# Ultrastructural Stereological Analysis of Absolute Parameters of Cardiomyocytes Exposed to Contrasting Temperature Effects

**E. L. Lushnikova and L. M. Nepomnyashchikh**

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It is shown that moderate low and superlow temperatures as well as general overheating of the organism cause a marked decrease in the total volume of mitochondria and sarcoplasmic matrix in cardiomyocytes of the left ventricle. These alterations develop in all cases and do not depend on changes (an increase or decrease) in the heart mass. The most pronounced alterations in the total volume and area of the main organelles of cardiomyocytes are noted after general cooling of the organism.

**Key Words**: *general cooling; general overheating; cardiomyocyte ultrastructure; stereology; absolute indexes*

General cooling and overheating of homoiothermic animals induces pronounced morphofunctional reorganizations in the cardiovascular system [1,8]. Adaptive-compensatory processes developing under these conditions involve spatial tissue and intracellular reorganization of the myocardium [2,4,7]. This is manifested in unbalanced changes of the volume and area of the main tissue and cell components. For an assessment of such changes the volume and surface density of the appropriate structures must be analyzed. However, these quantitative indexes reflect just the relative changes of structures in cells or in an entire organ. Calculation of the absolute parameters is required in order to elucidate the changes of total volume and area of the cell and tissue structural components, especially in the case of diverse changes in the volume (mass) of an organ and relative quantitative indexes of structures. Only thus is it possible to judge the extent of the reorganizations at the organ level and to obtain information on the direction of adaptive-compensatory reactions.

The pattern of changes of the absolute quantitative indexes of the principal organelles is not yet understood for cardiomyocytes (CM) exposed to contrasting temperature effects. This was the goal of our study.

## MATERIALS AND METHODS

Alterations in the total volume and area of the main sarcoplasmic organelles were assessed in CM of Wistar rats using the following experimental models: under conditions of a moderate general cooling at 3-4°C for 8 weeks, after a general supercooling at -7°C for 16 days, and, on the 3rd and 7th, day after a one-time general overheating at 43°C for 45 min.

The weight of the heart and of the left ventricle was determined for each experimental group of rats. Tissue samples from the left ventricle myocardium were then fixed in 4% paraformaldehyde, postfixed in 1% osmium tetroxide, and, after dehydration, embedded in an Epon 812 and Araldite M mixture. Ultrathin sections were prepared with an LKB III ultratome. After contrasting with uranyl

Laboratory of Histocytometry and Stereology, Laboratory of General Pathological Anatomy, Research Institute of Regional Pathology and Pathomorphology, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk (Presented by V. V. Lyakhovich, Member of the Russian Academy of Medical Sciences)

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acetate and lead citrate they were examined with a JEM 100B electron microscope.

The absolute total volume ($V_j$) and area ($S_j$) of the CM organelles were calculated for the left ventricle myocardium in each experimental group by the equations:

$$V_j = V^{cyt}_{vj} \times V^{ts}_{vcyt} \text{ and } S_j = S^{cyt}_{vj} \times V^{ts}_{vcyt},$$

where $V^{cyt}_{vj}$ and $S^{cyt}_{vj}$ are the volume and area of organelles per the unit of volume of the CM cytoplasm, respectively, and $V^{ts}_{vcyt}$ is the volume density of the CM cytoplasm in myocardial tissue. In this case $V^{cyt}_{vj}$ and $S^{cyt}_{vj}$ were determined by stereological methods during electron-microscopic study, whereas $V^{ts}_{vcyt}$ was estimated by tissue stereological analysis of the semithin sections using the calculated indexes of the volume of the left ventricle myocardium [3]. Volume and area were estimated for myofibrils, mitochondria, sarcoplasmic reticulum, T system, and sarcoplasmic matrix.

The results were subjected to statistical treatment and the reliability of differences was determined by the Student $t$ test.

## RESULTS

Under the conditions of the chosen temperature regimes the weight and volume of the left ventricle myocardium reliably decreased after general supercooling and general overheating (by 15 and 27%, respectively) and increased for moderate general cooling (by 10-14%, Fig. 1).

The most pronounced alterations in volume and area of the main CM sarcoplasmic organelles were found with general cooling, both moderate and excessive. The moderate general cooling caused an increase of the total volume and area of the majority of the sarcoplasmic organelles except for the mitochondria and sarcoplasmic matrix (Table 1).

