AN EXPLORATORY LITERATURE REVIEW OF MICROGRAVITY-INDUCED PERIPHERAL NERVE DAMAGE AND POTENTIAL NEURPROTECTANTS USING CONCEPTUAL RESEARCH

Naomi Carter[1], Juan Yakisich[2]

[1] Hampton University Department of Biology, Hampton VA
[2] Hampton University, Assistant Professor, Hampton VA

Abstract

A plethora of research studies have correlated changes in the gravitational vector with neuronal adaptation. Prolonged exposure to microgravity has been shown to induce physiological changes including depolarized afferent and efferent nerves, increased membrane fluidity and reduced nerve conduction velocities all of which contribute to downstream consequences in the musculoskeletal system. Current methods of astronaut preparation lack sufficient modeling of microgravity and investigating additional treatments that mediate symptoms of microgravity-induced peripheral nerve injury (MIPNI) will help astronauts adjust during long duration space missions. A thorough literature review on MIPNI was conducted using the PubMed and Hampton University William R. and Norman B. Harvey Library Databases. The results were indicative of a significant gap in the research literature in regards to the role of immune activation, diet and altered vasculature in MIPNI pathophysiology. Notwithstanding, oxidative stress and decreased membrane viscosity were identified as primary determinants in MIPNI progression. Oral cholesterol and antioxidants are thoroughly discussed as possible treatments which may mediate decreased membrane viscosity and apoptosis associated with MIPNI.

Key Words

Microgravity-Induced Peripheral Nerve Injury (MIPNI)
Peripheral Nerve Damage (PND)
Peripheral Nervous System (PNS)
Central Nervous System (CNS)

Introduction

Microgravity Induced Peripheral Nerve Injury and Factors in Development

Research studies have correlated changes in the gravitational vector to neuronal adaptation. Prolonged exposure to microgravity has been shown to induce physiological changes including sensory-motor distortions and neuromuscular atrophy that may pose a significant threat to astronaut safety during future long duration interplanetary space explorations [1,2]. Predominant symptoms seen in space cadets include reduced astronaut tactile sensitivity and difficulty controlling slow voluntary limb movements [3]. In rare cases, astronauts have also reported decreases or a complete absence of proprioception during space missions such as Apollo and Gemini [3].

Extended periods of microgravity exposure induce peripheral nerve injury by prolonging neuroplasticity-induced adaptations that occur in response to an abnormal gravitational field [4]. Short-term exposure to microgravity was not shown to influence baseline nervous function in embryos, due to the reversibility of the adaptive process [5]. At the early stages of development, organisms are prone to adapt to their environment and embryos are more likely to undergo significant physiological changes in comparison to adults if the gravitational vector is modified [5]. This suggests that the likelihood of adults demonstrating severe MIPNI during short term microgravity exposure is extremely low and inconsequential as the body can adequately reverse any adaptations to return to a normal state. However, over long periods of time, the transition back to Earth's associated physiological conditions becomes more challenging.

This principle is evident in another study featuring short, middle and long-term microgravity exposure to neuronal networks where neurons with minimal periods of microgravity exposure displayed reduced neurite network density and neuron size, altered β-tubulin distribution and some apoptosis yet displayed fast recovery mirroring the ground morphological state [4]. Neurons exposed to long term microgravity displayed longer re-adaptation periods and a higher degree of adaptation concomitant with increases in apoptosis [4]. This substantiates that the degree of physiological change in response to microgravity and the reversibility of these alterations is dependent on the duration of the exposure, where extended subjection complicates the return to baseline functionality due to maladaptive neuroplasticity.

Genetics may also play a role in an individual's neuroadaptive processes in space. Scott Kelly was the first American to spend more than a year in space, spending 340 days on the Soyuz TMA-18M spacecraft before returning September 4th, 2015 [6]. NASA researchers were able to observe how the human body adjusts to microgravity and stress in a new context, considering Kelly's identical twin brother on Earth [6]. The twin studies revealed that roughly 93% of astronaut Scott Kelly's genes returned to normal after his space mission, and the other 7% remained changed, even after Kelly returned to earth, suggesting that space travel has prolonged effects on the body and that gene expression helps mediate the acclimation to a microgravity environment [7,8,9]. In simulated microgravity conditions, adipose derived stem cells demonstrated increased neural differentiation and upregulated expression of *GNL3*, *MAP-2*, *BDNF*, *NT-3* genes [10]. Post differentiation of these neurotrophic factors, cell viability was shown to decrease [10]. Investigating genetic disposition and altered gene expression in microgravity may explain why in rare cases, astronauts have experienced significant decreases or even a complete absence of proprioception on space flights [3].

Radiation exposure, alongside microgravity, ranks amongst the top five list of concerns for human spaceflight according to NASA and can directly moderate the functionality of the peripheral nervous system [11]. Long duration space missions expose crew-members to 50 to 2,000 mSv of ionizing radiation that easily exceeds the maximum human dosage with impactful repercussions to neuronal network integrity [12]. Galactic cosmic rays and solar proton events in earth's atmosphere place astronauts at risk for degenerative diseases [13,14]. Post-irradiation swelling of the myelin sheath in the sciatic nerve was concomitant with necrotic and degenerative changes [15]. This suggests that neuronal conductivity is also moderated by radiation levels in space. The constant exposure to high doses of radiation places astronauts at risk of developing Radiation Fibrosis Syndrome and further neuromuscular and musculoskeletal fibrosis [16]. While specialized space wear and technology helps limit the radiation exposure, radiation is still a significant factor in peripheral nerve functionality and MIPNI.

Microgravity induces systemic changes that alter hydrostatic pressure, fluid shear stress and cellular polarity that may have secondary consequences on MIPNI progression [10,17,18,19]. Further investigation is required to understand how these factors mediate MIPNI presentation.

Current Standards of Astronaut Preparation for Microgravity Environment and Microgravity Management in Space

According to NASA, microgravity is one of the top five threats to astronaut health and safety [20]. Aspiring astronauts undergo rigorous training to prepare for the microgravity environment. The Sonny Carter training facility features the largest indoor pool in the world where astronauts spend up to 10 uninterrupted hours in a simulated low gravity environment [21,22]. At about 32-49 ft or ⅓ of the depth of the total body of water, the average diver becomes neutrally buoyant and the forces actively pulling up or down on the body are greatly reduced [23]. At a depth of roughly 40ft, the Sonny Carter facility can simulate weightlessness [24]. However, there are significant differences between the underwater training facility and real microgravity environments. Primarily, the air density at the edge of space is almost zero which vastly differs from to the density of water at 1g/cm^3 [25]. Water adds drag to the body and astronauts can recognize this hindrance while completing tasks [24]. While training in such underwater environments provides an excellent simulation, it has many limitations and cannot prepare astronauts for prolonged exposure to microgravity nor elicit MIPNI symptoms.

