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Left ventricular remodeling in normotensive Wistar rats exposed to intermittent hypoxia of different duration

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

Intermittent hypoxia has been studied for many years as a promising non-pharmacological method of cardiovascular disease prevention. Hypoxic effects are accompanied by structural and functional changes in the myocardium. There is a direct link between the duration of hypoxic exposures and the severity of left ventricular myocardial remodeling. A range of histochemical markers of myocardial remodeling (cardiotrophin-1, titin, collagen type 1, annexin V) characterizing parenchymal-stromal relationships in the myocardium has shown high informativeness and prognostic value.

The aim of the study was to examine cardiotrophin-1, titin, collagen type 1, annexin V and the morphofunctional state of the left ventricle of the heart in experimental rats exposed to intermittent 15-day (IH15) and 60-day hypoxia (IH60).

Materials and methods. Intermittent hypoxia was modeled using 30 normotensive male Wistar rats, 7–8 months old, which were randomly assigned to 3 experimental groups of 10 animals each: 1) INT – a control group – intact animals (196.3 ± 6.8 g); 2) IH15 – 15-day hypoxia (205.6 ± 4.1 g); 3) IH60 – 60-day hypoxia (201.1 ± 5.5 g). The study compared the effects of intermittent hypoxia of varying duration: 15-day and 60-day hypoxia. Experimental modeling of intermittent hypoxia of 2 terms revealed a number of differences between the effects dependent on this factor duration through functional (blood pressure measurement, echocardiography) and immunofluorescent studies.

Results. Blood pressure in rats of both groups was in the normotensive range, but an increase in systolic by 10 % and diastolic by 19 % was found in IH60 group compared to IH15 group ($p < 0.05$). In IH15 group, there was a significant decrease in end-diastolic dimension by 20 %, end-systolic dimension by 22 %, an increase in the thickness of left ventricular posterior wall by 44 % and interventricular septum by 33 % as well as left ventricular mass by 12 %, indicating concentric remodeling of the left ventricle, the development of which was confirmed by a 76 % increase in relative wall thickness compared to that in the control group ($p < 0.05$). Along with these changes, a decrease in end-diastolic volume by 47 %, end-systolic volume by 48 %, stroke volume by 49 % and cardiac output by 50 % with preserved ejection fraction was revealed ($p < 0.05$). While the parameters of IH60 rats were characterized by an increase in the thickness of interventricular septum by 33 % and left ventricular posterior wall by 17 %, as well as left ventricle mass by 23 %, relative left ventricular wall thickness was 15 % higher than the control value ($p < 0.05$). At the same time, diastolic volume was 9 % decreased and systolic volume was 24 % increased ($p < 0.05$). Also, cardiac output was increased by 58 % compared to that in 15-day hypoxic rats with an 8 % decrease in ejection fraction ($p < 0.05$). The concentrations of markers in IH60 group exceeded those in IH15, namely: cardiotrophin-1 by 39 %, titin by 70 %, collagen type 1 by 60 % and annexin V by 130 % ($p < 0.05$).

Conclusions. 15-day hypoxia forms concentric left ventricular hypertrophy according to echocardiography findings; the study of marker profile of myocardial remodeling has revealed the development of moderate hypertrophy with increased resilient-elastic properties and decreased intensity of cardiomyocyte death. Remodeling caused by 60-day hypoxia is characterized by the eccentric pattern of changes with severe hypertrophy, significant fibrosis associated with apoptosis of cardiomyocytes. Such morphofunctional state of the myocardium may indicate the initial stages of maladaptation, increasing the risk of heart failure development.

Key words: myocardial remodeling, left ventricular, markers of myocardial remodeling, cardiotrophin-1, intermittent hypoxic hypoxia, rats.

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Ремоделювання лівого шлуночка в нормотензивних щурів лінії Вістар, які зазнали інтермітуючої гіпоксії різної тривалості

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Переривчасту гіпоксію вже багато років вивчають як перспективний метод немедикаментозної профілактики серцево-судинних захворювань. Гіпоксичні впливи супроводжуються структурно-функціональними змінами в міокарді. Виявили прямий зв'язок між тривалістю гіпоксичних експозицій і вираженістю ремоделювання міокарда лівого шлуночка. Високу інформативність і прогностичну цінність показав комплекс гістохімічних маркерів ремоделювання міокарда (кардіотрофіну-1, тайтину, колагену 1 типу, анексину V), що характеризує паренхіматозно-стромальні зв'язки в міокарді.

Мета роботи – вивчення маркерів кардіотрофіну-1, тайтину, колагену 1 типу, анексину V та морфофункціонального стану лівого шлуночка серця в експериментальних щурів при впливі переривчастої 15- (ІН15) та 60-денної гіпоксії (ІН60).

