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Influence of type 2 diabetes mellitus on the development of hypoxic pulmonary vasoconstriction

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Key words: diabetes mellitus, intrapulmonary arteries, hypoxia, hypoxic pulmonary vasoconstriction

A study of complex interaction of factors involved in development of type 2 diabetes mellitus (T2DM) provides the basis for developing strategies for the prevention, treatment of complications, and for reduction of disability and mortality from this disease.

Hypoxia is currently considered to be an universal factor involved in complications development of many diseases including diabetes mellitus. Acute hypoxia causes a body's systemic reaction manifested as vasodilatation in the large circulatory system and hypoxic pulmonary vasoconstriction (HPV) in the arteries of the pulmonary circulation [1]. Under physiological conditions, such a vasoconstriction optimizes the respiratory function of the lungs and demonstrates an ability of arteries to regulate pulmonary circulation and maintain a normal ventilation-perfusion ratio. Lack of the adequate response to hypoxic effects may be an additional mechanism for the development of diabetic complications in the cardiovascular system. Despite years of study, the mechanisms of HPV remain largely unclear, as does the effect of diabetes on the pulmonary artery reactivity. Since currently known mechanisms for HPV development are based on changes in the glycolytic pathways of metabolism, a significant deterioration in the condition of patients with diabetes is quite predictable. In such cases, taking into account the specific features of pulmonary artery reactivity is very important for both the adequate correction of circulatory disorders and treatment of concomitant diseases [2–4]. Many researchers consider pulmonary hypertension (PH) to be one of these diseases [5, 6]. Although the link between diabetes and PH is being actively debated, there is a need for more reliable experimental studies that would show the modifying effect of diabetes on regulation of the pulmonary circulation.

Alveolar hypoxia is currently considered to be the main factor that causes HPV, with extra and intra-acinar arteries being the main vascular segment that ensures HPV development [7, 8]. These arteries are located in that part of the lung that cannot be locally studied using most of the common approaches. Therefore, the method of video microscopic analysis of changes in the tone of the intrapulmonary segment of the arteries was used in the presented work.

The aim of the study was to evaluate the effect of high-fat diet and T2DM on the development of the HPV reaction *in vivo* and *in vitro*.

Materials and methods. *Animals used in the experiments*. The experiments were conducted on 75 adult white Wistar male rats weighing (180 ± 15) g, kept on the vivarium standard diet, consisting of dry briquetted compound feeds (vivarium of SI «Institute of Pharmacology and Toxicology of NAMS of Ukraine»). All manipulations with animals were carried out in accordance with the Law of Ukraine № 3447-IV «On Protection of Animals from Cruel Treatment» [9] and the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes [10]. The animals were euthanized by intravenous injection of urethane in the lethal dose (400 mg per 100 g of body mass).

All animals were randomly divided into three groups of 20 animals each in 2 groups and 35 in group 3. The first group was a control one, the animals of the second group were treated on a high-fat diet (HFD), and the animals of the third group were simulated with T2DM.

Simulation of diabetes in rats. Simulation of diabetes was performed according to the previously described method [11]. The development of T2DM was caused by keeping rats on a high-fat diet (animal fat in the amount of 10 % of the total feed weight was added to the vivarium standard granulated feed containing 6 % vegetable fat) for 21 days followed by a single intraperitoneal administration of streptozotocin (STZ) at a dose of 40 mg/kg. For further experiments, the rats with hyperglycemia were used, having blood glucose levels above 15 mM/L 11 days after STZ administration (group T2DM) [12]. Blood glucose level was measured using Bionime glucometer (BIONIME Rightest GM 300, Switzerland).

Operational preparation. The experiments were performed on animals under intraperitoneal chloralose-urethane anesthesia (1:10; based on 30 mg of urethane per 100 g of body mass). After a tracheostomy, the left jugular vein and the left common carotid artery were catheterized with Teflon catheters; heparin was administered in a dose of 50 units per 100 g of body weight. Given the complexity of pulmonary artery catheterization in small animals, the retrograde catheterization of the right ventricular cavity through the right jugular vein was performed. It is known that systolic pressure in the pulmonary trunk is fully consistent with pressure in the right ventricle, and its measurement is often used when studying the pulmonary circulation in both experimental and clinical settings [13].

Hypoxic hypoxia (HH) was caused by transferring the animals to respiration with a gas mixture containing 10 % O_2 and 90 % N_2. The duration of hypoxia was 30 min.

Registration and calculation of the primary cardiac and hemodynamic parameters.

A non-invasive measurement of systolic blood pressure (BPs) in the caudal artery was performed with S-2 sphygmomanometer (Hugo-Sachs Elektronik, Germany).

Blood pressure in the right ventricle (RVP) was measured with Millar's pressure microcatheter (Millar Instruments, USA), and in the left common carotid artery – with ISOTEC pressure sensors (HSE, Germany) using a DBA amplifier, type 660 (HSE, Germany). The data was digitized with analog-to-digital converters PowerLab 4/30 (AD Instruments; Australia) and ADC (HSE, Germany). Signal processing was performed using the Chart 5 program (AD Instruments, Australia).

