RESTORATION OF ADAPTIVE CARDIOPROTECTION IMPAIRED BY METABOLIC SYNDROME IN RATS BY THE PPARα ACTIVATION

Naryzhnaya N.V.,
Derkachev I.A.,
Kurbatov B.K.,
Sirotina M.A.,
Kilin M.,
Maslov L.N.

Cardiology Research Institute, Tomsk National Research Medical Center of the Russian Academy of Sciences (Kievskaya str. 111A, Tomsk 634012, Russian Federation)

Corresponding author:
Natalia V. Naryzhnaya,
e-mail: natalynar@yandex.ru

ABSTRACT

Background. *It is known that the protective effect of adaptation and conditioning influence is weakened in animals with metabolic syndrome. Metabolic syndrome may be the basis for the failure of cardioprotection in clinical settings.*

The aim of the study. *To identify the relationship between disorder in carbohydrate and lipid metabolism and a decrease in the effectiveness of the infarct-limiting effect of moderate chronic normobaric hypoxia; to check the possibility of correcting reduced cardioprotection by normalizing carbohydrate and lipid metabolism.*

Materials and methods. *The study included 64 Wistar rats. Metabolic syndrome was induced by feeding animals a high-carbohydrate, high-fat diet for 84 days. Chronic normobaric hypoxia was carried out for 21 days in the following mode: 12 % O_2 : 0.3 % CO_2. Metformin at a dose of 200 mg/kg/day or PPARα agonist WY14643 at a dose of 1 mg/kg/day were added to the drinking water of rats with metabolic syndrome during adaptation period to hypoxia. A 45-minute coronary occlusion and 120-minute reperfusion were performed, and the infarct size was determined. Indicators of lipid and carbohydrate metabolism, leptin, and adiponectin were studied in the blood serum.*

Results. *The infarct-limiting effect of chronic normobaric hypoxia was weakened in animals with metabolic syndrome. Infarct size showed a direct correlation with decreased glucose tolerance and serum triglyceride levels. Using metformin therapy did not lead to the restoration of the infarct-limiting effect of chronic normobaric hypoxia, while the normalization of lipid metabolism with the use of the PPARα agonist WY14643 corrected the impairment of adaptive cardioprotection in rats with metabolic syndrome.*

Conclusion. *The lack of cardioprotection at chronic normobaric hypoxia in rats with metabolic syndrome is associated with impaired carbohydrate and lipid metabolism. The PPARα agonist restores impaired lipid metabolism and adaptive cardioprotection.*

Key words: *myocardium, infarction, adaptation to hypoxia, metabolic syndrome, metformin, PPARα*

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ВОССТАНОВЛЕНИЕ НАРУШЕННОЙ МЕТАБОЛИЧЕСКИМ СИНДРОМОМ АДАПТАЦИОННОЙ КАРДИОПРОТЕКЦИИ У КРЫС ПУТЁМ АКТИВАЦИИ PPARα

Нарыжная Н.В.,
Деркачев И.А.,
Курбатов Б.К.,
Сиротина М.А.,
Килин М.,
Маслов Л.Н.

Научно-исследовательский институт кардиологии, Томский национальный исследовательский медицинский центр Российской академии наук (634012, г. Томск, ул. Киевская, 111а, Россия)

Автор, ответственный за переписку: **Нарыжная Наталья Владимировна,** e-mail: natalynar@yandex.ru

РЕЗЮМЕ

Обоснование. *Известно, что протекторное действие адаптационных и кондиционирующих воздействий ослаблено у животных с метаболическим синдромом (МетС). МетС может лежать в основе неэффективности кардиопротекции в клинических условиях.*

Цель исследования. *Выявить взаимосвязь между нарушением углеводного и липидного обмена и снижением эффективности инфаркт-лимитирующего влияния умеренной хронической нормобарической гипоксии (ХНГ); проверить возможность коррекции сниженной кардиопротекции путём нормализации углеводного и липидного обменов.*

Методы. *В исследование включено 64 крысы линии Wistar. МетС вызывали кормлением животных высокоуглеводной высокожировой диетой в течение 84 дней. ХНГ проводили в течение 21 дня в режиме: 12 % O_2 : 0,3 % CO_2. В питьевую воду крысам с МетС добавляли метформин в дозе 200 мг/кг/ сут. или агонист PPARα WY14643 в дозе 1 мг/кг/сут. в течение адаптации к гипоксии. Проводили 45-минутную коронарооккклюзию и 120-минутную реперфузию, определяли размер инфаркта. В сыворотке крови исследовали показатели липидного и углеводного обменов, лептин, адипонектин.*

Результаты. *Инфаркт-лимитирующий эффект ХНГ оказался ослаблен у животных с МетС. Размер инфаркта показал прямую корреляционную взаимосвязь со снижением толерантности к глюкозе и содержанием триглицеридов в сыворотке крови. Применение терапии метформином не привело к восстановлению инфаркт-лимитирующего эффекта ХНГ, в то время как нормализация липидного обмена при использовании агониста PPARα WY14643 скорректировала нарушение адаптационной кардиопротекции при метаболическом синдроме у крыс.*

