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Topical: Liver, immune and stem cell function during spaceflight exposure

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Long-duration spaceflight is associated with significant risks including prolonged exposure to microgravity and low-dose/low-dose rate radiation. Previous studies in mice exposed to both spaceflight and simulated spaceflight have identified increased oxidative stress and activation of the innate inflammatory response as major contributors to physiological changes associated with spaceflight, including alterations to the cardiovascular and musculoskeletal system, metabolism, and the liver[1-3]. Furthermore, studies have shown significant alterations in the proliferation and differentiation potential of hematopoietic and mesenchymal populations in response to both short and long-duration spaceflight. Molecular pathway analysis suggested spaceflight led to down-regulation of the hematological system and of cellular development, with significant alterations in NF-kB, glucocorticoid receptor, and peroxisome proliferator-activated receptor (PPAR) signaling. Additionally, increased populations of pro-inflammatory macrophages have been observed in multiple spaceflight experiments[4,5] therefore indicating a significant role for bone marrow-derived cell populations in peripheral tissue conditions, including liver fibrosis. As such, this white paper is focused on identifying the key gaps in knowledge that exist relating to liver, immune and stem cell health in microgravity.

Non-alcoholic fatty liver disease (NAFLD) is a rising public health issue in both the United States and across the globe, affecting nearly 1 in 4 adults and 10% of all children[1]. Furthermore, NAFLD is highly prevalent in subjects >60 years old and promotes metabolic disorders such as type 2 diabetes and dyslipidemia[2]. NAFLD is characterized by accumulation of fat in the liver and is the earliest stage of progressive liver disease. Consequent aggravation of the immune response and activation of inflammation contributes to non-alcoholic steatohepatitis (NASH), resulting in fibrotic scarring and loss of liver function, which may progress to cirrhosis[3]. NASH is now the leading etiology for liver transplant in the US[6]. Numerous investigations suggest that perturbations in the environment and physiology can contribute to NAFLD, and disease and loss of function in the central metabolic organ adversely affects multiple physiological systems. Of particular interest is the impact of aging on liver health. Investigations of accelerated aging conducted on the International Space Station (ISS) have shown that spaceflight induces rapid liver injury in as little as two weeks in mice[4]. Currently, the average mission length for astronauts onboard the ISS is six months and NASA plans to send humans to the Moon and Mars, thereby significantly extending mission duration and increasing exposure to negative spaceflight health factors implicated in accelerated aging. The rapid liver injury seen in spaceflight experiments provides insight into potential mechanisms that have yet to be explored in current studies on Earth. In fact, the relationship between aging and the progression of disease is a critical knowledge gap that can be addressed by exposure to spaceflight stressors, warranting further investigation. As elderly populations demonstrate increased incidence of NAFLD, and cellular aging is thought to drive degenerative decline in multiple physiological systems including the metabolic system, studies aiming to investigate the relationship between aging, spaceflight and metabolic dysfunction are critical to astronaut health during long-duration spaceflight.

Previous collaborative studies by our team and others have shown significant alterations to oxidative stress, inflammation, and metabolism in response to spaceflight, impairing stem-cell based tissue regeneration[3,7-9]. Specifically, previous studies have demonstrated significant elevations in lipid content and numbers of cytoplasmic lipid droplets in livers of spaceflown mice compared to ground controls[10], presaging NAFLD, suggesting potential for a new major health hazard for long-duration spaceflight. Previous investigations have also suggested that

perturbations in the brain-liver-gut axis could contribute to NAFLD, however, the mechanism and the connection with aging, which increases NAFLD risk in humans and mice[11], has not been fully elucidated and remains a critical knowledge gap.

In addition to NAFLD-like liver impairments, it was recently discovered that 13.5 days of exposure to space were sufficient to cause oxidative stress and senescent phenotypes in the liver, including impaired oxidative defense, activation of PPAR signaling, increase in senescence-associated mitochondrial dysfunction, (SAMD), retinoid loss from hepatic stellate cells, and up-regulation of markers of extracellular matrix remodeling and fibrosis[1,10,12]. Spaceflight, simulated spaceflight, and aging (up to 24 months) data from rodents has also shown significant increases in the senescence-inducing molecule CDKN1a/p21 in multiple tissues. Cellular senescence is known to lead to senescence associated secretory phenotype (SASP) inflammation, which induces neighboring cells to become senescent. SASP promotes mitochondrial dysfunction, resulting in impaired fatty acid metabolism and accumulation of cytosolic lipid droplets[13]. Moreover, SASP promotes systemic inflammation, particularly macrophage activation via interleukin 1 beta (IL-1β), IL-6, macrophage inflammatory protein (MIP) 1α and MIP 3α[14]. In addition, mitochondrial dysfunction contributes to tissue degeneration[15], increased release of reactive oxygen species (ROS) and subsequent oxidative damage[16,17], further exacerbating inflammation and fibrosis[15]. Thus, liver senescence caused by ROS and mitochondrial dysfunction in spaceflight may lead to systemic inflammation that exacerbates spaceflight-mediated damage in and to other organs. These studies suggest that spaceflight-induced liver damage may be mediated in part by oxidative stress, as well as by activation of inflammatory pathways and lipogenesis. These results raise the concern that long duration exposure to the space environment may result in progressive liver damage, elevating the risk for fibrotic liver disease. However, the precise molecular mechanisms by which this occurs have yet to be elucidated and remain a critical knowledge gap that must be investigated in depth.