In the KC-135 plane, astronauts can spend up to 25 minutes in a truly weightless environment [22]. However, this exposure is too brief to observe MIPNI development. Currently, NASA offers no training for astronauts that would mimic the effects of microgravity on neuronal adaptation or sensory communication and trainees do not have the opportunity to become familiar with physical effects of MIPNI before entering space.

To address the consequences of gravitational changes in space, astronauts exercise regularly and have specialized suits that can help increase the load on the body [2,26]. These methods can reduce symptoms associated with MIPNI including sensory-muscle latency, but does not address the mechanisms driving MIPNI progression or symptom presentation [2].

Objectives and Relevance of Research

During expeditions to the international space station, astronauts can be in space for roughly six months, with the longest US space mission being 215 days by Mike Lopez-Alegria [27,28]. Still, this is less than one third of the duration of the NASA Mars exploration trip that is much farther, and requires another lift off [20,27]. While the body's acute changes to neural afferent input in response to microgravity have shown to be reversible in short term missions typically under three weeks, long term exposure to microgravity during spaceflight will have deleterious impacts to human function upon returning to earth [26]. Given the difference in space mission length, astronauts will likely present with significantly more severe MIPNI symptoms than those in previous astronaut's missions. The transition from earth to space, space to Mars and back will induce excessive stress on neuronal adaptive mechanisms. Investigation of MIPNI pathophysiology and potential treatments is essential to protecting interstellar astronauts from the effects of microgravity.

The mechanism by which microgravity alters peripheral nerve integrity is still being elucidated. Currently there are no available treatments for MIPNI and preventative attempts to prepare astronauts for the microgravity environment are inadequate. An urgent need arises to evaluate MIPNI in order to protect astronauts during space travel and aid in readjustment upon returning to earth's climate. By

condensing the data on MIPNI pathophysiology using a thorough literature review, we hope to clarify mechanisms fueling MIPNI development. This paper aims to use systematic literature searches and conceptual research methods to summarize MIPNI pathophysiology and propose therapeutics that will inform future clinical research.

Methods for Literature Review

Scientific Journals and Key Words Involved in Search

PubMed and the William R and Norman B Harvey Library Databases were utilized in the search. Scientific articles within the first 100 search results that reference MIPNI pathophysiology were considered in the review. The results were filtered to only include journal articles with the full text accessible online for both PubMed and Harvey Library Database. Microgravity Induced Peripheral Nerve Injury Keywords include "Microgravity induced peripheral nerve injury" "microgravity influence on peripheral nervous system", "microgravity neurology" and "microgravity nerve injury pathology".

Evaluation of MIPNI

A primary determinant is experimentally defined as a driving factor in the pathogenesis demonstrated in the literature that is onset directly by the etiology, influences the outcome of the peripheral nerves and mediates the severity or progression of the nerve damage. Primary determinants involved in MIPNI were conceptually investigated to predict possible treatments that could serve as a therapeutic.

Literature Search of MIPNI

Microgravity Induced Peripheral Nerve Injury

MIPNI stems from prolonged neuronal adaptation in microgravity that alters the normal electrophysiology of afferent and efferent neurons and disrupts synaptic communication [4,29,30]. Decreases in axon membrane viscosity under microgravity conditions have been observed in multiple neuronal cell lines including SH-SY5Y [1,17,31]. As the membrane becomes more fluid under microgravity, membrane resistance is diminished and current could potentially escape through the membrane reducing the axon potential. The closed-state probability of ion channels increases in response to these conditions due to a pressure-dependent relationship with the axon membrane [1,32,33,34].

During microgravity exposure in *Xenopus Laevis*, axon currents were significantly decreased [1,35]. This implies that length constant, or the distance required for an axon potential to drop to 36% of its initial value, contracts, reducing signal output. This is substantiated in a 2016 Ritzmann study where peripheral nerve stimulation output signals and peak to peak amplitudes are decreased in microgravity conditions [36]. Collectively these factors indicate reduced action potential velocities as shown in previous studies with earthworms [1,17].

The resting potential has been shown to be slightly depolarized under microgravity condition in multiple human neuronal cell lines, including SF-21 [1,17,35,37,38]. The threshold potential is also increased under microgravity and higher currents were required to depolarize efferent and afferent peripheral neurons [1,36]. Interestingly, research studies revealed a clear increase in the rate of action potentials under microgravity conditions suggesting that these alterations ultimately decrease the distance between the resting potential and membrane threshold allowing for axons to depolarize easier and fire potentials at faster rates [1,36]. This hyperexcitability observed may serve to counteract the increased probability of closed ion channels and improve successful action potential propagation.

Sensory information collected by the PNS is reviewed by the central nervous system (CNS) to direct efferent stimuli and muscular response. Given the close relationship between the PNS and the musculoskeletal system, MIPNI may play a significant role in not only the sensory-related symptoms seen in astronauts but also indirectly contribute to musculoskeletal changes in space. Axonal nerve conduction relies on the ability of the signal to travel rapidly down the membrane, a process shown to be diminished in microgravity conditions due to reduced membrane viscosity [1]. Successful synaptic nerve conduction relies on interneuronal communication and is likely inhibited by the increased closed-state probability of ion channels [1,34]. Although peripheral neurons are pushed towards hyperexcitability, closed ion channels still reduce propagation speeds of axon potentials and ultimately slow neuromuscular communication [1]. This may explain microgravity-associated neuromuscular alterations. Peripheral nerve stimulation in the M. *soleus* under microgravity conditions revealed increased H-reflex latencies, prolonged sensory-motor inter-peak intervals and elevated stimulation thresholds of H-reflexes and M waves [1]. The closed state of nicotinic acetylcholine receptors, which modulate neuromuscular communication, also increase under microgravity conditions and may serve as the predominant force behind the muscular latency symptoms observed in astronauts [17,34]. The data indicates that MIPNI contributes to attenuated musculoskeletal response.

