Hypercapnia augments resistive exercise-induced elevations in intraocular pressure in older individuals

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NEW FINDINGS

What is the central question of this study?

Astronauts onboard the International Space Station (ISS) perform daily exercises designed to prevent muscle atrophy and bone demineralisation. The present study assessed the effect of resistive exercise performed by subjects while exposed to the same level of hypercapnia as on the ISS on intraocular pressure (IOP).

What is the main finding and its importance?

The static exercise-induced IOP elevation in intraocular pressure during 6° prone head down tilt (simulating the headward shift of body fluids in microgravity) is augmented by hypercapnia, and exceeds the ocular hypertension threshold.

ABSTRACT

The present study assessed the effect of 6° head down (establishing the cephalad displacement noted in astronauts in microgravity) prone (simulating the effect on the eye) tilt during rest and exercise (simulating exercise performed by astronauts to mitigate the sarcopenia induced by unloading of weight-bearing limbs), in normocapnic and hypercapnic conditions (the latter simulating conditions on the International Space Station) on IOP.

Volunteers (average age = 57.8 ± 6 yrs.; N=10) participated in two experimental sessions, each comprising: i) 10-min rest, ii) 3-min handgrip dynamometry (30% max), and iii) 2-min recovery, inspiring either room air (NCAP), or a hypercapnic mixture (1% CO_2, HCAP). We measured IOP in the right eye, cardiac output (CO), stroke volume (SV), heart rate (HR) and mean arterial pressure (MAP) at regular intervals.

Baseline IOP in the upright seated position while breathing room air was 14.1 ± 2.9 mmHg. Prone 6° HDT significantly ($p < 0.01$) elevated IOP in all three phases of the NCAP (rest: 27.9 ± 3.7 mmHg; exercise: 32.3 ± 4.9 mmHg; recovery: 29.1 ± 5.8 mmHg) and HCAP (rest: 27.3 ± 4.3 mmHg; exercise: 34.2 ± 6.0 mmHg; recovery: 29.1) trials, with hypercapnia augmenting the exercise-induced elevation in IOP ($p < 0.01$). CO, SV, HR and MAP were significantly increased during handgrip dynamometry, but there was no effect of hypercapnia.

The observed IOP measured during prone 6°HDT in all phases of the NCAP and HCAP trials exceeded the threshold pressure defining ocular hypertension. The exercise-induced increase in IOP is exacerbated by hypercapnia.

INTRODUCTION

A major hindrance to future long-term missions on the International Space Station (ISS), and to the exploration of the Moon and Mars are the unexplained ophthalmic changes observed in a subset of astronauts. Recently, it has been reported that the majority of astronauts participating in long-term missions on the ISS experience significant visual impairment, which manifests as blurred vision for near and distance (Mader *et al.,* 2011), as well as significant morphological changes in the eye and optic nerve, including optic disc oedema, choroidal folds, and retinal haemorrhages (Mader *et al.,* 2011; Nelson *et al*., 2014). Globe flattening and optic nerve protrusion (Nelson *et al.,* 2014; Kramer *et al.,* 2012) have also been described in some individuals. Some of these symptoms and signs are not fully reversible. Although the functional degradation of vision in astronauts exposed to microgravity has been known for some time, the associated morphological changes have only been observed recently. That is, because these ocular changes occurred more frequently, and worsened with longer durations, and repetitive exposure to microgravity. The unresolved aetiology and high incidence of these morphological and visual changes among astronauts jeopardises all future long-term missions on the ISS, as well as planned missions to the Moon and Mars.

This, as yet unresolved, vision impairment has been attributed primarily to the increase in intracranial pressure (ICP) associated with spaceflight, which impacts on the vision neuro-axis. As a consequence, the phenomenon is termed Spaceflight Associated Neuro-ocular Syndrome (SANS). Numerous studies have provided evidence regarding the manner in which different stressors impact on ICP and intraocular pressure (IOP). The effects reported so far do not suggest that these factors would affect vision in the long term (cf. Stenger and Tarver 2017). One issue may be that the bed rest models used in the investigations, namely either horizontal or 6° head down tilt (HDT) supine bed rest, may not adequately represent the fluid shifts in the eye. Another issue is that many of the factors identified as affecting ICP and IOP may occur simultaneously, and their synergistic effect may be of clinical significance, although each of these alone have only minimal effects.

Ground-based studies simulate the effects of weightlessness with the experimental bed rest model, since adaptation of physiological systems to microgravity are similar to the adaptations observed during inactivity and unloading of the weight bearing limbs (for review see Pavy-Le Traon *et al.,* 2007). With the exception of the report by Drozdova and Nesterenko (1969), deterioration in visual function during the course of prolonged experimental or clinical bed rest has not been previously observed (Jaki Mekjavic *et al.,* 2002). It would therefore appear that the current bed rest protocols do not completely mimic the effects of weightlessness on the eye, and may be of limited value as a ground-based model for studying the effects of vision deterioration in Space. Previous longer duration bed rest studies have extensively documented the effect on cardiovascular, locomotor, haematologic and neurovestibular systems (Pavy-Le Traon *et al.,* 2007; Jost, 2008). Earlier studies on the effect of

simulated microgravity on the eye have mainly focused on IOP, the results of which were equivocal (Chiquet *et al.,* 2003; Jaki Mekjavic *et al.,* 2002). Ocular changes in these studies, when observed, were subclinical, and did not significantly affect visual function (Jaki Mekjavic *et al.,* 2002; Taibbi *et al.,* 2014). With newer diagnostic methods, especially optical coherence tomography (OCT) some significant morphological changes in the eye were observed also under similar experimental conditions. Subtle increases in peripapillary retinal thickness and peripapillary retinal volume measured by OCT were reported in a subject after a 30-day 6° head-down-tilt bed rest (HDT BR). After 6 months, the OCT measurements matched baseline findings (Taibbi *et al.,* 2013). Increased subfoveal choroidal thickness without significant change in foveal retinal thickness, but increased IOP after 30 minutes of 10° HDT (Shinojima *et al.,* 2012) has also been previously reported. Recently, an increase in retinal nerve fibre layer (RNFL) thickness was observed (but not in peripapillary retinal thickness and volume), after 10 days in 16 participants enrolled in 14-day HDT BR by the same group of investigators (Taibbi *et al.,* 2014). Subsequent studies by Taibbi *et al.* (2016) reported that ocular changes in 14 and 70-day HDT were comparable.

