9 (14.4–15.4) *	22.2 (21.0–23.2) #	27.0 (26.5–27.9) */#/^
Rat weight, g	214.5 (211.0–217.3)	245.5 (243.3–247.8) *	243.5 (242.3–247.8) *	265.0 (261.8–266.8) */#/^
BMI, g/cm^2	0.55 (0.47–0.55)	0.66 (0.66–0.67) *	0.57 (0.57–0.59) */#	0.59 (0.586–0.587) */#
TyG index	8.0 (7.8–8.0)	9.8 (9.80–9.81) *	7.9 (7.8–7.9) #	8.4 (8.39–8.43) */#/^
TG/HDL–C index	3.9 (3.1–4.2)	16.0 (15.1–17.3) *	3.7 (3.4–4.2) #	4.1 (3.7–4.2) #
TyG–BMI index	42.9 (36.8–44.4)	64.7 (64.4–65.7) *	45.2 (44.4–46.7) #	49.4 (49.2–50.2) */#/^
METS–IR index	5.6 (5.5–5.7)	6.8 (6.75–6.82) *	5.7 (5.6–5.7) #	5.9 (5.87–5.92) */#/^

[a] * – 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 CRPIC administration (p<0.05)

Administration of CRPIC during MetS modelling increased blood sugar level by 21.2%, TG by 32.3%, TC by 16.9%, HDL-C by 25.0%, while the LDL-C content remained unchanged compared to the control group. Rat weight and BMI were elevated by 23.5% and 7.3%, respectively, compared to control. TyG, TyG-BMI and METS-IR increased by 5.0%, 15.2% and 5.4%, respectively. The TG / HDL-C index did not change compared to the control group.

Administration of CRPIC during MetS modelling modeling increased blood sugar level by 24.2%, TG by 36.1%, TC by 28.4%, HDL-C by 21.6%, LDL-C content increased by 47.9% in the CRPIC group. Rat weight increased by 8.8%, compared to the CRPIC group, while BMI remained unchanged. TyG, TyG-BMI and METS-IR increased by 6.3%, 9.3% and 3.5%, respectively. The TG/HDL-C index did not change compared to the CRPIC group.

Administration of CRPIC during MetS modelling decreased blood sugar level by 42.5%, TG by 55.1%, TC by 22.9%, LDL-C by 31.2%, while the HDL-C content elevated by 81.2% compared to the CRPIC group. Rat weight increased by 7.9%, while BMI decreased by 10.6% compared to the CRPIC group. TyG, TG/HDL-C, TyG-BMI and METS-IR decreased by 14.3%, 74.0%, 23.6% and 13.2%, respectively.

Changes in NO-cycle enzymes activities in rat heart

The MetS led to increase of total NOS activity by 46.9% compared to the control group (Fig. 1A). iNOS activity in these circumstances elevated by 49.0%, while cNOS activity decreased by 11.3% (Fig. 1B, Fig. 1C). Arginase activity increased by 29.9% compared to the control group (Fig. 1D). Analysis on the nitrate-nitrite pathway of NO production revealed that MetS modeling led to an increase of nitrate reductase activity by 27.9% and nitrite reductases activity was elevated by 155.9% compared to control group (Fig. 2A, Fig. 2B).

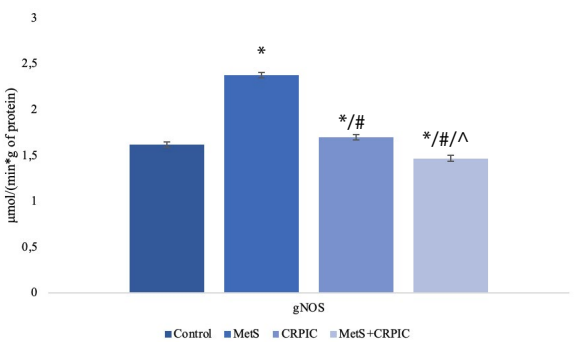

Fig. 1A. Total NO synthase (gNOS) activity in rat heart during introduction of chromium picolinate in the background of metabolic syndrome modelling, note: * – p<0.05 vs. control; # – p<0.05 vs. MetS; ^ – p<0.05 vs. CRPIC

