

# Effects of meldonium on cardiomyocyte hypertrophy and myocardial polyploidy in physically exercised rats

Malyshev I.I.<sup>1</sup>, Alpidovskaja O.V.<sup>2</sup>, Romanova L.P.<sup>2</sup>

<sup>1</sup> Mari State University, Yoshkar-Ola, Russia.

<sup>2</sup> I.N. Ulianov Chuvash State University, Cheboksary, Russia.

## AUTHORS

**Igor I. Malyshev**, MD, PhD, Professor, Department of Physiology and Pathology, Mari State University, Yoshkar-Ola, Russia. ORCID: 0000-0001-8930-5537

**Olga V. Alpidovskaja**, MD, PhD, Associate Professor, Department of General and Clinical Morphology and Forensic Medicine, I. N. Ulianov Chuvash State University, Cheboksary, Russia. ORCID: 0009-0004-0232-3193

**Lubov P. Romanova**, PhD, Associate Professor, Department of Dermatovenerology with a Course in Hygiene, I. N. Ulianov Chuvash State University, Cheboksary, Russia. ORCID: 0000-0003-0556-8490

Hypertrophy of cardiomyocytes can be considered an adaptive response that enhances cardiac performance by increasing the myocardial contractility under conditions of physical exercise. However, myocardial hypertrophy can lead to persistent decompensation of cardiac function.

**The aim of the study** was to determine the effect of meldonium on reproduction of cardiomyocytes against the background of their hypertrophy and polyploidy, which could expand the range and reliability of adaptation to overload.

**Methods.** The experimental animals were male Wistar rats, weighing 180–210 g, divided into three groups (18 rats in total). The animals swam for 15 minutes (Group 1 — light exercise), 30 minutes (Group 2 — moderate exercise), and 55–59 minutes (Group 3 — heavy ex-

ercise). During the experiment, meldonium was added to the rats' diet at a dose of 100–120 mg/kg of body weight.

**Results.** Moderate mode of physical exercise was the most beneficial for the rat myocardium. Heavy physical exercise led to structural impairments in the myocardium, its polyploidy, accompanied by persistent cardiomyocyte hypertrophy and a decrease in the proliferative potential of cardiomyocytes. Meldonium significantly altered the morphological parameters of the heart under heavy physical exercise. Its administration led to an increase in the number of binucleated cells, which became polyploid, while simultaneously reducing the number of hypertrophied tetraploid mononucleated cells.

**Conclusion.** The use of meldonium during heavy physical exercise reduces cell hypertrophy, increases the percent-

age of binucleated cardiomyocytes, and decreases the number of tetraploid mononucleated cells.

**Keywords:** physical exercise, polyploidy, binucleated cardiomyocytes, tetraploid cells, proliferation, apoptosis, meldonium.

**Conflict of interest:** none declared.

Received: 19.09.2025

Accepted: 14.11.2025



**For citation:** Malyshev I.I., Alpidovskaja O.V., Romanova L.P. Effects of meldonium on cardiomyocyte hypertrophy and myocardial polyploidy in physically exercised rats. *International Heart and Vascular Disease Journal*. 2025; 13(48):16-21. DOI: 10.24412/2311-16232025-47-21-28

## Introduction

Heavy physical exercise leads to organ alterations [1–3]. According to authors' data, intensive exercise can contribute to the risk of sudden cardiac death (SCA) [4]. Achievement of outstanding and constantly improving sportive results is commonly accompanied with heavy physical exercise, which blurs the line between the normal adaptive mechanisms of the cardiovascular system and pathologic shifts, caused by the dystrophy in cardiomyocytes. Hypertrophy of cardiomyocytes is considered an adaptive reaction of the organism, when muscular fibers thicken and the structure of the heart alters. It leads to enhanced cardiac performance, improving the myocardial contractility during the increased load. However, beyond a certain point, myocardial hypertrophy might lead to persistent decompensation of cardiac function. This is why the identification of the range of the effective hypertrophy and the pharmacologic ways of prevention of decompensation of cardiac function, are of practical interest.

