# Hypergravity Effects on the Retina and Intraocular Pressure in Mice

Young Jun Kim; Jae Seung Chung; Tae Young Jang; Young Hyo Kim; Hee Seung Chin

**BACKGROUND:** We investigated the effect of exposure to +10.0 G for 4 h on the intraocular pressure and the retina of mice.

**METHODS:** We exposed 10 mice to +10.0 $G_z$ for 4 h by using a centrifugal acceleration test facility for animals. Intraocular changes were compared before and after hypergravity exposure. The eyeballs of the mice were enucleated after measuring the intraocular pressure. Tissue slides of the retina were prepared with hematoxylin and eosin (H&E) for histological examination and immunohistochemical analyses for vascular endothelial growth factor-A (VEGF-A), VEGF receptor 1 (VEGF-R1), VEGF-R2, glial fibrillary acidic protein (GFAP), and glutamine synthetase (GS).

**RESULTS:** The average intraocular pressure was 7.7 $\pm$ 0.86 mmHg before the hypergravity exposure and 6.65 $\pm$ 0.67 mmHg after the exposure. No histological difference was observed between the retinas in the two groups. The levels of VEGF-A, VEGF-R1, VEGF-R2, GFAP, and GS as assessed by immunohistochemistry were increased in the group exposed to hypergravity compared to the control group.

**DISCUSSION:** Repeated exposure to a high level of hypergravity could cause elevation of intraocular pressure and hypoxic damage to the retina.

**KEYWORDS:** hypergravity, retina, vascular endothelial growth factor, intraocular pressure, glial fibrillary acidic protein, glutamine synthetase.

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Jet fighter pilots or astronauts experience hypergravity and microgravity due to the acceleration and deceleration of the aircraft or the gravitational changes of the environment. Jet fighter pilots can be exposed to accelerated hypergravity as high as 7.0 $G_z$ temporarily.[7] Sudden acceleration is known to decrease ocular blood flow and to cause blackouts or loss of peripheral vision.[5,19] Exposure to microgravity for a prolonged period caused optic disc swelling or an increase in intraocular pressure in astronauts in previous studies.[6,21]

An experimental study in rats showed damage of the rod photoreceptors of the retina after exposure to hypergravity.[3] However, only a few studies evaluated the effects of hypergravity in the field of ophthalmology and most of these studies were performed under relatively low gravity (~2-4 $G_z$) for an extended duration. The sudden acceleration of jet fighters or the launching or landing of the space shuttle occurs during a relatively short period with high hypergravity exposure. While experimental models with high hypergravity and a short duration of exposure would simulate these real-life situations more precisely, in this study we evaluated the eyes of mice in extreme circumstances with high hypergravity and a long duration of exposure.

Decreased ocular blood flow causes hypoxia of the retina. Retinal hypoxia can induce an increase in vascular endothelial growth factor (VEGF), which causes neovascularization. Neovascularization of the choroid or the retina can cause vision-threatening ocular diseases such as exudative age-related macular degeneration or proliferative diabetic retinopathy.[15,16,20] Oshima et al. showed that exposure to hypergravity induced increased expression of VEGF in the heart vessels of mice.[17]

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Further, a prolonged hypoxic state of the retina induces ischemic injuries, which could lead to glial cell death. Glial fibrillary acidic protein (GFAP) and glutamine synthetase (GS) are known markers of glial reactivity in the retina.[4,12] Muller glial cells show increased GFAP expression after retinal injuries due to ischemia.[13] Glutamate is a neurotransmitter that is regulated by GS in glial cells. Neuronal injuries cause an increase in glutamate, which leads to neuronal cell death by overexcitation of postsynaptic receptors. Increased GS was shown to have protective effects against neuronal degeneration in injured retinal tissue.[8] Further, a previous study suggested that GS activity is transiently elevated after hypoxic injury, but before apparent neuronal death.[10,22]

The purpose of this study was to evaluate the effect of high levels of hypergravity on intraocular pressure and the retina in mice. Further, we determined whether long-term exposure to hypergravity alters the expression of VEGF, GFAP, and GS in the mouse retina.

## METHODS

### Animals

We used 6-wk-old female BALB/C mice for this study. All animal experiments followed the guidelines established by the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. The protocol was approved by the Institutional Animal Care and Use Committee of Inha University.

