

THE EFFECT OF SPACEFLIGHT ASSOCIATED FACTORS ON THE EYE

Tinkara Mlinar

Doctoral Dissertation
Jožef Stefan International Postgraduate School
Ljubljana, Slovenia

Supervisor: Professor Igor B. Mekjavić
Department of Automatics, Biocybernetics and Robotics
Jožef Stefan Institute, Ljubljana, Slovenia

Co-Supervisor: Professor Polona Jaki Mekjavić
Faculty of Medicine, University of Ljubljana &
Eye Hospital, University Medical Centre Ljubljana,
Ljubljana, Slovenia

Evaluation Board:

Chair: Dr. Adam C. McDonnell
Department of Automatics, Biocybernetics and Robotics
Jožef Stefan Institute, Ljubljana, Slovenia

Members: Associate Professor Nataša Vidović Valentinčič
Faculty of Medicine, University of Ljubljana &
Eye Hospital, University Medical Centre Ljubljana,
Ljubljana, Slovenia

Professor Pierre Denise
Faculty of Medicine, Université de Caen Normandie,
Caen, France

MEDNARODNA PODIPLOMSKA ŠOLA JOŽEFA STEFANA
JOŽEF STEFAN INTERNATIONAL POSTGRADUATE SCHOOL



Tinkara Mlinar

THE EFFECT OF SPACEFLIGHT-ASSOCIATED
FACTORS ON THE EYE

Doctoral Dissertation

VPLIV DEJAVNIKOV, POVEZANIH Z VESOLJSKIMI
POLETI NA OČI

Doktorska disertacija

Supervisor: Prof. Igor B. Mekjavić

Co-Supervisor: Prof. Polona Jaki Mekjavić

Ljubljana, Slovenia, March 2023

*Dear grandad Tone,
unfortunately you are not with us anymore to celebrate the completion
of my doctoral thesis. Therefore, I would like to dedicate this body of
work to you because I know how important education was to you.
Thank you for everything!*

*Dragi ata Tone,
žal nisi več med nami, da bi lahko skupaj proslavili zaključek mojega
doktorata, zato to delo posvečam tebi, saj vem, kaj ti je pomenila
izobrazba.
Hvala ti za vse!*

Acknowledgements

As with any research work of this size, the completion of this doctoral thesis could not have been possible without the assistance, support, and guidance of many people in my life.

I would like to express my utmost gratitude to my PhD supervisor Professor Igor B. Mekjavić, and co-supervisor Professor Polona Jaki Mekjavić. Without their guidance, sincere and selfless support, patience, and all the knowledge they generously shared with me, this thesis would not exist.

I am grateful to Dr. Urša Ciuha, Dr. Adam C. McDonnell, and Dr. Tadej Debevec for their warm welcome to the laboratory when I first joined it during my placement year, invaluable advice, and for teaching me how to become a good researcher and scientist. Urša, Adam, Tadej, and Igor were a great inspiration to me and played a major role in my decision to pursue a PhD in sports and extreme environments physiology.

I would like to thank Miro Vrhovc for his technical assistance throughout the studies, and Jason Fisher and Tamara Valenčič for their help with the testing. I would also like to thank the rest of the laboratory and department for all the laughs and new friendships that have emerged in the last few years.

I would also like to express thanks to the Slovenian Research Agency for their financial support throughout the entirety of my PhD.

I would like to express my deepest gratitude to my parents Judita and Marko Mlinar, my sister Laura and her family, and my grandparents Ida and Tone Oblak for their love and constant support of my educational endeavours.

Most of all I would like to thank Josh Royal. Josh, thank you for all the words of encouragement, endless hugs, and unconditional love in good and bad times throughout the last four years.

Abstract

The unexplained aetiology of Spaceflight Associated Neuro-ocular Syndrome (SANS) jeopardises all planned future missions to the Moon and Mars. Various intrinsic and extrinsic potential risk factors have been proposed to contribute to its manifestation. Specifically, the present thesis compared the effect of static exercise (simulating the exercise performed by astronauts on the International Space Station; ISS) and hypercapnia (simulating the ambient conditions on the ISS) on intraocular pressure (IOP) in younger and astronaut-aged individuals. Additionally, the effect of hypoxia (the anticipated ambient in future space habitats) on retinal vessels was also investigated.

These objectives were achieved in a series of four studies:

Study 1: The effect of posture and hypercapnia on IOP during handgrip exercise in young males. To compare the effect of posture (i.e., seated, supine 6° head-down tilt (HDT), and prone 6°HDT), exercise, and hypercapnia on IOP responses, 10 younger (age = 22.5 ± 3.1 yrs) males participated in six interventions consisting of rest and submaximal isometric handgrip exercise while inspiring either room air or a hypercapnic ($F_i\text{CO}_2 = 0.01$) gas mixture.

Study 2: The effect of hypercapnia on IOP during handgrip exercise in older males. To assess the effect of age, exercise, and hypercapnia on IOP responses in an astronaut-aged population, 10 older (age: 57.8 ± 6 yrs) male participants underwent trials consisting of bouts of rest and isometric handgrip exercise in a prone 6°HDT position while breathing room air or a hypercapnic ($F_i\text{CO}_2 = 0.01$) gas mixture.

Study 3: The effect of sex on IOP during static handgrip exercise in astronaut-aged individuals. To investigate the effect of sex and exercise on IOP responses in an astronaut-aged population, 12 older males (age: 57.7 ± 5.2 yrs) and 12 older females (age: 53.4 ± 5.1 yrs) performed isometric handgrip exercise in a prone 6°HDT position.

Study 4: The effect of age on hypoxia-induced changes in retinal blood vessel diameter. To assess the effect of age and hypoxia on retinal vessel diameters, 11 adults (age: 40.1 ± 4.1 yrs) and 8 children (age: 9.3 ± 1.3 yrs) spent one day in normobaric normoxia and one day in normobaric hypoxia at an equivalent altitude of 3,000 m elevation.

The principal findings of these studies are that IOP was significantly higher in a prone 6°HDT position, compared to a seated or supine 6°HDT position, and that neither exercise nor hypercapnia had any significant effect on IOP responses in younger males in either of the three positions (Study 1). Isometric exercise significantly elevated IOP in older males in the prone 6°HDT position, with the exercise-induced elevations being further augmented by hypercapnia (Study 2). The prone 6°HDT exercise-induced IOP responses were similar in males and females (Study 3). Normobaric hypoxia significantly increased the diameter of the retinal vessels in both adults and children, with no difference between the two groups (Study 4).

We conclude that IOP is significantly affected by posture, but not by sex. Additionally, IOP responses observed in the prone 6°HDT position were similar to those reported for astronauts shortly after entering microgravity. It would therefore appear that the prone, rather than supine 6°HDT position is more suitable for the simulation of ocular changes that occur in acute microgravity. Resistive exercise and hypercapnia have a significant effect on IOP in older but not in younger males, indicating the potential presence of age-related effects on SANS manifestation. Lastly, adults and children appear to be similarly sensitive to changes in ambient partial pressure of oxygen.

Povzetek

Vesoljski očesni sindrom (ang. Spaceflight-Associated Neuro-ocular Syndrome; SANS) ogroža načrtovane prihodnje misije na Luno in Mars. Natančne etiologije sindroma še ne poznamo, a obstaja več dejavnikov, povezanih z vesoljskimi leti, ki bi lahko prispevali k njegovemu nastanku. V doktorski disertaciji smo s simulacijo ambientalnih razmer na Mednarodni vesoljski postaji (ang. International Space Station; ISS) opredelili učinek statične vadbe in hiperkapnije na vrednost očesnega tlaka (ang. intraocular pressure; IOP) pri mlajših moških ter moških in ženskah povprečne starosti astronautov. S simulacijo pričakovanega okolja v prihodnjih vesoljskih habitatih smo ovrednotili tudi vpliv hipoksije na mrežnične žile.

Delo smo izvedli v seriji štirih raziskav:

Raziskava 1: Vpliv telesnega položaja in hiperkapnije na IOP med vadbo pri mlajših moških. Za primerjavo učinka položaja telesa [tj. sede, leže na hrbtu z nagnjenostjo glave 6° navzdol (6°HDT); leže na trebuhu 6°HDT], vadbe in hiperkapnije na IOP smo preiskovancem ($N = 10$; starost = $22,5 \pm 3,1$ leta) izmerili IOP med submaksimalno izometrično vadbo s stiskom pesti, medtem ko so dihali normokapnično ali hiperkapnično ($F_i\text{CO}_2 = 0,01$) plinsko mešanico.

Raziskava 2: Vpliv vadbe in hiperkapnije na IOP pri starejših moških. Da bi ocenili učinek starosti, vadbe in hiperkapnije na spremembe IOP pri osebah povprečne starosti astronautov, smo preiskovancem ($N = 10$; starost: $57,8 \pm 6$ let) izmerili IOP med počitkom in med izometrično vadbo (stisk pesti) v položaju leže na trebuhu 6°HDT , medtem ko so dihali normokapnično in hiperkapnično ($F_i\text{CO}_2 = 0,01$) plinsko mešanico.

Raziskava 3: Vpliv spola na IOP med vadbo pri osebah povprečne starosti astronautov. Preiskovankam ($N = 12$; starost: $53,4 \pm 5,1$ let) in preiskovancem ($N = 12$; starost: $57,7 \pm 5,2$ let) smo izmerili IOP med akutno izometrično vadbo (stisk pesti) v položaju leže na trebuhu 6°HDT .

Raziskava 4: Vpliv starosti in hipoksije na premer mrežničnih žil. Da bi ocenili učinek starosti in hipoksije na premer mrežničnih žil, je 11 odraslih (starost: $40,1 \pm 4,1$ leta) in 8 otrok (starost: $9,3 \pm 1,3$ leta) en dan preživel v normobarični normoksiji in en dan v normobarični hipoksiji (približno 3000 m nadmorske višine).

Z raziskavami smo ugotovili: i) da je IOP znatno višji v položaju leže na trebuhu 6°HDT kot v položaju sede ali v položaju leže na hrbtu 6°HDT in ii) da niti vadba niti hiperkapnija ne vplivata pomembno na IOP pri mlajših moških v katerem koli od preučevanih treh položajev (raziskava 1). Izkazalo se je, da je IOP pri starejših moških zaradi izometrične vadbe v položaju leže na trebuhu 6°HDT znatno višji in da ga hiperkapnija dodatno zviša (raziskava 2). Vrednost IOP med vadbo v položaju leže na trebuhu 6°HDT je bila pri moških in ženskah primerljiva (raziskava 3). Normobarična hipoksija je znatno povečala premer mrežničnih žil tako pri odraslih kot pri otrocih, a med skupinama ni bilo statistično značilnih razlik (raziskava 4).

Na podlagi rezultatov zaključujemo, da na IOP pomembno vpliva položaj telesa, medtem ko vpliva spola ne moremo potrditi. Vrednost IOP je v položaju leže na trebuhu 6°HDT podobna vrednosti IOP, izmerjeni kmalu po vstopu v mikrotežnost, kar nakazuje, da je za simulacijo očesnih sprememb, ki se pojavijo v akutni mikrotežnosti, bolj primeren položaj leže na trebuhu 6°HDT kot položaj leže na hrbtu 6°HDT . Izometrična vadba in hiperkapnija pomembno vplivata na IOP pri starejših moških, medtem ko na IOP pri mlajših moških nimata vpliva. To nakazuje, da na nastanek vesoljskega očesnega sindroma morda vpliva starost. Izsledki raziskave 4 potrjujejo tudi, da so odrasli in otroci podobno občutljivi na spremembe delnega tlaka kisika v okolju.

Contents

List of Figures	xvii
List of Tables	xix
Abbreviations	xxi
Preface	xxiii
1 Spaceflight-Associated Neuro-ocular Syndrome (SANS): A Brief Review of Potential Contributing Factors within the Framework of the Present Thesis	1
1.1 The Aetiology of SANS Could Be Explained by a Neural Mechanism Regulating Intraocular Pressure.....	2
1.2 Intraocular and Intracranial Pressure	3
1.2.1 Intraocular pressure regulation and types of tonometry.....	3
1.2.1.1 Contact tonometry	3
1.2.1.2 Non-contact tonometry.....	4
1.2.2 Effects of intraocular and intracranial pressure on the lamina cribrosa ...	5
1.2.3 Problems associated with measuring intracranial pressure.....	5
1.2.3.1 Transcranial Doppler ultrasonography	6
1.2.3.2 Tympanic membrane displacement	7
1.2.3.3 Otoacoustic emission	7
1.2.3.4 Magnetic resonance imaging.....	7
1.3 Spaceflight-Associated Factors Which May Contribute to SANS.....	8
1.3.1 Gravitational vector	8
1.3.2 Exercise	9
1.3.2.1 Resistive exercise.....	9
1.3.2.2 Aerobic exercise.....	9
1.3.3 Hypercapnia	9
1.3.4 Hypoxia.....	10
1.3.5 Age	10
1.3.6 Sex.....	11
1.3.7 Mission duration.....	11
1.4 Ground-Based Simulations of Spaceflight	12
2 Problem Identification And Thesis Structure	15
2.1 Problem Identification	15
2.2 Thesis Structure.....	16
2.3 Hypotheses.....	17
3 The Effect of Posture and Hypercapnia on Intraocular Pressure During Handgrip Exercise in Young Males	19

3.1	Methods.....	20
3.1.1	Participants	20
3.1.2	Experimental protocol.....	20
3.1.3	Intraocular pressure measurements in a standard clinical position.....	21
3.1.4	Maximal strength measurement.....	21
3.1.5	Normocapnic and hypercapnic exercise trials.....	22
3.1.6	Statistical analyses.....	23
3.2	Results.....	23
3.2.1	Heart rate	24
3.2.2	Systolic, diastolic and mean arterial pressure	25
3.2.3	Intraocular pressure	27
3.3	Discussion.....	29
3.3.1	Posture.....	29
3.3.2	Resistive exercise	30
3.3.3	Hypercapnia.....	31
3.3.4	Limitations.....	31
4	The Effect of Hypercapnia on Intraocular Pressure During Handgrip Exercise in Older Males	33
4.1	Methods.....	34
4.1.1	Ethical approval.....	35
4.1.2	Participants	35
4.1.3	Protocol	35
4.1.4	Normocapnic and hypercapnic trials.....	36
4.1.5	Intraocular pressure measurement in a standard clinical seated position.....	36
4.1.6	Maximal handgrip strength measurement.....	36
4.1.7	Impedance plethysmography and sphygmomanometry.....	37
4.1.8	Statistical considerations	37
4.2	Results.....	38
4.3	Discussion.....	41
4.3.1	Intracranial pressure	42
4.3.2	Gravitational vector.....	42
4.3.3	Posture: supine vs. prone.....	43
4.3.4	Hypercapnia.....	43
4.3.5	Resistive exercise	44
4.3.6	Retinal and choroidal vessels	44
4.3.7	Translaminal cribrosa pressure.....	45
4.3.8	Age.....	45
4.3.9	Benefits for humans in space and on Earth	46
4.3.10	Limitations.....	46
5	Effect of Sex on Intraocular Pressure During Static Handgrip Exercise in Astronaut-Aged Individuals	47
5.1	Methods.....	48
5.1.1	Participants	48
5.1.2	Experimental protocol.....	49
5.1.3	Statistical analyses.....	49
5.2	Results.....	50
5.2.1	Participants	50
5.2.2	Heart rate	50
5.2.3	Mean arterial pressure	50

