HIGHLIGHTED TOPIC | Analogs of Microgravity: Space Research without Leaving the Planet

Hindlimb unloading: rodent analog for microgravity

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Globus RK, Morey-Holton E. Hindlimb unloading: rodent analog for microgravity. J Appl Physiol 120: 1196–1206, 2016. First published February 11, 2016; doi:10.1152/japplphysiol.00997.2015.—The rodent hindlimb unloading (HU) model was developed in the 1980s to make it possible to study mechanisms, responses, and treatments for the adverse consequences of spaceflight. Decades before development of the HU model, weightlessness was predicted to yield deficits in the principal tissues responsible for structure and movement on Earth, primarily muscle and bone. Indeed, results from early spaceflight and HU experiments confirmed the expected sensitivity of the musculoskeletal system to gravity loading. Results from human and animal spaceflight and HU experiments show that nearly all organ systems and tissues studied display some measurable changes, albeit sometimes minor and of uncertain relevance to astronaut health. The focus of this review is to examine key HU results for various organ systems including those related to stress; the immune, cardiovascular, and nervous systems; vision changes; and wound healing. Analysis of the validity of the HU model is important given its potential value for both hypothesis testing and countermeasure development.

spaceflight; hindlimb unloading; gravity; physiology; adaptation

THE MODEL OF HINDLIMB UNLOADING (HU) was developed to explore musculoskeletal responses to the space environment and now also is used extensively to investigate muscle atrophy and disuse osteopenia (reduced bone density) caused by various Earth-based conditions such as muscle wasting disease, inactivity, bed rest, and immobilization. In addition, the HU model confers the ability to evaluate recovery responses, an important aspect of determining the long-term effect of spaceflight on astronaut crews. Rodent models of HU show site-specific effects on both bone and muscle, and the model already has proven its value for unraveling detailed mechanisms and testing potential interventions for muscle and bone.

Brief introductory comments on the HU model and the musculoskeletal system set the stage for the main objective of this minireview, which is to analyze the main attributes, limitations, and applicability of the rodent HU analog of spaceflight, with a particular focus on specific areas currently of interest, including stress; the immune, cardiovascular (CV), and nervous systems; wound healing; and vision. For each organ system, we will address two essential questions that define both the validity and value of experiments that use the HU analog: first, how well does the model mimic spaceflight observations; and second, are HU animal results similar to human findings? Thus it is useful first to evaluate the relative differences in key aspects of HU model and spaceflight related to the unique environment’s influence on physical aspects of the organism (Table 1).

Rodent HU and the Musculoskeletal System

To mimic weightlessness, the HU model selectively unloads the hindquarters of rats or mice using tail traction, surgical pins, or body harness to cause a cephalad fluid shift and removal of ground reaction forces from the hindlimbs (Table 1) as occurs in astronauts (23). Gravity is still present in the HU model, thus responses reflect only partial unloading of the musculoskeletal system with a headward shift of internal organs and fluid. The internal organs still exert pressure against each other with HU, unlike in spaceflight, during which organs free float. Although the prevalent method to unload rodents is designed to avoid surgery with resulting stress [i.e., by applying tail traction using orthopedic tape (75)], methods that entail placement of a surgical pin may afford the advantage of enabling longer-duration studies. Additional recent modifications of the model include use of a body harness to impose partial gravitational loading (118). Whether or not the specific technique selected to unload animals substantively contributes to observed results is not known, although an early study comparing the back harness and tail traction methods side-by-side revealed that rats unloaded by back harness display more adverse stress and skeletal responses than animals unloaded by tail traction (129). Thus technical details related to the HU method selected for a specific investigation is an important...
HU studies clearly show that many independent variables can influence results obtained and, ultimately, the analog’s validity with respect to understanding the mechanisms and physiological responses to spaceflight. Just as with spaceflight studies, timing of HU exposure is a critical variable, and acute responses can differ dramatically from those observed after longer durations of HU with chronic adaptation. Additional HU variables known to influence experimental results include age (growing vs. adult), sex, species (rat vs. mouse), and strain. Results obtained from using the HU model over the past 4 decades demonstrate the complexity of the physiological adaptations and cascading consequences of what would first seem to be single, simple perturbation of body position relative to the gravity vector. For example, unloading of the hindlimbs causes selective atrophy of musculature in the weight-bearing hindquarters, which in turn, reduces forces exerted on bone where the muscles attach via tendons; this likely contributes to bone loss at that site. The vascular supply and neural innervation adapt to the cephalad fluid shift and changes in hindlimb mass, and so proceed a cascade of changes in multiple physiological systems throughout the body, including metabolism. HU and spaceflight in rats, and also bed rest and spaceflight in humans, cause a reduction in overall physical activity levels that lead to both a shift away from fatty acids toward glucose in oxidative muscle metabolism and insulin resistance (11, 73, 91, 111). Thus the physical challenges posed by both HU and spaceflight lead to both metabolic and structural adaptations that are related (111).