Заключение. *Отсутствие кардиопротекции при ХНГ у крыс с МетС связано с нарушением углеводного и липидного обменов. Агонист PPARα восстанавливает нарушенный липидный обмен и адаптационную кардиопротекцию.*

Ключевые слова: *миокард, инфаркт, адаптация к гипоксии, метаболический синдром, метформин, PPARα*

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INTRODUCTION

A number of strategies have been developed in recent years to protect the myocardium against ischaemic reperfusion injury, such as remote postconditioning, adaptation to chronic moderate hypoxia [1, 2]. However, translating the results of experimental studies into the clinic is difficult since most patients have metabolic disorders [2]. The results of studies conducted in recent years have shown that the metabolic syndrome (MetS), a symptom complex that combines a number of clinical and laboratory indicators of the patient: obesity, arterial hypertension, dyslipidaemia, and carbohydrate metabolism disorders, may underlie the ineffectiveness of adaptation and conditioning in clinical settings [2]. Experimental studies have revealed that a long-term high-fructose diet results in a significant reduction, but not complete prevention, of the beneficial inotropic effects of chronic intermittent hypoxia in an ischaemia-reperfusion model of the isolated heart [3]. The infarct-limiting effect of chronic normobaric hypoxia (CNH) is reduced in animals with diet-induced metabolic syndrome [4]. Meanwhile, it is revealed that the detected decrease in cardioprotection is accompanied by impaired carbohydrate and lipid metabolism [4]. It can be assumed that correction of carbohydrate or lipid metabolism disorders leads to the restoration of CNH cardioprotection that has been lost as a result of metabolic syndrome. Verification of this hypothesis was the aim of the present study.

METHODS

Experiments were performed on 64 female Wistar rats. The study was approved by the local ethical committee (Protocol No. 201 dated June 30, 2020) and was performed in accordance with the provision of Regulation 2010/63/EU of the European Parliament and of the Council of the European Union on the protection of animals used for scientific purposes.

The animals were randomly divided into six groups; the initial weights of rats of all groups were equal and were 203 ± 5 g. Group 1 rats (n = 12) were kept on a standard diet for laboratory animals with free access to drinking water.

Group 2 rats were adapted to CNH for 21 days in a chamber with a continuous supply of a gas mixture consisting of 12 % O_2, 0.3 % CO_2, 87.7% N_2, at normal atmospheric pressure [5]. The gas environment was monitored by TCOD-IR and OLC 20 sensors (Oldham France S.A., France) and Bio-Nova-204G4R1 apparatus (Bio-Nova STS (Scientific and Technical Society), Russia) via MX 32 control unit (Oldham France S.A., France). Exposure to hypoxia was stopped 24 h before the start of the experiment.

Group 3 rats (n = 12; diet-induced metabolic syndrome, MetS) were kept for 84 days on a high-carbohydrate, high-fat diet (HCHFD); drinking water was replaced with a 20 % fructose solution. HCHFD composition: proteins – 16 %, fats – 21 %, carbohydrates – 46 % (including 17 % – fructose), cholesterol – 0.125 %, cholic acid – 0.5 % [6]. After HCHFD, animals were kept on a standard diet and normal drinking water for 7 days to exclude exaggerated blood pressure (BP) readings caused by the osmotic effect of fructose consumption.

Group 4 rats (n = 12) were kept for 70 days on HCHFD, after which they were placed in a hypoxic chamber to simulate CNH and continued HCHFD for 2 weeks of adaptation to CNH; the last week of adaptation to CNH, group 4 rats were kept on a standard diet and drinking water without additives.

Group 5 animals (n = 8) as well as group 4 rats were kept on HCHFD, then were exposed to CNH and received the AMPK activator metformin in drinking form at a dose of 200 mg/kg/day throughout the period of adaptation to hypoxia.

Group 6 animals (n = 9) received the PPARα agonist WY14643 at a dose of 1 mg/kg/day in drinking form under the same conditions.

After the end of diet and/or adaptation to hypoxia 1 day before coronary occlusion modelling, all animals had their tail blood pressure measured by non-invasive volumetric plethysmography using MP35 device with NIBP200A pressure measuring device (Biopac System Inc., USA) and glucose tolerance test (GTT) was performed using standard method, area under curve (AUC, area under curve) was calculated. The infarct-limiting effect of adaptation to CNH was evaluated by the infarct size formed during 45-minute coronary occlusion and 120-minute reperfusion in an *in vivo* experiment. Coronary occlusion was performed during the diestrus phase, which was verified by vaginal mucus microscopy. Anesthesia with α-chloralose (80 mg/kg) and artificial lung ventilation with SAR-830/P (CWE, Inc., USA) were used to perform coronary occlusion. Ligation of the left coronary artery was performed 2 mm below the aortic outlet. After 45 min of ischaemia, the ligature was relieved and the onset of reperfusion was verified by hyperaemia of the ischaemic area. The experiment was terminated without taking the animals out of anaesthesia by sampling blood from the external carotid artery. Myocardium was eviscerated, washed through the aorta with physiological solution. The ligature previously applied to the coronary artery area was re-tightened, and the myocardium was stained through the aorta with 5 % potassium permanganate solution to identify the Area at risk, AAR – the area of myocardium exposed to ischaemia. 1 mm thick transverse sections of the left ventricle were made and stained with 1 % 2,3,5-triphenyltetrazolium solution for 30 min at 37 °C, then fixed for 1 day in 10 % neutral formalin solution and scanned (HP Scanjet G2710; HP Inc., USA). The area of myocardial tissue necrosis (infarction size, IS) on sections was revealed as unstained areas with 2,3,5-triphenyltetrazolium. The size of the necrosis area and risk zone was determined planimetrically using the Ellipse 2.02 software (ViDiTo, Czech Republic).