Preparation of the thin slices of the lung tissue. The preparation was carried out according to the previously described method [14]. Under general anesthesia, thoracotomy was performed followed by extirpation of the heart and lungs *en bloc*. The heart and lungs were immersed in Hanks solution with following composition (mM/L): 1,26 $CaCl_2 \cdot 6H_2O$; 5,33 KCl; 0,44 KH_2PO_4; 0,8 $MgSO_4 \cdot 7\ H_2O$; 138 NaCl; 4,2 $NaHCO_3$; 0,3 Na_2HPO_4; 5,6 glucose. In an additional lobe of the lung, a section was made perpendicular to the distal part of the artery and transferred to a chamber with Hanks solution at 37 °C. The chamber was placed on the objective table of the inverted microscope Biolam P-1 (Lomo, Russia). The examinations were conducted at 100x magnification. On the section, the lumen of the lobar artery segment with a diameter of 100–200 microns was found. During the experiment, images were taken at regular intervals with a rate of 5 per min using a digital camera with a resolution of 5 megapixels (eTrek, China).

An ability of the intrapulmonary artery (iPA) to contract was determined using a 7-min reaction to Hanks solution containing 40 mM KCl (depolarizing solution). Further changes in the lumen area of the iPA segment were calculated as a percentage of amplitude of the maximum contraction in response to the depolarizing solution.

To create hypoxia, the chamber with lung tissue slices was perfused with Hanks solution preliminarily bubbled with a gas mixture containing O_2 1 %, CO_2 5 %, N_2 94 % for 20 min.

The solutions in the chamber were changed using peristaltic pump IPS (Ismatec, Switzerland) at a rate of 3,5 mL/min.

Calculation of iPA lumen area. The obtained images were processed in real time with Image J program (National Institutes of Health, USA) using the algorithm written in Image J Macro Language – the image was converted to 8-bit format (gray scale) and owing to the Canny-Deriche edge detector algorithm [15], the VLA lumen boundaries were determined with subsequent calculating the area of this lumen in pixels. Based on the obtained values, a graph was constructed and updated after each image taking.

A study of contractile activity of the pulmonary artery circular segments. Immediately after extraction from the chest, the lungs were placed in the cooled Krebs solution with the following composition (mM/L): 132 NaCl, 4,7 KCl, 1,4 NaH_2PO_4, 1,0 $MgCl_2$, 1,8 $CaCl_2$, 25 $NaHCO_3$, 6,5 glucose, pH 7,4. The iPA rings were placed in the flow chamber (4 ml) with Krebs solution (35 °C) and stretched on the metal hooks with preliminary load up to 0,5 g (5 mN). A contractile activity of the iPA rings was recorded in isometric mode using the tension sensors (FTK-0.1, C.K.K., Ukraine), and oxygen concentration in the chamber for a study of blood vessels was measured using E-5046 electrode (Radiometer, Denmark). The experimental data were recorded with LabTrax 4-CDA adapter (WPI, USA) and DataTrax 2 software (WPI, USA).

A study of the blood vessel responses was carried out according to the following two protocols:

1 – for the iPA rings, contraction to phenylephrine (PE) and relaxation to acetylcholine (Ach) were determined;

2 – a magnitude of the iPA rings reaction to decrease in oxygen content in Krebs solution was estimated. The iPA rings preliminarily contracted with PE ($3 \cdot 10^{-6}$ mM/L) were kept for 10 min in the oxygenated Krebs solution (pO_2 ~ 350 mm Hg) with subsequent 20-min perfusion with Krebs solution having low partial oxygen pressure (pO_2 ~ 1 mm Hg). The hypoxic solution was obtained by sparging the gas mixture with the following composition: CO_2 5 %, N_2 95 %.

Data analysis and statistical processing. Statistical analysis and graphic presentation of the study results were performed using the programs Exel (Microsoft, USA) and Statistica 8 (Stat Soft Inc., USA).

The distribution normality was evaluated with Shapiro-Wilk test. The data are presented as follows: as means ± standard errors of means (m ± SE) for the normal distribution, as medians and the first and third quartiles (Med, Q_{25} and Q_{75}) for the non-parametric distribution. The Wilcoxon test was used to compare the dependent samples. Multiple comparisons were performed using ANOVA Kruskal-Wallis test. The differences were considered statistically significant if p-value was less than 0,05.

Results and its discussion. The general characteristic of the type 2 diabetes mellitus model is shown in Table 1 and corresponds to the data presented previously [12].

In vivo studies. When measuring pressure in the right ventricular pressure (RVP), the significant differences were revealed between rats of different groups. In the control group rats RVP mean was (10,13 ± 0,54) mm Hg. In animals of the second and third groups, its values were twice as many: (20,58 ± 2,81) and (22,33 ± 2,69) mm Hg respectively (Tabl. 2).

