Intraocular Pressure and Cardiovascular Alterations Investigated in Artificial Gravity as a Countermeasure to Spaceflight Associated Neuro-ocular Syndrome

Allison P. Anderson[1], Joseph S. Butterfield[1,2], Prem S. Subramanian[3], Torin K. Clark[1]

[1]Smead Department of Aerospace Engineering Sciences, University of Colorado – Boulder
[2]Department of Integrative Physiology, University of Colorado - Boulder
[3]Department of Ophthalmology, University of Colorado – Denver

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Corresponding Author:
Allison Anderson
University of Colorado - Boulder
429 UCB
ECAE 115
Boulder, CO 80309

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ABSTRACT

INTRODUCTION: Artificial gravity (AG) has been proposed as a countermeasure to spaceflight associated neuro-ocular syndrome (SANS). The etiology of SANS is unknown, but mimicking gravitational loading through AG may mitigate these negative adaptations. *METHODS:* Seventeen subjects (9M, 8F, 18-32 years) were analyzed in four experimental conditions: 1) standing, 2) supine, 3) AG with the center of rotation at the eye (AGEC), 4) AG with 2G's at the feet (AG2G). In both AG conditions, subjects were spun to produce 1G at their center of mass. Data included self-administered intraocular pressure (IOP, Tonopen AVIA), heart rate (HR), and mean arterial blood pressure (MAP, Omron Series 10). Data were analyzed with repeated measures ANOVAs, with Tukey-Kramer corrections for multiple pairwise comparisons. *RESULTS:* IOP was 15.7 ± 1.4 mmHg (mean±95% confidence interval) standing, 18.8 ± 1.3 mmHg supine, 18.5 ± 1.7 mmHg in AGEC, and 17.5 ± 1.5 mmHg in AG2G. Postures showed a main effect (F(3,48)=11.0, p<0.0005), with standing significantly lower than supine (p=0.0009), AGEC (p=0.002), and AG2G (0.036). Supine, AGEC, and AG2G were not statistically different. HR and MAP were lower in supine compared to all other postures (p=0.002 to p<0.0005), but there were no differences between standing, AGEC, and AG2G. *DISCUSSION:* IOP in supine and standing was consistent with previous studies, but contrary to our hypothesis, remained elevated in both AG conditions. Cardiovascular parameters and hydrostatic gradients determine IOP, which remain unchanged compared to standing. These results suggest additional influence on IOP from previously unconsidered factors.

NEW AND NOTEWORTHY: This is the first study, to the authors' knowledge, to measure intraocular pressure in short-radius centrifuge artificial gravity (AG), which has been proposed

as a countermeasure to the spaceflight associated neuro-ocular syndrome (SANS). If the etiology of SANS is related to intraocular pressure, these results have implications for whether or not short-radius AG can be used to prevent ocular changes relevant to it. Our results indicate this proposed countermeasure merits further investigation.

INTRODUCTION

The spaceflight associated neuro-ocular syndrome (SANS) affects between 38% and 51% of long-duration spaceflight flyers (46). Astronauts return from microgravity with globe flattening, choroidal folds, cotton wool spots, optic disc edema, and optic nerve sheath distention. For some, this results in degraded near visual acuity (37, 40). The etiology of SANS is unknown. Elevated intracranial pressure (ICP) was originally hypothesized to cause SANS, as the observed ocular changes resemble those seen with terrestrial idiopathic intracranial hypertension. However, these astronauts do not have the expected corresponding symptoms of high ICP (37, 46). Additionally, invasive measures of ICP in parabolic flight microgravity were found to be lower than supine values, suggesting ICP may not be clinically elevated immediately during flight (32).

Although we do not know the cause of SANS, long-term exposure to the microgravity environment is a contributing factor since these changes do not occur on Earth. It is possible the removal of gravitational force acting on the body is a contributing factor. It has been hypothesized the removal of hydrostatic gradients result in cephalad fluid shifts that change the fluid pressures in the eye, cerebral cardiovascular system (specifically cerebral venous congestion), cerebral spinal fluid (CSF) production and/or absorption, and/or compartmentalized subarachnoid CSF pressures in the optic nerve sheath (38, 40, 46, 47). Tissue weight may also be relevant, since it has been shown to alter central venous pressure (7–9). Additional factors unrelated to gravitational loading, such as straining in exercise (40), sodium intake (38),radiation (46), genetic predisposition (51, 52), or carbon dioxide (31), may contribute to SANS, but are not unique to the microgravity environment (with the exception of radiation and carbon dioxide on the International Space Station). Therefore, mimicking gravitational loading through artificial gravity (AG) may mitigate the development of SANS (16).

AG, as produced through centrifugation, has been proposed as a "comprehensive" countermeasure for human physiological deconditioning resulting from extended exposure to microgravity (14). To date, AG has not been experimentally validated as a human countermeasure in space (12). Several ground-based studies, however, have shown benefits of AG in other physiological systems (29). In such studies, typically subjects are exposed to continuous head-down bed rest as a spaceflight analog, with a subset of subjects also experiencing intermittent (~1 hour per day) AG through centrifugation (typically 1G's of centripetal acceleration at a specified location along the subject's body). In the cardiovascular system, several studies have found AG to beneficially mitigate deconditioning resulting from otherwise continuous bed rest (27). Fewer studies have investigated mitigation of musculoskeletal deconditioning (45), but some have shown beneficial effects (30), including functional benefits (43). Head-down bed rest fails to replicate the altered vestibular simulation of microgravity (42). Thus it is not surprising few differences have been found between bed rest and AG+bed rest subjects in metrics of balance, locomotion (13, 39), and neurovestibular function. Nonetheless, AG is a promising countermeasure for sensorimotor deconditioning since, unlike exercise in microgravity, the gravitational stimulation to the vestibular system is reproduced.

To the authors' knowledge, the only paper to investigate AG and its effects on the eye was a study by Chung in 2001 (10). Three subjects were spun in a long-radius centrifuge to +3 Gz, +2

Gz, and -1 Gz. Subjects self-measured intraocular pressure (IOP) using a handheld applanation tonometer. Surprisingly, all AG conditions (+2, +3, -1 Gz) had significantly elevated IOP compared to baseline. This indicates hydrostatic gradients under centrifugation were not the primary determinant of IOP, as was hypothesized, since the +Gz conditions would have caused a fluid shift into the lower body. Exposure, though, was limited in duration, with 45 seconds at each experimental condition (10). These short-term effects may not be indicative of ocular status once the eye has reached steady state under AG exposure. A study by Anderson et al. found IOP has a time constant of 5.3 minutes, reaching steady state pressure in approximately 10 minutes when going from the seated to the prone position (1). Further, the Chung study used long-radius centrifugation, while current investigations for AG to mitigate SANS have been proposed as short-radius centrifugation, where head-to-toe hydrostatic gradients are larger (14, 21).

The objective of this study is to investigate short-radius AG as a potential countermeasure to mitigate SANS. The presentation of SANS is highly individualized and develops over microgravity exposure timescales not feasible for Earth-based analog studies. In this research, we investigated whether AG can mitigate changes in IOP, as this would indicate AG could mitigate relevant factors contributing to SANS. In the current study, we quantify IOP with subjects standing, supine, and in two conditions in which the subject is supine but experiencing footward AG loading through centrifugation (Figure 1). When going from standing to supine, it is known that IOP increases by 2-4 mmHg (34, 35, 41). We hypothesized our subjects would show similar changes between standing and supine positioning. Further, we hypothesized IOP in AG would be reduced relative to supine and be similar to the standing posture. The basis of this hypothesis is that short-radius AG produces a cardiovascular environment consistent with standing, with heart rate and blood pressure elevated compared to supine (28), but with a hydrostatic gradient greater than standing (16). Cardiovascular pressures, specifically venous pressure, are a determinant of IOP (6). If AG is able to counteract the ocular physiologic response when going from standing to supine, it would suggest that AG reduces cephalad fluid shifts and venous pressures at eye level and thus may be a potential countermeasure to mitigate SANS in long-duration astronauts.

METHODS

Subjects

The protocol was approved by the University of Colorado – Boulder Institutional Review Board, and informed consent was obtained from all subjects. Nineteen subjects (10 male, 9 female) participated in the study. Subjects ranged between 18 and 32 years of age. To participate in the study, there were several screening criteria, however none of the volunteers were excluded due to these. Seated intraocular pressure was exclusionary if above 20 mmHg. Subjects were required to have systolic blood pressure between 100 and 140 mmHg and diastolic blood pressure between 70 and 90 mmHg, weight between 110 and 225 lbs, height at or between 5’2’’ and 6’3’’, and a capacity to stand upright unsupported for 15 minutes. Subjects were also screened based on ocular history, cardiovascular health, overall health history, vestibular pathology, motion sickness susceptibility, and a history of orthostatic hypotension or other condition that could cause low blood pressure. Any history of allergy to proparacaine hydrochloride (see Protocol) was also exclusionary. Finally, pregnant women or women actively trying to become pregnant were excluded from this study.