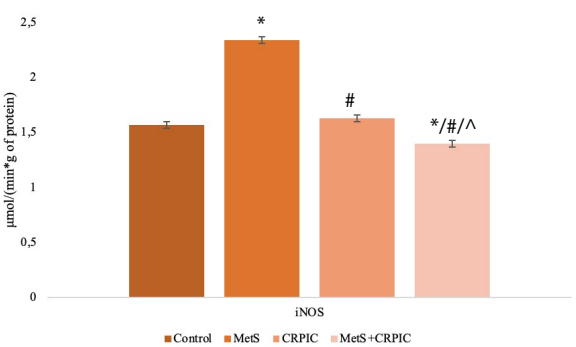

Fig. 1B. Inducible NOsynthase (iNOS) activity in rat heart during introduction of chromium picolinate in the background of metabolic syndrome modelling, note: * – p<0.05 vs. control; # – p<0.05 vs. MetS; ^ – p<0.05 vs. CRPIC

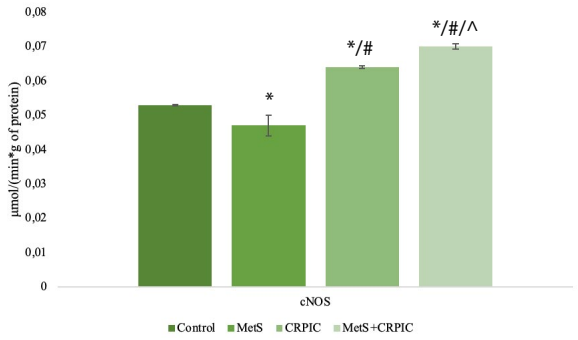

Fig. 1C. Constitutive NOsynthase (cNOS) activity in rat heart during the introduction of chromium picolinate in the background of metabolic syndrome modelling, note: * – p<0.05 vs. control; # – p<0.05 vs. MetS; ^ – p<0.05 vs. CRPIC

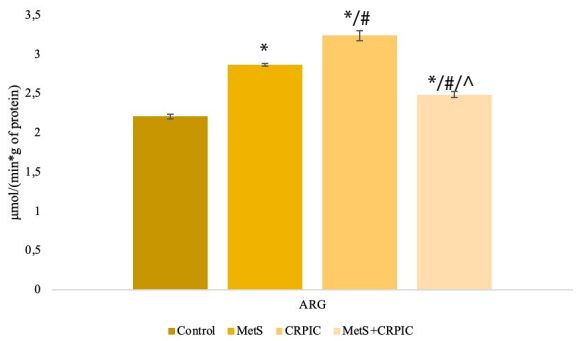

Fig. 1D. Total arginase (ARG) activity in the rat heart during the introduction of chromium picolinate in the background of metabolic syndrome modelling, note: * – p<0.05 vs. control; # – p<0.05 vs. MetS; ^ –p<0.05 vs. CRPIC

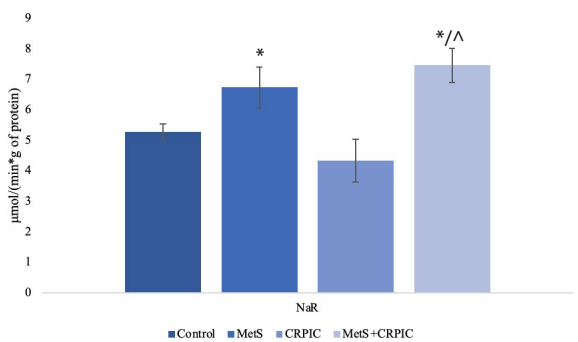

Fig. 2A. Activity of nitrate reductases (NaR) in rat heart during introduction of chromium picolinate on the background of metabolic syndrome modelling, note: * – p<0.05 vs. control; # – p<0.05 vs. MetS; ^ – p<0.05 vs. CRPIC

Administration of CRPIC to healthy animals resulted in a 4.9% increase in gNOS activity, attributable to a 20.8% increase in cNOS activity, whereas iNOS activity in the rat heart did not differ from that of the control group. Administration of CRPIC increased arginase activity in the rat heart by 46.6%. The activity of nitrate reductases did not change after CRPIC administration to healthy animals, but the activity of nitrite reductases increased by 144.8% compared to the control group.

