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# ISOPTWPO Today

## PEGGY A. WHITSON (PH.D.) NASA ASTRONAUT

*She graduated from Mt. Ayr Community High School, Mt. Ayr, Iowa, in 1978; received a Bachelor of Science degree in Biology/Chemistry from Iowa Wesleyan College in 1981 and a Doctorate in Biochemistry from Rice University in 1985. Whitson worked as a Research Biochemist in the Biomedical Operations and Research Branch at NASA-JSC. From 1991 to 1993, she served as Technical Monitor of the Biochemistry Research Laboratories in the Biomedical Operations and Research Branch. From 1991 to 1992, she was the Payload Element Developer for the Bone Cell Research Experiment (E10) aboard SL-J (STS-47) and was a member of the U.S.-USSR Joint Working Group in Space Medicine and Biology. In 1992, she was named the Project Scientist of the Shuttle-Mir Program (STS-60, STS-63, STS-71, Mir 18, Mir 19) and served in this capacity until the conclusion of the Phase 1A Program in 1995. From 1993 to 1996, Whitson held the additional responsibilities of the Deputy Division Chief of the Medical Sciences Division at NASA-JSC. From 1995 to 1996, she served as Co-Chair of the U.S.-Russian Mission Science Working Group. In April 1996, she was selected as an Astronaut Candidate and started training in August 1996. Upon completing two years of training and evaluation, she was assigned technical duties in the Astronaut Office Operations Planning Branch and served as the lead for the Crew Test Support Team in Russia from 1998 to 1999. From November 2003 to March 2005, she served as Deputy Chief of the Astronaut Office. Also in 2003, she served as commander of the fifth NASA Extreme Environment Mission Operations (NEEMO) mission. From March 2005 to November 2005, she served as Chief of the Station Operations Branch, Astronaut Office. Whitson trained as the backup ISS commander for Expedition 14 from November 2005 to September 2006. Whitson also was a member of the 2004 Astronaut Selection Board and chaired the Astronaut Selection Board in 2009.*

*Whitson completed two six-month tours of duty aboard the International Space Station, the second as the station commander for Expedition 16 in April 2008. This was Whitson's second long-duration spaceflight. She has accumulated 377 days in space between the two missions, the most for any woman. Whitson has also performed a total of six career spacewalks, adding up to 39 hours and 46 minutes. From October 2009 to July 2012, Whitson served as Chief of the Astronaut Corps and was responsible for the mission preparation activities and on-orbit support of all International Space Station crews and their support personnel.She was also responsible for organizing the crew interface support for future heavy launch and commercially-provided transport vehicles.*

— Image Credit:NASA

## Muscle atrophy in microgravity

After a few days of exposure to microgravity, muscle atrophy begins and the urinary excretion of nitrogen compounds increases. This atrophy is characterized by structural and functional alterations in the muscle tissue. There is a decrease in muscle fiber size, with no apparent change in fiber number. Atrophy is considerably greater for postural muscles, i.e., those muscles that support activities such as walking, lifting objects, and standing on Earth, as compared to the non-postural muscles, which undergo only marginal changes. Astronauts lose 10-20% of their muscle mass on short missions. On long-duration flights, the muscle mass loss might rise to 50% in the absence of countermeasures. The visible reduction in the leg circumference has been used as an indicator of muscle atrophy.

The muscle loss is presumably caused by changes in the muscle metabolism, i.e., the process of building and breaking down muscle proteins. Experiments performed during long-duration missions on board Mir have revealed a decrease of about 15% in the rate of protein synthesis in humans[1].

In addition to pure muscle loss, the fibers involved in muscle contractions change their contractile properties and are weakened. Significant decreases in strength of the trunk, knee and shoulder muscles have been found in as few as 6 days in microgravity.

Extensor muscles are more affected than flexor muscles. Animal studies also revealed that muscle fiber regeneration is less successful in space. The associated continued excretion of nitrogen may also have deleterious hormonal and nutritional effects.

Spaceflight also results in increased susceptibility of skeletal muscle to contraction damage, which occurs in muscular atrophies on Earth-bound patients. These effects may compromise the ability of astronauts to perform some of their activities in orbit. Likewise, they may not be able to withstand the stress of 1-g upon return to Earth.

### Muscle physiology

There are several types of muscle tissue in the human body. The muscles that are the most affected by spaceflight are the skeletal muscles, which are those directly attached to the skeleton. Skeletal muscles are the largest tissues in the body, accounting for 40-45% of the total body weight. These muscles are attached to the bones by tendons. Their contraction allows for the movement of joints in everyday activities, like walking, lifting objects and standing. The anti-gravity muscles, also known as postural muscles, owe their importance and strength to the presence of gravity.

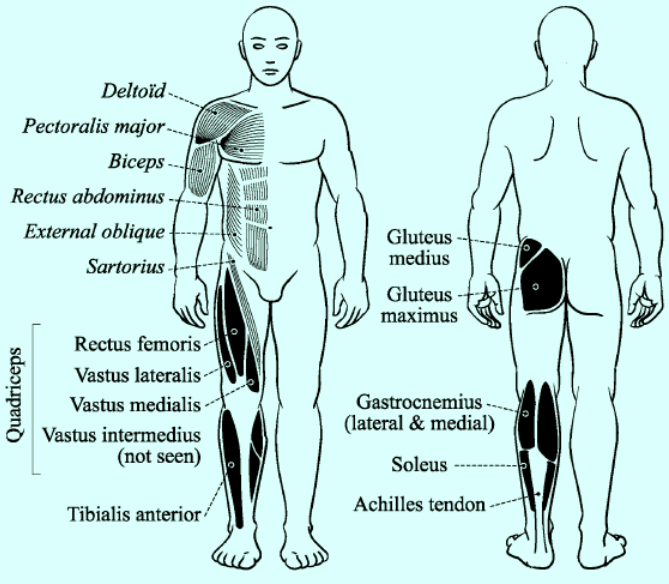
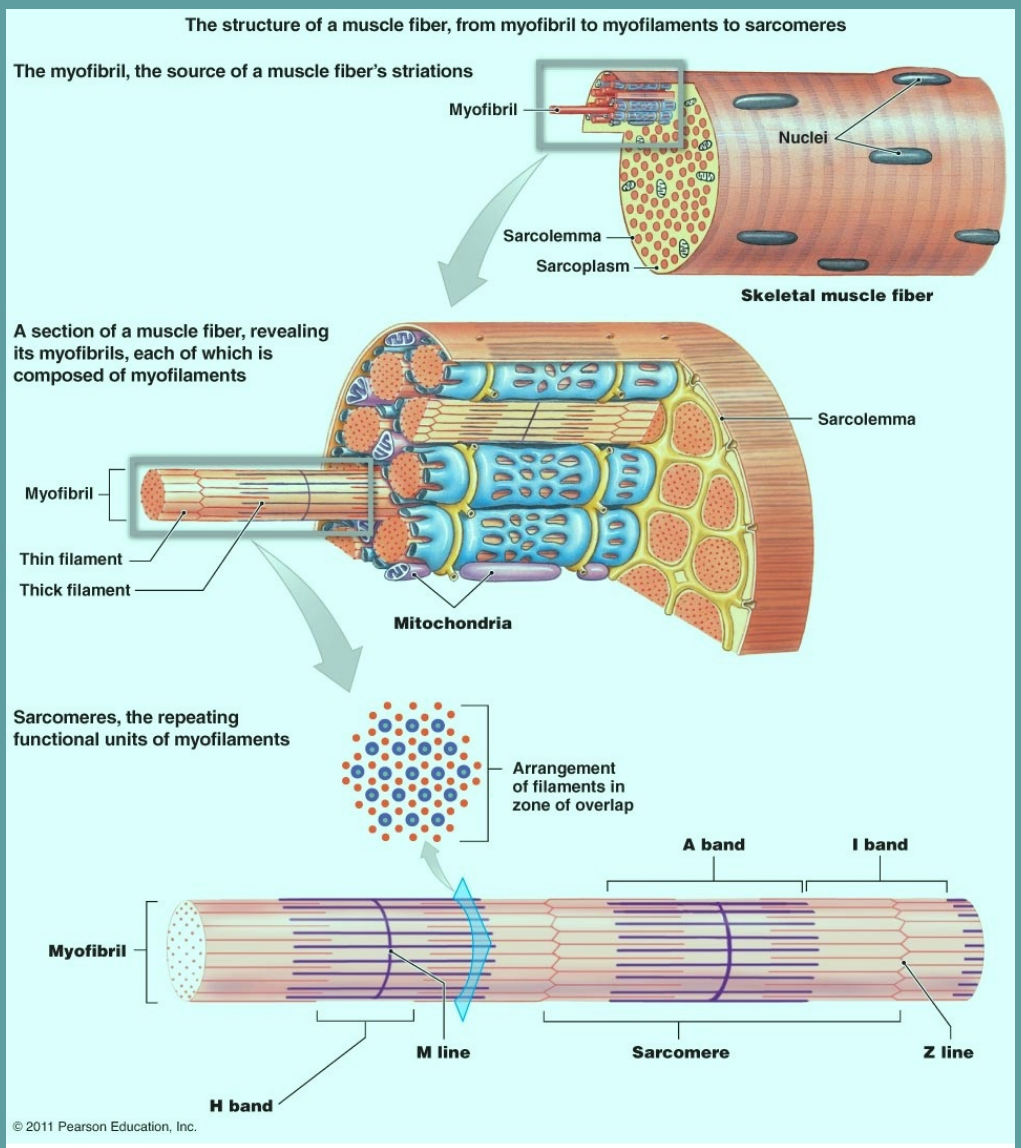



**Figure 1. Major Skeletal Muscles in the Body.** The Postural Muscles (in *Black*) Are Used to Counteract the Acceleration of Gravity During Standing on Earth.

**Adapted from Lujan BF, White RJ (1994) Human Physiology in Space. Teacher's Manual. A Curriculum Supplement for Secondary Schools. Houston, TX: Universities Space Research Association [2].**

Skeletal muscle cells, called fibers, are cylindrical cells, about 50 $\mu$ m in diameter.Each muscle fiber contains several hundred myofibrils, about 1 mm in diameter as well as many mitochondria for adenosine triphosphate (ATP) production and a complex system of internal membranes called the sarcoplasmic reticulum, which regulates calcium ion levels in the fiber. A myofibril consists of many filaments of myosin and actin, the structural unit of contraction[3].




Adapted from The Muscular System: Micro and Macro Anatomy [3].

ATP is the basic source of chemical energy for muscle contraction. However, the amount of ATP present in the muscle cells is only sufficient to sustain maximal muscle power for 5-6 s. Consequently, new ATP must be formed continuously.

Three processes can be used:
(a) the phosphagen system can sustain 10-15 more s of muscle activity;
(b) the glycogen-lactic acid system (anaerobic step of glucose breakdown) allows another 30-40 s "bursts" of energy;
(c) the aerobic system provides muscle activity that is only limited by the oxygen and nutrients supplies.

Each muscle fiber is supplied by a motor nerve (axon), and contracts when that axon "fires" an action potential. Muscle action potentials are fast (1-2 ms in duration) and are all-or-nothing, i.e., not graded. When a single stimulus is applied to the muscle fiber, it responds by a twitch. The twitch force is a weak force and is very slow compared to the duration of the action potential. There is a latent period between the start of the action potential and the time

when the fiber begins to develop contractile force, during which the muscle fiber cannot be stimulated again. The duration of the twitch for any one muscle fiber is constant but it can be shorter (e.g., 10 ms in large, fast fibers) or longer (e.g., 50 ms in small, slow fibers). The latent period is about the same for both slow and fast types of muscle fibers.

Slow (oxidative) fibers, also called Type I, are characterized by a relatively slow development of force but are able to maintain this force relatively long. Marathon runners typically develop those in the Soleus muscle in the calf for prolonged lower leg muscle activity. Fast (glycolytic) fibers, also called Type II, are able to develop force faster. Sprinters and weight lifters typically develop those in the Gastocnemius muscle in the calf and in the biceps muscle for quick, powerful "bursts" of movement.The downside of fast fibers is that they fatigue rapidly.