Supine bed rest studies conducted to date have not yielded any significant clinical evidence of the source of vision impairment in astronauts. The duration of any prone bed rest studies will probably be limited by the discomfort this may cause. Such discomfort includes backache, difficulty with eating, hygiene, etc. Parabolic flight studies have provided valuable data regarding the brief acute effect of microgravity on ICP and IOP (Lawley *et al*., 2017); however, these are of limited benefit in predicting long-term exposures to microgravity. There is no doubt that SANS is a result of a microgravity-induced adaptive response, which can only be determined with long-term exposures to microgravity, or relevant simulations thereof.

Our previous work demonstrated that the vessels in the neuroretina are vasoactive, reacting to both changes in the oxygen and carbon dioxide (CO_2) partial pressure in the arterial blood, and that the vessels in the choroid are predominantly affected by the hydrostatic component (Jaki Mekjavic *et al*., 2016, Louwies *et al*., 2016). In the study of Jaki Mekjavic *et al.* (2016) the hypercapnic breathing mixture mimicked the level of hypercapnia present on the ISS (Taylor et al., 2013).

In the present ground-based study we assessed the effect of 6° head down (establishing the cephalad displacement noted in astronauts in microgravity) prone (simulating the effect on the eye) tilt during rest and exercise (simulating exercise performed by astronauts to mitigate the sarcopenia induced by unloading of weight-bearing limbs), in normocapnic and hypercapnic conditions (the latter simulating conditions on the ISS) on IOP. In the event of a significant effect, it would then be warranted in future studies to assess the contribution of any adaptive mechanisms of daily hypercapnic exercise to SANS, during prolonged exposures to microgravity.

METHODS

The establishment of a suitable protocol for assessing the potential effect of exercise in the hypercapnic environment of the ISS was predicated on the fact that astronauts conduct strenuous resistive and aerobic exercise on a daily basis in a normoxic microgravity environment with the average fraction of CO_2 in excess of 0.008 (i.e. 0.8% CO_2 in the normobaric environment of the ISS). To mimic the microgravity-induced cephalad displacement of fluid during exposure to microgravity and its effect on the eye, subjects were prone in the 6° HDT position during the experimental trial. Finally, to match the age range of the astronauts working on the ISS, the subject inclusion criteria was age between 45 and 65.

A further aim was to establish a protocol, which could be replicated by astronauts during a mission on the ISS.

Ethical approval

The study conformed to the standards set by the Declaration of Helsinki, except for registration in a data base. The procedures were approved by the University of Portsmouth (United Kingdom) Science Faculty Ethics Committee (approval no. SFEC 2019-040). Subjects were informed regarding the nature of the study and details of the experimental protocol and measurements. Prior to participating in the study they gave their written informed consent. They were aware that they could terminate the trial, and withdraw from the study, at any time.

Subjects

Ten older and healthy male volunteers (age: 57.8 ± 6.0 years, range: 48 to 65 years; weight: 80.9 ± 8.2 kg; height: 178.9 ± 4.9 cm; max handgrip strength: 40.2 ± 6.7 kg) gave their informed consent to participate in the study. Exclusion criteria included high blood pressure, any acute or chronic ophthalmic disorders, and any condition which would render participants incapable of conducting the hand grip dynamometry in the 6°HDT prone position.

Protocol

Participants were requested to participate in two experimental sessions separated by a minimum of 24 hrs. Prior to the first experimental session we measured each participant's IOP in a standard clinical sitting position using 2 tonometry methods.

Each experimental session comprised two exercise trials. The two exercise trials conducted on the same day were separated by 30 minutes. All exercise trials were conducted in the 6°HDT prone position and each comprised 3 phases: i) 10-min rest phase, ii) a 3-min static handgrip exercise (dynamometry), and iii) 2-min recovery phase, as seen in Figure 1. During the static handgrip dynamometry phase, subjects maintained a grip force which was 30% of their maximum grip force measured previously. In each experimental session, subjects conducted one trial breathing normocapnic normoxic room air (NCAP), and the other breathing a hypercapnic normoxic mixture containing 1% CO_2 (HCAP). The order of NCAP and HCAP trials was counterbalanced and for each subject, the order of the trials was switched in the second experimental session conducted on a different day. The reason for conducting the NCAP and HCAP trials in one session was twofold. We wished to assess the magnitude of the carryover effect, if any, of fatigue. Furthermore, in the sessions where HCAP trial was the first trial, we wished to observe any carryover effect of the HCAP trial on the responses in the succeeding NCAP trial. As mentioned above, these additional objectives were included as a prelude to the possibility of having the opportunity to conduct the protocol on astronauts prior to and upon return from a short duration mission on the ISS, as well as having the astronauts conduct such a protocol on the ISS.

Normocapnic and hypercapnic trials

Upon instrumentation, participants assumed the prone 6° HDT position, and were fitted with a nose clip and a mouthpiece connected to a two-way non-rebreathing valve (Hans Rudolph Inc, Shawnee, Kansas, USA). Resting IOP and MAP measurements were obtained at 5 minute intervals during the 10-min rest period (BL, R5, R10), each minute of the 3-min exercise period (T1–T3; isometric handgrip exercise at 30% of their maximum, using a hand-held dynamometer) and 2-min recovery (P1-P2) period (Fig. 1). To avoid any influence of Valsalva manoeuvres on any of the measured variables, all subjects were instructed to maintain normal respiratory patterns.

The second trial was conducted following a 30 min rest period during which subjects were required to be upright. The protocol for both trials was identical, with the exception of the gas inhaled. In the NCAP trial the inspired gas was normocapnic and normoxic, whereas in the HCAP trial it was hypercapnic and normoxic.