Muscle and bone share two general characteristics with respect to adaptive structural responses to spaceflight and HU. First, muscle atrophy and osteopenia occur in select regions related to loading; and second, both can result from diminished synthesis and enhanced degradation. In skeletal muscle, spaceflight and HU exert the greatest atrophic effects on unloaded, predominantly slow-twitch, postural muscles such as the soleus, sometimes referred to as an “antigravity muscle” (see reviews in (10, 19, 87)). Substantial atrophy of the soleus (to 50% of controls) occurs rapidly (within 2 wk of initiating HU), primarily via a reduction in synthesis of myosin heavy chain (MHC) and actin proteins via regulation of gene expression. Both spaceflight and HU lead to a switch in expression of MHC isoforms, reduced glucose tolerance, and increased markers of oxidative stress. A particularly interesting recent finding is that both HU mice and humans subjected to bed rest display reduced glucose tolerance and activation of specific molecular inflammatory pathways in muscle (54). In rodents, HU activates multiple molecular pathways responsible for proteolysis and degradation, although the importance of degradation for muscle atrophy in a human crew is not yet known (19).

In bone, spaceflight and HU of rodents exert rapid effects on high-turnover, cancellous (spongy) tissue, but they also can cause decrements in slow-turnover, cortical (compact) tissue, typically affecting those skeletal regions that are subjected to mechanical loading during normal ambulation on Earth (see reviews in (18, 45, 74)). The main skeletal regions where HU appears less valid for simulating spaceflight include the upper spine, forelimbs, and the mandible which closes against gravity, unlike in orbit (Table 1). In growing rats, which were the subjects for nearly all early spaceflight and HU experiments on the musculoskeletal system, unloading primarily reduces bone formation rate in the hindquarters, whereas indices of bone resorption (degradation) most often remain unaffected. Furthermore, in growing rats, the decrements in bone mass due to spaceflight and HU occur due to a failure to grow rather than a net loss. Interestingly, adult mice subjected to spaceflight or HU show consistent increases in indices of bone resorption and net bone loss. The negative calcium balance and decrements in bone mineral density observed in astronauts is attributed to increased bone resorption, whereas changes in expression of markers of bone formation typically remain unchanged in human crews during flight (104); therefore, adult mice may provide better test subjects than growing rats for interrogating spaceflight-induced osteopenia (i.e., bone loss) of human crews.

In summary, the findings from musculoskeletal studies performed to date using the HU analog have advanced scientific understanding of the cellular and molecular mechanisms that mediate disuse muscle atrophy and osteopenia, although limitations of the model should be considered when designing experiments, interpreting results, and extrapolating the findings to human crews. In particular, possible influences of systemic endocrine, metabolic, inflammatory, and stress-related factors on HU experimental outcomes should be considered, as discussed below.

**Stress Response**

An important question related to both spaceflight and HU is to what extent are the observed changes in given organ systems mediated by activation of the hypothalamic-pituitary-adrenal (HPA) axis and/or sympathetic nervous system as a stress

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### Table 1. Comparison of major characteristics

<table>
<thead>
<tr>
<th>System</th>
<th>Rodent, Hindlimb Unloading</th>
<th>Rodent, Spaceflight</th>
<th>Human, Spaceflight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluid distribution</td>
<td>Cephalad fluid shift</td>
<td>Unknown, but not preferentially cephalad</td>
<td>Cephalad fluid shift</td>
</tr>
<tr>
<td>Physical activity level/mobility</td>
<td>Reduced</td>
<td>Sometimes increased in mice*</td>
<td>Reduced</td>
</tr>
<tr>
<td>Cell/tissue loading directly from 1G</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Related to weight-bearing</td>
<td>Lower jaw needs action to close</td>
<td>Lower jaw needs action to open</td>
<td>Lower jaw needs action to open</td>
</tr>
<tr>
<td>Head/lower jaw</td>
<td>Load depends on angle of unloading</td>
<td>Flight cage design-dependent*</td>
<td>Similar to Earth</td>
</tr>
<tr>
<td>Forelimbs</td>
<td>Unloaded with foot drop</td>
<td>Unloaded</td>
<td>Unloaded</td>
</tr>
<tr>
<td>Spine</td>
<td>Inward curvature</td>
<td>Unloaded</td>
<td>Unloaded</td>
</tr>
<tr>
<td>Hindquarters</td>
<td>Unloaded</td>
<td>Unloaded</td>
<td>Unloaded</td>
</tr>
</tbody>
</table>