5.2.4	Intraocular pressure.....	51
5.3	Discussion	52
5.3.1	Ground-based results.....	52
5.3.2	Microgravity	53
5.3.3	Limitations	54
6	Effect of Age on Hypoxia-Induced Changes in Retinal Blood Vessel Diameter	55
6.1	Methods	56
6.1.1	Study design.....	56
6.1.2	Participants.....	57
6.1.3	General adaptation to hypoxia.....	58
6.1.4	Retinal fundus examination.....	58
6.1.5	Data processing and analysis.....	58
6.2	Results	59
6.2.1	General adaptation to hypoxia.....	59
6.2.2	Retinal fundus	60
6.2.2.1	High altitude retinopathy	60
6.2.2.2	Central retinal arteriolar equivalent (CRAE).....	60
6.2.2.3	Central retinal venular equivalent (CRVE).....	60
6.2.2.4	Central retinal arteriolar vs venular equivalent.....	61
6.3	Discussion	61
6.3.1	The effect of hypoxia on retinal vessel diameters.....	61
6.3.2	Adults vs. children	62
6.3.3	High-altitude headache, high-altitude retinopathy, and acute mountain sickness.....	63
6.3.4	Clinical implications	63
6.3.5	Limitations	64
7	Conclusions and Future Work	65
7.1	Conclusions	65
7.2	Future Work.....	66
	References	67
	Bibliography	89
	Biography	91
	Appendix A Ethics Approval	93
	Appendix B Publications Related to the Thesis	95

List of Figures

Figure 1.1: A cross-sectional diagram of the eye.....	5
Figure 1.2: IOP responses of the AGBRESA Control group	13
Figure 3.1: Graphic depiction of the protocol.....	23
Figure 3.2: Heart rate response during normocapnic and hypercapnic trials.....	24
Figure 3.3: Mean arterial pressure response during normocapnic and hypercapnic trials.....	27
Figure 3.4: Intraocular pressure response during normocapnic and hypercapnic trials.....	28
Figure 4.1: Schematic representation of the protocol.....	35
Figure 4.2: Intraocular pressure (IOP) during prone 6° head-down tilt	40
Figure 4.3: 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	40
Figure 4.4: Intraocular pressure response of males from the present study compared to younger males from Chapter 3.....	41
Figure 5.1: Intraocular pressure response to rest and isometric handgrip exercise	51
Figure 5.2: Relationships between seated IOP (IOP _{Control}) and BMI.....	52
Figure 6.1: Testing schedule for Day 1 (normoxia) and Day 2 (hypoxia)	57
Figure 6.2: Adults' and children's central retinal arteriolar equivalents.....	60
Figure 6.3: Adults' and children's central retinal venular equivalents.....	61

List of Tables

Table 3.1: Participants' anthropometric characteristics	20
Table 3.2: Example of the sequence of the trials for one of the participants.....	21
Table 3.3: Average (SD) responses of systolic and diastolic pressure	26
Table 3.4: Participants' IOP measured in a standard clinical seated position	27
Table 4.1: Intraocular pressure measured in the upright seated position	38
Table 4.2: Mean (SD) responses of the mean (MAP), systolic (SAP) and diastolic (DAP) arterial pressures, heart rate (HR), cardiac output (CO) and stroke volume (SV)	39
Table 5.1: Participants' physical characteristics.....	48
Table 5.2: Participants' heart rate and mean arterial pressure responses.....	50
Table 6.1: Participants' physical characteristics.....	58
Table 6.2: Adults' and children's morning HR, S _p O ₂ and LLS	59

Abbreviations

AMS	...	Acute mountain sickness
ANOVA	...	Analysis of variance
ARED	...	Advanced Resistive Exercise Device
ARIA	...	Automated Retinal Image Analyser
BMI	...	Body mass index
CO	...	Cardiac output
CO ₂	...	Carbon dioxide
CBFV	...	Cerebral blood flow velocity
CRAE	...	Central retinal arteriolar equivalent
CRVE	...	Central retinal venular equivalent
CSF	...	Cerebrospinal fluid
DAP	...	Diastolic arterial pressure
DPOAE	...	Distortion product otoacoustic emission
EOA	...	Extracranial segments of the ophthalmic artery
ESA	...	European Space Agency
EVA	...	Extravehicular activities
F _I CO ₂	...	Fraction of inspired carbon dioxide
GAT	...	Goldmann Applanation Tonometer
HAH	...	High-altitude headache
HAR	...	High-altitude retinopathy
HCAP	...	The normoxic hypercapnic gas mixture
HDT	...	Head-down tilt
HR	...	Heart rate
ICP	...	Intracranial pressure
IIH	...	Idiopathic intracranial hypertension
IOA	...	Intracranial segments of the ophthalmic artery
IOP	...	Intraocular Pressure
IPS	...	International Postgraduate School
ISS	...	International Space Station
JSI	...	Jožef Stefan Institute
LLS	...	Lake Louise score
LP	...	Lumbar puncture
MAP	...	Mean arterial pressure
MRI	...	Magnetic resonance imaging
NASA	...	National Aeronautics and Space Administration
NCAP	...	Normoxic normocapnic room air
nICP	...	Non-invasively measured intracranial pressure
OAE	...	Otoacoustic emission
OCT	...	Optical coherence tomography
ONH	...	Optic nerve head
ONS	...	Optic nerve sheath
PO ₂	...	Partial pressure of oxygen

RM	...	Repetition maximum
RNFL	...	Retinal nerve fibre layer
SANS	...	Spaceflight Associated Neuro-ocular Syndrome
SAP	...	Systolic arterial pressure
SD	...	Standard deviation
SMAC	...	Spacecraft Maximum Allowable Concentrations
S _p O ₂	...	Peripheral blood oxygenation
SV	...	Stroke volume
TCD	...	Transcranial Doppler
TLCPD	...	Translaminar cribrosa pressure difference
TMD	...	Tympanic membrane displacement
VIIP	...	Visual Impairment Intracranial Pressure Syndrome
VPSA	...	Vacuum-pressure swing adsorption

Preface

This thesis is based on the following publications:

- Mlinar, T.**, Jaki Mekjavic, P., & Mekjavic, I. B. (2023). Effect of sex on intraocular pressure during static handgrip exercise in astronaut-aged individuals. *Acta Astronautica*, submitted.
- Mlinar, T.**, Debevec, T., Kapus, J., McDonnell, A. C., Najdenov, P., Ušaj, A., Mekjavic, I.B. and Jaki Mekjavic, P. (2023). Retinal blood vessel diameters in children and adults exposed to a simulated altitude of 3,000 m. *Frontiers in Physiology*, 14:1026987. doi: 10.3389/fphys.2023.1026987
- Mlinar, T.**, Jaki Mekjavic, P., Royal, J. T., Valencic, T., & Mekjavic, I. B. (2021). Intraocular pressure during handgrip exercise: The effect of posture and hypercapnia in young males. *Physiological Reports*, 9(20), e15035. doi: 10.14814/phy2.15035
- Mekjavic, I. B., Amoaku, W., **Mlinar, T.**, & Jaki Mekjavic, P. (2020). Hypercapnia augments resistive exercise-induced elevations in intraocular pressure in older individuals. *Experimental Physiology*, 105(4), 641-651. doi: 0.1113/EP088236
- Jaki Mekjavic, P., Amoaku, W., **Mlinar, T.**, & Mekjavic, I. B. (2020). The aetiology of Spaceflight-Associated Neuro-ocular Syndrome (SANS) might be explained by a neural mechanism regulating intraocular pressure. *The Journal of Physiology*, 598(8). doi: 10.1113/jp279699

Chapter 1

Spaceflight-Associated Neuro-ocular Syndrome (SANS): A Brief Review of Potential Contributing Factors within the Framework of the Present Thesis

Anecdotal evidence of vision changes during spaceflight have been reported sporadically since the early days of the space programme. In the late 1980s, to better study eye problems during short- and long-duration missions, the National Aeronautics and Space Administration (NASA) introduced a questionnaire as part of their postflight eye examination. The results of the survey revealed that of approximately 300 astronauts, near visual acuity declined in 23% of short-duration shuttle fliers and nearly 50% of astronauts on long-duration missions. The postflight hyperopic shifts were observed in 11% of astronauts following short-duration missions and in 34% of astronauts upon completing long-duration missions. These changes were more prevalent in older astronauts with decreased accommodation (Mader et al., 2011). With technological advancements and more detailed pre- and post-mission examinations, additional ocular changes were discovered and identified. In 2011, Mader et al. (2011) were the first to publish the results of a complete eye examination of seven astronauts following a six month long mission on the International Space Station (ISS), including cycloplegic and manifest refraction, fundus photography, optical coherence tomography (OCT), magnetic resonance imaging (MRI), and lumbar puncture. Visual performance decrements were observed in a subset of astronauts and were often accompanied by one or more of the following ophthalmic findings with varying degrees of severity and permanence: hyperopic shift, optic disc oedema, cotton wool spots, choroidal folds, and optic nerve sheath (ONS) distention.

Visual impairment associated with prolonged spaceflight was originally thought to be a consequence of the microgravity-induced increase in intracranial pressure (ICP), thus the problem was termed visual impairment/intracranial pressure (VIIP) syndrome (Alexander et al., 2012). Since then, it has been demonstrated that ICP in microgravity is not elevated pathologically (Lawley et al., 2017). Consequently, due to the, as yet, unresolved aetiology of this syndrome, VIIP has been redefined as Spaceflight-Associated Neuro-ocular Syndrome (SANS). The cephalad displacement of body fluids due to the absence of a gravitational vector during exposure to microgravity has been implicated as the main contributing factor in the aetiology of SANS. Other potential risk factors include increased ICP, and intraocular pressure (IOP), radiation exposure retinopathy, daily resistive exercise, high sodium diet, and elevated levels of carbon dioxide (CO₂) onboard the ISS

(Mader et al., 2013; Marshall-Bowman et al., 2013). Most likely, SANS is not caused by a single factor, but rather by a combination of the aforementioned factors.

1.1 The Aetiology of SANS Could Be Explained by a Neural Mechanism Regulating Intraocular Pressure

The recent study by Ficarrota and Passaglia (2020), particularly their theory of an as yet undetermined neural feedback loop regulating IOP, provides an exciting new perspective towards explaining the aetiology of SANS.

Fundamental to the evolution of the human species, in particular the ability to maintain appropriate perfusion of vital organs during rest and activity in the upright position, is the evolution of the structure and function of the cardiovascular system. The remarkable feature of cardiovascular regulation is that in the upright posture, 70 to 75% of the blood volume is below the heart (i.e., pump). As remarked by Loring B. Rowell in his 1993 book *Human Cardiovascular Control*:

“If an engineering analysis of the aeronautical features of the bumblebee could lead to the conclusion that these insects cannot fly, then a hemodynamic analysis of the human circulation could also lead to the conclusion that human beings cannot stand up.”

The maintenance of an upright posture is possible due to the regulation of blood pressure, which ensures adequate perfusion of all tissues, including the eye.

Withdrawal of the head-to-foot gravitational vector, either by changing body position or exposure to microgravity, will induce changes in the perfusion of several tissues, particularly if acute exposure is concomitant with increased physical exertion. Chronic exposure to microgravity has been shown to cause impairment in visual function, which was initially attributed to the increase in ICP, but recently perfusion-induced modifications of the retinal circulation have also been implicated. The majority of astronauts participating in long-term missions on the ISS experience visual impairment.

Ficarrota and Passaglia (2020) demonstrate quite elegantly that ICP is the afferent arm of a central neural feedback mechanism, which provides an efferent signal modulating the outflow facility (aqueous humour), thus regulating IOP. As they point out, IOP has a much greater influence on the biomechanics of the optic nerve head than ICP, and the principal aim of IOP regulation is the maintenance of the translaminal pressure across the lamina cribrosa.

Their experiments were performed with a rat model (prone position), and the fluid dynamics are different to those of an upright human. Nevertheless, their theory would suggest that factors other than ICP might elevate IOP, and may also pose a threat to retinal cellular structure and function. Using self-tonometry, Draeger et al. (1993) were the first to report a transient elevation of IOP in microgravity, whereas Chung et al. (2011) reported a persistent elevation of IOP measured by the first Korean astronaut during her sojourn on the ISS. In female participants confined to bed rest for 10 days, Jaki Mekjavic et al. (2016) observed that choroidal thickness is influenced by hydrostatic pressure (i.e. comparison of measurements taken in the seated and supine positions), whilst hypoxia and hypercapnia affect the neural fibre layer of the retina. The increased blood flow in the retinal circulation will not affect ICP, and will thus presumably not initiate the feedback mechanism postulated by Ficarrota and Passaglia (2020). It may, however, affect IOP. Based on our results (Mekjavic et al., 2020; Mlinar et al., 2021) and those of Anderson et al. (2016), we hypothesised (Mekjavic et al., 2020) that the supine 6° head-down tilt (HDT)

model may not be an appropriate simulation of the microgravity-induced effects on the ocular circulation. In contrast to the supine position, the prone 6°HDT position is most likely a better model, as it elevates IOP substantially above the threshold for ocular hypertension. The prone 6°HDT position in humans elevates the episcleral venous pressure due to a hydrostatic effect (Anderson et al., 2016; Jaki Mekjavic et al., 2016; Mekjavic et al., 2020; Mlinar et al., 2021). This may result in an IOP increase through a different mechanism to that described in the prone rat model used by Ficarrotta and Passaglia (2020). Such contributions of reduced outflow in microgravity models require evaluation. Furthermore, the daily static exercise performed by astronauts on the ISS to mitigate microgravity-induced musculoskeletal degeneration may cause IOP elevations. Interestingly, the substantial static exercise-induced elevations in IOP observed in older (astronaut-aged) individuals are not observed in younger adults (Mekjavic et al., 2020; Mlinar et al., 2021), perhaps due to the known age-related modifications in the biomechanical properties of the lamina cribrosa.

The focus of research to explain the aetiology of SANS, thus far, has been on the effects of microgravity on ICP. However, it would appear that increased IOP, particularly by factors influencing ocular fluid dynamics, may be the prime cause of the impaired vision observed in astronauts.

1.2 Intraocular and Intracranial Pressure

1.2.1 Intraocular pressure regulation and types of tonometry

IOP is a balance between the inflow and outflow of aqueous humour in the anterior part of the eye and is measured in millimetres of mercury (mmHg). Aqueous humour is produced in the ciliary body through two pathways. The majority (80%) is produced by active secretion mechanisms that are independent of IOP, although the production may be reduced by a decrease in blood flow to the ciliary body. The second pathway (accounting for about 20% of ciliary body production) is through the ultrafiltration of plasma. This pathway is influenced by the blood pressure in the ciliary body capillaries, plasma oncotic pressure and IOP. The aqueous humour is drained from the anterior chamber by two mechanisms: the minority of resorption occurs through the uveoscleral route into the lymphatic system while the majority of resorption is through the trabecular meshwork and Schlemm's canal into the episcleral venous system. This canal is directly connected to the episcleral veins, therefore absorption through this pathway is influenced by the gradient between IOP and episcleral venous pressure (Murgatroyd & Bembridge, 2008).

In contrast to aqueous production and outflow maintenance, which appear to be relatively independent of variations in body position, episcleral venous pressure does react to gravitational changes and therefore, headward fluid shifts result in elevated episcleral venous pressure (Friberg et al., 1987). A relationship between episcleral venous pressure and IOP has been reported in glaucoma studies (Selbach et al., 2005). Any increase in episcleral venous pressure leads to decreases in trabecular meshwork outflow which consequently results in increased IOP (Craven, 2008).

1.2.1.1 Contact tonometry

Most IOP measurements are indirect and non-invasive. The international gold standard ocular tonometer used in clinical settings is the Goldman applanation tonometer (GAT; Haag-Streit Holding, Switzerland). Following the application of an eye anaesthetic and a diagnostic dye such as fluorescein, a small probe is applied against the cornea. The IOP measured by GAT corresponds to 10 times the force required to flatten a corneal area 3.06

mm in diameter (Moses, 1958). The Perkins tonometer (Haag-Streit Holding, Switzerland) is a portable version of the GAT that does not require the use of a slit lamp. Whereas the GAT only allows measurements in a seated position, the Perkins tonometer can also be used with a patient in a supine position (Jablonski et al., 2013).