Intraocular pressure (IOP, mmHg) measurement in a standard clinical sitting position

Prior to commencing the experimental protocol (described above), baseline measurements of IOP were obtained from each subject in the standard clinical sitting position using two tonometry methods: i) rebound tonometry with an Icare® model ic200 tonometer (Icare Finland Oy, Vantaa, Finland) and, ii) pneumotonometry with a Pulsair IntelliPuff Tonometer (Keeler, Windsor, United Kingdom). Triplicate measurements were obtained from the right eye with each tonometer. The pneumotonometer was adopted in the subsequent experimental trials, as the rebound tonometer was not capable of measuring IOP with participants in the prone position. Icare tonometer results obtained in the seated position were correlated with the seated Pulsair measurements to confirm that the measurements obtained with pneumotonometry would be comparable to those obtained with rebound tonometry. All measurements of IOP were conducted by the same investigator.

Maximal handgrip strength measurement

To measure maximal handgrip strength, participants assumed a prone 6°HDT position on a table with their humerus parallel to the torso, elbow maintained at 90° angle, and the Jamar (JLW Instruments, Chicago, IL, USA) hand-held hydraulic dynamometer in their right hand. On instruction, participants maximally squeezed the dynamometer twice for 5 seconds, with a 2 min rest between the exertions. Strong verbal encouragement was provided throughout both trials. Participant's maximum handgrip strength was determined as the highest force maintained in the two trials.

Impedance plethysmography and sphygmomanometry

On arrival to the laboratory, participants were instrumented with 6 electrodes for the measurement of impedance electrocardiography (Physioflow Q-Link, Manatec Biomedical, Paris, France). Two electrodes were positioned on the left side of the participant's neck, one in the middle of the sternum, one on the rib closest to V6, and two next to the midpoint of the spine. The Physioflow device provided continuous recording of heart rate (HR, min^{-1}), cardiac output (CO, $L \cdot min^{-1}$), and stroke volume (ml). Impedance electrocardiography has previously been validated against the direct Fick method during exercise in healthy participants (Richard *et al*., 2001; Siebenmann *et al*., 2015). Blood pressure (BP) was measured using a Withings (Issy-les-Moulineaux, France) model BP-800 automated sphygmomanometer, from which mean arterial pressure (MAP) was calculated from values of systolic (SAP) and diastolic (DAP) pressures. Withings BP-800 device fulfills the validation criteria of the European Society of Hypertension International Protocol Revision 2010 (Topouchian et al., 2014).

Statistical considerations

The sample size determination in the current study was based on the following criteria: a) power (1 - β) = .80, b) α = .05, c) two groups (normocapnic and hypercapnic), d) four repeated measures (baseline measure, resting measure, hand grip dynamometry, recovery) and e) effect size = 0.25. The analysis showed that the minimum total sample for the specific study was 24 cases (Faul et al., 2007).

All data were assessed for normality using the Shapiro-Wilk and Kolmogorov-Smirnov test of normality. A paired samples t-test was conducted to assess the difference between IOP measured in the standard clinical sitting position using two tonometry methods. Two-way repeated measures analysis of variance (ANOVA) with a Bonferroni correction was conducted to assess the impact of hypercapnia and normocapnia on 3 min isometric handgrip exercise in prone 6°HDT position at 8 different time points, and to determine, if differences existed between the said exercise being conducted in normocapnic or hypercapnic conditions. A paired samples t-test was used to further investigate the significant differences between. In order to observe the effect of time on each of the

measured variables, one-way ANOVA was conducted. To assess the magnitude of the carryover effect of performing both NCAP and HCAP trials in one session, a paired t-test with a Bonferroni adjustment was performed. The significance level for all statistical tests in this study was set at $p \leq 0.05$. Only participants with full data sets for each measured variable were included in the statistical analysis, therefore for some variables *n* is not equal to the total number (20) of the tests.

Descriptive statistics were expressed as mean ± SD. All statistical analysis was performed using SSPS (v.25, Chicago, IL, USA) computer software. Cohen's standardised-mean difference test was used to assess the effect sizes of the change in variables, and defined as small when $d \leq 0.2$, moderate when $d \leq 0.6$, large when $d \leq 1.2$, very large when $d \leq 2.0$, and extremely large effect when $d \leq 4.0$ (Hopkins *et al*., 2009).

RESULTS

The IOP measurements derived with rebound tonometry were significantly ($p = 0.008$) lower (11.8 ± 1.9 mmHg) than the measurements derived with pneumotonometry (14.1 ± 2.9 mmHg), obtained with the subjects breathing room air in the seated position, as evident from Table 1. When the subjects assumed the prone 6° HDT position IOP obtained with the pneumotonometer increased to 27.9 ± 3.7 mmHg.

As shown in Table 2, normocapnic isometric exercise (NCAP trial) caused a significant increase in MAP (from 99 ± 6 at rest to 137 ± 12 mmHg upon completion of the 3-min handgrip), with a corresponding increase in IOP (from 27.9 ± 3.7 mmHg during rest to 32.3 ± mmHg upon completion of the exercise; Fig. 2). Hypercapnia (HCAP trial) did not significantly modify the responses of MAP, HR, CO, and SV (Table 1), but did enhance the IOP response, which increased from resting levels of 27.3 ± 4.3 mmHg to 34.2 ± 6.0 mmHg (Fig. 2).

Results of a two-way repeated measures ANOVA revealed a significant time ($F(4.225, 80.277) = 35.066$; $p < 0.001$) and condition effect ($F(1, 19) = 8.351$; $p = 0.009$), and significant interaction between time and condition for IOP ($F(7, 133) = 0.873$; $p = 0.044$). Further tests (Fig. 2) revealed that the IOP level during NCAP trial was significantly lower than that in the HCAP trial at BL (23.4 ± 3.3 mmHg vs. 25.0 ± 3.8 mmHg; $p = 0.029$; $d = 0.45$), T1 (28.6 ± 3.8 mmHg vs. 30.1 ± 5.4 mmHg; $p = 0.042$; $d = 0.32$), T2 (30.0 ± 4.3 vs. 31.9 ± 4.8 mmHg; $p = 0.049$; $d = 0.42$) and T3 (32.2 ± 4.8 vs. 34.2 ± 6.0 mmHg; $p = 0.017$; $d = 0.37$). Additionally, IOP during both HCAP and NCAP were significantly higher at T2 and T3 compared to R10 (NCAP: $p = 0.044$ and $p = 0.005$ respectively; HCAP: $p = 0.001$, $p < 0.001$ respectively).

