ORIGINAL RESEARCH

Effects of Long-Term Simulated Weightlessness on Retinal Microcirculation and Visual Electrophysiology

Yan Huo, MD; Lancao Hao, MM; Minghao Sun, MM; Wei Dai, MD; Guanghan Kan, MM; Zhou Yang, MD; Siquan Zhu, MD

ABSTRACT

Objective • To investigate the mechanisms of ocular injuries in astronauts due to gravity deficit by examining changes in retinal microcirculation and visual electrophysiology in macaques subjected to simulated weightlessness.

Methods • The head-down recumbency of macaques was used to simulate the movement of blood to the side of the head that occurs without microgravity. Head-down recumbency was performed with the head tilted downwards at a recommended angle of 10°. The macaques in the control group were similarly tethered to the rope but could be held in a normal position. The whole experiment lasted for 6 weeks and retinal microcirculation and visual electrophysiology information was collected at weeks 0, 3 and 6.

Results • The retinal microcirculation of macaques was affected by 3 weeks of weightlessness. This includes morphological changes, such as dilation and tortuosity of the retinal microvasculature in macaques at day 21. OCT and OCTA results showed an increase in retinal and choroidal thickness and a significant decrease in vessel length density within 6×6 mm of the macula. Sustained simulated weightlessness (42 days) significantly exacerbated retina-related damage. This was evidenced by a significant decrease in the perfusion density of microcirculatory vessels, such as the macular 3×3 mm mesial vessels and the macular 6*6 mm central and medial vessels. The FAZ density in the macula 3×3 mm area began to increase. Retinal oxygen saturation testing showed a slight increase in arterial oxygen saturation. Simultaneous changes in visual electrophysiology occurred, including a significant decrease in a- and b-wave amplitudes on the dark-vision electroretinogram and a significant decrease in the amplitude of the bright-vision negative wave response. The peak timing of the flash visual evoked potential component P1 was significantly delayed compared to its baseline and time-matched control.

Conclusions • Sustained simulated weightlessness (42 days) significantly exacerbated retina-related damage, with both reduced macular blood supply and increased FAZ density suggesting the development of retinal ischemic changes, which disrupt visual electrophysiology. Retinal damage in human astronauts under long-term outer space conditions may be prevented by intervening in ischemic changes in the retina during the early stages of weightlessness. (*Altern Ther Health Med.* [E-pub ahead of print.])

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INTRODUCTION

Advances in space exploration technology have opened the door to understanding the mysterious realm of outer space. However, physiological adaptations and even pathological damage during space flight or simulated microgravity involve multi-system responses in the human body, including cardiovascular, metabolic and musculoskeletal. Spaceflight-associated neuro-ocular syndrome (SANS) is one of the key manifestations, first described in 2011.[1,2] Prolonged space activity can lead to optic disc edema, choroidal congestion, hyperopic deviation and ocular equalization.[3] After returning to the earth environment, it takes about 1 year for these structural changes to fully recover.[4] Even astronauts on short-term (<30 days) missions developed ocular defects, with healthy individuals showing changes in intraocular pressure and

ocular blood vessels within 20 seconds of exposure to microgravity.[5] Considering the current increase in the frequency and duration of space missions, it is urgent to address the potential health risks associated with SANS.

Multiple factors are involved in the ocular alterations brought about by this microgravity, including head fluid shifts, increased cerebrospinal fluid pressure, reduced venous compliance and altered cerebrospinal fluid dynamics.[6] Cephalic fluid shifts are the main mechanism triggering neurophysiological changes under microgravity conditions, referring to the redistribution of blood and body fluids to the upper body under microgravity, resulting in venous stasis in the head and neck, affecting the blood-brain barrier, intraocular and intracranial pressure, and persistent microgravity can even lead to changes in ocular structures and the optic nerve.[7] Altered retinal structure and function may affect astronaut vision, impacting mission accomplishment and long-term quality of life. The health risks posed by this microgravity situation urgently need to be addressed. SANS can significantly affect space exploration and missions. The syndrome's effects on astronauts' visual acuity, intracranial pressure, and overall neurological health can impact their performance during extended space missions. Understanding and mitigating the risks associated with SANS is crucial for ensuring the safety and well-being of astronauts during prolonged space travel. Moreover, addressing the impact of SANS is essential for planning future long-duration space missions, including potential missions to Mars or other deep-space destinations. The potential mechanisms of SANS during and after spaceflight are the subject of ongoing research, but the exact pathophysiology remains unclear.