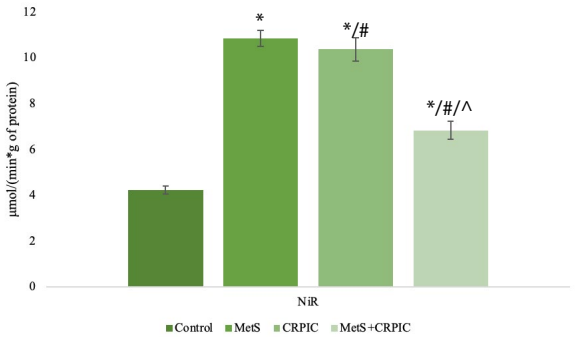

Fig. 2B. Activity of nitrite reductases (NiR) in rat heart during introduction of chromium picolinate on the background of metabolic syndrome modelling, note: * – p<0.05 vs. control; # – p<0.05 vs. MetS; ^ – p<0.05 vs. CRPIC

CRPIC administration resulted in a 28.6% reduction in gNOS activity, primarily due to a 30.3% decrease in iNOS activity, despite a 36.2% increase in cNOS activity in the rat heart relative to the MetS group. In addition, CRPIC treatment was associated with a 12.9% elevation in cardiac arginase activity compared with the MetS group. The activities of nitrate and nitrite reductases remained unchanged following CRPIC administration, showing no significant differences relative to the MetS group.

Administration of CRPIC during MetS modelling led to a decrease in gNOS activity by 9.3%, which happened due to a decrease in iNOS activity by 10.8% since cNOS activity increased by 32.1% relative to control. Administration of CRPIC increased arginase activity in the rat heart by 12.7%. The activity of nitrate and nitrite reductases after administration of CRPIC to animals with MetS increased by 41.6% and 61.3%, respectively, compared to the control group.

CRPIC administration during MetS modeling led to decrease in total NOS activity by 38.2%, which happened due to a decrease in iNOS activity by 40.2%, since cNOS activity increased by 48.9% compared to the MetS group. Administration of CRPIC decreased arginase activity in the rat heart by 13.2% compared to the MetS group. The activity of the nitrate reductases did not change and the activity of the nitrite reductases decreased by 37.0% compared to the MetS group.

Administration of CRPIC during MetS modeling was associated with a 13.5% reduction in gNOS activity, driven by a 14.1% decrease in iNOS activity, while cNOS activity in the rat heart increased by 9.4% relative to the CRPIC group. Cardiac arginase activity decreased by 23.1% compared to the CRPIC group. Nitrate reductase activity increased by 72.3%, whereas nitrite reductase activity decreased by 34.1% relative to the CRPIC group.

Changes in NO derivates content in heart of rats

During metabolic syndrome modelling nitrite and nitrosothiols content in rat heart decreased by 60.0% and 21.9%, respectively, compared to control group (Fig. 3A, Fig. 3C). Under these conditions peroxynitrite content increased by 320.0% (Fig. 3B). Analysis of concentration of nitric oxide metabolites revealed that administration of CRPIC to healthy animals decreased nitrite content by 11.0%, increased peroxynitrite content by 208.0%, and decreased nitrosothiols content by 15.1% in compared to control. Nitrite content increased by 122.6%, peroxynitrite content decreased by 26.7%, but nitrosothiols content did not change in comparison to the results of the MetS group.

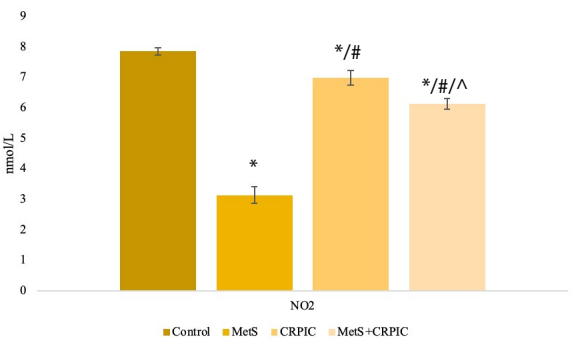

Fig. 3A. Nitrite (NO_2) content in the rat heart during the introduction of chromium picolinate in the background of metabolic syndrome modelling, note: * – p<0.05 vs. control; # – p<0.05 vs. MetS; ^ – p<0.05 vs. CRPIC