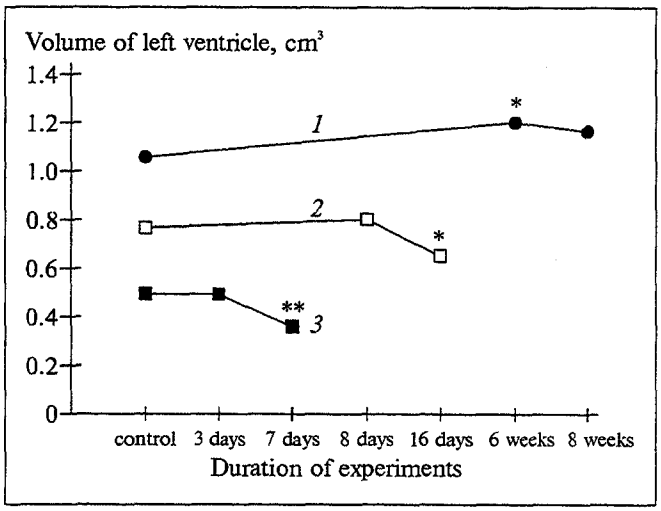


**Fig. 1.** Changes of total volume of the left ventricle of Wistar rats exposed to moderate general cooling (*1*), general supercooling (*2*), and after a one-time general overheating (*3*). $^*p<0.05$, $^{**}p<0.01$.

Whereas the volume and surface characteristics of the organelles were maximally increased after 6 weeks of general cooling, the corresponding indexes for the mitochondria and sarcoplasmic matrix were lowest after 8 weeks of the same temperature effect.

The general supercooling induced an increase of the total volume and area of the myofibrils (by 34 and 46%, respectively) on the 8th day of experiment. Toward the 16th day these indexes were no different from the control values. The volume and area of the other sarcoplasmic organelles (mitochondria, sarcoplasmic reticulum, and T system) and sarcoplasmic matrix decreased successively to the 16th day of cooling (Table 2). With this mode of action the largest drop was noted for the volume and area of the T system (by 62 and 60%, respectively) and for the total volume of sarcoplasmic matrix (by 66%).

The one-time general overheating caused a drop of the total volume and area of the myofibrils and

**TABLE 1.** Absolute Stereological Parameters of Wistar Rat CM after Moderate General Cooling of the Organism ($M \pm m$)

| Index | Control | Duration of cooling | |
|---|---|---|---|
| | | 6 weeks | 8 weeks |
| Total volume, $cm^3$: | | | |
| myofibrils | 0.463±0.014 | 0.623±0.026* | 0.602±0.045 |
| mitochondria | 0.301±0.013 | 0.278±0.010 | 0.254±0.016 |
| sarcoplasmic reticulum | 0.018±0.003 | 0.030±0.005 | 0.022±0.004 |
| T system | 0.014±0.0006 | 0.021±0.004 | 0.017±0.001 |
| sarcoplasmic matrix | 0.116±0.014 | 0.114±0.011 | 0.101±0.009 |
| Total area, $m^2$: | | | |
| myofibrils | 1.707±0.184 | 1.916±0.172 | 2.105±0.168 |
| mitochondria | 1.929±0.023 | 1.653±0.089 | 1.812±0.223 |
| sarcoplasmic reticulum | 0.253±0.044 | 0.445±0.061 | 0.330±0.042 |
| T system | 0.184±0.002 | 0.253±0.036 | 0.199±0.036 |

**Note.** Here and in Tables 2 and 3: $^*p<0.05$, $^{**}p<0.01$.

**TABLE 2.** Absolute Stereological Parameters of Wistar Rat CM after General Supercooling of the Organism ($M \pm m$)

| Index | Control | Duration of cooling | |
|---|---|---|---|
| | | 8 days | 16 days |
| Total volume, $cm^3$: | | | |
| myofibrils | 0.321±0.006 | 0.431±0.031* | 0.321±0.005 |
| mitochondria | 0.221±0.020 | 0.171±0.008 | 0.157±0.013* |
| sarcoplasmic reticulum | 0.014±0.002 | 0.010±0.001 | 0.008±0.0005 |
| T system | 0.013±0.001 | 0.006±0.001* | 0.005±0.0005* |
| sarcoplasmic matrix | 0.077±0.010 | 0.056±0.005 | 0.026±0.002* |
| Total area, $m^2$: | | | |
| myofibrils | 0.942±0.098 | 1.376±0.127* | 0.969±0.035 |
| mitochondria | 0.999±0.119 | 1.086±0.102 | 0.833±0.105 |
| sarcoplasmic reticulum | 0.203±0.033 | 0.212±0.025 | 0.174±0.023 |
| T system | 0.195±0.007 | 0.097±0.017** | 0.078±0.009** |

mitochondria. These quantitative indexes were reduced by 21 and 13%, respectively, for the myofibrils and by 33 and 11%, respectively, for the mitochondria (Table 3) toward the 7th day of the experiment. The total volume of the sarcoplasmic reticulum and T system did not change markedly in the course of the experiment, whereas the total area of these cellular compartments increased, the increase being most pronounced on the 3rd day after overheating. Under this regime of thermal action a gradual decrease was noted for the total volume of sarcoplasmic matrix with a reliable 44% reduction on the 7th day after overheating.

