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Short-term, daily exposure to cold temperature may be an efficient way to prevent muscle atrophy and bone loss in a microgravity environment

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ABSTRACT

Microgravity induces less pressure on muscle/bone, which is a major reason for muscle atrophy as well as bone loss. Currently, physical exercise is the only countermeasure used consistently in the U.S. human space program to counteract the microgravity-induced skeletal muscle atrophy and bone loss. However, the routinely almost daily time commitment is significant and represents a potential risk to the accomplishment of other mission operational tasks. Therefore, development of more efficient exercise programs (with less time) to prevent astronauts from muscle atrophy and bone loss are needed. Consider the two types of muscle contraction: exercising forces muscle contraction and prevents microgravity-induced muscle atrophy/bone loss, which is a voluntary response through the motor nervous system; and cold temperature exposure-induced muscle contraction is an involuntary response through the vegetative nervous system, we formed a new hypothesis. The main purpose of this pilot study was to test our hypothesis that exercise at 4 °C is more efficient than at room temperature to prevent microgravity-induced muscle atrophy/bone loss and, consequently reduces physical exercise time. Twenty mice were divided into two groups with or without daily short-term (10 min × 2, at 12 h interval) cold temperature (4 °C) exposure for 30 days. The whole bodyweight, muscle strength and bone density were measured after terminating the experiments. The results from the one-month pilot study support our hypothesis and suggest that it would be reasonable to use more mice, in a microgravity environment and observe for a longer period to obtain a conclusion. We believe that the results from such a study will help to develop efficient exercise, which will finally benefit astronauts' heath and NASA's missions.

1. Introduction

Microgravity in space-induced muscle atrophy and bone loss are one major threat to astronauts' health (Salanova et al., 2008; Trappe et al., 2009; Sandona et al., 2012; Dabertrand et al., 2012; Fitts et al., 2013). The longer astronauts stay in a microgravity environment, such as a >6-month mission at the International Space Station (ISS) or other space explorations, the worse the muscle atrophy and bone loss is for astronauts. The major reason for microgravity-induced muscle atrophy and bone loss is due to less muscle contractions and less pressure stimulation on bone, which is similar to the muscle atrophy and osteoporosis of the patients who have to lay in bed for several months. Muscle contraction does not only strengthen muscle but also provides pressure on the bone. Therefore, exercise is the most efficient way so far to circumvent microgravity-induced muscle atrophy and bone loss. Until now, an abundance of fitness equipment has been developed for astronauts, and is continually improved upon and used at ISS. On the International Space Station (ISS), each U.S. crewmember exercises for as many as 2.5 hours per day for 6 days per week. This almost daily time commitment is significant and represents a potential risk to the accomplishment of other mission operational tasks (NASA Program Announcement "Spaceflight Research Opportunities in Space Biology" (NNH14ZTT001N NRA) by Space Life and Physical Sciences Research and Applications Division). Therefore, more efficient exercise programs are required for reducing the exercise time, which will allow more time for astronauts to focus on their mission tasks. Here, based on our medical knowledge and research experience, we formed a hypothesis

Abbreviations: ISS, International Space Station; MHC, Myosin heavy chain.

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that exercise with current fitness equipment at a cold temperature (4 °C) would be more efficient than at room temperature (20 °C) to prevent microgravity-induced muscle atrophy and bone loss, which could reduce physical exercise time.

Brief low temperature exposure can cause the body to experience goose bumps that are due to the contraction of tiny muscles attached to each hair follicle. This contraction causes the hair strands to literally "stand on end". At the same time, the tiny muscle contractions cause "bunching" of the skin that surrounds the hairs, which results in the "goose bumps". Goose bumps are very natural, and are the body's way of preserving its own heat by causing the hairs on the skin to stand up. When exposed to a low temperature (4 °C), people will develop goose bumps through their entire body, which spontaneously stimulates body movement by muscle contraction to generate heat. In general, exercise at room temperature is more efficient than at low temperature (4 °C) for reducing bodyweight due to the evaporation of more heat via sweating at room temperature; however, in contrast, the main purpose for astronauts to exercise at the ISS is to prevent losing bodyweight against microgravity-induced muscle atrophy and bone loss. Therefore, exercise at a low temperature (4 °C) should be more efficient than at room temperature for astronauts because cold temperature exposure will stimulate spontaneous muscle contraction in addition to the astronaut's intentional exercise. When the astronauts exercise at a cold temperature, it will feel more comfortable than at room temperature because cold temperature balances the muscle contraction-induced heat, which will also stimulate the astronaut's appetite and improve their health without apparent side effects. The main purpose for this pilot study is to test our hypothesis that exercise at 4 °C is more efficient than at room temperature to prevent microgravity-induced muscle atrophy/bone loss and, consequently reduces physical exercise time. Our one-month pilot study provided positive results to support our hypothesis and suggest that it would be reasonable to use more mice, in a microgravity environment and observe for a longer time to obtain a conclusion. We believe that the results from such a study will help to develop efficient exercise, which will finally benefit astronauts' heath and NASA's mission.

