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

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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%. H₂S 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

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 $(1-\beta)=0.80$ 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 $\mu\text{g}/\text{kg}$ 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 $\mu\text{g}/\text{kg}$ 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: $\text{iNOS} = \text{NOS} - \text{cNOS}$ ($\mu\text{mol}/\text{min}$ 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 ($\lambda=540$ 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

H₂S concentration was determined colorimetrically based on the formation of a chromogenic complex resulting from the reaction of H₂S with a specific sulfide-detecting reagent composed of N,N-dimethyl-p-phenylenediamine (0.4 g) and iron(III) chloride hexahydrate (0.6 g FeCl₃·6H₂O) 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 Diagnostika” (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). $TyG = \ln [TG (mg/dL) \times FPG (mg/dL) \div 2]$

Triglyceride/high-density lipoproteins index (TG/HDL-C). $TG/HDL-C = TG (mg/dL) \div HDL-C (mg/dL)$

Triglyceride glucose body mass index (TyG-BMI). $TyG-BMI = \ln [TG (mg/dL) \times Glucose (mg/dL) \div 2] \times BMI (kg/m^2)$.¹²

Metabolic score for insulin resistance (METS-IR) index. $METS-IR = \ln [(2 \times Glucose (mg/dL)) + TG (mg/dL)] \times BMI (kg / m^2) \ln [HDL-C (mg/dL)]$.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%, respectively. 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).

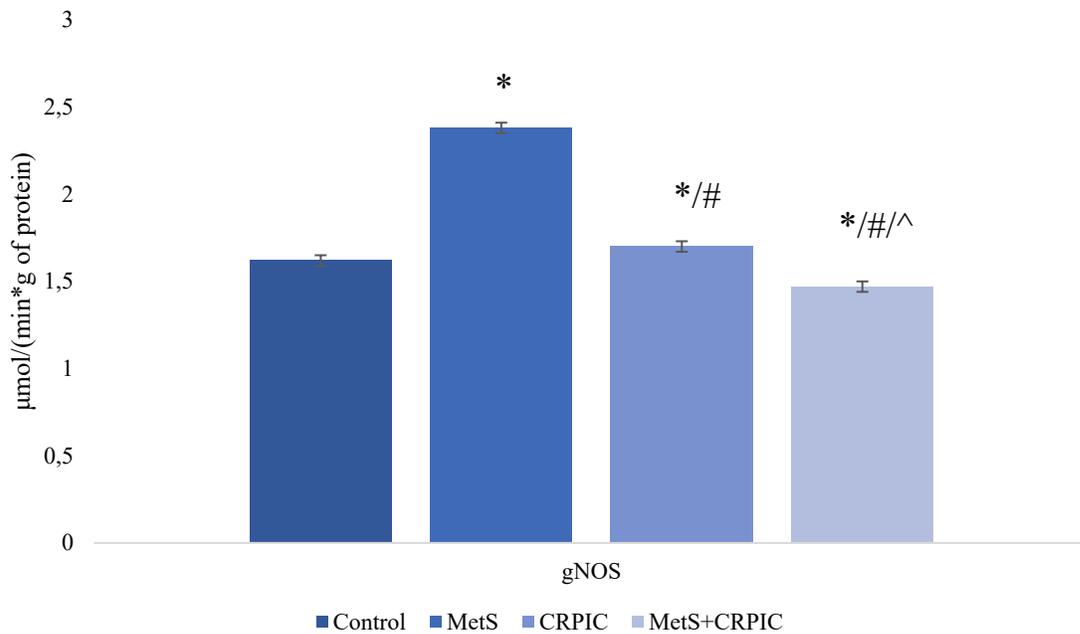


Fig. 1A. Total NO synthase (gNOS) activity in rat heart during introduction of chromium picolinate in the background of metabolic syndrome modelling, note: * – $p < 0.05$ vs. control; # – $p < 0.05$ vs. MetS; ^ – $p < 0.05$ vs. CRPIC