The transfer of the control animals to breathing with the gas mixture with low oxygen content (10 %) led to the typical reaction of the circulatory system. Hypoxic hypoxia in rats of this group was accompanied by decrease in blood pressure in the systemic circulation and increase in pressure in the right ventricle cavity (Fig. 1, 2). It should be noted that increased pressure in the right ventricle cavity, as well as the hypotensive reaction on the part of the systemic circulation persisted throughout the period of hypoxia. The restoration of breathing by air with the normal oxygen content caused a gradual decrease in pressure in the right ventricle and increase in blood pressure in the carotid artery.

As in animals of the control group, in

Table 1

General characteristics of the diabetes model (m ± SE)

Parameter	Time period			
	Baseline (n = 60)	33 days		
		Control (n = 20)	High-fat diet (n = 20)	Type 2 diabetes mellitus (n = 18)
Body mass, g	187 ± 9	280 ± 7 *p < 0,001*	298 ± 7 *p < 0,001* *p1 > 0,05*	247 ± 6 *p < 0,001* *p1 < 0,01* *p2 < 0,001*
Glucose level, mM/L	5,6 ± 0,1	6,0 ± 0,2 *p > 0,05*	5,6 ± 0,2 *p > 0,05* *p1 > 0,05*	20,7 ± 0,9 *p < 0,001* *p1 < 0,001* *p2 < 0,001*
Systolic blood pressure, mm Hg	112,7 ± 1,6	123,5 ± 1,9 *p < 0,05*	127,3 ± 1,8 *p < 0,01* *p1 > 0,05*	128,2 ± 2,0 *p < 0,01* *p1 > 0,05* *p2 > 0,05*

Notes. p – compared to the baseline value, p1 – compared to the control group, p2 – compared to the HFD group.

Table 2

The effect of a high-fat diet and streptozotocin injection on changes in the right ventricular pressure (m ± SE)

Group	Right ventricular pressure, mm Hg
Control (n = 9)	10,13 ± 0,54
High-fat diet (n = 6)	20,58 ± 2,81
	p1 < 0,01
Type 2 diabetes mellitus (n = 11)	22,33 ± 2,96
	p1 < 0,01
	p2 > 0,05

Notes. Here and on Table 4: p1 – compared to the control group, p2 – compared to the HFD group.

rats kept on the high-fat diet and in rats with T2DM model, hypoxic hypoxia was accompanied by development of a hypotonic reaction in the systemic circulation. Blood pressure decreased by 15–20 % and remained at that level throughout the period of hypoxia. In animals of all groups, mean arterial pressur (MAP) values at the respective time intervals did not differ from each other (Fig. 1).

In rats of the second group, lung ventilation with hypoxic gas mixture was not accompanied by significant changes in pressure in the right ventricle cavity (Fig. 2).

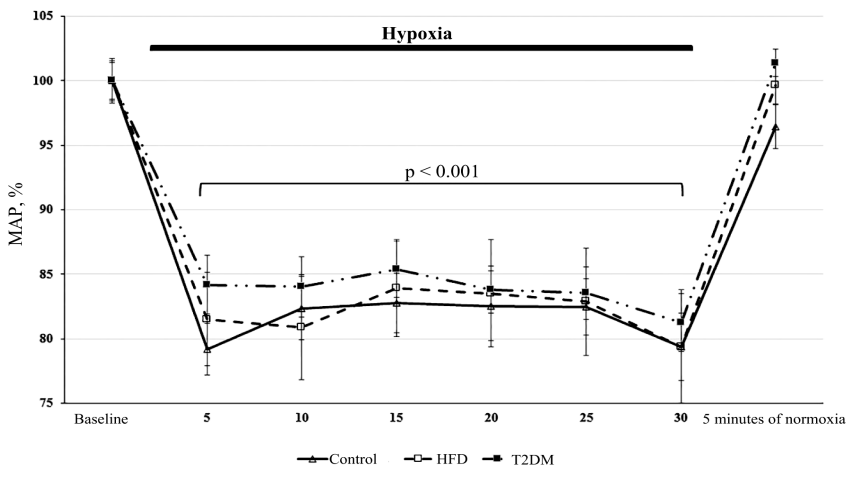

Fig. 1. Changes in pressure in the carotid artery under hypoxic hypoxia

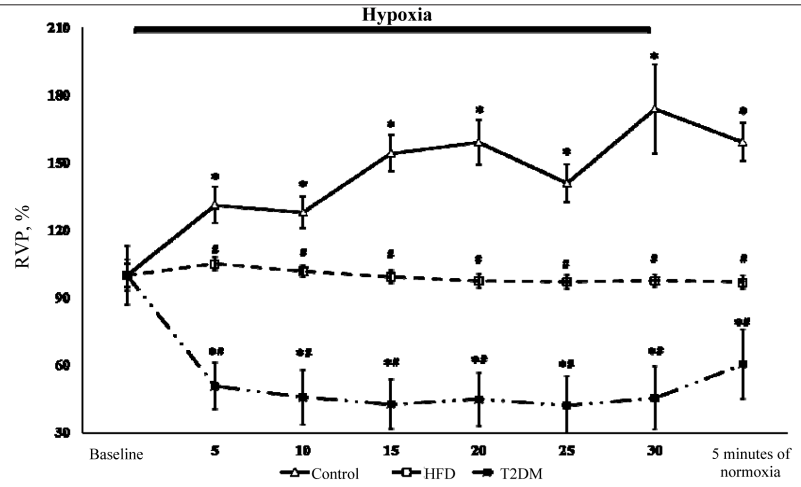

Fig. 2. Dynamics of pressure changes in the right ventricle of the heart during hypoxic hypoxia
*Notes. *p < 0,05 compared to the baseline, #p < 0,05 compared to the control group.*

