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PI Name:	Turner, Russell T Ph.D.		
Project Title:	Housing Temperature: An Important Variable for Simulated Spaceflight Studies Using Mice		
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Program/Discipline--Element/Subdiscipline:			
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Space Biology Cross-Element Discipline:	(1) Musculoskeletal Biology		
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Task Description:	Spaceflight, by altering the differentiation program of hematopoietic and mesenchymal stem cells residing within bone marrow, results in bone loss, increased bone marrow adiposity, anemia, and impaired immune function. These closely associated disturbances may compromise the success of long-term missions. Thus, there exists an urgent need to identify the underlying mechanisms and implement effective countermeasures. Mice are currently the preferred animal model for evaluating adaptive responses to microgravity experienced during spaceflight and simulated spaceflight (e.g., hindlimb unloading). Mice have important advantages over other animal models for spaceflight studies, including small size and ease of genetic manipulation. Although mice and humans share many common characteristics, fundamental species differences in thermoregulation may contraindicate the mouse, as currently applied, as a model for human spaceflight. Humans are homeotherms and when exposed to a cold environment defend their core body temperature, whereas mice are obligatory daily heterotherms and experience cyclic changes in core temperature when subjected to cold stress (i.e., temperature below thermoneutral). Mice are typically housed at or near room temperature (~22°C), which is well below the thermoneutral zone for the species (~32°C). Therefore, mice must expend energy to maintain core body temperature. Cold stress induced by sub-thermoneutral housing increases sympathetic outflow to peripheral tissues, including brown adipose tissue, and has profound effects on metabolism. We have recently shown that cold stress induced by room temperature housing results in rapid cancellous bone loss in mice. Based on this finding, we hypothesize that activation of adaptive thermogenesis in mice housed at room temperature introduces unrecognized and uncontrolled confounding variables into mouse studies. Strategies used by weight-bearing mice to minimize heat loss during room temperature housing (e.g., huddling or postural adjustments) are less effective during spaceflight and simulated spaceflight. This results in increased dependence on adaptive thermogenesis, likely exaggerating the negative physiological effects of skeletal unloading on bone and immune cells. Additionally, the thermogenic mechanisms mediating cold stress-induced changes in metabolism in mice are unlikely to be directly translatable to astronauts and could therefore confound interpretation of experimental results as applicable to humans. This proposal will explore the individual and combined effects of mild cold stress induced by room temperature housing and hindlimb unloading (HLU) on the skeleton in C57BL6 (B6) mice, a strain commonly used in spaceflight and HLU studies. To accomplish our goal, we propose 2 Specific Aims: Specific Aim 1: Determine the contribution of increased adaptive thermogenesis to bone loss during HLU in mice housed at room temperature. We will accomplish this aim by comparing HLU-induced bone loss in mice housed at room temperature (22°C) with mice housed at thermoneutral (32°C). Specific Aim 2: Determine the lowest sub-thermoneutral housing temperature able to prevent adaptive thermogenesis-associated bone loss. We will accomplish this aim by performing temperature response studies (20-32°C) to evaluate induction of adaptive thermogenesis. Once we identify the lowest sub-thermoneutral housing temperature that does not induce adaptive thermogenesis, we will perform a long-term study to verify that minimal premature bone loss occurs and that magnitude of HLU-induced bone loss does not differ from mice housed at thermoneutral. Successful completion of the proposed research will provide guidance to investigators for insuring housing conditions minimize the confounding effects of species-specific differences in thermoregulation on experimental outcomes with the ultimate goal of optimizing the mouse to model human responses to spaceflight.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	One of our goals is to establish mouse models that more accurately replicate human physiology. Mice, because of their small size, short lifespan, and ease of genetic manipulation, are a mainstay for cutting edge research in fundamental skeletal biology, and are increasingly being used as preclinical models for skeletal disuse and metabolic bone disease. There is, however, a critical need to identify and address major limitations of mice as models for human physiology. For example, in contrast to humans, mice experience bone- and bone compartment-specific cancellous bone loss while growing. This premature age-related bone loss is a concern because the most common metabolic bone diseases preferentially target the aged or during conditions such as long duration spaceflight believed to accelerate aging. An important difference between mouse and human physiology that may contribute to premature age-related bone loss is the tight coupling of bone metabolism to thermoregulation in mice. If our hypothesis is correct, mild temperature stress induced by room temperature housing is responsible for premature cancellous bone loss in mice and factors that influence thermoregulation in mice may further compromise studies designed to model human responses by independently altering bone metabolism.