In microgravity, proprioception is shown to diminish and paresthesia can develop, although fine touch is not disturbed [39,40]. This implies that both motor and sensory nerves experience microgravity induced alterations however, it is unclear whether or not this mechanism is identical and further investigation is required.

Radiation exposure exacerbates the negative effects of microgravity [39,40,41]. On its own, radiation can arrest neurite outgrowth [41]. Radiation exposure had the most pronounced influence on soma size while microgravity exposure predominantly decreased total neurite area [41]. Additionally, microgravity alone can increase apoptosis in neurons depending on the duration of exposure [36]. Combined radiation and microgravity exposed neurons demonstrated a retraction in neurite growth and a reduction in neurite area [41]. The

combined effects of radiation and microgravity resulted in the most severe changes in cell morphology [41]. Delayed apoptosis of irradiated neurons was doubled by microgravity exposure [41]. Furthermore, microgravity and radiation treated cells demonstrated increased gene expression of Vglut2, a regulator of excitatory glutamate neurotransmitter and Gabrb2, an inhibitory postsynaptic GABA receptor [41]. This substantiates the reduction in synaptic transmission and potentiation observed in previous studies.

Discussion

A primary determinant is experimentally defined as a driving factor in the pathogenesis demonstrated in the literature that is onset directly by the etiology, influences the outcome of the peripheral nerves and mediates the severity or progression of the nerve damage. Three primary determinants were identified in MIPNI including hyperexcitability, morphological changes and oxidative stress. More specifically, the closed state of ion channels observed in MIPNI literature and data may induce compensatory mechanisms that contribute to hyperexcitability. Decreases in axon membrane viscosity can be characterized as a morphological change. Given the presence of apoptosis in MIPNI, oxidative stress likely plays a role in the pathogenesis. These three factors appear to drive MIPNI and will be considered as potential targets in the following section.

Surprisingly, immune response, vasculature changes and diet did not appear to play a large role in MIPNI. This may be attributable to microgravity's ability to evoke wide-scale cellular responses using a traverse mechanism not limited to physical dispersion anatomy such as immune infiltration or vasculature alterations and yet influence local tissues. Rather the etiology can create a plethora of systematic micro-changes instantaneously, that if continued for prolonged periods drive the body away from homeostasis and lead to PNI development [4]. Aforementioned radiation and microgravity are intuitively synergistic likely because of their pervasive mechanisms and demonstrate severe alterations to neuron function when combined [39,40,41]. The apparent lack of data connecting immune response, vasculature changes and diet in MIPNI that may be explained by the ability of microgravity to induce cellular changes directly without the intermediate of immune activation or blood vessel transport. These additional factors may only serve as secondary determinants.

However, significant data exists on the influence of microgravity on immune alternations within human physiology. Microgravity induced immune suppression has been observed in inflammatory cytokines, macrophages and associated antigen-presenting cells [42,43]. Osteoblastic differentiation of human mesenchymal stem cells was inhibited in modeled microgravity conditions via the MAPK pathway where altered cytokine functionality within the immune system drives bone formation and resorption [44,45,46]. Inappropriate neurotrophin and MAPK function plays a role in inflammation, a function of both immune and vasculature adaptation, in distal symmetric neuropathy pathogenesis and is downregulated in symptomatic patients [47,48]. More than half of Apollo mission astronauts develop opportunistic infections upon returning to earth, furthering the relevance of immune activation in microgravity pathophysiology's [49,50].

Microgravity induced alterations that generate hypovolemia, orthostatic intolerance, shift fluid pressure and contribute to uneven perfusion thorough vascular modification, have also not been evidenced to play a role in MIPNI, but may present systemic changes that indirectly influence progression [51]. Similarly, dried plum powder and soy protein can mediate some of the symptoms from microgravity [52,53]. This implicates that diet may mediate microgravity induced changes, although this system is not well understood in the context of peripheral nerve functionality.

Many systemic changes from microgravity exposure have been observed in particular organ systems and cannot be directly translated to MIPNI without further investigation. Immune response, altered vasculature and diet appear relatively unrelated to MIPNI pathology, but the aperture in the literature could be attributed to a lack of data available exploring this factor in the context of MIPNI pathophysiology. Whether or not immune response, vasculature changes and diet serve as a primary or secondary determinants in MIPNI has yet to be elucidated in the literature. This review demonstrates a wide gap exists elaborating the pathophysiological features in MIPNI progression, where factors that typically play a large role in pathophysiology are not demonstrated in MIPNI related literature.

MIPNI Targets for Treatment and Research Proposals

Cholesterol Diet May Counter MIPNI Associated Decrease in Membrane Viscosity

MIPNI pathophysiology can be distinguished, by the abrupt decrease in membrane viscosity under microgravity conditions [1,17,31]. Typically, the cholesterol and phospholipid ratios determine membrane viscosity and plasma membrane integrity is modulated by cholesterol in the CNS [54,55]. Nerve lesions, altered nerve conduction and severe clinical symptoms associated with diabetic polyneuropathy in diabetes type 2 patients was correlated with low serum cholesterol and low-density lipoprotein C suggesting that mediating lipid metabolism, specifically cholesterol, can moderate nerve damage in PNS focused pathologies [56]. Increasing membrane viscosity may serve as an excellent target to reversing reduced afferent communication associated with MIPNI as membrane fluidity can be transiently altered with cholesterol diet, allowing for astronauts to opt into this potential therapeutic as needed during spaceflight. Additionally, the original study investigating the relationship between microgravity and neuronal membrane viscosity also featured asolectin vesicles which are representative of a broad range of cell types and indicate that investigating cholesterol treatment may have beneficial systemic effects [31]. To test this hypothesis, we suggest a multifaceted study evaluating lipid distribution,

lipid composition and cholesterol treatment in peripheral neurons that will be evaluated after short and prolonged exposure to microgravity.

Undifferentiated and differentiated SH-SH5Y neuroblastoma cell lines were used as a model in the original study [31]. The study was performed in 1g and the nondifferentiated cell line demonstrated more significant changes [34]. While this CNS derived model cell line successfully demonstrated reduced lipid membrane viscosity in microgravity, SH-SH5Y are typically used to assess differentiation or neurite growth and considering the use of differentiated cell lines from human embryonic stem cells may be more accurate [57,58,60,61]. More specifically, human derived stem cells treated with dual-SMAD inhibitors, WNT activation, small molecular notch inhibitors and VEGF/FGF/PDGF signaling pathways have been demonstrated to successfully mimic morphological and electrophysiological aspects of peripheral sensory neurons with minimal caveats as well as coalesce with Schwann cells and fibroblast when grown together in culture [61]. This model will serve as an ideal archetype of peripheral neurons for the preliminary study and should be used for the experiment [61].