Blood samples were centrifuged at 3000 rpm, serum was collected and stored at -70 °C. Glucose, triacylglycerides, and cholesterol were determined in serum by enzymatic colorimetric method using B-8054, B-8322, and B-8069 kits (Vektor-Best, Novosibirsk, Russia). The content of leptin, adiponectin, corticosterone, and insulin in serum was determined by enzyme immunoassay using SEA084Ra Leptin, SEA605Ra Adiponectin, CEA448Ra Insulin (Cloud-Clone, China); RE52211 Corticosterone (Human, Rat, Mouse) (IBL International GmbH, Germany) kits. Samples were measured using an Infinite 200 PRO microplate reader (Tecan GmbH, Austria).

Statistical data processing was performed using Statistica 13.0 software (StatSoft Inc., USA). The data obtained were verified for agreement of the distribution with the normal law using the Shapiro – Wilk criterion, showed a distribution satisfying the normality criterion, and are presented as mean ± standard error of the mean (M ± SEM). Homogeneity of variance was checked using Levene's criterion. The numerical values of the studied parameters in the groups were comparable in terms of variance, so two-way ANOVA followed by Fisher's posterior criterion was used in their comparison. Correlations between parameters were investigated using Spearman's coefficient. The threshold value of the achieved significance level p was assumed to be 0.05.

RESULTS

Adaptation of rats to chronic normobaric hypoxia did not affect rat mass and organ mass except for an increase in the mass of the right ventricle of the heart, which is characteristic of the state of chronic hypoxia (Table 1).

TABLE 1

CHANGES IN ORGAN WEIGHTS OF RATS DURING ADAPTATION TO CHRONIC NORMOBARIC HYPOXIA AND METABOLIC SYNDROME

Indicators	Control (n = 12)	CNH (n = 11)	MetS (n = 12)	MetS + CNH (n = 12)	MetS + CNH + metformin (n = 8)	MetS + CNH + WY14643 (n = 9)
	1	2	3	4	5	6
Weight of rat initial, g	204.21 ± 1.57	201.20 ± 1.98	200.00 ± 2.83	208.54 ± 1.86	212.00 ± 2.97	205.82 ± 1.86
Rat weight final, g	286.37 ± 4.71	277.92 ± 6.62	298.67 ± 6.55 p_1 = 0.023	279.23 ± 6.98 p_3 = 0.033	284.88 ± 4.89 p_3 < 0.001	273.56 ± 3.25 p_3 < 0.001
Heart, g	1.08 ± 0.02	1.09 ± 0.04	1.27 ± 0.05 p_1 < 0.001 p_2 = 0.001	1.12 ± 0.02 p_3 = 0.005	1.19 ± 0.02	1.20 ± 0.01
Left ventricular mass, g	0.859 ± 0.016	0.797 ± 0.024	0.980 ± 0.036 p_1 < 0.001 p_2 < 0.001	0.818 ± 0.014 p_3 < 0.001	0.850 ± 0.01 p_3 < 0.001	0.840 ± 0.014 p_3 < 0.001
Right ventricular mass, g	0.221 ± 0.009	0.294 ± 0.023 p_1 = 0.002	0.291 ± 0.021 p_1 = 0.002	0.304 ± 0.013 p_1 < 0.001	0.34 ± 0.02 p_1 < 0.001	0.304 ± 0.013 p_1 < 0.001
Liver, g	10.63 ± 0.27	10.69 ± 0.32	13.12 ± 0.45 p_1 < 0.001 p_2 < 0.001	11.92 ± 0.41 p_1 = 0.012 p_2 = 0.030 p_3 = 0.027	11.67 ± 0.49 p_1 = 0.01 p_2 = 0.009 p_3 = 0.047	11.38 ± 0.33 p_1 = 0.01 p_2 = 0.005 p_3 = 0.029
Kidneys, g	1.83 ± 0.05	1.85 ± 0.05	2.04 ± 0.05 p_1 = 0.002 p_2 = 0.01	1.83 ± 0.05 p_3 = 0.005	1.96 ± 0.04	1.89 ± 0.07
Abdominal fat, g	11.32 ± 0.56	11.52 ± 0.94	15.56 ± 1.24 p_1 = 0.002 p_2 = 0.006	13.97 ± 1.17 p_1 = 0.043 p_2 = 0.088 p_3 = 0.266	14.74 ± 2.86 p_1 < 0.001	14.25 ± 2.15 p_1 = 0.01
Spleen, g	0.67 ± 0.05	0.54 ± 0.03	0.68 ± 0.02	0.64 ± 0.03	0.69 ± 0.04	0.62 ± 0.02
Adrenal glands, mg	42 ± 3	37 ± 2	41 ± 1	37 ± 1	40 ± 1	37 ± 1

Note. n – number of animals in the group; p – statistical significance of differences in relation to the corresponding group (two way ANOVA, Fisher's posterior test).