Various imaging techniques have been used to assess ocular changes in the microgravity environment, including optical coherence tomography (OCT), ultrasound, etc. OCT provides non-contact, non-invasive tomographic imaging of microscopic structures in living ocular tissues, including the retina, retinal nerve fiber layer, macula and optic disc.[8] Optical coherence tomography angiography (OCTA) is a new non-invasive fundus imaging technique that allows high-resolution identification of retinal choroidal blood flow information and imaging of the retinal choroidal microvascular circulation in living tissue.[9] Electroretinography (ERG) is an important test to assess the functional integrity of the retina and may indicate abnormalities in blood circulation within the inner layers of the retina.[10] All these techniques are widely used to diagnose fundus diseases.

Cardiovascular, metabolic and musculoskeletal responses during spaceflight or simulated microgravity have been well studied, but alterations in the central and peripheral nervous system using experimental methods of clinical neurophysiology are still poorly studied. And with advances in space technology and the progressively longer duration of exposure to the spaceflight environment, it is important to explore the effects of long-term such microgravity adaptation on the eye and its mechanisms. In this paper, we investigate the specific mechanisms underlying the physiopathological changes in the eye brought about by gravity deprivation by comparing changes in retinal OCT, OTCA, visual electrophysiology and ocular oxygen saturation in macaques before and after simulated weightlessness, to provide a basis for preventing and treating the occurrence of SANS.

MATERIALS AND METHODS

Animals

Macaques (n=9), weighing 8.45±0.95 kg and aged 5-10 years old, were purchased from Beijing Zhongke Lingrui Biotechnology Company Limited. All experimental animals were housed in specific-pathogen-free (SPF) animal rooms with temperatures maintained between 20°C and 24°C and relative humidity levels between 50% and 70%. Light and dark lighting alternated for 12 hours, and normal ventilation was maintained. The study began with one week of acclimatization to feeding. In this study, the macaques were divided into a control group (0 weeks), a short-term simulated weight loss group (3 weeks) and a long-term simulated weight loss group (6 weeks) by collecting various ocular parameters at different time points of simulated weightlessness. Our experiments adhered strictly to the American Association for Research in Vision and Ophthalmology (ARVO) statement regarding the use of animals in research. The study protocol was approved by the Experimental Animal Ethics Committee of the China Astronaut Research and Training Center (Beijing, China, Approval No. ACC-IACUC-2019-002).

Suspension simulation weightlessness model

According to the previously described method,[11] head-down recumbency was used to construct this study's simulated weightlessness animal model. Each macaque was individually placed on a specially designed device, secured by abdominal straps, with the head tilted downward at an angle of -10° to simulate the weightlessness effect (Figure 1). This angle avoids unnecessary stress and does not interfere with feeding and drinking. The limbs of the macaques are free to move. Ocular retinal microcirculation and visual electrophysiology data were collected at 0, 3 and 6 weeks of the experiment.

Figure 1. A schematic diagram of a simulated weightlessness apparatus for macaques. The angle (α) between the longitudinal axis of the macaque's body and the horizontal plane is 10°, and the limbs can move freely and can eat and drink freely.

