The indicators of cardiorespiratory system in rats in conditions of chronic hypokinetic stress on the background of normal and restricted nutrition

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***Abstract*—The article is devoted to the study of indicators of the cardiorespiratory system in rats of different sexes under conditions of chronic hypokinetic stress (HK), on the background of normal nutrition, and also in conditions of long-term restricted nutrition (RN). It was shown that the 21-day exposure to hypokinetic stress, both with a normal type of diet and in conditions of long-term restricted nutrition, has an evident effect on the indicators of the cardiorespiratory system in laboratory animals, which is expressed in the increase of the heart rate on the background of the decreased respiratory rate and depth, and also the decrease in the percentage of oxygen in the rats blood, both males and females.**

Keywords — cardiorespiratory system, blood pressure, heart rate, respiratory rate, plethysmography, saturation

I. Introduction

It is widely known that one of the necessary conditions for the normal functioning of the body is to maintain high motor activity [1]. Some authors have shown that along with hypokinesia (HA), an unbalanced diet is a powerful stress factor that negatively affects the human and animal body [2]. Nowadays, there were described in detail the changes that occur with the human and animal body under hypokinetic stress [3]; in particular, the works of some authors are devoted to the study of the blood system, nociception, and behavior of laboratory animals [4, 5].

II. Purpose of the Study

Assessment in changes of the indicators of the cardiorespiratory system in rats of different sexes in conditions of chronic hypokinesia, on the background of normal nutrition, as well as in conditions of long-term restricted nutrition.

III. Experimental

The studies were carried out on the basis of the laboratory of cellular physiology and biophysics of the Center for the collective use of scientific equipment “Experimental physiology and biophysics” of the Taurida Academy of V.I. Vernadsky Crimean Federal University

During the experiment, all the principles of bioethics were observed in accordance with the international principles of the European Convention for the Protection of Vertebrate Animals, which are used for scientific purposes.

The experimental part of the work was performed on 20 white Wistar male rats and 20 female rats aged 3-4 months, weighing 180-200 g. Rats of each sex were equally divided into groups: 1 - females (n = 10) and 2 - males (n = 10), which were subjected to long-term hypokinetic stress (21 days of mobility restriction). 3 - females (n = 10) and 4 - males (n = 10), who 3 weeks before the start of the study, as well as during the 21-day hypokinesia were under conditions of limited nutrition. 3 weeks before the start and during stressing, these animals were given grain, which was preliminarily calcined in an autoclave at a temperature of 132 degrees Celsius and a pressure of 2.1 atmospheres for 30 minutes, and distilled water. Thus, this chemical in the finished solution exceeded the maximum permissible concentration by 10 times.

To create conditions for limiting the motor activity, special boxes made of organic glass 140 x 60 x 60 mm in size were used. The walls and bottom of each case had the special openings that provided ventilation and removal of animal waste. In the boxes rats of all groups were 18 hours a day. At the same time, with the help of drinking bowls, animals were provided a free access to water. In the boxes, rats were placed at peak activity: from 4 p.m. to 10 a.m. For 6 hours, animals were fed and cared for.

The heart rate (HR), respiratory rate (BR) and saturation (SAT) in rats were recorded using the Biopac MP150 measuring complex (USA) on the first day of the study, as well as on the 7th, 14th and 21st days of the experiment. Recording and processing of data was carried out on a computer using the program "AcqKnowledge 4.2 for MP150".

Statistical analysis and processing of the obtained data was carried out using nonparametric statistical methods. Intra-group differences were assessed using the Wilcoxon test, and the significance of differences between the groups was evaluated using the Mann-Whitney test. Calculations and graphic presentation of the results were carried out using the “Microsoft Excel” program and “STATISTICA - 8.0” software.

IV. Results and discussion

On the first day of the study, the laboratory animals of the determined groups did not show significant differences in the values of all studied indices of the cardiorespiratory system (Table I).

A. Changes in heart rate in laboratory animals of determined groups

The significant increase in heart rate throughout the study was recorded in laboratory animals subjected to long-term hypokinetic stress. So, in the group of males on the 7th day of the study, the values of this indicator increased by 1.9% ($p > 0.05$); it should be noted that on the 14th day of the study, these changes were expressed even more (2.0% ($p > 0.05$)), and by 21 days of the study, an increase in heart rate values reached a statistical significance level and amounted to 107.5% ($p < 0.05$), relative to the values of this indicator obtained from males of this group on the first day of the study (Table I).