*Physical activity level and loading of forelimbs is likely to depend on cage design (i.e., ability of rodent to grab onto cage for movement) and if the animals are group-housed.
response? This is relevant to address because neuroendocrine stress may mask, mimic, or augment adaptive responses to spaceflight or HU, thus confounding interpretation of the results. In addition, stress is known to disrupt circadian rhythms, which can have a profound effects on physiological function (2). Stress responses typically are assessed by findings of one or more of the following: increased levels of adrenal corticosterone, growth hormone, epinephrine or norepinephrine, reduced body mass or lack of growth, lymphoid organ atrophy, or increased adrenal gland mass. Both for astronauts and animals returning from spaceflight, reentry and landing are known to be stressful, and in animals, the time of tissue harvest may be sufficient to mitigate or mask spaceflight effects. For determining whether or not there are stress responses, blood corticosteroid levels alone are particularly problematic because levels rapidly change due to conditions at the time of sample recovery (e.g., landing), whereas lymphoid organ atrophy and body mass decrements are non-specific to activation of the HPA axis and occur in response to other conditions such as exercise and immunodeficiency.

Rodent spaceflight. Results from rodent spaceflight experiments have not yielded consistent results with respect to activation of the HPA axis. After landing from relatively short-duration Shuttle experiments (all less than 3 wk long) rodents can show increased serum corticosterone levels and also reduced body weight, adrenal hypertrophy, and lymphoid organ atrophy, consistent with activation of the HPA axis (27, 86). In a short-duration (17 days) Shuttle mission that was dedicated to determining whether specific spaceflight effects are due to elevated adrenal corticoids, 6-wk-old rats were adenalecromized, and adrenal steroids were replaced by implant to maintain constant blood levels. Replacement of corticosterone in flight rats extinguishes spaceflight-induced changes in the central serotonergic system, indicating that these effects are probably due to activation of the HPA axis (17). In contrast, in this experiment, spaceflight inhibits bone formation in the thoracic vertebrae and pelvis relative to ground controls despite glucocorticoid status (132). These findings were interpreted to mean that changes in the thoracic vertebrae and pelvis occur independent of stress, although the general applicability of these findings is questionable due to the young age of the test subjects and the absence of similar spaceflight responses at other relevant skeletal sites (128).

Rodent HU. Despite considerable effort devoted to determining whether observed responses in various organ systems are caused by stress, general agreement among investigators is elusive, in large part because of the wide range in animal variables (age, sex, species, strain), experimental conditions (short- or long-term), and technical details selected, including HU method and diet (116). HU in some experiments increases catecholamine production (8) and also can increase corticosterone levels in both short- and long-duration experiments. An early increase in serum corticosterone (59, 122) and atrophy of lymphoid organs atrophy occur, which are absent in osteopontin-null mice (122). In contrast, investigators have reported the absence of these stress indices at later time points in long-duration experiments (42), although often the study designs did not preclude the possibility that transient changes occurred prior to measurement. Better insight may be gained in the future by the use of transgenic animal strains with specific deficiencies in responsiveness to glucocorticoids (3, 122, 123) and pathway analyses from global expression arrays.

Human relevance. Launch and landing can pose an acute stress to human crews during short-duration missions as indicated by elevated salivary cortisol levels and other stress markers preflight and postflight (67), although some studies from long-duration missions reported no differences (105). There is considerable individual variation reported in cortisol levels, and changes are often transient (112), as with the rodent spaceflight and HU studies. Thus findings that HU can cause acute stress in adult rodents conceivably may be interpreted as an indication that the model may simulate some aspects of human responses to spaceflight that occur near launch, even though findings such as these may complicate data interpretation. Experimental conditions that cause chronic activation of the HPA axis in HU rodents, however, are less likely to prove relevant to human crews.

Immune System

Understanding immune system responses to spaceflight is of unquestionable importance for both pathogen defense and the potential impact of inflammation on other organ systems. From the earliest manned spaceflight missions, anecdotal reports indicated that spaceflight leads to increased incidence of infections (33). Given the challenging and novel environment of space, psychological stress has long been suspected to contribute to impaired immune function and may account solely for some responses (107). Nonetheless, experiments using cultured immune cells demonstrate convincingly that microgravity can exert profound and very rapid effects on immune cell activation, cytokine secretion, and gene expression even in the absence of systemic factors (5, 26, 30, 51).

Rodent spaceflight. Rodent experiments have demonstrated that spaceflight can affect both the innate and acquired immune systems including the depletion and redistribution of B and T cells, altered gene expression and cytokine profiles, and lymphoid gland masses, although results can vary widely (48, 86). Differing results between spaceflight experiments may be due in part to the relatively short duration of most experiments (Shuttle missions) when changes occur rapidly and transiently, and to stresses associated with landing. Landing-associated stress reproducibly increases neutrophils (innate immunity) in both humans and rodents (33).