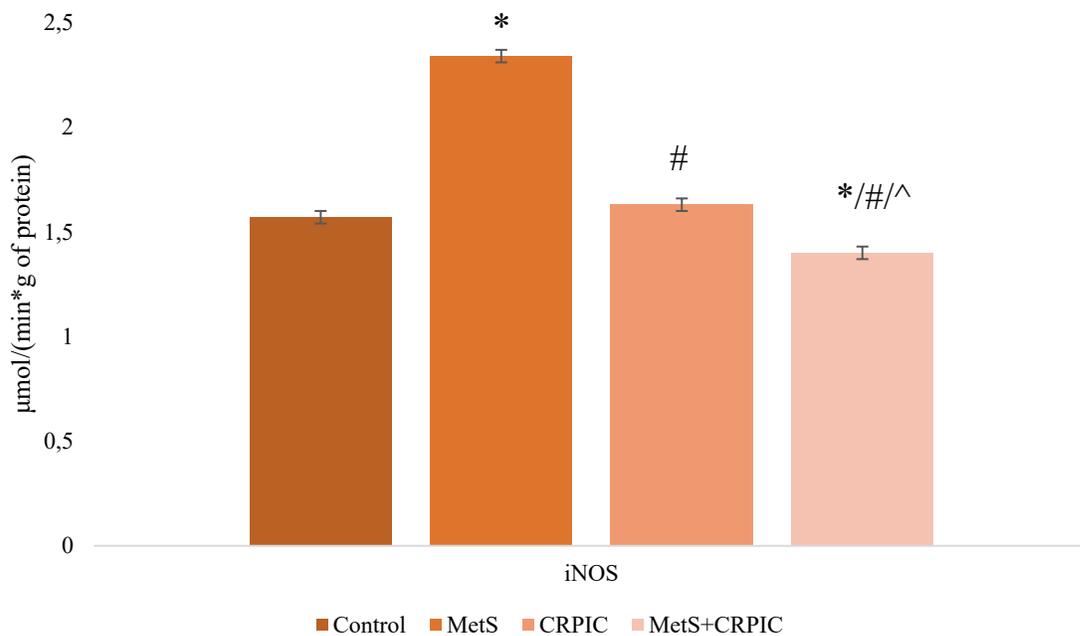


Fig. 1B. Inducible NOsynthase (iNOS) activity in rat heart during introduction of chromium picolinate in the background of metabolic syndrome modelling, note: * – $p < 0.05$ vs. control; # – $p < 0.05$ vs. MetS; ^ – $p < 0.05$ vs. CRPIC

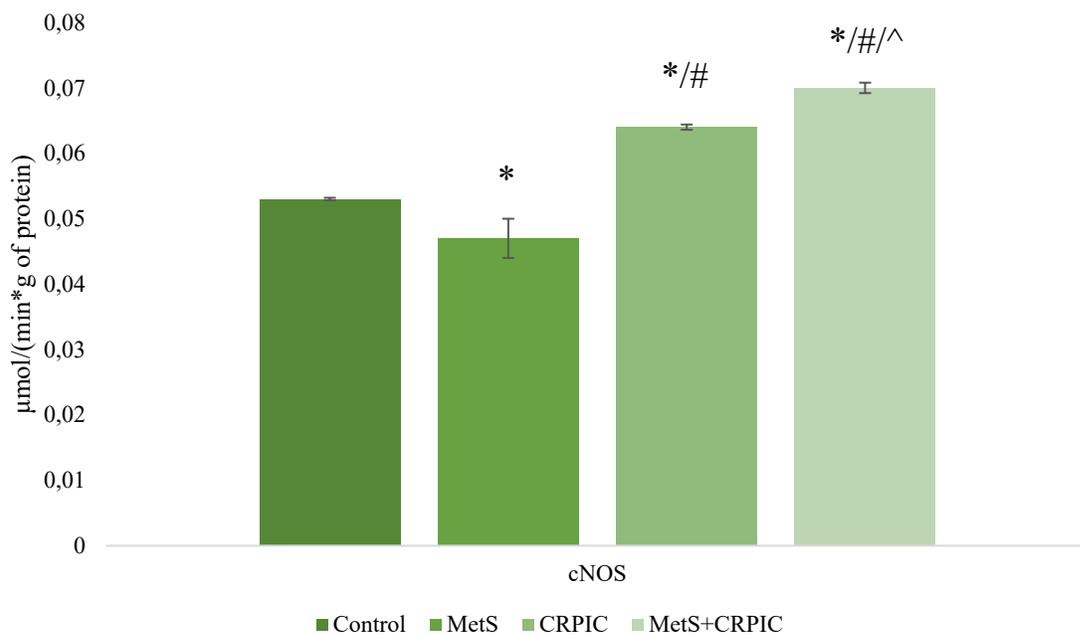


Fig. 1C. Constitutive NOsynthase (cNOS) activity in rat heart during the introduction of chromium picolinate in the background of metabolic syndrome modelling, note: * – $p < 0.05$ vs. control; # – $p < 0.05$ vs. MetS; ^ – $p < 0.05$ vs. CRPIC