In addition, the data obtained in the group of females subjected to long-term hypokinetic stress indicate similar changes with the group of males. So, on the 7th day of the study, the heart rate values in these animals tended to increase by 2.6% ($p > 0.05$), on the 14th day the values of this indicator also increased by 2.5% ($p > 0.05$) (table I).

However, these changes did not reach the level of statistical significance. It should be noted that by 21 days in the group of female HK we recorded a significant increase in heart rate by 10.2% ($p < 0.05$), compared to the values of that indicator in the same animals on the first day of the study (Table I).

TABLE I. The Heart Rate Values (Per Minute) in Laboratory Animals of Esdetermined Groups Under the Influence of Long-Term Hypokinetic Stress on the Background of Normal and Rtricted Nutrition

Group	**HK**		**HK+ RN**	
Sex of animals	***females***	***males***	***females***	***males***
background	341,3±8,0	340,4±8,7	332,3±7,7	336,8±7,1
7th day	350,1±4,8	347,8±9,4	336,5±9,5	350,9±7,4
14th	349,9±9,6	348,0±13,6	359,1±9,5	359,6±9,5
21st day	376,3±7,6 **p_1<0,05**	367,1±12,7 **p_1<0,05 p_2<0,05**	409,1±5,8 **p_1<0,05 p_2<0,05**	392,0±8,5 **p_1<0,05**

p1 – the significance of differences according to the Wilcoxon criterion with respect to the values obtained on the first day of the study; p2 - by the Mann-Whitney criterion with respect to the values obtained in the HK group.

In animals that were under conditions of hypokinesia and limited nutrition during the study, the similar dynamics of changes in heart rate values was recorded. However, we noted that those changes were even more expressed than in laboratory animals subjected only to hypokinesia. So, in the group of males on the 7th day, and also on the 14th day of the study, the tendency of heart rate increasing was recorded - by 2.8% ($p > 0.05$) and 5.3% ($p > 0.05$), respectively (table 1). It was noted that by 21 days of the study in the group of males (HK + RN), an increase in heart rate reached the level of statistical significance and amounted to 114.8% ($p < 0.05$), compared to the heart rate values that were obtained on the first day of the study in animals of those groups (table I).

It should be noted that in the group of females rats subjected to the long-term hypokinetic stress due to restricted nutrition, on the 21st day of the study, the maximum increase in the indicator values (by 19.9% ($p < 0.05$)) was recorded, compared to the values of the heart rate in rats of the same group on the first day of the study (table I).

It is well known that the cardiovascular system of animals is one of the most sensitive body systems to the changes of the parameters of both the internal and external environment [6]. Moreover, it was shown [7] that the most reactive parameter of the response to the exposure is the change in the characteristics of the heart rhythm. According to published data [8], normal heart rate values in laboratory animals are in the range of 300-600 beats per minute. In different sources [9, 10] the cases of increasing heart rate under the influence of both acute and chronic stress on the animal body have been repeatedly described. In particular, it was shown [10] that acute stress caused a significant increase in heart rate values, which in turn can lead to the development of tachycardia.

Thus, the data obtained by us in laboratory animals in all four groups confirmed the previous studies and indicate that long-term hypokinesia had a significant chronotropic effect on the body of laboratory animals, and caused functional changes associated with the stress of regulatory systems. It should be noted that the maximum increase in heart rate was recorded in animals that were under conditions of limited movement and nutrition, which indicates that the lack of vital chemical elements in food further exacerbates the negative effects of mobility restrictions.

B. Change in respiratory rate in laboratory animals of selected groups

In laboratory animals that were subjected to long-term immobilization stress, the significant changes in the dynamics of the values of the breath rate (BR) indicator were recorded during the study. Thus, in the analysis of the values obtained in the group of male HK on the 7th day of the study, the BR values tended to decrease by 6.1% (p>

0.05), compared to the values obtained in animals of this group on the first day of the study. Note that on the 14th day of mobility limitation, the values of this indicator in these animals significantly decreased by 14.4% (p <0.05) (Table II), and by 21 days of the study, the maximum decrease in the BR index values was recorded by 20, 6% (p <0.05), compared to the values obtained on the first day of the experiment (table II).