The influence of decreased membrane viscosity in simulated microgravity has previously been demonstrated in the literature [31]. While a true microgravity environment would be most ideal for this study, because this experiment is still preliminary, the application of simulated microgravity may be required before benefactors are willing to fund financially demanding projects. The RCCS Bioreactor microgravity model allows 3D cell cultures to be grown in constant orientation with the fluid, allowing the cytoarchitecture and microenvironment to be observed in microgravity-like conditions.

Lipid density must be examined after microgravity exposure to reconfirm previous studies in the new model of peripheral neurons derived from stem cells and can be evaluated using fluorescence polarization anisotrophy that increases as solvent viscosity increases [62]. This method was used in the original investigation of microgravity induced changes in membrane fluidity [31]. A simple 96-well plate reader can detect cells labeled with 1,6-Diphenyl-1,3,5-hexatriene (DPH), a fluorescent dye often used in fluorescence polarization anisotrophy [31,63]. This experiment should include side by side comparison of SH-SH5Y cells and any significant differences between the results should be noted. Differences in lipid viscosity may explain alterations in the post-synaptic cleft activity and this should be further evaluated in a separate investigation [4,30,64].

Subsequently, evaluating lipid composition of microgravity exposed and control cell populations, will determine if cholesterol concentrations are modified after microgravity exposure. This study should include positive controls where cells are treated with statins, which lower cholesterol, fibrates, which target fatty acids and triglycerides as well as niacin, that modifies lipoproteins in order to provide comparison alongside the microgravity treatment group and normal cells [65]. Lipid content from treated cells, positive controls and negative controls can be extracted into fractions after homogenization using differential and discontinuous density gradient centrifugation and quantified with an enzymatic assay [66,67,68]. Depending on the results of the previous experiments, cholesterol treatment after microgravity exposure can be evaluated using similar methods described for analyzing lipid density composition. If cholesterol treatment progresses to clinical trials, evaluating nerve conduction and muscular latency would he highly beneficial to understanding MIPNI pathogenesis.

Why Not Target Ion Channels for MIPNI Treatment?

The closed-state probability of ion channels was shown to increase under microgravity conditions, however this may not be an ideal target for treating MIPNI [1,32,33,34]. Microgravity directly influences the lipid membrane which can indirectly modify membrane protein function and transmembrane proteins such as sodium and potassium ion channels [17,69,70]. In response to altered ion channel function, neuroadaptive mechanisms allow for the membrane potential to be adjusted in order to preserve communication [17]. This is evident in MIPNI as the resting potential is depolarized and the threshold potential is increased indicating that the body possesses compensatory mechanisms that mediate this process leading to hyperexcitability [1,17,36,35,37,38]. Targeting membrane viscosity may aggravate a wide range of downstream compensatory events and further investigation of ion channel alteration is required before potential treatments can be identified.

Antioxidants to Mediate Neuronal Apoptosis in MIPNI

Further evaluation of oxidative stress pathways in neuronal injury and apoptosis would be an excellent starting point in clarifying MIPNI pathophysiology. More specifically, if oxidative stress is a predominant mechanism within MIPNI, antioxidants may serve as a potential treatment. In any case, exploring antioxidant treatment may be beneficial to reducing the effects of radiation that increase reactive oxygen species and reactive nitrogen species which consequentially mediate the combined effects of radiation and microgravity [71].

During oxidative stress, reactive free radicals damage cellular proteins, lipids and nucleic acids [72]. Innate antioxidant mechanisms including superoxide dismutase and glutathione reductase typically modulate reactive oxygen and nitrogen species and convert them into compounds safer for the body to manipulate [72]. Antioxidants assist in this process and regulate the production and degradation of radicals [72]. In neuropathy pathologies such as distal symmetric polyneuropathy and radiation induced brachial plexopathy, it is not uncommon for patients to be administered antioxidants, indicating that this therapeutic has the potential to address excessive reactive free radicals and oxidative stress [73,74,75]. Similar to cholesterol, antioxidants can be given orally, and serve at as optional treatment that can be taken as needed when astronauts experience symptoms of MIPNI [76,77]. Additionally, microgravity induced oxidative stress was originally found to be systemic, having primary, secondary and tertiary implications in other microgravity induce

physiological changes [78]. Oral antioxidant has the potential to mediate oxidative stress not only in the PNS, but on a broad scale benefiting multiple organ systems.

Non-enzymatic sources of free radicals, enzymatic sources of free radicals and non-enzymatic antioxidants should be evaluated in the context of MIPNI to shed light on the mechanism of microgravity-induced oxidative stress. Aforementioned in the review of cholesterol, in vitro cultures of differentiated human derived stem cells, Schwann cells and fibroblast will serve as an excellent model for evaluating normal functions in sensory neurons.

Oxidative stress must be confirmed in the PNS by comparing the presence of radical species and their concentrations in healthy and microgravity exposed tissues. Fluorogenic probes such as 5-(and -6)-carboxy-2′,7′-dichlorodihydrofluorescein diacetate (DCFDA) can diffuse into cells, bind to hydrogen peroxide and form dichlorofluorescein (DCF) [79]. This can be analyzed with flow cytometry or fluorescent microscopy [79]. In a similar process, superoxides can be detected with dihydroethidium (DHE) [79]. This experiment will clarify the relationship between the production of radical species, oxidative stress and apoptosis in MIPNI.

Sequentially, the mechanism of oxidative stress must be investigated. The two predominant pathways of oxidative stress either require or function without enzymes that convert oxygen into deleterious oxygen and nitrogen species [72]. Non-enzymatic sources of radicals include the mitochondrial respiratory chain, glycolysis, glycation and the polyol pathway while enzymatic sources require endothelial and NADPH oxidases, Xanthine, cyclooxygenase and uncoupled nitrogen oxygen species [72]. Measuring the end products of the respective pathways is one method of investigating oxidative stress mechanisms in MIPNI, however, this process would be extremely tedious for preliminary studies. Rather evaluating protein, lipid and DNA damage may provide more applicable data to the MIPNI pathophysiology. Quantification of protein carbonyl context is relatively simple and can be performed using gel electrophoresis and western blot [79,80,81]. Malondialdehyde (MDA) is an end product from polyunsaturated fatty acid peroxidation and can be extracted after reaction with using thiobarbituric acid reactive substances (TBARS) [79,82,83]. Thymidine glycol (TG) can assess DNA damage and can be examined using the streptavidin-biotin-peroxidase complex method and immunohistochemistry [79,84].