Visual electrophysiology

Macaques were fasted from food and drink for 6 h and anesthetized by intramuscular injection of ketamine hydrochloride and xylazine hydrochloride (1:1) 0.1 mL/kg in the buttocks. All testing parameters were set with reference to the latest standards of the International Society for Electrophysiology (MONPACK3, METROVISION, France), anesthesia was performed in the same manner as before and the electrode site was disinfected with 70% alcohol. The recording electrode was placed 2-3 cm above the occipital ridge, the reference electrode was placed in the middle of the forehead, the ground electrode was placed in the earlobe, the non-test eye was covered, and the lid was opened with a child's lid opener. The PVEP was recorded in the order of right then left eye. The reference electrode was connected to the outer canthus of both eyes, and the ground electrode was connected to the middle of the forehead. The eyelids were opened with a pediatric lid opener. The eyes were surface anesthetized with oxybuprocaine hydrochloride and then placed in both eyes with "bright vision" drops (sodium hydroxymethylcellulose, Allergan Pharmaceuticals, Ireland) on the corneal contact electrodes, with complete contact with the cornea, and PERG was recorded. The FVEP electrode position was the same as PVEP, and the macaque was fixed in front of the stimulator and recorded in one eye. After recording the FVEP, the pupils were dilated with Medrol and dark-adapted for 30 min in a dark room environment. The dark-adapted 0.01 ERG, dark-adapted 3.0 ERG, and bright-adapted 3.0 ERG and bright-adapted 30 Hz oscillation potentials were recorded sequentially after 10 min of bright adaptation.

Retinal arteriovenous oximetry

After the macaque was anesthetized (in the same manner as before), the eye was fully dilated with compound tropicamide drops. The eye was then surface anesthetized with oxybuprocaine hydrochloride drops, and the eye position was adjusted to the appropriate distance from the lens for optic disc positioning. A retinal arteriovenous oximeter (Sichuan Hesheng Vision Retinal Arteriovenous Oximeter) was used to take fundus oximetry images.

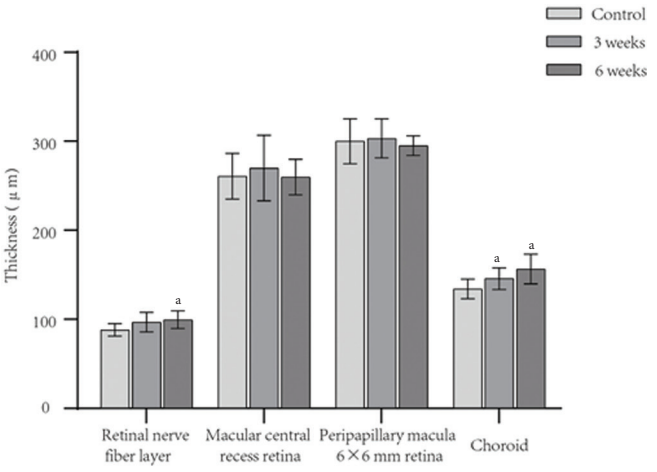
Retinal optical coherence tomography and Optical coherence tomography angiography

After completion of photography, an optical coherence tomography scanner (Carl Zeiss cirrus HD-OCT 5000) was used to scan the fundus of the macaques for optical coherence tomography and optical coherence tomography angiography to detect changes in the macula, retina and ocular vasculature. Two stationary physicians performed all examinations, one to fix the macaque collaboration and one to operate.

Statistical analysis

All data are expressed as mean ± standard deviation. Statistical analysis was performed using Statistic Package for Social Science (SPSS) 26.0 (IBM, Armonk, NY, USA) and plotted using GraphPad Prism 7 (La Jolla, CA, USA). The measurement data conformed to a normal distribution and were compared between two groups using the independent samples t test. A non-parametric rank sum test was used if the data did not follow a normal distribution. $P < .05$ was considered statistically significant.

Figure 2. Effects of long-term simulated weightlessness on OCT in rhesus macaques. The horizontal coordinates represent the four sites examined, namely the retinal nerve fiber layer, the macular central recess retina, the peri-macular 6×6 mm retina, and the choroid. The vertical coordinate represent the thickness (μm). Light gray represents the control group, brown gray represents the 3-week simulated weightlessness group, and dark gray represents the 6-week simulated weightlessness group.



[a]indicates a statistically significant difference compared to the control group ($P < .05$).