Protocol

The protocol was performed over 2 days: a training day and a testing day. On the training day, subjects were screened to determine eligibility and consented. Anthropometric information (height, weight, chest and waist circumference, and eye and armpit height) were recorded. Subjects were trained on the procedure to collect the IOP measurement, first by video demonstration, then by the trained experimenter, and finally seated in front of a mirror to practice self-administered measurements until the subjects felt comfortable using the device (Tonopen AVIA). Finally, subjects were spun on a short-radius centrifuge (Figure 1) to determine the personalized spin-rate and distance from the center of rotation to achieve the desired AG conditions and practice self-administered IOP measurements under centrifugation.

Two AG conditions were investigated in the study (Figure 1). In one, the subject was situated such that the eye was placed in the center of rotation (AGEC). In the other, 2G's was produced at the feet (AG2G, causing the eye to be radially displaced from the center of rotation). In both AG conditions, subjects were spun to produce 1G at their center of mass in order to mimic the net loading created while standing. We tested two AG conditions to evaluate positioning and the associated centripetal acceleration loading (i.e., presence of loading at the eye location) and to match future experiments (see Discussion). It was not anticipated that loading on the eye itself in the G_z direction will alter IOP, since IOP is a function of venous pressure at the eye level and drainage mechanisms in the eye (6). This required different centrifuge spin rates and positioning for each AG condition and subject anthropometry. Subjects laid supine, and were safely secured with a four-point harness, on a low-friction bed, such that they would support their centripetal acceleration "weight" in AG against a footplate, mimicking a standing loading. As a result, in AG the subjects also supported the "weight" of the bed upon which they were lying (approximately 45 lbs, see Discussion). On the training day, subjects began in the AGEC condition, with the footplate adjusted to ensure the eye position was at the center of rotation. A scale was mounted on the footplate to empirically measure the “weight” resulting from centrifugation of the subject and bed. With the centrifuge spinning, the rate was adjusted in one rotation per minute (rpm) increments until the scale matched the subject’s body weight and the weight of the bed within 10% (producing 1G at the subject’s center of mass). Depending upon subject anthropometry, spin rates ranged from 36-40 rotations per minute (rpm) (mean=37.5 rpm), theoretically yielding G-levels at the feet from 2.37-2.71 G’s (mean=2.51 G’s). Thus, an empirical measurement of the appropriate spin rate, rather than a theoretical approximation, was achieved.

This process was repeated for the AG2G condition, with adjustments in subject position as well, if needed. In this condition, the subject’s eye location ranged from 8-22.5 inches (mean=12.8 inches) radially displaced from the center of rotation (Figure 1). The required spin rates ranged from 28-33 rpm (mean=30.4 rpm), which theoretically yielded 0.21-0.49 G’s (mean=0.33 G’s) at the eye location. After the AG conditions had been determined (spin rate and positioning), subjects were given an opportunity to practice taking IOP measurements during centrifugation until they felt comfortable with the procedure.

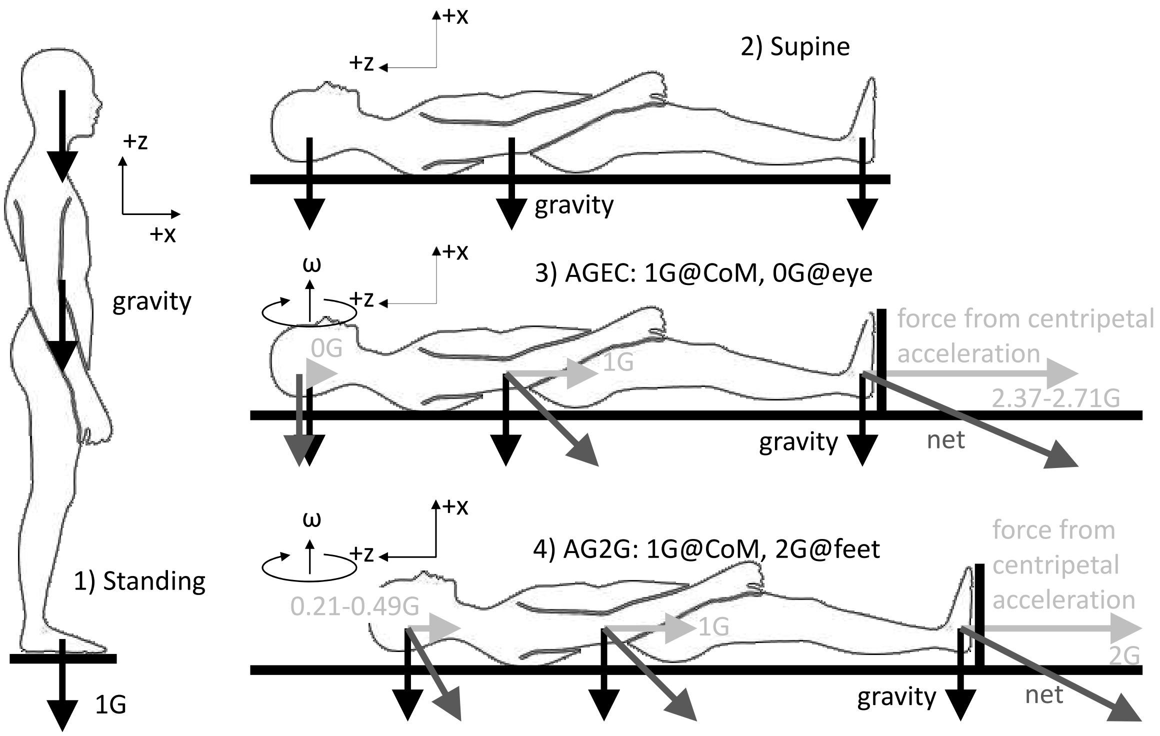

Figure 1: The four experimental postural conditions: 1) Standing creates 1G in the –z-direction (footward) from gravity; 2) Supine removes the –z-direction loading, but still has –x-direction loading from gravity; 3) artificial gravity with the eye-centered (AGEC) has the subject supine, positioned with their eye at the center of rotation of the centrifuge, and spun at a rate to produce 1G of centripetal acceleration (the force from which is in the –z-direction) at the subject's center of mass. This produced 2.37 – 2.71 G at the subject's feet; 4) AG2G has the subject positioned and spun at a rate to produce 1G of centripetal acceleration at the subject's center of mass (as in AGEC), but now positioned to produce 2G at the subject's feet. AG2G required the subject's eye to be radially displaced from the centrifuge center of rotation causing a –z-direction force from centripetal acceleration with a magnitude ranging from 0.21 – 0.49 G. Of course, in both AG conditions, the –x-direction loading from gravity remains, yielding a net loading in the x-z plane that varies in direction and magnitude along the length of the subject's body.

On the testing day, subjects experienced each of the four experimental conditions in a randomized order: 1) standing, 2) supine, 3) AGEC, and 4) AG2G. In each condition, participants first acclimatized for 10 minutes in the posture prior to taking measurements, to allow the cardiovascular system and IOP to equilibrate (1). Subjects remained stationary, feet shoulder width apart, hands at their side. In the AG conditions, subjects supported their weight against a footplate, lying down with feet shoulder width apart. Lighting conditions were standardized in each condition and across all subjects. A mirror was provided approximately 25 cm in front of the subject's face to assist in self-administering IOP measurements. In the AG conditions, two-way audio communication was maintained and researchers had video surveillance of the subject to monitor well-being and ensure the protocol was followed correctly. Motion sickness was also reported on a 0-20 scale (0=no symptoms, 20=vomiting) every 5 minutes in the AG conditions, though no subjects experienced substantial motion sickness. The total duration of the experiment, including training and testing across both days, was always less than 5 hours per subject.

Subjects self-administered IOP using their dominant hand to assess that eye (e.g., right handed subjects measured in their right eye). Subjects' corneas were anesthetized using proparacaine hydrochloride 0.5%, just prior to the 10-minute equilibration period. To collect the measure, subjects press the AVIA lightly against the center of their cornea. The AVIA collects the first 10 high quality pulsed measurements for a single aggregated reading of IOP. The AVIA reports an estimated accuracy, ranging from 80-95% confidence. Subjects verbally reported the reading to the experimenter to record. In each postural condition, subjects collected their own IOP readings twice, one immediately after another. If there was more than a 2 mmHg difference between the two readings, or measurement confidence was less than 90%, additional readings were taken until two readings with 90% confidence or greater were consistent within 2mmHg from each other. After IOP was collected, heart rate (HR), and blood pressure were collected (Omron Series

10) with the cuff on the subject's left arm. Mean arterial blood pressure (MAP) was calculated as the sum of one third systolic blood pressure and two thirds diastolic blood pressure.

