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The effects of simulated microgravity on skeletal muscle of Japanese quail: transmission electron microscopic study

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Abstract

The aim of the present study was to investigate the effects of simulated microgravity (hypodynamia) on the structure of the skeletal muscle (m. gastrocnemius) in developing Japanese quail by transmission electron microscopy. Samples of muscle tissues from experimental (n = 28) and control (n = 28) birds were collected at day 7, 14, 28, 42 and 56 of age.

The structure of m. gastrocnenmius was changed depending on hypodynamia length. The first extensive structural changes were found on day 14 of age. The mitochondria were enlarged and the spaces between the myofibrils were slightly extended compared to control. The sarcomeres were irregular and lipid droplets occurred in the sarcoplasm. Further developmental changes occurred on day 28 of age. Mitochondria fused into the giant mitochondria which frequently exceeded the length of one sarcomere. Moreover, at 42 days of age, beside the above mentioned changes, sarcoplasmic reticulum was dilated and the number of mitochondrial cristae was reduced. However, the structure of m. gastrocnemius on day 56 was less damaged compared to the damage observed on day 42 of age.

Presented results indicate that the continuous stay of male Japanese quail under simulated microgravity has a negative impact on the structure of m. gastrocnemius, but also the ability of muscle tissue to cope with these specific conditions.

Hypodynamia, m. gastrocnemius, mitochondria, sarcoplasmic reticulum

Simulated and real microgravity affect considerably the skeletal muscles and bones. With regard to the considerable mass of skeletal muscles, even relatively slight damage inducing changes to the permeability of cellular membranes results in marked physiological and biochemical changes. Muscle atrophy is an accompanying feature related to a long-term stay of an organism under conditions of weightlessness (Riley et al. 2005; Mazzatti et al. 2008; Tesch et al. 2008). Animal muscles show distinct signs of atrophy, and in the final stage, destruction of muscle proteins inducing decline in muscle volume can be observed (Lebedeva et al. 1998; Litvinova et al. 2007). While during the first stage of exposure to simulated weightlessness, the ion transport through sarcolemma in the rat hind limb is impaired, during the later stages an increased content of proteolytic myofibrils-damaging enzymes can be observed (Reznicka et al. 1995). Changes could be seen even in the capillary network of immobilized muscles. Vascular endothelium is very thin and discontinuous nuclei exhibit an irregular shape with condensed chromatin (Oki et al. 1995). The density of capillary network is reduced and capillary lumina diameter is also smaller (Fujino et al. 2005).

In future long-term space missions, Japanese quail is a suitable animal model representing higher heterotrophic link of the proposed autonomous closed ecosystem at the spaceship or space stations. Low body weight, high productive and reproductive abilities, short individual development as well as a high ability to cope with crowded conditions aboard orbital stations and planetary bases are some of the many reasons for this choice (Boďa 1993). The first space experiments showed that microgravity does not have a dramatic

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impact on embryogenesis of Japanese quail (Guryeva et al. 1993). Nevertheless, the question how microgravity affects further development after hatching remains still open. Ground-based experiments with simulated microgravity can contribute to answering this question.

The aim of the present study was to investigate the effects of simulated microgravity on the structure of skeletal muscle (m. gastrocnemius) in Japanese quail males from day 2 post-hatch to 56 days of age.

Materials and Methods

Hypodynamia is a method to simulate weightlessness in the laboratory conditions on the Earth. The experiment was carried out at the Institute of Animal Biochemistry and Genetics of the Slovak Academy of Sciences in Ivanka pri Dunaji. Fifty-six newly hatched Japanese quail males were used in the present study. On the second day after hatching, 28 chicks of the experimental group were exposed to hypodynamia as described by Škrobánek et al. (2004). At the same time 28 chicks (control group) were placed in a rearing box. Birds from both experimental and control groups were kept under these conditions until day 56 of age in a windowless poultry room with controlled ventilation and electrical heating by infrared lamps. The temperature was adjusted to 35-36 °C for the first few days after hatching and gradually declined to 20 °C at 4 weeks and remained at this level until the end of the experiment. A commercial started mash HYD-13 and water were available *ad libitum*. The diet was granular and contained 260 $g \cdot kg^{-1}$ protein and 11.5 MJ metabolisable $energy \cdot kg^{-1}$. The lighting in the rearing room was continuous. The care and use of animals were in accordance with laws and regulations of the Slovak Republic and approved by the Ethics Committee of the Institute of Animal Biochemistry and Genetics, Slovak Academy of Sciences, Ivanka pri Dunaji and the State Veterinary and Food Agency (ŠVPS SR Č. K. Ro-7879/04-220/3).