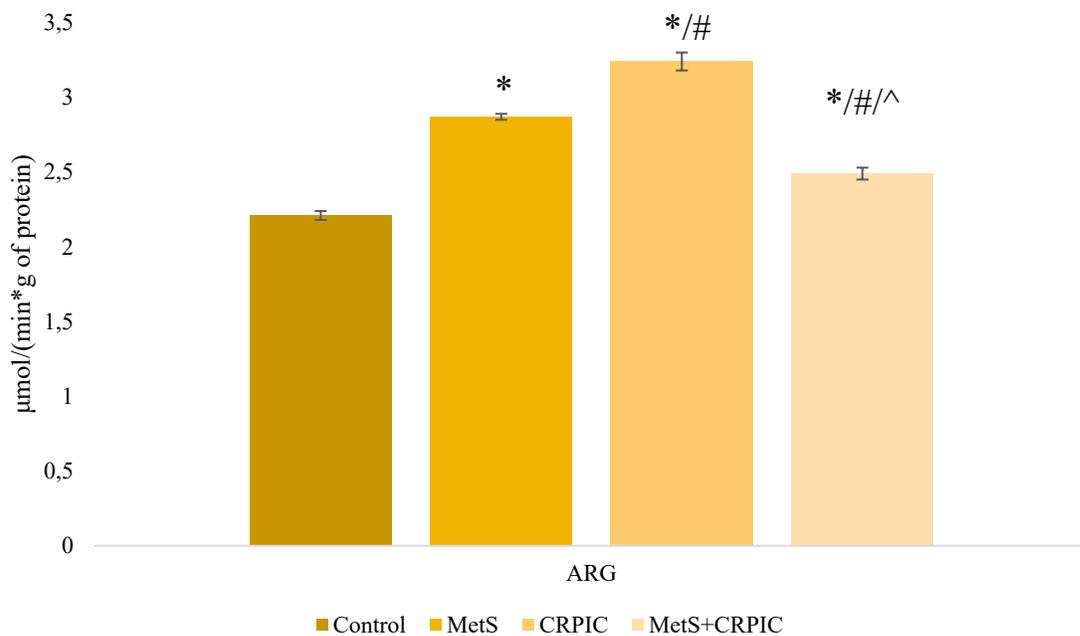


Fig. 1D. Total arginase (ARG) activity in the rat heart during the introduction of chromium picolinate in the background of metabolic syndrome modelling, note: * – $p < 0.05$ vs. control; # – $p < 0.05$ vs. MetS; ^ – $p < 0.05$ vs. CRPIC

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.

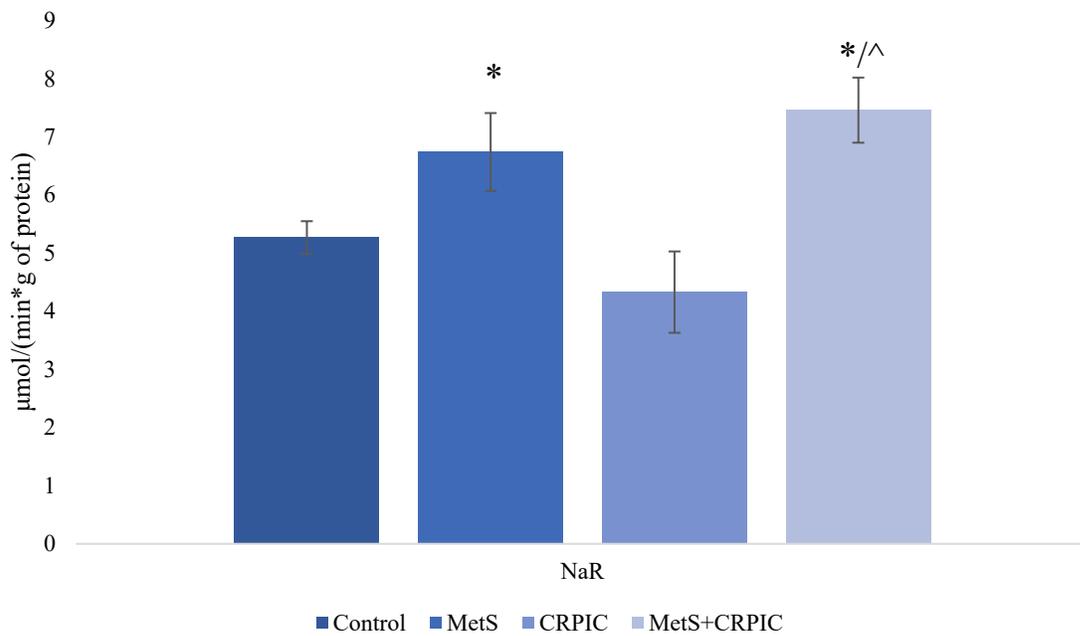


Fig. 2A. Activity of nitrate reductases (NaR) in rat heart during introduction of chromium picolinate on the background of metabolic syndrome modelling, note: * – p<0.05 vs. control; # – p<0.05 vs. MetS; ^ – p<0.05 vs. CRPIC