### Equipment

A centrifugal acceleration test facility for animals at the Aerospace Medical Center (Cheongju, Korea) was used for the experimental conditions. Centrifugation with a 2.0-m arm and a rotation speed of 67.0 rpm were used to produce hypergravity of 10.0 $G_z$. A cage was attached to the end of the arm and the mice were monitored through a high-resolution video camera inside the cage throughout the experiments. There were 10 mice that underwent centrifugation at 10.0 G for 4 h; 10 other mice served as controls under normal gravity with no manipulation. In the cage, mice were fixed in body-sized clear cylindrical plastic tubes with the body rolled with gauze, exposing their heads upright. The intraocular pressure of the mice was measured 24 h before centrifugation and right after centrifugation by using an Icare Labs (Icare, Helsinki, Finland) contact tonometer made for animals. The resolution of the measuring apparatus is 1 mmHg. The mice were anesthetized during this procedure with a 1:1 mixture of ketamine (100 $mg \cdot kg^{-1}$) and xylazine (10 $mg \cdot kg^{-1}$).

### Procedure

After 4 h of centrifugation at 10.0 $G_z$, the mice were anesthetized immediately for measurement of intraocular pressure and subsequent enucleation. The enucleated eyeballs were fixed at 4°C for 24 h in Davison's fixation solution. Thereafter, the cornea and lens were removed, and the remaining cup of the eyeball was fixed again for another 24 h under the same conditions. The fixed eyecup was dehydrated, embedded in paraffin wax, and sectioned at 4-μm thickness. The tissue slides were stained with hematoxylin and eosin (H&E) for histological examination and were photographed using a photomicroscope (Olympus BX43, Tokyo, Japan). The tissue slides were deparaffinized and were subsequently blocked at room temperature for 1 h with antibody diluent reagent solution (Invitrogen, Waltham, MA). After the slides were washed three times for 5 min in phosphate buffer saline (PBS), the tissue slides were incubated with a primary antibody for VEGF-A (dilution, 1:200; Proteintech Group, Chicago, IL), VEGF-R1 (dilution, 1:100; Abcam, Cambridge, UK), VEGF-R2 (dilution, 1:100; Santa Cruz Biotechnology, Dallas, TX), GFAP (dilution, 1:1000; Abcam), or GS (dilution, 1:100; Santa Cruz Biotechnology, Dallas, TX) at 4°C overnight. After antibody incubation, the slides were washed five times for 5 min with PBS in 0.05% Tween-20 (PBS-T, Sigma-Aldrich, St. Louis, MO). After washing with PBS-T, the slides were incubated in a dark room at room temperature for 2 h with affinity-purified fluorescein-labeled goat antirabbit IgG (H + L) (dilution, 1:200; KPL, Gaithersburg, MD). Subsequently, the samples were washed five times for 5 min with PBS-T. The samples were examined by confocal microscopy (Olympus IX 81). The staining results were independently evaluated by two experienced observers who were masked to the exposure conditions of the eyes. A retinal specitalist reviewed the staining results in cases of disagreement.

### Statistical Analysis

The results were analyzed using the Student's paired *t*-test according to the variables. The results are expressed as mean ± SD. $P < 0.05$ was considered statistically significant. Statistical analysis was performed using SPSS for Windows version 18.0 (SPSS, Inc., Chicago, IL).

## RESULTS

Intraocular pressure was measured in both eyes of all 10 mice (**Fig. 1**). The average intraocular pressure of the mice measured