Spaceflight findings may be further complicated by variable stress responses to launch and introduction of the animals into a novel environment, independent of microgravity effects per se. For example, in one study, spaceflight reduced splenic T cells (CD4+) and neutrophils/macrophages (CD11b+) but the study did not record typical stress responses (i.e., body mass and thymus mass decrements) (122). In contrast, another study showed that spaceflight decreased lymphoid organ mass and splenic cell counts, altered cytokine secretion and upregulated expression of antioxidant genes, suggesting that spaceflight stimulates anti-inflammatory mechanisms (12). Furthermore, spaceflight impairs antigen-specific tolerance and increases expression of proinflammatory cytokines (24). In bone marrow, short-duration spaceflight yields a shift in granulocyte lineage from less differentiated to more differentiated cells in mice (83), as well as T-cell redistribution in the thymus and spleen (47). A longer-duration experiment (i.e., 30 days) also

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showed thymus and spleen atrophy and changes in expression of cytokines and heat shock proteins (79), although the lengthy period (13 h) between landing and sample recovery likely affected gene expression findings in that study. A more recent experiment on the International Space Station (ISS) of similar duration (37 days) but using different hardware and on-orbit sample recovery showed no indications of lymphoid organ atrophy compared with ground controls (R.K. Globus, unpublished observations). Consistent with the finding that antioxidant enzymes are induced by spaceflight, the rodent spaceflight experiment of longest duration to date (90 days) showed elevated erythrocyte lipid peroxidation, a marker of oxidative damage (101), which may result from generalized inflammation. Taken together, these findings show that immune system function is temporally sensitive to duration of exposure to the spaceflight environment, and that stress responses at various phases of a typical rodent spaceflight experiment (launch, on-orbit, landing) may contribute to immune dysfunction.

**Rodent HU.** HU causes a generalized and possibly transient activation of the immune system. Factors that may mediate HU-induced changes in immune system function include anti-orthostasis, stress (activation of the sympathetic nervous system and/or HPA axis), and disuse/inactivity. HU can disrupt the integrity of the gastrointestinal (GI) epithelium with leakage of pathogens from the GI tract, first shown to occur into the portal system causing endoporal toxemia in both rats and mice (100). In young mice, HU alone causes bacterial translocation out of the intestinal lumen, activates the innate immune system, and impairs the ability to clear bacterial infections, which is markedly worsened by radiation exposures designed to simulate solar particle events (59, 137). Additional evidence for a contribution of inflammation to dysfunction of various organ systems includes altered vascular reactivity of the carotid artery (48, 136), impaired insulin and toll-like receptor/myeloid differentiation primary response MyD88 signaling in muscle (54), and increased expression of oxidative stress markers in multiple tissues (28, 56).

HU alters immune cell distribution and impairs B-cell lymphopoiesis in the bone marrow (57) and T-cell function (76). HU improves resistance to primary infection with *Listeria monocytogenes* but impairs long-term resistance via T-cell-mediated memory (69, 70). HU mice infected with pathogens more relevant to humans (e.g., *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) show increased mortality, reduced time to death, reduced bacterial clearance, and increased corticosterone levels (7, 15); thus stress may be an important component of these responses. Interestingly, direct comparison of results from analysis of splenic subpopulations in rats comparing groups of spaceflight, HU, and launch and landing controls reveals that landing loads alone (not HU) most closely mimics spaceflight-induced changes in CD4+ T cells (86), emphasizing the importance of controlling for the various environmental factors that may influence outcome.

**Human relevance.** Spaceflight affects the innate and acquired immune systems in astronaut crew members. As with rodent spaceflight and HU studies, activation of the sympathetic nervous system and HPA axis is likely to contribute to at least some of the human immune responses (112). Both human crew and animals show changes in peripheral blood leukocytes and, in general, the leukocyte changes observed in HU experiments resemble those observed in humans postflight, which are most likely associated with the stress of landing (25, 33, 48, 112). However, analysis of blood collected during (not after) flight shows that leukocyte distribution, T-cell function, and cytokine production profiles are altered by short-duration spaceflight (34). Furthermore, in vivo skin tests of astronauts following a short-duration spaceflight show reduced delayed hypersensitive responses (114). Results from these human studies do not preclude the possibility that activation of the HPA axis in orbit has a persistent effect to alter cellular immune responses. Despite notable differences in findings on the specifics of immune dysfunction between rodent HU and astronauts (33, 48), the HU model allows direct testing of both resistance to pathogens and candidate countermeasures (4, 6, 9, 58, 73), which has not been ethically possible with human crews.