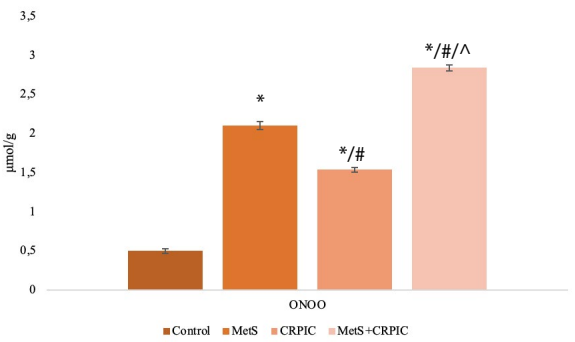

Fig. 3B. Peroxynitrite (ONOO) content in the rat heart during introduction of chromium picolinate in the background of metabolic syndrome modelling, note: * – p<0.05 vs. control; # – p<0.05 vs. MetS; ^ –p<0.05 vs. CRPIC

CRPIC administration during MetS modeling decreased nitrite content by 21.9%, increased the peroxynitrite content by 468.0% and decreased nitrosothiols content by 60.3% compared to the results of the control group. The nitrite content increased by 95.2%, the peroxynitrite content increased by 35.2%, and the nitrosothiol content decreased by 49.1% compared to MetS. However, the nitrite content decreased by 12.3%, the peroxynitrite content increased by 84.4% and nitrosothiols content decreased by 53.2% compared to the results of the CRPIC group.

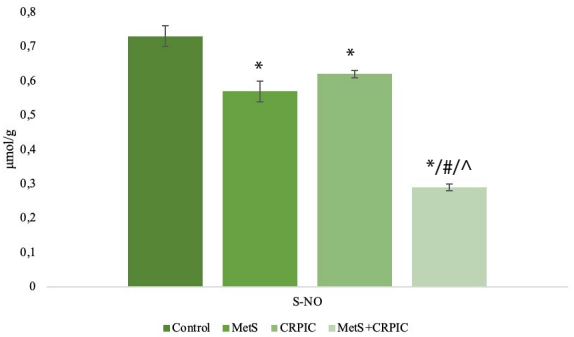

Fig. 3C. Nitrosothiols (S-NO) content in the rat heart during the introduction of chromium picolinate in the background of metabolic syndrome modelling, note: * – p<0.05 vs. control; # – p<0.05 vs. MetS; ^ –p<0.05 vs. CRPIC

H2S content in rat heart

MetS was associated with a 16.5% increase in myocardial H_2S content compared to the control group (Fig. 3D). In contrast, H_2S levels in the CRPIC-treated group were reduced by 15.5% compared to controls. Administration of CRPIC to healthy animals resulted in a 27.5% decrease in cardiac H_2S content compared to the MetS group. During MetS modeling, CRPIC treatment did not alter myocardial H_2S levels compared to controls; however, H_2S content was reduced by 16.8% compared to the MetS group and increased by 14.6% compared with the CRPIC group.

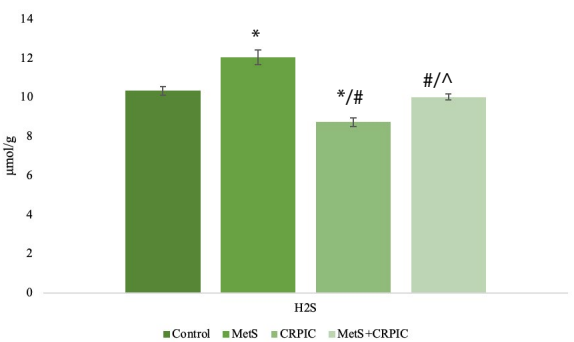

Fig. 3D. Hydrogen sulfide (H_2S) content in rat heart during introduction of chromium picolinate on the background of metabolic syndrome modelling, note: * – p<0.05 vs. control; # – p<0.05 vs. MetS; ^ –p<0.05 vs. CRPIC