In rats with T2DM model, the inversion of the hypoxic pulmonary vasoconstriction reaction was observed. Unlike animals in the control group, there was a decrease in pressure in the right ventricle cavity. Such a change in pressure occurred almost from the first minute of hypoxia and persisted throughout its period. Restoring ventilation with room air with the normal oxygen content led to a gradual return of pressure to its initial value (Fig. 2).

In vitro studies. Contractile activity of iPA on the lung sections

In the control group rats, perfusion of lung sections with hypoxic solution reduced iPA lumen by 25,6 %, which indicated the development of the reaction of hypoxic pulmonary vasoconstriction. In rats fed with HFD, hypoxic pulmonary vasoconstriction in response to hypoxia was maintained, but the lumen area decreased by only 8,2 %. In rats with T2DM model, perfusion of the lung sections with the hypoxic solution led to significant increase in the iPA lumen area (Tabl. 3, Fig. 3).

Such a change in iPA lumen under hypoxia on the lung sections of rats with the T2DM model indicates an inversion of HPV reaction, and it should be regarded as a disturbance of one of the fundamental adaptive reactions in the body.

Table 3

Changes in intrapulmonary artery lumen under hypoxia in type 2 diabetes mellitus group of rats (% of the maximal decrease lumen in area in response to Hanks solution with 40 mM/L KCl)

Group	m ± SE
Control (n = 17)	-25,64 ± 5,38
High-fat diet (n = 8)	-8,16 ± 1,55
	p1 < 0,05
Type 2 diabetes mellitus (n = 12)	20,78 ± 5,19
	p1 < 0,001
	p2 < 0,01

Notes. The symbol «-» means a decrease in the lumen area, p1 – compared to the control group, p2 – compared to the HFD group.

Contractile activity of the pulmonary artery circular segments. Given the absence of the normal distribution of the values obtained as a result of measurements in this series of experiments, we presented means and standard errors only in the text for a better perception of the data.

As can be seen in Figure 4, the vascular preparations of the intraorgan segments of the pulmonary artery preserved their functional activity. At the same time, the obtained results indicate a moderate decrease in the amplitude of the phenylephrine-induced contraction of the pulmonary artery segments iso-

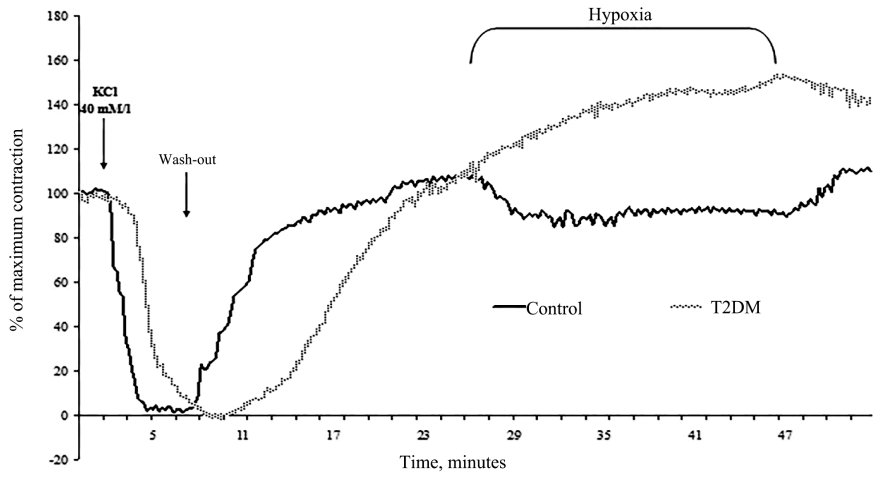

Fig. 3. Typical changes in lumen area of the intrapulmonary artery of a rat in the control group and a rat with type II diabetes

lated in rats with T2DM model in comparison with vessels of animals in HFD group.

A decrease in oxygen content in Krebs solution led to appearance of the typical reaction of PE-contracted pulmonary artery (PA) rings from all experimental groups. In the control group animals, the contraction of the vascular preparations averaged (47,1 ± 5,1) % of the maximum contraction was observed. The response of the vessels from rats kept on the high-fat diet was significantly less averaging (18,2 ± 4,5) %. In the group of animals with diabetes mellitus model, this reaction was practically absent, and its value was only (2,3 ± 0,9) % (Tabl. 4, Fig. 5).