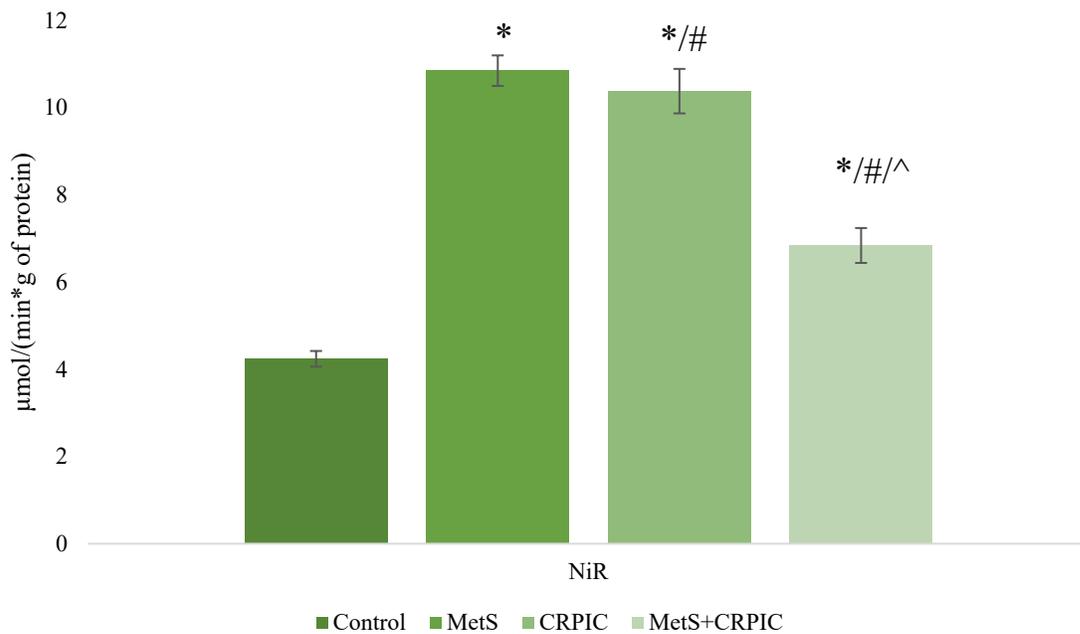


Fig. 2B. Activity of nitrite reductases (NiR) in rat heart during introduction of chromium picolinate on the background of metabolic syndrome modelling, note: * – p<0.05 vs. control; # – p<0.05 vs. MetS; ^ – p<0.05 vs. CRPIC

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 derivatives 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.

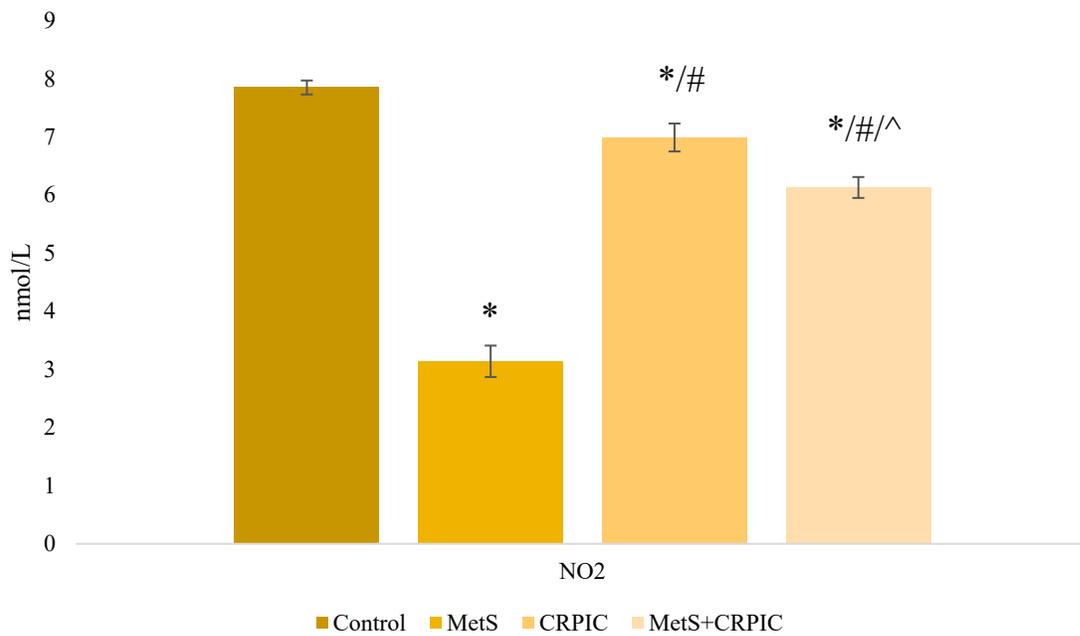


Fig. 3A. Nitrite (NO₂) content in the rat heart during the introduction of chromium picolinate in the background of metabolic syndrome modelling, note: * – p<0.05 vs. control; # – p<0.05 vs. MetS; ^ – p<0.05 vs. CRPIC