At the same time, in the group of female rats that were in conditions of limited locomotor activity (HK), similar changes in the BR values were recorded. On the 7th and 14th day of the study, we recorded the tendency to a decrease in the BR indicator values by 14.4 (p> 0.05) and 16.3% (p> 0.05), respectively, and by 21 days the decrease in the values of this indicator was maximally expressed and reached the level of statistical significance - by 23.1% (p <0.05), compared to the values obtained from the same animals on the first day of the study (Table II).

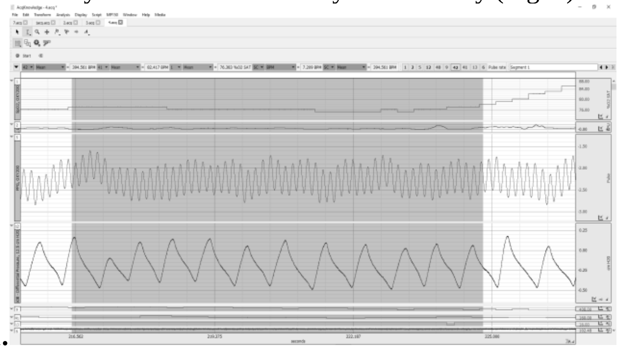
It should be noted that while studying the effects of immobilization stress and limited nutrition on the body of laboratory animals, it was shown that the recorded values of the BR indicator had the dynamics similar to the groups of males and females with the isolated exposure of HK, however, the obtained changes of this indicator were even more expressed (Table II). So, in males of the group HK + RN, on the 7th day of the study, we recorded the significant decrease in the BR value by 10.6% (p <0.05), and the decrease in the BR reached the maximum level by the 21st day of the study - by 19, 5% (p <0.05), compared to the values obtained on the first day of the study (table II). It should be noted that in females who were under conditions of long-term hypokinesia and restricted nutrition, it was recorded that the significant decrease in BR values occurred from the 14th day of the study by 13.2% (p <0.05), and the maximum decrease in the values of this indicator were recorded on the 21st day of the study - by 28.0% (p <0.05), compared to the values obtained on the first day of the study (table II).

It is known that the respiratory system is one of the main systems that ensure normal functioning and adaptation of the body to environmental factors. Also the respiratory system, like the cardiovascular system, is one of the first to respond to various stressful effects by changing the respiratory rate. Thus, the data recorded by us indicate the development of an acute stress reaction that develops in laboratory animals in response to both mono-hypokinetic stress and its combination with nutritional stress. It is likely that the obtained data on the decrease in BR values in animals of all groups under the influence of both hypokinetic stress and in combination with a limited diet can be explained by the development of non-cardiogenic pulmonary edema from 14-21 days of the study (Fig. 1).

TABLE II. THE BREATH RATE VALUES IN LABORATORY ANIMALS OF DETERMINED GROUPS UNDER THE INFLUENCE OF LONG-TERM HYPOKINETIC STRESS ON THE BACKGROUND OF NORMAL AND RESTRICTED NUTRITION

Group	**HK**		**HK+ RN**	
Sex of animals	***females***	***males***	***females***	***males***
background	113,2±2,6	115,8±2,2	116,9 ±2,5	112,8±3,0
7th day	111,5 ±4,0	106,3±3,0	103,1 ±6,3	101,2±3,4 **p_1<0,05**
14th	94,8±8,1	97,0±4,6 **p_1<0,05**	98,3 ±6,4	103,6±4,1 **p_1<0,05**
21st day	87,1±1,8 **p_1<0,05**	89,9 ±2,0 **p_1<0,05**	81,5±1,4 **p_1<0,05** **p_2<0,05**	91,2 ±3,7 **p_1<0,05**

