Pulmonary vascular smooth muscle contraction in the rat model of metabolic syndrome

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Abstract. Metabolic syndrome is defined as a constellation of many metabolic disorders such as hypertension, impaired glucose tolerance, dyslipidemia and obesity, being this last disorder a key factor in the etiology of the syndrome. Animal model of metabolic syndrome allows us to study the phenomenology of disorders of cooperative interactions of effector cells responsible for regulating of the blood vessel tone. Here, we have characterized the metabolic, vascular changes in male Wistar rats fed on a high-fat, high-carbohydrate diet including lard (17%), fructose (17%) together with 20% fructose in drinking water, control rats were fed a standard rat chow. During 12 weeks on this diet, rats showed a change in body weight with impaired glucose tolerance. Vascular disorders included endothelial dysfunction in pulmonary artery.

1. Introduction

Vascular tone is regulated by the contractile activity of smooth muscle cells (SMCs) located in the walls of blood vessels and plays an important role in the regulation of blood flow in tissues and organs. Abdominal obesity, hyperglycemia, insulin resistance, decreased glucose tolerance, dysregulation of lipid metabolism in Metabolic syndrome (MetS) contribute to the development of endothelial dysfunction and alternation of SMCs of blood vessels [1]. Chronic inflammation plays a significant role in MetS and vascular diseases including pulmonary hypertension [2].

The contractile reactions of SMCs are determined by their membrane potential, the activity of ion-transporting systems, the set of receptors, signaling molecules and contractile proteins necessary to maintain arterial tone. As a result of changes in their contractile function, an imbalance in the effects of vasoconstrictor (e.g. Angiotensin II (Ang II), prostanoids, endothelin-1) and vasodilation endothelial factors (e.g. NO, prostaglandins (PGI2), endothelium-derived hyperpolarization factor (EDHF)), the regulation of vascular tone is impaired, intravascular resistance increases, and the sensitivity of the vascular wall to pressure influences of catecholamines [3]. The aim of the study was to investigate the contractile activity of smooth muscle segments of the pulmonary artery of rats with MetS.
2. Materials and methods

2.1. Animals
Male Wistar rats (n = 20) were fed ad libitum and housed in a 12-h light/dark cycle. Experiments were performed in strict accordance with the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (1986) and approved by the Ethics Committee of Siberian State Medical University (№7793, 2019).

Two groups were formed: 1) rats on a standard chow diet (n = 8) – control group; 2) rats on a high-fat, high-carbohydrates diet (HFHC diet, n = 12) – experimental group. The control group was fed with food for laboratory animals Delta Feeds (Biopro, Russia), in which fats accounted for 18% from the total calories intake. For the experimental group, we developed a diet that included the Delta Feeds feed described above (66%), lard (17%), fructose (17%), cholesterol (0.125%) with 20% fructose in drinking water. The diet complied with the following criteria: 54 % of the calories accounted for fats. The experiment continued within 12 weeks. The body weight test was carried out every 4 weeks. Before and at the end of the study, animals were measured weight and blood pressure (non-invasive blood pressure measurement system "Systola", Neurobotics, Russia). The glucose tolerance test was conducted on the 12th week. On the day of the experiment rats were euthanized by an overdose of CO2 inhalation.

2.2. Glucose tolerance test
Concentration of plasma glucose were measured by a glucose-oxidase enzymatic commercial kit (Glucose-Nov v-8054, Vector-Best, Russia) and determined at 510 nm with a spectrophotometer (SP-2000, Russia). Load of 2.0 g/kg 20% glucose solution was administered through oral gavage. Blood glucose levels were measured, and tail blood was collected at 15, 30, 60, 90 and 120 min post glucose gavage [4].

2.3. Pulmonary artery ring preparation and organ bath tension measurements
The pulmonary artery (PA) was rapidly removed and immersed in Krebs’ solution (in mM: 120.4 NaCl, 5.9 KCl, 2.5 CaCl2, 1.2 MgCl2, 5.5 glucose, 15 NH2C(CH2OH)3; pH 7.35-7.40, 37 ºC). The PA was dissected to remove fat and connective tissue and sliced into rings (2-3 mm in length). Mechanical tension of PA segments was measured by mechanography (Myobath Tissue Bath System II, World Precision Instruments, Germany). The rings are exposed to basal tension (500 mg) and aerated continuously with 95% O2/5% CO2 for 40-50 min. Contractions of segments were induced by modified Krebs solution with high K+ (we used 30 mM KCl) or 1 µM phenylephrine (PE) until a maximum stable contraction is reached. Rings were cocontracted at least two more times before each experiment until a reproducible contractile response was obtained.