Discussion

Changes observed in rat blood in MetS group are typical for development of metabolic syndrome (hyperglycemia, hyperlipemia, dyslipidemia and insulin resistance). These changes are caused by excessive calorie intake, which is the core essence of our model.[7] Such results correspond to data obtained by other scientists, who used the same experimental model.[13,14]

Administration of CRPIC to healthy animals revealed its ability to lower LDL-C and increase animal mass and BMI. Several literature sources show ability of chromium picolinate to lower LDL and very low-density lipoprotein (VLDL) content.[15,16] Such ability of CRPIC may be attributed to activation of p38 MAPK cascade and stimulation of cell division.[17] Since cholesterol is an element of cell membranes, new cells, formed due to stimulation of division by p38 MAPK activation, will require additional cholesterol, which is absent in the diet, leading lower LDL content in blood. BMI elevation of rats fed with CRPIC may be connected with ability of chromium ion to increase average daily BMI gain.[18]

Combination of CRPIC administration and MetS modelling revealed that CRPIC is effective in lowering glucose, triglycerides, total cholesterol, and LDL-C levels, which were elevated by excessive fructose intake.

In MetS group we observed increased NO production from NO-synthases, which was characterized by increased activity of iNOS and decreased cNOS activity. Increased iNOS activity was also observed by other scientists, who studied metabolic syndrome.[19,20] The reason for increased iNOS activity during MetS development lies in NF-κB activation, which controls iNOS gene expression.[21] Decreased cNOS activity in rat heart in MetS group may be associated with lowered activity of endothelial isoform of NOS (eNOS). During MetS lipid droplets in endothelium can inhibit eNOS activity, leading to endothelial dysfunction.[22]

MetS also increased production of nitric oxide from L-arginine-independent pathway, namely from nitrate and nitrite reductases. One of the known potent nitrate-nitrite reduction enzymes is xanthine oxidoreductase (XOR), which consists of two domains: xanthine dehydrogenase (XDH, EC 1.17.1.4) and xanthine oxidase (XO, EC 1.17.3.2).[23] Main substrate of XOR is hypoxanthine, which is transformed by XDH domain to uric acid. Since during MetS purine catabolism is severely enhanced, increased nitrate and nitrite reductases activity observed in MetS group can be attributed to XOR activation.[24,25]

Increased peroxynitrite content in rat heart may be indication of nitrosative stress development. The reasons for the increase in the formation of peroxynitrite during metabolic syndrome can be associated with eNOS uncoupling.[26] Uncoupling of eNOS provides superoxide, while the sources of NO necessary for peroxynitrite formation of peroxynitrites may vary from iNOS to NiR.

The increase in H_2S content in the rat heart observed in the present study can be attributed to enhanced activity of cystathionine-β-synthase (CBS, EC 4.2.1.22), which is known to be induced by excessive fructose consumption.[27] The increase in myocardial H_2S content observed during metabolic syndrome may be interpreted as an adaptive compensatory response to the increased conversion of nitric oxide to peroxynitrite, a process that attenuates NO-dependent smooth muscle relaxation. In this context, elevated H_2S may partially compensate for impaired NO bioavailability, as hydrogen sulfide is capable of directly targeting vascular smooth muscle cells and inducing vasorelaxation.[28,29] The scientific literature provides evidence that H_2S can act as peroxynitrite scavenger forming sulfenic acid (HSOH).[30]

Administration of CRPIC to healthy animals revealed a sharp increase in nitrite reduction activity, which can be explained by the action of chromium ions of chromium picolinate, because some nitrite reductases contain Cr (IV) in their active center; therefore, excessive chromium intake can increase their activity.[31] Elevation of the peroxynitrite content in the rat heart during administration of CRPIC to healthy animals can cause damage to heart tissue and requires further study. The dose chosen for rats in our study is much higher than the one used in humans, therefore peroxynitrite elevation may be the result of CRPIC overdosage. And, it is worth mentioning, that longitudinal studies of lower doses in humans revealed no harmful effects.[32] The source of NO needed to form peroxynitrite is increased NiR activity, observed in CRPIC administration group, while superoxide may come from toxic effects of excessive chromium ions accumulation.[33] Increased H_2S content under these conditions is an adoptive response towards peroxynitrite accumulation. Elevated cNOS activity may be attributed to the ability of CRPIC to enhance endothelial functions.[34]