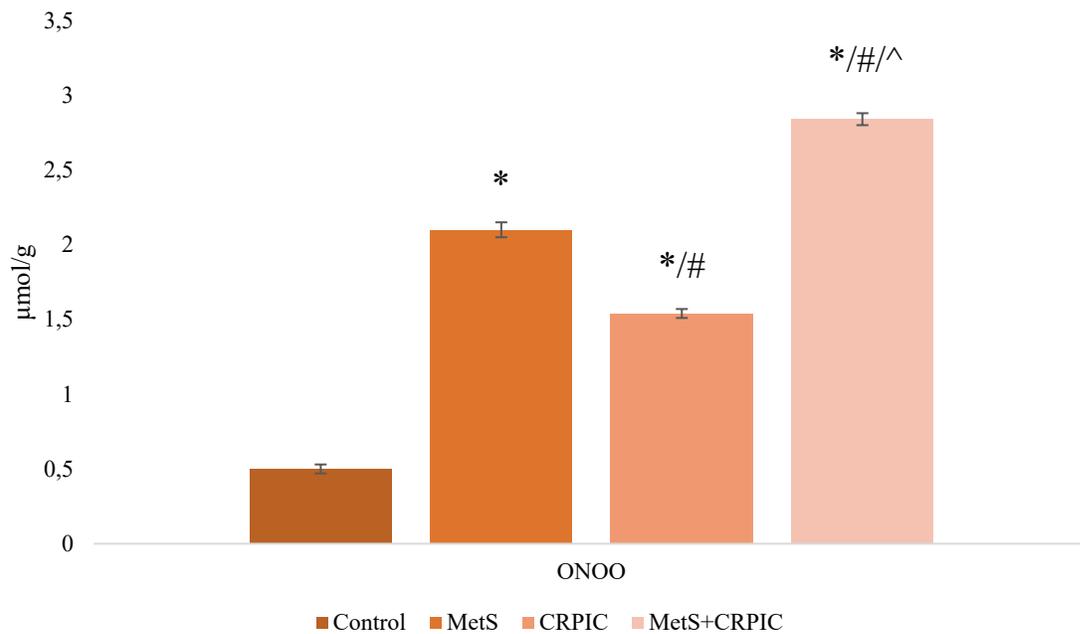


Fig. 3B. Peroxynitrite (ONOO) content in the rat heart during introduction of chromium picolinate in the background of metabolic syndrome modelling, note: * – p<0.05 vs. control; # – p<0.05 vs. MetS; ^ – p<0.05 vs. CRPIC

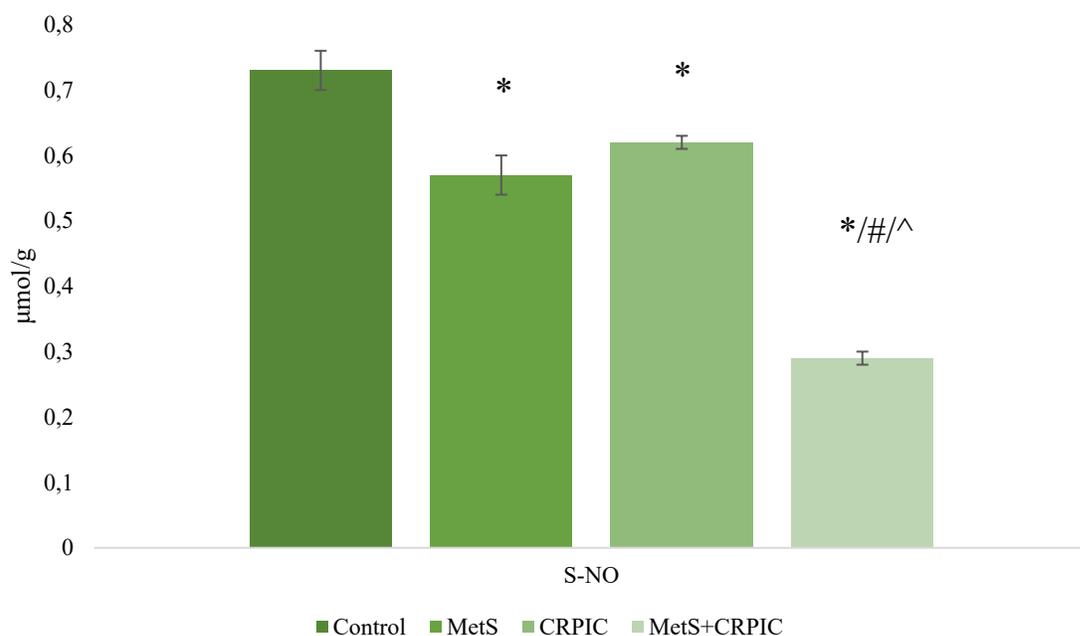


Fig. 3C. Nitrosothiols (S-NO) content in the rat heart during the introduction of chromium picolinate in the background of metabolic syndrome modelling, note: * – $p < 0.05$ vs. control; # – $p < 0.05$ vs. MetS; ^ – $p < 0.05$ vs. CRPIC

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.