Meldonium is known to modulate myocardial energy metabolism by enhancing aerobic glycolysis and the malate-aspartate shuttle, thereby potentially improving energy balance. Its antiischemic, vasoprotective, antioxidative activity and ability to restrict apoptosis and prevent the alterations of electric activity of the heart is also identified. These mechanisms are the reason for an increased scientific interest towards the study of the effects of meldonium on myocardial hypertrophy in physical exercise.

**The aim of the study** was to determine the effect of meldonium on reproduction of cardiomyocytes against the background of their hypertrophy and polyploidy, which could expand the range and reliability of adaptation to overload.

## Methods

The study was conducted at I. N. Ulianov Chuvash State University. The animals were kept under standard vivarium conditions.

Rats were divided into three groups ( $n=6$  per group). Animals in each group underwent a physical exercise regimen (swimming) in water at a comfortable temperature of 32–34 °C for 10 days. Group 1 swam for 15 minutes daily (light exercise), Group 2 for 30 minutes (moderate exercise), and Group 3 swam to exhaustion, defined as the point of drowning (55–59 minutes after the start of the experiment), representing heavy exercise. Throughout the 10-day experiment, meldonium was added to the animals' diet at a daily dose of 100–120 mg/kg of body weight. The experimental rats ( $n=18$ ) were euthanized immediately after the final exercise session, and another cohort ( $n=18$ ) was euthanized 30 days post-exercise. For comparison, three control groups of six rats were subjected to the same graded exercise regimens, but did not receive meldonium in their diet. An additional control group of intact animals ( $n=3$  per group), which were not exercised, was used. Upon completion of the experiment, animals were euthanized by decapitation using a guillotine, and the hearts were non-traumatically extracted.

## Outcome registration methods

During autopsy, the heart was excised in full and placed in 10% formalin for primary fixation for 24 hours. This was followed by secondary fixation and dehydration. The resulting fragments of the left ventricle (LV) were embedded in paraffin, and 5  $\mu\text{m}$  thick histological sections were prepared using a "Microm" sled microtome.

The sections were stained with hematoxylin and eosin. The amount of deoxyribonucleic acid in the

cardiomyocyte nuclei was determined using a Biolam-70 microscope equipped for photometry with a MFEL-1 microphoto attachment and a FEU-79A photometer. Measurements were taken in transmitted light with a bandpass filter having a maximum transmittance at a wavelength of 570 nm and an applied voltage of 900 V. Peripheral blood lymphocytes and small lymphocytes from lymph nodes served as the diploid reference. Binucleated cardiomyocytes were counted per 7000 nuclei at a magnification of 900x. To assess the degree of hypertrophy, the diameter of the cardiomyocytes was measured. The data were processed using the SIGMA SCAN PRO software.

Immunohistochemical analysis was performed using the proliferation marker Ki-67 (Santa Cruz Biotechnology) according to a standard protocol. For this, 3 µm thick sections were placed on highly adhesive glass slides coated with L-polylysine and dried at room temperature for 24 hours.

Staining was carried out both manually and automatically using the AUTOSTAINER-360 (THERMO, UK) and Leica BOND-MAX (Germany) automated stainers with the EnVision (Dako, Denmark) and NovoLink™ Polymer (Novocastra, UK) detection systems, respectively. Controls included non-immune rabbit and mouse sera, as well as sections of rat heart tissue from control groups.

The staining results were assessed by counting 200 cardiomyocyte nuclei across six random fields of view at 400x magnification.

Inclusion criteria: Male Wistar rats weighing 180–210 g, with no external defects, injuries, or behavioral abnormalities.

Exclusion criteria: Presence of concomitant pathology in the internal organs or brain of the rats.

### Ethical approval

The study was conducted in compliance with the Federal Law of the Russian Federation “On the Protection of Animals from Cruel Treatment” dated January 1, 1997. The study protocol was approved by the Local Ethical Committee of Mari State University, Ministry of Health of Russia (Protocol No. 1, April 28, 2023).

### Statistical analysis

Descriptive statistical analysis was performed using Statistica 10 (USA) and Microsoft Excel 2016 (USA). The Kruskal-Wallis test was used to assess the

equality of medians across multiple samples, with results considered significant at  $p < 0.05$ . Data for each animal group were averaged, and the standard error and standard deviation were calculated.