Матеріали та методи. Для моделювання інтермітуючої гіпоксії використовували 30 нормотензивних щурів-самців лінії Вістар віком 7–8 місяців, яких випадковим чином поділили на 3 експериментальні групи по 10 тварин у кожній: INT – контрольна група – інтактні тварини (196,3 ± 6,8 г); ІН15 – 15-добова гіпоксія (205,6 ± 4,1 г); ІН60 – 60-денна гіпоксія (201,1 ± 5,5 г). Порівняли наслідки періодичної гіпоксії різної тривалості (15- та 60-денної). Експериментальне моделювання переривчастої гіпоксії 2 термінів виявило низку відмінностей між ефектами, що залежать від

Ключові слова: ремоделювання міокарда, лівий шлуночок, маркери ремоделювання міокарда, кардіотрофін-1, переривчаста гіпоксія, щури.

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тривалості цього фактора, за допомогою функціональних (вимірювання артеріального тиску (АТ), ехокардіографія) та імунофлуоресцентних досліджень.

Результати. АТ у щурів обох дослідних груп відповідав нормотензивному діапазону, виявили збільшення систолічного (на 10 %) і діастолічного (на 19 %) тиску в групі ІН60 порівняно з ІН15 ($p < 0,05$). У групі ІН15 зареєстрували достовірне зменшення кінцевого діастолічного розміру (на 20 %), кінцевого систолічного розміру (на 22 %), збільшення товщини задньої стінки лівого шлуночка (на 44 %), міжшлуночкової перетинки (на 33 %) та збільшення маси лівого шлуночка (на 12 %). Це свідчить про концентричне ремоделювання лівого шлуночка, виникнення якого підтверджене збільшенням індексу відносної товщини стінки на 76 % порівняно з контрольною групою ($p < 0,05$). На тлі цих змін виявили зменшення кінцевого діастолічного об'єму (на 47 %), кінцевого систолічного об'єму (на 48 %), ударного об'єму (на 49 %) та серцевого викиду (на 50 %) зі збереженою фракцією викиду ($p < 0,05$). Показники щурів ІН60 характеризувалися збільшенням міжшлуночкової перетинки (на 33 %), задньої стінки лівого шлуночка (на 17 %) і збільшенням маси лівого шлуночка серця (на 23 %), а індекс відносної товщини стінки лівого шлуночка був більшим, ніж у контролі (на 15 %, $p < 0,05$). Встановили зниження кінцевого діастолічного об'єму на 9 %, що супроводжувалось збільшенням систолічного об'єму на 24 % ($p < 0,05$). Серцевий викид також збільшений порівняно з щурами з 15-денною гіпоксією на 58 % зі зниженням фракції викиду на 8 % ($p < 0,05$). Показники концентрації маркерів групи ІН60 перевищували ІН15: кардіотрофін-1 – на 39 %, тайтин – 70 %, колаген 1 типу – на 60 %, анексин V – на 130 % ($p < 0,05$).

Висновки. За даними ехокардіографії, 15-денна гіпоксія формує концентричну гіпертрофію лівого шлуночка серця, маркерна характеристика ремоделювання міокарда виявила розвиток помірної гіпертрофії з підвищенням пружно-еластичних властивостей і зниження інтенсивності загибелі кардіоміоцитів. Ремоделювання, спричинене 60-денною гіпоксією, характеризується ексцентричною спрямованістю змін із вираженою гіпертрофією, значним фіброзом, що асоційований з апоптозом кардіоміоцитів. Такий морфофункціональний стан міокарда свідчить про початкові етапи дезадаптації, що підвищують ризик серцевої недостатності.

Intermittent hypoxia is studied as a promising method of non-pharmacological prevention of cardiovascular diseases. Its cardioprotective effect, that increases myocardial resistance to acute and chronic ischemic damage, ischemia-reperfusion, toxic effects and many other influences is considered [1,2]. Hypoxic influences are accompanied by structural and functional changes in myocardium – its remodeling. The nature and severity of this process depends on many parameters: exposure time, oxygen concentration, gas-transport systems and substrate-enzyme interactions [3].

The efficiency of hypoxic training is determined by the pattern of myocardial remodeling, the degree of its expression and the direction of cardiac geometry transformations [4]. The complex of histochemical markers of myocardial remodeling (cardiotrophin-1, titin, collagen type 1, annexin V), quantitatively characterizing parenchymatous-stromal interrelations in myocardium, has demonstrated high informative and prognostic value. Cardiotrophin-1 provides the first line of myocardium defense by promoting cell survival and proliferation, it also possesses hemodynamic and endocrine properties. At the same time, overexpression of this cytokine leads to pathological hypertrophy, therefore it is considered as a marker of cardiovascular diseases [5].

Fibrosis is an important component of remodeling; it can develop because of extracellular space expansion (reactive) and/or replacement of cells that died by apoptosis (replacement fibrosis). The ratio of titin to type 1 collagen can be considered a prospective approach to assessing the severity of fibrosis. Titin is a protein maintaining sarcomere integrity by modeling passive tension, changing its conformation, isoform balance, as well as interacting with other myofilament components [6]. Physiological role of collagen is to provide mechanical power of myocardial matrix as well as the ability to transmit force generated by cardiomyocytes. In pathological conditions, its accumulation promotes differentiation and proliferation of myofibroblasts, forming a "vicious circle" – increasing collagen synthesis [7].