Keeping rats on a high-carbohydrate, high-fat diet (metabolic syndrome) resulted with an increase in rat weight, abdominal fat mass relative to age-adequate controls, indicating the formation of obesity (Table 1).

In addition, an increase in myocardial mass by 20 %, liver by 30 % and kidneys by 10 % was observed (Table 1). The increase in heart mass in the MetS group was attributable to an increase in both left and right ventricular masses. No statistically significant changes in spleen and adrenal gland masses were observed.

In MetS rats, chronic hypoxia modelling resulted in a decrease in body weight, heart, liver and kidney weights, but not abdominal fat mass to the level of control rats (Table 1). No decrease in the mass of the right ventricular myocardium was observed during the simulation of chronic hypoxia in rats with MetS, indicating the preservation of its hypertrophy, consistent with adaptation to chronic hypoxia [1]. Administration of metformin or WY14643 did not result in statistically significant changes in organ weights in CNH-adapted rats on the MetS background (Table 1).

The formation of metabolic syndrome was characterised by increased blood glucose and insulin content, decreased glucose tolerance (increased area under the curve of glucose dynamics in the glucose tolerance test), insulin resistance (increased HOMA-IR (Homeostasis Model Assessment of Insulin Resistance)), development of hypercholesterolemia, increased triglyceride content in the blood of rats by 1.5 times (Table 2). Adaptation to CNH against MetS background prevented the increase in triglycerides, cholesterol, glucose, glucose and insulin tolerance (HOMA-IR) formation. Along with this, the concentration of insulin in the rat serum under combined modelling of CNH and MetS remained at a high level (Table 2).

Metabolic syndrome caused an increase in serum leptin and adiponectin relative to the control group equally in the groups of non-adapted and CNH-adapted rats (Table 2).

The metabolic syndrome was accompanied by an increase in serum corticosterone levels from 394 ± 6.1 to 475 ± 3.7 ($p_1 < 0.001$), which indicates moderate stress (data are not presented in the table). Adaptation to chronic normobaric hypoxia did not increase corticosterone elevation during MetS. It should be emphasised that the absence of changes in the mass of target organs (adrenal glands, spleen; Table 1) indicates a small severity of the stress response.

TABLE 2

BIOCHEMICAL INDICES OF METABOLIC SYNDROME FORMATION IN RATS UNDER THE INFLUENCE OF METFORMIN AND WY14643

Indicators	Control (*n* = 12)	CNH (*n* = 11)	MetS (*n* = 12)	MetS + CNH (*n* = 12)	MetS + CNH + metformin (*n* = 8)	MetS + CNH + WY14643 (*n* = 9)
	1	2	3	4	5	6
Glucose, mmol/L	4.55 ± 0.34	5.02 ± 0.46 $p_1 > 0.05$	5.32 ± 0.30 $p_1 = 0.05$	4.36 ± 0.39 $p_2 > 0.05$	4.6 ± 0.29 $p_4 > 0.05$	4.95 ± 0.15 $p_1 = 0.05$
Glucose tolerance test (AUC)	709 ± 13	723 ± 26 $p_1 > 0.05$	761 ± 12 $p1 = 0.012$	725 ± 13 $p_2 > 0.05$	725 ± 25 $p_4 > 0.05$	759 ± 22 $p_1 = 0.012$
Insulin, pmol/L	8.02 ± 0.57	9.78 ± 0.71 $p_1 > 0.05$	10.37 ± 0.45 $p_1 = 0.05$	11.80 ± 0.98 $p_1 = 0.002$	10.58 ± 1.26 $p_4 > 0.05$	10.42 ± 0.22 $p1 < 0.05$
HOMA-IR	1.78 ± 0.14	2.14 ± 0.14 $p_1 > 0.05$	2.48 ± 0.13 $p_1 = 0.03$	2.25 ± 0.26 $p_2 > 0.05$	2.20 ± 0.30 $p_4 > 0.05$	2.32 ± 0.11 $p_1 = 0.04$
TG, mmol/L	1.01 ± 0.15	1.25 ± 0.16 $p_1 > 0.05$	1.57 ± 0.29 $p_1 = 0.036$	1.31 ± 0.15 $p_2 > 0.05$	1.30 ± 0.16 $p_4 > 0.05$	1.0 ± 0.09 $p_3 = 0.036$
Cholesterol, mmol/L	4.30 ± 0.44	5.34 ± 0.69 $p_1 > 0.05$	6.71 ± 1.24 $p_1 = 0.034$	5.62 ± 0.68 $p_2 > 0.05$	5.56 ± 0.68 $p_4 > 0.05$	4.51 ± 0.94 $p_3 = 0.034$
Leptin, ng/mL	1.77 ± 0.26	1.34 ± 0.11 $p_1 > 0.05$	5.37 ± 0.74 $p_1 < 0.001$	5.89 ± 0.55 $p_1 < 0.001$ $p_2 < 0.001$	2.50 ± 0.41 $p_3 < 0.001$ $p_4 < 0.001$	1.25 ± 0.14 $p_3 < 0.001$
Adiponectin, μg/mL	1.77 ± 0.26	1.34 ± 0.11 $p_1 > 0.05$	5.37 ± 0.74 $p_1 < 0.001$	5.89 ± 0.55 $p_1 < 0.001$ $p_2 < 0.001$	6.96 ± 0.84 $p_3 < 0.001$ $p_4 = 0.009$	8.85 ± 1.17 $p_3 < 0.001$

Note. TG – triglycerides; *n* – number of animals in the group; *p* – statistical significance of differences in relation to the respective group (two-way ANOVA, Fisher's posterior test).