Fig. 3 compares the last minute of the three phases in both NCAP and HCAP trials, namely the 10-minute rest, 3-min exercise, and 2-min recovery. As evident, HCAP significantly augmented the IOP

during the handgrip dynamometry, but did not have a significant effect during rest and recovery. Noteworthy is the substantial individual variation observed in the IOP response.

DISCUSSION

The principal finding of the present study is of the substantial elevation of IOP in the prone 6° HDT position compared to the seated upright position, which was further augmented by both hypercapnia in combination with static exercise. Our results confirm the findings of Laurie *et al.* (2017) of an elevation in resting IOP during supine 6°HDT. However, in the present study, the prone 6°HDT elevated IOP much more substantially than was observed in the supine position. Whereas Laurie *et al.* (2017) observed a significant effect of CO_2 during resting conditions (supine 6°HDT), we did not (prone 6°HDT). Rather, we observed the hypercapnic-augmentation of IOP during the static exercise.

The baseline (seated upright) value of IOP observed in our subjects (14.1 ± 2.9 mmHg) was similar to that previously reported for a slightly older (64 ± 9.6 years) population of 3135 participants in the Beijing Eye study 2011 (Wang *et al.,* 2018). In both studies IOP was obtained using pneumotonometry, which gives higher values of IOP compared to Goldmann Applanation Tonometry (GAT; Mohan *et al.,* 2014). During the rest period in the 6° HDT position, IOP increased to 27.9 ± 3.7 mmHg in the NCAP and 27.3 ± 4.3 mmHg in the HACP, there being no significant difference in the IOP between the two trials. Clinically, IOP measured with GAT has a normal upper level of 21 mmHg, and values above this are considered as indicative of ocular hypertension (The Royal College of Ophthalmologists, 2015), if there is no associated optic disc damage or visual field defect. IOP recordings above 30 mmHg are considered detrimental, and are treated. The resting values of IOP in these trials already exceeded the threshold IOP of 21 mmHg (Fig. 2).

Assuming that the prone 6° HDT position is a better analogue of the conditions within the eye experienced by astronauts on the ISS, it is clear from Figs. 2 and 3 that their IOP may be consistently elevated above the threshold for open angle glaucoma (OAG), and recurrently even further elevated during the exercise conducted on the advanced resistive exercise device (ARED).

Despite the CO_2-potentiation of the exercise-induced increase in IOP in older subjects, there was no difference in the MAP response between the NCAP and HCAP trials. This supports our previous finding that the diameter of the vessels in the choroid are affected by CO_2, and this effect may contribute to the elevated IOP. The mechanisms for these responses and their potential contribution to SANS are discussed below. In particular, the manner in which a combination of these factors, as experienced by astronauts on the ISS may maintain a significant trans-lamina cribrosa pressure difference above the threshold for glaucoma damage for the duration of their mission, may expose them to the risk of glaucomatous pathology.

Intracranial pressure

Increased ICP has been postulated as contributing to the aetiology of SANS (Alexander *et al.,* 2012), due to its direct effect on IOP (Salman, 1997; Lashutka *et al.,* 2004). During exposure to microgravity, the cephalad displacement of fluids coupled with the hypercapnia-induced increase in cerebral blood flow (Sato *et al.,* 2012; Willie *et al.* 2012), and exercise-induced elevation in arterial blood pressure (Mcdougall *et al.,* 1992) most likely causes a substantial increase in ICP and consequently IOP.

Gravitational vector

Gravity, or rather the hydrostatic pressure gradients resulting from changes in the gravitational vector, is the major culprit in the aetiology of the observed hyperopic shift in astronauts during prolonged exposure to weightlessness. The source of the changes in hydrostatic pressures within the eye (IOP) and brain (ICP) is the headward shift of body fluids. However, bed rest studies simulating the effects of exposure to microgravity have, so far, not been successful in uncovering any changes that could lead to the hyperopic shift and vision deterioration observed in astronauts, specifically hyperopia. The first, and most extensive programme of research regarding the effect of gravity acting on the eye was initiated by Levinsohn in 1912 (for review of Levinsohn's work see Young 1964, 1973), who exposed monkeys to a 10° prone HDT for several hours each day for up to 90 days, and reported the development of significant axial length myopia in his subjects. Interestingly, HDT BR, but in the supine position, is an experimental model used by NASA and ESA to mimic the cephalad displacement of body fluids, as observed in Space, with the exception that the angle is only 6°. The hydrostatic pressure changes caused by bed rest have been shown to increase the diameter of the retinal venules and arterioles (Louwies *et al.,* 2016). However, more importantly, this hydrostatic effect also increased the thickness of the choroid layer within the posterior eye segment (Jaki Mekjavic *et al*., 2016). These changes, although significant, would not be expected to lead to such vision changes observed by Levinsohn in primates (see Young 1964, 1973), and Mader *et al.* (2011, 2013) in astronauts. There is, however a striking difference between the HDT studies conducted by Levinsohn and those currently conducted by ESA and NASA, which is most likely the key for ground-based studies on space hyperopic shift. The primates in Levinsohn's experiment were only exposed to the HDT for several hours a day, but they were in the prone position. All bed rest studies to date require the subjects to be in bed rest continuously, and in the supine position. Another crucial aspect of Levinsohn's study is that during the 10° head down tilt the monkeys' heads were positioned at a distance of 14 cm from the table surface. Food was placed on the table to ensure that the majority of time was spent accommodating, in order to focus on the food. This arrangement was designed to mimic the distance in humans from the eyes to a surface, when reading and writing. As a consequence of the accommodation effort, the experiments resulted in an increase in axial length. In the absence of

the accommodation effort, the prone HDT may induce the changes observed as a result of prolonged exposure to microgravity.