H₂S content in rat heart

MetS was associated with a 16.5% increase in myocardial H₂S content compared to the control group (Fig. 3D). In contrast, H₂S 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 H₂S content compared to the MetS group. During MetS modeling, CRPIC treatment did not alter myocardial H₂S levels compared to controls; however, H₂S content was reduced by 16.8% compared to the MetS group and increased by 14.6% compared with the CRPIC group.

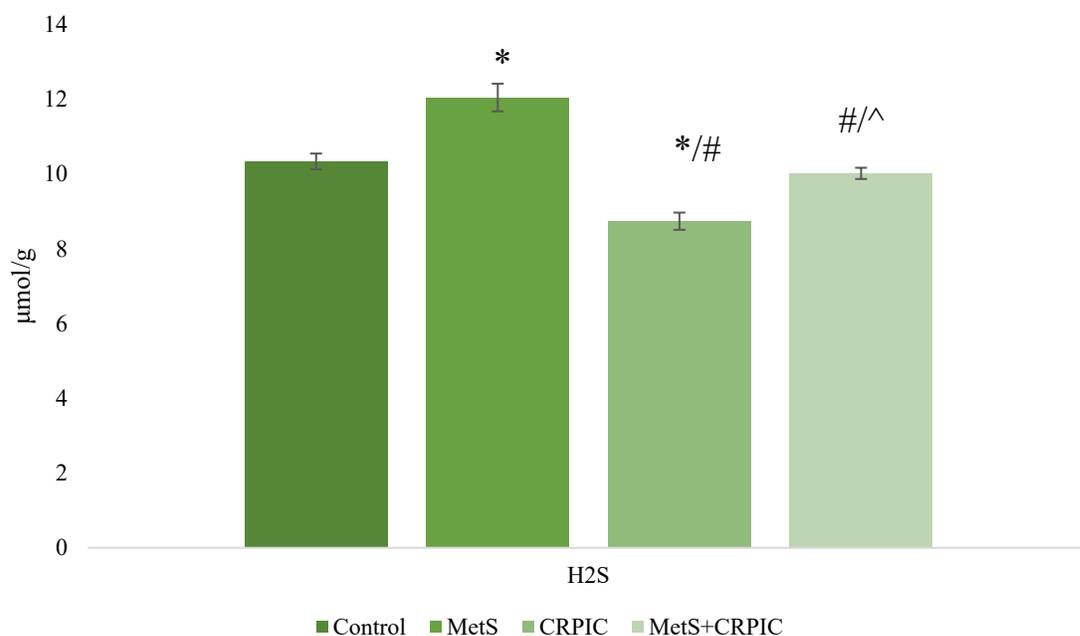


Fig. 3D. Hydrogen sulfide (H₂S) content in rat heart during introduction of chromium picolinate on the background of metabolic syndrome modelling, note: * – p<0.05 vs. control; # – p<0.05 vs. MetS; ^ – p<0.05 vs. CRPIC

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- κ B 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 H₂S 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 H₂S 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 H₂S 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 H₂S 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 H₂S 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- κ B, 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 H₂S 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, H₂S 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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Authors' 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).

References

1. Lushchak VI, Covasa M, Abrat OB, et al. Risks of obesity and diabetes development in the population of the Ivano-Frankivsk region in Ukraine. *EXCLI J.* 2023;22:1047-1054. doi:10.17179/excli2023-6296
2. Chandrasekaran P, Weiskirchen R. The Role of Obesity in Type 2 Diabetes Mellitus-An Overview. *Int J Mol Sci.* 2024;25(3):1882. doi:10.3390/ijms25031882
3. Sanz RL, Inserra F, García Menéndez S, Mazzei L, Ferder L, Manucha W. Metabolic Syndrome and Cardiac Remodeling Due to Mitochondrial Oxidative Stress Involving Gliflozins and Sirtuins. *Curr Hypertens Rep.* 2023;25(6):91-106. doi:10.1007/s11906-023-01240-w
4. Liu J, Aylor KW, Chai W, Barrett EJ, Liu Z. Metformin prevents endothelial oxidative stress and microvascular insulin resistance during obesity development in male rats. *Am J Physiol Endocrinol Metab.* 2022;322(3):E293-E306. doi:10.1152/ajpendo.00240.2021
5. Ruan G, Wu F, Shi D, Sun H, Wang F, Xu C. Metformin: update on mechanisms of action on liver diseases. *Front Nutr.* 2023;10:1327814. doi:10.3389/fnut.2023.1327814
6. Moradi F, Kooshki F, Nokhostin F, Khoshbaten M, Bazyar H, Pourghassem Gargari B. A pilot study of the effects of chromium picolinate supplementation on serum fetuin-A, metabolic and inflammatory factors in patients with nonalcoholic fatty liver disease: A double-blind, placebo-controlled trial. *J Trace Elem Med Biol.* 2021;63:126659. doi:10.1016/j.jtemb.2020.126659
7. Mamikutty N, Thent ZC, Sapri SR, Sahrudin NN, Mohd Yusof MR, Haji Suhaimi F. The establishment of metabolic syndrome model by induction of fructose drinking water in male Wistar rats. *Biomed Res Int.* 2014;2014:263897. doi:10.1155/2014/263897
8. Sahin K, Tuzcu M, Orhan C, et al. Anti-diabetic activity of chromium picolinate and biotin in rats with type 2 diabetes induced by high-fat diet and streptozotocin. *Br J Nutr.* 2013;110(2):197-205. doi:10.1017/S0007114512004850
9. Yelins'ka AM, Akimov OYe, Kostenko VO. Role of AP-1 transcriptional factor in development of oxidative and nitrosative stress in periodontal tissues during systemic inflammatory response. *Ukr Biochem J.* 2019;91(1):80-85. doi:10.15407/ubj91.01.080
10. Mykytenko A, Akimov O, Yeroshenko G, Neporada K. Phenformin attenuates the oxidative-nitrosative stress in the liver of rats under long-term ethanol administration. *Ukr Biochem J.* 2024;96(3):22-30. doi:10.15407/ubj96.03.022
11. El-Kafoury BMA, Bahgat NM, Abdel-Hady EA, Samad AAAE, Shawky MK, Mohamed FA. Impaired metabolic and hepatic functions following subcutaneous lipectomy in adult obese rats. *Exp Physiol.* 2019;104(11):1661-1677. doi:10.1113/EP087670