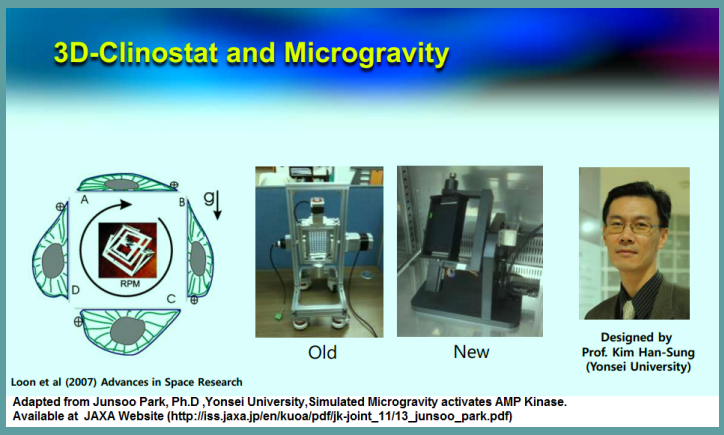
Contraction refers to the active process of generating a force in a muscle. The force exerted by a contracting muscle on an object is the muscle tension. The force exerted on a muscle by the weight of an object is the load. When a muscle shortens and lifts a load, the muscle contraction is isotonic (constant tension). When shortening is prevented by a load that is greater than muscle tension, the muscle contraction is isometric (constant length).

Another classification of muscle contraction is into concentric or eccentric contractions. Concentric contraction means that the muscle fibers decrease in length. Under the influence of external forces, muscle fibers can increase in length while contracting. This is called an eccentric contraction. An example of an eccentric contraction is walking downstairs, when the force of gravity causes the muscle to lengthen while contracting. During eccentric contraction, the force that is produced by the muscle is even greater than during isometric contraction. This greater production of force is still unexplained, but is surprisingly at the cost of hardly any ATP. When gravity is absent, eccentric contractions rarely occur, which has been suggested to be an important reason why muscles atrophy in microgravity[4].

During muscle contraction, there is a strict relationship between force and length.Because of this relationship it is important to standardize the angles of the relevant joints (i.e., standardization of the length of the muscle) when comparing muscle strength production before and after a certain period of time. In addition, the highest forces are developed at slower velocities of contraction. Consequently, it is also important to compare muscle strength production at identical angular velocities (i.e., standardization of the velocity of contraction).

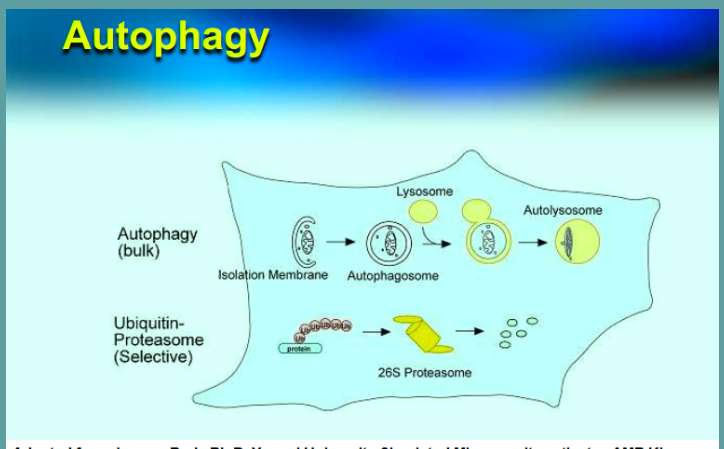
The power a muscle can generate is largely dependent on the amount of actinmyosin filaments that can be used. More filaments mean more potential to generate muscular pull. The length and size of a muscle fiber can vary considerably between various muscles in the body and between individuals of different gender, fitness, and age. The length of a muscle fiber can vary between several millimeters and approximately 15 cm, and is mainly responsible for the maximum velocity of contraction. The strength of a muscle is mainly determined by the size of myofilaments, which is often indicated by the surface area of a perpendicular slice of the muscle, the crosssectional area. There is generally a high correlation between maximal strength and the cross-sectional area of a specific muscle.

**Simulated Microgravity Contributes to Autophagy Induction by Regulating AMP-Activated Protein Kinase**
A clinostat is considered a useful model system for simulating microgravity in biological experiments. A clinostat is equipped with two independent rotating axes to disperse the gravity vector uniformly to a whole steric angle. Thus far, simulated microgravity using the clinostat has provided insight into biological processes and has been used to examine the biological changes.



Autophagy is an intracellular degradation and recycling pathway, conserved from yeast to human life forms. While the ubiquitin-proteasomal degradation system targets most soluble short-lived proteins, autophagy targets long-lived proteins and organelles such as mitochondria.

Autophagic degradation is mediated by the formation of autophagosomes, which are double-membrane vesicles that engulf the cytoplasmic organelles and macromolecules. The autophagosome is then fused with a lysosome to form an autolysosome, in which the targeted protein is degraded via lysosomal hydrolases at a low pH. The degradation products such as amino acids can then be recycled.




Adapted from Junsoo Park, Ph.D ,Yonsei University,Simulated Microgravity activates AMP Kinase
Available at JAXA website (http://iss.jaxa.jp/en/kuoa/pdf/jk-joint_11/13_junsoo_park.pdf)

Autophagy is induced by various stimuli, including nutrient depletion, accumulation of damaged organelles, and infection of cytoplasmic pathogens. A recent study identified the cellular signaling pathways of autophagy and autophagy regulation by nutrient depletion stimulus. Nutrient depletion promotes autophagy by activating AMP-dependent protein kinase (AMPK), a key energy sensor that responds to an increased AMP/ATP ratio. AMPK is also activated by various cellular stresses, such as hypoxia and heat shock. AMPK activation results to the activation of FoxO3, which leads to an increase in the expression of Beclin, LC3-II, and Gabarap11. In addition, autophagy is negatively regulated by mammalian target of rapamycin (mTOR), which responds to the nutrient signal. Both AMPK activation and mTOR suppression activates the autophagy-initiating kinase Ulk1 (ATG1 in yeast).

Muscle atrophy is a decrease in muscle mass that is typically caused by a variety of diseases or disuse. A decrease in muscle mass is caused by loss of muscle protein. The proteasomes degrade myofibrillar proteins via the ubiquitin-proteasome pathway, and autophagy is responsible for long-lived protein and organelle degradation.

To sensitively examine autophagy induction due to microgravity, HEK293 cells with stable expression of GFP-LC3 (GFPLC3 cells), an autophagosomal marker, and used the clinostat to evaluate the effect of microgravity on autophagy induction. Incubation of GFP-LC3 cells in the clinostat resulted in the alteration of autophagosomal marker expression, which was accompanied by AMPK activation and mTOR suppression.

For sensitive autophagy monitoring, authors used the HEK293 cell lines that stably express GFP-LC3, an autophagosomal marker. Various autophagy signals stimulate the formation of cytoplasmic GFP-LC3 punctuates, which reflect autophagy induction. GFP-LC3 cells were set on the clinostat to simulate microgravity and then rotated for 24 or 72 h. GFP-LC3 cells exposed to microgravity for 72 h showed cytoplasmic GFPLC3 punctuates, which are reflective of the autophagosomes, while cells exposed to other conditions did not (Fig. 2A, B). For motion control, authors rotated GFP-LC3 cells using the laboratory platform rocker at 5 rpm for 72 h; however, we did not observe the cytoplasmic GFP-LC3 punctuates (Fig. 2C, D). These results indicate that simulated microgravity positively regulates autophagosome formation.

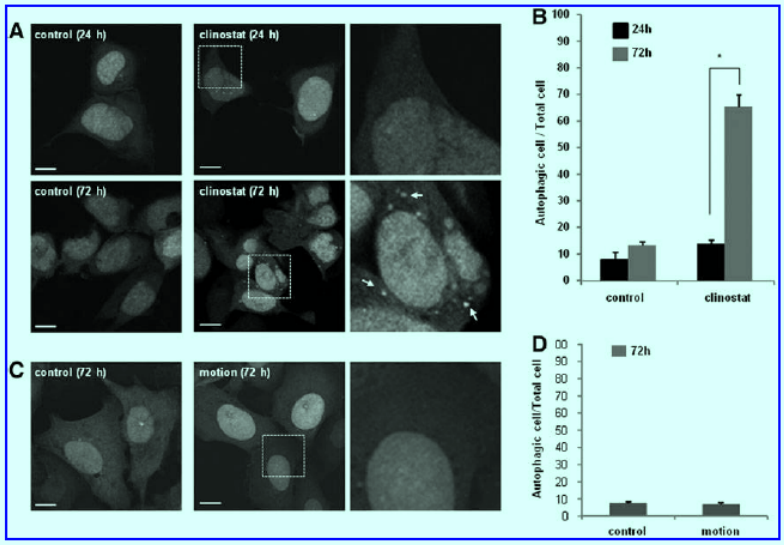



**FIG. 2.** Simulated microgravity-induced autophagy. **(A)** Clinorotation for 72 h resulted in GFP-LC3 punctuates in the cytoplasm. GFP-LC3 cells were set in the clinostat designed to simulate microgravity and rotated for 24 or 72 h, and control cells were kept under the same environment without rotation. After clinorotation, cells were fixed and examined by confocal microscopy (White arrows indicate autophagosome, scale bars =10 μM). **(B)** The number of GFP-LC3-dot–positive cells ( ≥ 5 dots per cell) was counted using a fluorescent microscope ($n = 300$, $^*p < 0.005$). **(C)** For motion control, GFP-LC3 cells were set in the laboratory platform rocker and rotated at 5 rpm for 72 h, and control cells were kept under the same environment without rocking. **(D)** The number of GFP-LC3-dot–positive cells was counted using a fluorescent microscope ($n = 300$).

**Adapted from Ryu Hyun-Wook, Choi Sang-Hun, Namkoong Sim, Jang Ik-Soon, Seo Dong Hyun, Choi Inho, Kim Han-Sung, and Park Junsoo. DNA and Cell Biology. March 2014, 33(3): 128-135. doi:10.1089/dna.2013.2089.**

To biochemically analyze autophagy induction under simulated microgravity condition, authors examined the expression patterns of LC3 and p62, which are widely used as markers of autophagosomes. LC3 is initially produced in an unprocessed form and then converted into LC3-I by Cterminal domain cleavage. LC3-I is then modified into PEconjugated LC3-II, the autophagosomal marker.GFP-LC3 cells placed in the clinostat for 24 or 72 h and untreated control cells were collected and the cell lysates were subjected to western blot analysis with anti-LC3 antibody. While other conditions did not show a significant change in the density of GFP-LC3-II bands, clinorotation for 72 h resulted in an elevation of GFP-LC3-II level (Fig. 3A).

Authors then examined the level of p62, another autophagy marker. Because p62 (also called SQSTM1) is degraded by autophagy and inhibition of autophagy upregulates p62, p62 has been implicated as a potential autophagic marker.Western blot analysis with anti-p62 antibody showed reduced levels of endogenous p62 after 72 h of clinorotation (Fig. 3A). Western blot results were consistent with the microscopy data, and these results collectively indicate that simulated microgravity positively regulates autophagy induction. Inhibition of autophagic flux using bafilomycin A1 resulted in the elevated level of endogenous LC3-II, indicating that autophagosomes were not formed due to inhibition of lysosomal degradation (Fig. 3B). Since autophagy is induced by simulated microgravity conditions, we attempted to determine the cellular signaling pathway involved in autophagy induction. Recent studies showed that AMPK and mTOR regulate autophagy directly via phosphorylation of Ulk1 (yeast ATG1).

Authors examined phosphorylation at two sites on AMPK (T172 and S485) and mTOR. AMPK is activated by phosphorylation at threonine 172 (T172) within the a subunit activation loop and is suppressed by phosphorylation at serine 485 (S485). Clinorotation for 24 and 72 h increased the level of phosphorylation of AMPK at T172 when compared with the control (Fig. 3C). The phosphorylation of AMPK at S485 is reduced by exposure to microgravity for 24 h; however, there was no difference between the control and cells exposed to clinorotation at 72 h. Clinorotation decreased the level of phosphorylation at serine 2448 of mTOR in the same manner (Fig. 3C). In addition, we examined the activation of AMPK in the motion control; however, the level of phosphorylation of AMPK at T172 was not significantly changed by rocking for 72 h (Fig. 3D). These results suggest that simulated microgravity activates AMPK and suppresses mTOR, and these events are potentially involved in autophagy regulation.