IOP measurements using the GAT have relatively low intra- and inter-observer variability (Dielemans et al., 1994). GAT is based on empirical experimentation and the Imbert-Flick principle, which states that the pressure in an ideal sphere with an infinitely thin and flexible membrane is equal to the force applied necessary to flatten its surface to a known size (Goldmann & Schmidt, 1957). However, the eye is not a perfect sphere and the cornea is not infinitely thin, so the GAT measurements are affected by the corneal curvature and central corneal thickness. In individuals with significantly thinner ($<525\ \mu\text{m}$) corneas, GAT can produce artificially low readings, and in individuals with significantly thicker ($>555\ \mu\text{m}$) corneas artificially high readings. Despite this, GAT is still considered the gold standard in clinical practice worldwide and all the other tonometers are evaluated in comparison to it.

The Tono-Pen (Reichert Technologies, New York, USA) is an example of an applanation tonometer that does require superficial eye anaesthesia but does not require the use of a contrast dye. IOP measured using the Tono-Pen corresponds to the resistance produced by the cornea when the tonometer makes contact with the eye. IOP is calculated as the average of multiple readings. Unlike most tonometers, Tono-Pen can be used in any position, and in weightlessness (Greenwald et al., 2021). Due to the smaller contact area of the Tono-Pen, it is recommended over GAT for IOP measurements in participants with irregular corneas (Rootman et al., 1988). Within normal IOP ranges, Tono-Pen correlates well with GAT (Bhan et al., 2002), whereas at the lower and higher ends of the range, it can over- or underestimate IOP (Frenkel et al., 1988; Mollan et al., 2008; Salvetat et al., 2007).

Contrary to other contact tonometers, a portable iCare rebound tonometer (Icare Finland Oy, Vantaa, Finland) does not require the use of a topical anaesthetic. To measure IOP, a light magnetised probe rapidly touches the cornea several times. IOP is calculated from the speed at which the probe is decelerated when it touches the eye. Faster deceleration is indicative of high IOP, and slower deceleration is indicative of lower IOP (De Moraes et al., 2008). Whereas some studies have shown good agreement between iCare and GAT (Stamper, 2011; Van der Jagt & Jansonius, 2005), others reported an overestimation of IOP by iCare compared to GAT (Fernandes et al., 2005; Nakamura et al., 2006). Additionally, the iCare is affected by different biomechanical corneal factors, such as the central corneal thickness and corneal hysteresis (Jorge et al., 2008; Nakamura et al., 2006), and cannot measure IOP in a participant lying in a prone position.

1.2.1.2 Non-contact tonometry

The most common type of non-contact tonometer is an air puff tonometer, either portable or static, such as the Pulsair (Keeler, Windsor, United Kingdom). The measurement consists of a column of air released at the eye across a range of pressures, and an infrared light beam shone at the cornea. The air pressure at which the cornea flattens, as measured by the change in the beam reflection, corresponds to the IOP. As there is no direct contact with the cornea, there is no risk of corneal abrasion and no need for a topical anaesthetic (De Moraes et al., 2008).

In the present thesis, IOP was measured using a portable Pulsair IntelliPuff Tonometer (Keeler, Windsor, United Kingdom), because it is quick, can be used in any position, and does not require the use of a local anaesthetic. Hubanova et al. (2015) reported that although Pulsair IntelliPuff overestimated measured IOP values (mainly at IOP >40

mmHg) in normo- and hypertensive eyes compared to GAT, a strong correlation coefficient was found for both populations. Additionally, an excellent agreement between Pulsair and GAT was reported in normotensive (intraclass correlation coefficient (ICC) = 0.75) and hypertensive (ICC = 0.86) eyes.

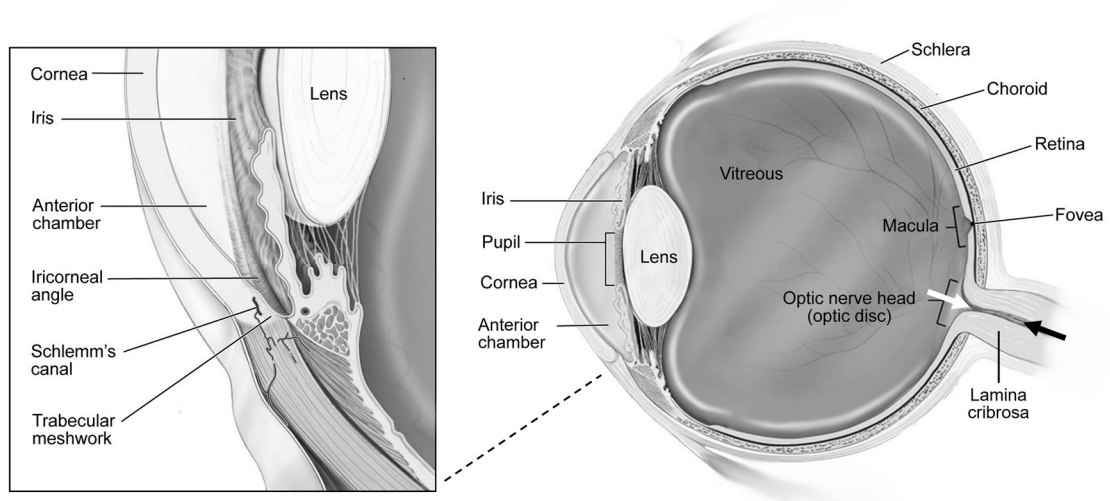


Figure 1.1: A cross-sectional diagram of the eye. The white and small black arrows represent the direction of intraocular and intracranial pressures, respectively, on the lamina cribrosa. Adapted from a diagram by the National Eye Institute, United States of America.

1.2.2 Effects of intraocular and intracranial pressure on the lamina cribrosa

Recent evidence suggests that an imbalance between the ICP and IOP across the lamina cribrosa may give rise to the morphological changes observed in SANS. Namely, IOP and ICP are closely interconnected, and both play a critical role in the development of optic disc nerve damage (Berdahl et al., 2008; Jonas & Wang, 2012; B. Wang et al., 2017). IOP and ICP exert opposing forces on the lamina cribrosa located in the posterior of the eye, as seen in Figure 1.1. The difference between these two forces, called translaminar pressure difference (TLPD), is extremely important in maintaining a healthy optic nerve head (Berdahl et al., 2012). Therefore, monitoring both IOP and ICP during exposure to microgravity is extremely important to gain a better understanding of their combined impact on the lamina cribrosa.

Lamina cribrosa stiffens with age, exerting more pressure on nerve axons and damaging them. Conversely, the resilience of lamina cribrosa decreases with age, leaving it more susceptible to permanent deformation (Albon et al., 2000). These findings might be of importance in the explanation of ocular changes primarily manifesting in older astronauts.

1.2.3 Problems associated with measuring intracranial pressure

Common to most of the initial studies investigating SANS is that they consider vision impairment to result from an increase in ICP. One problem with investigating this issue is the difficulty in obtaining a measure of ICP. In individuals with communicating cerebrospinal fluid (CSF) pathways, the most widespread method of measuring ICP is indirect via lumbar puncture (LP). This invasive procedure is typically performed under

local anaesthesia with the individual positioned in either a seated or lateral recumbent position. ICP is measured by inserting a needle connected to an external pressure transducer, into a lumbar subarachnoid space (Raboel et al., 2012).

LP is regarded as a safe procedure, but certain limitations exist. In cases when CSF does not circulate freely, ICP will not be measured correctly. Furthermore, LP is not appropriate to use in individuals with very high ICP due to the possibility of brain herniation (Speck et al., 2011). Lastly, the estimation of ICP via LP is conducted in a sterile environment, with participants not allowed to move, therefore it is not possible to apply in a laboratory setting with other interventions being administered (i.e., exercise). To circumvent this problem, several methods which proclaim to non-invasively provide an index of ICP (nICP), have been developed.

1.2.3.1 Transcranial Doppler ultrasonography

The use of Transcranial Doppler (TCD) ultrasonography as a non-invasive measure of ICP is based on the effect of ICP on cerebral blood flow. Specifically, increases in ICP impede blood flow, which results in decreased cerebral blood flow velocity (CBFV). The TCD measures CBFV in the middle cerebral artery by using an ultrasound probe emitting a high-frequency (> 2 MHz) wave. The detected frequency shift between the incident and reflected wave (the so-called Doppler effect), resulting from blood movement, correlates directly to CBFV. The measurement is usually performed over the regions of the skull with thinner walls, such as at the back of the head, through the eye or in the temporal region, as the bones greatly weaken the transmitted ultrasound frequencies (Kristiansson et al., 2013). The relationship between increasing ICP and decreasing TCD-derived blood flow velocities was first described by Klingelhöfer et al. (1987).

Besides the mean velocity, other approaches for nICP estimation from TCD, such as the pulsatility index (difference between peak systolic and end-diastolic blood flow velocity, divided by the mean blood flow velocity), segments of the cycles in systole and slopes of the TCD waveforms, have been correlated with ICP (Bellner et al., 2004; Popovic et al., 2009). However, these estimates were reported to be inadequately accurate with a margin of error from ± 10 to ± 15 mmHg (Schmidt et al., 2003).

The main limitation of the TCD method, besides the insufficient accuracy of ICP estimates, is the need for a trained and skilled operator to find the correct cerebral vessel and accurately interpret the results (Shen et al., 1999). Furthermore, significant intra- and interobserver variations have been reported previously (Baumgartner et al., 1994; McMahan et al., 2007; Shen et al., 1999). The use of TCD is not possible in certain subgroups due to the lack of a TCD window, resulting in the inability of the ultrasound to penetrate the skull (Tsivgoulis et al., 2009). Lastly, CBFV is significantly affected by certain medications, autoregulation, and other physiological factors, therefore, the use of the TCD method for the estimation of nICP is faced with many unresolved challenges (Evensen & Eide, 2020).

A more promising approach for estimating nICP based on the TCD technique is the two-depth ophthalmic artery Doppler ultrasonography developed by Ragauskas et al. (2005). The blood flow velocity in the intracranial segments of the ophthalmic artery (IOA) depends on the ICP. The blood flow velocity in the extracranial segments of the ophthalmic artery (EOA) is affected by the surrounding intra-orbital pressure (Koskinen et al., 2017). In this method, pressure is applied on the tissue surrounding the eyeball, while simultaneously measuring the flow velocities in the intra- and extracranial portions of the eye artery using a two-depth trans-orbital Doppler ultrasonography. When the blood flow in IOA matches the blood flow in EOA, the corresponding external pressure is taken as the estimate of ICP (Ragauskas et al., 2012).

Two-depth TCD technique was validated against invasive ICP pressure monitors and LP (Ragauskas et al., 2005; Ragauskas et al., 2012). In both cases, low mean systematic errors and low standard deviations of the paired invasive and non-invasive recordings were observed. The main limitation of this technique is that it can only be utilised on individuals with healthy eyes and orbits and that it has an upper ICP pressure range of 25 mmHg (Kristiansson et al., 2013).

1.2.3.2 Tympanic membrane displacement

The tympanic membrane displacement (TMD) technique developed by Marchbanks (1989) was the first audiological method for assessing ICP noninvasively. Cochlear fluid pressure, a direct measure of ICP, can be assessed indirectly by measuring the displacement of the tympanic membrane during the acoustic reflex contraction elicited by a loud sound (Reid et al., 1989).

This non-invasive technique for the measurement of ICP is possible due to the communication of the CSF and the perilymph via the perilymphatic duct. Stimulation of the stapedial reflex results in a movement of the tympanic membrane, which was shown to correlate to ICP (Lang et al., 2003; Reid et al., 1990). An acoustic stimulus above the acoustic reflex threshold initiates a stapedius muscle contraction, resulting in a displacement of the tympanic membrane which can be measured with common tympanometry used for impedance audiometry. An outward displacement is indicative of low or normal ICP, and an inward displacement is suggestive of high ICP (Marchbanks, 1993; Shimbles et al., 2005).

It is important to note that the direction and magnitude of the TMD depends not only on the initial position of the stapes but also on numerous other factors affecting the acoustic impedance or the acoustic reflex strength (Popovic et al., 2009). The TMD technique cannot be used in patients with a compromised cochlear aqueduct, which is often the case in elderly individuals (Phillips & Marchbanks, 1989; Shimbles et al., 2005). Furthermore, due to the high interindividual variability and the predictive limits of the regression analysis greater than the normal ICP range, the TMD method is not a reliable quantitative assessment of ICP in clinical practice (Shimbles et al., 2005).

1.2.3.3 Otoacoustic emission

An evoked otoacoustic emission (OAE), first measured by Kemp (1978), is a sound emitted by subtle oscillations of the endo- and perilymph caused by contractions of the outer hairy cells of the inner ear in response to external acoustic stimuli. OAE travels to the stapes, and through the ossicles to the tympanic membrane, and can be recorded by sensitive microphones inserted in the external meatus. As mentioned earlier, CSF pressure changes in the inner ear alter the position of the stapes. As a result, OAEs generated in the inner ear in response to acoustic excitation change when ICP changes (Büki et al., 1996).

Similarly to TMD, the use of OAE is limited by the need for a patent cochlear aqueduct and has previously shown large interindividual variability (X. Zhang et al., 2017). Furthermore, OAE cannot be used on patients with sensorineural or conductive hearing loss (Büki et al., 2009).

1.2.3.4 Magnetic resonance imaging

The magnetic resonance imaging (MRI) method, developed by Alperin et al. (2000), is based on the intracranial pressure-volume relationship. A motion-sensitive MRI is used to measure pulsatile arterial inflow, venous outflow, and CSF flow between the cranium and the vertebrospinal compartment, from which intracranial volume and ICP are calculated.

The ratio between the pressure and volume from which the ICP is derived is called the elastance index. A strong correlation ($r = 0.98$; $p < 0.005$) has previously been found between an elastance index and invasively measured ICP (Alperin et al., 2000).

When using the MRI method, special care is required in the selection of representative image slides as well as choosing the representative blood vessels. Furthermore, this method is very sensitive to differences in heart rate measured in individual cases as well as CSF measurements. Even when the above were addressed, significant intraindividual variability was observed in some of the participants (Marshall et al., 2008). Lastly, despite an excellent agreement between the invasive ICP measurements and MRI estimates, the MRI method is impractical, especially for continuous ICP monitoring, due to the high costs, energy consumption, and non-portability (Kristiansson et al., 2013; Popovic et al., 2009).

To summarise, invasive methods of measuring ICP carry a risk of injury and possible complications, such as infection. Additionally, invasive ICP methods cannot be performed in microgravity or in combination with other research interventions, such as exercise. Several non-invasive techniques for the estimation of ICP have been developed, however, they lack the accuracy of their invasive counterparts. Furthermore, none of the non-invasive techniques available today are suitable for continuous monitoring or cannot be applied to a sub-group of individuals due to anatomical variations (Kristiansson et al., 2013).

1.3 Spaceflight-Associated Factors Which May Contribute to SANS

Astronauts are exposed to many external (i.e., ambient conditions) and internal (i.e., microgravity-induced adjustments in organ systems) factors during their missions on the ISS. Some of the factors that may contribute to the development of SANS are reviewed below, primarily in relation to their effects on IOP.

1.3.1 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.

In space, astronauts' IOP upon initial exposure to microgravity increases substantially (Chung et al., 2011; Draeger et al., 1993; Draeger et al., 1995; Draeger et al., 1997; Stenger et al., 2017) compared to pre-flight values in a seated position. However, when monitoring IOP for longer time periods, the results are inconclusive. Chung et al. (2011) reported that an astronaut's IOP, following an initial spike upon entering microgravity, remained elevated above the baseline ground values throughout the 8-day mission, while other researchers (Draeger et al., 1993; Draeger et al., 1997; Stenger et al., 2017) observed that IOP during space missions initially increased, but returned to pre-flight values within a few days.