RESULTS

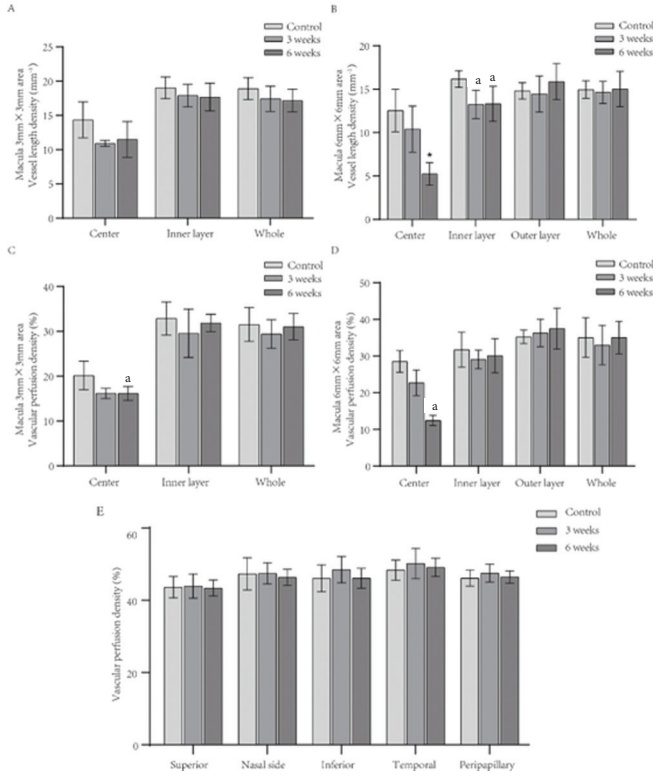
Effects of long-term simulated weightlessness on OCT

Our study examined the effects of different durations of simulated microgravity treatment on retinal nerve fiber layer thickness, macular central recess retinal thickness, peripapillary macula 6×6 mm retinal thickness, and choroidal thickness in macaques using OCT. The results showed that 3 weeks of simulated weightlessness increased choroidal thickness in macaques. In comparison, 6 weeks of simulated weightlessness treatment increased both retinal nerve fiber layer thickness and choroidal thickness in macaques ($P < .05$) (Figure 2). No significant changes were seen in macula central recess retinal thickness and peri-macula 6×6 mm retinal thickness ($P > .05$).

Effect of long-term simulated weightlessness on OCTA

We next examined the effects of different durations of simulated microgravity treatment on macular blood flow density and peri-optic disc perfusion density in macaques. Simulated weightlessness reduced the vessel length density in the macula 6 mm × 6 mm region of macaques ($P < .05$), but no significant changes were seen in the macula 3 mm × 3 mm region ($P > .05$) (Figure 3A-B). Among them, the 3-week simulated weightlessness treatment only reduced the vessel length density in the inner layer of macula 6 mm × 6 mm

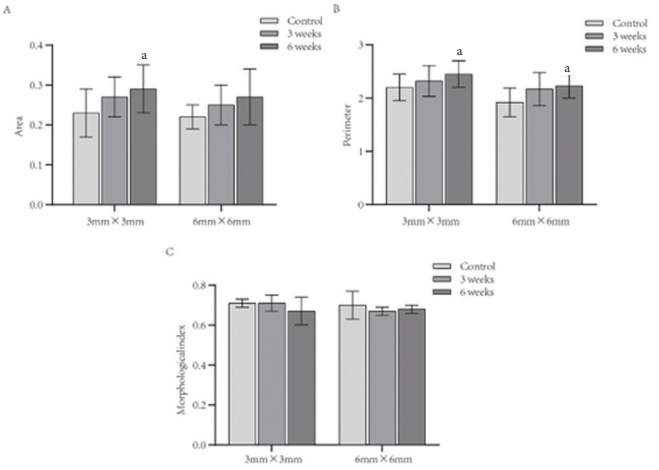
Figure 3. Effect of long-term simulated weightlessness on OCTA in rhesus macaques. A: Vascular length density in the macula 3mm×3mm area in the control group and simulated weightlessness treatment groups (3 weeks and 6 weeks). B: Vascular length density in the macula 6mm×6mm area in the control group and simulated weightlessness treatment groups (3 weeks and 6 weeks). C: Vascular perfusion density in the 3mm×3mm area of the macula in the control group and simulated weightlessness treatment groups (3 weeks and 6 weeks). D: Vascular perfusion density in the macula 6mm×6mm area in the control group and simulated weightlessness treatment groups (3 weeks and 6 weeks). E: Vascular perfusion density of the peri-optic disc in the control group and simulated weightlessness treatment groups (3 weeks and 6 weeks). Light gray represents the control group, brown gray represents the 3-week simulated weightlessness group, and dark gray represents the 6-week simulated weightlessness group.