Posture: supine v. prone

The eye, containing the gelatinous vitreous fluid in the posterior segment is encased in a cavity, the orbit, where it is surrounded by tissue (extraocular muscles and fat). Despite the evidence that bed rest induces changes in the choroid (Jaki Mekjavic *et al.,* 2016) and in the retina (Taibbi *et al.,* 2014), no morphological or functional changes in the vitreous have been noted (for review see Stenger and Tarver, 2017). There is no reason to suspect that the minor changes in hydrostatic pressure would have profound effects on the form of a gelatinous mass within a cavity, with the gravitational vector in the anterior to posterior direction, although these changes may modify the production and drainage of aqueous humour (cf. Chang and Hargen, 2018).

Based on the work of Levinsohn, it would appear that the supine horizontal or 6° HDT BR may not be the best experimental model for studying SANS. The supine 6°HDT may serve a purpose for studying the effects on brain activity. However, the anatomical position of the eye, within the orbit, probably prevents any significant gravity-induced changes in its axial length. The head-down prone position obviously allows the hydrostatic pressure due to the body fluid shifts to have a more significant influence on eye morphology. The cephalad displacement of fluid will affect the tissue of the orbit, most likely resulting in oedema, which will impinge on the optic nerve causing the observed optic disc oedema.

Hypercapnia

The effect of a fluid shift on IOP and on the morphology and function of the eyes may be exacerbated by the elevated levels of CO_2 on the ISS and the exercise countermeasures conducted by the astronauts on a daily basis. Both of these factors are a consequence of the life support system design. In particular, although the CO_2 removal system on the ISS is at maximum capacity, it is not capable of maintaining the CO_2 concentration in the space habitat at the same level as on Earth. The CO_2 levels on the ISS are 15 times greater than on Earth, occasionally reaching values 20 times greater. CO_2 is vasoactive and has a marked effect on brain blood flow (Willie *et al.* 2012, Sato *et al.* 2012), which may exacerbate the hydrostatic effect discussed earlier on ocular structures, particularly during long-term exposure to such high levels (Jaki Mekjavic *et al.,* 2016).

Resistive exercise

Even a short-duration space flight results in significant musculoskeletal and cardiovascular deconditioning (Hayes *et al.,* 2013; Levine *et al.,* 1996; Trappe *et al.,* 1985), and thus exercise countermeasures have been a cornerstone of crew medical health care since the first ISS expedition. Exercise countermeasures on both Mir and ISS included treadmill walking/running, cycle ergometry,

and resistive exercise (Moore *et al.,* 2010). Recent upgrades to the resistive (Loehr *et al.,* 2011) and treadmill exercise capabilities, coupled with enhanced nutritional practices, have resulted in improved health outcomes (Smith *et al.,* 2012). Currently, ISS astronauts are scheduled for up to 2.5 h/day for exercise preparation, execution, and clean-up, which typically includes ~30 min of aerobic exercise (choice of treadmill or cycle) and ~45 min of resistive exercise (Moore *et al.,* 2014). The ISS exercise prescriptions are based on the demonstrated efficacy of countermeasures that combat musculoskeletal and cardiovascular deconditioning in bed rest models (Pavy-Le Traon *et al.,* 2007; Lee *et al.,* 2010; Ploutz-Snyder *et al.,* 2014; Shackelford *et al.,* 2004; Trappe *et al.,* 2007), and include both continuous and interval aerobic exercise training and resistive exercises, focussing on the trunk and lower body, where the majority of the musculoskeletal losses occur (LeBlanc *et al.,* 2000a,b). However, during this same time period that the ARED came on-board, ocular structural and functional symptoms began to emerge.

Resistive exercise can cause substantial increases in arterial pressure, with systolic and diastolic arterial pressures of 480 mmHg and 350 mmHg measured during weightlifting (Mcdougall *et al.,* 1992). These arterial pressure responses during weightlifting have been implicated in a variety of pathological responses (cf. Dickerman *et al.,* 1999), particularly in the brain (cerebral haemorrhage) and eye (subarachnoid haemorrhage, retinal haemorrhage and detachment, foveal haemorrhage). Macdougall (1999) reported that during a maximal isometric contraction of the leg muscles in the seated position, with subjects performing a concomitant Vasalva maneuver, intraocular pressure increased to 28 ± 9.3 mmHg, with one subject generating an IOP of 46 mmHg. The IOP elevations during prone 6°HDT observed in the present study during a submaximal (30% maximum) hand grip exercise were higher than those observed during a maximal contraction of muscles in both legs, mimicking the contraction observed during a maximal weightlifting effort, with hypercapnia significantly augmenting the IOP response.

It is thus possible that the high intensity resistive/static exercise in microgravity may be a contributing factor to the development of SANS (Marshal-Bowman, 2013), particularly considering that any effects of exercise would be superimposed on the gravitational effects discussed earlier.

Retinal and choroidal vessels

Embryologically, the retina is an extension of the diencephalon, and both organs share a similar pattern of vascularization during development (Dorrell *et al.,* 2002; Risau, 1997). There is a close anatomical correlation between both the macrovascular and the microvascular blood supply to the brain and the retina, and both vascular networks share similar vascular regulatory processes (Delaey *et al.,* 2000; Hardy *et al.,* 1997). The retina receives its nutrients from two separate circulations: the retinal and the choroidal circulation. Although the retinal and choroidal vessels are all derived from the ophthalmic artery, a branch of the internal carotid, they differ morphologically and functionally.

To understand the different responses of the vessels in the choroid and retina layers, it is also necessary to review the manner of their regulation (Pournaras *et al.,* 2008). Studies investigating ICP have done so, and extended their observations to the retinal circulation. Observing the changes in the retinal and choroidal circulation may provide insight into the effects of a stressor on the brain circulation.