In multi-cellular organisms, metabolism and the immune system influence each other to accelerate aging[18]. Aging-related immunometabolic changes, also termed “inflammaging”, are low-grade pro-inflammatory shifts in innate and adaptive immunity[19]. Monocytes are one of the most prevalent cell types implicated in tissue inflammation, including in digestive tissues, and are associated with chronic inflammatory states[20-23]. ROS play a critical role in the development of tissue injury by facilitating monocyte recruitment to peripheral tissues and by inducing differentiation of monocytes into pro-inflammatory macrophages[24-26]. Furthermore, ROS, senescence and SASP inflammation induce polarization of tissue macrophages into a pro-inflammatory phenotype. Both pro-inflammatory and aging macrophages undergo changes in metabolism resulting in increased mitochondrial ROS and dysfunction[27-33]. Therefore, investigation into the mechanisms causing activation of a pro-inflammatory macrophage phenotype during spaceflight are critically important. Such studies are needed to elucidate the impact of inflammatory metabolic reprogramming on mitochondrial health, tissue degeneration, and the development of age-based diseases.

The brain-liver-gut axis is a highly connected system whose roles include the processing of gut-derived products, regulation of metabolic homeostasis and stability of systemic immune and neuronal function[34]. It operates as a bi-directional communication system integrating the central nervous system, endocrine, metabolic and immune signaling pathways[35]. Communication between the brain and liver can occur through both direct nerve stimulation and circulating factors

such as brain derived neurotrophic factor (BDNF) and glial cell line derived neurotrophic factor (GDNF), regulating homeostasis[36], as well as hypothalamic neurotransmitters that regulate metabolic function in response to circulating nutrients[37-39]. Aging has been proposed to cause shifts in sympathetic nervous system signaling activity that induces liver fat accumulation, though the mechanism by which this occurs, and whether it is shared by spaceflight responses, remains unknown[36,40]. Accumulating data also implicate dysregulation of the brain-liver-gut axis in a variety of concurrent pathologies ranging from inflammatory bowel disease (IBD) to neurodegenerative diseases[41,42,43]. The manifestations of these interconnected pathologies are relatively well described, yet the "cause and effect" paradox remains to be solved. This is in part due to the complexity of animal models offering limited control of experimental parameters[44] and on the other hand lack of *in vitro* models that would take into account the complex, long-term organ-organ dynamics needed for accurate representation of organ function[45]. This therefore drives a need for mechanism-focused, human-based, preclinical models coupled with new platform technologies Hence, for multi-organ studies to be conducted, there needs to be increased focus on both organoid and microphysiological systems (MPS) studies during long-duration spaceflight.

The emergence of neuropsychiatric-neurodevelopmental disorders and NAFLD in children born to obese mothers suggests that developmental changes induced by an adverse intrauterine environment in the fetus have permanent epigenetic or molecular effects *linked to maternal metabolic history*[46-52]. The developing hematopoietic system consists of fetal precursors of monocytes and macrophages found in adult tissues, therefore epigenetic modulation of fetal hematopoiesis has lifelong implications that may program offspring toward an increased risk for inflammation and metabolic disease. We[53,54], and others[55] showed that maternal consumption of an obesogenic diet in rodents drives progression to NASH in adult mouse liver through metabolic reprogramming of non-reparative hepatic macrophages and BM-derived macrophages (BMDMs). The innate and adaptive immune system is immature in the fetus[56,57], with a limited capacity for antioxidant response, making the fetus uniquely vulnerable to fuel overload[58-60] and cell death. Studies in rodent models further demonstrate that maternal obesogenic diets rich in saturated fat or fructose are strongly linked to inflammatory and metabolic disorders in the liver[61], and implicate changes in immune cell function, epigenetic reprogramming, and the microbiome[21,62,63]. HSPCs are responsible for producing all blood cells, including lymphoid cells and myeloid cells; both promote and resolve inflammation[64,65]. Adverse early-life exposures may induce life-long changes in functional identity of immune cells (known as immune reprogramming), leading to enhanced susceptibility to immune and inflammatory disorders later in life[66]. Mφ function is driven by stimuli such as diet or infection; it is unknown whether changes in HSPC activity directly contribute to progeny Mφ dysfunction. In utero, populations of HSPCs migrate from the yolk sac to the liver, then seed the BM where they remain for life[67]. HSPCs require a specialized habitat and appropriate resources, which, in turn, contribute to the control of their fate and their roles. HSPC proliferation and differentiation can be modulated by consumption of a high-fat diet[68], changes in microbiome composition[69] and even exercise[70]. These data demonstrate the need for multigenerational animal studies to be conducted during long duration spaceflight to understand how maternal liver pathologies may affect fetal offspring.