Statistics

To assess the hypothesis that postural condition (standing, supine, AGEC, AG2G) impacted IOP and cardiovascular status (HR and MAP), we performed repeated measures Analysis of Variances (RMANOVA), checking the residuals for normality (Kolmogorov-Smirnov test) and homoscedasticity (Bartlett's test). We then performed post-hoc pairwise t-tests between each of the four conditions, with Tukey-Kramer corrections for multiple comparisons (6 total comparisons). Finally, exploratory analysis was performed to identify potential differences between males and females, effects of subject height and body-mass index, and the presentation order of the four conditions. All tests were performed as two-tailed tests, with the level of significance set to $\alpha = 0.05$. Data processing and statistical analysis was performed in MATLAB 2017a.

Some individual IOP readings were believed to be erroneous due to any number of factors, including operator experience, misalignment, and application pressure of the device. To reduce the impact of erroneous readings on the aggregated single measurement of IOP within each postural condition, we used a robust averaging approach. Individual readings differing from the median of all readings by more than 5 mmHg were deemed erroneous and excluded (11.6% of individual readings were excluded). This resulted in an average of 2.7 readings included per subject per condition, which were then averaged to yield a single IOP measurement for each subject in each condition. This approach balanced removing erroneous readings and utilizing multiple readings to efficiently yield a central estimate with high precision and accuracy. As elaborated upon in the Results section, alternative criteria to excluding erroneous IOP readings did not impact the conclusions.

After fitting the RMANOVA, if the standardized residuals exceeded three, the subject was considered an outlier and was removed from analysis. For completeness, we also present the analysis with all subjects included and found the overall conclusions were very similar, with the exception of the significance of one pairwise comparison (detailed in the Results).

HR and MAP measurements were missing for two subjects in the AGEC condition and for one subject in the AG2G condition due to hardware malfunction, presumably caused by cuff misalignment. While HR and MAP measurements did exist for these subjects in the remaining postural conditions, to be conservative, we excluded these subjects entirely from our RMANOVA statistical analyses for these parameters (n = 17). Data for these subjects in the conditions for which it exists is shown (gray shapes), in Figure 3a and is consistent with other subjects.

RESULTS

Intraocular pressure remains elevated in AG

The IOP in each of the four postural conditions is shown in Figure 2. On average 3.05 measurements were taken to reach the stopping criteria (max of 10) with a similar number in each postural condition (standing: 2.9, supine: 2.8, EC2G: 3.4, AG2G: 3.05). When fitting a RMANOVA, two subjects (1M/1F) had measurements that yielded standardized residuals exceeding three. In Figure 2a, the measurement highlighted with a triangle had a standardized residual of 3.71. Once this subject was removed, the measurement highlighted with a diamond yielded 3.28 and this subject was also removed from the analysis (subjects shown in gray in Figure 2a). IOP when standing averaged 15.7 mmHg (95% confidence interval (CI)=14.3-17.1, range=10.5-21.5). The RMANOVA found a significant main effect of posture on IOP (F(3,48)=11.0, $p<0.0005$). Post-hoc tests indicated IOP in the supine posture was significantly higher than standing (change=3.12 mmHg, 95% CI: 1.29-4.95, p=0.0009), as expected (34, 35, 41). Contrary to our hypothesis, IOP in AGEC was significantly elevated relative to standing (change=2.78 mmHg, 95% CI:0.97-4.59, p=0.002) and was not significantly different than supine (p=0.86). IOP in AG2G was also contrary to our hypothesis, as it was also significantly higher than in standing (change=1.85, 95% CI:0.10-3.61, p=0.036) and not significantly different than supine (p=0.21). Finally, there was no significant difference between the two AG conditions (p=0.46).

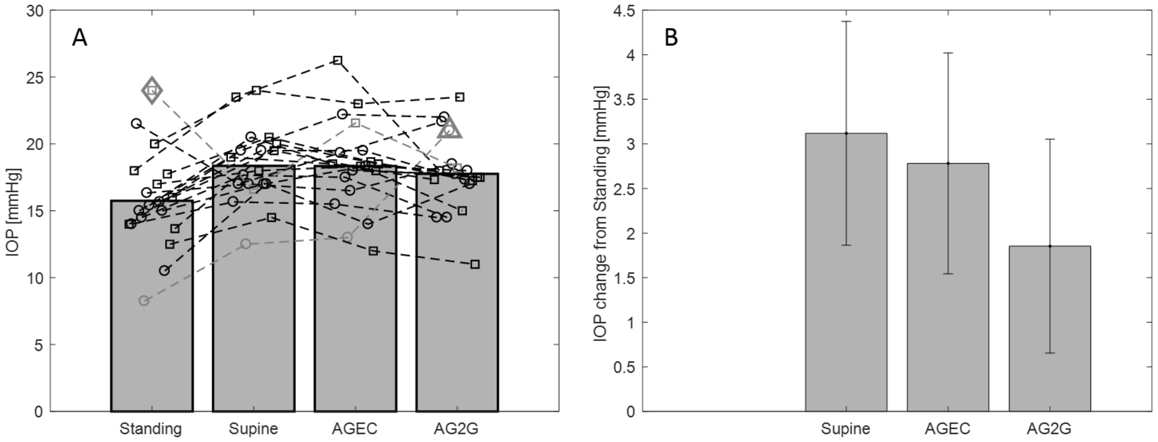

Figure 2: IOP response in standing, supine, and the two AG conditions. Panel A shows the raw data for each subject (squares=females, circles=males). The bold shapes highlight measurements with large standardized residuals from the initial RMANOVA model fit ($\Delta=3.71$, $\Diamond=2.99$ originally, but when the data of the subject with the first outlier is removed, increased to 3.28). Each subject is shifted laterally slightly to avoid

overlapping. The bars represent means in each condition (including all measurements). Panel B shows the change in IOP from the standing condition (positive values correspond to an increase in IOP relative to standing). The bars depict the average change across subjects and the error bars are 95% confidence intervals (excluding two outlier subjects), without any correction for multiple comparisons.

To ensure our conclusions were not due to excluding the subjects with outliers, we re-ran the RMANOVA again and with all 19 subjects and found a nearly identical outcome. The one exception was a pairwise comparison, in which the IOP in AG2G was no longer significantly different from standing ($p=0.15$). If only the one subject with the largest outlier was excluded, the statistical outcome was the same as including all subjects.

Furthermore, we considered alternative approaches to identifying erroneous IOP readings for removal. For example, we repeated the analysis with a conservative approach in which all readings were included in each average (i.e., no readings removed as erroneous). This tended to increase the IOP measurements (~1mmHg for each condition) as erroneous readings tended to err with high values. However, in our statistical analysis, we found identical results (the data for the same two subjects were found to be outliers and excluded and the pairwise differences that were significant were identical). Alternatively, we considered a more aggressive approach in which readings that differed from the median by more than 3 mmHg were excluded prior to averaging (20.1% of individual readings were excluded, compared to 11.6% for our previous approach of ±5 mmHg). Again, the same subjects' data were found as outliers and the statistical analysis yielded identical results. It is possible that either good readings were excluded or that erroneous readings were still included. These methods, however, center on the median since outlier readings would heavily skew an average, and therefore would not be resilient to erroneous measurements. Using this method, confidence can be gained since the statistical conclusions do not deviate with both conservative and liberal analysis methodologies.

Thus, we remain confident in our primary conclusions that 1) as expected, IOP was elevated in supine relative to standing, and 2) contrary to our hypothesis, IOP was not reduced in either AG condition relative to supine and was typically elevated relative to standing.

Heart rate and blood pressure respond to AG

In summary, HR (Figure 3) and MAP (Figure 4) both responded as expected in response to the presence of hydrostatic gradient, or lack thereof, for each postural condition.

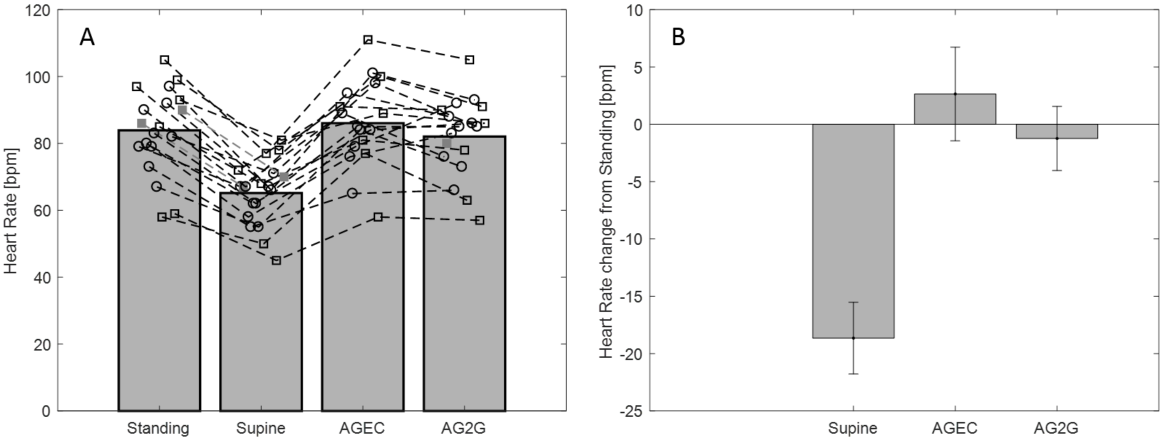

Figure 3: Heart rate (HR) response in standing, supine, and the two AG conditions. Panel A shows the raw data for each subject (squares=females, circles=males). The filled gray shapes are for the two subjects in which there was missing data for another condition(s). Each subject is shifted laterally slightly to avoid overlapping. The bars represent means in each condition (including all measurements). Panel B shows the change in HR from the standing condition (positive values correspond to an increase in HR relative to standing). The bars depict the average change across subjects and the error bars are 95% confidence intervals (excluding two subjects with missing data), without any correction for multiple comparisons.

