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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 $\times$ 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' health and NASA's mission.

Keywords

exercise at 4°C; muscle atrophy; bone loss; microgravity

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Conflict of interest statement

The authors state that they have no conflict of interests.

1. Introduction

Microgravity in space-induced muscle atrophy and bone loss are one major threat to astronauts' health [1-5]. 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 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' health 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 seconds) 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 (7:00am and 7:00pm) 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.

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 [1, 4, 6-9]. 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 seconds. The samples were heated at 100°C for 10 minutes and then centrifuged (12000 rpm) for 10 min. Ten μ l per sample was loaded in 12% SDS-gel for electrophoreses. Partial gel containing the MHC was directly stained by Coomassie 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 (μ CT) analysis. μ CT analysis was performed as we reported previously [10, 11]. 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. μ CT scanning and analysis were performed by a technician blinded to the grouping of animals using a Scanco μ CT-40 (Scanco Medical, Bassersdorf, Switzerland). The primary spongiosa (0.5 mm from the growth plate and extending 0.8 mm proximally) and secondary spongiosa (0.5 mm from the growth plate and extending 0.5 mm distally) within the endosteal borders of distal femurs were selected for trabecular analysis. Bones were scanned at a resolution of 12 μ m. Tomographic images were obtained at conditions of 70 KV and 114 μ A by collecting 500 projections for each rotation at 200-ms integration time. Reconstruction of 3D images was performed using standard convolution back-projection algorithms with Shepp and Logan filtering and rendered within a 12.3-mm field of view at a discrete density of 578,704 voxels/mm³. BV/TV was calculated by measuring 3D distances directly in the trabecular network. One high-resolution (6 μ m) 3D image was generated for 1 representative sample from each group clustering closest to the average indices in the histomorphometric analysis.

2.5. Statistical Analysis

Data were analyzed using χ^2 test or Student's *t*-test. Statistical significance was determined as $P < 0.05$ (*).

3. Results

3.1. Exposing the mice to 4°C, two times/day, 10 min/each time, for 30 days increased whole bodyweight and did not show significant side effects

To study whether a short period (10 min $\times$ 2) of cold temperature (4°C) exposure each day up to 30 days is an efficient way of exercise, we first wanted to know whether such a cold temperature exposure affected the mice physiological conditions. For this purpose, we compared the whole bodyweight between the two groups of mice with or without cold temperature exposure. The results showed that exposing the mice to 4°C, two times/day, 10 min/each time, for 30 days did not induce apparent side effects, the whole bodyweight of the mice exposed to cold temperature did not decrease, but increased when compared with that of the mice without cold temperature exposure (Fig. 2). At day 30 after daily short periods of cold temperature exposure, the whole bodyweight of the mice exposed to a cold temperature significantly heavier than that of the mice without cold temperature exposure (Fig. 2). These results indicate that the 30-day experiments have no side effects for the cold temperature exposed mice, suggesting that daily short period of exposure to 4°C is safe for mice.

3.2. Exposing the mice to 4°C, two times/day, 10 min/each time, for 30 days seemed to increase the muscle strength

We then were interested in determining whether the increased bodyweight was associated with the increased muscle strength. For this purpose, after euthanizing the mice at day 30 after finishing the pilot study, we first measured the perimeter of each leg (with muscle but without skin) of the two hind legs of each mouse (Fig. 3A). The results showed that the sum of the perimeter from two hind legs (mainly representing the volume of musculus gastrocnemius/calf muscle) each mouse increased in the group that was exposed to cold

temperature when compared with the mice that were never exposed to cold temperature (Fig. 3B). Although we did not find a statistically significant difference between the two groups due to the sample size that is relatively small, we believed that such results reflected positive effects of cold temperature on the muscle's strength. We then measured the myosin heavy chain (MHC) levels as described by different groups [1, 4, 6-9] since this parameter indirectly indicates the muscle contraction status. The results showed that the myosin heavy chain up-regulated in the muscle from the mice with cold temperature exposure when compared with the mice without cold temperature exposure (Fig. 4). These data also support the data shown in Fig. 3, suggesting that the cold temperature exposure strengthens the muscle contraction ability.

3.3. Exposing the mice to 4°C, two times/day, 10 min/each time, for 30 days seemed to increase the bone density

Next, we wanted to see whether the strength muscles affect the bone densities in cold temperature exposed mice. To compare the bone density between the two groups of mice, we measured the femur density from these mice using μ CT. The results showed that the bone was relatively denser from the mice with cold temperature than the mice without cold temperature exposure (Fig. 5) although the difference is not significant due to the small size of animal. Combining all the results shown in this manuscript, we believe that cold temperature exposure should increase bone density.

