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Structural Changes in Lung Tissue of Young Rats after Exposure to Restricted Nutrition

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Abstract: *The aim of the work was to investigate the structural changes in the lung tissue of young rats after exposure to restricted nutrition (RN). The study was conducted on 24 male Wistar rats at the age of 3 months. Experimental rats, during 28 days, received a reduced calorie diet by 30% compared to the control. Histological preparations of the lungs were prepared according to the standard method. In the lungs of rats exposed to RN, an increase in the relative area of air spaces, an increase in the number of alveoli per unit area, a decrease in the area of parenchyma and connective tissue elements and a decrease in the thickness of the interalveolar septum were found. These structural changes indicate the presence of signs of increased lung air filling and improved gas exchange processes.*

Keywords: *restricted nutrition, lungs, rats*

1. Introduction

The positive effect of restricted nutrition (RN) on the body has been proven by numerous scientific works. It is known that RN improves the metabolic state of the body, reducing inflammation and oxidative stress, contributes to the treatment and prevention of chronic diseases, etc. [1]. RN is able to increase the body's ability to resist internal stresses [2]. It can be assumed that RN will be noted in the recommendations of doctors as an additional treatment method in the near future [3].

However, the literature data on the effect of RN on the structure of the lungs are insufficient, and the results obtained are often contradictory. This may be due to the different type of RN, the duration of the experiments, the age and sex of the animals, etc. All this emphasizes the relevance of conducting research in this direction. There is data in the scientific literature that RN prevents the onset of asthma symptoms, reduces the development of carcinogenesis and reduces a number of age-related lung diseases [4].

The aim of the work was to investigate the structural changes in the lung tissue of young rats after exposure to a restricted nutrition.

2. Materials and Methods

The study was conducted on 24 male Wistar rats at the age of 3 months. The rats were divided into 2 groups: I – control; II – rats that received vivarium feed reduced in weight by 30% compared to the control. The control rat received 20 g of balanced feed (65 kcal) daily, and the experimental rat – 14 g (45 kcal). Access to water was free. The duration of the experiment was 28 days. All protocols were approved by the Biomedical Ethics Committee for the Care and Use of Animals of the Bogomoletz Institute of Physiology, NAS of Ukraine.

Histological preparations were prepared from lung tissue using standard methods: fixed in Bouin's fluid, dehydrated in alcohols of increasing concentration and dioxane, and embedded in paraffin. Paraffin sections (5-6 μm thick) were prepared on a sledge microtome. Sections were stained using the Van Gieson method [5].

Photographs were taken of the micropreparations using a "Nikon Eclipse E100" microscope (Japan), which is equipped with a digital camera. Histomorphometry was performed using the "Image J" computer program. Statistical analysis was performed using variational statistics methods. The normality of the distribution of digital arrays was checked using the Pearson test. In the case of normal distribution, the Student's t-test was used to assess the probability of the difference between the control and experimental groups. Differences were considered significant at $p < 0.05$.

3. RESULTS

In rats exposed to RN, was found a smaller alveolar area by 15% ($p < 0.05$), a tendency to a decrease in alveolar depth and width of the entrance to them by 8% compared to the control. However, the smaller size of the alveoli was compensated by an increase in their number per unit area, which generally increased lung airiness. Also in these rats, a tendency to an increase (by 10%) in the width of the lumen of the respiratory bronchioles, alveolar ducts, and alveolar sacs was found and a significant increase in the ratio of this indicator to double the depth of the alveoli by 20%. In the lungs of rats exposed to RN, a significantly smaller relative area of the parenchyma and connective tissue was found by 16% and a larger area of the air spaces by 11% compared to the control. This led to a smaller ratio of the area of the parenchyma and connective tissue to the area of the air spaces by 25% ($p < 0.05$).

The thickness of the alveolar septum in rats after exposure to RN was significantly reduced by 16% compared to the control. This may indicate increased lung air filling and improved gas exchange processes (Table).

Table 1. *Morphometric indicators of the lungs (M ± m, n=12)*

Indicators	Control	Restricted nutrition
Alveoli cross-sectional area, μm^2	746±22	633±21*
Alveoli depth, μm	22.6±0.6	20.8±0.5
Width of the alveoli entrance, μm	13.5±0.5	12.4±0.3
Width of the lumen of RB, AD and AS, μm	63.1±2.9	69.7±3.2
Ratio of diameter of RB, AD and AS to double depth of alveolus	1.40±0.05	1.68±0.06*
Thickness of the interalveolar septum, μm	3.40±0.16	2.86±0.14*
Relative area of parenchyma and connective tissue, %	41.8±1.7	35.2±1.9*
Relative area of air spaces, %	58.2±1.3	64.8±1.4*
Ratio of parenchyma-connective tissue area to airspace area	0.72±0.04	0.54±0.06*

Note: ** $p < 0.05$ – significant differences compared to control.*

RB – respiratory bronchioles, AD – alveolar ducts, AS – alveolar sacs

4. DISCUSSION

We have found that caloric restriction by 30% of the standard diet leads to the appearance of morphological signs of increased lung activity in rats. This is evidenced by an increase in the relative area of air spaces and a decrease in the relative area of parenchyma and connective tissue in the lungs. A decrease in the thickness of the interalveolar septum may indicate a decrease in the number of connective tissue elements, which contributes to the improvement of alveolar-capillary gas exchange [6].

The literature data on the effect of RN on the condition of the lungs are ambiguous. This is most likely due to the different modes of its exposure. There is literature data indicating the development of pulmonary emphysema after exposure to severe RN [7]. Other researchers have found that RN prevents the onset of asthma symptoms [8], and a 40% diet restriction can reduce carcinogenesis in the lungs of mice [9]. It has also been studied that long-term exposure to RN helps reduce the risk of a number of lung diseases that are closely associated with old age [4].

5. CONCLUSION

It was found that rats that received a 30% reduced diet for 28 days had structural signs of increased lung air volume and functional activity.

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