The first demonstration of the structural changes in the pulmonary vasculature in diabetes was a study of 1984, which evaluated the angiogenesis in the lungs of rat fetuses with STZ-induced diabetes mellitus [16]. In fetuses obtained from females with diabetes, it was shown that the capillary network development is much less than in lungs of fetuses from rats in the control group, and it affects the efficiency of gas exchange in the lungs. A decrease in density of lung capillaries in the adult rats with STZ-induced DM has been shown by other researchers [17], but M. Pan et al. found a significant increase in vascular density in the lungs of mice with both an induced

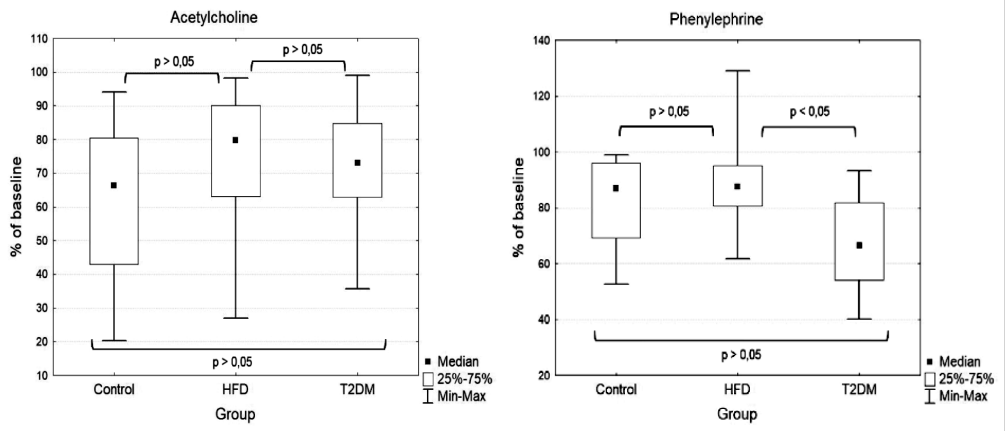

Fig. 4. Functional activity of the pulmonary artery vascular preparations under influence of the test substances

Notes. Left – relaxation in response to acetylcholine, right – contraction in response to phenylephrine.

Table 4

Contraction of intrapulmonary artery vascular preparations of rats with type 2 diabetes mellitus model under influence of hypoxia

Group	Median	Q_{25}	Q_{75}
Control	31,30	23,27	56,93
High-fat diet	6,71	2,38	23,41
	p1 < 0,001		
Type 2 diabetes mellitus	2,64	1,33	4,26
	p1 < 0,001		
	p2 < 0,01		

and genetically determined model of T2DM [18].

However, the changes caused by diabetes in the lungs are not limited to purely morphological ones. Lopez-Lopez et al. [19] showed a decrease in endothelium-dependent relaxation of the pulmonary arteries due to increased production of the reactive oxygen species (ROS) in the lungs of rats with STZ-induced type 1 diabetes. Excessive ROS production was the result of increased expression of p47phox (regulatory NADPH subunit) in rats with diabetes. Later, this group published another study devoted to investigation of the LA response to vasoconstrictors in rats with T1DM. The authors showed increased 5-HT2A receptor expression and increased cyclooxygenase metabolite levels, which led to increase in 5-HT-induced LA contraction in rats with T1DM [20].

Data on the development of peripheral endothelial dysfunction in the pulmonary vessels as an universally recognized characteristic of the peripheral diabetic macro- and microangiopathies are rather contradictory. Both the presence and the absence of impaired NO-dependent pulmonary endothelial function have been reported [19, 21–23]. Moral-Sanz et al. demonstrated that endothelium-dependent relaxation was not altered, and the contraction was significantly reduced in resistive PAs in obese Zucker rats compared to thin rats of the same line. The decrease in response to vasoconstrictors is explained by increased expression of inducible NO-synthase (iNOS) in PA in Zucker rats with obesity [24]. The downward trend in PA constricting responses is also confirmed by this study: there is a moderate decrease in PE-induced contraction of pulmonary artery segments isolated in rats with T2DM. At the same time, it is known that both endothelium-dependent and independent relaxation of LA in mice with T2DM is not changed compared to the control [18].

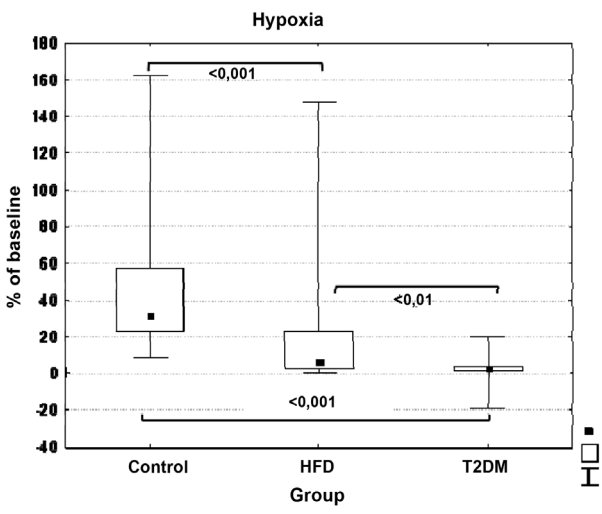

Fig. 5. Contractile reactions of the pulmonary artery rings in response to decrease in oxygen content