### Results

In control rats of the first two groups, the myocardium macroscopically showed almost no differences from that of the intact animals. In the third group, noticeable flabbiness of the heart and dilation of its chambers were observed.

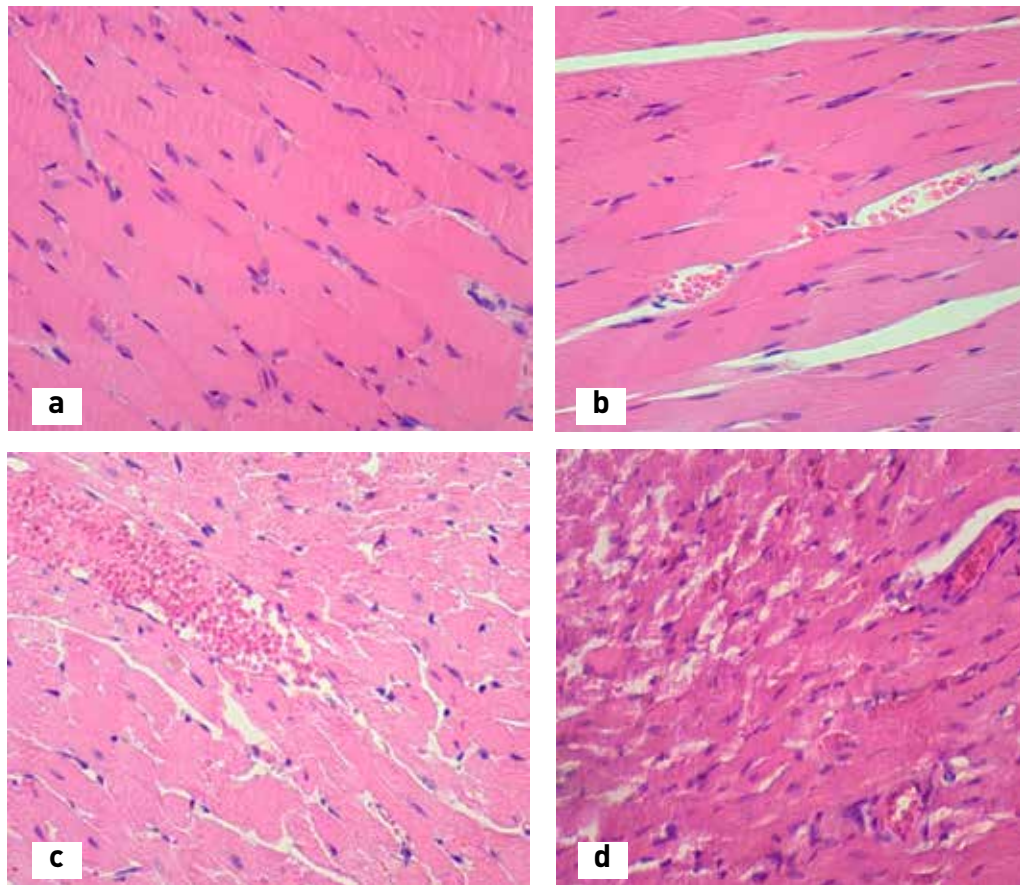
Histological examination of the left ventricular (LV) myocardium in the first group revealed vascular congestion (Fig. 1a-b); in the second group, hemorrhages (Fig. 1c); and in the third group, marked dystrophic changes in cardiomyocytes and edema. Signs of cytoplasmic homogenization and interstitial edema with vascular congestion were also identified (Fig. 1d).

Histological examination of the LV myocardium in all groups showed varying degrees of cardiomyocyte hypertrophy, ranging from mild in the first group to severe in the third group. The cytoplasm of the enlarged cardiomyocytes was hypereosinophilic with hyperchromatic nuclei. Binucleated cardiomyocytes, as well as diploid and tetraploid cells, were detected in all rat groups. In the first group, diploid cardiomyocytes were the predominant cell type in the myocardium. Against the background of moderate cardiomyocyte hypertrophy, polyploidy developed primarily due to binucleated cells.