Four randomly selected birds of each group were euthanized by cervical dislocation on day 7, 14, 28, 42 and 56 of age. Tissue samples of m. gastrocnemius were collected by small excision. Thereafter, the samples were processed for transmission electron microscopy (TEM).

Samples of m. gastrocnemius were fixed by immersion in 3% glutaraldehyde in cacodylate buffer, pH 7.2, and post-fixed in 1% OsO_4, dehydrated in acetone and embedded in the Durcupan. Ultrathin sections were contrasted with uranyl acetate and lead citrate. Photographs were taken using the TESLA BS 500 electron microscope.

Results

The ultrastructure of normal skeletal muscle is shown in Fig. 1 (Plate XI). Myofibrils in longitudinal sections showed cross-striations formed by alternating segments of dark anisotropic (A band) and light isotropic (I band) bands. Each anisotropic band contained centrally located Hensen disc (H band) with dark transverse plate, mesophragm. The isotropic bands were subdivided by dark thin Z lines demarcating regular segments, sarcomeres. Mitochondria were situated between the myofibrils, beneath the sarcolemma and adjacent to the nucleus. The transverse tubules formed triads with cisterns of sarcoplasmic reticulum near the A and I bands. The oval pail nuclei were located under the sarcoplasma.

The ultrastructural changes of the Japanese quail m. gastrocnemius depended on the length of hypodynamia. There were no marked structural changes in the skeletal muscles observed on day 7 of age. The myofibrils appeared unaltered and sarcomeres were regular, prominently containing A and I bands. The sarcoplasmic reticulum was well developed and the glycogen granules occurred in the spaces between myofibrils. The cristae-rich mitochondria were larger than those in control.

At day 14 of age there were marked changes in mitochondria in terms of their number, size and structure in the group subjected to hypodynamia. In addition to changes in the internal structure, mitochondria were accumulated in bigger areas between the myofibrils. The cristae were less distinct that in control group. The spaces between the myofibrils were slightly extended. In some regions the sarcomeres were irregular and lipid droplets occurred in the sarcoplasm (Plate XI, Fig. 1[2]).

At day 28 of age the mitochondria of the hypodynamia group fused, forming the giant mitochondria which frequently exceeded the length of one sarcomere. They were densely packed with cristae and few electron-dense granules. Dilated spaces between the myofibrils were filled with accumulated mitochondria and lipid droplets. The boundaries between I and A bands were vague and the Z lines were uneven. In some regions, the sarcomeres were narrowed at the level of Z lines (Plate XI, Fig. 1[3]).

At day 42 of age the sarcoplasmic reticulum of the birds from the hypodynamia group showed slightly irregular swelling and the numerous apparently segmented small vesicles. The mitochondria varied in shape and size and showed signs of damage. They were swollen, some cristae were destructed and the mitochondrial matrix contained electron-dense material. The irregular sarcomeres were at the level of Z lines damaged (Plate XI, Fig. 1[4]).

At day 56 of age the structure of m. gastrocnemius in hypodynamia group did not differ significantly from control. However, in some regions of the muscle tissue, the changes were detected. The swollen mitochondria contained disorganized cristae and, in some cases, their usual arrangement was lost. The sarcoplasmic reticulum was irregularly dilated, forming large electron lucent areas. There were changes in the pattern of Z lines that was discontinuous or uneven. Damaged regions were characteristic by myofibrillar lysis with disrupted and frayed sarcomeres. Dilated spaces between adjacent myofibrils contained residua of myofilaments, sarcoplasmic reticulum and damaged mitochondria without the cristae (Plate XII, Fig. 2).

