EFFECT OF 12-DAY SPACE FLIGHT ON THE TITIN ISOFORM COMPOSITION AND SARCOMERIC ORGANIZATION OF SKELETAL MUSCLE OF MONGOLIAN GERBIL (*MERIONES UNGUICULATES*)

A.D. Okuneva [1,2], I.M. Vikhlyantsev [1], V.V. Rogachevsky [3], N.N. Salmov[1], Z.A. Podlubnaya [1,2], A.I. Grigor'ev[4]

[1]*Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino, Russia;*

[2] *Pushchino State Institute of Natural Sciences; Pushchino, Russia;*

[3] *Institute of Cell Biophysics, Russian Academy of Sciences, Pushchino, Russia*

[4]*SSC RF Institute for Biomedical Problems, Russian Academy of Sciences, Moscow*

The locomotory system of animals and humans develops under gravity conditions and organized with regard to the action of gravitational forces. The absence of support loads in microgravity conditions is accompanied by changes in the functions of vestibular and locomotor systems [1]. In particular, it leads to the development of structural and functional changes in skeletal muscles, known as "hypogravitational muscle syndrome" (HMS) [1]. HMS manifests in the reduction of muscle tone, decrease of strength of muscle contractions, muscle hyperreflection and coordination disorders in the short-term (2-5 days) influence of microgravity. These disorders are accompanied by the development of atrophy of muscle fibers when the influence of microgravity is more long. Atrophy is accompanied by destructive changes in the thick and thin filaments, in particular, the degradation of myosin heavy chains is increased, the content of titin, nebulin, X-protein is decreased, integrity of dystrophin layer of sarkolemmal membrane is disturbed, myosin phenotype is transformed toward to the increase the proportion of fast isoforms of myosin heavy chains. Later the muscle tone, endurance and the general muscle performance are reduced. The most changes are observed in the muscle soleus, because it consists of slow fibers by 85-90%.

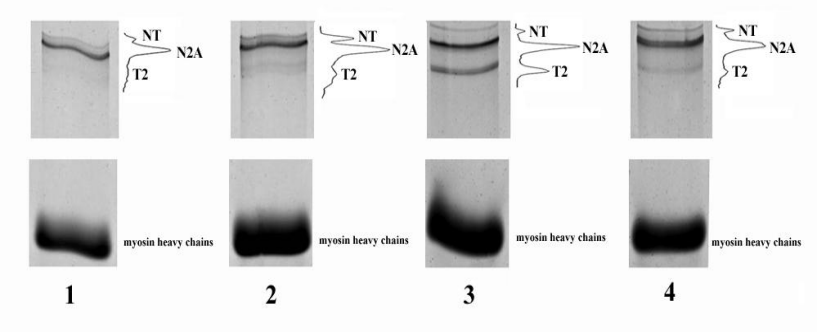
In earlier studies we observed decrease in the content of N2A and a more high molecular weight (NT) titin isoforms in m. soleus of human and rat under conditions of simulated microgravity [2,3]. These changes contribute to the development of the HMS.

In this paper we present the results of the study of changes in titin isoform composition and sarcomeric structure in *m. soleus, m. tibialis anterior* and *m. gastrocnemius* of Mongolian gerbils (*Meriones unguiculates*) after the 12-day flight on the Russian spacecraft "Photon M3".

Methods. 2.0-2.3% SDS-PAGE with the addition of agarose (0.5-0.6%) were used for electrophoretic studies of titin isoformes composition by method [4]. The content of titin was evaluated in relation to the content of myosin heavy chains. Western blotting of titin was performed by the method [2]. Antibodies AB5 to titin was used as the primary antibodies. These

antibodies bind to the titin molecule in the A-disk around the M-line of the sarcomere. Muscle tissue samples were fixed for 2 hours in 1.25% glutaraldehyde with 3% formaledgide in 0.1 M Na-kakodilat buffer for electron microscopic studies. The samples were transferred to 1% solution of OsO4 for 1 hour. Dehydration of the samples was carried out by ethanol of different concentrations and the acetone. Fixed and dehydrated samples were filled by a mixture of Araldite M. and Epon 812 resins. Cutting of samples was carried out on ultratome with slope angle of the knife 5˚. Negative staining of samples was performed by 1%-aqueous solution of uranyl acetate and lead citrate.