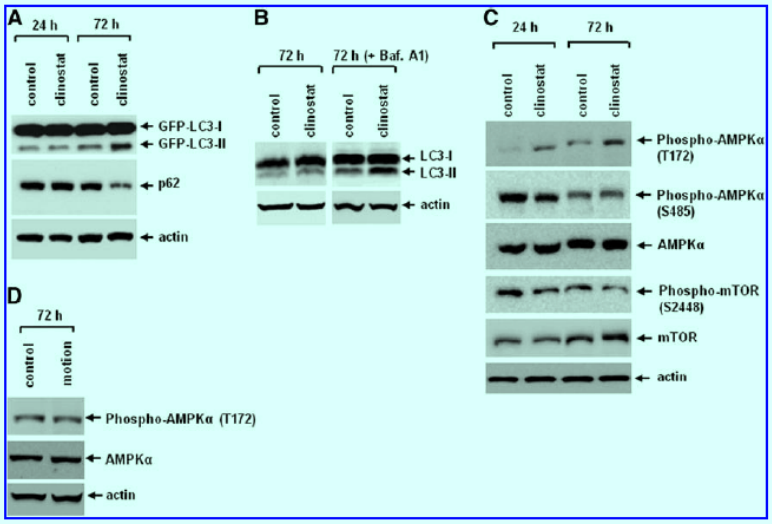



**FIG. 3.** Regulation of AMPK and mammalian target of rapamycin (mTOR) under simulated microgravity. **(A)** Expression of an autophagosome marker under simulated microgravity. The level of GFP-LC3-II was increased and the level of p62 was decreased in cells subjected to simulated microgravity. After clinorotation, cells were collected for lysis and equal amounts of cell lysates were subjected to western blot analysis with anti-LC3 antibody, anti-p62 antibody, and anti-actin antibody. **(B)** GFP-LC3 cells were rotated for 72 h and autophagic flux was inhibited by bafilomycin A1 treatment (100 nM, 4 h). **(C)** The phosphorylation of AMPKα and mTOR was changed by clinorotation. GFP-LC3 cells were set in the clinostat and rotated for 24 or 72 h, and control cells were kept under the same conditions without rotation. After clinorotation, cells were collected for lysis and equal amounts of cell lysates were subjected to western blot analysis using the corresponding antibodies. **(D)** For motion control, GFP-LC3 cells were set in the laboratory platform rocker and incubated for 72 h, cells were collected for lysis, and equal amounts of cell lysates were subjected to western blot analysis.

**Adapted from Ryu Hyun-Wook, Choi Sang-Hun, Namkoong Sim, Jang Ik-Soon, Seo Dong Hyun, Choi Inho, Kim Han-Sung, and Park Junsoo. DNA and Cell Biology. March 2014, 33(3): 128-135. doi:10.1089/dna.2013.2089.**

## AMPK inhibition interferes with microgravity-induced autophagy

To examine the role of AMPK in autophagy induction, authors used AMPK siRNA to suppress AMPK expression. GFP-LC3 cells were transfected with control siRNA, AMPK siRNA No. 1, or AMPK siRNA No. 2, and the transfected cells were set in the clinostat to simulate microgravity and rotated for 72 h. Authors examined the level of AMPK expression and AMPK expression was efficiently silenced by AMPK siRNA transfection (Fig. 4A). While the control siRNA cells exhibited autophagy induction by clinorotation, the AMPK-depleted cells did not (Fig. 4B, C). AMPK-depleted cells did not show autophagosome formation in the cytoplasm nor did they exhibit changes in the expression of autophagosome markers, GFP-LC3-II and p62 (Fig. 4B). These results indicate that AMPK is involved in microgravity-induced autophagy induction.

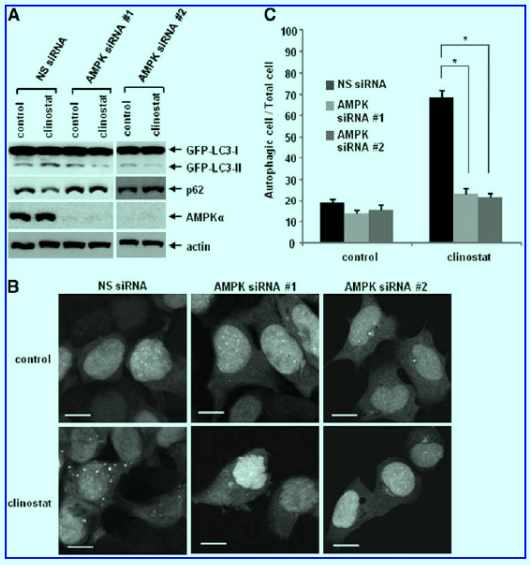



**FIG. 4.** Knockdown of AMPK interferes with the autophagosome formation under microgravity. **(A)** GFP-LC3 cells were transfected with siRNA against AMPK and autophagy marker (GFP-LC3-II and p62) expression was examined by western blot. The expression of AMPK was suppressed by two different siRNAs, AMPK siRNA No. 1 and AMPK siRNA No. 2. **(B)** Autophagosome formation in GFP-LC3 cells transfected with AMPK siRNA. GFP-LC3 cells were transfected with control, AMPK siRNA No. 1, or AMPK siRNA No. 2, fixed, and examined by confocal microscopy (scale bars = 10 μm). **(C)** The number of GFP-LC3-dot–positive cells (≥5 dots per cell) was counted using a fluorescent microscope ($n = 300$, $^*p < 0.01$).

**Adapted from Ryu Hyun-Wook, Choi Sang-Hun, Namkoong Sim, Jang Ik-Soon, Seo Dong Hyun, Choi Inho, Kim Han-Sung, and Park Junsoo. DNA and Cell Biology. March 2014, 33(3): 128-135. doi:10.1089/dna.2013.2089.**

**Simulated microgravity-induced autophagy in C2C12 myotube cells**

Authors examined whether simulated microgravity induced autophagy in C2C12 myotube cells. C2C12 myoblast cells were incubated in the differentiation medium to form the multinucleated myotube cells, and the cells were clinorotated for 72 h to simulate microgravity. Multinucleated myotube cells exposed to microgravity showed the enlarged and distinctive LC3 spots in the cytoplasm, suggesting that the simulated microgravity induced autophagy in C2C12 myotube cells (Fig. 5A). To confirm the results, the C2C12 myotube cells exposed to microgravity were collected and analyzed by western blots. The level of LC3-II was elevated, suggesting that simulated microgravity induced autophagy in C2C12 myotube cells (Fig. 5B). In addition, clinorotation for 72 h increased the level of phosphorylation of AMPK at T172 when compared with the control (Fig. 5B).

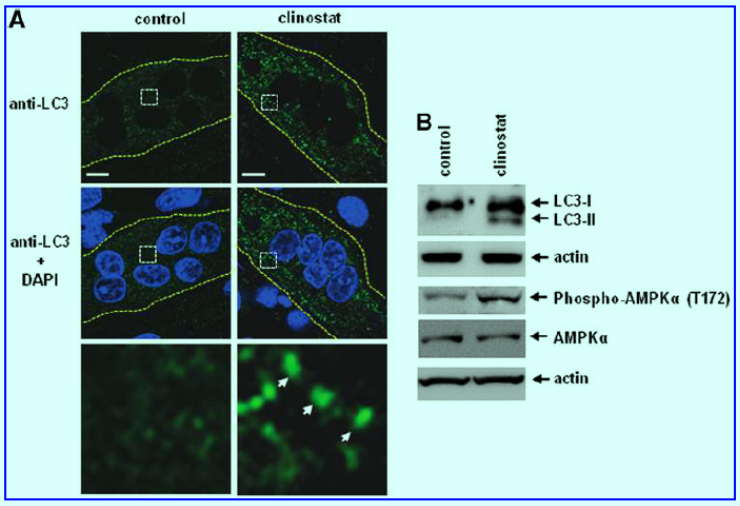



**FIG. 5.** Simulated microgravity induced autophagy in C2C12 myotube cells. **(A)** C2C12 myoblast cells were incubated in the differentiation medium to form the multinucleated myotubes, and the differentiated cells were rotated for 72 h. After clinorotation, cells were fixed and stained with anti-LC3 antibody. Scale bar = 10 μm. White arrows indicate autophagosome. **(B)** After rotation for 72 h, C2C12 myotube cells were collected for lysis and equal amounts of cell lysates were subjected to western blot analysis using the corresponding antibodies.

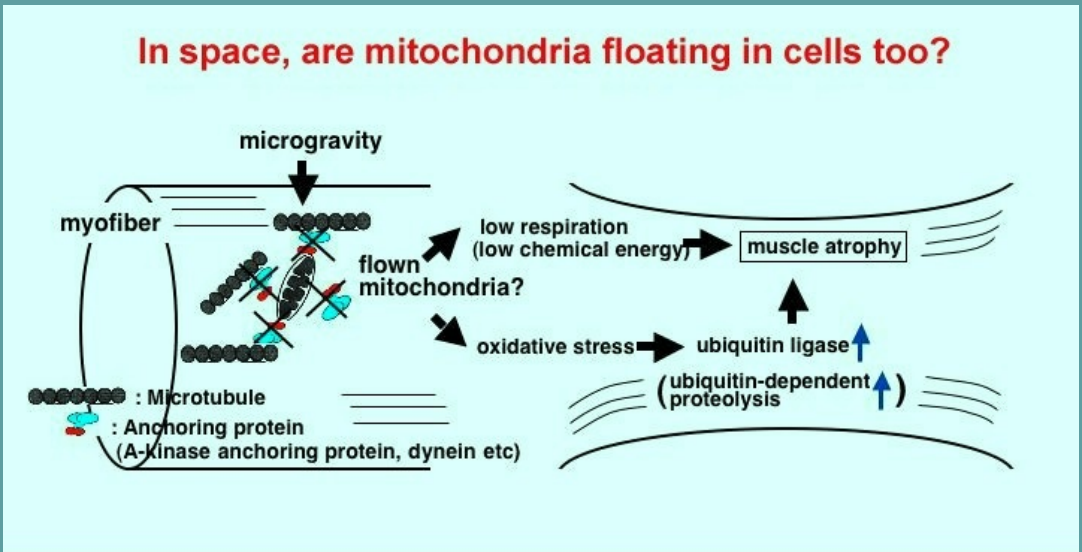
Adapted from Ryu Hyun-Wook, Choi Sang-Hun, Namkoong Sim, Jang Ik-Soon, Seo Dong Hyun, Choi Inho, Kim Han-Sung, and Park Junsoo. DNA and Cell Biology. March 2014, 33(3): 128-135. doi:10.1089/dna.2013.2089.

**Why do muscles atrophy in space?**

Muscles are made up primarily of proteins. Muscle proteins are continuously in a process of degradation and synthesis. A balanced muscle protein mass sustains a certain muscle mass, but when you are in space or laid up in bed for a long period of time, that balance is lost. This happens because of decreased synthesis and increased degradation of muscle proteins, which causes muscle loss.

In 1998, about 100 rats were sent to space on the Space Shuttle, and experiments revealed that their muscles became drastically atrophied. Even though these rats were floating in a microgravity environment, they had continued to move around. Yet, in spite of that, some of their muscles atrophied by almost half in their roughly two-week stay in space. After the rats were returned to Earth, we tested the proteins in their muscles (gastrocnemius muscle), and found that ubiquitinated proteins were drastically increased. Let me explain what ubiquitins are. When you check in your luggage at the airport, they put a label on it that indicates the destination. Similarly, a ubiquitin is like a label put on a protein that's to be broken down. When ubiquitins are added, we call it ubiquitination.

In summary, author discovered that in space skeletal muscle atrophy is induced by an increase in the number of proteins that are tagged for degradation. Author analyzed genes that were expressed in the space environment, and genes that were largely expressed on the ground. When the environment changes, the expression of some genes increases while that of other genes decreases.When skeletal muscle cells are exposed to the space environment, there is a dramatic increase of expression of the gene for the enzyme that induces ubiquitination (ubiquitin ligase). On the other hand, expression of the gene that regulates mitochondria organelles is abnormally decreased. Mitochondria are sustained by proteins, so when proteins are decreased, mitochondria alignment is disordered. When we stained mitochondria, it became apparent that the local existence of mitochondria in the space rats' skeletal muscles was in disorder. It's possible that in space, mitochondria are floating within their cells - just like people float in a spacecraft. Furthermore, mitochondria generate energy (ATP) from oxygen, but when they are improperly aligned, they cannot function properly. This results in a malfunction in the production of ATP. And not only that, it creates active oxygen (oxidative stress), because oxygen cannot be metabolized as normal. Author presume that oxidative stress increases expression of ubiquitin ligase, which then induces muscle atrophy.