Discussion

Many studies have shown that simulation of weightlessness in animals results in metabolic, functional and structural changes in tissues and organs (Booth 1977; Kočišová et al. 1996; Cigánková et al. 2001; Zibrín et al. 2001; Hudson and Franklin 2002). These changes are not surprising, since hypodynamia and microgravity are known to be stressful (Juráni et al. 1991). Several papers demonstrated significant reduction of body weight, food consumption and size of leg of quail hens induced by hypodynamia from hatching to maturity (Škrobánek et al. 2004, 2005). Microgravity caused also changes in quail behaviour (Košťál et al. 1993).

Our experiments support the idea that hypodynamia causes structural changes in skeletal muscles of Japanese quail. The first extensive structural changes in m. gastrocnemius were observed on day 14 of age. We assume that these morphological changes represent the first stage of adaptation to hypodynamia. The most marked differences were observed in mitochondria that were larger and accumulated on larger areas. At this stage the body mobilized its energy reserves. ATP as the source of energy is produced in the process of degradation of saccharides and other nutrients. Škrobánek et al. (2007) investigated the influence of hypodynamia on growth and development of breast and thigh muscles of Japanese quail and found a significant increase in glycogen on day 14 of age. On the other hand, Wu et al. (2002) observed no changes in the ultrastructure of m. soleus of rats on day 14 of simulated weightlessness (tail suspension) compared to control. According to their study, the most extensive changes particularly enlarged mitochondria and extended terminal cisternae could be observed already on day 4 and 7 of the experiment. These differences can be interpreted in term of difference in the simulation method or in term of species differences.

Further ultrastructural changes of skeletal muscles corresponding to strong stress were detected in our experiment on day 28 of age. Stress in general (hunger, cold, environmental conditions, etc.) results in mobilisation of lipid reserves and their more efficient use (Baranyiová and Standara 1980). Increased content of lipid droplets in the sarcoplasm as a consequence of endurance exercise training was also observed by Hoppeler and Fluck (2003).

Formation of giant mitochondria represents also one of the forms of adaptation to stress situations. Free radicals that arise under physiological conditions, and in an increased amount under pathological conditions related to stress, probably play a crucial role in production of so-called giant mitochondria. Free radicals damage mitochondrial membranes and result in fusion of neighbouring mitochondria and production of giant mitochondria. The formation of giant mitochondria is the consequence of an effort to decrease concentration of oxygen and thus intracellular concentration of oxygen radicals (Wakabayashi 2002). Lawler et al. (2003) and Sui et al. (2008) observed changes in oxidative stress markers and reduction of mitochondrial Mn superoxide dismutase protein content in response to hind limb unloading in rats. Oxidative stress results in damage of sarcoplasmic reticulum, subsequent increase of intracellular calcium (Castilho et al. 1997) and in the end in hydrolysis of phospholipids and proteins (Pascoe and Reed 1989). Free radicals damage lysosomes which lead to the release of lysosomal enzymes and causes subsequent muscle atrophy (Mak et al. 1983). Proteins can be directly damaged by free radicals (Reid 1996). Damage to muscle fibres causes release of iron from myoglobin and the loose iron acts as a catalyst for oxidative processes and accelerates atrophy (Kondo et al. 1993).

Several authors referred to the ability of muscular tissue to cope with the specific conditions of simulated weightlessness. Kočišová et al. (1996) found a similar response of muscle tissue of adult Japanese quail to hypodynamia as we observed in this study on growing birds. These authors described the progress of dystrophic changes in mitochondria, myofibrils and sarcotubular system. Our results indicate that gradual structural changes in muscle tissue depend on length of hypodynamia. The degree of damage varies from slight to more substantial.