Recommendations:

- Increased focus on systemic effects of spaceflight on liver physiology and interrelationships with other organ systems. It is well known that the liver is a central metabolic organ that affects and is affected by multiple physiological systems. Therefore, we need to understand the mechanisms for these effects and the driving factors causing dysregulation of lipid metabolism in the liver.
- The establishment of facilities and hardware to enable multigenerational studies in spaceflight. This is a critical need for multiple physiological systems and an area that has not been well studied in mammals. Multiple studies demonstrate the effects of maternal liver disease on fetal offspring that remains through adulthood. This area of research needs to be prioritized in spaceflight research for long-duration human presence to be feasible.
- Increased focus on the impact of spaceflight on aging and the progression of age-based diseases, including liver fibrosis. Presently there has been limited focus on the connection between the influence of aging on progression of liver disease. The basic pathology of fatty liver disease is well known, yet no cures or treatments are currently available.[11,12] More specifically, the role of external factors including cellular aging and circadian disruption on liver function have not been studied in great detail.
- Ensuring funding levels are adequate to enable multi-organ, multi-omics approaches including bulk RNA-sequencing, single cell RNA sequencing, spatial transcriptomics, epigenetics, and metabolomics. Access to new sequencing technologies has improved greatly over the last decade and we are now able to conduct multiple high throughput studies from very small samples. However, the cost of these studies is still incredibly high and funding levels generally offered by NASA grants is not sufficient to keep up with advances in sequencing and omics technologies. Therefore, funding levels need to be improved to reflect these increased assay costs.
- Increased focus on the mechanisms by which spaceflight factors affects the bone marrow microenvironment and bone marrow stem cells and how this links to peripheral tissue conditions. The hypoxic microenvironment within immunological niches, such as bone marrow, critically regulates immune cell function, modulating the balance between “effective immunity” and the development of pathological inflammation[71]. Alterations due to aberrant ROS signaling within the bone marrow niche might therefore lead to dysregulation of innate immune function and needs to be studied in greater detail.
- Increased analysis on how changes in circadian rhythms and metabolism affect stem cell and liver function. Disruption of the circadian rhythm can be caused by several factors, including light exposure, sleep deprivation, and aging[6,7]. As metabolic pathways are synchronized to a diurnal schedule, this is known to relate to changes in metabolic function with impacts such as weight gain, inflammation, and organ injury being prevalent in those with disrupted circadian rhythms[8,9].
- Understanding the role of cellular senescence in progression of fibrotic liver disease during spaceflight. The senescent phenotype is characterized by an irreversible arrest of proliferation and release of inflammatory signals caused by multiple factors including telomere attrition and damage to DNA. Persistent secretion of interleukin and chemokine

signaling molecules compose the senescence-associated secretory phenotype (SASP), which directly contributes to inflammation and increased lipid deposition in the liver[5].

- Determining the role of mitochondria on liver fibrosis. Several recent studies have demonstrated that spaceflight impacts mitochondrial function and decline in mitochondrial activity and efficiency is a classic hallmark of aging. Senescent cells also have increased reactive oxygen species content because of mitochondrial dysfunction (known as senescence associated mitochondrial dysfunction) that contributes to impaired liver function.
- Increased investigations focusing on understanding the impact of liver fibrosis and early-onset NAFLD on other organ systems and systemic metabolism. Specific focus on the brain and gut is recommended.
- Investigations into the impact of partial gravity and 1g spaceflight controls must also be conducted to elucidate the impact of lowered gravity levels on liver pathophysiology during Lunar or Martian missions.

As the focus of space exploration expands to the Moon and Mars, understanding the effects of spaceflight and spaceflight factors on systemic physiology is of critical importance. The liver and bone marrow microenvironment/bone marrow stem cell niche are two physiological systems that are now known to be significantly affected by spaceflight in low-Earth Orbit and may be of critical importance to astronaut health during long duration spaceflight exposure. The recommendations listed above our aimed to increase the mechanistic understanding of how spaceflight affects these systems and the effects of these changes on mammalian physiology.

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