Researchers are more unanimous in that diabetes leads to impaired respiratory function of the lungs [22, 25, 26]. The structural changes in the airborne barrier and impaired *in vivo* diffusion have also been reported [27, 28]. It is noteworthy that under conditions of metabolic syndrome and diabetes, hyperglycemia itself increases vascular permeability due to increase in superoxide level in the vascular wall and perhaps due to the activity of NADPH-oxidase [29]. Another reason for the increased permeability of endotheliocyte membranes may be ET-1, since it increases permeability of the endothelial membrane [30] and increases adhesion of leukocytes [31]. This is confirmed by data on overexpression of ET-1, ET-A and ET-B receptors in lungs of rats with STZ-induced T1DM [32]. Several groups of researchers have shown increased adhesion of macrophages and leukocytes in the lung capillaries of animals with diabetes, suggesting that inflammation develops in the pulmonary vasculature [27, 33–36].

Hypoxia also actively affects the pulmonary blood flow. Alveolar hypoxia is a consequence of many diseases caused by inadequate alveolar ventilation, and is observed in pulmonary edema, acute respiratory distress-syndrome or chronic obstructive pulmonary diseases [37]. In this regard, metabolic syndrome and/or diabetes mellitus (DM) is no exception [5, 38–40]. There are few publications devoted to the study of the body's response to hypoxia in diabetes mellitus, and they mainly consider models of the prolonged hypoxia. In mice with a T2DM model compared to the control group animals, 4-week hypoxia led to increase in pressure in the right ventricle. This fact suggested a higher susceptibility of mice with diabetes to hypoxia. The explanation for this was an increase in ROS production by mitochondria and a decrease in superoxide dismutase content in mitochondria [19]. El-Habashy et al. observed a decrease in the forced expiratory volume in the first second, forced expiratory flow, peak expiratory flow and maximal oxygen consumption in patients with DM. Such disorders were more pronounced in cases of uncontrolled diabetes, which allowed the authors to consider the lungs as a target organ for DM [41]. In addition, STZ-induced diabetes exacerbates damage caused by hypoxia (8 % O_2 for 12 h) due to enhancing the inflammation reactions [42].

The most discussed topic is the relationship of diabetes mellitus, metabolic syndrome, insulin resistance with dyslipidemia and PH. For example, R. T. Zamanian et al. published the first clinical report on the relationship between insulin resistance and PH [43]. It is believed that patients with DM are at increased risk of PH and pulmonary embolism [5, 44]. It has been shown that DM is an independent factor in PH development in patients with chronic obstructive pulmonary disease and interstitial pneumonia [38, 45]. The experimental data generally confirm the existence of such a relationship [17, 46]. This study also revealed a significant increase in pressure in the right ventricle of the heart of rats on a HFD and with T2DM model compared to the pressure in rats of the control group. On the other hand, between rats with T1DM model and animals of the control group, there were no significant differences in this parameter values [20, 34]. In our previous work, when simulating T1DM, the pressure in the right ventricle of the heart was also significantly lower than in animals of the respective control group [47]. Such conflicting results may be due to different models of DM and different species of animals.

Our data presented in this article indicate an inversion of response in rats with T2DM model. This phenomenon was observed both at the whole organism level, and on the pulmonary vessel preparations. A probable explanation of this fact may be a change in the pathway of glucose metabolism in PA of animals with DM under hypoxia influence. It was shown that in healthy animals, selective glycolysis blockade may change this reaction from constriction to dilatation [48]. The transition from oxidative to glycolytic metabolism during hypoxia is provided with HIF-1 due to both inhibiting the conversion of pyruvate to acetyl

coenzyme A or lactate [49, 50], and increasing efficiency of the electron transfer when switching subunits in cytochrome c oxidase [51]. It is known that in hyperglycemia, dysfunction of this factor is observed [52–54], and it can lead to disturbances in the metabolic and therefore contractile responses of smooth muscle cells to hypoxia.

When analyzing the results of the study, some differences were found in PA reaction *in vitro*: inversion of HPV reaction was observed on the lung sections, and only the absence of contraction – on the pulmonary artery rings. It can be assumed that such an inversion of the reaction depends on the presence of tissues surrounding PA. It is known that bronchi can enhance relaxation of the nearest arteries. It was found in newborn rats, that bronchial epithelium produces a factor that can reduce the muscle tone of the pulmonary vessels [55]. A detailed study of the role of various lung tissues in regulation of PA tone would make more grounded conclusions.

Conclusion

Thus, in T2DM, there is an increase in pressure in the right ventricle of the heart of both rats on a HFD and rats with T2DM model, as well as the absence of HPV reaction in animals of the HFD group and inversion of the reaction to hypoxia in rats with T2DM model. This is confirmed by the results obtained in the experiments *in vitro*. Unfortunately, the available data do not allow us to judge, with an acceptable degree of certainty, about the possible mechanisms of the inversion of HPV reaction in DM.