With a RMANOVA, we found a significant main effect of posture on HR (F(3,48)=59.1, p<0.0005). With Tukey-Kramer corrections for multiple comparisons, as expected, we found the standing posture to have significantly higher HR than supine (change=18.6 bpm, 95% CI: 14.1-23.2, p<0.0005). Relative to supine, HR was significantly higher for AGEC (change=21.3 bpm, 95% CI: 15.8-26.8, p<0.0005) and AG2G (change=17.4 bpm, 95% CI: 12.3-22.5, p<0.0005). There was no difference in HR between any of the postural conditions in which there was a hydrostatic gradient (standing vs. AGEC: p=0.59, standing vs. AG2G: p=0.82, AGEC vs. AG2G: p=0.17).

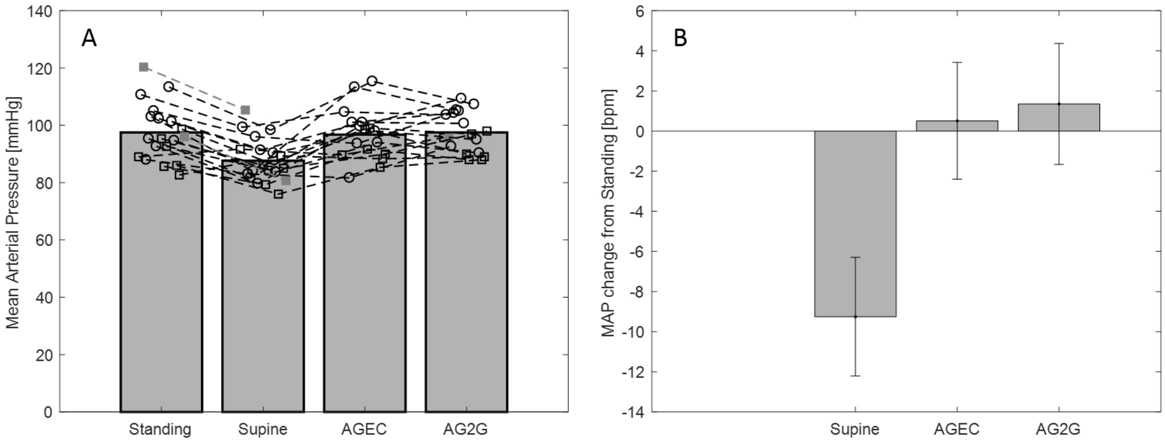

Figure 4: Mean arterial pressure (MAP) response in standing, supine, and the two AG conditions. Panel A shows the raw data for each subject (squares=females, circles=males). The filled gray shapes are for the two subjects in which there was missing data for another condition(s). Each subject is shifted laterally slightly to avoid overlapping. The bars represent means in each condition (including all measurements). Panel B shows the change in MAP from the standing condition (positive values correspond to an increase in HR relative to standing). The bars depict the average change across subjects and the error bars are 95% confidence intervals (excluding two subjects with missing data), without any correction for multiple comparisons.

The MAP response follows the same pattern as HR. A RMANOVA found a significant main effect of posture on MAP (F(3,48)=17.0, $p<0.0005$). With Tukey-Kramer corrections for multiple comparisons, the standing posture had significantly higher MAP than supine (change=9.3 mmHg, 95% CI: 4.9-13.6, $p<0.0005$). Relative to supine, MAP was significantly elevated for AGEC (change=9.8 mmHg, 95% CI: 3.7-15.9, p=0.002) and AG2G (change=10.6 mmHg, 95% CI: 5.3-15.9, $p<0.0005$). Again, there was no difference in MAP between any of the postural conditions in which there was a hydrostatic gradient (standing vs. AGEC: p=0.99, standing vs. AG2G: p=0.82, AGEC vs. AG2G: p=0.95).

Exploratory Analysis

We performed some post-hoc exploratory analysis to identify potential effects worth further investigation (n = 19). There were no differences due to gender (despite not applying a correction for multiple comparisons, as this analysis was exploratory). Unpaired t-tests in each postural condition on the IOP values for males vs. females were each not significant (standing: p=0.96, supine: p=0.63, AGEC: p=0.96, AG2G: p=0.23). Similarly, there were no gender differences in the *changes* in IOP from standing to any other postural condition (standing vs. supine: p=0.73, Standing vs. AGEC (p=0.99), standing vs. AG2G (p=0.35)).

Anthropometry was not found to be a relevant factor. Subject height was not significantly correlated with IOP in each condition (standing: p=0.18, supine: p=0.11, AGEC: p=0.18, AG2G: p=0.80). Similarly, an individual's change in IOP from standing to each of the other postural conditions was not significantly correlated with their height, (supine vs. standing: p=0.92, AGEC vs. standing: p=0.94, AG2G vs. standing: p=0.30). Each subject's body-mass index (BMI) was calculated as weight [kg] / sqrt(height [m]), which ranged from 20.1 to 34.5 kg/m^2 (mean=24.0). BMI was not significantly correlated with IOP in each postural condition (standing: p=0.28, supine: p=0.61, AGEC: p=0.43, AG2G: p=0.93). The correlations between BMI and *changes* in IOP relative to standing were not significant for supine vs. standing (p=0.12) and AG2G vs. standing (p=0.35). However, there was a significant, positive correlation between BMI and change in IOP from AGEC vs. standing (coefficient=0.46 mmHg/(kg/m^2), R=0.58, p=0.0095). This corresponds to subjects with higher BMIs having a greater increase in IOP in AGEC compared to when standing. Although IOP was not correlated to BMI, this finding should be investigated in future studies since this could imply that tissue weight is a factor in causing elevated IOP under centrifugation, as it has been shown to influence cardiovascular parameters in microgravity (7). We reiterate, though, that since this analysis was exploratory, we did not apply any correction for the multiple statistical tests being performed.

Finally, we assessed whether the order of the presentation of the postural conditions impacted IOP. Repeated contact to the cornea required for each measurement might lead to altered IOP. A RMANOVA with IOP as the dependent variable and presentation order as a categorical independent variable (to allow for any form of an order effect) found no significance (p=0.60).

DISCUSSION

This study measured IOP in AG and reports findings contrary to our initial hypothesis. Despite major differences between our experimental design and that investigated by Chung, our results are consistent with those found previously in very short duration AG exposure (10). We hypothesized IOP during AG would be consistent with standing values and below those in supine due to its dependency on cardiovascular parameters and hydrostatic gradients. This was not shown in the data; IOP in both AG conditions was consistent with supine and was statistically elevated from standing. But, cardiovascular status in AG was consistent with standing values and elevated from supine. This suggests IOP in AG is influenced by previously unconsidered factors.

The study yielded results in the standing and supine position consistent with the literature, indicating subjects were able to self-administer IOP measures accurately. IOP in the standing position was 15.7±1.4 mmHg, and 18.8±1.7 mmHg in the supine position, consistent with expected values (35, 41). Further, the change in IOP going between standing and supine positions was consistent with other studies. The change in IOP going from seated to supine has been reported to be approximately 2 mmHg (34, 41). Linder et al. also report typical IOP changes for subjects going from seated or standing to supine are between 2-4 mmHg (35). Since the self-administered IOP measures in standing and supine and in the transition between these postures match published values, it is likely that measurements in AG were reliable.

The TonoPen AVIA is an accurate device to measure changes in IOP with changes in posture. Compared to Goldmann applanation tonometry, the AVIA measures are similar (on average 0.5 mmHg higher in the seated position) with minimal bias on repeated measures (0.1 mmHg in the seated position and 0 mmHg in the supine position) (44). In another study involving 180 eyes (50 with glaucoma), the AVIA was found to be within 3 mmHg in 85.2% of subjects (5). A device that operates on a similar principle has also been shown to be accurate with novice users under self-administration, as compared to both a trained operator-made measurement (82% of measures within 3 mmHg) and Goldmann applanation tonometry (75% of measures within 3 mmHg) (3).

Self-tonometry has been performed in microgravity using automated devices (11, 23, 24). The TonoPen XL, which uses the same technology as the AVIA but averages fewer readings, has also been used extensively in spaceflight (46). In our evaluation of the device, the readings by a novice user were within 1mmHg of the trained operator's measures. In the experiment, subjects' self-administered readings were consistent with those taken by a trained operator during the screening process. Auditory cues were also used by the experimenter to verify in real-time the device was performing the measures properly. Therefore, it is unlikely that use of the AVIA or aspects of self-administration would have led to erroneous readings.