In a recent review, Zhang and Hargens (2018) emphasised that autoregulation of IOP is also influenced by the flow of aqueous humour in the anterior part of the eye, and any factors that modify this flow will consequently also influence IOP. The effect of factors associated with spaceflight on aqueous humour flow remains unresolved.

Trans-lamina cibrosa pressure

The elevation in ICP as a result of prolonged exposure to microgravity has been implicated as a contributing factor in SANS (Berdahl *et al*., 2012), by virtue of its effect on IOP. A barrier between the forces exerted by IOP and ICP is the lamina cribrosa in the optic nerve head which maintains a trans-lamina cribrosa pressure difference (TLCPD). The TLCPD is known to increase significantly with age, with increased posterior displacement of the lamina (Fleischman & Allingham 2013). It is most likely that the altered TLCPD leads to irreversible damage of the retinal ganglion cells and consequently glaucomatous optic neuropathy (McMonnies, 2016). Jonas *et al.* (2015) have previously shown that higher TLCPD was associated with open angle glaucoma. The significant increase in IOP to above clinically acceptable limits in this study would suggest an increased risk of glaucomatous vision loss in astronauts in space. A variety of normal daily activities will cause an increase in IOP (for review see McMonnies, 2016), such as long duration prone sleeping (Cheng *et al.,* 2001), inverted body position (Freiberg and Weinreb, 1985), and static exercise (O'Connor and Poirier, 1985). In particular, during exercise, the increased intrathoracic pressure causes an increase in central venous pressure, whereas IOP and ICP are elevated by the reduced outflow (McMonnies, 2016). Similarly, the development of choroidal folds described as part of SANS may represent structural manifestations of altered stress at the lamina cribrosa and surrounding sclera resulting from TLCP alterations (Sibony *et al.* 2015).

Interestingly, no significant impairment in vision was observed when space missions were of shorter duration, undertaken by astronauts of younger age, and did not include the daily rigour of physical exercise.

Benefits for humans in space and on Earth

The exercise pressor reflex is a well-known response to exercise, resulting in an increase in HR and MAP (cf. Rowell, 1986). Due to efficient autoregulatory mechanisms, IOP decreases during dynamic (aerobic) exercise and returns to baseline levels during the recovery period (Marcus *et al*., 1970). In contrast, resistance (anaerobic) exercise, such as weight lifting, may cause a slight transient elevation in IOP, followed by a post-exercise drop in IOP, which then returns to baseline levels (Vieira *et al*., 2006). The post-exercise decrease in IOP is not yet resolved, but is most likely a consequence of autoregulation of retinal blood flow in response to elevated MAP (Robinson *et al*., 1986). The prevailing consensus is that the most likely exercise-induced factors that contribute to this autoregulatory response are decreased blood pH, elevated plasma osmolarity and elevated blood lactate (cf. Risner *et al*., 2009), which could stimulate the exercise pressor response. The dynamic exercise-induced lowering of IOP supports the potential of exercise reducing this risk factor in glaucoma patients. Most exercise studies to date have been performed in the seated position, and thus

the results are different to those of the present study in which the static exercise was performed in the prone 6°HDT position. Whereas CO_2 has been considered as a potential contributing factor (Risner *et al*., 2009), this is the first study, to our knowledge, providing evidence of its action on IOP. The present study on older subjects suggests that hypercapnia causes an increase in the diameter of the vasoactive retinal vessels, and that the cephalad shift due to posture causes an increase in the thickness of the choroidal vessel; consequently, the resistive exercise-induced elevation in IOP is enhanced. The known age-related changes in the structure of the lamina cribrosa (TLCPD), may cause a strain on the optic nerve head, leading to glaucomatous pathology. Although there is significant individual variation in the observed IOP response to hypercapnic exercise, and variability in the TLCPD between individuals, it might be prudent to consider individuals with an IOP response exceeding certain threshold limits in experimental prone microgravity as being at greater risk for SANS than others.

Limitations

The main limitation of this study is the reliance on non-contact pneumotonometry rather than GAT. However, the reliability of the adopted tonometer was established in preliminary experiments. The duration of experiments was short, and only included older participants. Future experiments will evaluate changes after longer duration exposure to prone HDT in different age groups. Finally, the current study included only male participants. The astronaut corps comprises both genders. The IOP responses to hypercapnic static exercise in the prone position reported for male subjects will be compared to the responses observed in young and older females in a further study.

Conclusions

To minimize the sarcopenia resulting from extended exposures to microgravity on the ISS, astronauts are required to adhere to a daily regimen of exercise. The present results demonstrate that the hypercapnic environment of the ISS augments the elevations in IOP induced by resistive/static exercise. This may be a contributing factor to the development of SANS.

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AUTHOR CONTRIBUTIONS

The experiments were performed at the Jozef Stefan Institute (Ljubljana, Slovenia). IM and PJM conceived the study. All authors contributed to the design of the protocol, data acquisition and interpretation of the data. IM and PJM drafted the manuscript, which was revised and the final version approved by all authors. All authors agree to be accountable for all aspects of the work. All authors qualify for authorship, and all those who qualify for authorship are listed.

