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The Impact of Weightlessness on Lymphatic and Endothelial Glycocalyx Function;

A New Era in Ground Based Spaceflight and Weightlessness Research

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Introduction

Within the context of space exploration, lymphatic function and the vascular endothelial glycocalyx (GCX) remain under-researched areas. Within the past 20 years, lymphatic research and the understanding of lymphatic function within human health has clinically accelerated and gained increased recognition[1]. A missing element to incorporate into the paradoxes of many biological and physiologic pathologies associated with extreme environments may reside within the response and adaptation of the lymphatic system and the GCX[2-5]. To gain this important perspective, research will first be required in ground-based analogues for spaceflight[6], then progressing to true weightlessness in low earth orbit (LEO), as we begin to understand the impact on human physiology within the context of lymphatic and GCX function during exposure to extreme environments[7-14]. Spaceflight biology is advancing the understanding of genetics and epigenetics, metabolism, mitochondria, microbiome, digestive health, micro- to macro-vascular function, fluid shifts, immune function, neural and glial plasticity, ect., within the confines of an isolating spaceship with elevated radiation exposure[15]. Lymphatic function/contractility is now noted to be regionally, organ and tissue function specific[16]. The GCX lines the luminal surface of the 60-70,000 miles of the arterial, venous, and lymphatic vasculature to the 5-micron level. The new pathways of research into both areas are a robust opportunity to correlate nominal function, pathophysiology, and adaptability. On Earth, body fluid compartments are maintained and balanced by the nano-scaled architectural integration of the endothelial GCX, the vasculature, the integument, and the lymphatic system. The GCX is a carbohydrate-rich matrix composed of proteoglycans, glycoproteins, glycosaminoglycans, coagulation pathway and anti-inflammatory components[2,3,5,17,18]. The GCX is a dynamic, functional, multi-level, integrated matrix that is adhered to cell membranes and the structural cellular cytoskeleton, regulating fluid, solute, and macromolecule transfer from the vessels into the sub-glycocalyx and interstitial spaces[19-22]. The GCX, stimulated by luminal oscillatory shear, results in mechanacotransduction and eNO (endothelial nitric oxide) production through coupling of eNOS (endothelial nitric oxide synthetase) and radical oxygen species (ROS) quenching[23-27].

Lymphatic system function is gravity dependent, GCX shedding in weightlessness is a current unknown

The lymphatic system's capability to transport fluid from the lower extremities and torso, against gravity and soft tissue gradients, occurs via a combination of lymphangion contractility, leg muscle contraction, and respiratory/chest wall function in the setting of "primed" local/regional subatmospheric tissues. This creates a "suction effect" within the subatmospheric tissue distribution zones for lymphatic fluid movement within the lymphatic vasculature, lined with lymphatic endothelial cells and the GCX (the Guyton principle)[28,29]. The head, neck, and upper torso, however, are gravity gradient dependent for venous and lymphatic drainage, and must be balanced with arterial in-flow to this region[30]. In the

weightlessness of space, astronauts experience a dramatic fluid redistribution of ~ 2 liters from the legs to the head, neck and upper torso within the first 24-48 hours of flight[31]; this may be due in part to the persistence of pedal to cephalad lymphatic fluid flow due to "Guyton forces" and loss of gravity induced head and neck lymphatic drainage, resulting in an imbalance of soft tissue fluid gradients. A contributing physiologic component is the loss of "tissue weight" which reduces tissue hydrostatic pressure further, resulting in a higher transmural pressure which increases fluid flow into tissues[14]. Compromise of teleologically less robust head and neck lymphatic contractility (standard environment is "1G" with gravity assist drainage) may accentuate cephalad fluid shifts due to compromised drainage. Cervical lymphatic contractile inhibition was identified in a rat head down tail suspension model [32] and within the gastrointestinal mesentery of space flown mice[33]. A NASA Task book listing provides partial details of male C57BL/6 mice after 30-days aboard ISS . The cervical lymphatic vessels (CLVs) showed increased lymphatic tone and enhanced stretch-dependent changes in contraction frequency, while the flow/shear-induced inhibition on lymphatic phasic contraction was significantly impaired. The authors concluded that there was more constricted lymphatic vessel status overall and a loss in flow-sensitivity in spaceflight, suggesting a functional adaptation of CLVs in response to the loss of gravitational force and cephalic fluid accumulation (full data embargoed)[34]. Mouse CLVs may not be an accurate representative of human CLVs given ontological differences.

GCX shedding is known to occur with elevated atrial naturetic peptide (ANP)[35] levels. ANP levels and bioactivity are elevated for the first 24 hours after launch in the setting of a decreased CVP, then ANP levels decline[36-38]. GCX shedding, because of elevated ANP, would result in diffuse vascular hyperpermeability and fluid shifts, though not necessarily result in a diuresis given the subsequent associated decrease in plasma volume. GCX shedding remains undetermined and has not been researched in weightlessness. A 5-day HDT (head down tilt) study measuring limited GCX components of heparan sulfate (HS) and hyaluronate (HA) (non-carbon dioxide elevated testing environment), noted no elevated HS or HA that would be indictive of shed GCX. L-Selectin levels, however, were noted to be elevated[39]. Over the first 2 weeks, a 10-17% plasma volume reduction occurs without associated notable change in crew mass[12,40], which potentially could be accounted for by shedding of the GCX resulting in diffuse microvascular hyperpermeability and may correlate with the noted decreased systemic vascular resistance (SVR) of 14-39% and increased cardiac output and stroke volume (Doppler technique on ISS, 23-25% and 19-21%, respectively). The phenomenon of "neocytolysis" resulting in young red blood cell (RBC) culling and relative decline in total RBC mass[41-44], may also impact GCX maintance and restoration given RBC contribution to GCX integrity through delivery of sphingosine 1 phosphate (S1P) in conjunction with albumin, both significant components of a functional GCX[45,46]. Increased oxidative stress and inflammation with the potential to impact arterial function and shear may also impact GCX thickness and function, potentially contributing to eNOS uncoupling and decreased eNO production[26,47,48], especially in at risk crew based on single nucleotide polymorphisms (see SANS discussion).