2. Materials and methods

2.1. Mice and low temperature (4 °C) exposure

All animal related experiments were approved by Emory Institutional Animal Care and Use Committee (IACUC) and followed the American Association for Laboratory Animal Science policies. Twenty female C57BL6 mice (6-week old) were purchased from Jackson Laboratory. These mice were divided into two groups: 10 mice for each group. One group of mice was exposed to a cold temperature (4 °C) and the other group of mice was never exposed to cold. The cold temperature exposure experiments were carried out as follows: put 2 cages of mice (10 mice in total, 5 mice/cage) in a cold room (4 °C). The mice were given a brief time (20–30 s) to adapt to the cold when the temperature inside the cage dropped to 4 °C. The mice were kept in the same cage; however, the filtered lid was replaced with a lid that enables easy air/temperature exchange (Fig. 1). After 10 min when the mice started to huddle up, we changed back to the filtered lid and placed the mice back to room temperature, two times/day (7:00 am and 7:00 pm) for 30 days.

2.2. Whole bodyweight measurement

The mice were weighed before the experiments and weighted every week for 4 weeks until the experiments were terminated.

Fig. 1. Image of the caged mice on the ground in a cold room. When the mice were placed in the cold room (4 °C), the mice were kept in the same cage; however, the filtered lid (top photo) was changed to a lid that enables easy air/temperature exchange (bottom photo). The mice had 20–30 s to adapt to the cold temperature and they immediately began to move actively. After 10 min, we changed the lid back to the original filtered lid and placed the mice back in the animal facility at room temperature.

2.3. Muscle strength examination

To compare the muscle strength between the two groups of mice, we euthanized the mice and first measured the perimeter of each leg (with muscle but without skin) of the two hind legs of each mouse. The calf muscles were removed from the hind legs and immediately frozen in liquid nitrogen. The myosin heavy chain (MHC) levels were measured as described by different groups (Salanova et al., 2008; Dabertrand et al., 2012; Hamalainen and Pette, 1997; Stevens et al., 1999; Widrick et al., 1999; Fitts et al., 2010). When all the samples were collected, the frozen muscles were minced with scissors in 9 vol of ice-cold homogenization buffer (250 mM sucrose, 100 mM KCl, 5 mM EDTA, and 20 mM hydroxymethyl aminomethane in Tris, pH 6.81) and were subsequently homogenized by hand in glass tissue grinders. The samples were centrifuged (1000 rpm) for 5 min and the pellets (0.1 g muscle tissue) were mixed with 1 ml of gel loading buffer and sonicated for 10 s. The samples were heated at 100 °C for 10 min and then centrifuged (12 000 rpm) for 10 min. Ten µl per sample was loaded in 12% SDS-gel for electrophoreses. Partial gel containing the MHC was directly stained by Coomasie G-250. The MHC marker (Catalog number: M7659) was purchased from Sigma-Aldrich Inc.

2.4. Bone density measurement

The bone density was measured with micro computed tomography (µCT) analysis. µCT analysis was performed as we reported previously (Gao et al., 2007, 2008). Briefly, after removing soft tissues, the femurs were fixed overnight in 10% neutral buffered formalin, washed twice in PBS, and stored in 70% ethanol at 4 °C until analysis. µCT scanning and analysis were performed by a technician blinded to the grouping of animals using a Scanco µCT-40 (Scanco Medical, Bassersdorf, Switzerland). The primary spongiosa