Several mechanisms have been proposed to contribute to the sudden elevation in IOP observed upon entering microgravity, such as choroidal engorgement and expansion, and increased episcleral venous pressure brought about by headward fluid shifts (Mader et al., 1993; Mader et al., 1990; Thornton et al., 1977).

1.3.2 Exercise

Even short-duration spaceflight results in significant musculoskeletal and cardiovascular deconditioning (Hayes et al., 2013; Levine et al., 1996; T. Trappe et al., 2006) and thus exercise countermeasures have been a cornerstone of crew medical health care since the first ISS expedition. In an effort to mitigate musculoskeletal atrophy, astronauts on the ISS conduct daily training, using equipment specially designed for microgravity. The crew are allocated a daily two-hour window in which to conduct their exercise training regime of a combination of aerobic and resistive exercises. Crew members have access to a cycle ergometer, a treadmill, and an Advanced Resistive Exercise Device (ARED) (Marshall-Bowman et al., 2013; S. Trappe et al., 2009). Exercise prescription is based on the demonstrated efficacy of countermeasures to combat musculoskeletal and cardiovascular deconditioning in bed rest models (S. Lee et al., 2010; Pavy-Le Traon et al., 2007; Ploutz-Snyder et al., 2014; Shackelford et al., 2004; T. A. Trappe et al., 2007) and primarily focus on the trunk and lower body, where the majority of the musculoskeletal losses occur (LeBlanc, Lin, et al., 2000; LeBlanc, Schneider, et al., 2000).

1.3.2.1 Resistive exercise

Reports regarding the behaviour of IOP during static exercise are contradictory. The majority of studies reported significant increases in IOP with exercise (Bakke et al., 2009; Dickerman et al., 1999; Vieira et al., 2006), while others reported decreases (Lanigan et al., 1989), or even no change (Marcus et al., 1974; Robinson et al., 1986). When IOP did increase with static exercise, this increase was observed both in the presence (Dickerman et al., 1999; Vieira et al., 2006) and absence (Bakke et al., 2009; Vieira et al., 2006) of a Valsalva manoeuvre. If Valsalva manoeuvres are performed during a strength training exercise on the ARED onboard the ISS, high-intensity resistive exercise, particularly in conjunction with the gravitational effects described earlier, may be a contributing factor to the development of SANS (Barisano et al., 2022; Marshall-Bowman et al., 2013).

1.3.2.2 Aerobic exercise

In contrast to resistive exercise, the results of research into the effects of aerobic exercise on IOP are clear. Aerobic exercise has been repeatedly shown to produce transient reductions in IOP both during and post-exercise (Natsis et al., 2009; Roddy et al., 2014).

Exercise-induced reductions in IOP were observed at exercise intensities ranging from light, such as a brisk walk, to maximal, such as volitional exhaustion exercise on treadmills or cycle ergometers (Risner et al., 2009). It has been suggested that the magnitude of the IOP reduction is driven solely by the intensity, and not also the duration of the exercise (Kiuchi et al., 1994; Qureshi et al., 1996).

1.3.3 Hypercapnia

The ISS has no natural supply of fresh air. The existing air is reconditioned by scrubbing CO₂ and replenishing the oxygen. On Earth, the CO₂ concentration in ambient air is approximately 0.03%, whereas, on the ISS, this number ranges from 10 to more than 23 times higher (0.3 – 0.7 %) (Alexander et al., 2012), indicating that the CO₂ scrubbing system on the ISS is incapable of maintaining the CO₂ levels at levels similar to that on Earth.

CO₂ is a potent vasodilator that has been shown to cause increases in IOP (Laurie et al., 2017), and in the diameter of the vessels located in the retinal nerve fibre layer (RNFL) (Jaki Mekjavic et al., 2016). Additionally, the headaches often reported by otherwise

healthy astronauts (Law et al., 2014) onboard the ISS have been attributed to the elevated levels of ambient CO₂. Consequently, it was speculated that this ambient hypercapnia-induced cerebral vasodilation in conjunction with decreased venous drainage that occurs due to the loss of hydrostatic fluid gradient, might be a contributing factor towards the development of ocular changes observed in astronauts (Marshall-Bowman et al., 2013; Marshall-Goebel et al., 2017).

1.3.4 Hypoxia

When preparing for extra-vehicular activities (EVAs; i.e., activities external to the ISS), astronauts undergo a lengthy procedure in order to safely transfer between the environment of the ISS (~101.3 kPa) and the pressurised space suit (~29.7 kPa). The risk of decompression sickness is minimised by breathing oxygen combined with a slow decompression profile to prevent the inert nitrogen from forming gas emboli (Conkin et al., 2016).

To eliminate the need for such lengthy and complex decompression procedures and reduce the fire hazard, it is anticipated that future space vehicles and planetary habitats will be hypobaric with an enriched oxygen ambient. The increased oxygen levels will not be of sufficient magnitude to maintain normoxia under the anticipated hypobaric conditions. It is currently predicted that the ambient pressure in the habitats will be substantially reduced, with a partial pressure of oxygen (PO₂) corresponding to that at an altitude in excess of 2,000 m (Bacal et al., 2008; Bodkin et al., 2006).

The ocular apparatus is a commonly affected system during exposure to hypoxia (Jaki Mekjavic et al., 2021). As a consequence of the retina's high oxygen demand as a highly metabolically active tissue (Eshaq et al., 2014), adequate retinal oxygenation is essential for the maintenance of normal visual function. Similarly to CO₂, hypoxia also has a vasodilatory effect, leading to increased IOP (Karadag et al., 2008), and RNFL thickness (Jaki Mekjavic et al., 2016). Additionally, to match the increased retinal oxygen demand during hypoxic exposure, retinal vessels dilate and become more tortuous to allow for the required increases in blood flow (Neumann et al., 2016). Therefore, hypoxia may play a role in the development of ocular and cerebral changes associated with SANS.

1.3.5 Age

Ageing is associated with a variety of morphological and functional changes in all structures of the eye. Most common age-related eye problems include presbyopia, macular degeneration, cataracts, and dry eyes (Salvi et al., 2006). The relationship between age and IOP appears to be geographically dependent. Most American and European studies (Bonomi et al., 1998; Klein et al., 1992) reported an increase in IOP with age, whereas an inverse relationship was reported in Korean and Japanese studies (J. S. Lee et al., 2002; Nomura et al., 2002). It has been suggested that this is due to the lower incidence of obesity and hypertension in the older Asian population compared to European and American (Nomura et al., 2002), since both obesity and hypertension have previously been shown to have a positive association with IOP (Mori et al., 2000; Shiose, 1990).

Another age-related change to the ocular apparatus is the stiffening of the lamina cribrosa, a mesh-like structure at the optic nerve head. Consequently, its resilience decreases, making it more prone to permanent deformation of the retinal ganglion cells (Albon et al., 2000).

The age of the astronauts on their first expedition is increasing (Smith et al., 2020). Prior to the establishment of ISS in 2000, the mean astronaut age was 38 years, and by 2011, it increased to 46.7 years (Marshall-Bowman et al., 2013). It has been reported that

hyperopic shift is more commonly observed in older astronauts with decreased accommodation. To tackle this issue, NASA astronauts over the age of 40 years routinely bring ‘Space Anticipation Glasses’ on their missions on the ISS (Mader et al., 2011). It is possible that also other SANS-associated morphological and structural microgravity-induced ocular changes are potentiated by the ageing of the active astronaut population.

1.3.6 Sex

Sex-related discrepancies in ocular disorders occur due to a multitude of causes, from behavioural, biological, societal, and cultural. Worldwide, most eye illnesses are more prevalent in women than in men (Clayton & Davis, 2015; Korpole et al., 2022; Zetterberg, 2016). Results of studies investigating the relationship between IOP and sex are inconclusive (Bulpitt et al., 1975; Dane et al., 2003; Hashemi et al., 2005). It has been suggested that this might be due to the fluctuations in oestrogen throughout the females’ lifetime. Oestrogen has previously been shown to reduce sympathetic activity (Ebeigbe et al., 2012; Qureshi, 1997).

Female astronauts have flown and worked in outer space since the beginning of human spaceflight. Only two years after the first successful flight of Yuri Gagarin in 1961, cosmonaut Valentina Tereshkova became the first woman to orbit the Earth (Uri, 2018). Despite that, female astronauts have been significantly underrepresented in space missions. As of 2020, only 64 (11.4 %) out of 562 astronauts ever in space were female, with no female astronauts involved in spaceflights in the 1971–1980 decade (Smith et al., 2020).

Based on current data, SANS appears to predominantly affect male astronauts (Marshall-Bowman et al., 2013; Platts et al., 2014). Platts et al. (2014) reported that out of 25 ISS crew members who underwent complete ocular examination pre- and post-spaceflight, symptoms of SANS were manifested in five of the eight evaluated females (62.5%), and in 14 out of 17 evaluated males (82.3%). The rate of occurrence of SANS signs and symptoms, such as globe flattening, optic disc protrusion, ONS diameter, and refraction changes, between the sexes was insignificant ($p = 0.09$). However, out of the 19 astronauts where symptoms were present, females exhibited milder symptoms than males. It has been speculated that the observed sex differences might be related to higher vascular compliance in women (Waters et al., 2002), and lower age and weight of the female astronauts (Buckey et al., 2018; Platts et al., 2014). Due to a high underrepresentation of female astronauts, definite conclusions regarding the involvement of sex-related factors implied in the manifestation of SANS cannot yet be drawn.

1.3.7 Mission duration

As mentioned earlier, results of the post-flight questionnaire showed that spaceflight-induced ocular changes were observed in astronauts participating in short- and long-duration missions. Postflight hyperopic shift and decline in near visual acuity were observed in 11% and 23% of short-duration shuttle fliers, respectively, and 34% and 48% of astronauts on long-duration missions, respectively (Mader et al., 2011). Visual impairment is more frequent and severe in astronauts participating in long-duration missions (longer than 6 months) compared to short missions (up to 2 weeks), suggesting a potential dose-dependent effect of mission duration (Marshall-Bowman et al., 2013).

Recent evidence suggests that only some, but not all, of the ocular changes observed in astronauts worsen with the duration of the spaceflight. Macias et al. (2021) reported a progressively greater global total retinal thickness and development of macular choroidal folds in two astronauts during a 1-year mission to the ISS. The progression of folds was characterised by the development of new and deformation of existing folds. On the

contrary, the axial length, anterior chamber depth, and peripapillary choroid engorgement observed in the same two astronauts at the end of the 1-year mission did not differ from the cohort of 11 astronauts participating in a 6-month mission (Macias et al., 2020). Furthermore, a positive relationship between optic nerve head displacement and mission duration has been observed in a cohort of 22 astronauts (Wählin et al., 2021).

1.4 Ground-Based Simulations of Spaceflight

Ground-based studies simulate the effects of weightlessness with the experimental bed rest model, since the adaptation of physiological systems to microgravity is similar to the adaptations observed during inactivity and unloading of the weight-bearing limbs (for review see Pavy-Le Traon et al., 2007). This model, in which the participants are supine in the horizontal or 6°HDT position for different durations, is acceptable to study the adaptation of most physiological systems, it has not yet, however, yielded ophthalmological results similar to those observed on the ISS.

Since the most commonly noted symptom of SANS among astronauts is a change in visual function, similar observations would be anticipated during prolonged bed rests, were it a valid ground-based simulation. With the exception of one study (Drozdova & Nesterenko, 1970), such changes have not been observed during prolonged bed rest (Jaki Mekjavic et al., 2002; Taibbi et al., 2014, 2016). Other ocular changes observed during and post prolonged bed rest include subtle increases in peripapillary retinal thickness and peripapillary retinal volume (Taibbi et al., 2013), increased retinal nerve fibre layer thickness (Jaki Mekjavic et al., 2016; Taibbi et al., 2014), increased choroidal thickness (Jaki Mekjavic et al., 2016), and more recently also optic disc oedema (Laurie et al., 2019, 2020). Traditionally, bed rest studies allow the participants to use a pillow throughout the day and night, and to lift their head and upper torso during mealtimes (Taibbi et al., 2014, 2016). This may limit the cephalad fluid shift and lead to differences compared to that observed in weightlessness. Furthermore, the majority of bed rest studies fail to adequately replicate the elevation in CO₂ levels on board the ISS, therefore, Laurie et al. (2019, 2020) designed a study to include the mild hypercapnic environment as experienced by astronauts (F_iCO₂ = 0.5%), and limit lifting of the head to maintain cephalad fluid redistribution. Following 30 days of strict 6°HDT bed rest in such an environment resulted in Frisen grade 1 and 2 optic disc oedema (Frisén, 1982) in 5 out of 11 healthy participants, and a total retinal thickness increase that was ~4 to 5 times greater than following a 70-day HDT bed rest in a normal environment and with a standard pillow (Laurie et al., 2020; Taibbi et al., 2016). The incidence of optic disc oedema in astronauts following a spaceflight is lower than observed in the mentioned bed rest study and frequently presents with retinal and/or choroidal folds, which were not observed by Laurie et al. (2020). Therefore, whether the underlying mechanism of optic disc oedema formed during bed rest is the same as during spaceflight, remains unresolved.

Results from studies measuring IOP during prolonged exposures to simulated microgravity in the supine position are equivocal. Chiquet et al. (2003) reported that during supine bed rest, IOP initially spiked and then gradually decreased back to pre-bed rest values measured in a seated position. This is in line with results from studies that only measured IOP pre and post prolonged bed rest exposure (Jaki Mekjavic et al., 2016; Louwies et al., 2016). A tendency for an increase in IOP in the initial few days of bed rest compared to seated values measured pre-bed rest was observed also during the latest 60-day supine 6°HDT bed rest organised by ESA, NASA, and the German Aerospace Centre (Mekjavic, personal correspondence). As seen in Figure 1.2, individual IOP values for each participant tended to increase with a supine position compared to a seated position,

however, as a group, this increase was non-significant. On the other hand, Taibbi et al. (2016) reported a constant and sustained increase in IOP throughout 14- and 70-day bed rest, and Drozdova and Nesterenko (1970) observed a decrease in IOP following 70-day bed rest.

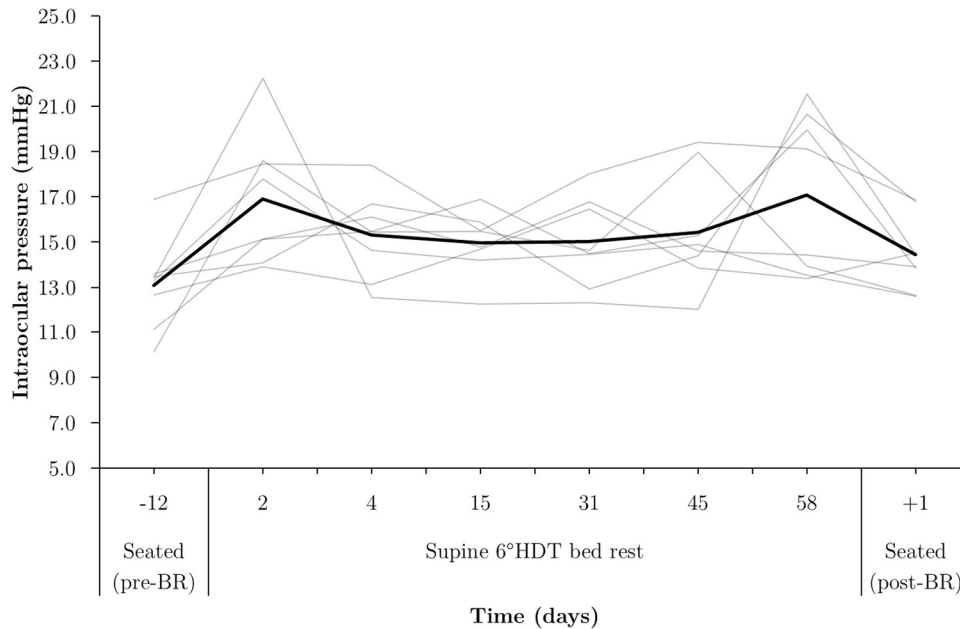


Figure 1.2: IOP responses of the AGBRESA Control group (6 males, 2 females; non-exercising) during the 60-day supine 6°HDT bed rest. Mean group response is shown in black and individual responses are shown in gray (Mekjavic, personal correspondence).