The marker of molecular imaging of cardiomyocyte apoptosis is annexin V due to its high affinity for phosphatidylserine [8]. High invasiveness of endomyocardial biopsy limits wide use of this technique in patients. The distinctive feature of the above-named markers is the possibility to detect them in blood plasma, that makes this method available for wide use in clinical practice. Previous studies have shown a high correlation between the plasma concentrations of the presented markers and their expression in the myocardium.

For example, in a study by Asparuh Nikolov and Nikola Popovski, collagen type 1 is considered as an indicator of myocardial fibrosis and prognostic marker for heart failure [9]. At the same time, experimental study on hypoxia effect of different duration will allow to ascertain the nature of morpho-structural and hemodynamic changes directly in myocardium. Thus, the aim of the study was to examine cardiotrophin-1, titin, collagen type 1, annexin V and morphofunctional state of the left heart ventricle in experimental rats exposed to intermittent 15-day and 60-day hypoxia.

Materials and methods

The study was conducted at the Laboratory of Experimental Pathophysiology (license 2CK2 YMK2 T6PB SG5N SJLS4) in the Training Scientific Medical and Laboratory Center with a vivarium at Zaporizhzhia State Medical University (certificate of technical competence of the Ministry of Health of Ukraine No. 033/18 dated December 25, 2018, valid until December 25, 2023).

Intermittent hypoxia was modeled using 30 normotensive male Wistar rats, 7–8 months old, which were randomly assigned to 3 experimental groups of 10 animals each: 1) INT – a control group – intact animals (196.3 ± 6.8 g); 2) IH15 – 15-day hypoxia (205.6 ± 4.1 g); 3) IH60 – 60-day hypoxia (201.1 ± 5.5 g).

An experimental part of the study was carried out in strict accordance with the national "General Ethical

Principles of Animal Experiments" (Ukraine, 2001) aligned with the provisions of the "European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes" (Strasbourg, 1985), "Provisions on the Use of Animals in Biomedical Research" and the Directive of the European Parliament and the Council of the European Union 2010/63/EU of September 22, 2010 on the protection of animals used for scientific purposes [10].

The study compared the effects of intermittent hypoxia of varying duration: 15-day and 60-day hypoxia [11–12].

Hypoxic training was carried out in a 1.0 m^3 ventilated pressure chamber at an "altitude" of 6000 m measured using an altimeter (pO_2 = 9.8 %). The animals were placed in a pressure chamber daily from 10 am to 4 pm. Hypoxic exposures were conducted in the following mode (*Table 1*) [13,14].

To obtain correct blood pressure (BP) measurement data using Blood Pressure Analysis System TM BP-2000 Series II (Visitech Systems, USA), the rats underwent a two-week adaptation to immobilizing protected tunnels. The measurement was done using a tail-cuff technique in total silence. The first measurement of BP was carried out at the stage of group formation and then according to the needs of the experiment, namely, in rats exposed to intermittent hypoxia of IH15 group – on the 1st and 15th day, and of group IH60 – on the 1st and 60th day. After a series of BP measurements (at least 7–10 BP readings with an interval of 1.5–2.0 minutes), mean BP was calculated automatically by the device according to the formula:

MBP = diastolic BP + 1/3 [systolic BP – diastolic BP].

The chest of the rats was shaved prior to cardiac ultrasound examination. Telazol was chosen as an anesthetic agent (composition of 1 bottle: tiletamine hydrochloride – 250 mg, zolazepam hydrochloride – 250 mg), which has no cardiodepressant effect. The study was conducted in the position of the animal on its back with the forelimbs fixed, as well as on the left side. A Vivid E9 scanner (GE Healthcare, USA) with a high-frequency linear matrix transducer ML6-15-D (scanning frequency of 15 MHz) was used after applying an ultrasound gel. Cardiohemodynamic parameters were calculated using the "Rodent" package (GE Healthcare, USA), recommended for small objects with a high heart rate. The correctness of the ultrasound beam positioning perpendicularly to the examined structures was verified using "anatomical" M-mode. The short axis view of the left ventricle (LV) at the level of papillary muscles enabled standard measurements of the interventricular septum (IVS) and LV posterior wall (LVPW) thickness, end-systolic (ESD) and end-diastolic dimensions (EDD) and calculation of LV ejection fraction (EF) and fractional shortening (FS). The study was performed in the B-mode grey-scale imaging in the parasternal and apical views.

The following parameters were calculated:

1. Left ventricular mass (g) = [0.8 × (1.04 × (IVSd + + LVIDd +LVPWd) × 3 – (LVIDd) × 3) + 0.14]/1000;
2. Diastolic volume (mL) = 1.047 × (LVIDd) × 3;
3. Systolic volume (mL) = 1.047 × (LVIDs) × 3;
4. Stroke volume (mL) = diastolic volume – systolic volume;

Table 1. Hypoxic exposure mode

Day of experiment		Altitude, m
IH15	IH60	
1		1000
2		2000
3		3000
4		4000
5		5000
6		6000
7–15	7–60	6000

5. Cardiac output (mL/min) = stroke volume × heart rate, where IVS, LVID and LVPW are all in mm;
6. Relative wall thickness = 2 × LVPWd / LVIDd [15].