Once the products of oxidative stress are confirmed and more is understood relative to the pathophysiology, the effect of non-enzymatic antioxidant treatments such as Glutathione, Vitamin A, Vitamin C and Vitamin E can be evaluated under simulated microgravity conditions in the RCCS bioreactor [77]. Depending on the results of the experimental therapeutics, cholesterol and antioxidants should be tested in combination to observe their potential for treatment in tandem.

Summary

Under microgravity conditions human efferent and afferent nerve cells are slightly depolarized, lipid membranes become more permeable, nerve conduction speeds are altered and single neurons have an increased likelihood of undergoing apoptosis depending on the duration of exposure. These pathological characteristics are characteristics of MIPNI are considered as potential targets for treatment. Based on the current literature, cholesterol may mediate lipid membrane viscosity and has been justified as a potential treatment for MIPNI that can be investigated by NASA researchers. Additionally, antioxidants may reduce signs of oxidative stress in MIPNI. The series of experiments described would provide a feasible and relatively inexpensive method of investigating MIPNI pathophysiology that furthers NASA's ability to address sensory complications experienced by astronauts and advances in understanding the impact of microgravity in astronaut physiology will be pertinent to future long duration space missions such as the highly anticipated exploration to Mars.

Acknowledgments

Financial support was provided by the Virginia Space Grant Consortium Undergraduate Scholarship sponsored by the National Aeronautics and Space Administration. I would like to thank Dr. Juan Yakisich for his continued guidance during the paper. Thank you, Dr. Ricks-Santi and Dr. Eric Lewallen, for sparking my interest in research and providing me with the initial opportunity to work in a lab. To Dr. Charles Carmada, thank you for sharing your personal experiences as an astronaut. Many thanks to Millard Reschke for providing additional clarification on his publication Neuroscience in Space.

Conflict of Interest

Research funding was contingent on completion of the research project by April 1st.

References

1. Kohn, F. P. M., & Ritzmann, R. (2017). Gravity and neuronal adaptation, in vitro and in vivo—from neuronal cells up to neuromuscular responses: a first model. *European Biophysics Journal*, *47*(2), 97–107. https://doi.org/10.1007/s00249-017-1233-7
2. *Astronaut Health and Performance*. (n.d.). National Aeronautics and Space Administration. https://www.nasa.gov/centers/johnson/pdf/584739main_Wings-ch5d-pgs370-407.pdf
3. Clément, G., & Reschke, M. F. (2008). Neuroscience in Space. *Springer*, 109. https://doi.org/10.1007/978-0-387-78950-7

4. Pani, G., Samari, N., Quintens, R., de Saint-Georges, L., Meloni, M., Baatout, S., Van Oostveldt, P., & Benotmane, M. A. (2013). Morphological and Physiological Changes in Mature In Vitro Neuronal Networks towards Exposure to Short-, Middle- or Long-Term Simulated Microgravity. *PLoS ONE*, *8*(9), e73857. https://doi.org/10.1371/journal.pone.0073857
5. Horn, E. R., Dournon, C., Frippiat, J. P., Marco, R., Böser, S., & Kirschnick, U. (2007). Development of neuronal and sensorimotor systems in the absence of gravity: Neurobiological research on four soyuz taxi flights to the international space station. *Microgravity Science and Technology*, *19*(5–6), 164–169. https://doi.org/10.1007/bf02919474
6. *NASA Astronaut Scott Kelly Safely Back on Earth after One-Year Mission*. (2016, March 2). NASA. https://www.nasa.gov/press-release/nasa-astronaut-scott-kelly-returns-safely-to-earth-after-one-year-mission/
7. Mars, K. (2018, August 16). *First Look at Findings of NASA Twins Study*. NASA. https://www.nasa.gov/feature/how-stressful-will-a-trip-to-mars-be-on-the-human-body-we-now-have-a-peek-into-what-the-nasa/
8. Rossman, S. U. R. A. S. T. (2018, March 16). *"Space genes": NASA confirms space travel changes DNA — even upon return to Earth*. USA TODAY. https://eu.usatoday.com/story/news/nation-now/2018/03/15/space-genes-nasa-confirms-space-travel-changes-dna-perhaps-long-time/427402002/
9. Rossman, S. U. T. (2017, February 1). *First results on Scott Kelly after year in space reveal space travel changes DNA*. USA TODAY. https://eu.usatoday.com/story/news/nation-now/2017/01/31/going-into-space-affects-dna-astronaut-twins/97295132/
10. Zarrinpour, V., Hajebrahimi, Z., & Jafarinia, M. (2017). Expression pattern of neurotrophins and their receptors during neuronal differentiation of adipose-derived stem cells in simulated microgravity condition. *Iran J Basic Medical Science*, 178. https://doi.org/10.22038/ijbms.2017.8244
11. NASA. (2019). *5 Hazards of Human Spaceflight*. https://www.nasa.gov/hrp/5-hazards-of-human-spaceflight/
12. NASA. (2019b). *Why Space Radiation Matters*. https://www.nasa.gov/analogs/nsrl/why-space-radiation-matters/
13. Cucinotta, F. A., & Durante, M. (2006). Cancer risk from exposure to galactic cosmic rays: implications for space exploration by human beings. *The Lancet Oncology*, *7*(5), 431–435. https://doi.org/10.1016/s1470-2045(06)70695-7
14. Townsend, L., & Fry, R. (2002). Radiation protection guidance for activities in low-earth orbit. *Advances in Space Research*, *30*(4), 957–963. https://doi.org/10.1016/s0273-1177(02)00160-6
15. Shabeeb, D., Musa, A. E., Keshavarz, M., Esmaely, F., Hassanzadeh, G., Shirazi, A., & Najafi, M. (2019). Histopathological and Functional Evaluation of Radiation-Induced Sciatic Nerve Damage: Melatonin as Radioprotector. *Medicina*, *55*(8), 502. https://doi.org/10.3390/medicina55080502
16. Stubblefield, M. D. (2017). Clinical Evaluation and Management of Radiation Fibrosis Syndrome. *Physical Medicine and Rehabilitation Clinics of North America*, *28*(1), 89–100. https://doi.org/10.1016/j.pmr.2016.08.003
17. Mann, V., Sundaresan, A., & Chaganti, M. (2019). Cellular changes in the nervous system when exposed to gravitational variation. *Neurology India*, 684. https://doi.org/10.4103/0028-3886.263169
18. Ingber, D. (1998). In search of cellular control: Signal transduction in context. *Journal of Cellular Biochemistry*, *72*(S30-31), 232–237. https://pubmed.ncbi.nlm.nih.gov/9893275/
19. Zahir, N., & Weaver, V. M. (2004). Death in the third dimension: apoptosis regulation and tissue architecture. *Current Opinion in Genetics & Development*, *14*(1), 71–80. https://doi.org/10.1016/j.gde.2003.12.005
20. *How Investing in the Moon Prepares NASA for First Human Mission to Mars*. (n.d.). National Aeronautics and Space Administration. https://www.nasa.gov/sites/default/files/atoms/files/moon-investments-prepare-us-for-mars.pdf
21. *Johnson Space Center Training for space Astronaut training and mission preparation*. (n.d.). National Aeronautics and Space Administration. https://www.nasa.gov/centers/johnson/pdf/160410main_space_training_fact_sheet.pdf
22. *NASA - Astronauts in Training*. (2004). National Aeronautics and Space Administration. https://www.nasa.gov/audience/forstudents/5-8/features/F_Astronauts_in_Training.html
23. Zvaritch, K. (2019, February 17). *Guide To Freefalling In Freediving*. DeeperBlue.Com. https://www.deeperblue.com/guide-to-freefalling-in-freediving/
24. *Exploring Space Through Math*. (n.d.). NASA. https://www.nasa.gov/pdf/593848main_Alg2-ST_NBL-Newton_final.pdf
25. Hall, N. (2015). *Air Properties Definitions*. National Aeronautics and Space Administration. https://www.grc.nasa.gov/www/k-12/airplane/airprop.html
26. Carvil, P. A., Baptista, R. R., & Russomano, T. (2013). The human body in a microgravity environment: long term adaptations and countermeasures. *Journal of the Aeronautical Sciences*, 1. https://www.semanticscholar.org/paper/The-human-body-in-a-microgravity-environment%3A-long-Carvil-Baptista/5b557630f5f6cef6db903e69a078b1982584fcff
27. NASA. (2020). *Moon to Mars Overview*. National Aeronautics and Space Administration. https://www.nasa.gov/topics/moon-to-mars/overview/
28. *Astronauts Answer Student Questions*. (n.d.). National Aeronautics and Space Administration. https://www.nasa.gov/centers/johnson/pdf/569954main_astronaut%20_FAQ.pdf
29. Gruener, R., & Hoeger, G. (1990). Vector-free gravity disrupts synapse formation in cell culture. *American Journal of Physiology-Cell Physiology*, *258*(3), C489–C494. https://doi.org/10.1152/ajpcell.1990.258.3.c489