Finding out the exact mechanisms involved in the inversion of the hypoxic pulmonary vasoconstriction response in diabetes mellitus will contribute to a better understanding of the fundamental pathophysiological processes that will promote development of pathogenetically justified approaches to the treatment of this disease and its complications.

Conflict of interest
The authors declare no conflict of interests.

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Effect of type 2 diabetes mellitus on the development of hypoxic pulmonary vasoconstriction

Hypoxia is currently considered to be an universal factor involved in the development of complications in many diseases including diabetes mellitus (DM). Acute hypoxia causes a systemic reaction with the large circulatory system developing vasodilation and the lesser circulatory system arteries developing hypoxic pulmonary vasoconstriction (HPV), which demonstrates the arteries' ability to regulate pulmonary circulation and maintain normal ventilation-perfusion ratio. Lack of the adequate response to hypoxic effects may be an additional mechanism for the diabetic complications in the cardiovascular system. Despite years of study, the mechanisms of HPV remain largely unclear, as does the effect of diabetes on pulmonary artery reactivity.

The aim of the study was to evaluate the effect of high-fat diet and diabetes on the development of the HPV reaction *in vivo* and *in vitro*.

Studies in rats divided into three groups (control group, a group of rats on a high-fat diet (HFD), and a group of animals with experimental type 2 diabetes mellitus (T2DM)) showed that in rats kept on the high-fat diet, an injection of streptozotocin leads to significant increase in blood glucose levels. In rats kept on the high-fat diet only, this parameter did not differ from the levels in the control animals. In rats kept on HFD and in rats with T2DM model, the right ventricular pressure was significantly higher compared to the control rats.

The lung ventilation with a hypoxic gas mixture led to decrease in blood pressure in the systemic circulation in animals of all groups. In the control rats, the pressure in the right ventricular cavity of the heart increased indicating the development of hypoxic pulmonary vasoconstriction. In HFD rats, hypoxic hypoxia had virtually no effect on the right ventricular pressure. In T2DM rats, an inversion of the hypoxic pulmonary vasoconstriction reaction with decrease in the right ventricular pressure was observed.

These results have been confirmed in the *in vitro* experiments. In lung sections, the responses of the intrapulmonary arteries to hypoxic effects were multi-directional: the pulmonary artery luminal area

decreased in the control animals, almost did not change in HFD animals, and increased in T2DM animals. In the rats with experimental diabetes mellitus, the contractile responses of the pulmonary artery ring segments to hypoxia were significantly suppressed compared to the vessels from the control animals.

Finding out the exact mechanisms involved in the inversion of the pulmonary vasoconstriction response in diabetes will contribute to a better understanding of the fundamental pathophysiological processes that will promote development of pathogenetically justified approaches to the treatment of this disease and its complications.

Key words: diabetes mellitus, intrapulmonary arteries, hypoxia, hypoxic pulmonary vasoconstriction

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Вплив цукрового діабету 2-го типу на розвиток гіпоксичної легеневої вазоконстрикції

Гіпоксія наразі вважається універсальним фактором, залученим до розвитку ускладнень при багатьох захворюваннях, включаючи цукровий діабет (ЦД). Гостра гіпоксія викликає системну реакцію організму, за якої у великому колі кровообігу розвивається дилатація судин, а в артеріях малого кола кровообігу виникає гіпоксична легенева вазоконстрикція (ГЛВ), яка демонструє здатність артерій до регуляції легеневого кровообігу для підтримки нормального вентиляційно-перфузійного співвідношення. Відсутність адекватної реакції на гіпоксичний вплив може стати ще одним механізмом розвитку діабетичних ускладнень у серцево-судинній системі. Незважаючи на багаторічні дослідження, механізми ГЛВ залишаються майже не з'ясованими, як і вплив ЦД на реактивність легеневих артерій.

Мета дослідження – оцінити вплив споживання їжі з високим вмістом жирів і ЦД 2-го типу (ЦД2) на розвиток реакції ГЛВ в умовах *in vivo* та *in vitro*.

Дослідження на щурах, яких було розподілено на 3 групи (контрольна група, група щурів, що перебували на високожировій дієті (ВЖД), та група тварин, у яких викликали ЦД2), показали, що введення стрептозотоцину щурам, які утримуються на дієті з високим вмістом жирів, призводить до значного збільшення вмісту глюкози в крові. У щурів, яких тільки утримували на ВЖД, цей показник не відрізнявся від величини у тварин контрольної групи. Величина тиску в правому шлуночку серця щурів, що перебували на ВЖД, та в щурів з моделлю ЦД2 була достовірно вищою за цю величину в щурів контрольної групи.