The parameters were averaged over three consecutive measurements.

The animals were anesthetized before euthanasia using thiopental (45 mg/kg body weight). The objects of study were LV myocardium fragments of the experimental animals. Serial sections were prepared from standardly histologically fixed, paraplast-embedded material. The immunoreactive material (IRM) concentrations (fluorescence units / micrometer^2 (Uif/μM^2)) to the markers of remodeling were analyzed using immunofluorescent detection following the manufacturer's instructions.

To detect cardiotrophin-1 (CT), polyclonal goat anti-CT antibodies (N-20): sc-20867 were used, type I collagen (CL) – primary polyclonal goat anti-COL1A1 antibodies (C-18): sc-8784, anexin V (AN) – goat polyclonal anti-Annexin V antibodies (R-20): sc-1929. FITC-conjugated anti-goat mouse IgG secondary antibodies (sc-2356) were used to the above-described markers. To study titin (TT), primary mouse monoclonal anti-Titin (E-2): sc-271946 antibodies and FITC-conjugated anti-rabbit mouse IgG secondary antibodies (sc-2359) were used. All antibodies manufactured by Santa Cruz Biotechnology, Inc. were used at a 1:200 dilution.

To assess the severity of fibrosis degree in rats of the experimental groups, the titin / collagen ratio was calculated.

IRM in myocardial sections was quantified in the ultraviolet excitation spectrum of 390 nm with a high-emission 38HE light filter (Carl Zeiss, Germany) using an AxioImager-M2 microscope (Carl Zeiss, Germany) with the AxioVision 40 V 4.8 software 2.0 (license No. 3005339). When analyzing images in an interactive mode, zones with statistically significant fluorescence were captured. At least 100 visual fields in each series were subjected to study. The files were then analyzed using ImageJ software (National Institutes of Health, USA).

Statistical processing was performed using single-factor analysis of variance (ANOVA) in the program Statistica (License No. JPZ804I382130ARCN10-J). All continuous variables were tested for a normal distribution using the Shapiro–Wilk's W test. Continuous variables are presented as mean ± standard error mean or median (interquartile range) if non-normally distributed. All normally distributed parameters were compared using a one-way ANOVA, followed, in case of significance, by a two-side Tukey test for multiple comparisons. Differences in non-normally distributed variables between groups were assessed by

Table 2. Blood pressure in rats of experimental groups, M ± m

	Day	Systolic BP, mmHg	Diastolic BP, mmHg	Mean BP, mmHg
Intact	1-day, 15-day and 60-day averages	115.0 ± 1.8	68.1 ± 1.2	83.8 ± 0.9
IH15	1	114.6 ± 1.2	67.5 ± 2.0	83.2 ± 1.3
	15	123.4 ± 2.1*	69.3 ± 1.9	87.3 ± 1.4*
IH60	1	113.2 ± 1.1	65.8 ± 1.5	81.6 ± 0.9
	60	135.1 ± 1.7*#	82.6 ± 3.2*#	92.4 ± 3.5*

*: significant difference in the parameters of the experimental groups ($p < 0.05$) compared to the corresponding parameters of the control group; #: significant difference in IH60 parameters ($p < 0.05$) compared to the corresponding IH15 parameters.

Table 3. Echocardiographic assessment of rats

Parameter, units of measurement	INT	IH15	IH60
EDD (LVIDd, mm)	6.1 (6.0; 6.1)	4.90 (4.6; 5.1)*	5.9 (5.6; 6.0)#
ESD (LVIDs, mm)	3.2 (3.0; 3.3)	2.5 (2.2; 2.8)*	3.4 (3.3; 3.4)#
IVS (IVSd, mm)	1.5 (1.5; 1.6)	2.0 (1.8; 2.2)*	2.0 (1.9; 2.1)*
LVPW (LVPWd, mm)	1.8 (1.7; 1.9)	2.6 (2.3; 2.7)*	2.1 (1.9; 2.1)*#
EF, %	84.0 (84.0; 84.0)	84.5 (80.5; 89.0)	77.5 (75.3; 80.8)*#
FS, %	47.0 (46.3; 47.8)	50.0 (46.5; 53.5)*	39.5 (38.0; 41.8)*#
HR, bpm	478 (471; 483)	464 (437; 475)*	456 (435; 464)*#
Relative wall thickness	0,59 (0,57; 0,62)	1,04 (0,93; 1,11)*	0,68 (0,64; 0,74)*#
Left ventricular mass, g	0.514 (0.497; 0.526)	0.575 (0.509; 0.674)*	0.631 (0.603; 0.657)*
Diastolic volume, mL	0.232 (0.226; 0.238)	0.123 (0.104; 0.137)*	0.210 (0.186; 0.226)*#
Systolic volume, mL	0.033 (0.029; 0.038)	0.017 (0.011; 0.023)*	0.041 (0.038; 0.041)*#
Stroke volume, mL	0.199 (0.195; 0.203)	0.101 (0.091; 0.112)*	0.172 (0.144; 0.188)*#
Cardiac output, mL/min	95.6 (93.8; 97.2)	47.5 (43.0; 51.2)*	75.1 (67.1; 83.8)*#

*: significant difference in the parameters of the experimental groups ($p < 0.05$) compared to the corresponding parameters of the control group; #: significant difference in IH60 parameters ($p < 0.05$) compared to corresponding IH15 parameters.