Keeping rats on HCHFD resulted in an increase in systolic (SBP) but not diastolic blood pressure (DBP) (Table 3). Adaptation of rats with metabolic syndrome to hypoxia caused an increase in DBP, while SBP in this group had no statistically significant differences from rats of the control group (Table 3).

Coronary occlusion in all groups of animals induced the formation of a myocardial hypoperfusion zone (risk zone), the size of which was 30–33 % of the left ventricular mass (Fig. 1b; Table 4). 2,3,5-triphenyltetrazolium staining revealed that the infarction size was 46.92 % of the mass of the hypoperfused area. Statistically significant myocardial hypertrophy was observed in the MetS group (Table 1); the mass of myocardial hypoperfusion area (AAR) in this group in absolute terms was statistically significantly greater than in rats without metabolic disorders. However, since the infarct size was considered as the ratio of the necrosis zone and risk zone masses (IS/AAR, %), the infarct size in rats with MetS was not statistically significantly different from that in the control group (Table 4).

TABLE 3

BLOOD PRESSURE IN RATS WITH METABOLIC SYNDROME

Indicators	Control (n = 19)	CNH (n = 14)	MetS (n = 15)	MetS + CNH (n = 15)
	1	2	3	4
SBP, mmHg	129.2 ± 2.5	129.3 ± 1.9	142.0 ± 2.8 p_1 = 0.014 p_2 = 0.008	137.5 ± 1.6 $p_{1.2.3}$ ns
DBP, mmHg	96.1 ± 2.5	96.5 ± 2.6	98.8 ± 2.2	104.7 ± 1.5 p_1 = 0.039

Note. n – number of animals in the group; p – statistical significance of differences in relation to the corresponding group (two way ANOVA, Fisher's posterior test); ns – statistically non-significant..

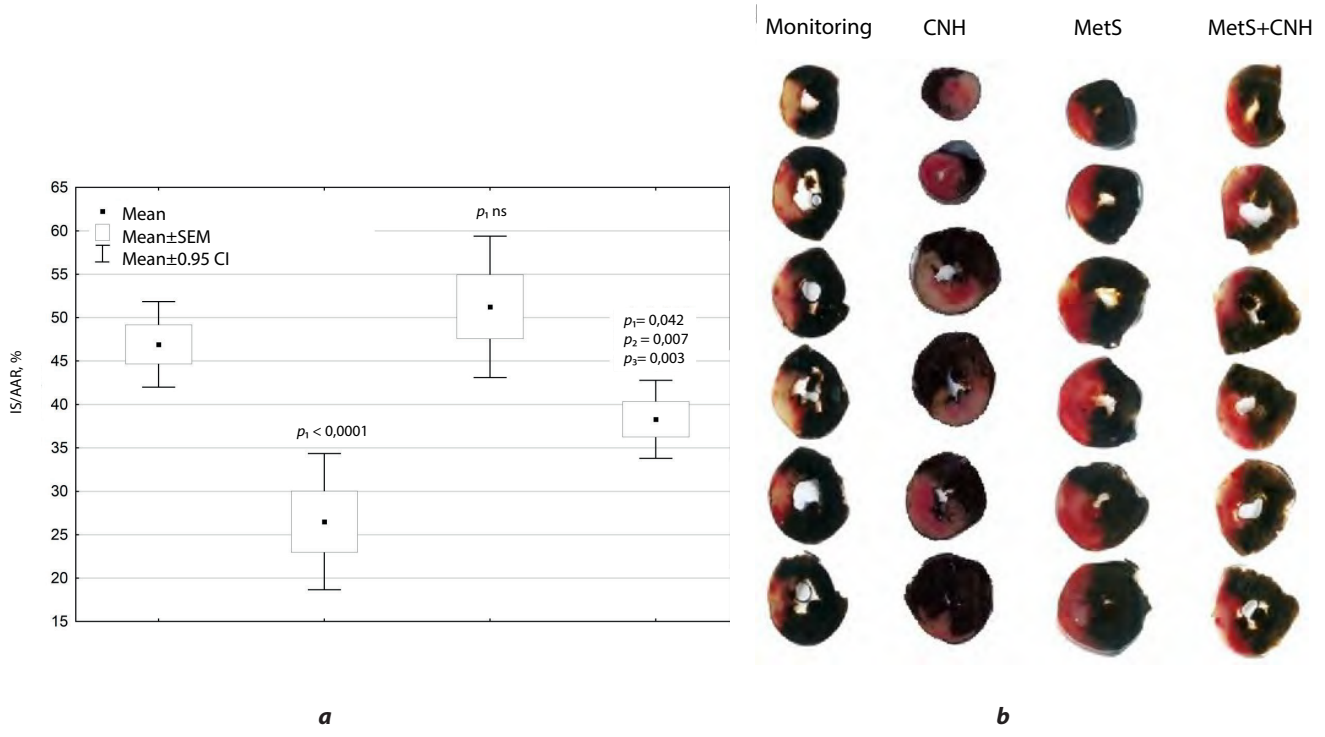

FIG. 1.