	Mice are currently the preferred animal model for evaluating adaptive responses to spaceflight. They have several important advantages over other animal models for microgravity studies, including small size and ease of genetic manipulation. Although mice and humans share many common characteristics, thermoregulation differs markedly between the two species. Because of their small size, maintaining body temperature in a cool environment is a greater challenge for mice than for humans. Humans are homeotherms. When exposed to cold environment humans resist decreases in interior body temperature. In contrast, mice are facultative daily heterotherms. Mice experience cyclic changes in body temperature when subjected to temperatures below thermoneutral, the temperature range where heat produced by metabolism is equal to heat loss to the environment. Mice are nocturnal and exhibit pronounced diurnal differences in activity, feeding and sleep. Mice are typically housed at room temperature (~22°C), which is comfortable for humans but cold for mice. Room temperature housing has profound effects on energy allocation. To maintain body temperature when awake, mice housed at room temperature eat more food, increase their heart rate, are physically more active, metabolize fat reserves, shiver, huddle together, and when inactive (e.g., sleep) lower their body temperature to reduce heat loss. Not suppressing, these adaptations are regulated by stress hormones and central nervous system. It has been known for some time that mice exhibit age related bone loss but in contrast to aging humans, much of the bone loss occurs in mice while they are still growing. Furthermore, we noticed that the bone loss in female mice is prevented if the mice are housed at 32°C, which is thermoneutral for a mouse. Based on our observation we hypothesized that the premature aging related bone loss noted in mice was a response to cold temperature stress.

Task Progress:	Based on this concept, we hypothesized that cold stress in mice housed at room temperature introduces unrecognized and uncontrolled confounding variables into mouse studies performed on the International Space Station or using Earth based models for spaceflight, such as hindlimb unloading (HLU). Strategies used by weight-bearing mice to minimize heat loss during room temperature housing (e.g., huddling or other postural adjustments) may be less effective during spaceflight and not possible during simulated spaceflight. This would results in increased dependence on increasing metabolism, likely exaggerating the negative physiological effects of skeletal unloading on bone and immune cells. Additionally, the heat generating mechanisms mediating cold stress-induced changes in metabolism in mice are unlikely to be directly translatable to astronauts and could therefore confound interpretation of experimental results as applicable to humans. The studies reported here were designed to explore the individual and combined effects of (1) mild cold stress induced by room temperature housing and (2) HLU on premature bone loss in C57BL6 (B6) mice, a strain commonly used in spaceflight/simulated spaceflight studies. To accomplish our goal, we proposed two Specific Aims: Specific Aim 1: Determine the contribution of increased adaptive thermogenesis to bone loss during HLU in mice housed at room temperature. Specific Aim 2: Determine the lowest sub-thermoneutral housing temperature able to prevent adaptive thermogenesis-associated bone loss. Progress on Specific Aim 1: We proposed to accomplish this aim by comparing HLU-induced bone loss in male and female mice housed at room temperature (22°C) with mice housed at thermoneutral (32°). We have completed the animal studies for female mice and are well into data collection and analysis. The findings to date demonstrate remarkable differences in response to HLU between growing female mice housed at 22°C and those housed at 32°C. Adaptation to cold stress was found to be responsible for ~50% of bone loss in the femur of growing HLU mice. When we evaluated a cancellous compartment (spongy bone located at the ends of long bones), the impact of housing temperature is even more striking; HLU prevented bone gain in growing mice at 32°C and resulted in bone loss at 22°C. In contrast, HLU prevented bone accrual in mice housed at 32°C but did not induce cancellous bone loss. Even more striking was our finding that HLU-induced bone loss in forearm (humerus), a bone subjected to near normal weight bearing in the HLU model. Taken together, these findings provide evidence that adaptation to cold stress induced by room temperature housing is an important modifier of the skeletal response to HLU in mice. Analyses of splenocytes (immune cells in spleen) suggests that increased cytokine secretion may contribute to excess bone loss induced by HLU in room temperature-housed mice. Progress on Specific Aim 2: As mentioned, the premature (prior to cessation of growth) cancellous bone loss in female B6 mice housed conventionally (room temperature) was prevented when the mice were housed at thermoneutral (32°C). We performed additional studies to determine whether these findings apply to males. Specifically, we compared growing mice housed at room temperature (22°C) to mice housed at 32°C. We found that room temperature housing-induced premature cancellous bone loss in growing mice is not sex specific; although male mice have higher peak bone mass, the magnitude of bone loss in room temperature-housed mice did not differ between male and female mice. The work is complete and a manuscript published. We are in the process of performing studies to establish the lowest housing temperature able to prevent premature bone loss. To date, we have completed the animal portion for one long duration study where male and female growing mice were housed at either 22°C or 26°C until adulthood. We chose 26°C because this is the highest temperature recommended in animal care and use guidelines. We have completed collection of data related to energy expenditure, oxygen consumption, carbon dioxide emission, respiratory exchange ratio, food consumption, and distance traveled but have not completed statistical analysis. We have also analyzed bone mass, density, and microarchitecture. The data clearly indicates that housing mice at 26°C reduced but did not prevent adaptive thermogenesis or premature bone loss. Importantly, the observed differences between mice housed at 22°C or 26°C indicate that even small differences in housing temperature influence bone mass in growing mice.
Bibliography Type:	Description: (Last Updated: 03/06/2024)
Articles in Peer-reviewed Journals	Martin SA, Philbrick K, Wong CP, Olson DA, Branscum AJ, Jump D, Marik C, DenHerder J, Sargent J, Turner RT, Iwaniec UT. "Thermoneutral housing attenuates premature cancellous bone loss in male C57BL/6J mice." Endocr Connect. 2019 Nov;8(11):1455-67. https://doi.org/10.1530/EC-19-0359 ; PubMed PMID: 31590144; PubMed Central PMCID: PMC6865368 , Nov-2019