Table 4. Concentration of markers, Uif/μm^2

Parameter	Intact	Hypoxia 15	Hypoxia 60
Cardiotrophin-1	0.09 (0.08; 0.10)	0.10 (0.09; 0.13)*	0.15 (0.14; 0.17)*#
Titin	0.08 (0.07; 0.09)	0.09 (0.08; 0.11)*	0.15 (0.13; 0.18)*#
Collagen type I	0.09 (0.07; 0.11)	0.10 (0.08; 0.11)	0.15 (0.13; 0.18)*#
Annexin V	0.09 (0.08; 0.11)	0.08 (0.07; 0.09)*	0.18 (0.14; 0.23)*#
Titin/collagen ratio	0.85	0.91	1.03

*: significant difference in the values of the experimental groups ($p < 0.05$) compared to the corresponding values of the control group; #: significant difference in IH60 values ($p < 0.05$) compared to corresponding IH15 values.

Kruskal–Wallis test with post hoc Mann–Whitney test. A two-sided p-value <0.05 was considered statistically significant for all the tests.

Results

BP in rats of the experimental groups was in the normotensive range for all measurements and no statistically significant changes were found between intermediate measurements (a concept of normotension was taken from normal BP in rats [16]). But on days 15 and 60, significant differences were revealed between the parameters, namely, an increase in systolic BP by 9.5 % and diastolic BP by 19.2 %, respectively, in IH60 group compared to those in IH15 group (*Table 2*).

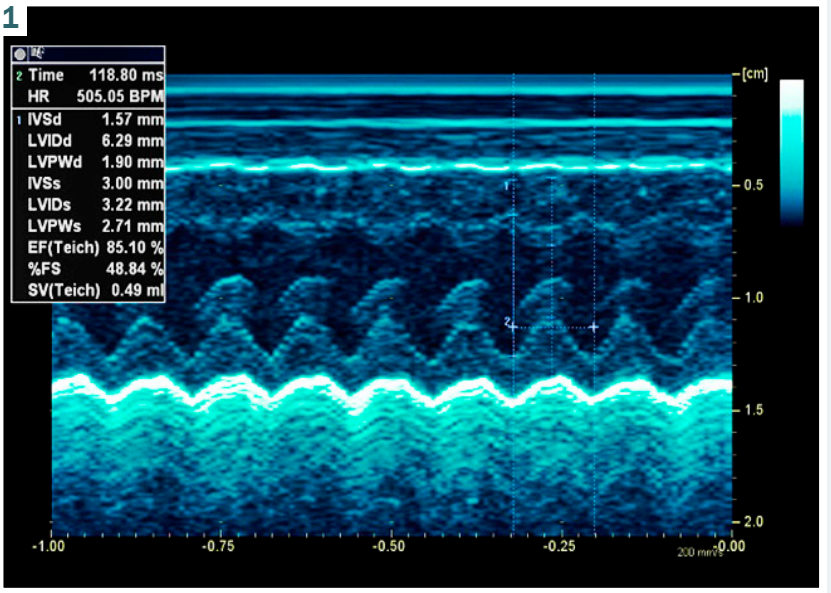
There were no baseline differences in the cardiac morphofunctional parameters between the groups. In IH15 group animals exposed to a 15-day course of intermittent hypoxia, a significant decrease in EDD by 20% and ESD by 22 % was detected ($p < 0.05$). At the same time, an increase in the thickness of IVS by 33 % and LVPW by 44 %, as well as LV mass by 12 % ($p < 0.05$) was recorded. Such changes were accompanied by a decrease in diastolic and systolic volumes by 47 %, as well as in stroke volume by 49 % and cardiac output by 50 % ($p < 0.05$). These data suggest the formation of concentric LV remodeling, which was confirmed by an increase in the LV relative wall thickness by 76 % compared to that of the control group ($p < 0.05$).

In IH60 group rats after a 60-day course of intermittent hypoxia, there was a relative increase in the size of the LV cavities compared to those in IH15 group animals. Meanwhile, the thickness of IVS was comparable, and of LVPW was 19 % less ($p < 0.05$). Such changes also affected LV relative wall thickness, which was only 15 % greater and LV mass by 23 % than those in the control, that taken together can demonstrate an eccentric direction of changes ($p < 0.05$). There was a decrease in LV EF by 8 % and LV FS by 16 % ($p < 0.05$) (*Table 3*, *Fig. 1–3*).