**Cardiovascular System**

The CV system evolved as life on Earth emerged from the sea requiring a system to transport fluids throughout all systems in the body to maintain blood perfusion to the brain against Earth’s gravity. As posture changed from crawling along the ground to walking, the position of heart moved toward the head and the vascular lungs became shorter (61, 62). The CV system is sensitive to positional changes and redistributes fluid as needed, and to physical activity, which regulates CV conditioning.

Vascular adaptation to changes in blood pressure or fluid flow is a result of vascular smooth muscle contractility and its regulation by the endothelium, hormones, metabolites, neurotransmitters, and mechanical forces. This adaptation is sustained by the equilibrium between contraction and relaxation pathways and involves Ca2+ signaling. All life on Earth is sensitive to changes in body position and gravity. Both during and following spaceflight, bipeds likely experience more dramatic CV changes than quadrupeds, although both show alterations.

**Rodent spaceflight.** Experiments with rodents in space demonstrate selective changes in the vascular system that nourishes various tissue beds throughout the body. Data from rodents in flight suggest that increased intracranial pressure (ICP) likely occurs given that mineral density in the skull of male rats and calvaria of female mice increases (55, 133). Spaceflight reduces production of cerebrospinal fluid (CSF) in male rats and pregnant female rats (37, 41). Decreased myogenic vasoconstrictor tone, with changes in arterial distensibility, are consistent with a lower cerebral vascular resistance and higher cerebral blood flow during spaceflight and imply that spaceflight impairs the ability of the cerebral circulation to precisely regulate brain blood flow through vasoconstriction and vasodilation in both female and male mice (106, 113). The difference in distensibility of cerebral arteries in mice following spaceflight might be due to several factors, including length of flight, postflight transportation, and delay in euthanasia (13–15 h vs. 3–5 h), sex of the animals, or the ambient CO2 levels on Bion vs. Shuttle (106, 113).

Relatively little is known about spaceflight effects on the rodent heart. Young male rats show no change in heart mass or function following spaceflight (98). Papillary muscle myofiber area is slightly smaller with no change in ventricular myofiber area in male rats following spaceflight (46). A recent long-
duration experiment (30 days, BionM1) revealed changes in the cortical cytoskeleton of cardiomyocytes from the left ventricle of flight mice compared with ground controls (82). Spaceflight impairs the arterial and venous systems in the body and limbs of male rats and female mice with decreased contraction and relaxation in both, which can function to lower vascular resistance and allow blood pooling in the lower extremities and gut (13, 36, 50, 102, 108).

**Rodent HU.** The HU rodent model leads to multiple CV adaptations that also occur in humans during spaceflight, arising as a consequence of both a cephalic fluid shift (32, 66, 103, 125) and physical deconditioning (93). In particular, diminished VO2max indicates the importance of physical deconditioning as a component of the CV response to HU (84). CV adaptations to HU include hypovolemia (21, 103), resting and exercise tachycardia (72, 127), reduced capacity to elevate peripheral vascular resistance (127), diminished aerobic exercise capacity (84), and orthostatic hypotension (125).

HU in rats causes region-specific changes in blood flow, and changes in vessel structure that in some cases are quite similar to those occurring in spaceflight animals. Changes in the ultrastructure of the choroid plexus (which produces CSF) due to HU are very similar to those of rats flown for the same duration (41), and there are indications of decreased production of CSF in male rats during HU similar to that during flight (37, 41). Beginning immediately and continuing throughout the unloading period, total blood-brain flow to the head diminishes, as does regional blood flow to all cerebral tissues including eyes, olfactory bulbs, left and right cerebrum, thalamic region, and the midbrain, with an increase in vascular resistance in all cerebral regions (124). Unlike soft tissues, blood flow in the bones of the head (skull and mandible) increases acutely after initiating HU (32). Increases in spontaneous tone and myogenic vasoconstriction of cerebral arteries (44, 95, 134) as well as a structural remodeling of cerebral arteries (126) correspond to increases in cerebral vascular resistance and decreases in cerebral blood flow in rats (124, 125). These results are very different than the flight data obtained from mice (106, 113). Surprisingly, HU mice show no change in basilar artery vasoconstrictor function (113), which differs from findings in HU rats and spaceflight mice. Thus the HU model does not seem to predict changes in the cerebral artery vasoconstrictor responsiveness found in mice during spaceflight. However, the impairment of endothelium-dependent vasodilation in cerebral arteries is found in both male flight mice and HU rats (95, 106).