The AG conditions did not differ significantly from the supine values, but they were slightly lower. IOP was 18.8±1.3 mmHg in supine, 18.5±1.7 mmHg in AGEC, and 17.5±1.5 mmHg in AG2G. Since both AG conditions were statistically greater than standing, though, it is unlikely these results did not find significance due to a small sample size or small effect sizes.

Furthermore, subjects did not demonstrate issues performing IOP measurements under AG conditions. The number of readings taken to collect consistent values was similar in all four conditions. Rarely, subjects indicated arm fatigue while counteracting the forces from centripetal acceleration and were instructed to rest their arm by their side before taking another IOP reading. Subjects also demonstrated they retained their skill in self-administering between the training and testing day. The experimenter monitored subjects with a live video feed to ensure they were not straining or holding their breath while taking the readings, which could have increased IOP. The operator also remained in communication with the subjects during all phases of the experiment, requiring them to report how they felt every 5 minutes during centrifugation.

Subjects reported being comfortable making the measures in the AG conditions and did not report being anxious about centrifugation. Subject anthropometry (i.e., height, weight, and BMI), and gender were generally not statistically associated with changes in IOP. Therefore, it is unlikely these factors contributed to finding elevated IOP in AG.

In short radius centrifugation, there is a more extreme hydrostatic gradient than when standing (Figure 5). Within a closed fluid system, with a given quantity of fluid and assuming a fixed volume, there is a hydrostatic indifference point above (relative to the direction of gravity) which pressures are lowered and below which pressures are elevated (7). The hydrostatic pressure is dependent on the density of the fluid, the position relative to the hydrostatic indifference point, and the gravitational force acting on the fluid. In short-radius AG, our "G level" is not uniform across the body, increasing linearly with distance from the center of rotation. Thus, the gravity gradient causes a nonlinear hydrostatic gradient, increasing with the square of the radius. As a result, we expected venous pressures at eye level to be lower in AG than in standing, subsequently causing lower IOP in AG, since venous pressure at eye level determines IOP (2, 22, 35).

Although we did not quantify venous pressures, during AG the experimenters noted jugular venous distention while supine, followed by collapse through the spin-up phase in both AG conditions. This suggests the drainage pathway was consistent with that when standing, likely draining through the vertebral venous plexus, which occurs when venous pressures reach the point of hydrostatic indifference (26).

Our cardiovascular measurements were consistent with anticipated findings in AG. Iwase (28) showed that HR and MAP were elevated above supine values in AG. HR was increased in the AG conditions compared to supine, likely due to the baroreflex response caused by the fluid shift into the lower body. MAP was also likely elevated due to increased peripheral resistance with the fluid shift into the legs. Diaz (21) similarly reported total peripheral resistance and HR were elevated in response to centrifugation compared to a supine resting condition. These HR and MAP responses in short-radius AG matched ours, therefore similar cardiovascular mechanisms were likely activated.

Given the exclusion of each of these factors on IOP in AG, alternative hypotheses must be considered.

Alternative Hypotheses

It is well established through the Goldmann equation that IOP is dependent on aqueous humor production and outflow facility through the uveouscleral and trabecular mechanisms. The latter is dependent on episcleral venous pressure (6). Further, increased choroidal blood volume may also influence IOP (46). Therefore, we propose four potential mechanisms, three by which venous pressures could be elevated in AG (due to vasoconstriction in the legs, altered venous return due to leg muscles, or increased thoracic pressures due to tissue weight), and one in which choroidal blood volume could be elevated in AG.

The gravity gradients during AG cause an extenuated hydrostatic gradient compared to standing throughout the cardiovascular system, which could influence IOP. Specifically, the linear gravity gradient in AG (proportional to radius) yields a quadratic hydrostatic gradient (proportional to the square of the radius). While the two AG conditions both have a hydrostatic gradient and 1G at the subject's center of mass (Figure 5), AGEC has the largest gravity gradient caused by the higher required spin rate and change in distance from center of rotation to the feet, resulting in 2.37-2.71 G's (mean=2.51 G's) at the feet. In contrast, the standing condition has no gravity gradient (Figure 5).

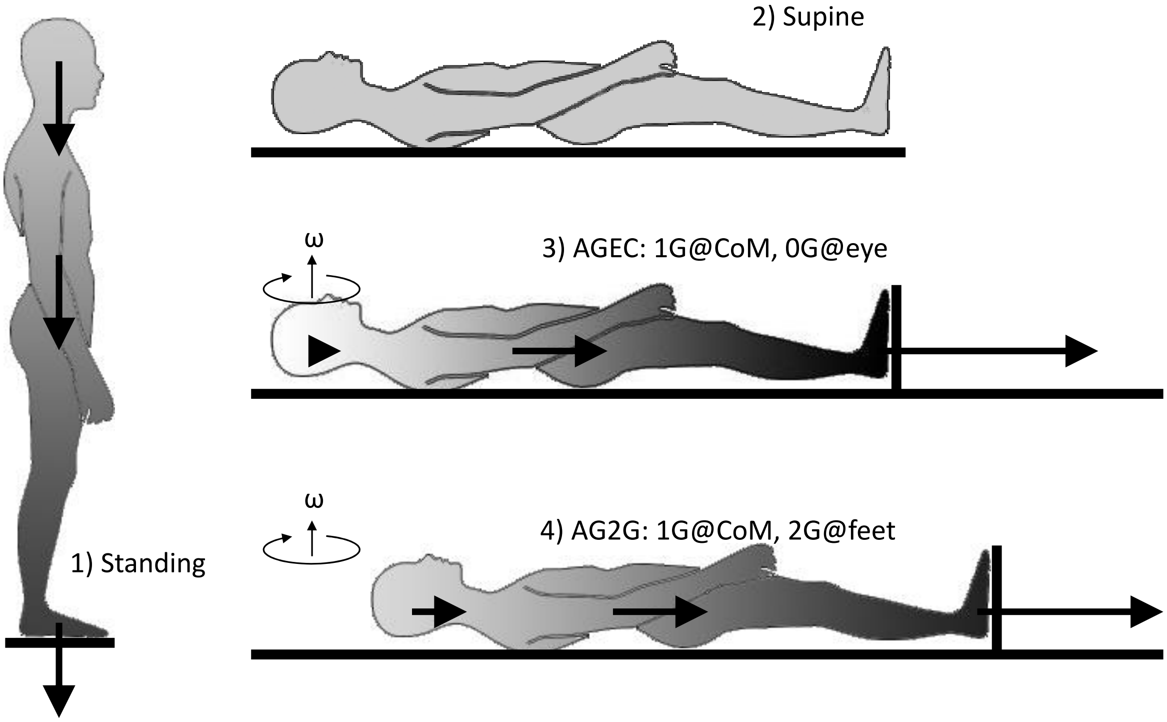

Figure 5: Pictorial representation of the longitudinal gravity gradients and hydrostatic pressure gradients of our four experimental postural conditions. For each condition, the longitudinal "gravity" loading at the eye, center of mass (CoM) and feet are shown with arrows. The resulting hydrostatic pressure gradients along the subject's longitudinal axis are shown with shading (darker gray corresponds to higher pressures).

Particularly for shorter subjects (i.e., those with higher BMI for a given weight), AGEC can have large gravity gradients (0 G's at the eye and up to 2.71 G's at the feet). This would lead to the pooling of blood in the legs to a greater extent than when standing. Cardiovascular regulation is governed by arterial baroreceptors in aortic arch, carotid sinus, and lungs to ensure arterial pressures are maintained. In AG, peripheral resistance is increased compared to supine resting conditions (21, 28). The body's regulatory systems in response to pooled blood in the legs could have increased systematic vasoconstriction, leading to unanticipated elevated venous pressures compared to supine. We also note that when standing there is 1 G of longitudinal loading at the eye location (Figure 5). In our short-radius AG conditions (Figure 5), at the eye there was either no loading (AGEC) or reduced loading (AG2G which produced 0.21-0.49 G's, mean=0.33 G's at the eye depending upon subject anthropometry). If the loading at the eye itself impacts IOP by some mechanism, it is possible these longitudinal loads were insufficient to reduce the IOP.

In the AG conditions of this study, the bed on which subject lay was on rails which allowed it to move radially with minimal friction (Figure 6A). This required subjects to support their own centripetal acceleration "weight" against the footplate, similar to standing. When supporting weight, the muscles in the legs are engaged, providing a similar experimental condition to standing, rather having the subjects supporting no weight under centrifugation. However, it also required subjects to support the centripetal acceleration "weight" of the bed (approximately 45 lbs). This is conceptually similar to supporting a heavy backpack when standing (Figure 6A). This required muscle activation to engage the lower body and maintain posture, even more so than in the standing posture. When engaged, the muscles in the legs alter blood flow and venous return, both through driving metabolic needs or by providing contractile resistance to outflow (20, 21) , making it desirable to achieve consistent muscle contraction with the standing condition. However, the additional "weight" of the bed could have altered venous return, beyond standing, leading to raised IOP in the AG conditions. Muscle contraction also influences parasympathetic withdrawal and sympathetic activity, which may also cause changes in heart rate and blood pressure (48).