Adapted from Takeshi Nikawa,Skeletal Muscle Cells in Response to Unloading Stress. Available at JAXA website (http://global.jaxa.jp/article/special/kibo/img/nikawa_photo_02_big_e.jpg)

**Musculoskeletal Health and Spaceflight**

The available data (Table 1) suggests that the rate of muscle mass loss is not linear across the duration of a mission and that the greatest losses occur in the antigravity muscles (lower back, abdominals, thighs, and lower legs).Significant atrophy of the knee extensors (-5% to -15%), knee flexors (-5% to -14%), plantar flexors (-6% to -15%) and muscles of the intrinsic lower back (-10%) has been documented even during short-duration missions of 9-17 days . During longer duration missions on Mir (115-197 days) and the ISS (161-192 days), it appears that the muscles of the intrinsic lower back (rotatores, multifidus, semispinalis, spinalis, longissimus, iliocostalis, -16%) and the ankle plantar flexors (soleus, gastrocnemius, -13% to -17%) are the most influenced by microgravity exposure (or the countermeasures for these muscles groups are not as efficacious). Of the plantar flexors, the soleus shows greater losses in fiber size and force production than the gastrocnemius.

A decrease in the rate of muscle protein synthesis (-46%) appears to be the primary mechanism underlying unloading-induced muscle atrophy. This causes rapid decreases in muscle fiber cross-sectional area (CSA) with short-duration spaceflight eliciting greater losses in type II fibers while type I fibers experience greater atrophy after long-duration missions.

For example, Edgerton et al.[7] showed that the CSA of vastus lateralis muscle fibers were 16%-36% smaller after 11 days of spaceflight, with the reductions in type IIb > type IIa > type 1. Similar observations were made in soleus (-26% in type IIa, -15% in type 1) and lateral gastrocnemius muscle fibers(-10% in type IIa, -7% in type I) after 17 days of spaceflight. However, more recent analyses from long-duration ISS crewmembers' plantar flexors suggest that fiber mass was most affected in the following order: soleus type I (-33%) > soleus type II (-29%), gastrocnemius type I (not reported), and gastrocnemius type II (-5%). The authors noted that there is a direct association between pre-flight fiber size and the degree of atrophy (r = 0.87), suggesting that larger fibers lose the most size during microgravity exposure. The functional consequences of these negative adaptations are reduced force, contractile velocity, and power, which ultimately result in less energy transfer to the tendon and skeleton for joint movement.

**Table 1.** Human Spaceflight and Skeletal Muscle Adaptations.

| Author | Yr | Space Era | N (Gender) | Flight Duration | Exercise Countermeasures | Highlighted Adaptations |
|---|---|---|---|---|---|---|
| Thorton [8] | 1974 | Skylab2 | 3(M) | 28 d | Bicycle Erg | M.Volume (Girth Circ.): ↓4.5% Legs; Strength. (Isok): ↓20% Legs |
| Thorton [8] | 1974 | Skylab 3 | 3(M) | 59 d | Bicycle Erg., MK-I, MK-II | M.Volume (Girth Circ.): ↓5% Legs, Strength (Isok): ↓20% Legs |
| Thorton [8] | 1974 | Skylab 4 | 3(M) | 84 d | Bicycle Erg., MK-I, MK-II, Thorton Passive Treadmill | M.Volume (Girth Circ): ↓2% Legs, Strength. (Isok): ↓7% Legs |
| Leblanc [9] | 1995 | STS | 4(2M2W) | 8 d | Shuttle Erg | M.Volume (MRI):↓6.3%, Gastr, ↓3.9% Ant. Calf. ↓8.3% Ham, ↓6.0% Quad, ↓10.3% L.Back |
| Edgerton [10] | 1995 | STS | 8(5M3W) | 5–11 d | Shuttle Treadmill, LBNP | F. CSA: ↓11% type I &, ↓24% type II VL in 5 d, ↓16%–36% type II loss > type I in 11 d |
| Widrick [11] | 1999 | STS/Mir | 4(M) | 17 d | NR | F. Diam/: ↓8% in Sol. type I |
| Akima [12] | 2000 | STS | 3(?) | 9–16 d | NR | Volume (MRI): ↓5.6% to 15.4% KE, ↓8.6% to 14.1% KF, ↓8.4% to 15.9% PF |
| Leblanc [13] | 2000 | STS/Mir | 4(M) | 17 d | NR | Volume (MRI): ↓10% AE & IB, ↓5%–7% Quad & Psoas, ↓3% in Ham & AL |
| Leblanc [13] | 2000 | STS/Mir | 16(15M1W) | 115–197 d | NR | Volume (MRI): 14.6% AL, ↓16.9% Gast, ↓16.8% Sol, ↓10.1% Quad, ↓12.7% Ham, ↓15.9% IB, and ↓4.4% Psoas |
| Riley [14] | 2000 | STS | 4(M) | 17 d | NR | F: A Band ↓17% Filaments, ↑9% Short Filaments |
| Koryak [15] | 2001 | Mir | 7(M) | 6 mo | NR | Strength (Isom): ↓42% TS, Strength: (Evoked Forces): ↓26% $P_o$ TS, ↑15% $P_t$ TS |
| Trappe [16] | 2001 | STS | 4(M) | 17 d | NR | F.CSA: ↓7.4% Type I Gast, ↓10.1 Type IIa Gast, ↓7.4 Type I Sol. ↔ Calf MVC or Force Velocity Relationship |
| Lambertz [17] | 2001 | Mir | 14(M) | 90–180 d | Cycle Erg., Treadmill | Strength (Isom): ↓2%–30% PF in 12 of 14 subjects |
| Narici [18] | 2003 | STS | 4(M) | 17 d | NR | M.CSA (Girth Circ.): ↓8% Calf., Strength (Evoked Forces) ↓24% Peak Twitch; ↓22% Tetanic Force at 50 Hz; ↓19.5% Specific Forces at 50 Hz of TS |
| Tesch [19] | 2005 | STS | 4(M) | 17 d | NR | M. CSA (MRI): ↓8% KE & Gluteal. Strength (Isom): ↓10% KE, Strength (Isok): ↓9% KE (CON), 11% KE (ECC) |
| Trappe [20] | 2009 | ISS | 9(?) | 6 mo | CEVIS, TVIS, VELO, iRED | M.Volume (MRI): ↓13% Calf, ↓15% Sol, ↓10% Gast. Calf Peak Power ↓32%, Force-Velocity ↓20%–29% across 30–300°/sec |
| Fitts [21] | 2010 | ISS | 9(?) | 6 mo | CEVIS, TVIS, VELO, iRED | F.CSA: ↓33% type 1 Sol > type II Sol > type I Gast. > type II Gas |
| Gopalakrishnan [22] | 2010 | ISS | 4(M) | 181 d | CEVIS, TVIS, VELO, iRED | M.Volume (MRI): ↓10 calf, ↓4 thigh |
| Smith [23] | 2012 | ISS | 8(6M2W) | 160 d | CEVIS, TVIS, iRED | Lean mass (DEXA): ↓2% total body |
| Smith [23] | 2012 | ISS | 5(3M2W) | 134 d | CVIS, TVIS, ARED | Lean mass (DEXA): ↑3% total body |

STS = Shuttle transport system, ISS = International space station, CEVIS = Cycle ergometer with isolation system, TVIS = Treadmill with isolation system, iRED = Interim resistive device, VELO = Russian velociped bicycle exercise device, MRI = Magnetic resonance imaging, CSA = Cross-sectional area, Pec/Lats = Pectoralis major/lattisimus dorsi, Gastr = Gastrocnemius, Ant = Anterior, Ham = Hamstring, Quad = Quadriceps, VL = Vastus lateralis, Sol. = Soleus, KF = Knee flexors, KE = Knee extensors, PF = Plantar flexors, IB = Intrinsic back, TS = Triceps surea, Circ. = Circumference, Isok = Isokinetic, Isom = Isometric, Erg = Egometer, LBNP = Lower body negative pressure, F. = Fiber, M. = Muscle, Diam. = Diameter, CON = Concentric, ECC = Eccentric, MVC = Maximal voluntary contraction, d = Days, Yr. = Year, mo = months, M = Men, W = Women. NR = Not reported.

## Human skeletal muscle after 6 months aboard the International Space Station

Ten crewmembers participated in this investigation. For the analysis presented, one crewmember had incomplete data sets and was not included. The subject population consisted of American astronauts and Russian cosmonauts. The subjects' (n=9) age, height, weight, and days in space were 45 ± 2 yr, 176 ± 2 cm, 81 ± 3 kg, and 177 ± 4 days (range=161-192 days), respectively. An overview of each crewmember's exercise history in the weeks preceding their launch is shown in Table 2.

Table 2. *Preflight conditioning biographies*

| Subject | Aerobic Exercise | Resistance Exercise |
|---|---|---|
| *A* | 3–6 days/wk: cycle (30 min), step (30 min) and arm ergometry (~3 min) | 2–3 days/wk: total body |
| *B* | 1–2 days/wk: cycle (15 min), walking and/or swimming (30–40 min) | No records |
| *C* | 5–6 days/wk: running (30 min) | 1 day/wk: total body |
| *D* | 2–3 days/wk: swimming (30–45 min) and fast walking (30–60 min) | 2–3 days/wk: total body |
| *E* | 5 days/wk: cycling (15 min) with progressive workloads | 5 days/wk: upper body only |
| *F* | No records | 3 days/wk: total body |
| *G* | 1–2 days/wk: variety of activities (swimming, biking, running) of low duration | Infrequent upper body routine |
| *H* | NA | NA |
| *I* | NA | NA |

NA, not available.

Adapted from Exercise in space: human skeletal muscle after 6 months aboard the International Space Station. J Appl Physiol 106: 1159 –1168, 2009.

**Exercise in space**: During the 6 mo the crewmembers were on the ISS, they had access to a treadmill (treadmill with vibration isolation system), two bicycle ergometers (cycle ergometer with vibration isolation system and a Velosiped, i.e., Russian bicycle exercise device), and an interim resistive exercise device (iRED). The crewmembers

also had access to bungee cords, which they could use to provide resistance-type exercise for various muscle groups.

**Muscle volume**: A summary of calf muscle volume before and after space flight is shown in Table 3. The gastrocnemius (medial and lateral) and soleus muscle were smaller ($P < 0.05$) after 6 mo in space. Combined, the gastrocnemius and soleus atrophied ($P < 0.05$) -13 ± 2% pre- to postflight. The soleus (-15 ±2%) atrophied more ($P < 0.05$) than the gastrocnemius (-10 ± 2%) pre- to postflight (Fig. 1). One crewmember (subject E) had insignificant (-1%) atrophy after the flight. Two of the crewmembers (subjects A and F) lost more than 20% of their calf muscle mass. Of the remaining six crewmembers, five lost more than 10% calf muscle mass.

At R+19 after landing, the gastrocnemius was still 5-6% atrophied, but this was not significant. Conversely, the soleus was still reduced ($P < 0.05$) compared with preflight, averaging -9 ± 1% for all crewmembers. Although calf muscle volume was still reduced -8 ±2%, it represented a partial recovery from the more immediate (R+4) flight measurement.