[a]indicates a statistically significant difference compared to the control group ($P < .05$).

region in macaques. In comparison, the 6-week simulated weightlessness treatment reduced the vessel length density in both the central and inner layer of macula 6 mm × 6 mm region ($P < .05$). In addition, 6 weeks of simulated weightlessness treatment decreased the vascular perfusion density in the central macula of macaques ($P < .05$), both in the macula 6 mm × 6 mm area and in the macula 3 mm × 3 mm area, but 3 weeks of simulated weightlessness treatment did not affect macular vascular perfusion density ($P > .05$) (Figure 3C-D). Peripapillary perfusion density did not change significantly after both 3 and 6 weeks of simulated weightlessness treatment ($P > .05$) (Figure 3E).

Figure 4. Effect of long-term simulated weightlessness on macular FAZ density in macula area of macaques. A: FAZ area in the macular 3 mm × 3 mm and 6 mm × 6 mm ranges in the control group and simulated weightlessness treatment groups (3 weeks and 6 weeks). B: FAZ perimeter of the macular 3 mm × 3 mm and 6 mm × 6 mm ranges in the control group and simulated weightlessness treatment groups (3 weeks and 6 weeks). C: FAZ morphological indices of the macular 3 mm × 3 mm and 6 mm × 6 mm ranges in the control group and simulated weightlessness treatment groups (3 weeks and 6 weeks). Light gray represents the control group, brown gray represents the 3-week simulated weightlessness group, and dark gray represents the 6-week simulated weightlessness group.



[a]indicates a statistically significant difference compared to the control group ($P < .05$).

Effects of long-term simulated weightlessness on macular FAZ density

The macular fovea is the most acutely visualized area, surrounded by capillaries, with a central avascular zone known as the foveal avascular zone (FAZ), and damage to this area can result in significant loss of fine vision. The density variation of the FAZ, including area, perimeter and morphological index, can reflect the extent of retinal ischemic lesions. We examined the effects of different simulated weightlessness durations on the density of the macula FAZ of macaques. The 6-week simulated weightlessness treatment significantly increased the FAZ area in the macula 3 mm × 3 mm area ($P < .05$). In comparison, the 3-week simulated weightlessness treatment did not affect the FAZ area either in the macula 6 mm × 6 mm area or in the macula 3 mm × 3 mm ($P > .05$) (Figure 4A). The 6-week simulated weightlessness treatment also significantly increased the FAZ perimeter in the macula 3 mm × 3 mm and 6 mm × 6 mm areas($P<0.05$), whereas the 3-week simulated weightlessness treatment did not affect it ($P > .05$) (Figure 4B). Neither the 3-week nor the 6-week simulated weightlessness treatment had any effect on the FAZ morphological index ($P > .05$) (Figure 4C).

Effects of long-term simulated weightlessness on visual electrophysiology

Examination of visual electrophysiology is a non-invasive visual function test that records the bioelectrical activity of the visual system during the vision formation to aid in the diagnosis of disease, mainly consisting of electroretinogram (ERG) and visual evoked potential (VEP). The results showed that 3 weeks of simulated weightlessness treatment had no significant effect on the visual electrophysiology of macaques ($P > .05$). The 6-week simulated weightlessness treatment significantly reduced the amplitudes of 0.01 ERG b-wave, 3.0 ERG a-wave, and 3.0 ERG b-wave under Dark adaptation, as well as the Amplitude of the photopic negative response (PhNR) ($P < .05$). Furthermore, 6 weeks of simulated weightlessness treatment also affected the formation of VEPs, with the peak time of flash visual evoked potential component P1 delayed compared to the control group (Table 1).