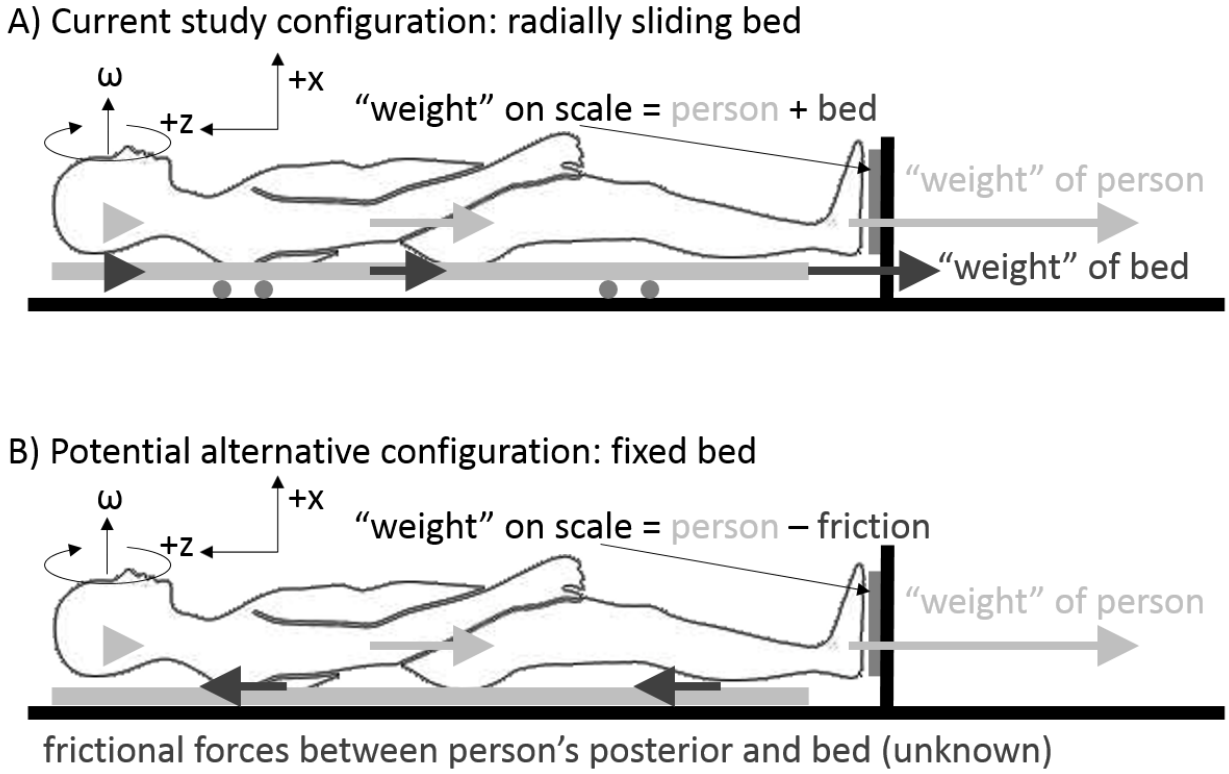

Figure 6: Configuration of the subject and bed in AG conditions. Panel A shows the configuration used in the current study. The bed (light gray) could move radially on rails (shown by dark gray circles between the bed and fixed centrifuge platform (black). This required the subject to support their own "weight" from centripetal acceleration, as well as that from the bed, against the footplate. A scale between their feet and the footplate reads this combined "weight". Panel B shows an alternate configuration in which the bed is fixed to the centrifuge platform. In this case, the subject does not support the weight of the bed. However, frictional forces between the subject's posterior and the bed, which are unknown, support at least some of the subject's "weight" from centripetal acceleration.

A future experiment could secure the bed radially such that subjects would not have to support it (Figure 6B). However, we note that friction between the bed and the subject’s posterior would at least partially support the “weight” of the subject radially. In this configuration, the required muscle engagement is unknown and would still not match that when standing. If subjects do not fully support their own weight, altering muscle activation, this would yield a notable difference in venous return between AG and standing conditions. Yet another alternative experimental configuration would be to have the bed side radially, but spin at a rate to create only the subject's weight on the scale (as opposed to the subject's weight and the bed's weight, like we did here). This approach would match the standing condition in terms of required supported weight. Although a consistent muscle activation of the legs would have been achieved, the hydrostatic gradients within the body’s fluids would not have been consistent with 1G at the subject's CoM.

Another important factor to consider is the effect of tissue weight, particularly in this Earth-based simulation of how AG would be implemented in microgravity. As shown in Figure 1, the forces acting on the body in AG on Earth are the force from centripetal acceleration and gravitational force. Combined, they create the net gravito-inertial force (GIF), which changes in magnitude and angle with increasing distance from the center of rotation. In other studies with increased acceleration negative x-direction (i.e., compressing the chest), central venous pressure increases (7). Central venous pressure also increases with increased body weight (7, 19). Similarly, when transitioning into microgravity, central venous pressure decreases compared to supine posture on Earth due to offloading of the chest compartment (7). In our study, the net GIF in AG could increase thoracic compression, increasing venous pressure, and subsequently elevating IOP. This effect, though, would not occur during AG in microgravity, since only the force of centripetal acceleration would be acting on the body, producing thoracic loading similar to standing on Earth. Future ground-based experiments could disambiguate the effect of tissue weight by positioning subjects in the lateral recumbent position under centrifugation.

IOP is also very sensitive to increases in choroidal blood volume. An increase of 20 μL of blood volume could lead to increases in IOP of 20 mmHg (46). The choroid does not have autoregulatory mechanisms. Increased blood volume could be caused by increased arterial or venous blood flow at eye level (46). Systemic vasoconstriction, which is likely consistent with subjects in AG, increases choroidal blood flow (25). But, since we did not measure choroidal blood flow and choroidal volume, advanced imaging, such as optical coherence tomography, is required to investigate this hypothesis (1, 2).

Implications for Spaceflight Associated Neuro-ocular Syndrome

The measured changes in IOP in each experimental condition are not clinically significant (i.e. they were consistent with those produced when going from standing or seated postures to supine postures). The importance of these findings, though, is that AG did not mitigate the increase in pressure in the supine position, which is caused by a fluid shift dominated by changes in hydrostatic gradients. These same factors influence IOP in microgravity, causing IOP to be initially elevated on orbit.

These findings are informative for future centrifuge AG designs in microgravity. Although this investigation involved short-term exposure to AG, this is consistent with proposed future implementations (12). Continuous AG created by a large-radius, rotating spacecraft is typically deemed infeasible due to the cost and engineering challenges. Prospective designs include short-radius centrifuges spun within the spacecraft modules or powered by human exercise to provide AG stimulus to the body (15, 21). The size and gravity gradients of these designs are similar to the centrifuge used in this study. In the near-term, AG could only be implemented in microgravity as an intermittent countermeasure to physiological deconditioning. Studies have demonstrated the effectiveness of AG for cardiovascular deconditioning when administered intermittently (28, 29, 49). Our findings, though, suggest short-radius centrifugation does not provide the same degree of intermittent alteration to the eye as it does to the cardiovascular system.

Not investigated in this research, though, was the effect of AG on ICP. Mechanisms by which ICP could contribute to SANS, while not necessarily being elevated to clinically relevant levels (such as in idiopathic intracranial hypertension) have been proposed. Mader et al. suggests local elevation of compartmentalized cerebrospinal fluid in the optic nerve in microgravity could create loading at the back of the eye (33, 36). Lawley et al. hypothesized that ICP may be mildly, chronically elevated in microgravity compared to aggregated ICP during typical activities on Earth, which are dominated by upright and seated postures (where ICP is lowest) (32). A change in the translaminar pressure gradient across the lamina cribrosa has been cited as one potential etiology for SANS (4, 50). IOP in microgravity increases initially, but then returns to pre-flight values (46). Therefore, future research should aim to quantify the effect of AG on both ICP and IOP, simultaneously.

Future bed rest studies that include an AG countermeasure are scheduled to begin at the :envihab research laboratory facilitated by the German Aerospace Center (DLR) and sponsored by NASA. Previous studies up to 70 days in head-down tilt bed rest performed by NASA did not find results consistent with SANS symptoms (17, 18). Notably, IOP was continuously elevated during bed rest, unlike in microgravity, and none of the SANS symptoms were reported, such as a shift in vision, globe flattening, or papilledema. The :envihab study will include 60 days of bed rest with intermittent AG to investigate its effectiveness to mitigate physiologic changes thought to contribute to SANS. This study, though, does not currently plan to measure IOP during centrifugation, but rather pre- and post-exposure. Chung found no statistical difference when standing between pre- and post- centrifugation, but their centrifuge exposure was brief and did not include subjects engaged in bedrest. The AG2G condition (i.e., creating 1G of centripetal acceleration at the subject's center of mass and 2G's at the feet) used here matches the proposed :envihab AG protocol. Our results will provide an additional data set which can be compared to the findings from long-duration bed rest with intermittent AG.