The highest level of polyploidy against the background of severe cardiomyocyte hypertrophy was observed in the third group, characterized by an increase in the number of mononucleated tetraploid cells to  $32.9 \pm 7.3\%$  and a decrease in diploid cardiomyocytes to  $67.1 \pm 8.0\%$ . A reduction in the number of binucleated cells to  $7.1 \pm 5.0\%$  was also noted (Table 1).

As shown in Table 2, even after 30 days, the morphological parameters in the first and second groups showed only minor differences from those observed immediately after the experiment: nuclear diameter and the number of binucleated cardiomyocytes were close to the levels in intact animals, while the number of tetraploid nuclei was somewhat higher than immediately post-experiment.

In the third group, marked hypertrophy, a reduced number of binucleated cardiomyocytes, and a decreased proportion of diploid cells ( $71.7 \pm 6.0\%$ ) per-



**Fig. 1.** Microscopic view of the heart: Cardiomyocytes of intact animals (a) and under light physical exercise (b): vascular congestion; c — under moderate physical exercise: edema, hemorrhagic site; d — under heavy physical exercise: cardiomyocytes cytoplasm homogenization, interstitial edema; vascular congestion. Hematoxylin and eosin stain,  $\times 400$

**Table 1. Comparative morphology of left ventricular cardiomyocytes in intact rats immediately after graded physical exercise**

Parameters	Intact animals	Group 1	Group 2	Group 3
Diameter of nuclei of cardiomyocytes, $\mu\text{m}$	$5.2 \pm 0.8$	$5.0 \pm 1.6$	$5.1 \pm 0.8$	$7.2 \pm 5.2$
Number of binucleated cardiomyocytes, ‰	$12.7 \pm 1.9$	$14.2 \pm 3.4$	$10.2 \pm 3.4$	$7.1 \pm 5.0^*$
Diploid/tetraploid cardiomyocytes nuclei, %	$91.6 \pm 7.4 / 8.2 \pm 6.3$	$90.9 \pm 4.2 / 9.1 \pm 4.2$	$93.8 \pm 3.2 / 6.2 \pm 6.0$	$67.1 \pm 8.0 / 32.9 \pm 7.3^*$
Ki-67 positive nuclei, %	0	0	0	0

**Note:** \* — indicates a statistically significant difference from the intact (control) group,  $p < 0.05$ .

sisted, due to an increase in tetraploid cells ( $28.3 \pm 6.3\%$ ). Thus, the cardiomyocyte hypertrophy and polyploidization via mononucleated cells, which developed under heavy load, persist for a considerable time.

Tables 3 and 4 present comparative characteristics of cardiomyocytes under physical exercise of varying

**Table 2. Morphological parameters of left ventricular cardiomyocytes in rats 30 days after graded exercise**

Parameters	Group 1	Group 2	Group 3
Diameter of nuclei of cardiomyocytes, $\mu\text{m}$	$5.1 \pm 1.8$	$5.2 \pm 0.8$	$7.2 \pm 6.23$
Number of binucleated cardiomyocytes, ‰	$12.6 \pm 2.7$	$18.0 \pm 3.5$	$8.1 \pm 7.0^*$
Diploid/tetraploid cardiomyocytes nuclei, %	$84.4 \pm 3.4 / 15.6 \pm 2.5$	$90.5 \pm 3.1 / 9.5 \pm 2.6$	$71.7 \pm 6.0 / 28.3 \pm 6.3^*$
Ki-67 positive nuclei, %	0	0	0

**Note:** \* — indicates a statistically significant difference from the intact (control) group,  $p < 0.05$ .

intensity, both immediately after the experiments and 30 days post-experiment, under conditions of meldonium administration.

The microscopic picture of the myocardium in rats subjected to physical exercise with meldonium was consistent across all groups: cardiomyocytes had clear boundaries and well-defined striations. The microscopic changes observed in the myocardium under heavy physical exercise are of particular interest.