In mice, selective carotid baroreceptor unloading stimulates heart rate, blood pressure, and myocardial contractility, and HU attenuates each response (53). Foley et al. (40), in comparing male and female rats following HU, found that baroreflex sympathetic reserve is reduced in female rats more than in males, suggesting a greater reduction in orthostatic tolerance in females. Adult HU rats show no difference in heart mass or function (98), similar to the findings by Cui et al. (35) in young male rats; however, the young HU rats show decreased left ventricular pressure with decreased response to isoproterenol, suggesting a depression of cardiac function with HU relative to controls. Atrophy of the cardiac papillary myofiber area in male rats is somewhat less in HU than during flight, although ventricular myocyte area is not altered by either flight or HU (46). In male rats, HU causes CV deconditioning, resulting in sympathovagal imbalance and anhedonia, a behavioral index of psychological depression (71). CV deconditioning also causes a predisposition to cardiac arrhythmias, which increases expression of the phosphorylated form of Connexin-43, a cardiac gap junction protein that ensures efficient cell-to-cell electrical coupling (72). Using measures of pulse-wave velocity to assess vascular stiffness in HU rats, Tudy et al. (117) report a greater pulse-wave velocity through the thoracic aorta, indicating stiffening, which they confirmed by an in vitro stiffness test. Data from venous cava strips in male rats suggest that HU induces a desensitization of α1B-adrenoceptors depending on increased protein kinase C activity. Thus findings indicate that the HU model is an appropriate model for studying the effects of the microgravity environment on receptor and ion-channel activities in venous myocytes (102). Using a cannula inserted into the right atrium of female rats, Shellock et al. (103) found that central venous pressure increases temporarily (20° HU tilt) or for an entire 24-h experimental period (45° tilt). Portal vein (36), abdominal aorta (85), and femoral (94) and pulmonary arteries (80) show decreased rather than increased vasoconstriction. In both mesenteric arteries (31) and veins (14), there is reduced adrenergic constriction in HU male rats. Mesenteric small arteries also lose their ability to actively stiffen the vessel wall during HU in male rats (43).

Blood flow to the femur and tibia is acutely reduced with the femoral shaft and marrow further diminished after 28 days of HU (32). HU also impairs perfusion of bone and marrow with reloading (109). Reductions in hindlimb bone and marrow blood flow with chronic HU and reloading appear to result from alterations in bone vascular structure (39, 109) and diminished endothelium-dependent vasodilation (94). Gastrocnemius feed arteries from male mice display impaired endothelium-independent and -dependent vasodilator responses relative to controls, which may contribute to musculoskeletal changes in the hindlimb (94).

**Human relevance.** Many recent reviews on human CV changes with spaceflight are available [e.g., (78, 135, 138)]. Currently, focus areas for CV research include 1) possible elevated ICP due to microgravity, which may be a factor in the visual impairment in some astronauts noted following long-duration flights; 2) postflight orthostatic intolerance, which seems to be more prevalent in women than men (92); 3) cardiovascular deconditioning; and 4) the ability of on-orbit exercise to mitigate adverse changes (49). The HU model seems appropriate for studies to determine potential mechanisms associated with human spaceflight related to the CV system and deconditioning (93), although it is less relevant for developing specific exercise regimens for human crews. The HU model also is a useful surrogate for investigating the influence of vascular flow through the hindlimb muscles and bones and to determine CV contributions to musculoskeletal changes with unloading. The difference in vasoconstrictor responsiveness of the cerebral artery in male vs. female mice during flight should be resolved. In addition, HU rats show an increase rather than a decrease in central venous pressure, which occurs in humans, likely because gravity is still acting on the head during HU. The HU model may prove useful for testing countermeasures against orthostatic intolerance if further research confirms similarities to humans in flight. In conclusion, findings suggest that the HU model has value for simulating some of the changes that occur with human space-
flight and for elucidating possible mechanisms for postflight orthostatic intolerance and deconditioning.

**Tissue Healing**

To date there have been a limited number of spaceflight and HU studies on tissue healing, although this is an active area of study given current interest in regenerative medicine and the influence of spaceflight on stem cells (16, 38).

**Rodent spaceflight and rodent HU.** All flight experiments investigating wound healing to date initiated the defect 2–5 days preflight rather than in flight. The Cosmos 2044 mission included both a flight and HU group and investigated changes in muscle or bone repair of a crush injury, during the 14-day flight of young male rats (38, 110). Repair of the gastrocnemius muscle in the Cosmos flight animals was minimal, with an easily detectable wounded area that was not as visible in the HU or control rats. Only in the flight rats did investigators find increased vascularity and numbers of macrophages, suggesting development of granulation tissue, which might indicate formation of scar tissue. In another experiment, a fibular osteotomy was performed 5 days before launch of a 5-day shuttle flight; both flight and HU adult male rats showed delayed callus formation with fewer blood vessels in the osteotomy gap primarily in flight animals, and the pattern of healing seemed different between flight and HU in these adult male rats. Unfortunately, the flight animals lost weight during the mission, and thus stress may contribute to reduced healing observed in flight animals. HU experiments were initiated within 24 h of wounding, with the exception of a study by Radek et al. (97), who began their study after a 2-wk HU period. In other experiments, HU was found to impair healing of wounds in the mouse cornea (60), the medial collateral ligaments (65, 96), and skin (97) of male rats.