CONCLUSION

Intraocular pressure and cardiovascular parameters were measured in human subjects while standing, supine, and in AG. Two AG conditions were investigated while maintaining 1G at the center of mass: with the eye at the center of rotation, and with the eye radially displaced from the center of rotation with 2G's at the feet. Our results indicate that IOP in AG was maintained at supine levels, while HR and MAP were maintained at standing levels. Given the dependency of IOP on cardiovascular parameters and hydrostatic gradients, these results were contrary to our initial hypothesis. We propose that either venous pressures were elevated or choroidal blood volume was increased in AG beyond what was expected, due to systemic vasoconstriction in the legs, altered venous return due to leg muscles, increased thoracic pressures due to tissue weight, and/or elevated chroroidal blood flow. Future AG investigations should evaluate these potential mechanisms and should also investigate changes in ICP in conjunction with these measures. Our results inform the potential for future implementation of AG in microgravity, which will likely be an intermittent, short-term exposure of centrifugation.

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REFERENCES

1. **Anderson AP**, **Babu G**, **Swan JG**, **Phillips SD**, **Knaus DA**, **Toutain-Kidd CM**, **Zegans ME**, **Fellows AM**, **Gui J**, **Buckey JC**. Ocular changes over 60 min in supine and prone postures. *J Appl Physiol* 123: 415–423, 2017.

2. **Anderson AP**, **Swan JG**, **Phillips SD**, **Knaus DA**, **Kattamis NT**, **Toutain-Kidd CM**, **Zegans ME**, **Fellows AM**, **Buckey JC**. Acute effects of changes to the gravitational vector on the eye. *J Appl Physiol 1985* (December 10, 2015). doi: 10.1152/japplphysiol.00730.2015.

3. **Asrani S**, **Chatterjee A**, **Wallace DK**, **Santiago-Turla C**, **Stinnett S**. Evaluation of the ICare Rebound Tonometer as a Home Intraocular Pressure Monitoring Device: *J Glaucoma* 20: 74–79, 2011.

4. **Berdahl JP**, **Yu DY**, **Morgan WH**. The translaminar pressure gradient in sustained zero gravity, idiopathic intracranial hypertension, and glaucoma. *Med Hypotheses* 79: 719–724, 2012.

5. **Bhartiya S**, **Bali SJ**, **James M**, **Panda A**, **Dada T**. Test retest variability of TonoPen AVIA. *Indian J Ophthalmol* 61: 129–131, 2013.

6. **Brubaker RF**. Goldmann's equation and clinical measures of aqueous dynamics. *Exp Eye Res* 78: 633–7, 2004.

7. **Buckey JC**. Central Venous Pressure. In: *Gravity and the Lung: Lessons from Microgravity*, edited by Prisk G.K. WJB Paiva M. New York: Marcel Dekker, 2001.

8. **Buckey JC Jr**, **Gaffney FA**, **Lane LD**, **Levine BD**, **Watenpaugh DE**, **Wright SJ**, **Yancy CW Jr**, **Meyer DM**, **Blomqvist CG**. Central venous pressure in space. *J Appl Physiol* 81: 19–25, 1996.

9. **Buckey Jr JC**, **Gaffney FA**, **Lane LD**, **Levine BD**, **Watenpaugh DE**, **Wright SJ**, **Yancy Jr CW**, **Meyer DM**, **Blomqvist CG**. Central venous pressure in space. *J Appl Physiol* 81: 19–25, 1996.

10. **Chung KY**. Effects of +3Gz +2Gz and -1Gz on Intraocular Pressure (IOP). *Korean J Aerosp Environ Med* 11: 200–207, 2001.

11. **Chung KY**, **Woo SJ**, **Yi S**, **Choi GH**, **Ahn CH**, **Hur GC**, **Lim JG**, **Kim TW**. Diurnal pattern of intraocular pressure is affected by microgravity when measured in space with the pressure phosphene tonometer (PPT). *J Glaucoma* 20: 488–91, 2011.

12. **Clément G**. International roadmap for artificial gravity research. *Npj Microgravity* 3: 29, 2017.

13. **Clement G**, **Bareille MP**, **Goel R**, **Linnarsson D**, **Mulder E**, **Paloski WH**, **Rittweger J**, **Wuyts FL**, **Zange J**. Effects of five days of bed rest with intermittent centrifugation on neurovestibular function. *J Musculoskelet Neuronal Interact* 15: 60–68, 2015.

14. **Clement G**, **Bukley A**, **Paloski WH**. Artificial Gravity as a Countermeasure for Mitigating Physiological Deconditioning during Long-Duration Space Missions. *Front Syst Neurosci* 9, 2015.

15. **Clément GR**, **Charles J**, **Norsk P**, **Paloski WH**. Artificial Gravity: Evidence Report. NASA Johnson Space Center Human Research Program: 2015.

16. **Clément GR**, **Charles JB**, **Paloski WH**. Revisiting the needs for artificial gravity during deep space missions. *REACH - Rev Hum Space Explor* 1: 1–10, 2016.

17. **Cromwell R**, **G. Taibbi**, **S. Zanello**, **Yarbough P**, **R. Ploutz-Snyder**, **Vizzeri G**. Ocular Outcomes Comparison Between 14- and 70-day Head-down Tilt Bed Rest. HRP Investigators Workshop. NASA, 2015.

18. **Cromwell R**, **Zanello S**, **Yarbough P**, **Ploutz-Snyder R**, **Taibbi G**, **Vizzeri G**. 70 Days of 6 Degrees-head Down Tilt Bed Rest and its Impact on Ocular Parameters. International Society for Gravitational Physiology. 2014.

19. **De Divitiis O**, **Fazio S**, **Petitto M**, **Maddalena G**, **Contaldo F**, **Mancini M**. Obesity and cardiac function. *Circulation* 64: 477–482, 1981.

20. **Delp MD**, **Laughlin MH**. Regulation of skeletal muscle perfusion during exercise. *Acta Physiol Scand* 162: 411–419, 1998.

21. **Diaz Artiles A**. Exercise under artificial gravity-experimental and computational approaches. Massachusetts Institute of Technology: 2015.

22. **Draeger J**, **Hanke K**. Postural variations of intraocular pressure--preflight experiments for the D1-mission. *Ophthalmic Res* 18: 55–60, 1986.

23. **Draeger J**, **Schwartz R**, **Groenhoff S**, **Stern C**. Self-tonometry under microgravity conditions. *Clin Investig* 71: 700–3, 1993.

24. **Draeger J**, **Schwartz R**, **Groenhoff S**, **Stern C**. [Self tonometry during the German 1993 Spacelab D2 mission]. *Ophthalmologe* 91: 697–9, 1994.

25. **Friedman E**. Choroidal Blood Flow: Pressure-Flow Relationships. *Arch Ophthalmol* 83: 95, 1970.

26. **Gisolf J**, **van Lieshout JJ**, **van Heusden K**, **Pott F**, **Stok WJ**, **Karemaker JM**. Human cerebral venous outflow pathway depends on posture and central venous pressure. *J Physiol* 560: 317–27, 2004.

27. **Iwasaki K**, **Shiozawa T**, **Kamiya A**, **Michikami D**, **Hirayanagi K**, **Yajima K**, **Iwase S**, **Mano T**. Hypergravity exercise against bed rest induced changes in cardiac autonomic control. *Eur J Appl Physiol* 94: 285–291, 2005.

28. **Iwase S**, **Fu Q**, **Narita K**, **Morimoto E**, **Takada H**, **Mano T**. Effects of Graded Load of Artificial Gravity on Cardiovascular Functions in Humans. Environmental Medicine: 2002.

29. **Kaderka J**, **Young LR**, **Paloski WH**. A critical benefit analysis of artificial gravity as a microgravity countermeasure. *Acta Astronaut* 67: 1090–1102, 2010.

30. **Kos O**, **Hughson RL**, **Hart DA**, **Clément G**, **Frings-Meuthen P**, **Linnarsson D**, **Paloski WH**, **Rittweger J**, **Wuyts F**, **Zange J**, **Gorczynski RM**. Elevated serum soluble CD200 and CD200R as surrogate markers of bone loss under bed rest conditions. *Bone* 60: 33–40, 2014.

31. **Law J**, **Van Baalen M**, **Foy M**, **Mason SS**, **Mendez C**, **Wear ML**, **Meyers VE**, **Alexander D**. Relationship Between Carbon Dioxide Levels and Reported Headaches on the International Space Station: *J Occup Environ Med* 56: 477–483, 2014.

32. **Lawley JS**, **Petersen LG**, **Howden EJ**, **Sarma S**, **Cornwell WK**, **Zhang R**, **Whitworth LA**, **Williams MA**, **Levine BD**. Effect of gravity and microgravity on intracranial pressure. *J Physiol* (January 16, 2017). doi: 10.1113/JP273557.

33. **Lee AG**, **Mader TH**, **Gibson CR**, **Tarver W**. Space Flight–Associated Neuro-ocular Syndrome. *JAMA Ophthalmol* 135: 992, 2017.