12. Zhang Y, Wang R, Fu X, Song H. Non-insulin-based insulin resistance indexes in predicting severity for coronary artery disease. *Diabetol Metab Syndr*. 2022;14(1):191. doi:10.1186/s13098-022-00967-x
13. Andrade N, Rodrigues I, Carmo F, et al. Sustainable Utilization of Coffee Pulp, a By-Product of Coffee Production: Effects on Metabolic Syndrome in Fructose-Fed Rats. *Antioxidants (Basel)*. 2025;14(3):266. doi:10.3390/antiox14030266
14. Mohammad-Sadeghipour M, Afsharinasab M, Mohamadi M, Mahmoodi M, Falahati-Pour SK, Hajizadeh MR. The Effects of Hydro-Alcoholic Extract of Fenugreek Seeds on the Lipid Profile and Oxidative Stress in Fructose-Fed Rats. *J Obes Metab Syndr*. 2020;29(3):198-207. doi:10.7570/jomes19051
15. Geohas J, Daly A, Juturu V, Finch M, Komorowski JR. Chromium picolinate and biotin combination reduces atherogenic index of plasma in patients with type 2 diabetes mellitus: a placebo-controlled, double-blinded, randomized clinical trial. *Am J Med Sci*. 2007;333(3):145-53. doi:10.1097/MAJ.0b013e318031b3c9
16. Paiva AN, Lima JG, Medeiros AC, et al. Beneficial effects of oral chromium picolinate supplementation on glycemic control in patients with type 2 diabetes: A randomized clinical study. *J Trace Elem Med Biol*. 2015;32:66-72. doi:10.1016/j.jtemb.2015.05.006
17. Moreira R, Martins AD, Alves MG, de Lourdes Pereira M, Oliveira PF. A Comprehensive Review of the Impact of Chromium Picolinate on Testicular Steroidogenesis and Antioxidant Balance. *Antioxidants (Basel)*. 2023;12(8):1572. doi:10.3390/antiox12081572
18. Zha LY, Wang MQ, Xu ZR, Gu LY. Efficacy of chromium(III) supplementation on growth, body composition, serum parameters, and tissue chromium in rats. *Biol Trace Elem Res*. 2007;119(1):42-50. doi:10.1007/s12011-007-0042-8
19. Kostić S, Tasić I, Stojanović N, et al. Impact of Obesity on Target Organ Damage in Patients with Metabolic Syndrome. *Diagnostics (Basel)*. 2024;14(14):1569. doi:10.3390/diagnostics14141569
20. Barbato JE, Zuckerbraun BS, Overhaus M, Raman KG, Tzeng E. Nitric oxide modulates vascular inflammation and intimal hyperplasia in insulin resistance and the metabolic syndrome. *Am J Physiol Heart Circ Physiol*. 2005;289(1):H228-36. doi:10.1152/ajpheart.00982.2004
21. Kostenko V, Akimov O, Gutnik O, et al. Modulation of redox-sensitive transcription factors with polyphenols as pathogenetically grounded approach in therapy of systemic inflammatory response. *Heliyon*. 2023;9(5):e15551. doi:10.1016/j.heliyon.2023.e15551
22. Kim B, Zhao W, Tang SY, et al. Endothelial lipid droplets suppress eNOS to link high fat consumption to blood pressure elevation. *J Clin Invest*. 2023;133(24):e173160. doi:10.1172/JCI173160