Вентиляція легень гіпоксичною газовою сумішшю призводила до зниження тиску крові у великому колі кровообігу в тварин усіх груп. У щурів контрольної групи тиск у порожнині правого шлуночка серця підвищувався, що свідчило про розвиток гіпоксичної легеневої вазоконстрикції. У щурів групи ВЖД гіпоксична гіпоксія практично не впливала на тиск у порожнині правого шлуночка серця. У щурів з моделлю ЦД2 спостерігалася інверсія реакції гіпоксичної легеневої вазоконстрикції зі зниженням тиску в порожнині правого шлуночка.

Ці результати підтверджувалися дослідами в умовах *in vitro*. На зрізах легень відповідь внутрішньолегеневих артерій на гіпоксичний вплив була різнонаправленою: у тварин контрольної групи площа просвіту артерії зменшувалася, у тварин, що перебували на ВЖД, площа просвіту артерії майже не змінювалася, а у тварин з моделлю ЦД2 відбувалося її збільшення. Скоротливі реакції кільцевих сегментів легеневої артерії на гіпоксію в щурів з моделлю ЦД2 були значно пригніченими порівняно з судинами тварин контрольної групи.

З'ясування точних механізмів, залучених до інверсії реакції легеневої вазоконстрикції за ЦД, дозволить краще зрозуміти фундаментальні патофізіологічні процеси, що сприятиме розробці патогенетично обґрунтованих підходів до лікування цього захворювання та його ускладнень.

Ключові слова: цукровий діабет, внутрішньолегеневі артерії, гіпоксія, гіпоксична легенева вазоконстрикція

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Влияние сахарного диабета 2-го типа на развитие гипоксической легочной вазоконстрикции

Гипоксия в настоящее время считается универсальным фактором, вовлеченным в развитие осложнений при многих заболеваниях, включая сахарный диабет (СД). Острая гипоксия вызывает системную реакцию организма, при которой в большом круге кровообращения развивается дилатация сосудов, а в артериях малого круга возникает гипоксическая легочная вазоконстрикция (ГЛВ), которая демонстрирует способность артерий к регуляции легочного кровообращения для поддержания нормального вентиляционно-перфузионного соотношения. Отсутствие адекватной реакции на гипоксическое воздействие может стать еще одним механизмом развития диабетических осложнений в сердечно-сосудистой системе. Несмотря на многолетние исследования, механизмы ГЛВ остаются почти не невыясненными, как и влияние СД на реактивность легочных артерий.

Цель исследования – оценка влияния потребления пищи с высоким содержанием жиров и СД 2-го типа (СД2) на развитие ГЛВ в условиях *in vivo* и *in vitro*.

Исследование проведенное на крысах, которые были распределены на три группы (контрольная группа, группа крыс, находившихся на высокожировой диете (ВЖД), и группа животных, у которых вызвали СД2), показали, что введение стрептозотоцина крысам, содержащившимся на диете с высоким содержанием жиров, приводит к значительному увеличению содержания глюкозы в крови. У крыс, которые находились только на ВЖД, этот показатель не отличался от величины у животных контрольной группы. Величина давления в правом желудочке сердца крыс, находившихся на ВЖД, и у крыс с моделью СД2 была достоверно выше его величины у крыс контрольной группы.

Вентиляция легких гипоксической газовой смесью приводила к снижению давления крови в большом круге кровообращения у животных всех групп. У крыс контрольной группы давление в полости правого желудочка сердца повышалось, что свидетельствовало о развитии гипоксической легочной вазоконстрикции. У крыс группы ВЖД гипоксическая гипоксия практически не влияла на давление в полости правого желудочка сердца. У крыс с моделью СД2 наблюдалась инверсия реакции гипоксической легочной вазоконстрикции – давление в полости правого желудочка снижалось.

Эти результаты подтверждались исследованиями в условиях *in vitro*. На срезах легких ответ внутрилегочной артерии на гипоксическое воздействие был разнонаправленным: у животных контрольной группы площадь просвета артерии уменьшалась, у животных группы ВЖД площадь просвета артерии почти не менялась, а у животных с моделью СД2 происходило ее увеличение. Сократительные реакции кольцевых сегментов легочной артерии на гипоксию у крыс с моделью СД2 были значительно подавленными по сравнению с сосудами животных контрольной группы.

Выяснение точных механизмов, вовлеченных в инверсию реакции легочной вазоконстрикции при сахарном диабете, позволит лучше понять фундаментальные патофизиологические процессы, способствовать разработке патогенетически обоснованных подходов к лечению этого заболевания и его осложнений.

Ключевые слова: сахарный диабет, внутрилегочные артерии, гипоксия, гипоксическая легочная вазоконстрикция

Author contributions
O. S. Khromov, N. V. Dobrelia, O. V. Parshikov contributed to the conception and design of the experiments, collection, analysis and interpretation of data, drafting the article or revising it critically for important intellectual content. L. V. Boytsova contributed to the conception and design of experiments. All authors approved the final submitted version. All experiments were carried out in the Department for Pharmacology of Cellular Signaling Systems and Experimental Therapeutics, Institute of Pharmacology and Toxicology, National Academy of Medical Sciences of Ukraine, Kyiv.

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