Table 3. *Calf muscle volume before and after spaceflight*

| Subject | Pre | Post (R+4) | Post (R+19) | % Δ (Pre to R+4) | % Δ (Pre to R+19) |
|---|---|---|---|---|---|
| | | *Gastocnemius (medial + lateral head)* | | | |
| *A* | 379 | 299 | 370 | −21 | −2 |
| *B* | 229 | 204 | 209 | −11 | −9 |
| *C* | 237 | 208 | 231 | −12 | −3 |
| *D* | 289 | 272 | 295 | −6 | 2 |
| *E* | 247 | 255 | 254 | 3 | 3 |
| *F* | 322 | 264 | 272 | −18 | −16 |
| *G* | 430 | 381 | 388 | −11 | −10 |
| *H* | 288 | 269 | 286 | −7 | −1 |
| *I* | 359 | 318 | 317 | −11 | −12 |
| Mean ± SE | 309±23 | 274±18* | 291±20 | −10±2 | −5±2 |
| | | *Soleus* | | | |
| *A* | 604 | 456 | 517 | −22 | −8 |
| *B* | 309 | 272 | 294 | −12 | −5 |
| *C* | 444 | 360 | 392 | −19 | −12 |
| *D* | 434 | 362 | 403 | −17 | −7 |
| *E* | 418 | 406 | 404 | −3 | −3 |
| *F* | 440 | 338 | 369 | −23 | −16 |
| *G* | 517 | 442 | 457 | −15 | −12 |
| *H* | 443 | 400 | 407 | −10 | −8 |
| *I* | 489 | 409 | 425 | −16 | −13 |
| Mean ± SE | 455±27 | 383±19* | 408±20*† | −15±2 | −9±1 |
| | | *Gastrocnemius + soleus* | | | |
| *A* | 983 | 755 | 887 | −21 | −6 |
| *B* | 538 | 476 | 503 | −12 | −7 |
| *C* | 681 | 568 | 632 | −17 | −9 |
| *D* | 723 | 634 | 698 | −12 | −3 |
| *E* | 665 | 661 | 658 | −1 | −1 |
| *F* | 762 | 602 | 641 | −21 | −16 |
| *G* | 947 | 823 | 845 | −13 | −11 |
| *H* | 731 | 669 | 693 | −8 | −5 |
| *I* | 848 | 727 | 742 | −14 | −13 |
| Mean ± SE | 764±47 | 657±35* | 699±39* | −13±2 | −8±2 |

All muscle volume data presented as $cm^3$. Pre, preflight; Post, postflight; R + 4 and R + 19, 4 and 19 days after return from space. *$P < 0.05$ compared with Pre. †$P < 0.05$ compared with R + 4.

**Adapted from Exercise in space: human skeletal muscle after 6 months aboard the International Space Station. J Appl Physiol 106: 1159 –1168, 2009.**

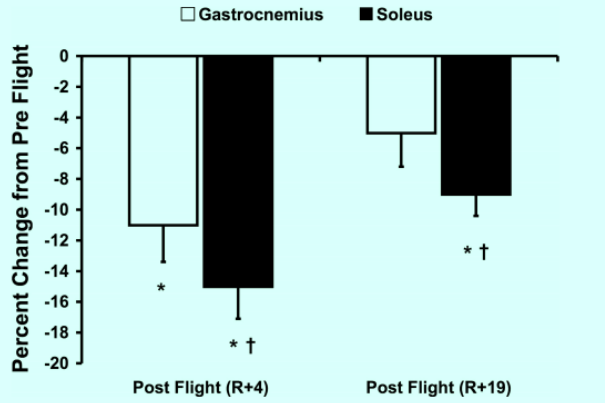



Fig. 1. Percent change in gastrocnemius and soleus muscle volume from preflight to postflight recovery *days 4* (R+4) and *19* (R+19). *$P < 0.05$ vs. preflight. †$P < 0.05$ vs. gastrocnemius.

**Adapted from Exercise in space: human skeletal muscle after 6 months aboard the International Space Station. J Appl Physiol 106: 1159 –1168, 2009.**

**Muscle performance**: A summary of each crewmember's calf muscle performance for MVC at one angle (neutral position) and a slow (60 °/s) and fast (180 °/s) isokinetic speed are shown in Table 4. MVC was reduced ($P < 0.05$) -14 ± 2% at R+7 and remained lower (-13 ± 5%; $P < 0.05$) at R+13. All nine crewmembers had a decline in MVC (range=-7 to -22%) with flight. At R+13, seven of the nine crewmembers were lower (range =-9 to -33%), with two crewmembers (subjects A and H) having a 5-10% increase compared with preflight.

At the slow isokinetic speed (60 °/s) there was a -20 ± 3% loss ($P < 0.05$), which was sustained (-19 ± 4; $P < 0.05$) at R+13. This pattern was also evident at the faster (180 °/s) isokinetic speed with a 25 ±10% reduction at R+7 and R+13. For both the slow and fast speeds, eight of the nine subjects had a decrease in muscle performance.

A force-velocity curve for all subjects from pre- to postflight(R+7) is shown in Fig. 2. On average, force-velocity characteristics were reduced -20 to -29% across the velocity spectrum ($P < 0.05$). Peak power was 134 ±11, 91 ±10, and 94 ±13 W preflight, R+7, and R+13, respectively. On average, peak power declined 32% with spaceflight ($P < 0.05$).

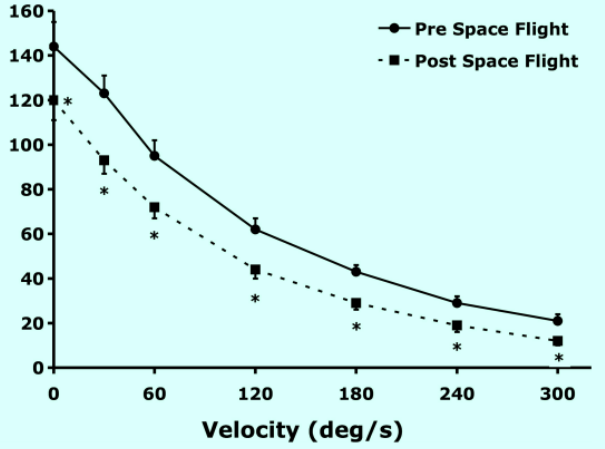



Fig. 2. Calf muscle force-velocity profile before and after spaceflight. *$P < 0.05$ vs. preflight.

**Adapted from Exercise in space: human skeletal muscle after 6 months aboard the International Space Station. J Appl Physiol 106: 1159 –1168, 2009.**

Table 4. *Calf muscle performance profile before and after spaceflight*

| | Isometric (at neutral ankle angle) | | |
|---|---|---|---|
| Subject | Pre | Post (R+7) | Post (R+13) |
| *A* | 119 | 108 | 128 |
| *B* | 114 | 102 | 87 |
| *C* | 128 | 100 | 86 |
| *D* | 157 | 121 | 126 |
| *E* | 135 | 114 | 119 |
| *F* | 117 | 100 | 89 |
| *G* | 218 | 187 | 185 |
| *H* | 138 | 115 | 137 |
| *I* | 171 | 133 | 134 |
| Mean ± SE | 144±11 | 120±9* | 121±11* |
| | Isokinetic (at 60°/s) | | |
| Subject | Pre | Post (R+7) | Post (R+13) |
| *A* | 103 | 83 | 91 |
| *B* | 71 | 67 | 65 |
| *C* | 76 | 60 | 54 |
| *D* | 100 | 78 | 83 |
| *E* | 117 | 78 | 65 |
| *F* | 64 | 41 | 41 |
| *G* | 117 | 93 | 89 |
| *H* | 99 | 76 | 86 |
| *I* | 110 | 74 | 80 |
| Mean ± SE | 95±7 | 72±5* | 73±6* |
| | Isokinetic (at 180°/s) | | |
| Subject | Pre | Post (R+7) | Post (R+13) |
| *A* | 54 | 46 | 48 |
| *B* | 24 | 35 | 26 |
| *C* | 41 | 21 | 22 |
| *D* | 48 | 28 | 32 |
| *E* | 51 | 26 | 23 |
| *F* | 29 | 15 | 12 |
| *G* | 47 | 36 | 29 |
| *H* | 40 | 31 | 50 |
| *I* | 50 | 23 | 26 |
| Mean ± SE | 43±3 | 29±3* | 30±4* |

All data are presented in Nm. Pre, preflight. *$P < 0.05$ compared with Pre.

**Adapted from Exercise in space: human skeletal muscle after 6 months aboard the International Space Station. J Appl Physiol 106: 1159 –1168, 2009.**

**Muscle fiber type**: Authors isolated and analyzed the MHC profile on a total of 4,328 single muscle fibers from the gastrocnemius and soleus muscles before and after flight. The breakdown was 1,960 muscle fibers (1,109 preflight, 851 postflight) for the gastrocnemius and 2,368 muscle fibers (1,277 preflight, 1,091 postflight) for the soleus.

The average MHC profile of the gastrocnemius and soleus muscles from the crewmembers before and after space flight is shown in Fig. 3. Individual data from the gastrocnemius and soleus of each crewmember are shown in Tables 5 and 6, respectively.

One individual (subject B) had a small muscle biopsy sample and therefore was not included in these analyses. The gastrocnemius had a 12% decrease ($P < 0.05$) in MHC I fibers and an increase ($P < 0.05$) in MHC I/IIa (-4%) hybrid fibers and MHC IIa fibers (9%). Seven of the eight subjects had a decrease in MHC I fibers (range=-6 to -31%). There were minimal MHC IIx and MHC I/IIa/IIx fibers detected in the pre- and postflight muscle samples. The 4% increase in hybrid muscle fibers appears to be the result of the MHC I/IIa hybrid fiber type. On average, the soleus had a 17% decrease ($P < 0.05$) in MHC I fibers. The shift away from MHC I fibers was distributed among the other fiber types (MHC I/IIa, IIa, IIa/IIx), with a nonsignificant increase of 4 -5% within each MHC phenotype. Combined, the soleus had a 12% increase ($P < 0.05$) in hybrid MHC isoforms. Three of the crewmembers (subjects D, E, and I) did not have any major alterations in fiber type of the soleus. Four of the crewmembers (subjects A, C, F, and G) had a decrease in soleus MHC I fibers that ranged from -20 to -44%.

Table 5. *Gastrocnemius fiber type distribution before and after spaceflight*

| | Preflight MHC Profile | | | | | | |
|---|---|---|---|---|---|---|---|
| Subject | I | I/IIa | IIa | IIa/IIx | IIx | I/IIa/IIx | Hybrids |
| *A* | 45 | 2 | 32 | 15 | 5 | 1 | 18 |
| *B*− | | | | | | | |
| *C* | 91 | 4 | 5 | 0 | 1 | 0 | 4 |
| *D* | 91 | 2 | 2 | 3 | 0 | 2 | 7 |
| *E* | 86 | 1 | 1 | 4 | 2 | 6 | 11 |
| *F* | 63 | 1 | 10 | 18 | 6 | 2 | 21 |
| *G* | 76 | 2 | 4 | 13 | 3 | 2 | 17 |
| *H* | 47 | 2 | 17 | 31 | 2 | 1 | 34 |
| *I* | 67 | 2 | 15 | 14 | 2 | 0 | 16 |
| Mean ± SE | 71±7 | 2±1 | 10±4 | 12±4 | 3±1 | 2±1 | 16±3 |
| | Postflight MHC Profile | | | | | | |
| Subject | I | I/IIa | IIa | IIa/IIx | IIx | I/IIa/IIx | Hybrids |
| *A* | 47 | 4 | 31 | 12 | 5 | 1 | 17 |
| *B*− | | | | | | | |
| *C* | 85 | 4 | 10 | 0 | 1 | 0 | 4 |
| *D* | 60 | 6 | 15 | 13 | 5 | 1 | 20 |
| *E* | 76 | 11 | 5 | 4 | 2 | 2 | 17 |
| *F* | 51 | 7 | 22 | 14 | 3 | 3 | 24 |
| *G* | 62 | 8 | 19 | 7 | 4 | 0 | 15 |
| *H* | 34 | 6 | 25 | 31 | 1 | 3 | 40 |
| *I* | 58 | 4 | 24 | 11 | 1 | 2 | 17 |
| Mean ± SE | 59±6* | 6±1* | 19±3* | 12±3 | 3±1 | 1±1 | 20±4 |

All data are expressed as percent distribution. *Subject B* (*B*−) had a small muscle biopsy sample, not included in analysis. MHC, myosin heavy chain. *$P < 0.05$ compared with preflight.