A visual analysis of the myocardium images of the control rats has shown that IRM to CT-1, TT, and AN was diffusely located solely in the cytoplasm of cardiomyocytes, while CL was seen in the intercellular interstitium (*Fig. 4*).

An analysis of the CT concentration has revealed its significant increase in both hypoxic groups by 11 % and 68 %, respectively, compared to that in the control (*Table 4*).

1



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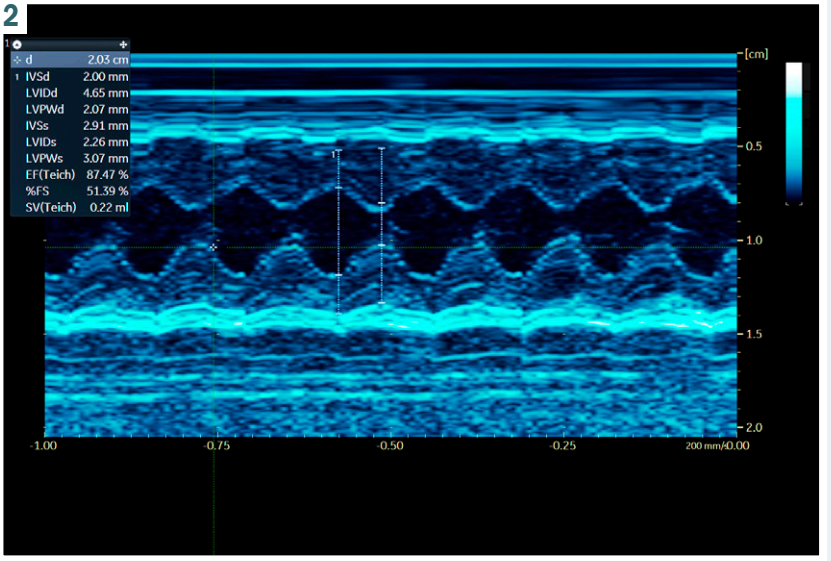

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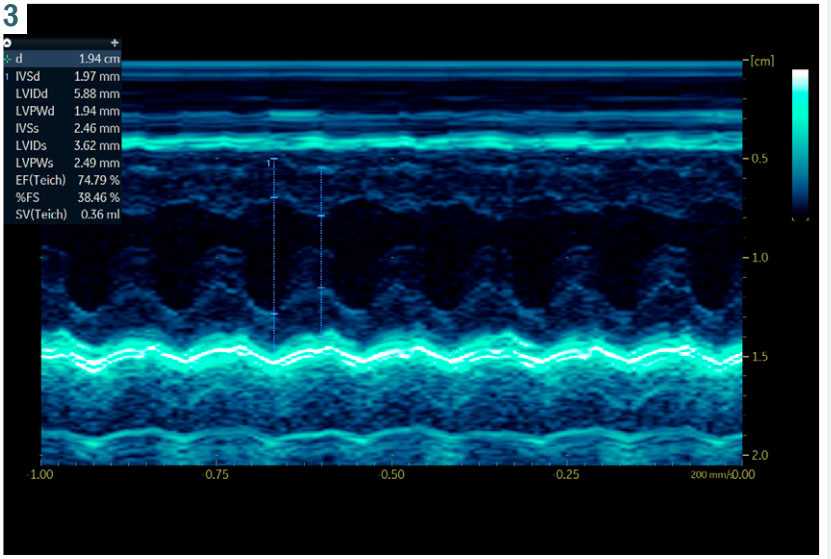

Fig. 1–3. Echocardiograms of experimental group rats (1-INT, 2-IH15, 3-IH60). M-mode scan. Parasternal position. Short axis view of the LV at the level of the papillary muscles.

IVSd: LV end-diastolic interventricular septal thickness;
LVIDd: LV end-diastolic internal dimension;
LVPWd: LV end-diastolic posterior wall thickness;
IVSs: LV end-systolic interventricular septal thickness;
LVIDs: LV internal diameter at end-systole;
LVPWs: LV systolic posterior wall thickness;
EF (Teich): ejection fraction calculated by Teichholz formula;
% FS: percentage of the fractional shortening calculated by Teichholz formula;
SV (Teich): stroke volume calculated by Teichholz formula.