**Relevance to humans.** There are anecdotal reports of slow healing of skin wounds by astronaut crew members, although controlled studies are not conducted for safety reasons. Thus the HU analog may be particularly useful for gaining information on potential decrements in healing during flight, assuming possible stress responses are assessed. HU experiments are useful for studying skin wound healing (97) and other tissue injury responses such as burns (60, 65, 90, 96), while keeping in mind the differential influence of HU on cephalic vs. caudal regions of the animals.

**Vision**

Visual problems recently were identified in a subset of astronauts after long-duration flights aboard the ISS. Due to the potential for long-term detrimental consequences of vision changes in crew members both during long missions and after return to Earth, HU offers a potentially useful analog for studying this risk (68, 77, 92).

**Rodent spaceflight.** Mao et al. (64) found that in female mice that were flown aboard the ISS for 13 days, expression of genes and proteins involved in oxidative stress and mitochondrial and endothelial cell biology are significantly modified, and that flight mice display increased apoptosis in the retina, suggesting that rodents might also exhibit visual changes as a result of spaceflight. Philpott et al. (88, 89) investigated eyes from rats flown on two early Cosmos missions and found that most of the flight eye tissue was normal; however, necrotic nuclei were found in the outer segment area while macrophages were observed between the pigment layer and outer segments. After a 25-day recovery period, some recovery from these histological changes did occur (89). A second Cosmos mission showed similar changes in the eyes in both the flight animals and flight-centrifuged (1 G) animals; the authors concluded that galactic cosmic radiation was the probable cause because similar histological findings were obtained from animals irradiated with high-energy particles on Earth. Thus various environmental factors, in addition to increased ICP, such as radiation and elevated ambient CO₂, may contribute to spaceflight-induced changes in the visual system.

**Rodent HU.** HU causes changes in blood flow to the eye and head of rodents (see Cardiovascular System). The National Aeronautics and Space Administration is currently funding research to determine whether vision changes occur following long-duration HU similar to those noted in humans following long-duration spaceflight.

**Relevance to humans.** Mader et al. (63) reported that after 6 mo of spaceflight on the ISS, some astronauts displayed opthalmic changes that included disc edema, posterior globe flattening, choroidal folds, cotton wool spots, nerve fiber layer thickening, and decrease in near vision and hyperopic shifts, along with indications of slightly elevated ICP. Some of the vision changes continued for longer than 1 yr postflight. Although emphasis is placed on increased ICP and the syndrome is called visual impairment ICP, other factors also may contribute to the observed deficits. A recent report demonstrated that a genetic predisposition to opthalmic changes during flight may account for the finding that only a subset of male astronauts become afflicted with opthalmic changes during spaceflight (139). All astronauts homozygous for the MTRR 66 GG genotype exhibit opthalmic changes (e.g., choroidal folds), whereas the SHMT 1420 TT genotype was protective against the optic disc edema found following long-duration flights (139). Future application of the HU model may provide a useful system for exploring the contribution of fluid shifts and ICP to visual system deficits caused by long-duration spaceflight.

**Nervous System**

The nervous system extends to and affects all systems in the body, thus it is not surprising that changes induced by spaceflight are reflected in altered neural activity both centrally and peripherally. In addition to the decreased gravity level, other factors in the space environment may contribute to the changes noted in the central nervous system, which includes the brain and spinal cord. For example, animal models reveal an unexpected sensitivity of mature neurons in the brain to low doses of charged particles typical of those encountered in space (77).

The vestibular system for gravity-sensing, located in the inner ear, evolved in all species under the influence of gravity. The chronic effects of reduced gravity on the vestibular system must be studied in microgravity and cannot be studied in a simulation model on Earth because the vestibular apparatus consists of a gravity-sensing system (i.e., otoconia and hair cells); acceleration on Earth due to gravity can be diminished only briefly by using drop towers or during rapid deceleration (52). HU studies are not expected to prove useful for studying
the influence of low gravity on the vestibular system, unless cephalad fluid shifts or unweighting of other parts of the body affect vestibular function, which has not been studied to date.

The peripheral nervous system includes the somatic or voluntary nervous system (SoNS) and the autonomic nervous system (ANS). The SoNS innervates the limbs and body wall, whereas the ANS controls heart rate, blood pressure, constriction and dilation of blood vessels, energy conservation, gastric motility and secretion, and bladder contractility. Aspects of changes in peripheral neural control during HU and spaceflight (e.g., CV, musculoskeletal) are addressed in previous sections. Therefore, the remainder of this section focuses on developmental aspects of the nervous system.