Results and discussion. Taking into account that the content of N2A and NT isoforms in m. soleus of human and rat decreased under conditions of simulated microgravity [2,3,5], we expected to find a similar decrease of the titin content in skeletal muscles of Mongolian gerbils after space flight. However, electrophoretic studies was found the increase of NT-titin isoform content in m. tibialis anterior, m gastrocnemius, m.soleus at 1,12-2,0 times after space flight. At the same time in m. tibialis anterior was observed conservation of the N2A-titin isoform content, but in m. soleus and m gastrocnemius was found reduction (in 1,2-1,25 times) of N2A-titin (figure). In all these muscles the content of T2-titin fragment was decreased in 1.3-1.4 times (table).



The changes in titin isoform composition in skeletal muscles of Mongolian gerbils under real microgravity conditions. Electrophoresis was performed in a vertical mini-gel containing 0.55% agarose and polyacrylamide 2.1-2.3%.
1 - m.soleus of control group gerbils, 2 - m. soleus of flight group gerbils, 3 - m. tibialis anterior of control group gerbils, 4 - m. tibialis anterior of flight group gerbils.

It is known that the decrease of N2A-titin isoform content is accompanied by disruption of the structure and contractile properties of rat soleus muscle under conditions of simulated microgravity [5]. Similar abnormalities of structure and functional properties of muscles as well as the decrease of titin content were observed in muscles after the influence of ionizing radiation [6]. We hypothesized that decrease of the titin content in m. soleus of gerbils after

Content of titin isoforms in gerbil skeletal muscles

Muscle	Group	NT/ТЦМ	N2A/ТЦМ	T2/ТЦМ	NT/N2A
m.soleus	control	0,025	0,127	0,020	0,188
	flight	0,028	0,102	0,015	0,247
m. tibialis anterior	control	0,016	0,146	0,071	0,108
	flight	0,031	0,145	0,051	0,126
m. gastrocnemius	control	0,015	0,157	0,087	0,101
	flight	0,030	0,133	0,062	0,163

space flight (table) could lead to a damage of muscle sarcomeric organization, because atrophic changes in this muscle were observed [7]. However, the results of our studies did not reveal any disorders of highly ordered sarcomeric structure in m. soleus of gerbils after space flight. It should be noted that the decrease of contractile ability and the reduction of the Ca2+-sensitivity of force development of m. soleus fibers of gerbils flight group was not found [7].

It is interesting that the changes in titin isoform composition in atrophied skeletal muscles of hibernating ground squirrels (Spermophilus undulatus) are similar to those in muscles of gerbils in conditions of space flight. In particular, it was found the preservation of the content of NT-titin isoform with a decrease in 1.3-1.5 times the content of N2A-titin isoform in ground squirrel skeletal muscles during hibernation [8,9]. These changes are not accompanied by disturbances of structural and functional properties of the skeletal muscle of ground squirrel [9-11]. Taking into account our results and published data about that the decrease of titin content is accompanied by disruption of the structure and contractile properties of muscle [5,6], we conclude that a main role in supporting of structural and functional properties of muscle plays the NT-isoform titin but not N2A-isoform of this protein. It is interesting that the decrease in N2BA- and N2B-titin isoformes content in ~ 1.5 times with preservation of the NT-isoform content in cardiac muscle of hibernating ground squirrels [8] did not lead to disturbances of the sarcomeric structure [9] and contractile properties of myocardium [12]. These data confirms our conclusion.

So, the changes in titin isoform composition in skeletal muscles of gerbils after a 12-day space flight have adaptive nature and are aimed at preserving the content of high molecular weight NT-titin isoforms for the support an ordered sarcomeric structure and contractile activity of muscles.