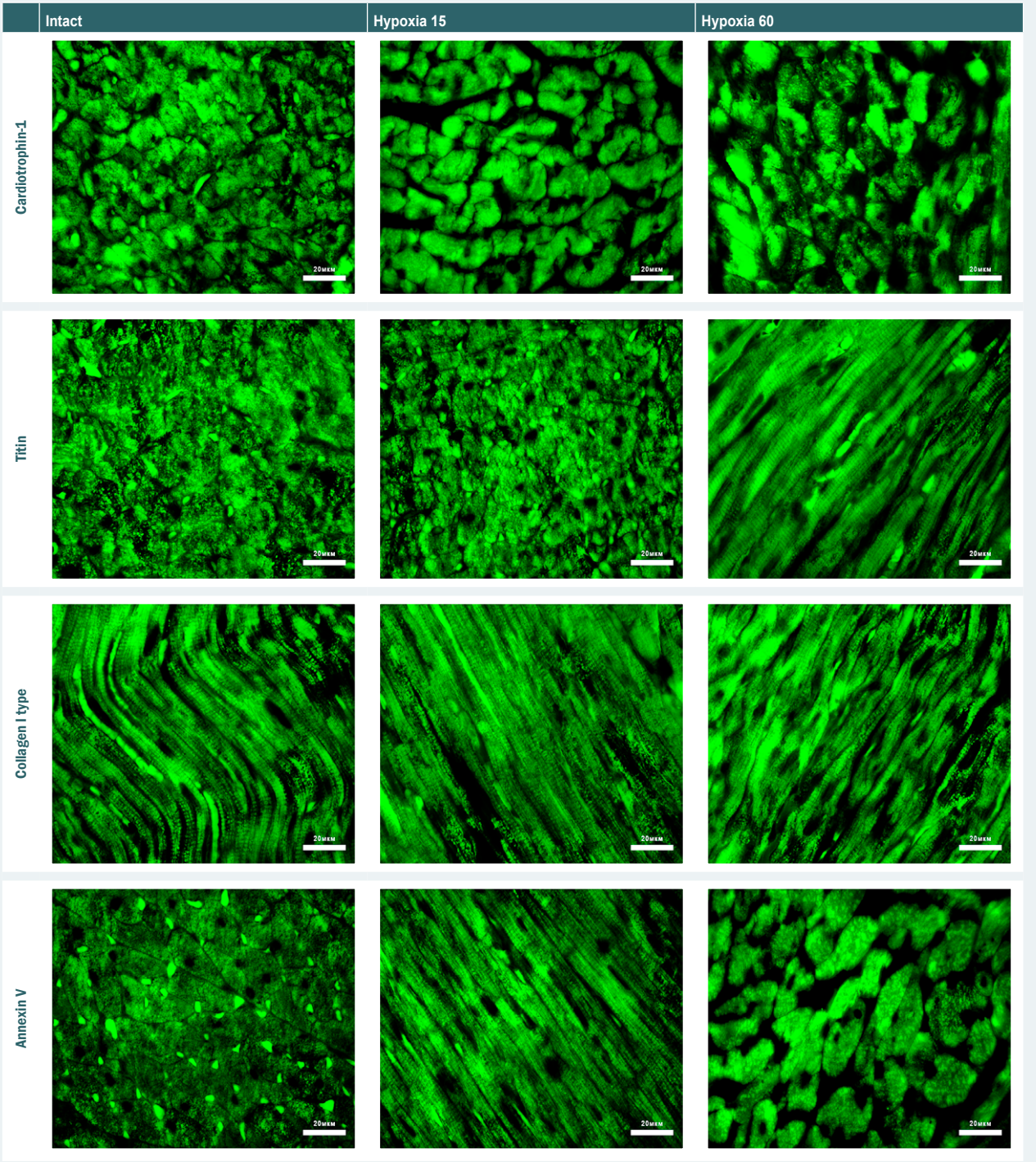

Fig. 4. Expression of remodeling markers in left ventricular myocardium. Indirect immunofluorescence reaction, magnification ×630.

A study of titin has found a statistically significant increase of its concentration in myocardium in both groups of rats, by 15 % in IH15 rats, and by 101 % in IH60 group ($p < 0.05$). CL concentration was significantly increased by 53 % in IH60 group compared to that in IH15 rats ($p < 0.05$). It is interesting to note that the AN concentration was significantly decreased by 11 % in rats exposed to short-term hypoxia compared to the control group, while the increase in this value reached 102 % ($p < 0.05$) in animals after long-term exposure.

Discussion

Analysis of Echo results in animals of both groups gives an insight into LV hypertrophy development. Meanwhile, the increase in LV mass was accompanied by decreased end-diastolic volume with increased end-systolic volume in rats subjected to prolonged hypoxia. Cardiac output improved in IH-60 rats compared to rats after short hypoxic exposure. Interesting results similar to ours were shared by František Papoušek et al., who found no changes in

LV cavity diastolic diameter, but significant thickening of the LV wall especially at 8000 m altitude [17].

The type of LV myocardial remodeling depends on the ratio of its components: hypertrophy, fibrosis and apoptosis. The analysis of marker profile indices revealed differences between the types of remodeling in the experimental rats, which was directly dependent on the duration of hypoxic exposure.

The starting process developing in response to any impact on the myocardium is hypertrophy, which can be assessed by CT. According to the literature, CT should be considered not only as a marker of hypertrophy, but also as a complete gp130-mediated cardioprotector under many influences, including reoxygenation [18], that would explain the increase in its myocardial concentration in rats of both experimental groups in our study.