CNH infarct-limiting effect in rats with and without metabolic syndrome: ***a*** *– mean values of infarct size in control group, with MetS, CNH and with the combination of CNH and MetS;* ***b*** *– representative images of myocardial infarction in experimental groups; p_1 – statistical significance of differences compared to control group; p_2 – statistical significance of differences compared to the group with chronic normobaric hypoxia; p_3 – statistical significance of differences compared to the group with metabolic syndrome (two-way ANOVA, Fisher's posterior test); ns – not significant*

Infarct size (IS/AAR, %) in CNH-adapted rats was 43 % smaller than in controls (Fig. 1a, b; Table 4). The obtained data indicate a pronounced CNH infarct-limiting effect. This effect was reduced in animals with MetS: the reduction in infarct size relative to the group of rats with MetS was 25 % (Fig. 1a, b; Table 4). Consequently, we can speak of an attenuation of the CNH infarct-limiting action in rats with diet-induced metabolic syndrome.

Correlation analysis (Spearman's *r*-criterion) revealed weak but statistically significant direct correlations between infarct size and glucose tolerance index (AUC), as well as between infarct size and serum triglyceride content (Table 5).

Metformin therapy (adding it to drinking water at a final dose of 200 mg/kg/day for 21 days of rats adaptation to CNH) did not affect glucose, insulin, triglycerides, cholesterol, glucose and insulin tolerance indices in MetS rats during adaptation to CNH (Table 2). At the same time, under the influence of metformin there was a decrease in leptin content and an increase in adiponectin content relative to the group of rats with metabolic syndrome, including relative to the group of rats adapted to CNH under MetS (Table 2).

Metformin administration did not change left ventricular mass and the size of the risk zone in rats with metabolic syndrome and CNH adaptation; infarct

TABLE 4

EFFECT OF METFORMIN AND WY14643 ON INFARCT SIZE IN RATS AFTER METS AND CNH MODELLING

Groups	Left ventricular mass, mg	Risk zone, mg	Necrosis zone, mg	NZ/RZ, %
1. Control (n = 12)	859 ± 16	257.25 ± 20.53	120.17 ± 10.34	46.92 ± 2.24
2. CNH (n = 11)	797 ± 24	268.89 ± 18.11	73.39 ± 12.29 p_1 = 0.023	26.50 ± 3.52 p_1 < 0.001
3. MetS (n = 12)	980 ± 36 p_1 = 0.0018	329.42 ± 25.51 p_1 = 0.019	170.96 ± 20.04 p_1 = 0.012	51.25 ± 3.70 p_1 ns
4. MetS + CNH (n = 12)	818 ± 14	241.34 ± 19.07 p_3 = 0.004	93.73 ± 9.92 p_3 < 0.001	38.29 ± 2.04 p_1 = 0.042 p_2 = 0.007 p_3 = 0.003
5. MetS + CNH + metformin (n = 8)	850 ± 10	250.0 ± 22.38 p_3 = 0.018	103.1 ± 18.99 p_1 ns p_2 ns p_3 = 0.0034 p4 ns	39.2 ± 4.75 p_1 ns p_2 = 0.013 p_3 = 0.016 p_4 ns
6. MetS + CNH + WY14643 (n = 9)	865 ± 29	247.2 ± 23.5 p_3 = 0.03	72.5 ± 19.2 p_3 = 0.002	29.3 ± 5.7 p_1 = 0.022 p_2 ns p_3 = 0.003

Note. n – number of animals in the group; p – statistical significance of differences in relation to the corresponding group (two way ANOVA, Fisher's posterior test); ns – statistically non-significant.

TABLE 5

CORRELATIONS OF BIOCHEMICAL PARAMETERS WITH INFARCT SIZE IN INDUCED METABOLIC SYNDROME AND ADAPTATION TO CHRONIC NORMOBARIC HYPOXIA

Indicators	Infarct size NZ/RZ, %	*p* value
GTT (AUC)	0.33	0.034
Triglycerides, mmol/L	0.39	0.017

Note. TG – triglycerides; n – number of animals in the group; p – statistical significance of differences in relation to the respective group (two-way ANOVA, Fisher's posterior test).

size was not altered by metformin in rats with combined metabolic syndrome and CNH modelling (Table 4).

Application of PPARα activator WY14643 did not change the indices of carbohydrate metabolism in CNH-adapted rats against MetS background. A decrease in serum triglycerides, cholesterol and leptin was observed under the influence of WY14643 (Table 2). Administration of PPARα activator did not change left ventricular mass and the size of the risk zone in rats with metabolic syndrome and CNH adaptation (Table 4). Simultaneously, the size of the necrosis zone and IS/AAR ratio in rats of this group were lower than in the groups of control animals, in the group of rats with metabolic syndrome and in the group with combined application of metabolic syndrome and CNH (Table 2).