Transient increases in IOP upon exposure to microgravity have been observed consistently. If supine 6°HDT bed rest was an appropriate ground-based model for the simulation of the effects of microgravity on the neuro-ocular system, similar increases would be expected. With the exception of Chiquet et al. (2003), such observations were not reported. Additionally, Anderson et al. (2016) showed with a series of measurements conducted in the horizontal supine and prone positions, and during parabolic flights, that IOP is not governed only by fluid shifts, but also by changes in hydrostatic gradients produced by changes in the direction of the gravity vector. Hence, it can be speculated that the supine horizontal or 6°HDT bed rest 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, and cardiovascular and musculoskeletal systems, however, the anatomical position of the eye, within the orbit, probably prevents any significant gravity-induced changes in its axial length.

Chapter 2

Problem Identification And Thesis Structure

2.1 Problem Identification

Visual impairment during spaceflight has been reported since the early days of the space programme. With advancements in technology, additional pathophysiological neuro-ocular changes have been identified, and since the first report in 2011 (Mader et al., 2011), extensively researched. Initially, these changes, observed in a subset of astronauts, were thought to arise due to increases in intracranial pressure (ICP), hence the malady was termed visual impairment/intracranial pressure (VIIP) syndrome. Early VIIP research focused heavily on ICP measurements. As discussed previously, invasive measurement of ICP in microgravity is not possible, and the non-invasive methods are unreliable. Due to the potential role of ICP in the manifestation of VIIP, and some of the common symptoms, scientists believed that idiopathic intracranial hypertension (IIH) provides a good terrestrial analogue. However, many of the clinical symptoms found in IIH, such as chronic severe headaches, tinnitus, transient visual obscuration, and diplopia, have not been reported by astronauts. Additionally, astronauts presenting with ocular changes do not fit the typical demographics of terrestrial IIH patients (e.g., obese, young, female) (A. Lee et al., 2018). It has since been demonstrated that ICP in microgravity is not elevated pathologically (Lawley et al., 2017), and due to yet unresolved aetiology of this syndrome, VIIP has been renamed as Spaceflight-Associated Neuro-ocular Syndrome (SANS). More likely, it is the interaction of ICP and intraocular pressure (IOP) at the lamina cribrosa that contributes to the development of SANS, along with a plethora of proposed potential internal and external factors. Likely, the exact SANS mechanism does not depend only on a single factor, but is rather multifactorial. An increased understanding of factors contributing to this syndrome is currently a top priority for ESA and NASA, especially since this medical obstacle could impact the visual health of astronauts as well as the success of future missions, including continued trips to the ISS, a return to the moon, or a future human mission to Mars. In the present thesis, the independent and interactive contribution of several current and future spaceflight-associated factors (i.e., hypercapnia, hypoxia, age, sex, and resistive exercise) on development of SANS was investigated in a series of four studies.

Given the time and cost limitations, as well as the low participant numbers (e.g., astronauts), of studying SANS development on board the ISS, it is imperative to find an adequate terrestrial analogue. Ground-based studies simulate microgravity and its effect on the body by using an experimental supine horizontal or 6°head-down tilt (HDT) bed rest model. This model is appropriate to investigate the effect of weightlessness on most

physiological systems, however, its effects on the neuro-ocular system do not adequately replicate the changes observed during spaceflight. To evaluate and potentially identify a more appropriate model, a study comparing three different positions, namely seated, supine 6°HDT and prone 6°HDT was performed.

2.2 Thesis Structure

The evaluation of different spaceflight-related factors on ocular structures, primarily IOP, and their potential involvement in the aetiology of SANS was investigated in a series of separate studies. All of the experimental protocols received appropriate ethical approval (Appendix A) and conformed to the Declaration of Helsinki guidelines. The contents of this thesis are based on the published papers (please, see Appendix B).

The Introduction (Chapter 1) presents a brief review of SANS and its symptoms observed in a subset of astronauts. The effect of IOP and ICP on lamina cribrosa, and several techniques of measuring IOP and ICP are also discussed. Furthermore, it addresses the shortcomings of current ground-based research protocols used to study the effects of simulated microgravity on ocular structures, and several factors that potentially contribute to the development of SANS.

Problem identification, thesis structure and hypotheses of each study are presented in Chapter 2.

Study I (Chapter 3) evaluated the effect of three different postures, namely seated, supine 6°HDT and prone 6°HDT, on IOP responses in younger males. Additionally, it examined the effect of hypercapnia on IOP during rest and isometric handgrip exercise in each of the mentioned positions. The suitability of using either supine or prone 6°HDT position as a ground-based equivalent to microgravity is also discussed in this chapter.

Study II (Chapter 4) explored the effect of hypercapnia on IOP during rest and isometric handgrip exercise in older, astronaut-aged males while in a prone 6°HDT position. Reasons, why age may or may not have an effect on SANS manifestation, are discussed in more detail, along with a comparison of the results from Studies I and II.

Study III (Chapter 5) examined the effect of sex on IOP responses in rest and exercise in a prone 6°HDT position in an older, astronaut-aged population. Current evidence suggests that the incidence and severity of SANS symptoms in female astronauts are lower than in male astronauts. However, due to the underrepresentation of female astronauts on space missions, no concrete conclusions have yet been made.

Study IV (Chapter 6) investigated the effect of a one-day exposure to normobaric hypoxia equivalent to the altitude of 3,000 m on retinal blood vessels in adults and children. According to current plans, future habitats and vehicles used during extra-vehicular activities on the Moon and Mars will be hypoxic, and it is expected that eventually, habitats will be populated with individuals of all ages.

The findings of the present thesis and suggestions for future work are summarised in Chapter 7.

The results of this thesis will help to elucidate how different factors affect the ocular system, particularly IOP, and potentially contribute to the understanding of the occurrence and pathophysiology of SANS. Currently, SANS jeopardises all future long-duration space missions, therefore a better understanding of SANS will be an important step toward the development of appropriate countermeasures to prevent its occurrence.

2.3 Hypotheses

The thesis consists of four studies. Each study addressed a set of null hypotheses. The null hypotheses and potential alternative hypotheses, should a null hypothesis not be accepted, are outlined below.

Study I (Chapter 3): The effect of posture and hypercapnia on intraocular pressure during handgrip exercise in young males

- Null hypothesis 1 (H_01): Body posture does not affect IOP in younger males.
- Alternative hypothesis 1 (H_A1): IOP in younger males is affected by changes in the body position.

- H_02 : Hypercapnia does not affect IOP response in younger males.
- H_A2 : IOP response in younger males is affected by hypercapnia.

- H_03 : Isometric handgrip exercise does not affect IOP in younger males.
- H_A3 : IOP in younger males is affected by isometric handgrip exercise.

- H_04 : Isometric handgrip exercise in hypercapnia does not affect IOP in younger males.
- H_A4 : IOP responses in younger males are affected by isometric exercise in hypercapnia.

Study II (Chapter 4): The effect of hypercapnia on intraocular pressure during handgrip exercise in older males

- H_05 : Hypercapnia does not affect IOP in older males.
- H_A5 : IOP in older males is affected by hypercapnia.

- H_06 : Isometric handgrip exercise does not affect IOP in older males.
- H_A6 : IOP in older males is affected by isometric handgrip exercise.

- H_07 : Isometric handgrip exercise in hypercapnia does not affect IOP in older males.
- H_A7 : IOP in older males is affected by isometric exercise in hypercapnia.

Study III (Chapter 5): Effect of sex on intraocular pressure during static handgrip exercise in astronaut-aged individuals

- H_08 : Sex does not affect the IOP responses to isometric handgrip exercise.
- H_A8 : IOP responses to isometric handgrip exercise are affected by sex.

Study IV (Chapter 6): Effect of age on hypoxia-induced changes in retinal blood vessel diameter

- H_09 : Hypoxia has no effect on retinal blood vessel diameters.
- H_A9 : Retinal blood vessel diameters are affected by hypoxia.

- H_010 : Age does not affect retinal blood vessel diameters.
- H_A10 : Retinal blood vessel diameters are affected by age.

Chapter 3

The Effect of Posture and Hypercapnia on Intraocular Pressure During Handgrip Exercise in Young Males

As with all environmental stressors, the human body adapts to the microgravity environment, as experienced during spaceflight. The time course of adaptation to microgravity is specific for each physiological system, and the adaptations are typically reversible upon return to Earth's gravity (Nicogossian & Parker Jr., 1982). An exception would appear to be certain ocular changes observed in Spaceflight-Associated Neuro-ocular Syndrome (SANS) (Mader et al., 2011; Mader et al., 2013; Marshall-Bowman et al., 2013; Nelson et al., 2014).

One of the factors that was identified as potentially playing a role in the development of SANS is elevated intraocular pressure (IOP). Whilst IOP has been reported to increase at the onset of exposure to microgravity (Draeger et al. 1993, 1997), it is doubtful whether the observed IOP of 25 mmHg would instigate the morphological changes observed during longer missions. However, the astronauts' daily exercise regimen to combat microgravity-induced sarcopenia may cause further substantial and deleterious increases in IOP. Namely, on Earth, resistive exercise and hypercapnia have both been confirmed to elevate IOP, either independently (Awad et al., 2009; Bakke et al., 2009; Dickerman et al., 1999; Hvidberg et al., 1981; Laurie et al., 2017; Vieira et al., 2006) or synergistically (Kiss et al., 2001). The elevations in IOP during resistive exercise observed on Earth might be greater when performed in the absence of gravity (e.g., in space), and further exacerbated by the hypercapnic environment experienced on the International Space Station (ISS) (Law et al., 2014; Taylor et al., 2013). Carbon dioxide (CO₂) levels on the ISS are significantly higher than on Earth. Since 2008, spacecraft maximum allowable concentrations (SMACs) for CO₂ are defined as 2.0% for 1 hour, 1.3% for 24 hours, 0.7% for 7 to 180 days, and 0.5% for 1000 days (James, 2008).

It is questionable whether supine or prone head-down tilt (HDT) positions are suitable analogues for studying the effects of microgravity on the ocular system, particularly given that IOP responses during parabolic flights were observed to be in the range between the values occurring in the prone and supine positions (Anderson et al., 2016). However, due to the insufficient time required for IOP to stabilise following the assumption of either supine or prone posture, the values reported by Anderson et al. (2016) likely underestimate the IOP that would have been observed over a longer period of time.

As a prelude to a study investigating the effects of the exercise of duration similar to that conducted on a near-daily basis by the astronauts on the ISS, we sought to identify the posture that would best mimic the IOP elevations observed in microgravity and to assess the effect of a short acute bout of resistance exercise on IOP. Specifically, the present study assesses the effect of posture (i.e., sitting, supine 6°HDT, and prone 6°HDT) on IOP at rest and during static handgrip exercise at 30% of the participant’s maximum strength. Submaximal isometric handgrip exercise was chosen because it has previously been shown to increase both IOP and mean arterial pressure (Bakke et al., 2009), and enables easier IOP measurements. Due to the reported prevalence of hypercapnia on the ISS (Law et al., 2014), we examined the IOP responses also during hypercapnic exercise. The novel approach to assessing IOP while combining and manipulating all three aforementioned factors (i.e., posture, exercise, and hypercapnia), as in the present study, has not yet been reported in the literature.

3.1 Methods

3.1.1 Participants

Ten healthy, non-smoking young male participants gave their written informed consent to partake in the study. Their physical characteristics are shown in Table 3.1. Exclusion criteria included hypertension, any acute or chronic ophthalmic disorders, and any condition which would render participants incapable of conducting isometric handgrip exercise in either seated (Seated), supine 6°HDT (Supine) or prone 6°HDT (Prone) positions. Except for registration in a database, this study conformed to the Declaration of Helsinki and was approved by the National Medical Ethics Committee (approval no. 0120-31/2020/9) at the Ministry of Health (Republic of Slovenia).

Table 3.1: Participants' anthropometric characteristics and maximum isometric grip strength obtained in the seated (Seated), supine 6° head-down tilt (Supine) and prone 6° head-down tilt (Prone) positions.

Variable	Mean	SD	Range	
			Min	Max
Age (years)	22.5	3.1	19	29
Height (cm)	179.6	5.8	169.0	188.2
Weight (kg)	78.7	12.9	60.2	107.9
Max grip (Seated; kg)	41.9	6.8	27.2	52.1
Max grip (Supine; kg)	43.1	7.1	29.3	53.9
Max grip (Prone; kg)	42.1	7.1	27.3	51.9

3.1.2 Experimental protocol

To assess the effect of the two factors, namely posture (seated, supine 6°HDT, and prone 6°HDT) and fraction CO₂ (FCO₂) in the breathing mixture (normocapnic and hypercapnic) on IOP during rest and static handgrip exercise, participants were required to visit the laboratory on six occasions, with a minimum of 48 hours between consecutive visits. During

two visits the trials were conducted in a seated position (Seated), during two in the supine 6°HDT position (Supine) and during two in the prone 6°HDT position (Prone), as seen in Table 3.2. The order of the visits, and thus trials, was randomized. Each visit comprised a control trial (Control), in which IOP was measured with the participant in the standard clinical seated position. The Control measurement was followed by two 23-min trials separated by 30 min. In one 23-min trial, the participants inspired normoxic normocapnic room air (NCAP) and in the other a normoxic hypercapnic ($F_i\text{CO}_2 = 0.01$) gas mixture (HCAP). The NCAP and HCAP trials had an identical protocol, comprising three phases: (i) 10-min rest, (ii) 3-min isometric handgrip exercise at 30% of the participant’s maximum, and (iii) 10-min recovery (Figure 3.1). The order of the trials during the second visit in a given position was reversed. The analysis of the results was conducted on the averaged data of the two trials. To avoid diurnal fluctuations in any of the measured physiological variables, participants were required to visit the laboratory at the same time of the day.

Table 3.2: Example of the sequence of the trials for one of the participants. Every participant visited the laboratory on six occasions, with a minimum of 48 hours between consecutive visits. During two visits the trials were conducted in a seated position (Seated), during two in the supine 6°HDT position (Supine) and during two in the prone 6°HDT position (Prone). On each visit, they completed one trial while breathing normoxic normocapnic air (NCAP) and one trial while breathing normoxic hypercapnic gas mixture (HCAP). The order of the visits, and thus trials, was randomized.

	Posture	Trial 1	Trial 2
Visit 1	Prone	NCAP	HCAP
Visit 2	Prone	HCAP	NCAP
Visit 3	Seated	HCAP	NCAP
Visit 4	Supine	NCAP	HCAP
Visit 5	Supine	HCAP	NCAP
Visit 6	Seated	NCAP	HCAP

3.1.3 Intraocular pressure measurements in a standard clinical position

On arrival at the laboratory, the participant’s IOP was measured in a standard clinical seated position. This was done to ensure that participants’ IOP on days they undertook trials in different positions ($\text{Control}_{\text{Seated}}$, $\text{Control}_{\text{Supine}}$, and $\text{Control}_{\text{Prone}}$) did not differ and, therefore, impact the trials’ results. Triplicate measurements were obtained from the right eye using Pulsair IntelliPuff Tonometer (Keeler, Windsor, United Kingdom). The three IOP measurements’ average was taken as a participant’s measured Control value in a given visit.