**Table 3. Comparative morphology of LV cardiomyocytes in control rats immediately after graded exercise (under meldonium treatment)**

Parameters	Group 1	Group 2	Group 3
Diameter of nuclei of cardiomyocytes, $\mu\text{m}$	4.3 $\pm$ 1.7	5.0 $\pm$ 1.9	5.4 $\pm$ 4.3
Number of binucleated cardiomyocytes, ‰	17.0 $\pm$ 2.6	24.3 $\pm$ 3.0*	15.6 $\pm$ 5.3*
Diploid/tetraploid cardiomyocytes nuclei, %	91.9 $\pm$ 3.5/ 8.1 $\pm$ 3.2	94.0 $\pm$ 3.1/ 6.0 $\pm$ 4.2	83.0 $\pm$ 3.6/ 17.0 $\pm$ 3.5*
Ki-67 positive nuclei, %	0	0	0

**Note:** \* — indicates a statistically significant difference from the intact (control) group,  $p < 0.05$ .

**Table 4. Morphological Parameters of LV cardiomyocytes in control rats at 30 days post-exercise (under meldonium treatment)**

Parameters	Group 1	Group 2	Group 3
Diameter of nuclei of cardiomyocytes, $\mu\text{m}$	4.6 $\pm$ 2.0	5.0 $\pm$ 1.6	5.3 $\pm$ 2.6
Number of binucleated cardiomyocytes, ‰	13.2 $\pm$ 1.8	17.1 $\pm$ 2.6	16.2 $\pm$ 3.0
Diploid/tetraploid cardiomyocytes nuclei, %	90.2 $\pm$ 3.5/ 9.8 $\pm$ 4.1	90.7 $\pm$ 3.4/ 9.3 $\pm$ 4.0	82.7 $\pm$ 3.1/ 17.3 $\pm$ 3.*
Ki-67 positive nuclei, %	0	0	0

**Note:** \* — indicates a statistically significant difference from the intact (control) group,  $p < 0.05$ .

Similar to the rats in the first two groups, the cardiomyocytes had clear boundaries without signs of dystrophic changes. The stroma showed no signs of edema, inflammatory cells, or hemorrhages, and the vessels were moderately congested. The absence of alterative changes in the myocardium of the third group rats can be objectively explained, in our view, by the morphometric parameters of the cardiomyocytes, which indicate that the heart was functioning without signs of overexertion.

In the groups with meldonium administration, the morphological parameters throughout the experiment (from the first to the 30th day) after a single exercise session did not change fundamentally compared to the baseline values; only a slight increase in the number of binucleated cardiomyocytes and diploid cells was observed.

The data from the third group are of particular interest. In contrast to the baseline data, cell hypertrophy was reduced, the number of binucleated cardiomyocytes increased, and the number of tetraploid mononucleated cells decreased.

## Discussion

This study establishes that physical exercise induces myocardial polyploidization. Polyploidy can occur in cells of various tissues, including liver parenchyma, cardiac muscle cells, bone marrow megakaryocytes, and placental trophoblasts. This phenomenon is observed during normal organ development [6–11]. Polyploidy is achieved either through nuclear division without cytokinesis, resulting in a binucleated polyploid cell, or through genome duplication within a single nucleus, resulting in a mononucleated cell of higher ploidy. It leads to an increase in cellular size, nuclear size, and the appearance of binucleated cells [8]. Increased functional load on an organ elevates its ploidy level [8]. Furthermore, polyploid cells exhibit higher metabolic activity compared to diploid cells [6, 8]. Consequently, the process of polyploidization results in cell hypertrophy, which constitutes an organ's response to increased functional demand.

In the control groups, myocardial polyploidization in rats of the first and second groups (light and moderate exercise) was achieved in two ways: through the formation of binucleated cardiomyocytes and through the accumulation of nuclear material in the genome, resulting in nuclei of higher ploidy (predominantly tetraploid). Heavy physical exercise (third group), against a background of alterative processes in the myocardium, increased the number of tetraploid cardiomyocytes while decreasing the number of binucleated cells. Under conditions of organ overexertion, such as heavy physical exercise, the process of polyploidy develops primarily through nuclear hypertrophy of cardiomyocytes via the formation of mononucleated polyploid cells.

In the experiment with meldonium administration during physical exercise, an increase in the number of binucleated cells was observed across all groups, with a corresponding decrease in mononucleated tetraploid cells. Notably, myocardial hypertrophy was absent in these cases.