Rodent spaceflight. The 16-day Neurolab Spacelab Mission STS-90, in 1998 was dedicated to studying how the nervous system develops and functions in space using multiple species (humans, rats, mice, crickets, snails, salish, and toadfish) (22). Due to constraints of spaceflight habitats for rodents, only the Neurolab mission has investigated the influence of spaceflight on the developing nervous system in any detail. This mission launched neonates [age, postnatal day (P) P7, P8, or P14] and returned them 16 days later, except for those euthanized in flight on P23. In general, the results obtained from the neonates that were flown then returned to Earth indicated that critical periods may exist for maturation of the nerves innervating particularly weight-bearing muscle because flight animals did not develop certain movements after they returned to Earth. These findings suggest that development of the neuromuscular system and possibly other systems in microgravity may not be sufficient for performing physical tasks in a 1-G environment, although results from this study must be interpreted with caution because of weight loss and reduced viability of the flight neonates (22).

Walton et al. (121) found that there are critical periods for development of certain sensory systems, and many aspects of motor behavior are preprogrammed into the young nervous system. In addition, several aspects of motor behavior are acquired as a function of the interaction of the developing organism and the rearing environment. Neuroanatomical differences between ground control and space-reared rats indicate that a structural basis for the adaptation to the rearing environment does exist. Soleus muscle development in space-reared rats is most disrupted compared with nonweight-bearing muscles such as the extensor digitorum longus. The absence of gravity when imposed at critical stages of development impairs body and skeletal muscle growth and alters expression of the MHC gene family of motor proteins, suggesting that normal weight-bearing activity is essential for establishing body and muscle growth in neonatal animals and for expressing the motor gene essential for supporting antigravity functions (1). Spaceflight affects the development of the aortic baroreflex. The sensitivity of the reflex may be suppressed; however, the function of the blood pressure control system can readapt to Earth’s gravity if the rats return before maturation. The structural differences in the input pathway of the reflex (i.e., the reduction in nerve fibers) may remain permanently (130). Within the developmental window studied, microgravity has minimal long-term effect on cognitive mapping function and cellular substrates important for this function (115).

HU studies. Developmental HU studies pose considerable practical challenges. Nonetheless, Walton et al. (119, 120) were able to conduct HU experiments using neonatal rats and to identify critical periods for motor system development using kinematic measures for swimming, walking, and righting reflex. The data suggest that the critical development period is P8–P13 for swimming, and walking begins after P13–P31, whereas air-righting occurs between P15 and P17. Thus these HU experiments conducted prior to the Neurolab mission were useful in defining sensitive developmental periods that were the focus of the more definitive spaceflight experiment on the Neurolab mission.

Human relevance. Space settlements and colonization of other planets are likely in the distant future. Further research is needed to confirm and extend the findings of developmental disturbances that were identified on the Neurolab mission and with the HU experiments to determine the potential for propagating multiple, healthy generations in space.

Concluding Comments

The HU model is used extensively to better understand the influence of the spaceflight environment on integrated physiologic, organ-specific, and mechanistic responses. Although the majority of the spaceflight rodent experiments reported in the literature did not include ground-based HU experiments that were carefully matched to spaceflight conditions, results obtained from those that did indicated that, in general, comparable results were obtained (10, 74). Strengths of the HU analog of spaceflight include 1) the ability to perform procedures (e.g., exposure to radiation and pathogens, drug treatments) that simply cannot occur with humans for ethical reasons or in spaceflight animals due to limited flight opportunities and other constraints, 2) well-characterized responses to HU for at least some organ systems, 3) availability of genetically modified strains, and 4) ability to study recovery from HU. Weaknesses of the HU model as an analog for spaceflight as it is currently implemented include 1) inconsistencies in stress responses to HU depending on the experiment, 2) continued gravitational loading of the forequarters, 3) lack of clarity about the influence of HU on the spine. Important emerging areas that are under active investigation include visual changes, tissue healing, stem cells, microflora/gastrointestinal permeability, and pathogen responses.

In addition to spaceflight relevance, HU is potentially a useful analog for studying physical inactivity, providing valuable new insight into the contribution of sedentary behaviors to chronic disease (20, 29, 54, 81, 99). Quantitative analysis of rat movements within HU and control cages demonstrates that HU causes a profound reduction in parameters reflecting movement and activity including total distance, number of movements, and vertical activity (131). Astronauts also show dramatic reduction in leg activity while in orbit, with little change in arm activity during nonexercise periods. Body weights of HU rodents are either unaffected, as in the study reporting movement (131), or lower than age-matched controls, depending on the particular study; in this respect, HU does not reproduce important aspects of inactivity that occur with a sedentary lifestyle. Thus further work is needed to confirm the validity of the HU model for these important clinical applications.

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AUTHOR CONTRIBUTIONS
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