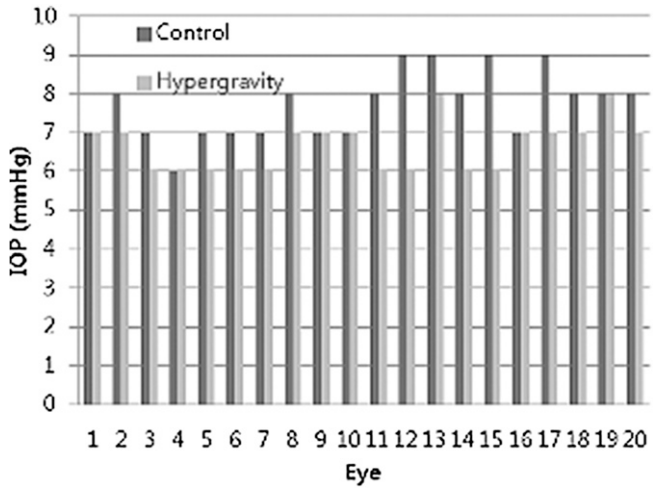


**Fig. 1.** Intraocular pressure of mice before and after exposure to 10.0 $G_z$.

24 h before exposure to hypergravity was 7.7 ± 0.86 mmHg, whereas the average intraocular pressure was 6.65 ± 0.67 mmHg after exposure to 10.0 $G_z$ for 4 h. A significant decrease in intraocular pressure was observed after exposure to hypergravity ($P = 0.01$). Histological examination of the retinas of the mice after H&E staining showed no difference between the control group and the experimental group (**Fig. 2**). Immunohistochemistry staining was performed with VEGF-A, VEGF-R1, VEGF-R2, GFAP, and GS. Compared with the control group, staining was increased for all of these markers in the group exposed to hypergravity (**Fig. 3** and **Fig. 4**).

## DISCUSSION

Most of studies about the effects of hypergravity on eyes were conducted under relatively low hypergravity, such as 2.0 $G_z$.[3,5] However, astronauts and jet fighter pilots experience much higher gravitational stress during a short period. In this study, we observed a decrease in intraocular pressure of the mice exposed to 10.0 $G_z$. Under microgravity, the shifting of body fluid toward the head causes a rise in episcleral venous pressure, which leads to increased outflow resistance of the aqueous humor and a subsequent increase in intraocular pressure.[14] Previous studies have shown that hypergravity decreases the blood circulation of the brain and the retina, leading to gray-out or blackout.[5,19] Therefore, we could assume that decreased ocular blood flow causes a decrease in episcleral venous pressure, which would result in the decreased intraocular pressure observed in this study. This phenomenon is the opposite of the increased intraocular pressure that occurs under microgravity.

Further, we compared the histology of retinas under normal gravity and those under hypergravity. Although Barnstable and colleagues showed severe damage to the outer retina in rats after hypergravity,[3] we did not observe any difference between the retinas exposed to hypergravity and those exposed to normal gravity. Barnstable and colleagues exposed the rats to hypergravity of 2.0 $G_z$ for 14 d by centrifugation, whereas we used a hypergravity of 10.0 $G_z$ for 4 h. Thus, a longer duration may be needed to cause histological changes in the retina.

Hypergravity causes body fluids to shift toward the lower body, which causes decreased ocular blood flow and leads to hypoxia.[18] Although we did not observe any histological changes in the retina after 4 h of centrifugation, we expected to see some physiological changes because hypergravity induces hypoxia of the retina. VEGF-A, which is a well-known proangiogenic factor, induces ocular neovascularization when the retina is in a hypoxic state.[1] Increased VEGF levels in the retina can induce vision-threatening diseases such as exudative age-related macular degeneration or proliferative diabetic retinopathy.[2,9] Hypoxia induces the expression of both VEGF-R1 and VEGF-R2, which are high-affinity receptors of VEGF-A.[11] Our immunohistochemistry results in the mouse retina showed increased expression of VEGF-A, VEGF-R1, and VEGF-R2 after exposure to hypergravity.

Further, we observed increased expression of both GFAP and GS in the retinas of the mice after exposure to hypergravity. GFAP and GS are usually found in astrocytes and Muller glial cells.[4,12] Muller cells normally express little GFAP, but expression is increased under conditions such as retinal ischemia;[13] GS is also increased under such circumstances, and it has neuroprotective effects.[8,22] We assumed that hypergravity caused a hypoxic impulse or, moreover, neuronal damage to the retinal glial cells; however, this is based only on our observation of GFAP and GS expression by immunohistochemistry.