Glycocalyx potential implications in weightlessness

The classic Starling forces as described in 1896[48] have been dogmatic for over 100 years. The endothelial GCX has both an anatomically defined layer and a functionally integrated layer resulting in endothelial cell expression. The recognition resulted in a "modified Starling forces" equation with an emphasis on the critical nature of the lymphatic system to interstitial fluid management and immune function, imparting dynamic crucial characteristics to tissue, organ and bodily health[49]. The recognition of modified Starling forces now confirms that the vast majority of interstitial fluid resulting from arterial perfusion re-enters the central venous system via the vast lymphatic network, and not via venules [4,50-52]. However, lymphatic education and recognition remains "paradoxically and unnecessarily ignored" at the medical school and residency levels[1]. Lymphatic research in space and astronaut health may "paradoxically" help to close this medical school curricula gap. Certified Lymphedema Therapists (CLT) and Physical Medicine and Rehabilitation (PMR) providers have been leaders in the arena of lymphatic evaluation and management. Given that chronic venous disease (CVD) and all venous ulcerations have associated GCX thinning or shedding[53], that associated lymphedema of venous etiology/phlebolymphedema is a common though vastly underrecognized and undertreated component[54,55], has a significant economic impact[56], and that there remain significant gaps in the understanding of topical treatment of venous leg ulcers and associated lymphedema develpoment[57], the potential for patient care improvement (medical "spinoff's) is significant. The focus on astronaut health and countermeasure development will expand understanding in preparation for exploration class missions[58].

With respect to the lymphatic system, a key role of the GCX must be considered: The GCX has been shown to be responsible for the transmission of extracellular forces into the cell[5,59-63]. For example, the endothelial GCX directly transmits the continuous force exerted by the blood stream, like the wind brushing through grass. Within the cell, the bending of the "glycocalyx grass" results in an integrated torque, causing a tailored response of cytoskeletal structures, cell adhesion foci, and junctional complexes[62,64], which is immediately abolished when glycocalyx-mediated mechanotransduction is abolished. Relative to these facts, several questions must be answered: First, how do the long-term systemic effects of weightlessness impact microvascular shear that maintains a healthy functional GCX?[24-26] Second, how is GCX mechanacotransduction impacted upon prolonged exposure to weightlessness and how are these changes reflected in cell morphology and intracellular function given cytoskeleton connectivity ("tensegrity") ?[5,17,19,21,23,24,25,62,65,66] Third, can the GCX be proactively targeted to reduce adverse systemic consequences of prolonged weightlessness if in fact the GCX is shed either in the initial 24- hours of weightlessness, during the initial 1-7 days of adaptability or after deorbit during the period of orthostatic hypotension?[67] Each of these questions offer a

rich, potentially high yield, area of new endeavors and study with potential "spinoffs" to standard medical cares.

Spaceflight Associated Neuroocular Syndrome: SANS

Approximately 40% of astronauts have experienced one or more ocular findings, now defined within the overarching term SANS (Spaceflight Associated Neuro-ocular Syndrome). Several non-exclusive theories have been proposed with overlapping validated data that contributes to a "multi-hit theory"[68-74] . One question specifically raised is *"What role, if any, does the lymphatic/glial lymphatic (glymphatic) circulatory system play in SANS?."* A 2020 study used a lymphatic imaging technique (near-infrared fluorescence lymphatic imaging, NIRFLI), to dynamically visualize the deep lymphatic drainage pathways shared by CSF outflow and disrupted during HDT. After validating CSF clearance into the lymph nodes of the neck in swine via intrathecal injections, a correlating pilot study was conducted in human volunteers to evaluate the effect of gravity on lymph flow through deep cervical lymphatics. IndoCyanine Green (ICG) was injected into the palatine tonsils and imaged draining into deep jugular lymphatic vessels and the cervical lymph nodes. NIRFLI was performed under HDT, sitting, and supine positions. NIRFLI demonstrated that lymphatic drainage through pathways shared by CSF outflow are dependent upon gravity assist and are impaired under short-term HDT[75].

Understanding "brain fluid mechanics" in weightlessness is an incomplete science as true ICP (intercranial pressure) has not been measured in long duration spaceflight[76]. The integrity of the blood brain barrier (BBB) and blood retinal barrier (BRB) GCX component and potential shedding have not been fully assessed and remains an opportunity for research in both ground-based analogues and true weightlessness[77-81], especially in light of the cerebral venous changes noted on MRI pre and post flight consistent with venous "congestion" and potentially venous hypertension[82]. The brain glymphatic system was first described in 2012[83], was recently recognized to compose a part of the optic nerve sheath and ocular drainage system[84,85]. The glymphatic system has a recognized primary function of metabolic waste clearance, functioning via "sweeping" beta amyloid and other metabolic waste products in nightly supine positioning, and is aligned with circadian function[86]. Given the lack of "supine" position sleeping on ISS and recognized circadian disruptions[87-88], glymphatic efficiency may be impaired as demonstrated in 5 cosmonauts during mean mission lengths of 169 days[89]. An elevation of amyloid proteins was noted upon return Earth and was felt to represent a washout phase after months of hindered protein waste clearance. An ocular glymphatic clearance system which removes β-amyloid from the rodent eye has also been described [90]. Given that the orbital optical nerve venous and lymphatic drainage systems may affected by venous and lymphatic stasis during cephalad fluid shifts, a hypothetical framework has been proposed by which optic disc edema may result, at least partly, from the forcing of peri-optic cerebrospinal fluid into the optic nerve and optic disc