p1 – the significance of differences according to the Wilcoxon criterion with respect to the values obtained on the first day of the study; p2 - by the Mann-Whitney criterion with respect to the values obtained in the HK group.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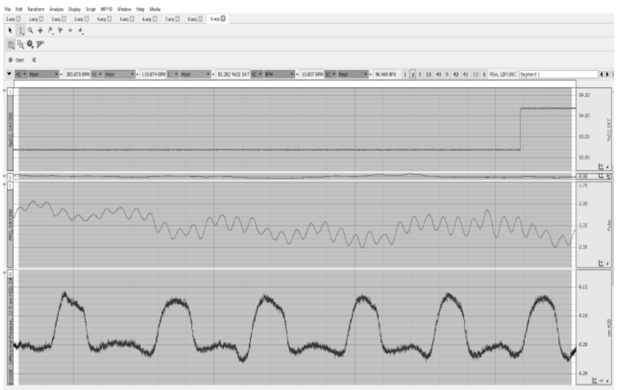
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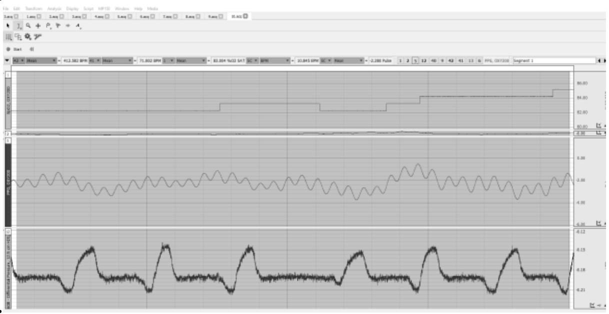
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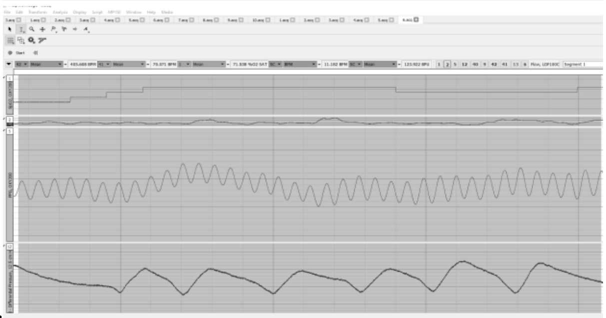
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Fig. 1 Patterns of heart rhythm and respiration in laboratory rats of the groups HK and HK + RN at different periods of the study (A - on 1st day of the study in the group with HK, B and C - on the 14th day and 21 days of the study in the group with HK, G - on the 21st day of the study in the group HK + RN).

In literature, the cases of the decrease in the respiratory rate with high heart rate in animals on the background of severe metabolic acidosis are described. According to [11], non-cardiogenic pulmonary edema can be based on damage to the alveoli-capillary membrane, followed by increased capillary permeability for large molecules due to the inflammatory process caused by stress.

The confirmations of this statement are the charts of respiratory rhythm in animals in the first and subsequent days of the study. So, from figure 1 you can see the change in the normal pattern of breathing with the appearance of apnea in animals from the 14th day of the study (figure 1).

The confirmation of the obtained data is the analysis of the saturation index in laboratory animals within 21 days of the study.

C. Change in saturation in laboratory animals of determined groups

Throughout the study, the animals exposed to long-term hypokinesia showed the significant decrease in saturation. That is, on the 7th day of the study, in the group of males, the values of this indicator decreased by 4.3% ($p > 0.05$) (table 3). We can note that on the 14th day of the study, the obtained changes were even more expressed and amounted to 90.6% ($p > 0.05$). By 21 days of the study, the decrease in SAT values reached the level of statistical significance and amounted to 84.7% ($p < 0.05$), compared to the values of that indicator that were obtained in males of the same group on the first day of the study (table III).

In females, who were also in conditions of limited movement, on the 7th day of the study, we recorded the significant decrease in SAT by 8.1% ($p < 0.05$). It should be noted that on day 14, the SAT index values had an unreliable increase of 6.8% ($p > 0.05$). On the 21st day of the study, this indicator reached the level of statistical significance and amounted to 86.7% ($p < 0.05$), compared to SAT values on the first day of the experiment in animals of that group (table III).

Under conditions of long-term hypokinetic stress and limited nutrition, similar changes in SAT values were recorded in animals throughout the study. In males of this group on the 7th day of the experiment it was recorded that this indicator decreased by 10.7% ($p > 0.05$), but by 14 days the SAT values had an unreliable increase of 6.9% ($p > 0.05$) (table III). It should be noted that on day 21 the SAT index was the smallest and amounted to 76.5% ($p < 0.05$), compared to the values of this indicator in males of the same group on the first day of the experiment.