DISCUSSION OF RESULTS

The results of the study revealed that the use of HCHFD leads to obesity, which is characterised by an increase in body weight and abdominal fat mass, accompanied by hyperglycaemia, impaired glucose tolerance, dyslipidaemia and the development of hypertension. The results obtained allow us to talk about the formation of a metabolic syndrome. Lipid accumulation was previously observed in the myocardium and aorta of rats kept on a diet similar to that used in our study [6]. We found no significant effect of MetS over infarct size, however. There is evidence in the literature of both increased myocardial resistance to ischaemia by a high-carbohydrate diet accompanied by hyperglycaemia [7] and increased infarct size in individuals with metabolic abnormalities such as hyperglycaemia and dyslipidaemia [8]. Accordingly, we can speak about unexpressed myocardial changes in MetS, which do not statistically significantly affect cardiac resistance to ischaemia-reperfusion.

Adaptation to chronic normobaric hypoxia demonstrated a pronounced infarct-limiting effect, which is consistent with the literature and our previous results [1, 5]. Our studies showed that the infarct-limiting effect of CNH is attenuated under MetS conditions. At the same time, adaptation to CNH of MetS rats results in reduction of MetS manifestations such as dyslipidaemia, impaired glucose tolerance. Therefore, we can conclude that CNH significantly but not completely prevents the formation of metabolic disorders, and its protective effect against ischaemic reperfusion injury is reduced. A study by J.J. Zhou et al. (2013) also revealed a decrease in serum glucose content in MetS rats when exposed to chronic hypoxia [3].

The failure of CNH adaptive mechanisms in MetS animals may be a consequence of a number of reasons. Correlation analysis revealed the relationship of infarct size with impaired carbohydrate metabolism. Myocardial insulin resistance in MetS may be considered as mechanisms of this correlation, caused, among others, by the endocrine influence of adipose tissue [9]. Under conditions of adaptation to hypoxia, myocardial metabolism becomes largely dependent from glucose oxidation [10]. Concurrently, decreased activity of the insulin signalling-associated glucose transporter GLUT4 prevents the use of sufficient carbohydrate to sustain the energy status of the cell. Along with this, the hypothesis about the role of myocardial insulin resistance in impairment of CNH infarct-limiting action formation needs additional verification.

The scientific literature discusses the decrease in the activity of adenosine monophosphate-activated protein kinase (AMPK), one of the key enzymes in the regulation of carbohydrate metabolism of the cell, during MetS. It has been revealed that lipotoxicity of fatty acids towards myocardium under high-fat diet is associated with decreased activation (phosphorylation) of AMPK, which leads to the development of myocardial contractility disorders, fibrosis, apoptosis, inflammation and oxidative stress [11].

The fact that AMPK is involved in myocardial defence against hypoxia should be considered [12]. Perhaps inhibition of AMPK signalling by MetS prevents the development of cardioprotection of CNH. These results, however, revealed a lack of metformin efficacy, an AMPK activator, to restore impaired cardioprotection. At the same time, metformin is known to show efficacy in myocardial ischaemia in obese rats induced by a high-fat diet [13]. The mechanisms of cardioprotection in this case are reduction of oxidative stress, anti-apoptotic effect of metformin, reduction of ferroptosis and necroptosis, improvement of contractility, increase of mitochondrial transmembrane potential, decrease of reactive oxygen species formation in mitochondria, and increase of mitochondrial fusion marker OPA1 [13]. According to other authors, however, metformin has no effect upon myocardial ischaemia-reperfusion resistance in rats with streptozotocin-induced diabetes mellitus, including infarct size and post-ischaemic recovery of myocardial contractility [14].

The literature data suggest a definite role in the reduced infarct-limiting efficacy of CNH in rats with MetS for the impairment of the intracellular mechanism of action of adiponectin signalling. It should be noted that our study revealed an increase of adiponectin in rat serum under MetS, which was persisted at a high level when CNH was modelled in these animals. In animals with unaltered carbohydrate metabolism, adiponectin, through interaction with AdipoR1 receptors, stimulates the intracellular APPL1-AMPK response of cardiomyocytes, providing anti-apoptotic and anti-necrotic effects during ischaemia/reoxygenation [15]. This protective effect of adiponectin in myocardial IR has been demonstrated to be reduced in cardiomyocytes of mice with type 2 diabetes mellitus, which may indicate impaired intracellular signalling of this adipokine [15]. Patients with metabolic syndrome were revealed to have decreased expression of both AMPK subunits (α1 and α2) in skeletal muscle, which is correlated with decreased myocyte sensitivity to adiponectin [16].

Additionally, failure of adaptive cardioprotection may be associated with impaired RISK-kinase signalling

during MetS, which is involved in the infarct-limiting effect of CNH. Specifically, it was found that activation of protein kinase B (Akt-kinase) and subsequent cardioprotection in response to preconditioning of rat myocardium did not occur when it was perfused with fatty acids [17]. Stimulation of Akt-kinase phosphorylation restores mitochondrial function impaired when cells are exposed to palmitic acid [9].