A limitation of our study is that experimental models with high hypergravity and a short duration of exposure would simulate those real-life situations related to pilots more precisely. It is true that 10 $G_z$ hypergravity for 4 h is a much more stressful condition than actual spaceflight. However, we also have to keep in mind that experimental animals like BALB/C mice are

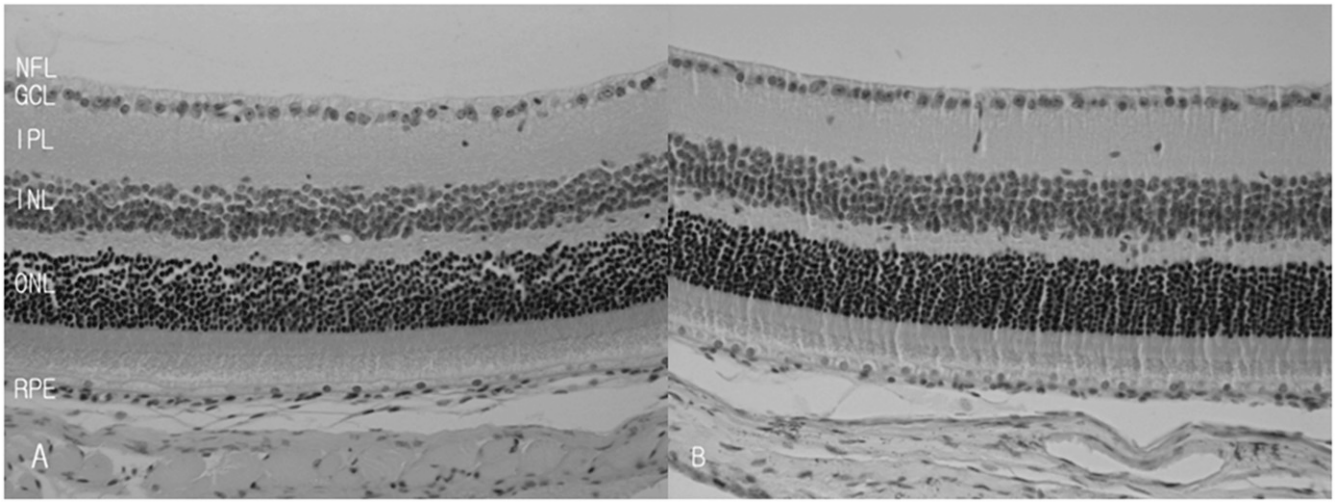


**Fig. 2.** Histology of mouse retina with hematoxylin & eosin staining (×400). A) Control and B) 10.0 $G_z$ (4 h) exposed mice. NFL: nerve fiber layer; GCL: ganglion cell layer; IPL: inner plexiform layer; INL: inner nuclear layer; ONL: outer nuclear layer; RPE: retinal pigment epithelium. See the online figure for color.