Table 1. Visual electrophysiological results of macaques in the experimental and control groups

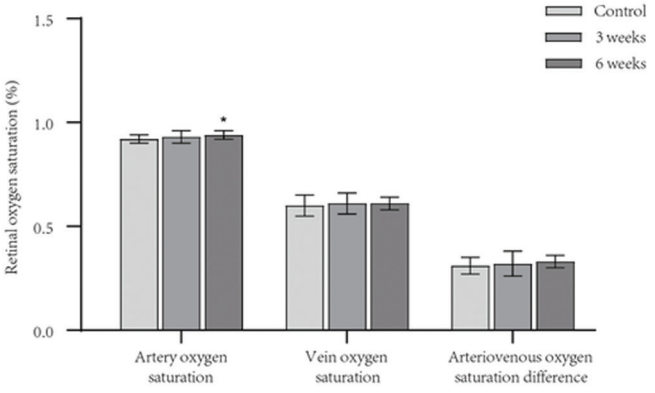
Items	Control	Simulated weightlessness for 3 weeks	Simulated weightlessness for 6 weeks
Dark adaptation 0.01ERG b-wave Peak time (ms)	70.04±6.28	70.32±4.79	69.16±5.93
Dark adaptation 0.01ERG b-wave Amplitude (uv)	73.69±7.83	52.74±4.62	22.38±1.02[a]
Dark adaptation 3.0ERG a-wave Peak time (ms)	17.70±1.15	17.66±1.40	17.02±1.57
Dark adaptation 3.0ERG a-wave Amplitude (uv)	131.75±19.46	135.20±21.92	89.89±7.31[a]
Dark adaptation 3.0ERG b-wave Peak time (ms)	38.45±3.85	38.77±2.06	39.49±3.26
Dark adaptation 3.0ERG b-wave Amplitude (uv)	239.26±15.99	225.88±18.68	159.78±16.39[a]
Bright adaptation 3.0ERG a-wave Peak time (ms)	14.57±0.73	14.24±0.96	14.51±0.51
Bright adaptation 3.0ERG a-wave Amplitude (uv)	20.32±2.47	21.62±3.11	19.17±3.41
Bright adaptation 3.0ERG b-wave Peak time (ms)	33.13±5.15	30.733±0.68	31.19±1.57
Bright adaptation 3.0ERG b-wave Amplitude (uv)	73.17±10.44	74.04±10.94	69.54±9.46
PHNR Amplitude (uv)	72.91±7.13	72.97±8.74	63.51±9.26[a]
VEP N1 wave Peak time (ms)	51.87±4.57	51.33±6.93	55.52±11.96
VEP N1 wave Amplitude (uv)	27.57±15.29	24.57±12.97	16.75±6.62
VEP P1 wave Peak time (ms)	88.03±7.10	93.43±1.95	103.89±12.53[a]
VEP P1 wave Amplitude (uv)	41.59±15.01	41.30±15.51	35.30±11.66

[a]indicates a statistically significant difference compared to the control group ($P < .05$).

Effects of long-term simulated weightlessness on retinal oxygen saturation

The retina is a highly differentiated neural tissue and is sensitive to hypoxia. Retinal oximetry provides direct and reliable information on retinal oxidative metabolism, reflects ocular and systemic microcirculatory status, and is widely used for ocular disease assessment. Therefore, we investigated the effect of long-term simulated weightlessness on retinal oxygen saturation in macaques. Our results showed that 3 weeks simulated weightlessness treatment had no significant effect on retinal oxygen saturation in rhesus macaques ($P > .05$). The 6-week simulated weightlessness treatment increased retinal arterial oxygen saturation ($P < .05$), but did not affect venous oxygen saturation or the arteriovenous oxygen saturation difference (Figure 5).