4. Discussion

In this pilot study, we examined the effects of daily short time ($10 \text{ min} \times 2$) of cold temperature (4°C) exposure on mouse bodyweight, muscle strength and bone density after 30-day experiments. The purpose of these experiments is to test our hypothesis that exercise with current fitness equipment at a cold temperature would be more efficient than at room temperature to prevent microgravity-induced muscle atrophy and bone loss, which could reduce physical exercise time. The results showed that the whole bodyweight in the group exposed to cold temperature significantly increased more than in the group without cold temperature exposure. Such results indicate that such short temperature exposure does not generate any apparent side effects, in contrast, it is better for the body health. The increased bodyweight could derive from increased fat cells or increased muscle volume and bone mass; however, our muscle strength and bone density data support the latter explanation. Since we believe that the increased bodyweight in the group with cold temperature exposure is due to both increased muscle strength and bone density, which explains why when we measured the muscle strength and bone separately, we could not get a significant difference between the two groups with or without cold temperature exposure.

To test our hypothesis that exercise with current fitness equipment at a cold temperature would be more efficient than at room temperature to prevent microgravity-induced muscle atrophy and bone loss, which could reduce physical exercise time, the ideal way is to combine such cold temperature exposure with special exercise under a microgravity environment. In our pilot study, we did not include any special exercise parameters and did not mimic a microgravity condition such as tail suspending for the mice due to a limited

budget; and, instead, just put the mice with or without daily short-term cold temperature exposure under normal gravity. However, we found that for the group without cold temperature exposure, they were at a normal movement status but for the group with cold temperature exposure, the mice immediately increased their movement (equivalent to exercise) for 10 min after they were exposed to a low temperature (4°C), and then slowed down and started to shrink. Since the 10 min cold temperature exposure promoted mice movement, we considered the mice during the cold temperature exposure period as exercise status. We believe that such cold temperature induced automatic exercise from both spontaneous muscle contraction and intentional muscle movement would also reduce the bored and uncomfortable feeling from the exercise at room temperature. Of course, to verify whether exercise with current fitness equipment at a cold temperature would be more efficient than at room temperature to prevent microgravity-induced muscle atrophy and bone loss needs more mice and longer time combining a microgravity mimic environment.

Taken together, the data from our pilot study support a novel, easy, cost-effective and practical idea that aims at potentially reducing astronauts' exercise time for preventing microgravity-induced muscle atrophy and bone loss.

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Abbreviations

ISS	International Space Station
MHC	myosin heavy chain

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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 seconds 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.

Fig. 2.

Bodyweight comparison between the mice with or without cold temperature exposure.

Twenty mice (C57BL6, female, 6 weeks old) were purchased from Jackson Lab. They were divided into two groups: 10 mice in each group, one group of mice with cold temperature exposure and another group of mice without cold temperature exposure. The mice were weighted every week for 4 weeks. The data represent mean $\pm$ SD from 10 mice for each group, *, $P < 0.05$.

Fig. 3.

Comparison of the sum of perimeters of two hind legs from each mouse between the two groups with or without cold temperature exposure. (A) Description of the measurement of the perimeter of one hind leg. After the mice were euthanized, the leg skin was removed and the perimeter of the leg was measured with waxed dental floss (Oral B Glide Pro Health Multi-protection Floss) (B) The sum of the perimeters of two hind legs per mouse from each group with or without cold temperature exposure. The data represent mean $\pm$ SD from 10 mice for each group, $P = 0.068$.

Fig. 4. Comparison of the Myosin Heavy Chain (MHC) levels between the mice with and without cold temperature exposure. (A) The MHC marker (Cat#: M7659) was purchased from Sigma-Aldrich Inc. The remaining gel was used for western blot with an Actin antibody as a loading control. M: a marker that indicates the protein size. Red numbers indicate the 10 samples from the mice without cold temperature exposure and blue numbers indicate 10 samples from the mice with cold temperature exposure. (B) Quantification of the band densities shown in (A). The data represent the mean $\pm$ SD from 10 samples, $P = 0.059$.

Fig. 5.

Bone density measurement. (A) Image of femurs from mouse with or without cold temperature was obtained with μ CT analysis. μ CT analysis was performed as we reported previously [10, 11]. Bone volume density/total volume density (BV/TV) was calculated by measuring 3D distances directly in the trabecular network. One high-resolution (6 μ m) 3D image was generated for 1 representative sample from each group clustering closest to the average indices in the histomorphometric analysis. (B) Quantity of the image data represent mean $\pm$ SD from 10 mice for each group, $P = 0.075$.