TABLE III. THE SATURATION VALUES IN LABORATORY ANIMALS OF DETERMINED GROUPS UNDER THE INFLUENCE OF LONG-TERM HYPOKINETIC STRESS ON THE BACKGROUND OF NORMAL AND RESTRICTED NUTRITION

Group	HK		HK+ RN	
Sex of animals	***females***	***males***	***Sex of animals***	***females***
background	93,3±1,5	87,8±2,1	86,3±1,6	87,8±2,3
7th day	85,8±3,6 **p_1<0,05**	89,3±1,2	85,7±2,5	83,3±3,4
14th	86,9±3,8	84,5±4,2	79,9±1,6 **p_1<0,05**	86,9±2,5
21st day	80,9±2,0 **p_1<0,05**	79,0±2,4 **p_1<0,05**	75,5±0,7 **p_1<0,05** **p_2<0,05**	71,4±4,3 **p_1<0,05**

p1 – the significance of differences according to the Wilcoxon criterion with respect to the values obtained on the first day of the study; p2 - by the Mann-Whitney criterion with respect to the values obtained in the HK group.

Females, who were also under long-term restricted nutrition and in conditions of limited locomotor activity, had a similar trend towards the decrease in SAT values throughout the study. So, on the 7th day of the experiment, it was recorded that the SAT index values began to decrease significantly and amounted to 8.2% ($p > 0.05$) (table III). However, these changes have not yet reached a statistically significant level. It should be noted that in females of this group on the 14th day, as well as on the 21st day, a significant decrease in SAT was recorded, respectively, by 14.3% ($p < 0.05$) and 19.1% ($p < 0.05$), compared to the values of this indicator on the first day of the study in animals of this group (table III).

From different sources [12] it is known that at the level of physiological processes, stress is accompanied by the development of hypoxia, proved by a significant decrease in the values of the saturation index. With a strong affinity of the hemoglobin and oxygen molecules, the transfer of the latter to the tissues of the body decreases. In addition, the lack of chemicals in food causes shifts in the functional state that are associated with the development of alkalosis. The obtained results indicate that hypokinetic stress, as well as the combination of hypokinetic stress and restricted nutrition, lead to significant shifts in the acid-base balance.

Thus, our data indicate that hypokinetic stress, both with normal nutrition and with limited nutrition, had a significant effect on the cardiorespiratory system, which was manifested in the increase in heart rate with the decrease in the frequency and depth of respiration, as well as the decrease in percentage the oxygen content in the blood of laboratory animals in the determined groups (table III). The results of this study indicate that changes in the parameters of the cardiorespiratory system were recorded in animals of both groups from the 7th day of the study; however, the maximum changes in all studied parameters were recorded on the 21st day of the study.

D. Differences in the performance of the cardiorespiratory system in animals of the HK and HK + NR groups

The results of our study indicate significant differences in the dynamics of all the studied parameters in different groups of animals that were under conditions of hypokinetic stress both on the background of normal nutrition and with restricted nutrition. We can note that on the 21st day of the study, the registered significant differences between the studied indices (Fig. 2, tables I-III). So on the 21st day of the study, males of the group HK + NR showed the tendency to increase the heart rate by 6.8% ($p < 0.05$), and a decrease in BR and SAT by 1.4% ($p > 0.05$) and 9 , 6% ($p > 0.05$), respectively, and in females of the group HK + RN, these changes reached statistical significance and amounted to heart rate - 108.7% (p <0.05), BR - 93.6% (p <0 , 05), SAT - 93.3% (p <0.05), compared to the values obtained on the same day of the study in the HK group (figure 2, tables 1-3).

Thus, the data obtained in laboratory animals of the GK + RN group indicate that restricted nutrition enhances the effect of hypokinetic stress, and changes in rats indices during course restriction of mobility and nutrition were more expressed in the group of females. Therefore, according to the results of the study, the females were more sensitive to nutritional deficiency conditions.