along the perivascular spaces surrounding the central retinal vessels[91-92]. The authors propose that prolonged exposure to weightlessness may predispose to an overload in the periarterial inflow of CSF into the optic nerve sheath, resulting in optic nerve cross sectional *reduction.* In those astronauts without pathologically elevated postflight CSF pressures in the optic nerve sheath, the rapid fluid redistribution upon return earth may lead to reduced CSF volume and pressure within the optic nerve sheath. This may decrease CSF inflow along the optic nerve periarterial glymphatic spaces, resulting in periarterial space reduction or disappearance. This could contribute to the observed decrease in optic nerve cross-sectional area. Further measurements, including in flight, would contribute to further evaluation of this hypothesis[93]. One high value target is determining the impact of venoconstrictive thigh cuffs and lower body negative pressure (LBNP)[94-97] upon the dermal lymphatic ventromedial bundles[98] in ground-based space analogues of both lower extremity models and head/neck transcapillary fluid shifts[99]. Understanding lymphangion contractility response and lymphatic ventromedial bundle flow patterns and lymphatic thoracic duct pre-/ during/post – cuff use, may explain the decreases noted in ocular pressure[100], ICP[97], jugular venous distention[101-104] and potential benefits for SANS[94-97].

A recent validated non-invasive, low mass/weight option for chronic venous insufficiency and lymphedema evaluation is Point of care Thermography (POCT)[105-107], which may further improve the evaluation and understanding of cephalad fluid shifts related to venous distension and suspected lymphatic dysfunction. Thermography has an established history in astronaut orbital use as a noncontact handheld extravehicular activity (EVA) inspection tool for Shuttle wing evaluations[108]. Subsequent POCT devices have continued to decrease in size and mass/weight while innovative engineering has achieved improved thermal image signal acquisition, tissue differentiation and increased pixel count for improved sensitivity and specificity. This may also be of benefit for core body temperature (CBT) evaluation of astronauts[109].

Post Flight Astronaut Orthostatic Intolerance

Post flight orthostatic intolerance has been extensively studied and countermeasures applied [110-113] . Lymphatic dysfunction and potential GCX shedding both may contribute to this condition through impaired interstial fluid clearance and vascular hyperpermeability. Age, pre-flight physiologic status, individualized response to training, nutritional intake, cardiovascular adaptability, and genetics may all play a contributing role[114].

Conclusion

Further detailed research regarding altered lymphatic and GCX function is indicated. Countermeasure incorporation into pre-launch, in-flight and post-flight segments may include lymphatic/GCX restorative checklists, consisting of manual lymphatic drainage (MLD), LBNP, Sulodexide[115], and Micronized Purified Flavonoid Fraction (MPFF)[116-118]/diosmin[119]. It is our hope that this new field of aerospace medicine will be prioritized as an essential research

element for nominal human health, as lymphatic and GCX functional restoration and maintenance will support improved human health in space and on Earth.

Textbooks

1. Handbook of venous and lymphatic disorders, Guidelines of the American Venous Forum, Fourth edition. Editor Gloviczki, P. Associate Editors Dalsing MC, Ekolf B, Lurie F, Wakefield TW. Assistant Editor Gloviczki ML. CRC Press, Taylor & Francis Group. 2017.
2. Principles of Clinical Medicine for Space Flight. Editors Barratt MR, Baker ES, Pool, SL. Springer publisher, 2019.
3. Fundamentals of Aerospace Medicine, 5th Edition. Editors Davis JR, Stepanek J, Fogarty JA, Blue RS. Wolters Luwer publisher, 2022.

References

1. Rockson SG. Lymphatic Medicine: Paradoxically and Unnecessarily Ignored. LYMPHATIC RESEARCH AND BIOLOGY Volume 15, Number 4, 2017 ª Mary Ann Liebert, Inc. https://doi.org/10.1089/lrb.2017.29033.sr
2. Möckl L. The Emerging Role of the Mammalian Glycocalyx in Functional Membrane Organization and Immune System Regulation. Front. Cell Dev. Biol., 15 April 2020 https://doi.org/10.3389/fcell.2020.00253
3. Mitra R, O'Neil GL, Harding IC, Cheng MJ, Mensah SA, Ebong EE. Glycocalyx in Atherosclerosis-Relevant Endothelium Function and as a Therapeutic Target. Curr Atheroscler Rep (2017) 19: 63 https://doi.org/10.1007/s11883-017-0691-9
4. Mortimer PS, Rockson SG. New developments in clinical aspects of lymphatic disease. J Clin Invest. 2014;124(3):915–921. https://doi.org/10.1172/JCI71608
5. Fu BM, Tarbell JM. Mechano-sensing and transduction by endothelial surface glycocalyx: composition, structure, and function. Wiley Interdiscip Rev Syst Biol Med. 2013; 5(3): 381–390. https://doi.org/10.1002/wsbm.1211
6. Pandiarajan M, Hargens AR. Ground-based analogs for spaceflight. Frontiers in Physiology 2020;11: article 716 https://doi.org/10.3389/fphys.2020.00716
7. Demontis GC, Germani MM, Caiani EG, Barravecchia I, et al. Human pathophysiological adaptations to the space environment. Front. Physiol., 02 August 2017 https://doi.org/10.3389/fphys.2017.00547.
8. Norsk, P. Adaptation of the cardiovascular system to weightlessness: Surprises, paradoxes and implications for deep space missions. Acta Physiologica 2020;228: e13434 http://dx.doi.org/10.1111/apha.13434
9. White R. Weightlessness and the Huan Body Scientific American September 1998:59-63. http://dx.doi.org/10.1038/scientificamerican0998-58