Observing the combined effects of administration of CRPIC and MetS modeling, we established that the ability of CRPIC to stimulate endothelium prevails over adverse effects on it caused by MetS modelling. The observed reduction in iNOS activity may be attributed to a complex regulatory interplay between CRPIC-induced activation of p38 signaling and MetS-associated activation of the transcription factor NF-κB, which together modulate the transcriptional and post-transcriptional control of iNOS expression.[35] We also observed a cumulative effect of administration of CRPIC and MetS modelling on peroxynitrite content in rat heart. A decrease in H_2S content compared to MetS group may be an indication of exhaustion of this adoptive mechanism.

CRPIC activating action on p38 MAPK cascade may be associated with stimulation of p38 alpha isoform of p38 family, since part of its effects can be alleviated by specific p38 alpha inhibitor SB203580.[36] Such activation may be beneficial for correction of metabolic changes, but may be harmful for myocardium. It is worth mentioning that CRPIC has a stimulation effect on L-arginine-independent nitric oxide production, which may be either due to influence on specific enzymes or due to non-enzymatic reduction of nitrates/nitrites to nitric oxide, which requires further study.

Hydrogen sulfide plays a significant role in maintaining cellular redox homeostasis by directly neutralizing reactive oxygen and nitrogen species and by regulating the function of redox-sensitive proteins via persulfidation-dependent post-translational modification.[37] In addition, H_2S reinforces antioxidant capacity by inducing the expression and activity of key enzymes, including superoxide dismutase, catalase, and glutathione peroxidase, while maintaining mitochondrial integrity and attenuating oxidative stress–mediated cellular injury.[38]

The present findings provide mechanistic insight into the cardiac consequences of MetS and the dualistic effects of chromium picolinate supplementation. The observed upregulation of iNOS activity and increased NO generation from nitrate–nitrite reductases during MetS suggest that excessive NO and subsequent peroxynitrite formation play a central role in myocardial oxidative-nitrosative injury.

Prolonged administration of CRPIC in metabolically healthy individuals may exert cardiotoxic effects by enhancing nitrosative stress. Administration of CRPIC during MetS revealed a modulatory role of CRPIC realized by attenuating excessive NO production from both NO synthases and nitrite reductases. However, the high ONOO content in the MetS + CRPIC group suggests the need to correct the dose or duration of treatment.

Study limitations

The principal limitation of the present study is the relatively small number of animals included in each experimental group. Additional limitations include the lack of direct markers of oxidative stress (ROS production, lipid peroxidation, antioxidant enzyme activity), and the absence of histological and functional cardiac assessments.

Conclusion

Metabolic syndrome changes the amount of nitric oxide produced in the rat heart by enhancing the inducible activity of NO-synthase and intensity of nitric oxide production from nitrate-nitrite reductases. Enhanced nitric oxide production in the rat heart under conditions of metabolic syndrome results in a shift toward the predominance of the peroxynitrite pathway of nitric oxide utilization.

Prolonged administration of chromium picolinate to healthy animals for 60 days can exert cardiotoxic effects, potentially mediated by enhanced generation of nitric oxide through nitrite reductase pathways and the concomitant increase in peroxynitrite levels.

Administration of chromium picolinate during metabolic syndrome modelling attenuates excessive nitric oxide production derived from both synthetic and reductive pathways, while simultaneously promoting increased peroxynitrite formation.

Declarations

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Author contributions

Conceptualization, O.A. and V.K.; Methodology, O.A.; Validation, O.A., A.M. and V.K.; Formal Analysis, O.A.; Investigation, O.A. and A.M.; Resources, O.A.; Data Curation, O.A. and V.K.; Writing – Original Draft Preparation, O.A. and A.M.; Writing – Review & Editing, O.A., A.M. and V.K.; Supervision, V.K.; Project Administration, O.A. and V.K.

Conflicts of interest

The authors declare no competing interests.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval

All experiments with laboratory animals were approved by Bioethical Committee of Poltava State Medical University (Record No. 206 from 24.06.2022).

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