30. Anken, R., Ibsch, M., & Rahmann, H. (2002). Microgravity (STS-90 neurolab-mission) influences synapse formation in a vestibular nucleus of fish brain. *Advances in Space Research*, *30*(4), 843–847. https://doi.org/10.1016/s0273-1177(01)00643-3
31. Sieber, M., Hanke, W., & Kohn, F. P. M. (2014). Modification of Membrane Fluidity by Gravity. *Open Journal of Biophysics*, *04*(04), 105–111. https://doi.org/10.4236/ojbiphy.2014.44012
32. Goldermann, M., & Hanke, W. (2001). Ion channel are sensitive to gravity changes. *Microgravity Science and Technology*, *13*(1), 35–38. https://doi.org/10.1007/bf02873330
33. Hanke, W., Schulue, W. R., & Sattelle, D. B. (1994). Planar Lipid Bilayers: Methods and Applications (Biological Techniques Series). Academic Press.
34. Zanello, L., Aztiria, E., Antollini, S., & Barrantes, F. (1996). Nicotinic acetylcholine receptor channels are influenced by the physical state of their membrane environment. *Biophysical Journal*, *70*(5), 2155–2164. https://doi.org/10.1016/s0006-3495(96)79781-3
35. Schaffhauser, D. F., Andrini, O., Ghezzi, C., Forster, I. C., Franco-Obregón, A., Egli, M., & Dittrich, P. S. (2011). Microfluidic platform for electrophysiological studies on Xenopus laevis oocytes under varying gravity levels. *Lab on a Chip*, *11*(20), 3471. https://doi.org/10.1039/c0lc00729c
36. Ritzmann, R., Krause, A., Freyler, K., & Gollhofer, A. (2016). Gravity and Neuronal Adaptation. *Microgravity Science and Technology*, *29*(1–2), 9–18. https://doi.org/10.1007/s12217-016-9519-4
37. Kohn, F. P. M. (2012). High Throughput Fluorescent Screening of Membrane Potential and Intracellular Calcium Concentration Under Variable Gravity Conditions. *Microgravity Science and Technology*, *25*(2), 113–120. https://doi.org/10.1007/s12217-012-9331-8
38. Wiedemann, M., Kohn, F. P. M., Roesner, H., & Hanke, W. R. L. (2011). Self-organization and Pattern-formation in Neuronal Systems Under Conditions of Variable Gravity. *Nonlinear Physical Science*, 1–93. https://doi.org/10.1007/978-3-642-14472-1
39. Ganapathy, K., Kandarpa, K., & Schneider, V. (2019). Human health during space travel: An overview. *Neurology India*, *67*(8), 176. https://doi.org/10.4103/0028-3886.259123
40. Ganapathy, K., da Rosa, M., & Russomano, T. (2019). Neurological changes in outer space. *Neurology India*, 37–43. https://doi.org/10.4103/0028-3886.253647
41. Pani, G., Verslegers, M., Quintens, R., Samari, N., de Saint-Georges, L., van Oostveldt, P., Baatout, S., & Benotmane, M. A. (2016). Combined Exposure to Simulated Microgravity and Acute or Chronic Radiation Reduces Neuronal Network Integrity and Survival. *PLOS ONE*, *11*(5), e0155260. https://doi.org/10.1371/journal.pone.0155260
42. Wang, J., Han, C., Lu, Z., Ge, P., Cui, Y., Zhao, D., Yang, X., Wu, B., Qiang, L., Zhang, Y., Chai, Q., Lei, Z., Li, L., Hua Liu, C., & Zhang, L. (2020). Simulated microgravity suppresses MAPK pathway-mediated innate immune response to bacterial infection and induces gut microbiota dysbiosis. *The FASEB Journal*, *34*(11), 14631–14644. https://doi.org/10.1096/fj.202001428r
43. Sun, Y., Kuang, Y., & Zuo, Z. (2021). The Emerging Role of Macrophages in Immune System Dysfunction under Real and Simulated Microgravity Conditions. *International Journal of Molecular Sciences*, *22*(5), 2333. https://doi.org/10.3390/ijms22052333
44. Zayzafoon, M., Meyers, V. E., & McDonald, J. M. (2005). Microgravity: the immune response and bone. *Immunological Reviews*, *208*(1), 267–280. https://doi.org/10.1111/j.0105-2896.2005.00330.x
45. Chapes, S. K., Simske, S. J., Sonnenfeld, G., Miller, E. S., & Zimmerman, R. J. (1999). Effects of spaceflight and PEG-IL-2 on rat physiological and immunological responses. *Journal of Applied Physiology*, *86*(6), 2065–2076. https://doi.org/10.1152/jappl.1999.86.6.2065
46. Kimzey, S., Ritzmann, S., Mengel, C., & Fischer, C. (1975). Skylab experiment results: Hematology studies. *Acta Astronautica*, *2*(1–2), 141–154. https://doi.org/10.1016/0094-5765(75)90050-8
47. Román-Pintos, L. M., Villegas-Rivera, G., Rodríguez-Carrizalez, A. D., Miranda-Díaz, A. G., & Cardona-Muñoz, E. G. (2016). Diabetic Polyneuropathy in Type 2 Diabetes Mellitus: Inflammation, Oxidative Stress, and Mitochondrial Function. *Journal of Diabetes Research*, *2016*, 1–16. https://doi.org/10.1155/2016/3425617
48. Luo, L., Zhou, W. H., Cai, J. J., Feng, M., Zhou, M., Hu, S. P., Xu, J., & Ji, L. D. (2017). Gene Expression Profiling Identifies Downregulation of the Neurotrophin-MAPK Signaling Pathway in Female Diabetic Peripheral Neuropathy Patients. *Journal of Diabetes Research*, *2017*, 1–11. https://doi.org/10.1155/2017/8103904
49. Green, M. J., Aylott, J. W., Williams, P., Ghaemmaghami, A. M., & Williams, P. M. (2021). Immunity in Space: Prokaryote Adaptations and Immune Response in Microgravity. *Life*, *11*(2), 112. https://doi.org/10.3390/life11020112
50. Johnston, R. S., & Dietlein, L. F. (1977). *Biomedical Results from Skylab NASA SP-377* (Hardcover ed.). Scientific and Technical Information Office,.
51. Zhang, L. F. (2001). Vascular adaptation to microgravity: what have we learned? *Journal of Applied Physiology*, *91*(6), 2415–2430. https://doi.org/10.1152/jappl.2001.91.6.2415
52. Steczina, S., Tahimic, C. G. T., Pendleton, M., M'Saad, O., Lowe, M., Alwood, J. S., Halloran, B. P., Globus, R. K., & Schreurs, A. S. (2020). Dietary countermeasure mitigates simulated spaceflight-induced osteopenia in mice. *Scientific Reports*, *10*(1), 1. https://doi.org/10.1038/s41598-020-63404-x
53. P, C., & M, S. (2013). Effects of Dietary Soy Protein on Hematological and Tissue Oxidant/Anti- Oxidant Levels in Rats Exposed to Simulated Microgravity. *Journal of Physical Chemistry & Biophysics*, *03*(01), 1. https://doi.org/10.4172/2161-0398.1000113
54. Feinstein, M., Fernandez, S., & Sha'afi, R. (1975). Fluidity of natural membranes and phosphatidylserine and ganglioside dispersions. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, *413*(3), 354–370. https://doi.org/10.1016/0005-2736(75)90121-2