Figure 5. The effect of long-term weightlessness on retinal oxygen saturation in rhesus macaques. The horizontal coordinates represent the three parameters tested, which are retinal artery oxygen saturation, retinal vein oxygen saturation and retinal arteriovenous oxygen saturation difference. The vertical coordinate are the specific values of retinal oxygen saturation. Light gray represents the control group, brown gray represents the 3-week simulated loss group, and dark gray represents the 6-week simulated loss group.



[a]indicates a statistically significant difference compared to the control group ($P < .05$).

DISCUSSION

Astronaut ophthalmopathy is a common adverse reaction observed in astronauts upon their return to Earth, and the mechanism is not yet fully understood. Our study used a macaque model to construct a gravity-deficient simulated environment. It assessed the effects of the simulated weightlessness environment on the eye using techniques such as OCT and OCTA. It was found that a 21-day simulated weightlessness experiment only altered the choroidal thickness and the vascular length density of the macula within the 6×6 mm zone in macaques. Simulated weightlessness for 42 days further exacerbated these changes but also significantly increased retinal nerve fiber layer thickness, decreased macular vascular perfusion density and significantly increased macular FAZ density (both 3×3 and 6×6 zones), disrupted visual electrophysiology and increased retinal arterial oxygen saturation.

Prolonged simulated weightlessness increases choroidal thickness. Our findings are supported by the work of Li et al.[12], who constructed a mouse model of simulated weightlessness by tail suspension for up to 12 weeks and found that the choroidal thickness increased gradually with increasing duration of simulated weightlessness. This structural alteration may affect microgravity conditions' ultrastructure and apoptosis of choroidal vascular endothelial cells (CVECs). Zhao et al.[13] used the Rotating Cell Culture System to simulate microgravity conditions. It exposed human CVECs to microgravity for 3 days and found that CVECs exhibited shrunken cell bodies, chromatin condensation and margination, mitochondrial vacuolization and apoptotic vesicles and that this damage may be associated with the Bcl-2 apoptotic pathway and the PI3K/AKT pathway. Hearon et al.[14] found that nightly lower body negative pressure reduced the increase in choroidal area and volume by restoring fluid transfer from the foot. This suggests that lower body negative pressure during sleep may be responsible for ocular remodeling during long-term space missions.

Prolonged simulated weightlessness significantly reduces macular blood flow density and increases FAZ density in the macula. Dai et al.[15] investigated altered retinal microcirculation in tail-suspended mice after simulated microgravity and observed dilated and tortuous retinal microvessels with relatively long fluorescence retention times in 15-day tail-suspended mice, but no significant change in the mean diameter of the major retinal vessels. Our results show that 6 weeks of simulated weightlessness causes a significant decrease in macular blood flow density, but no significant change in peri-optic disc perfusion density is seen. This alteration may be due to increased endothelial cell apoptosis in the presence of microgravity.[16] The microgravity environment may also cause such alterations by affecting endothelial cell metabolism, including increased mitochondrial autophagy, reduced mitochondrial content, oxygen consumption and maximum respiratory capacity.[17] The FAZ is the central visual avascular zone in the central macular recess and is essential for maintaining fine vision. When ischaemic changes to the retina occur, the retinal capillary network appears as an avascular zone, and the density of the capillary network as well as the morphology and size of the FAZ are altered. Significant increases in FAZ area have been found in ischaemic retinal diseases such as glaucoma,[18] diabetic eye disease[19] and retinal vein occlusion.[20] Our study explored for the first time the alteration of macular FAZ density under microgravity conditions. It showed that a 6-week simulated weightlessness experiment increased macular FAZ zone density, which was not observed in the 3-week group. To some extent, this explains the formation of retinal ischaemia in microgravity and the cause of visual acuity loss under microgravity conditions.