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Table 1: Intraocular pressure (IOP) measured in the upright seated position while breathing room air using contact (iPen) and non-contact (Pulsair) tonometry

Intraocular pressure (IOP, mmHg)		
Participant no.	**Rebound tonometry (Icare®)**	**Pneumotonometry (Pulsair®)**
1	11.7	11.7
2	14.3	16.7
3	10.4	11.0
4	13.1	12.3
5	15.4	21.0
6	11.5	13.0
7	10.7	15.0
8	9.6	14.0
9	10.0	13.7
10	11.2	12.3
Mean ± SD	**11.8 ± 1.9**	**14.1 ± 2.9***

*$p < 0.05$

Table 2: Average (SD) responses of mean (MAP), systolic (SAP), and diastolic (DAP) arterial pressures, heart rate (HR), cardiac output (CO) and stroke volume (SV) during Hypercapnic (HCAP) and normocapnic (NCAP) isometric hand grip exercise in the prone 6°HDT position.

	n	Trial	BL	R5	R10	T1	T2	T3	P1	P2
MAP (mmHg)	13	NCAP	96 (6)	96 (5)	98 (6)	108 (7)†	121 (9)†	135 (9)†	100 (6)	96 (6)
		HCAP	96 (5)	97 (5)	97 (7)	110 (6)†	120 (10)†	132 (7)†	100 (5)	98 (5)
SAP (mmHg)	13	NCAP	129.1 (6.4)	126.8 (6.1)	128.1 (6.0)	141.1 (6.6)†	157.3 (9.3)†	176.0 (12.5)†	135.1 (8.5)	129.8 (7.4)
		HCAP	126.0 (7.2)	128.3 (4.4)	127.5 (5.9)	142.7 (5.0)†	156.1 (10.5)†	175.0 (10.1)†	134.6 (8.4)	132.0 (7.1)
DAP (mmHg)	13	NCAP	79.3 (6.8)	80.2 (5.7)	82.8 (7.4)	92.1 (8.1)†	102.6 (9.9)†	113.8 (9.5)†	82.1 (6.9)	78.9 (5.9)
		HCAP	80.5 (5.7)	81.9 (5.2)	81.7 (7.7)	93.2 (7.0)†	102.0 (10.3)†	109.8 (6.3)†	83.0 (4.4)	81.5 (4.6)*
HR (bpm)	20	NCAP	62 (6)	60 (5)	62 (6)	69 (7)†	72 (7)†	75 (7)†	60 (5)	59 (6)
		HCAP	62 (6)	61 (6)	61 (5)	71 (7)†	73 (6)†	79 (9)†	62 (10)	59 (5)
CO ($L \cdot min^{-1}$)	18	NCAP	5.2 (0.8)	5.1 (0.7)	5.3 (0.8)	5.8 (0.9)†	6.0 (1.0)†	6.4 (1.4)†	5.0 (1.5)	4.8 (0.9)
		HCAP	5.3 (1.2)	5.1 (0.7)	4.8 (0.8)	5.7 (1.1)†	6.2 (1.4)	6.4 (1.6)†	5.0 (1.0)	4.7 (1.2)
SV (mL)	18	NCAP	83.1 (11.3)	83.8 (10.5)	81.7 (14.3)	83.6 (13.4)	82.4 (14.9)	83.3 (18.2)	82.0 (18.0)	80.6 (18.3)

HCAP	81.0 (22.1)	83.6 (10.8)	79.2 (14.5)	81.0 (14.7)	84.6 (17.9)	81.6 (18.5)	82.7 (15.3)	80.4 (20.2)

*HCAP significantly different from NCAP; † significantly different response during exercise compared to R10; $p \leq 0.05$).

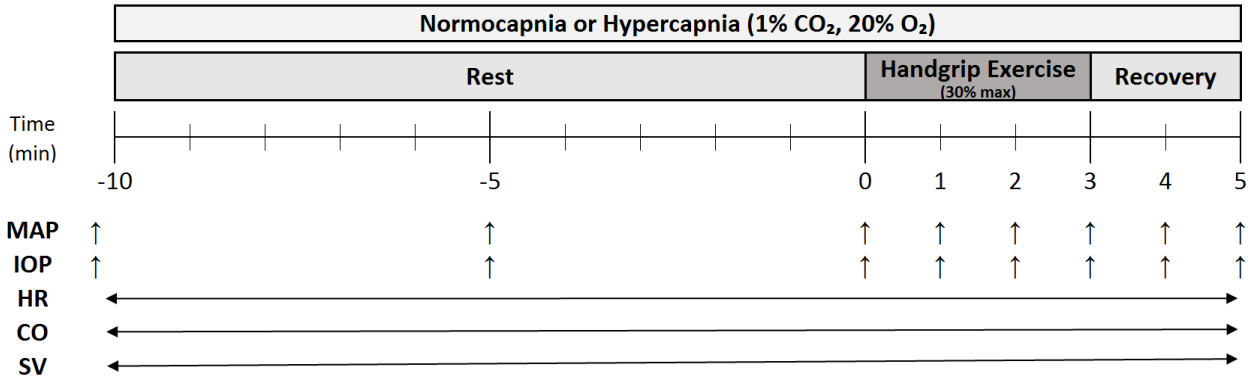

Figure 1: Schematic representation of protocol. In each experimental session, subjects conducted two trials in 6°HDT prone position. During one trial they inspired (normocapnic normoxic) room air (NCAP), and during the other they inspired a hypercapnic normoxic breathing mixture containing 1% CO_2 (HCAP). MAP: mean arterial pressure; IOP: intraocular pressure; HR: heart rate; CO: cardiac output; SV: stroke volume.