55. Fernández-Pérez, E. J., Sepúlveda, F. J., Peters, C., Bascuñán, D., Riffo-Lepe, N. O., González-Sanmiguel, J., Sánchez, S. A., Peoples, R. W., Vicente, B., & Aguayo, L. G. (2018). Effect of Cholesterol on Membrane Fluidity and Association of Aβ Oligomers and Subsequent Neuronal Damage: A Double-Edged Sword. *Frontiers in Aging Neuroscience*, *10*, 1. https://doi.org/10.3389/fnagi.2018.00226
56. Jende, J. M. E., Groener, J. B., Rother, C., Kender, Z., Hahn, A., Hilgenfeld, T., Juerchott, A., Preisner, F., Heiland, S., Kopf, S., Pham, M., Nawroth, P., Bendszus, M., & Kurz, F. T. (2019). Association of Serum Cholesterol Levels With Peripheral Nerve Damage in Patients With Type 2 Diabetes. *JAMA Network Open*, *2*(5), e194798. https://doi.org/10.1001/jamanetworkopen.2019.4798
57. *SH-SY5Y ATCC ® CRL-2266™ Homo sapiens bone marrow neuroblast*. (n.d.). ATCC. https://www.lgcstandards-atcc.org:443/products/all/CRL-2266.aspx?geo_country=nl#culturemethod
58. Shipley, M. M., Mangold, C. A., & Szpara, M. L. (2016). Differentiation of the SH-SY5Y Human Neuroblastoma Cell Line. *Journal of Visualized Experiments*, *108*, 1. https://doi.org/10.3791/53193
59. Kovalevich, J., & Langford, D. (2013). Considerations for the Use of SH-SY5Y Neuroblastoma Cells in Neurobiology. *Neuronal Cell Culture*, 9–21. https://doi.org/10.1007/978-1-62703-640-5_2
60. Han, A. M., Heo, H., & Kwon, Y. K. (2012). Berberine Promotes Axonal Regeneration in Injured Nerves of the Peripheral Nervous System. *Journal of Medicinal Food*, *15*(4), 413–417. https://doi.org/10.1089/jmf.2011.2029
61. Jones, I., Yelhekar, T. D., Wiberg, R., Kingham, P. J., Johansson, S., Wiberg, M., & Carlsson, L. (2018). Development and validation of an in vitro model system to study peripheral sensory neuron development and injury. *Scientific Reports*, *8*(1), 1. https://doi.org/10.1038/s41598-018-34280-3
62. *Fluorescence Polarization (FP)—Note 1.4 | Thermo Fisher Scientific - NL*. (n.d.). ThermoFisher Scientific. https://www.thermofisher.com/nl/en/home/references/molecular-probes-the-handbook/technical-notes-and-product-highlights/fluorescence-polarization-fp.html
63. Chazotte, B. (2011). Labeling the Plasma Membrane with TMA-DPH. *Cold Spring Harbor Protocols*, *2011*(5), pdb.prot5622. https://doi.org/10.1101/pdb.prot5622
64. Gruener, R., & Hoeger, G. (1990b). Vector-free gravity disrupts synapse formation in cell culture. *American Journal of Physiology-Cell Physiology*, *258*(3), C489–C494. https://doi.org/10.1152/ajpcell.1990.258.3.c489
65. Pahan, K. (2006). Lipid-lowering drugs. *Cellular and Molecular Life Sciences*, *63*(10), 1165–1178. https://doi.org/10.1007/s00018-005-5406-7
66. Baker, R. R. (1979). The isolation and lipid composition of subcellular fractions derived from neuronal perikarya isolated in bulk from rabbit cerebral cortex. *Brain Research*, *169*(1), 65–81. https://doi.org/10.1016/0006-8993(79)90374-3
67. Salè, F., Marchesini, S., Fishman, P., & Berra, B. (1984). A sensitive enzymatic assay for determination of cholesterol in lipid extracts. *Analytical Biochemistry*, *142*(2), 347–350. https://doi.org/10.1016/0003-2697(84)90475-5
68. Carr, T. P. (2010). Tissue Lipid Analysis with Enzymatic Reagents. *Clinical Chemistry*, *56*(6), 1026–1027. https://doi.org/10.1373/clinchem.2009.139360
69. Lee, A. G. (2004). How lipids affect the activities of integral membrane proteins. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, *1666*(1–2), 62–87. https://doi.org/10.1016/j.bbamem.2004.05.012
70. Heimburg, T. (2007). *Thermal Biophysics of Membranes* (1st ed.). Wiley-VCH.
71. Li, W., Wang, L., Shen, C., Xu, T., Chu, Y., & Hu, C. (2019). Radiation therapy-induced reactive oxygen species specifically eliminates $CD19^+$ IgA^+ B cells in nasopharyngeal carcinoma. *Cancer Management and Research*, *Volume 11*, 6299–6309. https://doi.org/10.2147/cmar.s202375
72. Johansen, J., Harris, A. K., Rychly, D. J., & Ergul, A. (2005). Oxidative stress and the use of antioxidants in diabetes: Linking basic science to clinical practice. *Cardiovascular Diabetology*, *4*(1), 5. https://doi.org/10.1186/1475-2840-4-5
73. *Prevention and Management of Radiation Side Effects*. (2013, February 1). Virginia Cancer Institute. https://www.vacancer.com/diagnosis-and-treatment/radiation-therapy/prevention-and-management-of-radiation-side-effects/
74. Kasznicki, J. (2014). State of the art papers Advances in the diagnosis and management of diabetic distal symmetric polyneuropathy. *Archives of Medical Science*, *2*, 345–354. https://doi.org/10.5114/aoms.2014.42588
75. Zotova, E. V., Chistiakov, D. A., Savost'ianov, K. V., Bursa, T. R., Galeev, I. V., Strokov, I. A., & Nosikov, V. V. (2016). Association of the SOD2 Ala(-9)Val and SOD3 Arg213Gly polymorphisms with diabetic polyneuropathy in patients with diabetes mellitus type 1. *Molecular Biology*, 1. https://pubmed.ncbi.nlm.nih.gov/12815947/
76. Slenzka, K. (2003). Neuroplasticity changes during space flight. *Advances in Space Research*, *31*(6), 1595–1604. https://doi.org/10.1016/s0273-1177(03)00011-5
77. Uva, B., Masini, M., Sturla, M., Bruzzone, F., Giuliani, M., Tagliafierro, G., & Strollo, F. (2010). Microgravity-induced apoptosis in cultured glial cells. *European Journal of Histochemistry*, *46*(3), 209. https://doi.org/10.4081/1681
78. Mao, X. W., Nishiyama, N. C., Pecaut, M. J., Campbell-Beachler, M., Gifford, P., Haynes, K. E., Becronis, C., & Gridley, D. S. (2016). Simulated Microgravity and Low-Dose/Low-Dose-Rate Radiation Induces Oxidative Damage in the Mouse Brain. *Radiation Research*, *185*(6), 647–657. https://doi.org/10.1667/rr14267.1
79. Katerji, M., Filippova, M., & Duerksen-Hughes, P. (2019). Approaches and Methods to Measure Oxidative Stress in Clinical Samples: Research Applications in the Cancer Field. *Oxidative Medicine and Cellular Longevity*, *2019*, 1–29. https://doi.org/10.1155/2019/1279250