Analysis of the dynamics of the volume and area of the main sarcoplasmic organelles of the CM under the contrasting temperature effects revealed some regularities in their structural reorganization, such as a diminution of the total volume of the mitochondria and sarcoplasmic matrix (Fig. 2). These alterations were noted for all experimental models, no matter how the weight and volume of the left ventricle changed.

A relative decrease of the volume of the mitochondria in the CM (in comparison with the volume of the myofibrils and sarcoplasm as a whole) was found in myocardial hypertrophy and atrophy of different genesis [5,6,15]. At the same time the absolute volume of the mitochondria as well as other organelles has always been found to decrease in myocardial dystrophy [5], while in hypertrophy it increases or does not change [6]. The results of our study suggest that an absolute decrease of the mass of the powerhouses of the cell - the mitochondria - occurs under temperature action both in hypertrophy and in atrophy of the myocardium. The intensification of the functional activity of mitochondria and their more rapid subsequent degradation may be a result of involvement of the heart in noncontractile thermogenesis under general cooling of the organism, calling for extra heat production. This is

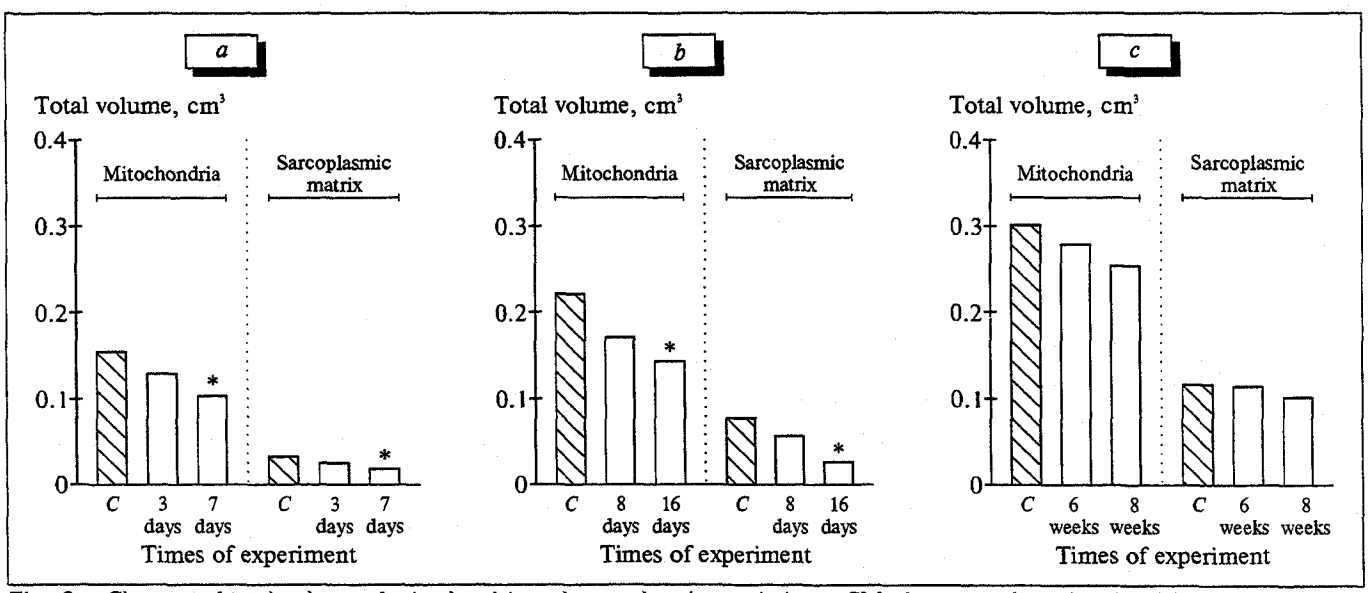


**Fig. 2.** Changes of total volume of mitochondria and sarcoplasmic matrix in rat CM after general overheating (*a*) and supercooling (*b*) and moderate cooling (*c*). *Differences are reliable in comparison to the corresponding control.