There was a significant increase of mitochondria size and number starting from day 14 of age. Their distribution in the muscle fibres was also altered. They tend to aggregate between myofibrils. On day 56 of age, however, the observed structural changes of skeletal muscles were less pronounced, except for some regions. Similar response of quail to hypodynamia was found in the study of total proteins and glycogen in the thigh and breast muscles (Škrobánek et al. 2007).

The obtained results indicate that hypodynamia has a significant impact on the ultrastructural changes in skeletal muscles of developing male Japanese quail and that there are signs indicating coping of skeletal muscles to the prolonged hypodynamia conditions. These data may contribute to our understanding of quail development under the condition of real microgravity.

Štúdium vplyvu simulovanej mikrogravitácie na kostrové svaly prepelice japonskej pomocou transmisnej elektrónovej mikroskopie

Cieľom našej práce bolo sledovať pomocou transmisnej elektrónovej mikroskopie vplyvy simulovanej mikrogravitácie (hypodynamia) na štruktúru kostrovej svaloviny (m. gastrocnemius) u vyvíjajúce sa prepelice japonskej. Vzorky svalového tkaniva boli od pokusných a kontrolných zvierat odobraté vo veku 7, 14, 28, 42 a 56 dní.

Štruktúra m. gastrocnemius bola zmenené v závislosti od trvania hypodynamie. Prvé výrazné štrukturálne zmeny boli zaznamenané vo veku 14 dní. Mitochondrie boli zväčšené a priestor medzi myofibrilami bol mierne rozšírený v porovnaní s kontrolou. Sarkoméry boli nepravidelné a v sarkoplazme sa objavovali tukové kvapky. Ďalšie vývinové zmeny boli pozorované vo veku 28 dní. Mitochondrie sa spájali do gigantických mitochondrií, ktoré boli často dlhšie ako sarkoméry. V 42 dňoch veku, okrem vyššie uvedených zmien, sa v svalovom tkanive objavovali dilatácie sarkoplazmatického retikula a taktiež bol redukovaný počet mitochondriálnych krist. Nakoniec, v 56 dňoch bola štruktúra m. gastrocnemius menej narušená ako vo veku 42 dní.

Dosiahnuté výsledky preukázali, že nepretržitý pobyt prepelice japonskej v simulovanej mikrogravitácii má negatívny vplyv na štruktúru m. gastrocnemius, ako aj na schopnosť svalového tkaniva prispôsobiť sa k týmto špecifickým podmienkam.

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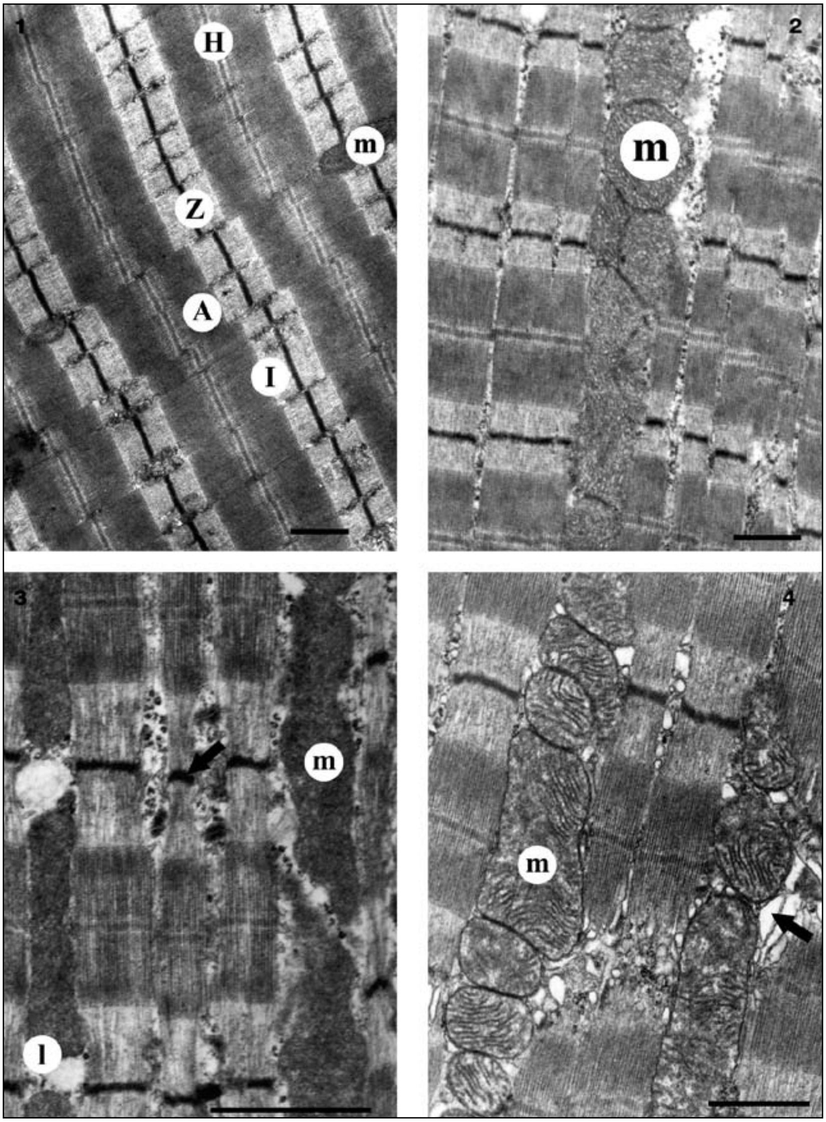