34. **Lee TE**, **Yoo C**, **Kim YY**. Effects of different sleeping postures on intraocular pressure and ocular perfusion pressure in healthy young subjects. *Ophthalmology* 120: 1565–70, 2013.

35. **Linder B**, **Trick G**, **Wolf M**. Altering Body Position Affects Intraocular Pressure and Visual Function. *Investig Opthalmology Vis Sci* 29: 6, 1988.

36. **Mader TH**, **Gibson CR**, **Otto CA**, **Sargsyan AE**, **Miller NR**, **Subramanian PS**, **Hart SF**, **Lipsky W**, **Patel NB**, **Lee AG**. Persistent Asymmetric Optic Disc Swelling After Long-Duration Space Flight: Implications for Pathogenesis. *J Neuroophthalmol* 37: 133–139, 2017.

37. **Mader TH**, **Gibson CR**, **Pass AF**, **Kramer LA**, **Lee AG**, **Fogarty J**, **Tarver WJ**, **Dervay JP**, **Hamilton DR**, **Sargsyan A**, **Phillips JL**, **Tran D**, **Lipsky W**, **Choi J**, **Stern C**, **Kuyumjian R**, **Polk JD**. Optic disc edema, globe flattening, choroidal folds, and hyperopic shifts observed in astronauts after long-duration space flight. *Ophthalmology* 118: 2058–69, 2011.

38. **Marshall-Bowman K**, **Barratt M**, **Gibson C**. Ophthalmic changes and increased intracranial pressure associated with long duration spaceflight: An emerging understanding. *Acta Astronaut* 87: 77–87, 2013.

39. **Mulder E**, **Linnarsson D**, **Paloski WH**, **Rittweger J**, **Wuyts FL**, **Zange J**, **Clement G**. Effects of five days of bed rest with and without exercise countermeasure on postural stability and gait. *J Musculoskelet Neuronal Interact* 14: 359–366, 2014.

40. **Otto C**, **Ploutz-Snyder R**, **Samuels B**, **Gibson C**, **Sargsyan A**, **Patel N**, **Riascos R**, **Garcia K**, **Kramer L**, **Alexander D**, **Lee S**. The Prospective Observational Study of Ocular Health in International Space Station (ISS) Astronauts: The Visual Impairment Intracranial Pressure Risk (VIIP) [Online]. NASA Human Research Program Investigator's Workshop http://ntrs.nasa.gov/archive/nasa/casi.ntrs.nasa.gov/20160001725.pdf.

41. **Ozcan MS**, **Praetel C**, **Bhatti MT**, **Gravenstein N**, **Mahla ME**, **Seubert CN**. The effect of body inclination during prone positioning on intraocular pressure in awake volunteers: a comparison of two operating tables. *Anesth Analg* 99: 1152–8, table of contents, 2004.

42. **Reschke MF**, **Bloomberg JJ**, **Paloski WH**, **Mulavara AP**, **Feiveson AH**, **Harm DL**. Postural Reflexes, Balance Control, and Functional Mobility with Long-Duration Head-Down Bed Rest. *Aviat Space Environ Med* 80: A45–A54, 2009.

43. **Rittweger J**, **Bareille MP**, **Clement G**, **Linnarsson D**, **Paloski WH**, **Wuyts F**, **Zange J**, **Angerer O**. Short-arm centrifugation as a partially effective musculoskeletal countermeasure during 5-day head-down tilt bed rest-results from the BRAG1 study. *Eur J Appl Physiol* 115: 1233–1244, 2015.

44. **Schweier C**, **Hanson JV**, **Funk J**, **Töteberg-Harms M**. Repeatability of intraocular pressure measurements with Icare PRO rebound, Tono-Pen AVIA, and Goldmann tonometers in sitting and reclining positions. *BMC Ophthalmol* 13: 44, 2013.

45. **Smith SM**, **Zwart SR**, **Heer MA**, **Baecker N**, **Evans HJ**, **Feiveson AH**, **Shackelford LC**, **LeBlanc AD**. Effects of artificial gravity during bed rest on bone metabolism in humans. *J Appl Physiol* 107: 47–53, 2009.

46. **Stenger M**, **Tarver W**. Risk of Spaceflight Associated Neuro-ocular Syndrome (SANS) [Online]. NASA Johnson Space Center Human Research Program: 2017. https://humanresearchroadmap.nasa.gov/evidence/reports/SANS.pdf.

47. **Taibbi G**, **Cromwell RL**, **Kapoor KG**, **Godley BF**, **Vizzeri G**. The effect of microgravity on ocular structures and visual function: a review. *Surv Ophthalmol* 58: 155–63, 2013.

48. **Victor RG**, **Pryor SL**, **Secher NH**, **Mitchell JH**. Effects of Partial Neuromuscular Blockade on Sympathetic Nerve Responses to Static Exercise in Humans. *Circ Res* 65: 10, 1989.

49. **Wang Y-C**, **Yang C-B**, **Wu Y-H**, **Gao Y**, **Lu D-Y**, **Shi F**, **Wei X-M**, **Sun X-Q**. Artificial gravity with ergometric exercise as a countermeasure against cardiovascular deconditioning during 4 days of head-down bed rest in humans. *Eur J Appl Physiol* 111: 2315–2325, 2011.

50. **Zhang LF**, **Hargens AR**. Intraocular/Intracranial pressure mismatch hypothesis for visual impairment syndrome in space. *Aviat Space Env Med* 85: 78–80, 2014.

51. **Zwart SR**, **Gibson CR**, **Gregory JF**, **Mader TH**, **Stover PJ**, **Zeisel SH**, **Smith SM**. Astronaut ophthalmic syndrome. *FASEB J* 31: 3746–3756, 2017.

52. **Zwart SR**, **Gibson CR**, **Mader TH**, **Ericson K**, **Ploutz-Snyder R**, **Heer M**, **Smith SM**. Vision Changes after Spaceflight Are Related to Alterations in Folate- and Vitamin B-12-Dependent One-Carbon Metabolism. *J Nutr* 142: 427–431, 2012.

FIGURE LEGENDS

Figure 1: The four experimental postural conditions: 1) Standing creates 1G in the –z-direction (footward) from gravity; 2) Supine removes the –z-direction loading, but still has –x-direction loading from gravity; 3) artificial gravity with the eye-centered (AGEC) has the subject supine, positioned with their eye at the center of rotation of the centrifuge, and spun at a rate to produce 1G of centripetal acceleration (the force from which is in the –z-direction) at the subject's center of mass; 4) AG2G has the subject positioned and spun at a rate to produce 1G of centripetal acceleration at the subject's center of mass (as in AGEC), but now positioned to produce 2G at the subject's feet. AG2G required the subject's eye to be radially displaced from the centrifuge center of rotation causing a –z-direction force from centripetal acceleration. Of course, in both AG conditions, the –x-direction loading from gravity remains, yielding a net loading in the x-z plane that varies in direction and magnitude along the length of the subject's body.

Figure 2: IOP response in Standing, Supine, and the two AG conditions. Panel A shows the raw data for each subject (squares=females, circles=males). The bold shapes highlight measurements with large standardized residuals from the initial RMANOVA model fit (Δ=3.71, ◊=2.99 originally, but when the data of the subject with the first outlier is removed, increased to 3.28). Each subject is shifted laterally slightly to avoid overlapping. The bars represent means in each condition (including all measurements). Panel B shows the change in IOP from the Standing condition (positive values correspond to an increase in IOP relative to Standing). The bars depict the average change across subjects and the error bars are 95% confidence intervals (excluding two outlier subjects), without any correction for multiple comparisons.

Figure 3: Heart rate (HR) response in Standing, Supine, and the two AG conditions. Panel A shows the raw data for each subject (squares=females, circles=males). The filled gray shapes are for the two subjects in which there was missing data for another condition(s). Each subject is shifted laterally slightly to avoid overlapping. The bars represent means in each condition (including all measurements). Panel B shows the change in HR from the Standing condition (positive values correspond to an increase in HR relative to Standing). The bars depict the average change across subjects and the error bars are 95% confidence intervals (excluding two subjects with missing data), without any correction for multiple comparisons.

Figure 4: Mean arterial pressure (MAP) response in Standing, Supine, and the two AG conditions. Panel A shows the raw data for each subject (squares=females, circles=males). The filled gray shapes are for the two subjects in which there was missing data for another condition(s). Each subject is shifted laterally slightly to avoid overlapping. The bars represent means in each condition (including all measurements). Panel B shows the change in MAP from the Standing condition (positive values correspond to an increase in HR relative to Standing). The bars depict the average change across subjects and the error bars are 95% confidence intervals (excluding two subjects with missing data), without any correction for multiple comparisons.

Figure 5: Pictorial representation of the longitudinal gravity gradients and hydrostatic pressure gradients of our four experimental postural conditions. For each condition, the longitudinal "gravity" loading at the eye, center of mass (CoM) and feet are shown with arrows. The resulting

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