**TABLE 3.** Absolute Stereological Parameters of Wistar Rat CM after a One-Time General Overheating of the Organism ($M \pm m$)

| Index | Control | Time after overheating, days | |
|---|---|---|---|
| | | 3rd | 7th |
| Total volume, $cm^3$: | | | |
| myofibrils | 0.196±0.014 | 0.187±0.008 | 0.155±0.016 |
| mitochondria | 0.154±0.006 | 0.127±0.014 | 0.103±0.012* |
| sarcoplasmic reticulum | 0.009±0.001 | 0.010±0.002 | 0.008±0.001 |
| T system | 0.004±0.001 | 0.005±0.0004 | 0.005±0.0006 |
| sarcoplasmic matrix | 0.032±0.003 | 0.025±0.005 | 0.018±0.002* |
| Total area, $m^2$: | | | |
| myofibrils | 0.757±0.076 | 0.794±0.075 | 0.660±0.090 |
| mitochondria | 0.647±0.079 | 0.659±0.043 | 0.575±0.064 |
| sarcoplasmic reticulum | 0.124±0.019 | 0.206±0.071 | 0.175±0.034 |
| T system | 0.054±0.009 | 0.083±0.020 | 0.063±0.012 |

confirmed by results of ultrastructural studies in rat CM subjected to general cooling. Such CM contain a myelinlike bodies which are mainly located in places where mitochondria are found and are specific autophagal vacuoles containing products of mitochondrial degradation. It is conceivable that new formation of mitochondria slows down due to a disturbed biosynthesis of certain structural proteins.

The similar pattern of alterations in the mitochondrial compartment of the CM after a one-time general overheating is probably due to the overall inhibition of intracellular regeneration. Since the half-life of the structural mitochondrial proteins (around 5.5 days) is less than that of myofibrils (6-9 days) [13], the mitochondrial population will be diminished faster against the background of lowered biosynthesis.

It should be taken into account that extreme factors may affect not only the biosynthesis of structures, but their degradation (lysis) as well. The destruction of myofibrils and mitochondria as well as other sarcoplasmic organelles reportedly occurs in different ways [9]. Degradation of myofibrilar structures is probably a two-step process: first they are destroyed by neutral and basic proteases [11,12] and then their residues are utilized by lysosomal enzymes [10]. Lysosomes alone accomplish the destruction of mitochondria [14]. The activity of lysosomal enzymes possibly increases in CM under extreme temperature effects, and as a result the decay of mitochondria is boosted. At the same time lytic changes of myofibrils are also noted in CM, but the intensity of these processes is ultimately lower than in the case of mitochondria.

Thus, extreme temperatures cause a significant decrease in the total volume of the mitochondria and sarcoplasmic matrix in the CM of the left ventricle probably due to the developing imbalance between catabolic and anabolic processes.

## REFERENCES


1. A. P. Avtsyn, A. A. Zhavoronkov, A. G. Marachev, and A. P. Milovanov, *Human Pathology in the Far North* [in Russian], Moscow (1985).
2. E. L. Lushnikova, L. M. Nepomnyashchikh, M. G. Klinnikova, and O. P. Molodykh, *Byull. Eksp. Biol. Med.*, **116**, № 7, 81-85 (1993).
3. L. M. Nepomnyashchikh, E. L. Lushnikova, L. V. Kolesnikova, *et al., Morphometric and Stereological Analysis of the Myocardium: Tissue and Ultrastructural Organization. Procedural Guidelines* [in Russian], Novosibirsk (1984).
4. L. M. Nepomnyashchikh, *Morphogenesis of the Main Pathological Processes in the Heart* [in Russian], Novosibirsk (1991).
5. L. M. Nepomnyashchikh, L. V. Kolesnikova, and G. I. Nepomnyashchikh, *Morphology of Heart Atrophy: Three-Dimensional Tissue and Ultrastructural Organization* [in Russian], Novosibirsk (1989).
6. L. M. Nepomnyashchikh, E. L. Lushnikova, and G. I. Nepomnyashchikh, *Morphometry and Stereoloy of Cardiac Hypertrophy* [in Russian], Novosibirsk (1986).
7. L. M. Nepomnyashchikh, E. L. Lushnikova, G. I. Nepomnyashchikh, *et al., Byull. Eksp. Biol. Med.*, **104**, № 11, 625-630 (1987).
8. F. F. Sultanov, B. I. Tkachenko, and G. F. Sultanov, *The Circulation in Hyperthermia* [in Russian], Ashkhabad (1988).
9. W. J. Carter, B. W. S. Van der Weijden, and F. H. Faas, *Metabolism*, **29**, 910-915 (1980).
10. M. G. Clark, C. J. Beinlich, E. E. McKee, *et al., Fed. Proc.*, **39**, 26-30 (1980).
11. J. Kay, R. F. Siemankowski, J. A. Greweling, *et al., Acta Biol. Med. Germ*, **40**, 1323-1331 (1981).
12. H. E. Morgan, B. Chua, and C. J. Beinlich, in: *Degradative Processes in the Heart and Skeletal Muscle*, Amsterdam (1980), pp. 87-113.
13. M. Rabinowitz, Amer. *J. Cardiol.*, **31**, 202-210 (1973).
14. M. Rabinowitz and R. Zak, *Circ. Res.*, **36**, 367-376 (1975).
15. E. W. Thompson, *Proc. Soc. Exp. Biol. Med.*, **205**, 294-305 (1994).