**Adapted from Exercise in space: human skeletal muscle after 6 months aboard the International Space Station. J Appl Physiol 106: 1159 –1168, 2009.**

Table 6. *Soleus fiber type distribution before and after spaceflight*

| | Preflight MHC Profile | | | | | | |
|---|---|---|---|---|---|---|---|
| Subject | I | I/IIa | IIa | IIa/IIx | IIx | I/IIa/IIx | Hybrids |
| *A* | 86 | 1 | 13 | 0 | 0 | 0 | 1 |
| *B*− | | | | | | | |
| *C* | 83 | 4 | 11 | 2 | 0 | 0 | 6 |
| *D* | 85 | 1 | 12 | 1 | 0 | 0 | 2 |
| *E* | 82 | 5 | 6 | 5 | 0 | 2 | 12 |
| *F* | 80 | 2 | 17 | 1 | 0 | 0 | 3 |
| *G* | 99 | 0 | 1 | 0 | 0 | 0 | 0 |
| *H* | 86 | 0 | 12 | 2 | 0 | 0 | 2 |
| *I* | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mean ± SE | 88±3 | 2±1 | 9±2 | 1±1 | 0±0 | 0±0 | 3±1 |
| | Postflight MHC Profile | | | | | | |
| Subject | I | I/IIa | IIa | IIa/IIx | IIx | I/IIa/IIx | Hybrids |
| *A* | 42 | 2 | 42 | 12 | 2 | 0 | 14 |
| *B*− | | | | | | | |
| *C* | 63 | 23 | 12 | 0 | 1 | 1 | 24 |
| *D* | 82 | 2 | 11 | 5 | 1 | 0 | 7 |
| *E* | 85 | 4 | 7 | 3 | 0 | 1 | 8 |
| *F* | 45 | 1 | 17 | 33 | 3 | 1 | 35 |
| *G* | 74 | 16 | 7 | 0 | 1 | 2 | 18 |
| *H* | 80 | 4 | 10 | 4 | 1 | 1 | 9 |
| *I* | 97 | 3 | 0 | 0 | 0 | 0 | 3 |
| Mean±SE | 71±7* | 7±3 | 13±4 | 7±4 | 1±1 | 1±1 | 15±4* |

All data are expressed as percent distribution; *$P < 0.05$ compared with preflight.

**Adapted from Exercise in space: human skeletal muscle after 6 months aboard the International Space Station. J Appl Physiol 106: 1159 –1168, 2009.**

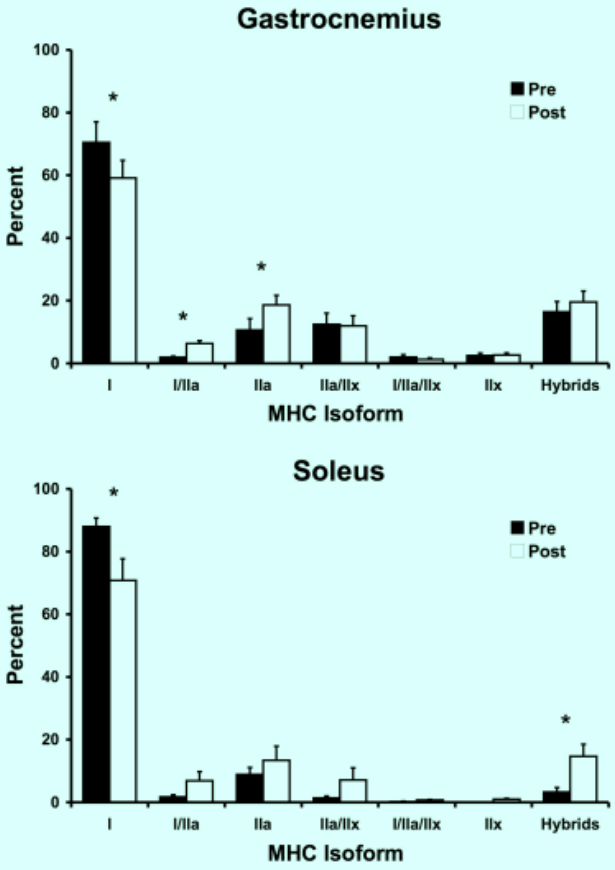



Fig. 3. Myosin heavy chain (MHC) composition of the gastrocnemius and soleus muscles before and after spaceflight. Hybrid, all fibers with more than one MHC isoform. *$P < 0.05$ vs. preflight.

**Adapted from Exercise in space: human skeletal muscle after 6 months aboard the International Space Station. J Appl Physiol 106: 1159 –1168, 2009.**

**The average amount of muscle mass lost (-13%) with spaceflight was slightly less than with previous long-duration stays on the Russian Space Station Mir (-17%). The current ISS and previous Mir calf muscle volume loss is about one-half that of long-duration (60- to 120-day) bed rest studies showing a 29% decrease among the control subjects without countermeasures. These data imply that the exercise in space is having a beneficial effect but is not complete, with the soleus being more difficult to protect than the gastrocnemius.**

**Data suggest that the crewmembers with larger calf muscles had a greater degree of atrophy with long-duration spaceflight. Second, the volume of treadmill exercise may have provided a level of protection for calf muscle mass. Third, when the treadmill is used in passive mode, more force is needed to drive the belt during walking/running activities and may help protect against calf muscle atrophy. Finally, inadequate caloric intake may have contributed to the muscle atrophy.**

**A substantial decline (-20 to -29%) in calf muscle performance with spaceflight was noted for both static (9 of 9 subjects) and dynamic (8 of 9 subjects) muscle actions. These data are in close agreement with the decline in muscle performance after 84 days on Skylab (-25%) and 180 days on the Mir (-35%). The decline in muscle performance following long-duration stays in space is less than in bed-rested subjects (40% loss) without countermeasures, suggesting that the ISS exercise program did provide a modest level of protection.**

**Microgravity-Induced Fiber Type Shift in Human Skeletal Muscle**

Spaceflight induces quantitative and qualitative modifications to skeletal muscle by markedly decreasing size, strength, and endurance Despite exercise countermeasures, muscle mass has been shown to decrease from -13% to -17% dur-

ing long-duration spaceflight. Furthermore, longmission studies conducted aboard the ISS, Skylab, and Mir have shown significant decreases (-20 - 35%) in muscle performance.

**MICROGRAVITY-INDUCED FIBER TYPE SHIFT**

Myosin heavy chain (MHC) protein composition determines mammalian skeletal muscle fiber classifications. Humans express three distinct fiber types (MHC I, IIa, and IIx) along with hybrids containing more than one phenotype (MHC I/IIa, IIa/IIx, and I/IIa/IIx). MHC I are slow-oxidative fibers (slow isoform contractile proteins, high mitochondrial density), MHC IIa are fast-oxidative fibers (fast contractile velocity, relatively fatigue resistance), and MHC IIx are fast-glycolytic fibers (fastest contractile proteins, low mitochondrial volume)[25].

Research supporting a MHC fiber type shift during spaceflight in humans has been increasing since the mid-1990s.After several ISS missions and long-term bed rest experiments in the last decade, enough data now exists to draw conclusions on the presence of spaceflight related fiber type shifts in humans.

Figure 4 contains compiled data from laboratory and others, lending support to the microgravity-induced fiber type shift paradigm in humans. Each of the studies report changes in fiber type from pre to post-spaceflight (or bed rest) in men and women measured via sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Unloading duration ranged from 11 to 177 days, with an average of ≈ 81 days. The studies investigated one of three lower limb muscles: the vastus lateralis (VL), soleus (Sol), or gastrocnemius (Gas). MHC I (slow) fiber composition decreased and total hybrid fiber proportion increased in all studies by an average of -13% and +14%, respectively. While unloading duration probably dictates the transition magnitude, trends were similar regardless of duration, unloading mode, or the muscle studied.

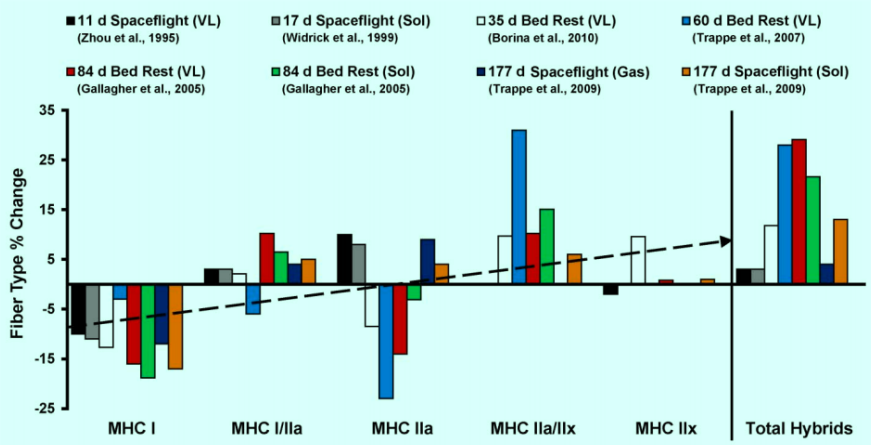



**Figure 4. Changes in myosin heavy chain (MHC) fiber type during unloading (spaceflight or bed rest). The linear trend-line is based on mean fiber type % change from all studies and illustrates a slow to fast fiber type shift (Plotted using Microsoft Excel 2010, trend-line equation: y = 3.51x - 10.408). All studies fiber typed using SDS-PAGE. Bed rest data is from control subjects. Total Hybrids represent fibers with multiple MHC isoforms. VL, vastus lateralis. Sol, soleus. Gas, gastrocnemius.**

Adapted from James R. Bagley, Kevin A. Murach, and Scott W. Trappe,Microgravity-Induced Fiber Type Shift in Human Skeletal Muscle, Gravitational and Space Biology Volume 26 (1) Apr 2012.

**LONG-DURATION SPACEFLIGHT EXERCISE COUNTERMEASURES**

Past exercise regimens onboard the ISS were varied among crewmembers, but generally included moderate intensity aerobic (≈5 days/wk) and resistance exercise (3-6 days/wk). The guidelines prescribed exercise for up to 2.5 h/day for 6-7 days/wk (time included hardware setup, stowage, and personal hygiene) utilizing a running treadmill, cycle ergometer, and resistance exercise device.

These previous exercise countermeasures failed to completely preserve skeletal muscle size and function, warranting modifications to longduration mission exercise prescription and/or hardware.

For decades, ground-based exercise physiology studies have shown chronic highintensity exercise promotes positive skeletal muscle adaptations (i.e. increases strength and endurance) and alters fiber type composition. Figure 3 illustrates fiber type changes (maintained MHC I, increased MHC IIa, decreased MHC IIx) following high-intensity and sprint cycle training in men and women ranging from 42 to 105 days in duration. These studies measured fiber type by SDS-PAGE or histochemical staining (standard technique of the 1970s and ’80s). Hybrid fibers were not

reported in these investigations. MHC I fiber percentage varied but was generally maintained (+1%), while MHC IIa composition increased (+6%) and MHC IIx composition decreased (-5%) on average. As opposed to spaceflight and bed rest, the trend-line compiled from these high-intensity/sprint cycling studies demonstrates a fast to relatively slower fiber type shift.

MHC I fibers significantly increased (+6%), MHC IIa fibers were maintained, and MHC IIb (IIx) fibers significantly decreased (- 6%) after 105 days of sprint cycling, suggesting lengthier training durations might induce increases in MHC I proportions as their transition may take longer to manifest.

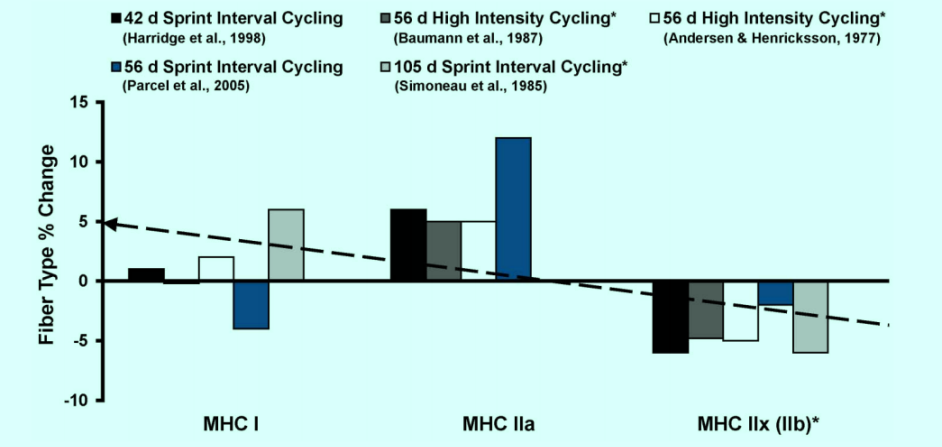



**Figure 5. Changes in myosin heavy chain (MHC) fiber type during high intensity exercise training. The linear trend-line is based on mean fiber type % change from all studies and illustrates a fast to slow fiber type shift (Plotted using Microsoft Excel 2010, trend-line equation: y = -2.86x + 6.32). *Fiber typing via ATPase histochemistry (IIb equivalent to IIx). VL, vastus lateralis.**

Adapted from James R. Bagley, Kevin A. Murach, and Scott W. Trappe,Microgravity-Induced Fiber Type Shift in Human Skeletal Muscle,Gravitational and Space Biology Volume 26 (1) Apr 2012.