10. Norsk P, Asmar A, Damgaard M, Christensen NJ. Fluid shifts, vasodilation and ambulatory blood pressure reduction during long duration spaceflight. J Physiol 2015;593(3):573-584. https://doi.org/10.1113/jphysiol.2014.284869
11. Garrett-Bakelman FE, Darshi M, Green SJ, et al. The NASA Twins Study: a multidimensional analysis of a year-long human spaceflight. Science. 2019 Apr 12;364(6436):eaau8650 https://doi.org/10.1126/science.aau8650
12. Drummer C, Gerzer R, Baisch F, Heer M. Body fluid regulation in μ-gravity differs from that on Earth: an overview. Pflügers Arch - Eur J Physiol (2000) 441 [Suppl]: R66–R72 https://doi.org/10.1007/s004240000335
13. Fortrat JO, de Holanda A, Zuj K, et al. Altered venous function during long-duration spaceflight. Front. Physiol., 12 September 2017 https://doi.org/10.3389/fphys.2017.00694
14. Hargens AR, Bhattacharya R, Schneid SM. Space physiology VI: exercise, artificial gravity, and countermeasure development for prolonged space flight. Eur J Appl Physiol https://doi.org/10.1007/s00421-012-2523-5
15. Afshinnekoo E, et al. Fundamental Biological Features of Spaceflight: Advancing the Field to Enable Deep-Space Exploration. Cell 183, November 25, 2020, https://doi.org/10.1016/j.cell.2020.10.050.
16. Breslin JW, Yang Y, Scallan JP, et al. Lymphatic Vessel Network Structure and Physiology. Compr Physiol.; 9(1): 207–299. https://doi.org/10.1002/cphy.c180015
17. Weinbaum S, Tarbell JM, Damiano ER. The Structure and Function of the Endothelial Glycocalyx Layer. Annu. Rev. Biomed. Eng. 2007. 9:121–67. https://doi.org/10.1146/annurev.bioeng.9.060906.151959
18. Hansen KC, D'Alessandro A, Clement CC, Santambrogio L. Lymph formation, composition and circulation: a proteomics perspective. International Immunology, Vol. 27, No. 5, pp. 219–227. Advance Access publication 18 March 2015. https://doi.org/10.1093/intimm/dxv012
19. Reitsma S, Slaaf DW, Vink H, et al. The endothelial glycocalyx: composition, functions, and visualization. Pflugers Arch - Eur J Physiol (2007) 454:345–359. https://doi.org/10.1007/s00424-007-0212-8
20. Woodcock TE, Woodcock TM. Revised Starling equation and the glycocalyx model of transvascular fluid exchange: an improved paradigm for prescribing intravenous fluid therapy. British Journal of Anaesthesia 108 (3): 384–94 (2012) Advance Access publication 29 January 2012. https://doi.org/10.1093/bja/aer515
21. Moore KH, Murphy HA, George EM. The glycocalyx: a central regulator of vascular function. Am J Physiol Regul Integr Comp Physiol 320: R508–R518, 2021. First published January 27, 2021; https://doi.org/10.1152/ajpregu.00340.2020
22. Hahn RG. Water content of the endothelial glycocalyx layer estimated by volume kinetic analysis. Intensive Care Medicine Experimental (2020) 8:29. https://doi.org/10.1186/s40635-020-00317-z.
23. Ebong EE, Lopez-Quintero SV, Rizzo V, et al. Shear-induced Endothelial NOS Activation and Remodeling via Heparan Sulfate, Glypican-1, and Syndecan-1. Integr Biol (Camb). 2014 March ; 6(3): 338–347. https://doi.org/10.1039/c3ib40199e
24. Fu BM, Tarbell JM. Mechano-sensing and transduction by endothelial surface glycocalyx: composition, structure, and function. WIREs Syst Biol Med 2013, 5:381–390. https://doi.org/10.1002/wsbm.1211
25. Potje SR, et al. The Role of Glycocalyx and Caveolae in Vascular Homeostasis and Diseases. Front. Physiol., 13 January 2021. https://doi.org/10.3389/fphys.2020.620840