Ten to fifteen min after this state was achieved acetylcholine (10 nM – 100 µM) or sodium nitroprusside (1 nM – 100 µM) were added. The amplitudes of contractile responses were calculated in percents of the controls contractions induced by 30 mM KCl or 1 µM PE. All chemicals were purchased from Sigma Aldrich (USA).

2.4. Statistics
Data were presented as means ± standard deviation (SD). For statistical significance between two groups was performed by the Student t-test using the IBM SPSS Statistics 21 software. The alpha level of significance for all experiments was set at p < 0.05.

3. Results and discussion
Experimental models of MetS are aimed at reproducing most of the characteristic features of the syndrome, such as overweight, visceral obesity, insulin resistance, impaired glucose tolerance, dyslipidemia, and arterial hypertension. It was shown that animal fats (lard or beef fat) in this case are more effective for animal model of MetS compared with vegetable fats, while glucose, fructose, or sucrose can be used as carbohydrates added to the diet [5, 6].
In this model, fat and sugars present in the diet provided more energy than required by the animals [7]. Thus, HFHC diet-fed rats displayed increased body weight by an average of 1.75 times (p<0.05), with no significant differences between the groups.

In animals on HFHC diet, there was an increase in blood pressure: 124.1 ± 9.2 mm Hg in the control group vs 136.2 ± 8.3 mm Hg in the experimental group (p<0.05).

In addition, 15 and 30 min after the carbohydrate load in rats of the experimental group, the level of blood glucose exceeded the control group by 25%. Within 60 min, the level of blood glucose in the control group began to decrease gradually, while in rats of the experimental group, the indicator increased and the differences with the control amounted to 32%. After 2 hours, the level of glucose in the control group almost returned to its initial level, while in the experimental group it remained elevated by 10% (figure 1). The area under the AUC curve in the control group was 585.5 ± 53.1 mmol / l × 120 min (p<0.05), in the experimental group - 809.9 ± 81.9 mmol / l × 120 min (p<0.05) (figure 2). Our finding of plasma glucose, indicate that in HFHC diet-fed rats, there was a violation of glucose tolerance.

![Figure 1](image1.png)  ![Figure 2](image2.png)

**Figure 1.** Glucose tolerance test: Plasma glucose levels.  **Figure 2.** Glucose tolerance test: AUC Standard chow vs. AUC HFHC diet.

Vascular endothelium acts as a modulator of myotropic reactions of most biologically active substances, however, contractile responses of SMC are caused not only by the effectiveness of endothelial-smooth muscle interactions, but also by the properties, mechanisms of reception and translation of signals of the vascular smooth muscles themselves. PA isolated from HFHC diet- or chow-fed rats showed reduced acetylcholine-induced vascular relaxation. But compared with PA from chow-fed rats in HFHC diet-fed rats a decrease in the relaxing effect of acetylcholine was observed (figures 3, 4). Acetylcholine-induced relaxation is caused by release NO, an endothelium-derived vasodilator factor. Therefore, the observed effects may be due to HFHC diet-fed rat PA endothelial dysfunction.

Application of sodium nitroprusside, a donor of nitric oxide, decreased contractile reactions of PA segments isolated from HFHC diet- or chow-fed rats caused by 30 mM KCl and PE (figures 5, 6). In rats on HFHC diet, the relaxing effect of the NO donor decreased.
Figure 3. Relaxation of PE-induced contraction of pulmonary artery rings to acetylcholine. Values are means ± SD and n = 6 for each group, * p < 0.05.

Figure 4. Relaxation of KCl-induced contraction of pulmonary artery rings to acetylcholine. Values are means ± SD and n = 6 for each group, * p < 0.05.

Figure 5. Relaxation of PE-induced contraction of pulmonary artery rings to sodium nitroprusside. Values are means ± SD and n = 6 for each group, * p < 0.05.

Figure 6. Relaxation of KCl-induced contraction of pulmonary artery rings to sodium nitroprusside. Values are means ± SD and n = 6 for each group, * p < 0.05.

NO released by sodium nitroprusside is a lipophilic substance and easily penetrates the SMCs membrane. In this case, there is an increase in the activity of soluble guanylate cyclase and an increase in the cytoplasmic concentration of cGMP, which leads to relaxation of smooth muscles. Therefore, a decrease in sodium nitroprusside-induced relaxation suggests a violation of the contractile properties of pulmonary artery SMCs of HFHC diet-fed rats.

4. Conclusion
HFHC diet feeding in rats reproduces most of the typical features of MetS and cardiovascular remodeling, endothelial dysfunction of pulmonary artery along with the moderate impairment in glucose
tolerance. This model of diet-induced MetS can be useful in studying the causes of the development and progression of metabolic and hemodynamic disorders in MetS, as well as in the study of potential approaches to its prevention and treatment.

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