Data from Figures 4 and 5 suggest mitigation of the microgravity-induced slow to fast shift is possible by employing high-intensity exercise during spaceflight. The idea of high-intensity exercise preventing a shift in MHC phenotype during long duration unloading was recently shown with bed rest (60 day), which has served as a guide for moving the exercise countermeasure program forward.

**Effect of Microgravity and Space Flight on the Chemical Senses**

Microgravity induces physiological changes including an upward shift of body fluids toward the head, which may lead to an attenuation of the olfactory component in the flavor of foods. Chemosensory changes may also relate to space sickness, Shuttle atmosphere, stress, radiation, and psychological factors.

The space environment can lead to alterations in the chemosensory perception of foods; these include diet, illness, and biochemical shifts.

Subjective reports from both Soviet and American astronauts report some attenuation of taste acuity (Rambaut et.al. 1977) and perception of an unpleasant taste in the mouth (Baranski et.al. 1983). A Russian study on the change in taste perception of astronauts in flight against the background of fatigue (Popov 1981) recommended adding different spices and condiments to food products to improve appetite under such conditions. On a similar note, a Russian cosmonaut on Vostok VI reported a reduced appetite for sweets and a desire for pungent food flavors (Oberg 1981).

In space travel, not only could the direct smell of the foods be partially lost, but part or all of the retronasal stimulation would be lost as well. This latter effect may be very important for the perception of some sensory qualities and the enjoyment of certain foods like juices because the perception of sweetness is increased by the retronasally perceived fruity flavor.

Astronauts frequently complain of the foods on the Shuttle being bland, and of a dislike for coffee while on orbit (Marshburn 1997).

**Space chemosensory experiments**

Russian and Polish scientists (Baranski et.al. 1983) conducted a study that followed a rather uncommon approach

of using an electrogustometer with electrodes to measure taste thresholds. The usefulness of electrogustometry in microgravity was discussed by Kubiczkowa and Skibnewski (1979) who argued that studies of thresholds of the "4 basic tastes" are not well suited for space because liquids and solutions behave differently in microgravity.

The study of Baranski et.al. (1983) included 2 astronauts on each of the Soyuz 30 and Soyuz 31 spacecrafts. A direct current of increasing intensity was applied to the tongue and the subjective perception of a slight pinching or a slightly acidic taste was indicative of the threshold of the taste organ. The measurements were replicated in different places on the tongue.

The results revealed a highly significant alteration in the taste threshold, reaching, in one instance, a change of 220% (preflight as compared to inflight, Figure 6). However, the direction of change was different for the 2 astronauts in both experiments.

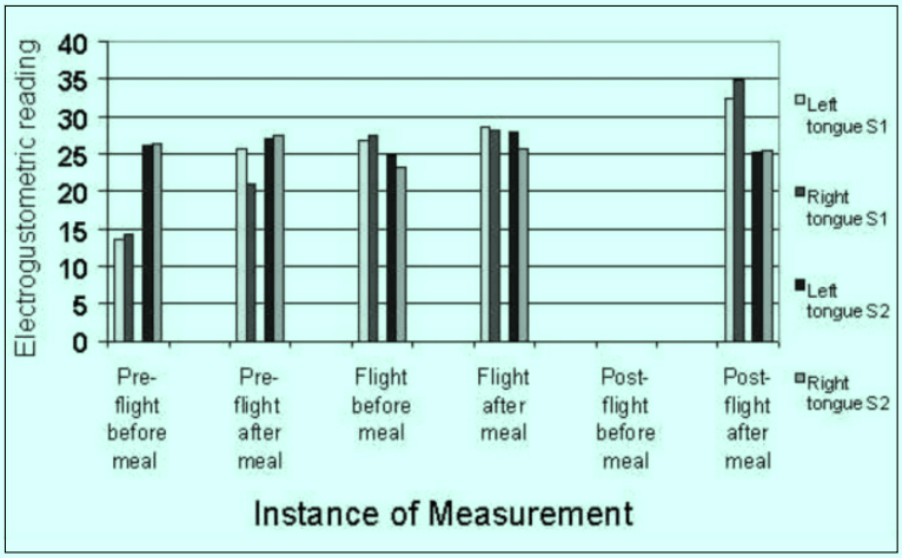



**Figure 6 —Electrogustometric measurements in Soyuz 30, adapted from Baranski and others (1983)**

Effect of Microgravity and Space Flight on the Chemical Senses, JOURNAL OF FOOD SCIENCE —Vol. 67, Nr. 2, 2002.

In Skylab-4 experiments, taste and odor thresholds were measured using slips of paper impregnated with different flavors. In this study, 3 crewmembers tasted orange and onion flavors in addition to the 4 basic tastes. Five concentrations were used with each flavor. The crewmembers were told in the beginning of the test about the different flavors and were asked to report the flavor when first detected and when definitely confirmed.

Odor identification thresholds were measured similarly for lemon, orange, onion, pepper, chicken, wintergreen, chocolate, cherry, spearmint, and cinnamon, in addition to a blank. The subjects' task was to identify the various aromas. The tests were conducted 10 d preflight, midflight, and 12 d postflight. The aroma tests gave no evidence of change in the ability to identify odors but the results of the taste tests were more varied, showing a shift in taste thresholds for certain sensations though results were highly individualized [31].

The findings of Skylab-4 prompted Canadian researchers to do a similar experiment during the 41-G Space Shuttle mission with 2 astronauts [32].The objectives were to compare preflight recognition thresholds for sucrose, urea, sodium chloride, and citric acid with the microgravity threshold, and to assess the recovery period of taste and olfaction postflight. Olfaction was assessed as all or none by identifying the smell of the following substances: lemon, mint, vanilla, and distilled water. In the taste experiment the samples were supplied at the concentration of preflight thresholds, along with a higher and another lower concentration for each category in addition to 2 blanks. The recognition thresholds were determined preflight, in-flight, landing day and fifth day postflight. This experiment showed no effect of microgravity on either the smell or taste thresholds of the astronauts.

The authors also mentioned that the 2 astronauts in the 41-G trip, which lasted 8 d, did not experience any space

sickness, which was not the case with the Skylab-4 astronauts who stayed for 84 d. The authors added that space sickness could have been a factor in altering taste thresholds. In a parallel example, Kekhayov (1974) found that healthy pilots exhibit changes in their taste sensitivity that can be the result of dynamic factors of flight. The space chemosensory studies and their results are summarized in Table 7.

**Table 7 —Space Chemosensory Experiments**

| Space Mission | Method | Sense(s) Assessed | Results | Reference |
|---|---|---|---|---|
| Soyuz 30-31 | Electrogustometry (anodic) | Taste | Significant Effect | Baranski and others 1983 |
| Skylab-4 | Impregnated paper | Taste/Smell | Effect /No Effect | Heidelbaugh and others 1975 |
| G-41 Shuttle | Taste solutions (detection + recognition thresholds) | Taste/Smell | No Effect/No Effect | Watt and others 1985 |
| Soyuz | Electrogustometry (anodic) | Taste | Conflicting results | Baranski and others 1979 |

Adapted from A.A. OLABI, H.T. LAWLESS, J.B. HUNTER, D.A. LEVITSKY, AND B.P. HALPERN,The Effect of Microgravity and Space Flight on the Chemical Senses,JOURNAL OF FOOD SCIENCE --Vol. 67, Nr. 2, 2002.

**Microgravity Simulation Studies**

Microgravity simulation studies and their results are summarized in Table 8. Rice et.al. (1996) observed in a microgravity simulation (n =6) that headward fluid shifts did not change subjects' sensitivity to taste (sodium chloride, sucrose, citric acid, quinine, monosodium glutamate, and capsaicin), odor (isoamylbutyrate and menthone) or the trigeminal system. It is unlikely that any change in the trigeminal system would have been found since this system only responds to "extreme" or painful stimulants such as chemical irritation.

**Table 8 —Microgravity Simulation Chemosensory Experiments**

| Experimental Conditions: Bed Rest | Method | Sense(s) Assessed | Results | Reference |
|---|---|---|---|---|
| 6 subjects laying on a –6° inclined bed | Taste + odor sensitivity (threshold) | Taste/Smell | No effect on Taste/Smell or Trigeminal system | Rice and others 1996 |
| Head tilt down condition vs. bed rest at normal angle | Taste sensitivity test (threshold) | Taste | No significant change of taste sensitivity between the 2 conditions | Kanda and others 1993 |
| 53 subjects with bed rest (–8°) vs normal bed rest | Electrogustometry (anodic) | Taste | Significant increase in gustatory thresholds | Yakovleva 1982 |
| Head inclined bed rest to produce hypokinesia | Functional mobility method | Taste | Decrease in functional activity of taste receptors of the tongue | Kurlyandski and others 1974 |
| Head inclined bed rest (+6°, –2° and –6°) | Taste sensitivity + functional mobility | Taste | Decreased taste sensitivity & increased mobilization of taste receptors | Budylina and others 1976 |
| Several levels of body tilt (0°, 90°, 135°, 180°) | Odor identification tests + nasal resistance | Smell | Decreased odor identification No effect on nasal resistance | Mester and others 1988 |
| A hypo-dynamia condition | Taste stimu-lation threshold + functional mobility | Taste | Decrease in taste sensitivity + decrease in mobilization of taste receptors | Volozhin and others 1974 |

Adapted from A.A. OLABI, H.T. LAWLESS, J.B. HUNTER, D.A. LEVITSKY, AND B.P. HALPERN, The Effect of Microgravity and Space Flight on the Chemical Senses,JOURNAL OF FOOD SCIENCE—Vol. 67, Nr. 2, 2002.

Kurlyandski et.al. (1974) studied changes in the taste of foods in 15 healthy men in a study that simulated weightlessness by using head inclined bed rest to produce hypokinesia. The method of functional mobility was used in this study and the results showed a reduction of the functional activity of taste receptors of the tongue (and disturbance of the gastro-lingual reflex).

In a study involving both cosmonauts and healthy male controls (62 men aged 25 to 45), Yakovleva (1982) observed altered taste perception in a weightlessness simulating condition using an electrogustometer. Fifty-three men were used as controls and 9 cosmonauts were examined during 5 d under bed rest with a bed head slope of -8 degrees. Statistically significant increases in gustatory thresholds were found.

Nakagawa and others (1996) studied the effect of mental or physical stress on taste perception. Mental stress was induced by performing a demanding letter search and physical stress was induced by performing an exercise using an ergometer. The taste intensity of solutions of sucrose, quinine sulfate and citric acid were measured using time intensity techniques. The mental task resulted in a reduction of the duration of aftertaste for all tastes. The authors assumed that this phenomenon is likely to be due to some type of central inhibition of taste perception. The physical task resulted in a reduction of the time intensity curve of sourness. The authors linked this outcome to an increase in the buffering capacity of saliva due to hard exercise. Hence mental stress appears to have a stronger effect on taste perception than pure physical stress.