26. Villalba N, Baby S, Yuan SY. The endothelial glycocalyx as a double-edge sword in microvascular homeostasis and pathogenesis. Front. Cell Dev. Biol., 14 July 2021. https://doi.org/10.3389/fcell.2021.711003
27. Jamalian S, Jafarnejad M, Zawieja SD, et al. Demonstration and analysis of the suction effect for pumping lymph fluid from tissue beds at subatmospheric pressure. Scientific Reports 2017 7:12080. https://doi.org/10.1038/s41598-017-11599-x
28. Guyton AC, Granger HJ, Taylor AE. Interstitial Fluid. Physiologic Reviews 1971;51(3): 527-563. https://doi.org/10.1152/physrev.1971.51.3.527
29. Wilson MH. Monro-Kellie 2.0: The dynamic vascular and venous pathophysiological components of intracranial pressure. Journal of Cerebral Blood Flow & Metabolism 2016, Vol. 36(8) 1338–1350. https://doi.org/10.1177%2F0271678X16648711
30. Moore TP, Thornton WE. Space shuttle inflight and postflight fluid shifts measured by leg volume changes. Aviat Space Environ Med 1987;58(9): A91-6. (No DOI in PubMed)
31. Gashev AA, Delp MD, Zawieja DC. Inhibition of active lymph pump by simulated microgravity in rats. Am J Physiol Heart Circ Physiol 290: H2295–H2308, 2006. First published January 6, 2006; https://doi.org/10.1152/ajpheart.00260.2005
32. Cromer WE, Zawieja DC. Acute exposure to space flight results in evidence of reduced lymph Transport, tissue fluid Shifts, and immune alterations in the rat gastrointestinal system. Life Sciences in Space Research 2018;17: 74–82. Epub 2018 Mar 28. https://doi.org/10.1016/j.lssr.2018.03.005
33. Zhang X, (2019). The Effects of Spaceflight on Cervical Lymphatic Vessel Function in Mice. Master's thesis, Texas A&M University. Available electronically from https: //hdl. handle.net /1969.1 /189244
34. Jacob M, Saller T, Chappell D, et al. Physiological levels of A-, B- and C-type natriuretic peptide shed the endothelial glycocalyx and enhance vascular permeability. Basic Res Cardiol (2013);108:347. https://doi.org/10.1007/s00395-013-0347-z
35. Drummer C, Gerzer R, Baisch F, Heer M. Body fluid regulation in μ-gravity differs from that on Earth: an overview. Pflügers Arch - Eur J Physiol (2000) 441 [Suppl]: R66–R72. https://doi.org/10.1007/s004240000335
36. Leach CS, Johnson PC, Cintron NM (1988) The endocrine system in space flight. Acta Astronaut 17:161–166. https://doi.org/10.1016/0094-5765(88)90017-3
37. Smith SM, Krauhs JM, Leach CS. Regulation of body fluid volume and electrolyte concentrations in spaceflight. Adv Space Biol Med (1997);6:123-65. https://doi.org/10.1016/s1569-2574(08)60081-7.
38. Feuerecker M, Feuerecker B, Matzel S, et al. Five days of head-down-tilt bed rest induces noninflammatory shedding of L-selectin. J Appl Physiol 115: 235–242, 2013. First published May 16, 2013; https://doi.org/10.1152/japplphysiol.00381.2013
39. Diedrich A, Paranjape SY, Robertson D. Plasma and blood volume in space. Am J Med Sci 2007;334(1):80-85. https://doi.org/10.1097/MAJ.0b013e318065b89b
40. Culliton K, Louati H, Laneuville O, et al. Six degrees head-down tilt bed rest caused low-grade hemolysis: a prospective randomized clinical trial. npj Microgravity (2021) 7:4; https://doi.org/10.1038/s41526-021-00132-0
41. Trudel G, Shafer J, Laneuville O, et al. Characterizing the effect of exposure to microgravity on anemia: more space is worse. Am J Hematol. 2020; 95:267–273. https://doi.org/10.1002/ajh.25699
42. Risso A, Ciana A, Achilli C, et al. Neocytolysis: none, one or many? A reprisal and future perspectives. Frontiers in Physiology 2014 5: article 54. https://doi.org/10.3389/fphys.2014.00054

43. Kunz H, Quiriate H, Simpson RJ, et al. Alterations in hematologic indices during long-duration spaceflight. Hematology 2017;17(2): 1-8. https://doi.org/10.1186/s12878-017-0083-y
44. Adamson RH, Clark JF, Radeva M, et al. Albumin modulates S1P delivery from red blood cells in perfused microvessels: mechanism of the protein effect. Am J Physiol Heart Circ Physiol 306: H1011–H1017, 2014. First published February 15, 2014; https://doi.org/10.1152/ajpheart.00829.2013
45. Zeng Y, Liu XH, Tarbell J, Fu B. Sphingosine 1-phosphate induced synthesis of glycocalyx on endothelial cells. Experimental Cell Research 339 (2015) 90-95 https://doi.org/10.1016/j.yexcr.2015.08.013
46. Lee SMC, Ribeiro LC, Martin DS, et al. Arterial structure and function during and after long-duration spaceflight. J Appl Physiol 2020; 129:108-123. https://doi.org/10.1152/japplphysiol.00550.2019
47. Hughson RL, Robertson AD, Shoemaker JK, et al. Increased postflight carotid artery stiffness and inflight insulin resistance resulting from 6 months spaceflight in male and female astronauts. Am J Physiol Heart Circ Physiol 2016 310: H628-H638. https://doi.org/10.1152/ajpheart.00802.2015
48. Starling E. J Physiol 1896;19;312-326. https://doi.org/10.1113/jphysiol.1896.sp000596
49. Oliver G, Kipnis J, Randolph GJ, Harvey NL. The Lymphatic Vasculature in the 21st Century: Novel Functional Roles in Homeostasis and Disease. Cell 182, July 23, 2020, https://doi.org/10.1016/j.cell.2020.06.039.
50. Michel CC. Starling: The formulation of his hypothesis of microvascular fluid exchange and its significance after 100 years. Experimental Physiology (1997), 82, 1-30. https://doi.org/10.1113/expphysiol.1997.sp004000
51. Zolla V, Nizamutdinova T, Scharf B, et al. Aging-related anatomical and biochemical changes in lymphatic collectors impair lymph transport, fluid homeostasis, and pathogen clearance. Aging Cell (2015) pp1–13. https://doi.org/10.1111/acel.12330 . Epub 2015 May 15.
52. Hansen KC, D'Alessandro A, Clement CC, Santambrogio L. Lymph formation, composition and circulation: a proteomics perspective. International Immunology 2015; Vol. 27, No. 5, pp. 219–227. https://doi.org/10.1093/intimm/dxv012 . Epub 2015 Mar 18
53. Castro-Ferreira R, Cardoso R, Leite-Moreira A, Mansilha A. The Role of Endothelial Dysfunction and Inflammation in Chronic Venous Disease. Ann Vasc Surg 2018; 46: 380–393 http://dx.doi.org/10.1016/j.avsg.2017.06.131.
54. Farrow W. Phlebolymphedema–A Common Underdiagnosed and Undertreated Problem in the Wound Care Clinic. Journal of the American College of Certified Wound Specialists (2010) 2, 14-23. https://doi.org/10.1016/j.jcws.2010.04.004 . eCollection 2010.
55. Dean SM, Valenti E, Hock K, et al. The clinical characteristics of lower extremity lymphedema in 440 patients. J Vasc Surg: Venous and Lym Dis 2020; 8:851-9. https://doi.org/10.1016/j.jvsv.2019.11.014
56. Melikian R, O'Donnell TF, Iafrati MD. The economic impact of infection requiring hospitalization on venous leg ulcers. Journal of Vascular Surgery: Venous and Lymphatic Disorders 2021, Journal Pre-Proof June 15, 2021, doi: https://doi.org/10.1016/j.jvsv.2021.06.012
57. Shaydakov ME, Ting W, Sadek M, Aziz F, Diaz JA, Raffetto JD, Marston WA, Lal BK, Welch HJ. The American Venous Forum Research Committee, Review of the Current Evidence for Topical Treatment for Venous Leg Ulcers, Journal of Vascular Surgery: Venous and Lymphatic Disorders (2021), Journal Pre-Proof doi: https://doi.org/10.1016/j.jvsv.2021.06.010.
58. Patel ZS, Brunstetter TJ, Tarver WJ, Whitmire AM, Zwart, SR, Smith SM, Huff JL. Red risks for a journey to the red planet: The highest priority human health risks for a mission to Mars. npj Microgravity (2020) 6:33; https://doi.org/10.1038/s41526-020-00124-6.