This work was supported by the Russian Foundation for Basic Research (grant No. 11-04-01026 and a grant of the Federal Targeted Program “Scientific and Scientific-Pedagogical Personal of Innovative Russia”, State Contract No. 02.740.11.0710.

References

1. Grigoriev A.I., Kozlovskaya I.B., Shenkman B.S. // Ros. Fiziol. Zh. im. Sechenova. 2004. V. 5. P. 508-521.

2. Vikhlyantsev I.M., Podlubnaya Z.A., Shenkman B.S., Kozlovskaya I.B. // Dokl. Biochem Biophys. 2006. V. 407 (5). P. 88-90.
3. Vikhlyantsev I.M., Podlubnaya Z.A. // Biofizika. 2008, V. 53(6). P. 1058-1065.
4. Vikhlyantsev I.M., Podlubnaya Z.A., Kozlovskaya I.B. // Doklady AN. 2004. V. 395(6). P. 828-831.
5. Udaka J., Ohmori S., Terui T., Ohtsuki I., Ishiwata S., Kurihara S., Fukuda N. // J. Gen. Physiol. 2008. V. 131(1). P. 33-41.
6. Horowits R., Kempner E.S., Bisher M.E., Podolsky R.J. // Nature. 1986. V. 323. P. 160-164.
7. Lipiec E.N, Ponomareva E.V. Ogneva I.V., Vikhlyantsev I.M, Karaduleva E.V., Kuznecov S.L., Podlubnaya Z.A, Shenkman B.S. // Aviakosm. i ecol. Med. 2009. V. 43. №3. P. 34-39. 8. Vikhlyantsev I.M., Karaduleva E.V., Podlubnaya Z.A. // Biofizika. 2008. V. 53(6). P. 1066-1072.
9. Trapeznikova K., Vikhlyantsev I., Kokoz Yu., Okuneva A., Rogachevsky V.,. Khutsyan S., Salmov N., Podlubnaya Z. // In book: Biological Motility: Fundamental and Applied Science, 2012.
10. Khromov A.S., Srebnickaya L.K., Rozdestvenskaya Z.E., Orlova A.A., Lednev V.V. // In book:"Mechanisms of hibernation", 1987, Pushchino, ONTI, P. 95-101.
11. Steffen J.M., Li Y., Steele P.S., Klueber K.M., Milsom W.K. // In: "Life in the Cold. Ecological, physiological, and molecular mechanisms" (edited by C. Carey, G. Florant, B. Wunder, B. Horwitz), Westview Press, Boulder, San Francisco, Oxford, 1993. p. 511-518.
12. Wang S.Q., Lakatta E.G., Cheng H., Zhou Z. // J. Exp. Biol. 2002. V. 205. P. 2957-2962.

CRYOELECTRON MICROSCOPY OF ACTIN: NEW INSIGHTS AND CONTROVERSY

Albina A. Orlova, Vitold E.Galkin, Edward H. Egelman

Department of Biochemistry and Molecular Genetics, University of Virginia Health System, Charlottesville, Virginia, USA

Electron cryo-microscopy has established itself as a valuable method for the structure determination of protein molecules, protein complexes, and cell organelles. Cryo-observation of vitrified samples allows the ultrastructural study of macromolecules, molecular assemblies and cells in their natural environment. Cryoelectron microscopy combined with single-particle reconstruction methods is a powerful technique to study the structure of biological assemblies at molecular resolution of 3-10A, it provides a means of capturing different conformational states and dynamic interactions of such assemblies. When the components of a macromolecular complex are known to atomic resolution, then the fitting and docking of X-ray coordinates into the lower-resolution cryo-EM maps can provide new insights into the structure of the complexes and their functions.

In cryo-EM the specimen, typically an unstained protein embedded in vitreous ice, is held at cryogenic temperatures while images are formed in the electron microscope. A modern cryo-EM specimen consists of aqueous film that spans holes in the thin carbon film. The protein film is blotted to very