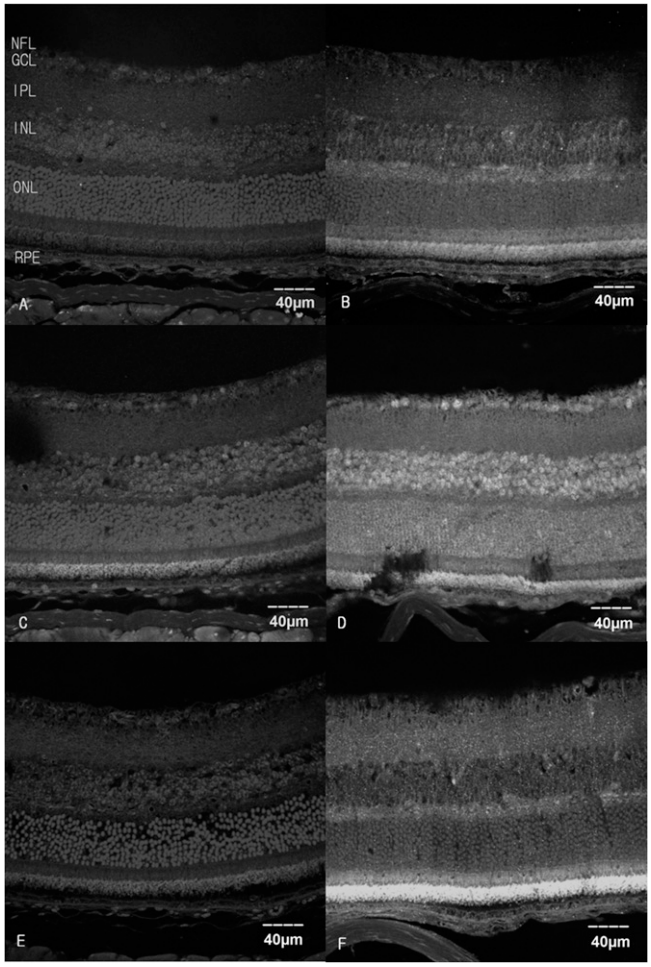


**Fig. 3.** Confocal images of immunostaining in mouse retina. Anti VEGF-A antibody A) before and B) after 10.0 $G_z$ exposure for 4 h. Anti-VEGF-R1 antibody C) before and D) after 10.0 $G_z$ exposure for 4 h. Anti-VEGF-R2 antibody E) before and F) after 10.0 $G_z$ exposure for 4 h. Nuclei were counterstained with DAPI and are shown in blue in the online figure. NFL: nerve fiber layer; GCL: ganglion cell layer; IPL: inner plexiform layer; INL: inner nuclear layer; ONL: outer nuclear layer; RPE: retinal pigment epithelium.

especially resistant to the harmful stimuli of hypergravity. After just a few seconds in 10 $G_z$ of hypergravity, every human pilot will faint and black out. However, all mice in our experiment were quite viable without any loss of consciousness. It is probable that mice are stronger and more resistant to hypergravity than human beings. The purpose of this study was to evaluate the eyes in an extreme circumstance with high hypergravity and a long duration of exposure and observe the histological changes in the retina. Hypoxia can be accurately measured with a real-time pulse oximeter for mice during exposure to high gravity. Further studies that measure blood oxygenation may be able to define the correlation between exposure to hypergravity and hypoxic retinal damage more precisely. The sample sizes of mice were relatively small. This may limit the statistical power in distinguishing the differences of intraocular pressure before and after exposure to high gravity.

In conclusion, after exposure of mice to hypergravity of 10.0 $G_z$, the intraocular pressure decreased and VEGF, VEGFR-1, VEGFR-2, GFAP, and GS expression increased in the retina. These results suggest that repeated exposure to a high level of hypergravity could cause damage to the retina.

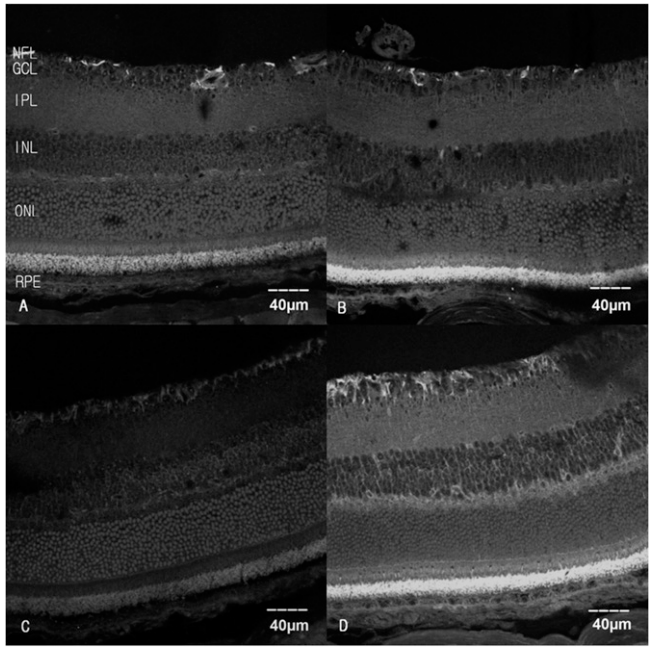


**Fig. 4.** Confocal images of immunostaining in mouse retina. Anti-GFAP antibody A) before and B) after 10.0 $G_z$ exposure for 4 h. Anti-GS antibody C) before and D) after 10.0 $G_z$ exposure for 4 h. Nuclei were counterstained with DAPI and are shown in blue in the online figure. NFL: nerve fiber layer; GCL: ganglion cell layer; IPL: inner plexiform layer; INL: inner nuclear layer; ONL: outer nuclear layer; RPE: retinal pigment epithelium.

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