59. Pinho SS, Reis CA. Glycosylation in cancer: mechanisms and clinical implications. NATURE REVIEWS | CANCER VOLUME 15 | SEPTEMBER 2015 | https://doi.org/10.1038/nrc3982
60. Smith BAH, Bertozzi CR. The clinical impact of glycobiology: targeting selectins, Siglecs and mammalian glycans. Nature Reviews | DRuG DIScoveRy Reviews volume 20 | March 2021 https://doi.org/10.1038/s41573-020-00093-1
61. Woods EC, Kai F, Barnes JM et al. A bulky glycocalyx fosters metastasis formation by promoting G1 cell cycle progression. eLife 2017;6: e25752. DOI: https://doi.org/10.7554/eLife.25752.
62. Thi MM, Tarbell JM, Weinbaum S, Spray DC. The role of the glycocalyx in reorganization of the actin cytoskeleton under fluid shear stress: A ‘‘bumper-car’’ model. PNAS November 23, 2004, CELL vol. 101 no. 47: 16483–16488. https://doi.org/10.1073/pnas.0407474101
63. Zeng Y, Tarbell JM. The Adaptive Remodeling of Endothelial Glycocalyx in Response to Fluid Shear Stress. PLOS ONE January 2014 | Volume 9 | Issue 1 | e86249 https://doi.org/10.1371/journal.pone.0086249
64. Chighizola M, Dini T, Marcotti S, et al. The glycocalyx affects force loading-dependent mechanotransductive topography sensing at the nanoscale. bioRxiv preprint posted March 2, 2021. doi: https://doi.org/10.1101/2021.03.02.433591.
65. Ingber DE. Tensegrity-based mechanosensing from macro to micro. Progress in Biophysics and Molecular Biology 2008. https://doi.org/10.1016/j.pbiomolbio.2008.02.005
66. Ingber DE. Cellular mechanotransduction: putting all the pieces together again. FASEB J. 20, 811– 827 (2006). https://doi.org/10.1096/fj.05-5424rev
67. Song JW, Zullo JA, Liveris D, et al. Therapeutic Restoration of Endothelial Glycocalyx in Sepsis. J Pharmacol Exp Ther 361:115–121, April 2017. DOI: https://doi.org/10.1124/jpet.116.239509.
68. Stenger MB, Tarver WJ, Brunstetter T, et al. Evidence Report: Risk of Spaceflight Associated Neuro-ocular Syndrome (SANS) Human Research Program, Human Health Countermeasures Element Approved for Public Release: November 30, 2017, National Aeronautics and Space Administration Lyndon B. Johnson Space Center Houston, Texas: p.6-109.
69. Lee AG, Mader TH, Gibson CR et al. Spaceflight associated neuro-ocular syndrome (SANS) and the neuro-ophthalmologic effects of microgravity: a review and an update. npj Microgravity (2020) 6:7; https://doi.org/10.1038/s41526-020-0097-9.
70. Zwart SR, Laurie SS, Chen JJ, et al. Association of genetics and B vitamin status with the magnitude of optic disc edema during 30-dat strict head-down tilt bed rest. JAMA Ophthalmol 2019;137(10):1195-1200. https://doi.org/10.1001/jamaophthalmol.2019.3124
71. Smith SM, Zwart SR. Spaceflight-related ocular changes: the potential role of genetics, and the potential of B vitamins as a countermeasure. Curr Opin Clin Nutr Metab Care 2018, 21:481–488. https://doi.org/10.1001/jamaophthalmol.2019.3124
72. Zwart SR, Gibson CR, Gregory JF, et al. Astronaut ophthalmic syndrome. FASEB J. 2017;31: 3746–3756. https://doi.org/10.1096/fj.201700294
73. Laurie SS, Vizzeri G, Taibbi G, et al. Effects of short-term mild hypercapnia during head-down tilt on intracranial pressure and ocular structures in healthy human subjects. Physiol Rep, 5 (11), 2017, e13302, https://doi.org/10.14814/phy2.13302
74. Khossravi EA, Hargens AR. Visual disturbances during prolonged space missions. Curr Opin Ophthalmol 2021, 32:69–73. https://doi.org/10.1097/icu.0000000000000724
75. Rasmussen JC, Kwon S, Pinal A, et al. Assessing lymphatic route of CSF outflow and peripheral lymphatic contractile activity during head-down tilt using near-infrared fluorescence imaging. Physiological Reports. 2020;8: e14375. https://doi.org/10.14814/phy2.14375
76. Goriely A, Geers MGD, Holzapfel GA, et al. Mechanics of the brain: perspectives, challenges, and opportunities. Biomech Model Mechanobiol (2015) 14:931–965. https://doi.org/10.1007/s10237-015-0662-4