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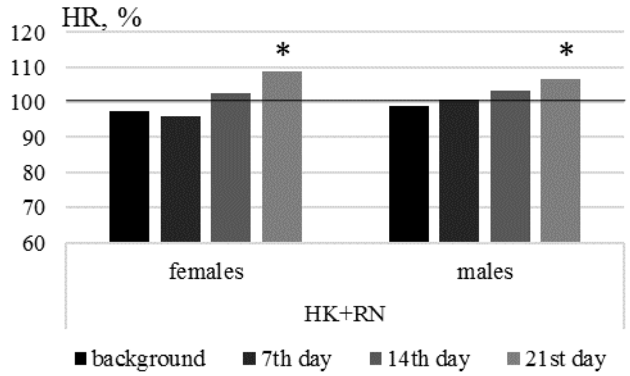

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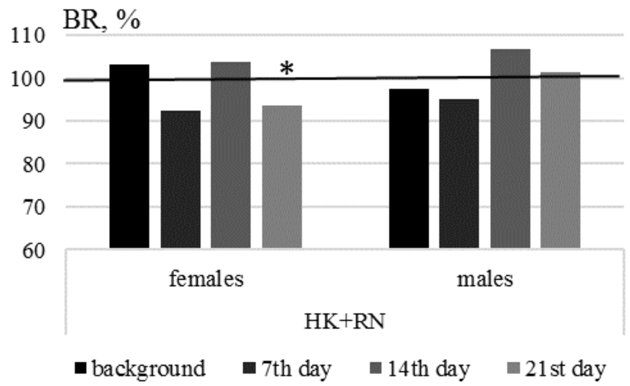

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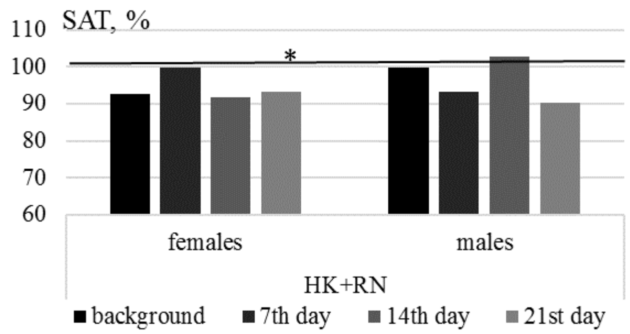

Fig. 2 Dynamics of changes in the values of heart rate (2-A), BR (2-B) and SAT (2-C) in laboratory animals of the determined groups under the influence of long-term hypokinetic stress under the normal nutrition and restricted nutrition (in %, compared to the values obtained in the group of HK taken as 100%).

Note: * - significance of differences according to the Mann Whitney criterion relative to the values obtained on the first day of the study; ▲ - relative to the values obtained in the group of HK.

V. Conclusion

1. The 21-day exposure to hypokinetic stress, both on the background of normal nutrition and with long-term restricted nutrition, has the expressed pathogenic effect on the indicators of the cardiorespiratory system in laboratory animals, which is evident by the increase in heart rate on the background of the decrease in the frequency and depth of breath, as well as the decrease in the percentage of oxygen in the blood of rats, both males and females.

2. Changes in the parameters of the cardiorespiratory system were recorded in laboratory animals of both groups from the 7th day of the study; however, the maximum changes in all studied parameters were recorded on the 21st day of the study. In the group of males and females with HK on the 21st day of the study, the significant increase in heart rate was recorded: in males by 7.5% (p <0.05), females by 10.5% (p <0.05); as well as the decrease in BR and SAT indicators by 20.6-23.1% (p <0.05) and 15.3-13.3% (p <0.05), respectively. In the group GK + RN, during these periods of the study, the increase in heart rate in males was recorded by 14.8% (p <0.05), in females by 19.9% (p <0.05), as well as the decrease in the values of indicators BR and SAT in males - by 19.5% (p <0.05) and 23.5% (p <0.05), and in females by 28.0% (p <0.05) and 19.1 % (p <0.05), respectively, compared to the values of these indicators on the first day of the study.

3. Hypokinetic stress on the background of restricted nutrition enhances the pathological changes in the cardiorespiratory system in animals of the GK + RN group and led to a maximum increase in heart rate, as well as the maximum decrease in respiratory rate and saturation under the influence of long-term exposure of that factor. So on the 21st day of the study, males of the group GK + RN showed the tendency to increase the heart rate by 6.8% (p> 0.05), and a decrease in BR and SAT by 1.4% (p> 0.05) and 9,6% (p> 0.05), respectively, and in females of the group GK + OD, these changes reached statistical significance and amounted to heart rate - 108.7% (p <0.05), BR - 93.6% (p <0 , 05), SAT - 93.3% (p <0.05), compared to the values obtained on this day of the study in the HK group.

Acknowledgment

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