3.1.4 Maximal strength measurement

Maximal handgrip strength in each of the three positions was measured on the days when the participants performed their first trial in a given position. For example, a participant whose schedule is presented in Table 3.2, performed a maximal strength measurement in a prone 6°HDT position at the beginning of visit 1, in a seated position at the beginning of visit 3, and in a supine 6°HDT position at the beginning of visit 4.

To measure participants’ maximal isometric handgrip strength, they assumed one of the three positions. For a measurement in the seated position, they sat on a stool with their back upright (90°), and for measurements in supine and prone 6°HDT positions, they

lay on a bespoke bed. The bed was at a 6° angle and had a padded horse-shoe-shaped headrest attached at one end. This allowed the face to protrude from the opening in the headrest while maintaining a relaxed neck and enabled IOP measurement in the prone 6°HDT position. During the maximal strength measurements, participants held the handgrip dynamometer (K-Force Grip, KINVENT, Montpellier, France) in their right hand with flexion at the elbow maintained at a 90° angle. On instruction, participants conducted maximal isometric handgrip with a handheld dynamometer twice for 5 s with a 60 s rest between the exertions. Strong verbal encouragement was provided throughout both trials. The participant's maximum handgrip strength was determined as the highest force obtained in the two trials.

3.1.5 Normocapnic and hypercapnic exercise trials

Prior to the trials, participants were instrumented for the measurement of impedance electrocardiography (Physioflow Q-Link, Manatec Biomedical, Paris, France), previously validated against the direct Fick method during exercise in healthy participants (Richard et al., 2001; Siebenmann et al., 2015). The Physioflow device provided continuous measurement of heart rate (HR, min⁻¹).

A blood pressure cuff (Withings model BP-800, Issy-les-Moulineaux, France) was fitted to the participant's left arm before assuming one of the three positions, for measurement of systolic (SAP, mmHg) and diastolic (DAP, mmHg) arterial pressure. Withings BP-800 sphygmomanometer fulfils the validation criteria of the European Society of Hypertension International Protocol Revision 2010 (Topouchian et al., 2014). Measures of SAP and DAP were used to calculate mean arterial pressure (MAP, mmHg), using Equation 3.1

$$\text{MAP} = \frac{\text{SAP} + 2\text{DAP}}{3} \quad (3.1)$$

Once instrumented, participants assumed one of the three positions (Seated, Supine or Prone) and were fitted with a nose clip and mouthpiece connected to a two-way non-rebreathing valve (Hans Rudolph Inc, Shawnee, Kansas, USA). During the trial, IOP, SAP, DAP, and HR were recorded towards the end of the 5th and 10th minutes of the rest period (R5 and R10, respectively), each minute of the 3-min exercise period (E1–E3; isometric handgrip exercise at 30% of their maximum, using a handheld dynamometer) and at the end of the 1st, 2nd, 5th, 7th and 10th minutes of the post-exercise recovery period (P1, P2, P5, P7, and P10, respectively), as seen in Figure 3.1. During the 3-min exercise period, the force exerted by the participants with the handgrip dynamometer was displayed on a screen (KAPA-INVENT, 2017), providing them continuous feedback. This was essential, as participants were instructed to maintain the exerted force at 30% of their previously measured maximal force. To avoid the influence of a Valsalva manoeuvre on any of the measured variables, all participants were regularly reminded to keep their neck relaxed and maintain normal respiratory patterns throughout the trial's entirety. Additionally, they were instructed to relax all the muscles not primarily involved in contraction to avoid the recruitment of accessory muscles and an increase in blood pressure. At every time point, IOP was measured three times on the right eye, and the average of three measurements was used for the analysis.

The second trial was conducted following a 30-min rest period. During the rest period, participants were requested to be seated in an upright position. The protocol for both trials was identical, with the exception of the gas inspired. In the NCAP condition, the inspired gas was normoxic normocapnic room air, whereas, in the HCAP condition, it was normoxic hypercapnic ($F_{\text{I}}\text{CO}_2 = 0.01$).

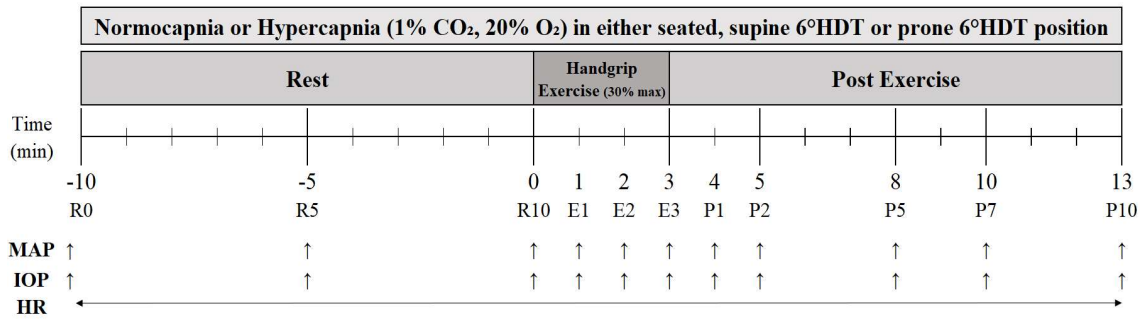


Figure 3.1: Graphic depiction of the protocol. Each participant underwent two experimental sessions in each of the three positions (seated, supine 6°HDT, and prone 6°HDT), six altogether. During each experimental session, participants conducted two trials; during one trial they breathed normocapnic normoxic air, and during the other normoxic hypercapnic gas mixture ($\text{FiCO}_2 = 0.01$). (Note: MAP: mean arterial pressure; IOP: intraocular pressure; HR: heart rate).

3.1.6 Statistical analyses

All data were assessed for normality using the Shapiro-Wilk and Kolmogorov-Smirnov test of normality. One-way repeated-measures analysis of variance (ANOVA) with a Bonferroni correction was used to investigate any significant difference in participants' IOP when measured in a standard clinical seated position on days when they underwent trials in different positions.

A two-way repeated-measures ANOVA was conducted to compare the main effects of all three positions, time, and their interaction effects on all measured variables (IOP, SAP, DAP, MAP, and HR) in each of the two conditions (NCAP and HCAP). When position*time interaction was significant, a one-way ANOVA with a Bonferroni correction was run as a *post hoc* test. The same *post hoc* test was used to observe the variables' temporal change in each position (Seated, Supine, or Prone) and each condition (HCAP or NCAP). A paired-samples *t*-test was used to assess if there was any difference between HCAP and NCAP trials in each position.

Descriptive statistics were expressed as mean \pm standard deviation (SD) unless stated otherwise, and the significance level for all statistical tests in this study was set at $p < 0.05$, *a priori*. Based on the results of a pilot test, I determined that for a required observed power of 0.8, a minimum of 8 participants needed to partake in the present study. To account for any potential drop-out, 10 participants were recruited. *A priori* power analysis was conducted using G*Power software (Faul et al., 2007). All other statistical analyses were performed using SPSS (v.25, IBM, NY, USA) software.

3.2 Results

All participants successfully completed the normocapnic and hypercapnic 3-min static handgrip exercise performed at 30% of their maximum handgrip strength during the three interventions (Seated, Supine, and Prone). The participants commented that they could not discern between the two breathing mixtures.

3.2.1 Heart rate

A significant main effect of position on HR (Figure 3.2) was observed in both conditions (NCAP: $F_{(2, 18)} = 22.829$, $p < 0.001$; HCAP: $F_{(2, 18)} = 12.739$, $p < 0.001$). *Post hoc* pairwise comparison analysis revealed that in both conditions HR in the Seated trials was significantly higher than in the Supine (NCAP: $p = 0.001$; HCAP $p = 0.005$) and Prone trials (NCAP: $p = 0.007$; HCAP: $p = 0.044$), while HR data in the Supine and Prone trials were statistically indifferent.

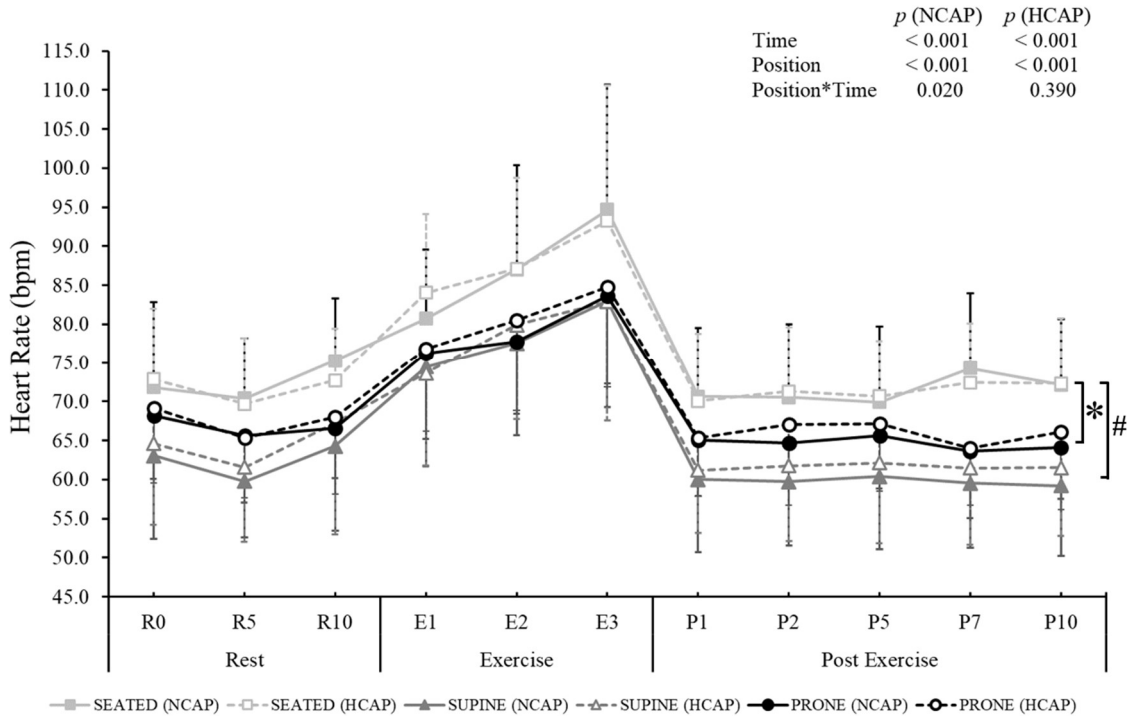


Figure 3.2: Heart rate response during normocapnic and hypercapnic trials (NCAP and HCAP, respectively) in seated (SEATED), supine 6°HDT (SUPINE) and prone 6°HDT (PRONE) positions. For clarity, the results of a two-way repeated-measures ANOVA are tabulated in the figure. Results on a one-way ANOVA are presented in the text (Note: *HR during trials in a SEATED position was statistically different to HR during trials in a PRONE position (NCAP and HCAP); #HR during trials in a SEATED position was significantly different to HR during trials in a SUPINE position (NCAP and HCAP); $p < 0.05$).

A significant main effect of time on HR was observed in all positions (Seated: $F_{(10, 90)} = 32.644$, $p = 0.002$; Supine: $F_{(10, 90)} = 35.163$, $p < 0.001$; Prone: $F_{(10, 90)} = 50.366$, $p < 0.001$). A significant exercise-induced elevation in HR (E3 compared to R10) was observed in all conditions and positions ($0.001 < p < 0.008$), except during the Seated HCAP trial ($p = 0.068$). A significant decrease in HR immediately upon cessation of exercise (E3 compared to P1) was observed in all positions and conditions ($0.001 < p < 0.015$) and persisted throughout the whole post-exercise period.

The main effect of the inhaled gas mixture on HR was significant only in the Supine trials ($F_{(1, 9)} = 10.065$, $p = 0.011$). A significant interaction effect of position and time on HR was observed in the NCAP condition ($F_{(20, 180)} = 26.085$, $p = 0.020$), where HR during

the Seated trials was higher compared to HR during the Supine trials at R5, R10, P1, P2, P7, and P10 ($0.002 < p < 0.035$), and higher than during the Prone trials at P7 ($p = 0.028$).

3.2.2 Systolic, diastolic and mean arterial pressure

A significant main effect of position on SAP was observed only in the NCAP condition (NCAP: $F_{(2, 18)} = 7.708$, $p = 0.004$). *Post hoc* pairwise comparison revealed that SAP in the Prone trials was significantly lower than in the Seated trials ($p = 0.018$). The main effect of position on DAP was observed in both NCAP and HCAP conditions (NCAP: $F_{(2, 18)} = 38.852$, $p < 0.001$; HCAP: $F_{(2, 18)} = 45.456$, $p < 0.001$) with DAP in the Seated trials being significantly higher than during the Supine (NCAP: $p < 0.001$; HCAP: $p < 0.001$) and Prone (NCAP: $p < 0.001$; HCAP: $p < 0.001$) trials.

The main effect of time on SAP and DAP was observed in all positions (Seated: $F_{(10, 90)} = 55.279$, $p < 0.001$ and $F_{(10, 90)} = 63.108$, $p < 0.001$ respectively; Supine: $F_{(10, 90)} = 41.150$, $p < 0.001$ and $F_{(10, 90)} = 49.332$, $p < 0.001$ respectively; Prone: $F_{(10, 90)} = 67.933$, $p < 0.001$ and $F_{(10, 90)} = 56.282$, $p < 0.001$ respectively), as seen in Table 3.3. Additionally, a significant effect of position and time interaction on DAP was observed only in HCAP trials ($F_{(20, 180)} = 1.925$, $p = 0.013$), and the main effect of the inhaled gas mixture on DAP was significant only in the Prone trials ($F_{(1, 9)} = 16.510$, $p = 0.003$), as seen in Table 3.3.

The main effect of position on MAP was observed in both HCAP and NCAP conditions (NCAP: $F_{(2, 18)} = 16.336$, $p < 0.001$; HCAP $F_{(2, 18)} = 24.528$, $p < 0.001$), as seen in Figure 3.3. MAP during the Seated trials was significantly higher compared to the Supine (NCAP: $p = 0.029$; HCAP: $p = 0.002$) and Prone (NCAP: $p < 0.001$; HCAP: $p = 0.001$) trials.

The main effect of time on MAP was present in all positions (Seated: $F_{(10, 90)} = 75.216$, $p < 0.001$; Supine: $F_{(10, 90)} = 8.890$, $p < 0.001$; Prone: $F_{(10, 90)} = 77.272$, $p < 0.001$). MAP increased significantly immediately with exercise (R10 compared to E1) in all positions and conditions ($0.001 < p < 0.020$), except in the Supine HCAP trial ($p = 0.138$). By the 3rd minute of exercise (E3) MAP in all conditions and positions was significantly higher than at R10 ($0.001 < p < 0.004$). Immediately upon cessation of exercise (E3 compared to P1), MAP significantly decreased ($p < 0.001$) and stayed decreased throughout the whole post-exercise period in all conditions ($p < 0.001$), except in the Supine NCAP trial at P5 ($p = 1.000$).

A significant interaction effect of position and time on MAP was observed only in the HCAP condition ($F_{(80, 180)} = 18.663$, $p = 0.011$). MAP during the Seated trials was significantly higher than during the Supine trials at R10, E1, E3, P2, P5, P7, and P10 ($0.012 < p < 0.041$), and higher than during the Prone trials at R5, E1, E3, P2, P5, P7, and P10 ($0.008 < p < 0.048$).

The main effect of the inhaled gas mixture on MAP was observed only in the Prone trials ($F_{(1, 9)} = 13.550$, $p = 0.005$). *Post hoc* tests revealed this difference was present at R5, R10, T1, T2, P2 and P7 ($0.002 < p \leq 0.037$).