77. Mao XW, Nishiyama NC, Byrum SD, et al. Characterization of mouse ocular response to a 35-day spaceflight mission: Evidence of blood-retinal barrier disruption and ocular adaptations. Nature Scientific Reports 2019; 9:8215 https://doi.org/10.1038/s41598-019-44696-0.
78. Marshall-Goebel K, Macias BR, Kramer LA, et al. Association of Structural Changes in the Brain and Retina After Long-Duration Spaceflight. JAMA Ophthalmol. 2021;139(7):781-784. https://doi.org/10.1001/jamaophthalmol.2021.1400
79. Marshall-Goebel, Rahul Damani KR, Bershad EM. Brain Physiological Response and Adaptation During Spaceflight. *Neurosurgery* 2019;85 (5): E815–E821. https://doi.org/10.1093/neuros/nyz203
80. McGregor HR, Lee JK, Mulder ER, et al. Ophthalmic changes in a spaceflight analog are associated with brain functional reorganization. Hum Brain Mapp. 2021;1–17. https://doi.org/10.1002/hbm.25546
81. Roy-O'Reilly M, Mulavara A, Williams T. A review of alterations to the brain during spaceflight and the potential relevance to crew in long-duration space exploration. npj Microgravity (2021) 7:5; https://doi.org/10.1038/s41526-021-00133-z.
82. Rosenberg MJ, Coker MA, Taylor JA, et al. Comparison of Dural Venous Sinus Volumes Before and After Flight in Astronauts With and Without Spaceflight-Associated Neuro-Ocular Syndrome. JAMA Network Open. 2021;4(10):e2131465. https://doi.org/10.1001/jamanetworkopen.2021.31465
83. Iliff JJ, Wang M, Liao Y, Plogg BA, Peng W, Gundersen GA, Benveniste H, Vates GE, Deane R, Goldman SA, Nagelhus EA, Nedergaard M. A Paravascular Pathway Facilitates CSF Flow Through the Brain Parenchyma and the Clearance of Interstitial Solutes, Including Amyloid β. Sci Transl Med. 2012 August 15; 4(147): 147ra111. https://doi.org/10.1126/scitranslmed.3003748
84. Thrane VR, Hynnekleiv L, Wang X, Thrane AS, Krohn J, Nedergaard M. Twists and turns of ocular glymphatic clearance – new study reveals surprising findings in glaucoma. Acta Ophthalmologica 2021 e283-e284. https://doi.org/10.1111/aos.14524
85. Mestre H, Mori Y, Nedergaard M. The brain's glymphatic system: current controversies. Trends in Neuroscience 2020;43(7):458-466. https://doi.org/10.1016/j.tins.2020.04.003
86. Hablitz LM, Plá V, Giannetto M, Vinitsky HS, Stæger FF, Metcalfe T, Nguyen R, Benrais A, Nedergaard M. Circadian control of brain glymphatic and lymphatic fluid flow. NATURE COMMUNICATIONS | (2020) 11:4411 | https://doi.org/10.1038/s41467-020-18115-2.
87. Wu B, Wang Y, Wu X, Liu D, Xu D, Wang F. On-orbit sleep problems of astronauts and countermeasures. Military Medical Research (2018) 5:17. https://doi.org/10.1186/s40779-018-0165-6
88. Flynn-Evans EE, Barger LK, Kubey AA. Circadian misalignment affects sleep and medication use before and during spaceflight. npj Microgravity (2016) 2, 15019. https://doi.org/10.1038/npjmgrav.2015.19
89. Eulenburg P, Buchheim JI, Ashton NJ et al. Changes in Blood Biomarkers of Brain Injury and Degeneration Following Long-Duration Spaceflight. JAMA Neurology Published online October 11, 2021. https://doi.org/10.1001/jamaneurol.2021.3589
90. Wang X, Lou N, Eberhardt A, Yang Y, Kusk P, Xu Q, Förstera B, Peng S, Shi M, Ladrón-de-Guevara A, Delle C, Sigurdsson B, Xavier ALR, Ertürk A, Libby RT, Chen L, Thrane AS, Nedergaard M. An ocular glymphatic clearance system removes β-amyloid from the rodent eye. Sci. Transl. Med. 12, eaaw3210 (2020) 25 March 2020: 1-14. https://doi.org/10.1126/scitranslmed.aaw3210
91. Wostyn P, Mader TH, Gibson CR, Killer HE. The escape of retrobulbar cerebrospinal fluid in the astronaut's eye: mission impossible? Eye (2019) 33:1519–1524. https://doi.org/10.1038/s41433-019-0453-8