It is acknowledged that one of the mechanisms of cardioprotection in chronic hypoxia is the development of the microvascular channel under the influence of hypoxia-induced factors (e.g., HIF1) [18]. This mechanism was revealed to be significantly impaired in patients with MetS, which may be one of the reasons for the impaired formation of cardioprotection [19]. The other mechanism of increased myocardial resistance to ischaemia by HIF-1 factor is the synthesis of microRNA miR-322, which is associated with cytoprotective and antiapoptotic effects of adaptation to hypoxia [20, 21]. This mechanism is affected by MetS [22].

Correlation analysis showed a relationship between infarct size and serum triacylglyceride levels. It can therefore be assumed that disorders of lipid metabolism may be responsible for the failure of adaptive cardioprotection in MetS. PPAR-α receptor is known to be one of the key structures regulating cellular lipid utilisation in cardiomyocytes [23]. PPAR-α is known to be involved in the control of transcription of genes involved in the capture and oxidation of fatty acids in cardiomyocytes [24]. In addition, PGC-1 to PPARα signalling has been also demonstrated to play an important role in the regulation of myocardial resistance to ischemia. For instance, the PPARα agonist clofibrate was revealed to have a direct antiapoptotic effect in ischaemia-reperfusion myocardium of rats with MetS [25], and the PPARα antagonist GW6471 prevented the cardioprotective effect of the cannabinoid anandamide in a model of chronic intermittent myocardial ischaemia (ischaemic cardiomyopathy) in mice [26]. However, the above signalling undergoes significant changes in both diabetes mellitus and chronic hypoxia. A significant decrease in the rate of fatty acid oxidation in rat myocardium was revealed in chronic hypoxia, and, on the contrary, an acceleration of this process in diabetes mellitus induced by the application of a high-fat diet and streptozotocin [10]. The combined state of chronic hypoxia and diabetes mellitus, according to these researchers, reveals a high rate of fatty acid oxidation by mitochondria [10]. No changes in PPARα mRNA in rat myocardium were observed by these authors, neither in isolated exposure to diabetes mellitus and hypoxia, nor in combined pathology. Other publication, however, revealed suppression of the expression of lipid metabolism regulatory protein genes, including PPARα, PPARγ, coactivator 1α (PGC1α), and carnitine palmitoyl transferase 1α (CPT1α), when exposed to hypoxia against a background of diabetes [27]. Meanwhile, PPARα activator WY14643 reduced obesity-induced myocardial lipid accumulation and improved left ventricular systolic function and mitochondrial respiration [27]. These data are consistent with our findings about the infarct-limiting effect of PPARα activator WY14643 in experimental myocardial infarction.

CONCLUSION

Studies have revealed that diet-induced metabolic syndrome reduces the infarct-limiting efficacy of adaptation to chronic normobaric hypoxia in rats. In this case, the decrease in the effectiveness of chronic normobaric hypoxia is correlated with impaired glucose tolerance and increased triglyceride levels. Correction of carbohydrate metabolism by metformin does not restore the infarct-limiting effect of CNH in metabolic syndrome, whereas the use of PPARα activator normalises lipid metabolism and completely restores the impairment of adaptive cardioprotection in metabolic syndrome in rats.

The obtained data allow us to conclude that for correction of adaptation cardioprotection disorders it is necessary to use means that improve not carbohydrate but lipid metabolism.

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Conflict of interest

The authors declare no conflict of interest.

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Information about the authors

Natalia V. Naryzhnaya – Dr. Sc. (Med.), Leading Research Officer at the Laboratory of Experimental Cardiology, Cardiology Research Institute, Tomsk National Research Medical Center of the Russian Academy of Sciences, e-mail: natalynar@yandex.ru, https://orcid.org/0000-0003-2264-1928

Ivan A. Derkachev – Postgraduate, Cardiology Research Institute, Tomsk National Research Medical Center of the Russian Academy of Sciences, e-mail: vanya.derkachev@mail.ru, https://orcid.org/0000-0003-1998-0873

Boris K. Kurbatov – Junior Research Officer at the Laboratory of Experimental Cardiology, Cardiology Research Institute, Tomsk National Research Medical Center of the Russian Academy of Sciences, e-mail: bobersanker@gmail.com, https://orcid.org/0000-0001-9603-822X

Maria A. Sirotina – Junior Research Officer at the Laboratory of Experimental Cardiology, Cardiology Research Institute, Tomsk National Research Medical Center of the Russian Academy of Sciences, e-mail: sirotina_maria@mail.ru, https://orcid.org/0000-0002-4502-0836

Mikhail Kilin – Laboratory Assistant at the Laboratory of Experimental Cardiology, Cardiology Research Institute, Tomsk National Research Medical Center of the Russian Academy of Sciences, e-mail: Kilin112233@gmail.com, https://orcid.org/0000-0002-4300-5763

Leonid N. Maslov – Dr. Sc. (Med.), Professor, Head of the Laboratory of Experimental Cardiology, Cardiology Research Institute, Tomsk National Research Medical Center of the Russian Academy of Sciences, e-mail: Maslov@cardio-tomsk.ru, https://orcid.org/0000-0002-6020-1598