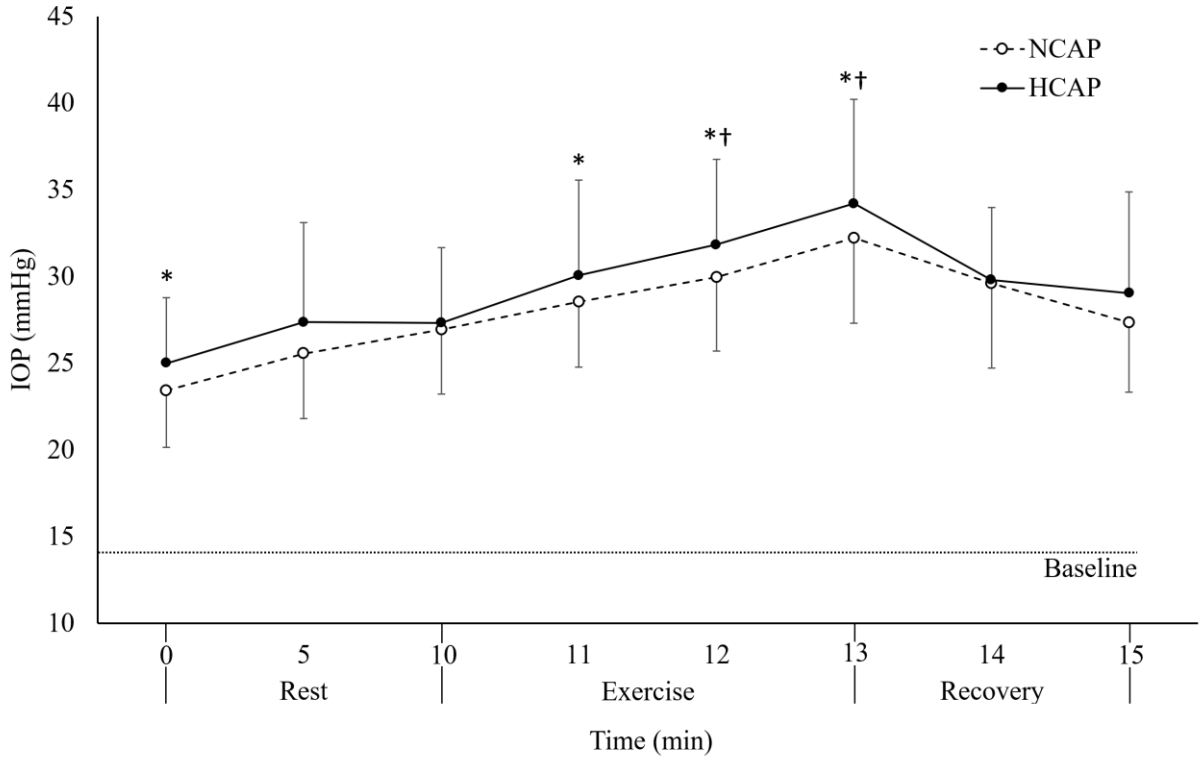

Figure 2: Intraocular pressure (IOP) during prone 6° head down tilt in the normocapnic (NCAP) and hypercapnic (HCAP) trials, each comprising a 10-min rest period, followed by a 3-min period of exercise during which the subject performed an isometric hand grip exercise at 30% of maximum, and a 2-min recovery period. The dotted line represents the average IOP of the group (14.1±2.9mmHg) obtained in the upright seated position while breathing room air. *HCAP trial significantly different to NCAP trial; † significantly different response during exercise compared to R10; $p \leq 0.05$.

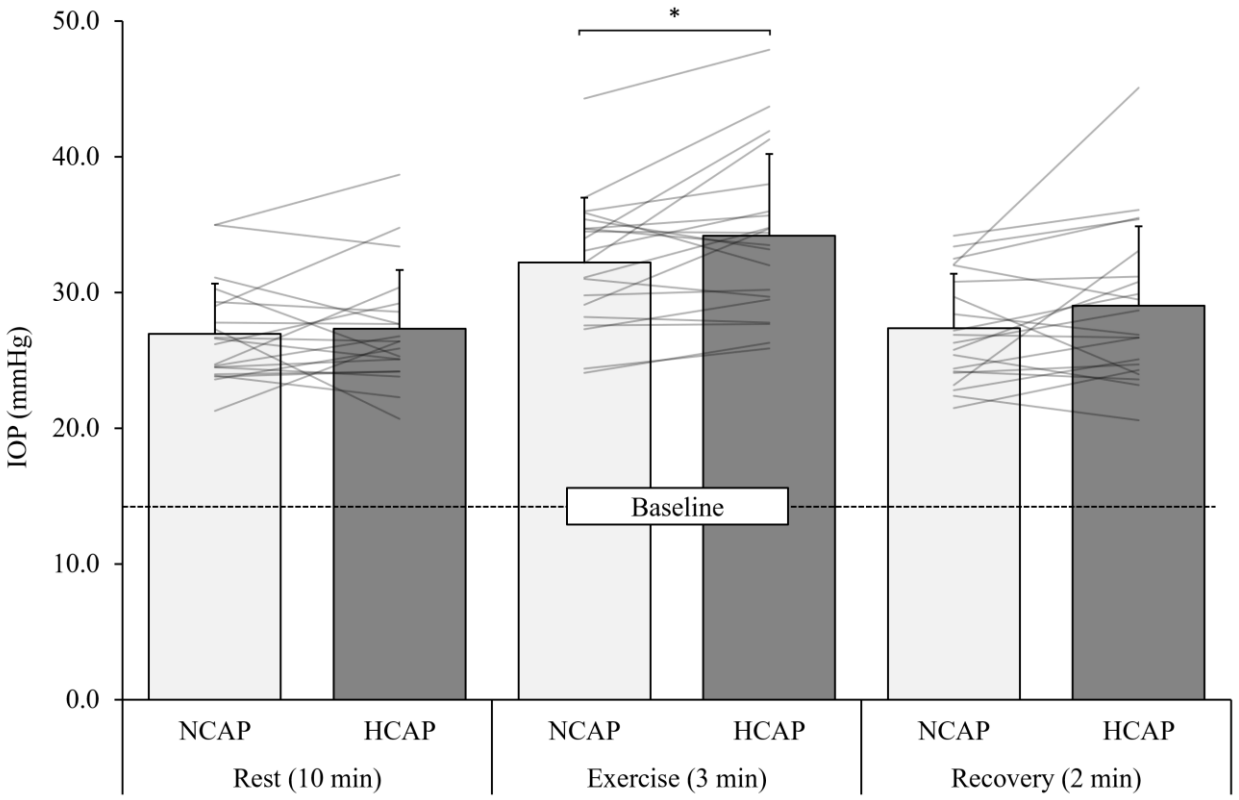

Figure 3: Mean and individual response of intraocular pressure (IOP) attained in the last minute of the 10-min rest, 3-min exercise and 2-min recovery, during the normocapnic (NCAP; open bars) and hypercapnic (HCAP; filled bars) trials. The Baseline indicates the average IOP obtained during the upright seated position while breathing (normocapnic normoxic)room air. * HCAP trial significantly different to NCAP trial; $p \leq 0.05$.