A clinical study by M. O. Matokhniuk et al. has revealed a correlation between plasma CT and LV myocardial mass index in the patients with heart failure [19]. The same result presented study by U. Polat et al. can be used as a new biomarker and correlated with standard markers of myocardial ischemia [20]. This may have led to the decrease in ejection fraction in rats after prolonged hypoxia.

Researchers E. Martínez-Martínez et al. suggest that CT activates Gal-3, which in turn mediates proinflammatory and profibrotic myocardial effects, and their mutual increase determines a subgroup of higher cardiovascular mortality risk among the patients with heart failure [21]. Thus, the increase in CT concentration in IH60 group of rats should be considered not only as a degree of hypertrophy severity but also as a probable stimulator of fibrosis. While the team of A. Raso believes that increased CT does not induce fibrosis but exhibits anti-apoptotic properties via gp130/LIFR and intracellular PI3K/Akt and p42/44-MAPK cascades [22]. The same effect was also proved with the decrease of annexin V concentration in IH15 rats, however the significant increase in apoptosis in IH60 rats could refute this conclusion.

The next component of myocardial remodeling is fibrosis, the severity of which is characterized by the level CL. It has been proved that progression of fibrotic changes aggravates cardiac dysfunction, impeding both systolic and diastolic function, potentiating arrhythmogenesis and increasing the probability of heart failure [23]. The problem of excessive fibrotic changes is not only in collagen overproduction, but also in apoptosis defect, inability to reverse transformation of myofibroblasts into fibroblasts or both mechanisms [24,25].

A "vicious circle" can form – large amounts of fibrillar collagens enhance differentiation and proliferation of myofibroblasts, which is especially clearly seen in the progression of remodeling [26]. Thus, the actual absence of reliable changes of CL concentration in myocardium of IH15 rats, in comparison with the control, and its significant increase in IH60 fits in well with the "vicious circle" described above, which is due to the duration of exposure. CL also serves as a marker of activity of some substances, correlating with their concentration.

An example is the revealed dependence of sodium-glucose co-transporter 1 gene expression level increase on collagen, atrial natriuretic peptide, brain natriuretic peptide, interleukin-18, connective tissue growth factor expression in response to chronic pressure overload and ischemia, which led to hypertrophy, fibrosis and impaired myocardial contractility in mice [27], that increases the risks of heart failure development in rats with prolonged hypoxia in our study, and the mechanism of its concentration increase is variable in IH60 rats.

In response to various hemodynamic demands, myocardial remodeling can develop through modulation of its stiffness by posttranslational modification of the TT protein and changes in the ratio of its isoforms. Such modulation of total myocardial stiffness affects cardiac chamber walls, diastolic filling, and systolic pump function due to autoregulation – Frank-Starling law [28].

The obtained results about the significant increase in TT concentration in the experimental groups with the maximum concentration in IH60 are fully consistent with these patterns. Modification of myocardial stiffness occurs in two ways. The first is through oxidation and phosphorylation of the TT protein itself. This is a fast, situational pathway of passive elasticity control [29]. The second pathway is realized by switching of two protein isoforms and is based on lower stiffness of N2BA isoform compared to N2B and their co-expression in the same sarcomere [30].

However, the concentrations of TT and CL should not be evaluated in isolation, but it is worth to analyze the coefficient of their correlation. Probably, the 15-day hypoxic influence contributes to the increase in the elasticity of the matrix scaffold as an element of adaptation to new conditions of functioning. Along with this, the increase in TT concentration during prolonged exposure to IH60 is a compensatory response to a significant increase in CL in rats to improve the resilient-elastic properties of the cardiac muscle.

Determination of AN content, a marker of cardiomyocyte apoptosis, demonstrated a significant decrease in its concentration at 15-day duration of hypoxic exposure, which may be due to suppression of apoptosis by CT or the effect of erythropoietin, a key transducer promoted by hypoxia [31]. It is possible that such changes are the result of anti-apoptotic activity of GRP78 recently revealed in an ischemia-reperfusion model [32]. In case of long-term hypoxia, there was found a 2-fold increase in AN concentration compared to control, which is a sign of death of a substantial number of cells and, possibly, the onset of cardiac myogenic dilatation development [33].

Conclusions

Thus, 15-day and 60-day hypoxia simulate different types of myocardial remodeling:

1. The model of 15-day hypoxia in normotensive rats forms concentric left ventricular hypertrophy. This is confirmed by the increase in left ventricle wall thickness while its cavity size decreases, with no changes of ejection fraction. These changes are accompanied with increased levels of the hypertrophy marker cardiotrophin-1 and titin, as well as decreased levels of the apoptosis marker annexin V.

2. Remodeling induced by 60-day hypoxia is characterized by eccentric orientation – progressive increase in cavity volumes with thickened left ventricle walls and sig-

nificantly decreased ejection fraction. The effect of long-term hypoxia is accompanied with marked hypertrophy, significant fibrosis, and apoptosis of cardiomyocytes. Such morphofunctional state of the myocardium may indicate the initial stages of maladaptation, increasing the risk of heart failure development.

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