80. Levine, R. L., Garland, D., Oliver, C. N., Amici, A., Climent, I., Lenz, A. G., Ahn, B. W., Shaltiel, S., & Stadtman, E. R. (1990). [49] Determination of carbonyl content in oxidatively modified proteins. *Oxygen Radicals in Biological Systems Part B: Oxygen Radicals and Antioxidants*, 464–478. https://doi.org/10.1016/0076-6879(90)86141-h
81. Mesquita, C. S., Oliveira, R., Bento, F., Geraldo, D., Rodrigues, J. V., & Marcos, J. C. (2014). Simplified 2,4-dinitrophenylhydrazine spectrophotometric assay for quantification of carbonyls in oxidized proteins. *Analytical Biochemistry*, *458*, 69–71. https://doi.org/10.1016/j.ab.2014.04.034
82. Halliwell, B., & Gutteridge, J. M. (1986). Oxygen free radicals and iron in relation to biology and medicine: Some problems and concepts. *Archives of Biochemistry and Biophysics*, *246*(2), 501–514. https://doi.org/10.1016/0003-9861(86)90305-x
83. Yagi, K. (1987). Lipid peroxides and human diseases. *Chemistry and Physics of Lipids*, *45*(2–4), 337–351. https://doi.org/10.1016/0009-3084(87)90071-5
84. Serum antioxidant capacity and oxidative injury to pulmonary DNA in never-smokers with primary lung cancer. (2012). *AntiCancer Research*, 1. https://pubmed.ncbi.nlm.nih.gov/22399633/