Fig. 1. Electron micrograph of m. gastrocnemius.
1: Control quails: A – anisotropic band, I – isotropic band, H – Hensen disc., Z – band. Bar = 1 µm. 2: Hypodynamia on day 14: spaces between the myofibrils are slightly extended and mitochondria (m) are interspersed among myofibrils. Bar = 1 µm. 3: On day 28 of hypodynamy: extended myofibrilar spaces are filled with giant mitochondria (m) and lipid droplets (l). Z-lines are narrowed (arrow). Bar = 1 µm. 4: On day 42 of age: cisternae of endoplasmic reticulum are dilated (arrow), giant mitochondria (m). Bar = 1 µm

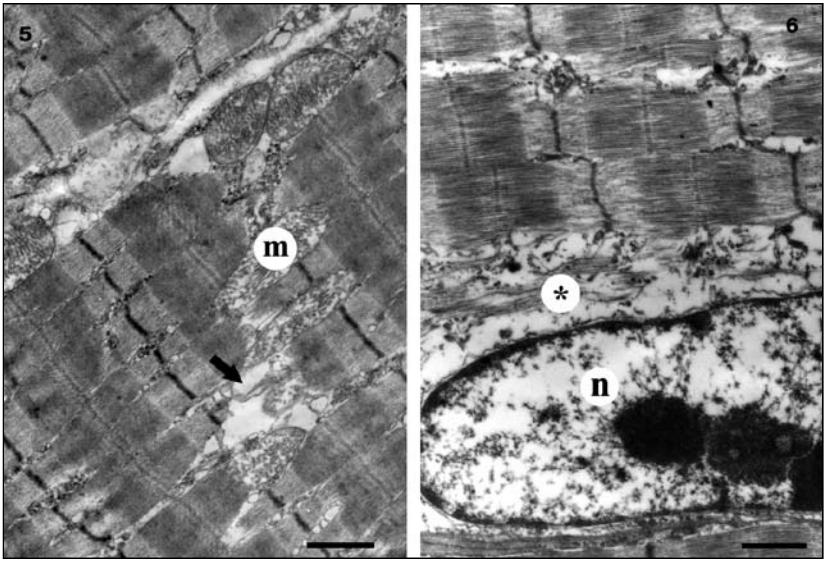

Fig. 2. Electron micrograph of m. gastrocnemius. Hypodynamia on day 56 of age.
5: Mitochondrial structure shows obvious damage and some mitochondria (m) show signs of disintegration, cisternae of endoplasmic reticulum are extended (arrow). Bar = 1 μm. 6: Pronounced changes in the structure of myofibrils (asterisk), nucleus (n). Bar = 1 μm