Table 3.3: Average (SD) responses of systolic and diastolic pressure during normoxic hypercapnic (HCAP) and normoxic normocapnic (NCAP) conditions in seated, supine 6°HDT, and prone 6°HDT positions (Seated, Supine and Prone, respectively).

		Systolic Pressure										
		R0	R5	R10	E1	E2	E3	P1	P2	P5	P7	P10
N	Seated	121	124	126	134	144	155	128	124	127	127	125
		(7)	(7)	(7)	(8)*	(11)*	(14)*	(8)†	(8)†	(9)†	(8)†	(9)†
		126	123	122	132	139	149	129	123	119	124	123
C	Supine	(10)	(9)	(7)	(9)*	(12)*	(10)*	(10)†	(8)†	(20)†	(7)†	(8)†
		124	120	120	126	133	142	124	122	121	120	121
P	Prone	(8)	(6)	(7)	(8)*	(8)*	(12)*	(8)†	(8)†	(6)†	(7)†	(8)†
		Diastolic Pressure										
		R0	R5	R10	E1	E2	E3	P1	P2	P5	P7	P10
N	Seated	77	77	77	83	92	100	74	75	76	76	78
		(6)	(8)	(10)	(11)*	(9)*	(7)*	(7)†	(10)†	(9)†	(7)†	(7)†
		68	66	65	74	80	90	65	61	65	64	67
C	Supine	(7)	(6)	(6)	(8)*	(11)*	(10)*	(6)†	(7)†	(8)†	(7)†	(4)†
		68	65	66	71	77	87	65	62	65	65	66
P	Prone	(7)	(6)	(7)	(9)	(10)*	(7)*	(7)†	(6)†	(7)†	(7)†	(5)†
H	Seated	76	76	78	84	93	104	77	76	77	77	79
		(8)	(8)	(9)	(7)*	(10)*	(11)*	(9)†	(10)†	(8)†	(7)†	(8)†
		67	69	66	72	81	89	67	62	67	66	66
C	Supine	(6)!!	(6)!!	(6)!!	(8)!!	(11)!!	(9)*!!	(8)!!!	(7)!!!	(5)!!!	(5)!!!	(6)!!!
		69	67	70	73	79	87	66	65	67	68	66
P	Prone	(6)	(4)!!	(6)	(7)!!	(8)*!!	(7)*!!	(6)!!!	(6)!!!	(6)!!!	(6)!!!	(6)!!!

*significantly different response during exercise compared to R10; † significantly different response during recovery period compared to last minute of exercise (E3); !! significantly different from Seated; $p < 0.05$

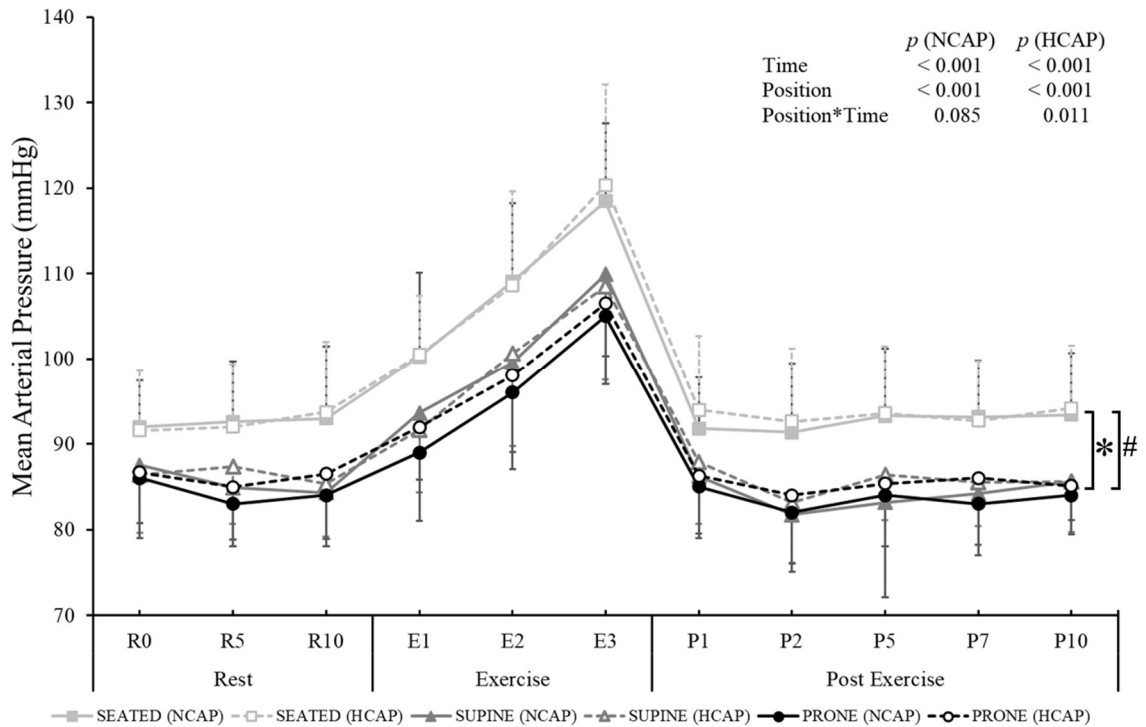


Figure 3.3: Mean arterial pressure response during normocapnic and hypercapnic trials (NCAP and HCAP, respectively) in seated (SEATED), supine 6°HDT (SUPINE) and prone 6°HDT (PRONE) positions. For clarity, the results of a two-way repeated-measures ANOVA are presented on a graph and the results of a one-way ANOVA are presented in the text (Note: dashed coloured error bars: HCAP condition; solid black error bars: NCAP condition; *MAP during trials in a SEATED position was statistically different to MAP during trials in a PRONE position (NCAP and HCAP); #MAP during trials in a SEATED position was significantly different to MAP during trials in a SUPINE position (NCAP and HCAP); $p < 0.05$).

3.2.3 Intraocular pressure

As shown in Table 3.4, no significant difference was found between participants' IOP measured in a standard clinical seated position on days when they undertook trials in different positions ($p = 0.189$).

Table 3.4: Participants' IOP measured in a standard clinical seated position on days when they undertook trials in different positions. (Note: $\text{Control}_{\text{Seated}}$ when trials were performed in a seated position, $\text{Control}_{\text{Supine}}$ when trials were performed in a supine 6°HDT (Supine) position, and $\text{Control}_{\text{Prone}}$ when trials were performed in a prone 6°HDT (Prone) position.)

Variable	Mean	SD	Range	
			Min	Max
$\text{Control}_{\text{Seated}}$	13.6	1.8	9.8	16.6
$\text{Control}_{\text{Supine}}$	13.8	2.1	10.6	17.3
$\text{Control}_{\text{Prone}}$	13.2	1.4	10.5	15.9

The main effect of position on IOP was present in both NCAP and HCAP conditions (NCAP: $F_{(2, 18)} = 267.485$, $p < 0.001$; HCAP: $F_{(2, 18)} = 155.436$, $p < 0.001$), as seen in Figure 3.4. In both conditions, IOP in the Prone trials was significantly higher compared to IOP in the Seated (NCAP and HCAP: $p < 0.001$) and Supine (NCAP and HCAP: $p < 0.001$) trials. Additionally, IOP in the Supine trials was higher than in the Seated trials (NCAP: $p = 0.005$; HCAP: $p = 0.003$).

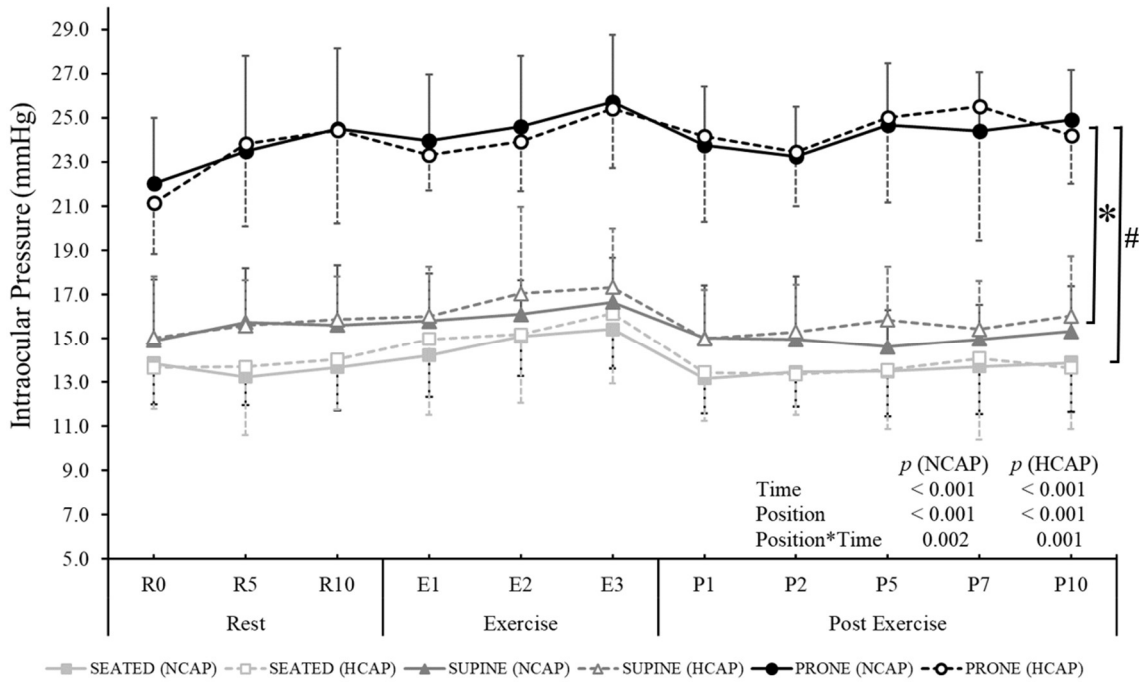


Figure 3.4: Intraocular pressure response during normocapnic and hypercapnic trials (NCAP and HCAP, respectively) in seated (SEATED), supine 6°HDT (SUPINE) and prone 6°HDT (PRONE) positions. For clarity, the results of a two-way repeated-measures ANOVA are presented on a graph and the results of a one-way ANOVA are presented in the text (Note: dashed coloured error bars: HCAP condition; solid black error bars: NCAP conditions; *IOP during trials in a PRONE position was statistically different to IOP during trials in a SUPINE position (NCAP and HCAP); #IOP during trials in a PRONE position was significantly different to IOP during trials in a SEATED position (NCAP and HCAP); $p < 0.05$).

A main effect of time on IOP was observed in all positions (Seated: $F_{(10, 90)} = 11.914$, $p < 0.001$; Supine: $F_{(10, 90)} = 8.507$, $p < 0.001$; Prone: $F_{(10, 90)} = 22.165$, $p < 0.001$), however, IOP increased significantly with exercise (R10 compared to E3) only during a HCAP Seated trial (from 14.0 ± 2.3 to 16.1 ± 3.2 mmHg; $p = 0.042$). Furthermore, immediate post-exercise drop (E3 compared to P1) in IOP was significant only in the Seated and Prone NCAP trials ($p < 0.048$ and $p < 0.021$, respectively).

A significant interaction effect of position and time was observed in both conditions (NCAP: $F_{(20, 180)} = 2.286$, $p = 0.002$; HCAP: $F_{(20, 180)} = 2.473$, $p = 0.001$). *Post hoc* pairwise comparison analysis showed that IOP in Prone trials was higher than IOP in the Seated and Supine trials at all time points ($p < 0.001$).

The main effect of the inhaled gas mixture on IOP was significant only in the Supine position ($F_{(1, 9)} = 10.932$, $p = 0.009$). IOP during the HCAP trial was significantly higher than during the NCAP trial only in the 5th minute of post-exercise rest (P5; $p = 0.002$).

3.3 Discussion

The main finding of the present study is that IOP during rest and static handgrip exercise was significantly greater in the prone 6°HDT position than in either the seated or supine 6°HDT positions. Additionally, IOP in the prone 6°HDT position was constantly elevated above 21 mmHg. Clinically, long-term values above 21 mmHg are considered indicative of ocular hypertension (The Royal College of Ophthalmologists, 2016). This was observed in both acute normoxic hypercapnia (HCAP) and normoxic normocapnia (NCAP). No significant effect of hypercapnia on IOP was observed in any of the positions in the present population of younger participants. IOP measured in a standard seated clinical position (13.5 ± 0.3 mmHg when combined for all three postures) was in line with the normative values for a given population (Martin, 1992; Shiose & Kawase, 1986).

In the present study, we did not replicate the length and type of resistance training as performed on ISS, since the aim was to observe the general effects of resistive exercise on IOP and not fitness. Additionally, we decided to only look at acute exposures to each condition since prolonged exposure to a prone 6°HDT position could result in too great of discomfort for the participants.

3.3.1 Posture

It has been suggested that initial and sudden increases in IOP observed during space missions are most likely due to choroidal engorgement and expansion brought about by headward fluid shifts (Mader et al., 1993; Mader et al., 1990). As reported by Draeger et al. (1993, 1997), IOP increased to 25 mmHg (a 92 – 114% increase compared to pre-flight data) within 16 min upon reaching Earth's orbit. Elevations in IOP were also observed during parabolic flights (Anderson et al., 2016; Frey et al., 1993) and bed rest in both the supine horizontal and 6°HDT positions (Anderson et al., 2017; Anderson et al., 2016; Carlson et al., 1987; Eklund et al., 2016; Lam & Douthwaite, 1997; Laurie et al., 2017), as well as a horizontal prone position (Anderson et al., 2017; Anderson et al., 2016; Lam & Douthwaite, 1997). Additionally, Anderson et al. (2016) showed that changes in hydrostatic gradients resulting from changes in the direction of the gravity vector play a significant role in IOP responses. During parabolic flights, Anderson et al. (2016) observed IOP values (16.3 ± 2.7 mmHg) that were between the values measured on the ground in the horizontal supine (13.7 ± 3.0 mmHg) and prone (20.3 ± 2.6 mmHg) positions. IOP values obtained by Anderson et al. (2016) in all three conditions were remarkably lower than IOP values measured during spaceflight (Draeger et al. 1993, 1997). This could be attributed to the fact that measurements in supine and prone positions were conducted in a horizontal and not 6°HDT position and insufficient time was allocated for the IOP to stabilise in each position. Although in the present study, IOP in supine 6°HDT increased to 15.7 ± 2.3 mmHg (NCAP and HCAP combined) by the end of the initial 10-min rest and was not significantly different from the seated values (13.8 ± 2.1 mmHg for NCAP and HCAP combined), IOP in the prone 6°HDT position increased to 24.5 ± 3.9 mmHg (NCAP and HCAP combined), values that are very similar to those reported by Draeger et al. (1993, 1997). It can therefore be speculated that fluid shifts and the direction of the gravity vector in prone 6°HDT position better mimic the effects of microgravity, hereby making it a more appropriate ground-based simulation model of acute ocular changes that occur in microgravity than supine 6°HDT.

3.3.2 Resistive exercise

A multitude of exercise countermeasures have proven to be only partially effective in preventing the adaptation of physiological systems to microgravity. Nevertheless, astronauts on the ISS perform daily training, consisting of aerobic and resistive exercises (Marshall-Bowman et al., 2013).