92. Wostyn P, DeWinne F, Mader TH, Gibson CR, DeDeyn PP. Potential involvement of the "Ocular Glymphatic System" in optic disc edema in astronauts. Aerosp Med Hum Perform 2020;91(12): 975-977. https://doi.org/10.3357/AMHP.5670.2020
93. Wostyn P, Gibson CR, Mader TH. The glymphatic pathway in the optic nerve: did astronauts already reveal signs of its existence? npj Microgravity (2021) 7:14; https://doi.org/10.1038/s41526-021-00142-y.
94. Ashari N, Hargens AR. The mobile lower body negative pressure gravity suit for long-duration spaceflight. Front. Physiol. 2020;11: article 977. https://doi.org/10.3389/fphys.2020.00977
95. Zhang LF, Hargens AR. Spaceflight-induced intracranial hypertension and visual impairment:, pathophysiology and countermeasure. Physiol Rev 98: 59 –87, 2018 Published November 22, 2017. https://doi.org/10.1152/physrev.00017.2016
96. Goswami N, Blaber AP, Hinghofer-Szalkay H, Convertino VA. Lower body negative pressure: physiological effects, applications, and implementation. Physiol Rev 99: 807–851, 2019 Published December 12, 2018. https://doi.org/10.1152/physrev.00006.2018
97. Petersen LG Lawley JS, Lilja-Cyron A, et al. Lower body negative pressure to safely reduce intracranial pressure. J Physiol 597.1 (2019) pp 237–248. https://doi.org/10.1113/JP276557
98. Kubiak S, Manestar M. Topographic Relationship of the Ventromedial Lymphatic Bundle and the Superficial Inguinal Nodes to the Subcutaneous Veins. Clinical Anatomy (1995) 8:25-28. https://doi.org/10.1002/ca.980080104
99. Parazynski SE, Hargens AR, Tucker B, Aratow M, Styf J, Crenshaw A. Transcapillary fluid shifts in tissues of the head and neck during and after simulated microgravity. J. Appl. Physiol. (1991) 71(6): 2469-2475. https://doi.org/10.1152/jappl.1991.71.6.2469
100. Hung AS, Stenger MB, Macias BR. Gravitational influence in intraocular pressure: implications for spaceflight and disease. J Glaucoma 2019;28(8): 756-764. https://doi.org/10.1097/ijg.0000000000001293
101. Arbeille P, Provost R, Zuj K, Vincent N. Measurements of jugular, portal, femoral, and calf vein cross-sectional area for the assessment of venous blood redistribution with long duration spaceflight (Vessel Imaging Experiment). Eur J Appl Physiol (2015) 115:2099–2106. https://doi.org/10.1007/s00421-015-3189-6
102. Fortrat JO, deHolanda A, Zuj K, et al. Altered venous function during long-duration spaceflights. Front. Physiol 2017;8: article 694. https://doi.org/10.3389/fphys.2017.00694
103. Marshall-Goebel K, Laurie SS, Alferova IV, et al. Assessment of jugular venous blood flow stasis and thrombosis during spaceflight. JAMA Network Open 2019;2(11): e1915011. https://doi.org/10.1001/jamanetworkopen.2019.15011
104. Auñón-Chancellor SM, Pattarini, JM, Moll, S, Sargsyan, A. Venous Thrombosis during Spaceflight. NEJM 2020. 382; 1:89-90. https://doi.org/10.1056/nejmc1905875
105. Soffer AD, Caine M, Hardigan PC, et al. Sensitivity and specificity of thermal imaging when used to detect superficial venous reflux as compared to duplex ultrasound. Vascular Disease Management (2021) 18(3), E45-E49. (No DOI in PubMed).
106. Debiec-Bak A, Skrzek A, Wozniewski M, et al. Using thermography in the diagnostics of lymphedema: pilot study. Lymphatic Research and Biology (2020) 18(3), 247-253. https://doi.org/10.1089/lrb.2019.0002
107. Kelly-Hope LA, Karim MJ, Mahmood ASM, et al. Infrared thermal imaging as a novel non-invasive point-of-care tool to assess filarial lymphedema. J Clin Med (2021) 10, 2301;1-17. https://doi.org/10.3390/jcm10112301
108. Howell PA, Winfree WP, Cramer KE. On-orbit passive thermography. Nondestructive Testing and Evaluation (2008) Sept., 1-18. https://doi.org/10.1080/10589750701855171

109. Stahn AC, Werner A, Opatz O, et al. Increased core body temperature in astronauts during long-duration space missions. Nature Scientific Reports (2017) 7, 16180 https://doi.org/10.1038/s41598-017-15560-w
110. Stenger MB, Lee SMC, Westby CM, et al. Abdomen-high elastic gradient compression garments during post-spaceflight stand tests. Aviation, Space, and Environment Medicine 2013;84(5): 459-466. https://doi.org/10.3357/asem.3528.2013
111. Lee SMC, Feiveson AH, Stein SP, Stenger MB, Platts SH. Orthostatic intolerance after International Space Station and Space Shuttle missions. Aerosp. Med. Hum. Perform. 2015;86: A54-A67. https://doi.org/10.3357/AMHP.EC08.2015
112. Lee SMC, Ribeiro LC, Laurie SS, et al. Efficacy of gradient compression garments in the hours after long-duration spaceflight. Front. Physiol., 17 July 2020 https://doi.org/10.3389/fphys.2020.00784
113. Fu Q, Shibata S, Hastings JL, Platts SH, et al. Impact of Prolonged Spaceflight on Orthostatic Tolerance During Ambulation and Blood Pressure Profiles in Astronauts. Circulation 2019; 140:729–738. https://doi.org/10.1161/CIRCULATIONAHA.119.041050
114. Scott JPR, Kramer A, Petersen N, Green DA. The role of long-term head-down bed rest in understanding inter-individual variation in response to the spaceflight environment: a perspective review. Front. Physiol., 11 February 2021 https://doi.org/10.3389/fphys.2021.614619
115. Broekhuizen LN, Lemkes BA, Mooij HL, et al. Effect of sulodexide on endothelial glycocalyx and vascular permeability in patients with type 2 diabetes mellitus. Diabetologia (2010) 53:2646–2655. https://doi.org/10.1007/s00125-010-1910-x
116. Nicolaides AN. The Benefits of Micronized Purified Flavonoid Fraction (MPFF) Throughout the Progression of Chronic Venous Disease. Adv Ther (2020) 37: S1–S5 http://dx.doi.org/10.1007/s12325-019-01218-8
117. Kakkos SK, Nicolaides AN. Efficacy of micronized purified flavonoid fraction (Daflon®) on improving individual symptoms, signs and quality of life in patients with chronic venous disease: a systematic review and meta-analysis of randomized double-blind placebo-controlled trials. International Angiology 2018 April;37(2):143-54. https://doi.org/:10.23736/S0392-9590.18.03975-5
118. Wirginiaa K, Agnieszkac C, Mariuszd K, Joanna K. Oxidative DNA Damage in Blood of CVD Patients Taking Detralex. The Open Cardiovascular Medicine Journal, 2011, 5, 179-187. http://dx.doi.org/10.2174/1874192401105010179
119. Smith SS, Zwart SR, Douglas GL, Heer M. Human adaptation to spaceflight: the role of food and nutrition. Second edition. 2021. https://www.nasa.gov/sites/default/files/atoms/files/human_adaptation_2021_final.pdf