ERG is an important test to assess the functional integrity of the retina, and prolonged simulated weightlessness can impair visual electrophysiology. Our study showed that 6 weeks of simulated microgravity significantly reduced a- and b-wave amplitudes under dark adaptation and significantly inflamed the timing of the P1 wave of the visual evoked potential. The results of Dai et al.[15] are similar to our findings, suggesting to some extent that such ERG-specific alterations may reflect abnormal retinal microcirculation.

Retinal oximetry is a non-invasive imaging technique that assesses retinal oxygenation and blood flow in vivo to characterise retinal ischaemia.[21] Our study examines for the first time the alteration of retinal oxygen saturation in macaques under different periods of simulated weightlessness. The results showed that 3 weeks of simulated weightlessness treatment had no effect on retinal oxygen saturation, but when the time was extended to 6 weeks, retinal arterial oxygen saturation increased. Jugular venous stasis and increased intracranial pressure caused by a persistent head-down position may lead to ischaemic perfusion damage to the eye.[22] Microgravity can also up-regulate the expression of hypoxia-inducible factor-1α and endothelial nitric oxide synthase, promote the release of NO, and activate the FAK/Erk1/2-MAPK signaling pathway to promote the proliferation and migration of vascular endothelial cells, leading to angiogenesis.[23] Prolonged ischaemic and hypoxic damage can lead to the induction of ocular angiogenesis, which leads to increased arterial oxygen saturation.[24,25] the increase in measured retinal vascular oxygen saturation may also be a technical artifact associated with a reduction in vessel diameter.[26] But more mechanistic studies are needed to validate our findings.

Mechanistically, various factors contribute to the development of SANS, including lateral cephalad fluid shifts, increased intracranial pressure, venous/lymphatic stasis, inflammation, metabolism, axoplasmic stasis and radiation exposure.[7,27] Li et al.[12] explored the mechanisms of ocular damage caused by microgravity at the cellular level and found that retinal ganglion cells survival was significantly reduced, optic nerve oligodendrocytes were reduced, and apoptotic factors and microglia-mediated inflammation-related factors were detected in both the retina and optic nerve. Kothiyal et al.[28] conducted a multi-omics longitudinal study under chronic low-dose radiation and simulated microgravity conditions, and sequencing of the mouse epigenome and transcriptome retinal maps yielded a total of 4178 differentially methylated sites or regions, and 457 differentially expressed genes. Autophagy and mitochondrial dysfunction are also involved in this pathogenic process.

There are also some limitations to this study. The first is that the number of samples was limited with 9 macaques and the number of animals for animal experiments could be appropriately increased. Secondly, environmental effects are more complex on the international space station or during spaceflight than in simulated microgravity on Earth. In our study (shown in Figure 1), although a head-down tilt model can simulate blood flow under weightlessness, it is unable to simulate the body's weightless floating under actual microgravity. In addition, there is a radiation environment in space consisting of highly charged and energetic particles, and this radiation environment is also involved in the development of retinal diseases, but the effects of these radiation factors were not modelled in our study.[29]

Animal models simulating blood transfer to the head under microgravity conditions show that 6 weeks of gravity deprivation impairs retinal microcirculation, macular FAZ density and visual electrophysiology in macaques, but 3 weeks has relatively little effect. This microgravity-induced ocular damage becomes more pronounced with time, and longer experiments are needed to monitor this alteration.

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DISCLOSURES

The authors have no conflicts of interest to disclose.

AUTHORS' CONTRIBUTIONS

All authors contributed to the conception and design of the study. Huo Yan and Zhu Siquan contributed to the design of the work and implementation of the experiments; Huo Yan was primarily responsible for writing the manuscript; Huo Yan and Hao Lancao contributed to the acquisition, analysis and interpretation of the data; Zhu Siquan critically revised the writing of the paper; Sun Minghao, Dai Wei, Kan Guanghan and Yang Zhou participated in the implementation of the experiments. All authors read and approved the final draft.

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This study did not receive any funding in any form.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The Animal Ethics Committee of Beijing Anzhen Hospital, Capital Medical University approved this study.

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