**Table 8 —Microgravity Simulation Chemosensory Experiments**

| Experimental Conditions: Bed Rest | Method | Sense(s) Assessed | Results | Reference |
|---|---|---|---|---|
| 6 subjects laying on a –6° inclined bed | Taste + odor sensitivity (threshold) | Taste/Smell | No effect on Taste/Smell or Trigeminal system | Rice and others 1996 |
| Head tilt down condition vs. bed rest at normal angle | Taste sensitivity test (threshold) | Taste | No significant change of taste sensitivity between the 2 conditions | Kanda and others 1993 |
| 53 subjects with bed rest (–8°) vs normal bed rest | Electrogustometry (anodic) | Taste | Significant increase in gustatory thresholds | Yakovleva 1982 |
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| Head inclined bed rest (+6°, –2° and –6°) | Taste sensitivity + functional mobility | Taste | Decreased taste sensitivity & increased mobilization of taste receptors | Budylina and others 1976 |
| Several levels of body tilt (0°, 90°, 135°, 180°) | Odor identification tests + nasal resistance | Smell | Decreased odor identification No effect on nasal resistance | Mester and others 1988 |
| A hypo-dynamia condition | Taste stimu-lation threshold + functional mobility | Taste | Decrease in taste sensitivity + decrease in mobilization of taste receptors | Volozhin and others 1974 |

**Adapted from A.A. OLABI, H.T. LAWLESS, J.B. HUNTER, D.A. LEVITSKY, AND B.P. HALPERN,The Effect of Microgravity and Space Flight on the Chemical Senses,JOURNAL OF FOOD SCIENCE-Vol. 67, Nr. 2, 2002.**

**Table 9 —High Altitude Chemosensory Experiments**

| Experimental Conditions | Method | Sense(s) Assessed | Results | Reference |
|---|---|---|---|---|
| 3500 m altitude | Taste threshold (4 basic tastes) | Taste | Thresholds shifted: increased glucose & NaCl; decreased quinine & citric acid | Singh and others 1997a |
| Simulated 5000 and 10000 ft | Taste threshold (4 basic tastes) | Taste | Significant increase in threshold (all solutions as one) between control & 5000 ft level | Maga & Lorenz 1972 |
| Simulated 5500 m + high $O_2$ | Functional mobility with sucrose, NaCl and citric acid | Taste | No difference between control and treatment | Zaiko and others 1963 |
| Simulated hypobaric hypoxia (7620 m) | Rat experiment with $H_2O$ + 4 basic fastes solutions | Taste | Changes in taste preferences | Singh and others 1997b |
| Hypobaric, normobaric hypoxia, hypobaric pressure + high $O_2$ | Rat experiment, feeding behavior | Taste | decrease in rate of feeding with 2 hypoxia conditions | Ettinger & Staddon 1982 |

**Adapted from A.A. OLABI, H.T. LAWLESS, J.B. HUNTER, D.A. LEVITSKY, AND B.P. HALPERN,The Effect of Microgravity and Space Flight on the Chemical Senses,JOURNAL OF FOOD SCIENCE -Vol. 67, Nr. 2, 2002.**

**Mental and Physical Workload, Salivary Stress Biomarkers and Taste Perception: Mars Desert Research Station Expedition**

It has been reported that that taste was altered in extreme condition during Mars Desert Research station crew-78 and simulated microgravity.

The Mars Desert Research Station (MDRS) is an analog to a Mars surface habitat, constructed for mission simulations according to Mars Reference Mission guidelines, and located in a US southwest desert region relevant to Mars analog geology, biology, and human research. The main aims of station are to develop field tactics based on environmental constraints (being mandatory to work in spacesuits), to test habitat design features and tools, and to evaluate crew selection protocols.

The 12 crew members were selected from two crews Euro Moon Mars by International Lunar Exploration Working Group and Vrije Universiteit Amsterdam. The ages for the crew members aged 20-26 (23.6 (2.4)) years. The average and calcium intake of the crew members during mission was 2400 kcal/day (range 2090-3200 kcal/day) and 1267 mg/day (1130-1400 mg/day), respectively. Dietary sodium and potassium intake were maintained at 98 (80-103) and 86 (75-120) mmol/day, respectively. Water intake was ad libitum 1236 (1200-1309) mL/day.

The taste stimuli were exemplars of the sensations of bitterness, sourness, and sweetness. The bitter sample was an aqueous solution of quinine sulfate ($1.82 \pm 10^{-5}$M). The sour sample was an aqueous solution of anhydrous citric acid ($1.37 \pm 10^{-2}$M), and the sweet sample was an aqueous solution of sucrose ($2.63 \pm 10^{-1}$M).

Saliva samples were collected before and after mental and physical tasks. The samples were immediately frozen at -4°C, centrifuged and analyzed for biomarkers. The CST was used for measuring stress[36]. Salivary cortisol (Salimetrics Inc., PA, USA) and alpha-amylase (alpha-amylase assay kit,Salimetrics Inc., State college, PA, USA) were measured.

| Parameters | Computer workload | | | | Physical workload | | | |
|---|---|---|---|---|---|---|---|---|
| | First day | | End of mission* | | First day | | End of mission* | |
| | Before | After** | Before | After** | Before | After** | Before | After** |
| Tension | 6.23 (1.22) | 24.68 (4.67) | 7.89 (2.33) | 30.67 (3.78) | 4.67 (1.04) | 1.89 (1.02) | 5.56 (1.23) | 2.34 (12.4) |
| Vigor | 20.68 (4.67) | 12.67 (5.34) | 21.02 (3.56) | 13.02 (4.56) | 24.67 (4.65) | 32.78 (3.78) | 28.78 (4.67) | 32.45 (5.36) |
| Fatigue | 11.45 (2.67) | 46.54 (3.46) | 13.78 (3.45) | 52.67 (3.67) | 14.78 (3.45) | 45.67 (6.45) | 15.89 (4.04) | 48.67 (5.89) |
| Vague | 12.67 (3.02) | 23.56 (5.78) | 14.56 (4.67) | 32.67 (3.57) | 10.96 (2.68) | 8.03 (2.04) | 9.12 (3.03) | 6.95 (3.05) |

Scores are a percentage (%) out of a possible 100 points possible for each factor; *P < 0.05 vs. first day; **P < 0.01 vs. before

Scores of mood state before and after mental and physical workload

**Rai, Balwant, and Jasdeep Kaur. "Mental and Physical Workload, Salivary Stress Biomarkers and Taste Perception: Mars Desert Research Station Expedition." North American Journal of Medical Sciences 4.11 (2012): 577–581.**

Duration of sleep, work and leisure was 482 (143), 542 (178), and 126 (34) minutes, respectively. Following the letter search task (tasks or workload), feelings of tension and fatigue increased while the sense of vigor decreased.

It was frequently reported in the subjects' self-examination after the test session that they felt irritable or very tired. Relative to the pre-stress baseline, the average TI function for bitterness showed a decrease in maximum intensity, a reduction in the duration of after-taste and a decrease in total bitterness (area). For sourness, there was change in maximum intensity, and there was a reduction in duration and a decrease in total sourness. The pattern for sweetness was similar to that for sourness and there was a reduction in the duration of after-taste and a decrease in the total amount of taste.

| Parameters | Sweetness | | | | Bitterness | | | | Sourness | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | First day | | End of mission * | | First day | | End of mission* | | First day | | End of mission* | |
| | Before | After** | Before** | After** | Before | After** | Before | After** | Before | After** | Before | After** |
| Total amount* (area) | 567 (45) | 468 (44) | 534 (67) | 460 (68) | 732 (64) | 453 (67) | 723 (62) | 420 (73) | 554 (54) | 420 (56) | 534 (64) | 402 (65) |
| Maximum intensity | 76 (4) | 74 (5) | 75 (6) | 72 (3) | 79 (5) | 65 (3) | 77 (6) | 62 (7) | 72 (5) | 70 (6) | 70 (7) | 67 (5) |
| Duration time (s) | 74 (12) | 54 (11) | 70 (12) | 52 (11) | 88 (8) | 55 (7) | 86 (7) | 52 (8) | 70 (6) | 46 (6) | 67 (5) | 43 (6) |

Total amount was calculated by summation every 5 s. *$P < 0.05$ vs. first day; **$P < 0.01$ vs. before

Taste perception following a period of physical stress

**Rai, Balwant, and Jasdeep Kaur. "Mental and Physical Workload, Salivary Stress Biomarkers and Taste Perception: Mars Desert Research Station Expedition." North American Journal of Medical Sciences 4.11 (2012): 577–581.**

The TI taste evaluation showed that the maximum intensity, the duration of after-taste, and the total amount of after-taste were changed for bitterness and sweetness. For sourness, however, there was a decrease in the intensity and the total amount of taste and the duration of after-taste tended to be reduced.

| Parameters | Sweetness | | | | Bitterness | | | | Sourness | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | First day | | End of mission* | | First day | | End of mission* | | First day | | End of mission* | |
| | Before | After** | Before | After** | Before | After** | Before | After** | Before | After** | Before | After** |
| Total amount[$] (area) | 523 (47) | 409 (43) | 511 (56) | 398 (54) | 702 (54) | 446 (52) | 678 (61) | 340 (64) | 489 (52) | 399 (52) | 478 (51) | 402 (65) |
| Maximum intensity | 68 (5) | 62 (7) | 65 (8) | 57 (4) | 70 (7) | 60 (5) | 68 (5) | 56 (6) | 64 (6) | 52 (7) | 63 (8) | 58 (6) |
| Duration time (s) | 67 (10) | 48 (12) | 64 (14) | 43 (10) | 70 (2) | 50 (3) | 67 (8) | 40 (9) | 63 (7) | 40 (8) | 61 (6) | 38 (7) |

[$]total amount was calculated by summation every 5 s.; *$P < 0.01$ vs. first day; **$P < 0.05$ vs. before

Taste perception following a period of mental stress

**Rai, Balwant, and Jasdeep Kaur. "Mental and Physical Workload, Salivary Stress Biomarkers and Taste Perception: Mars Desert Research Station Expedition." North American Journal of Medical Sciences 4.11 (2012): 577–581.**

CST scores, salivary alpha amylase, and cortisol levels were increased, although increased levels were more in physical tasks as compared to mental workload . So, taste affects more in physical as compared to mental tasks. There were good correlation between CST scores, salivary alpha amylase and cortisol (r = 0.89, r = 0.92). There were good correlation between average time intensity of sweetness, bitterness, sourness and cortisol levels (r = 0.89, r = 0.78, r = 0.84, respectively).

| Parameters | Mental workload | | | | Physical workload | | | |
|---|---|---|---|---|---|---|---|---|
| | First day | | End of mission * | | First day | | End of mission * | |
| | Before | After** | Before | After** | Before | After** | Before | After** |
| CST | 2.56 (0.23) | 3.02 (0.56) | 2.89 (0.45) | 3.45 (0.67) | 2.78 (0.89) | 3.24 (0.78) | 2.84 (0.68) | 3.35 (0.89) |
| Salivary alpha amylase (U/mL) | 59.6 (24.2) | 67.5 (23.2) | 60.5 (23.2) | 78.9 (23.6) | 65.9 (23.5) | 78.9 (24.6) | 67.8 (34.2) | 85.6 (23.5) |
| Salivary Cortisol (µg/dL) | 0.267 (0.112) | 0.289 (0.115) | 0.280 (0.117) | 0.304 (0.113) | 0.274 (0.114) | 0.296 (0.115) | 0.278 (0.115) | 0.312 (0.116) |

*$P < 0.05$ vs. first day ss; **$P < 0.01$ vs.before; CST: Current stress test

Scores of CST and salivary biomarkers levels before and after mental and physical workload

**Rai, Balwant, and Jasdeep Kaur. "Mental and Physical Workload, Salivary Stress Biomarkers and Taste Perception: Mars Desert Research Station Expedition." North American Journal of Medical Sciences 4.11 (2012): 577–581.**

The taste affects were more pronounced in physical as compared to mental tasks. It could be because of physical tasks leads to more stress as compared to mental tasks supported by the fact of higher level of CST scores, salivary alpha amylase, and cortisol levels in physical tasks. Furthermore, stress biomarker cortisol inhibits the neurotransmission of noradrenalin, dopamine, and serotonin, and/or a reduction in the sensitivity of their receptors.

Taste change is not due to sleep disturbance and leisure time as these are not contributing this study. Also, it has been reported that low levels of calcium and acidic condition leads to suppression of the taste responses. Normal calcium levels were reported in all crew members indicating this is not contributing factor. Decreased energy consumption is not linked to taste and smell loss and related complaints.

Microgravity induces physiological changes including an upward shift of body fluids toward the head, which may lead to an attenuation of the olfactory component in the flavor of foods. Chemosensory changes may also relate to space sickness, shuttle atmosphere, stress, radiation, and psychological factors.

High workload during performance of experiments and extravehicular activities, multicultural and international environments and working in spacesuits leads to stress. in simulated and real microgravity conditions.So, stress produced due to microgravity, physical, and mental tasks and extreme environment condition could affect the taste sensations as well.

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