Immunohistochemical analysis for the proliferation marker Ki-67 did not reveal positive nuclei under conditions of meldonium application. The absence of proliferation markers is generally accepted in the literature, however, some researchers have observed Ki-67-positive cardiomyocyte nuclei in the heart following myocardial infarction [7]. Thus, the use of meldonium during swimming exercise led to an increase in binucleated cells and a reduction in the

degree of cellular hypertrophy. This effect is likely attributable to meldonium's ability to reduce carnitine synthesis and the transport of long-chain fatty acids across cell membranes, preventing the accumulation of activated forms of non-oxidized fatty acids — acyl-carnitine and acyl-CoA derivatives. The decreased carnitine concentration promotes the synthesis of gamma-butyrobetaine, which possesses vasodilatory properties. Meldonium restores the balance between oxygen delivery and consumption in cells and prevents the impairment of adenosine triphosphate transport [5].

## Conclusion

This study revealed that heavy physical activity induces myocardial polyploidization accompanied by persistent nuclear hypertrophy. Microscopic examina-

tion of the heart showed signs of alterative damage. These changes persisted for up to 30 days after the swimming sessions concluded.

The administration of meldonium in the experiment significantly altered the morphological picture of the myocardium in a positive direction. This was particularly evident in the myocardium of rats subjected to heavy exercise. In these cases, the resulting myocardial polyploidy occurred largely through the formation of binucleated polyploid cells. This process took place concurrently with moderate hypertrophy and the formation of mononucleated polyploid cardiomyocytes. The microscopic architecture of the myocardium was virtually indistinguishable from the normal heart structure.

**Conflict of interest:** none declared.

## References

1. Alpidovskaya OV, Malyshev II, Romanova LP. Changes in the expression of the TGFB1 gene and the level of TGF- $\beta$ 1 in the liver during exercise load of varying degrees. *Bulletin of Experimental Biology and Medicine*. 2025;179(2):203–207. DOI:10.47056/0365-9615-2025-179-2-203-207
2. Malyshev II, Alpidovskaya OV, Romanova LP. Morphological changes of neurocytes in rats during physical exertion of the different intensity. *Medical News of North Caucasus*. 2024;19(1):49–52. DOI: 10.14300/mnnc.2024.19011
3. Sushchevich DS, Rudchenko IV, Kachnov VA. The influence of physical exercise on metabolism and remodeling of the cardiovascular system. *Science of the Young — Eruditio Juvenium*. 2020;3: 433–443. DOI: 10.23888/HMJ202083433-443
4. Smirnova AD, Novitsky AV, Shmoilova AS, Schwartz YuG. Risk of sudden cardiac death in those involved in strength training. *Russian Journal of Cardiology*. 2021;26(4S):4394. DOI:10.15829/1560-4071-2021-4394
5. Statsenko ME, Shilina NN, Turkina SV. Use of meldonium in the combination treatment of patients with heart failure in the early post-infarction period. *Therapeutic Archive*. 2014;86(4):3035.
6. Borodkina A. G. Polyploidy — an effective selection method. *SSSK*. 2021; 1–2: 18–20. DOI: 10.24411/2500–0454-2021-10105
7. Sukhcheva TV, Chudinovskikh YuA, Ereemeeva MV. Proliferative potential of cardiomyocytes in hypertrophic cardiomyopathy: relationship with myocardial remodeling. *Cellular technologies in biology and medicine*. 2016;3:196–207.
8. Brodsky VYa, Kudryavtsev BN, Bezbordkina NN. Journal of General Biology. Cellular polyploidy. Myocardium. Liver. Ontogenesis and regeneration. 2024;85(1):47–61.
9. Abouleisa, RRE, Farraj KA, Mehta SG et al. Cardiomyocyte maturation and proliferation is a flip coin. *BMC Cardiovasc Disord*. 2025. DOI: 10.1186/s12872-025-05360-w
10. Singh BN, Yucel D, Garay BI, Tolkacheva EG, Kyba M, Perlingeiro RCR, van Berlo JH, Ogle BM. Proliferation and maturation: Janus and the Art of cardiac tissue engineering. *Circ Res*. 2023;132(4):519–40.
11. Zhao MT, Ye S, Su J, Garg V. Cardiomyocyte proliferation and maturation: two sides of the same coin for heart regeneration. *Front Cell Dev Biol*. 2020;8:594226.