23. Bortolotti M, Polito L, Battelli MG, Bolognesi A. Xanthine oxidoreductase: One enzyme for multiple physiological tasks. *Redox Biol.* 2021;41:101882. doi:10.1016/j.redox.2021.101882
24. Lubawy M, Formanowicz D. High-Fructose Diet-Induced Hyperuricemia Accompanying Metabolic Syndrome-Mechanisms and Dietary Therapy Proposals. *Int J Environ Res Public Health.* 2023;20(4):3596. doi:10.3390/ijerph20043596
25. Yanai H, Adachi H, Hakoshima M, Katsuyama H. Molecular Biological and Clinical Understanding of the Pathophysiology and Treatments of Hyperuricemia and Its Association with Metabolic Syndrome, Cardiovascular Diseases and Chronic Kidney Disease. *Int J Mol Sci.* 2021;22(17):9221. doi:10.3390/ijms22179221
26. Engin A. Endothelial Dysfunction in Obesity and Therapeutic Targets. *Adv Exp Med Biol.* 2024;1460:489-538. doi:10.1007/978-3-031-63657-8_17
27. Berenyiova A, Cebova M, Aydemir BG, Golas S, Majzunova M, Cacanyiova S. Vasoactive Effects of Chronic Treatment with Fructose and Slow-Releasing H₂S Donor GYY-4137 in Spontaneously Hypertensive Rats: The Role of Nitroso and Sulfide Signaling. *Int J Mol Sci.* 2022;23(16):9215. doi:10.3390/ijms23169215
28. Smimmo M, Casale V, Casillo GM, et al. Hydrogen sulfide dysfunction in metabolic syndrome-associated vascular complications involves cGMP regulation through soluble guanylyl cyclase persulfidation. *Biomed Pharmacother.* 2024;174:116466. doi:10.1016/j.biopha.2024.116466
29. Bełtowski J, Wiórkowski K. Role of Hydrogen Sulfide and Polysulfides in the Regulation of Lipolysis in the Adipose Tissue: Possible Implications for the Pathogenesis of Metabolic Syndrome. *Int J Mol Sci.* 2022;23(3):1346. doi:10.3390/ijms23031346
30. Andrés CMC, Pérez de la Lastra JM, Andrés Juan C, Plou FJ, Pérez-Lebeña E. Chemistry of Hydrogen Sulfide-Pathological and Physiological Functions in Mammalian Cells. *Cells.* 2023;12(23):2684. doi:10.3390/cells12232684
31. Shi L, Liu B, Zhang X, et al. Cloning of Nitrate Reductase and Nitrite Reductase Genes and Their Functional Analysis in Regulating Cr(VI) Reduction in Ectomycorrhizal Fungus *Pisolithus* sp.1. *Front Microbiol.* 2022;13:926748. doi:10.3389/fmicb.2022.926748
32. Georgaki MN, Tsokkou S, Keramas A, Papamitsou T, Karachrysafi S, Kazakis N. Chromium supplementation and type 2 diabetes mellitus: an extensive systematic review. *Environ Geochem Health.* 2024;46(12):515. doi:10.1007/s10653-024-02297-5
33. Singh V, Singh N, Verma M, et al. Hexavalent-Chromium-Induced Oxidative Stress and the Protective Role of Antioxidants against Cellular Toxicity. *Antioxidants (Basel).* 2022;11(12):2375. doi:10.3390/antiox11122375
34. Imanparast F, Mashayekhi FJ, Kamankesh F, Rafiei F, Mohaghegh P, Alimoradian A. Improving the endothelial dysfunction in type 2 diabetes with chromium and vitamin D₃ by reducing

- homocysteine and oxidative stress: A randomized placebo-controlled trial. *J Trace Elem Med Biol.* 2020;62:126639. doi:10.1016/j.jtemb.2020.126639
35. Bansal A, Mostafa MM, Kooi C, et al. Interplay between nuclear factor- κ B, p38 MAPK, and glucocorticoid receptor signaling synergistically induces functional TLR2 in lung epithelial cells. *J Biol Chem.* 2022;298(4):101747. doi:10.1016/j.jbc.2022.101747
36. Wang YQ, Yao MH. Effects of chromium picolinate on glucose uptake in insulin-resistant 3T3-L1 adipocytes involve activation of p38 MAPK. *J Nutr Biochem.* 2009;20(12):982-91. doi:10.1016/j.jnutbio.2008.09.002
37. Murphy B, Bhattacharya R, Mukherjee P. Hydrogen sulfide signaling in mitochondria and disease. *FASEB J.* 2019;33(12):13098-13125. doi:10.1096/fj.201901304R
38. Soni P, Paswan S, Paul BD, Thomas B. Intersection of H2S and Nrf2 signaling: Therapeutic opportunities for neurodegenerative diseases. *Neurotherapeutics.* 2025;22(6):e00627. doi:10.1016/j.neurot.2025.e00627

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