During static exercise, blood flow to the active skeletal muscles is decreased due to increases in intramuscular pressure resulting from prolonged continuous contraction. Static exercise invokes marked increases in MAP (Avunduk et al., 1999; Lind, 1970), which are further exacerbated by a Valsalva manoeuvre (Narloch & Brandstater, 1995; Zebrowska et al., 2013). Narloch and Brandstater (1995) reported that during five repetition maximum (RM) leg press, MAP increased 110 mmHg more (183 vs. 293 mmHg) when the Valsalva manoeuvre was performed compared to slow exhalation during concentric contraction. In the present study, MAP increased significantly with exercise in all positions and conditions. Prior to testing, all the participants were told to avoid holding their breath, but since the breathing parameters were only observed visually and not monitored, we cannot ascertain whether all the participants successfully adhered to the instructions. Similarly, even though astronauts are trained to execute all exercise moves appropriately, it is likely that Valsalva manoeuvres are still occasionally performed during a strength training exercise on the Advanced Resistive Exercise Device (ARED) onboard the ISS.

The majority of studies investigating IOP responses during resistive exercise reported significant increases in IOP with exercise, either with (Dickerman et al., 1999; Vieira et al., 2006) or without (Bakke et al., 2009; Vieira et al., 2006) the presence of a Valsalva manoeuvre, while others reported decreases (Lanigan et al., 1989), or even no change (Marcus et al., 1974; Robinson et al., 1986), as observed in the present study. The reasons for these disparate results are unclear but are likely influenced by differences in exercise mode, participants' age, types of tonometry, and timings of the measurements. Studies investigating the relationship between MAP and IOP also report contradictory results. Some studies found that IOP increases parallel with MAP during static exercise (Bakke et al., 2009), whereas others reported a decrease (Lanigan et al., 1989) or found no change in IOP despite the increase in MAP (Marcus et al., 1974; Robinson et al., 1986). In the present study, MAP increased significantly in all positions and conditions; however, significant IOP elevations were only observed in the Seated position during hypercapnic exercise.

A drop in IOP has been observed following exercise, after short or prolonged bed rest and upon return to Earth. Whereas the post-exercise drop in IOP, as in our study, has been attributed to a decrease in plasma pH, and an increase in plasma osmolality and lactate (Marcus et al., 1970), the decrement in IOP following exposure to simulated and actual microgravity has been hypothesised to result from a sudden drop in choroidal volume, consequently resulting in a decreased aqueous volume and lower than expected IOP (Mader, 1991).

It has recently been reported (Fischman et al., 2018) that low-level resistance exercise does not affect ICP in either the seated or supine position. Even though IOP in the present study did not increase with exercise in any of the positions while in normocapnia, IOP values obtained in a Prone trial suggest that the prone 6°HDT rather than the supine 6°HDT position might be a better ground-based simulation of the effects of acute microgravity on IOP, therefore a similar analysis should be performed to investigate the ICP responses in a prone position.

3.3.3 Hypercapnia

Elevated levels of ambient CO₂ onboard the ISS, caused by insufficient CO₂ scrubbing systems, have been implicated in the frequently reported headaches by astronauts (Law et al., 2014). CO₂ is a potent vasodilator that has been shown to cause increases in IOP (Laurie et al., 2017), MAP (Sechzer et al., 1960) and the diameter of cerebral vessels (Faraci et al., 1994). Consequently, it has been speculated that hypercapnia-induced cerebral vasodilation is present also in astronauts on the ISS, and in combination with decreased venous drainage resulting from the absence of gravity vector exerts great stress on posterior ocular structures, potentially contributing to the development of SANS (Marshall-Bowman et al., 2013; Marshall-Goebel et al., 2017).

In the present study, a difference in the IOP response to hypercapnia and normocapnia during isometric handgrip exercise was observed only in a Seated position, where IOP increased significantly in the HCAP trials (from 14.0 ± 2.3 at R10 to 16.1 ± 3.2 mmHg at T3; $p = 0.042$), but not in the NCAP trials (from 13.7 ± 2.4 at R10 to 15.4 ± 2.2 mmHg at T3; $p = 0.303$). No such changes were observed in any other position, suggesting that IOP increases caused by cephalad fluid displacement in supine and prone 6°HDT positions potentially negated any changes that would have occurred due to hypercapnia.

Jaki Mekjavic et al. (2016) conducted examinations of the retina using OCT before and after 10-day bed rest, with participants inspiring either a hypoxic or a hypercapnic breathing mixture identical to the present study ($F_i\text{CO}_2 = 0.01$). Whereas a vasoactive effect of both hypercapnia and hypoxia was observed on the vessels in the neuroretina, the vessels in the choroid were predominantly affected by the hydrostatic component. In part, this may explain the observation in the present study where the highest IOP levels were recorded in the prone 6°HDT position.

3.3.4 Limitations

During the pilot phase of this study, we tested three portable tonometers (Pulsair IntelliPuff; iCare IC200, Icare Finland Oy, Finland; Tono-Pen, Reichert Technologies, USA) for the measurement of IOP in the seated position. Due to the nature of the experimental arrangement, Pulsair IntelliPuff was used in the study to avoid the use of an eye anaesthetic and to make it possible to measure IOP in all three positions. Considering the excellent agreements found between the data collected with Pulsair IntelliPuff and GAT, Pulsair IntelliPuff is an appropriate tool for measuring IOP in normo- and hypertensive individuals (Hubanova et al., 2015).

The second limitation of the present study was the lack of control resting trials in all three positions and both conditions during which participants would rest for the entire 23 minutes, assuming the postures tested. The 10-min rest prior to the exercise in the present study might not have been of sufficient duration to allow IOP to stabilise, especially in the prone 6°HDT position. A resting trial would allow us to observe any drift in the measured variables. If such data were available and indicated a significant drift, then the presented IOP data could have been corrected to reveal the true physiological responses of the variables.

Lastly, the present study only included younger male participants to avoid the presence of any age-related (ocular) illnesses. Data shows that female astronauts are less likely to develop SANS (Mader et al., 2011; Mark et al., 2014), potentially indicating that certain physiological differences between the sexes influence the manifestation of ocular changes observed in space. Therefore, to assess the influence of posture on IOP without the interference of these potential differences, we decided to include only younger male participants in the present study.

Chapter 4

The Effect of Hypercapnia on Intraocular Pressure During Handgrip Exercise in Older Males

A major hindrance to future long-term missions on the International Space Station (ISS), and the exploration of the Moon and Mars are the unexplained ophthalmic changes observed in a subset of astronauts. 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.

This, as yet unresolved, vision impairment has been attributed primarily to the increase in intracranial pressure (ICP) associated with spaceflight, which impacts the vision neuro-axis. Numerous ground-based studies have provided evidence regarding the manner in which different stressors impact 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 et al., 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 has only minimal effects.

With the exception of the report by Drozdova and Nesterenko (1970), deterioration in visual function during the ground-based studies simulating the effects of weightlessness with 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, haematological and neuro vestibular systems (Jost, 2008; Pavy-Le Traon et al., 2007). 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 participants after a 30-day 6°HDT bed rest. After 6 months, the OCT measurements matched baseline findings (Taibbi et al., 2013). Shinojima et al. (2012) reported an increase in IOP after 30 min of 10° HDT concomitant with a significant increase in subfoveal choroidal thickness, but in the absence of any significant change in the foveal retinal thickness. 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°HDT bed rest 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 bed rest 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 and difficulty with eating and hygiene. 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 Spaceflight-Associated Neuro-ocular Syndrome (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 changes in both the oxygen and the carbon dioxide (CO₂) 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, 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 was assessed. 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.

4.1 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 CO₂ in excess of 0.008 (i.e., 0.8% CO₂ 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, participants 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 participant inclusion criterion was age between 45 and 65 years.

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

4.1.1 Ethical approval

The study conformed to the standards set by the Declaration of Helsinki, except for registration in a database. The procedures were approved by the University of Portsmouth Science Faculty Ethics Committee (UK; approval no. SFEC 2019–040). Participants 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.

4.1.2 Participants

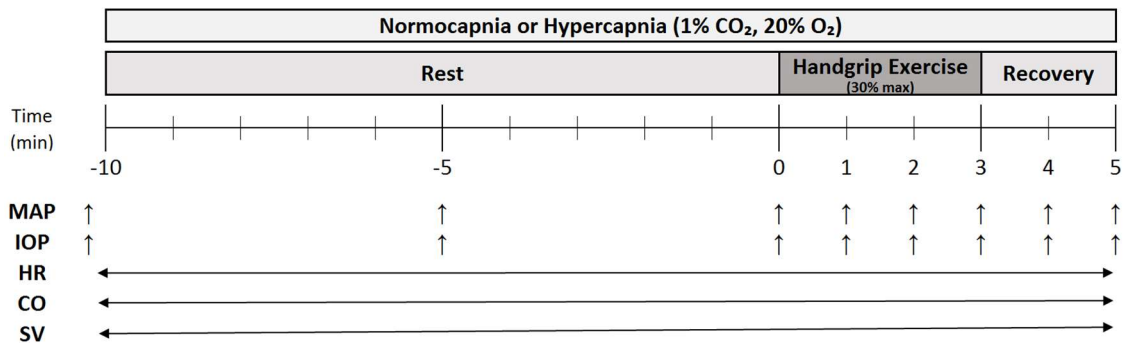
Ten older and healthy male volunteers (age: 57.8 ± 6.0 years, range: 48 – 65 years; weight: 80.9 ± 8.2 kg; height: 178.9 ± 4.9 cm; max prone handgrip strength: 40.2 ± 6.7 kg) gave their informed consent to participate in the study. Exclusion criteria included high blood pressure, any acute or chronic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

4.1.3 Protocol

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

Each experimental session comprised two exercise trials conducted on the same day and separated by 30 min. All exercise trials were conducted in the 6°HDT prone position and each comprised three phases: (i) a 10-min rest phase, (ii) a 3-min static handgrip exercise (dynamometry), and (iii) a 2-min recovery phase, as shown in Figure 4.1. During the static handgrip dynamometry phase, participants maintained a grip force which was 30% of their maximum grip force measured previously. In each experimental session, participants conducted one trial breathing normoxic normocapnic room air (NCAP), and the other breathing a normoxic hypercapnic mixture containing 1% CO₂ (HCAP). The order of NCAP and HCAP trials was counterbalanced and for each participant, the order of the trials was switched in the second experimental session conducted on a different day.

Figure 4.1: Schematic representation of the protocol. In each experimental session, participants conducted two trials in the 6°HDT prone position. During one trial they inspired (normoxic normocapnic) room air (NCAP), and during the other, they inspired a normoxic hypercapnic breathing mixture containing 1% CO₂ (HCAP). Abbreviations: CO, cardiac output; HR, heart rate; IOP, intraocular pressure; MAP, mean arterial pressure; SV, stroke volume.



The reason for conducting the NCAP and HCAP trials in one session was two-fold. I wished to assess the magnitude of the carryover effect, if any, of fatigue. Furthermore, in the sessions where the 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 protocol on the ISS.

4.1.4 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, KS, USA). Resting IOP and mean arterial pressure (MAP) measurements were obtained at minute 0 (R0), minute 5 (R5) and minute 10 (R10) of the rest period, each minute of the 3 min exercise period (T1–T3; isometric handgrip exercise at 30% of their maximum, using a handheld dynamometer) and at each minute of the 2 min recovery (P1–P2) period (Figure 4.1). To avoid any influence of Valsalva manoeuvres on any of the measured variables, all participants were instructed to maintain normal respiratory patterns.

The second trial was conducted following a 30 min rest period during which participants 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.

4.1.5 Intraocular pressure measurement in a standard clinical seated position

Prior to commencing the experimental protocol (described above), baseline measurements of IOP (mmHg) were obtained from each participant in the standard clinical seated 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, UK). Triplicate measurements were obtained from the right eye with each tonometer. The pneumotonometer was used 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.

4.1.6 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 a 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 s, with a 2 min rest between the exertions. Strong verbal encouragement was provided throughout both trials. The participant's maximum handgrip strength was determined as the highest force obtained in the two trials.

4.1.7 Impedance plethysmography and sphygmomanometry

On arrival to the laboratory, participants were instrumented with six 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 a continuous recording of heart rate (HR, min^{-1}), cardiac output (CO, $\text{L} \cdot \text{min}^{-1}$), and stroke volume (SV, 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 was measured using a Withings (Issy-les-Moulineaux, France) model BP-800 automated sphygmomanometer, from which MAP was calculated from values of systolic (SAP) and diastolic (DAP) pressures.

4.1.8 Statistical considerations

The sample size determination in the current study was based on the following criteria: (i) power $(1 - \beta) = 0.80$, (ii) $\alpha = 0.05$, (iii) two groups (normocapnic and hypercapnic), (iv) three repeated measures (resting, handgrip dynamometry, recovery), and (v) 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. Student's t -test for paired samples was conducted to assess the difference between IOP measured in the standard clinical sitting position using two tonometry methods. Two-way repeated measures 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 eight 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. 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 $p \leq 0.05$, *a priori*. Only participants with full datasets for each measured variable were included in the statistical analysis, and therefore for some variables, n is not equal to the total number (20) of the tests.

Age-related differences (comparison with younger men from Chapter 3) in participants' physical characteristics (age, height, weight, strength, and $\text{IOP}_{\text{Control}}$) were investigated using an independent samples t -test. The effect of age on IOP responses to rest and exercise in NCAP prone 6°HDT condition was assessed using a mixed model ANOVA, with the Bonferroni correction on subsequent *post hoc* analysis where the main effects were significant.

A relationship between age and $\text{IOP}_{\text{Control}}$ measured in a clinical seated position using Pulsair IntelliPuff Tonometer was investigated using Spearman's correlation analysis in GraphPad Prism (v.8.4, GraphPad Software, Inc., San Diego, USA). Correlation coefficients were applied as recommended by Cohen (2013): strong: $0.60 \leq r$; moderate: $0.40 \leq r < 0.59$; weak: $0.20 \leq r < 0.39$.

Descriptive statistics were expressed as means \pm standard deviation (SD), unless otherwise stated. All statistical analysis was performed using SSPS (v.25, IBM Corp., Armonk, NY, USA) software. Cohen's standardized-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).

4.2 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 participants breathing room air in the seated position, as evident from Table 4.1. When the participants assumed the prone 6°HDT position, IOP obtained with the pneumotonometer increased to 27.9 ± 3.7 mmHg.

Table 4.1: Intraocular pressure measured in the upright seated position while breathing room air using contact (iPen) and non-contact (Pulsair) tonometry.

Participant no.	Intraocular pressure (mmHg)	
	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 \pm SD	11.8 \pm 1.9	14.1 \pm 2.9*

* $p < 0.05$

As shown in Table 4.2, 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 $32.3 \pm$ mmHg upon completion of the exercise; Figure 4.2). Hypercapnia (HCAP trial) did not significantly modify the responses of MAP, HR, CO and SV (Table 4.2), but did enhance the IOP response, which increased from resting levels of 27.3 ± 4.3 mmHg to 34.2 ± 6.0 mmHg (Figure 4.2).

Results of a two-way repeated measures ANOVA revealed a significant time ($F_{(4,225, 80,277)} = 35.066$; $p < 0.001$) and condition ($F_{(1, 19)} = 8.351$; $p = 0.009$) effect, and significant interaction between time and condition ($F_{(7, 133)} = 0.873$; $p = 0.044$) for IOP. Further tests (Figure 4.2) revealed that the IOP level during the NCAP trial was significantly lower than that in the HCAP trial at R0 (23.4 ± 3.3 vs. 25.0 ± 3.8 mmHg; $p = 0.029$; $d = 0.45$), T1 (28.6 ± 3.8 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 was 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).

Figure 4.3 compares the last minute of the three phases in both NCAP and HCAP trials, namely the 10 min rest, 3 min exercise, and 2 min recovery. As is evident, HCAP significantly augmented the IOP during the handgrip dynamometry but did not have a significant effect during rest and recovery. Of note is the substantial individual variation observed in the IOP response.

Table 4.2: Mean (SD) responses of the 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 handgrip exercise in the prone 6°HDT position.

	<i>n</i>	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 · 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; $p \leq 0.05$.

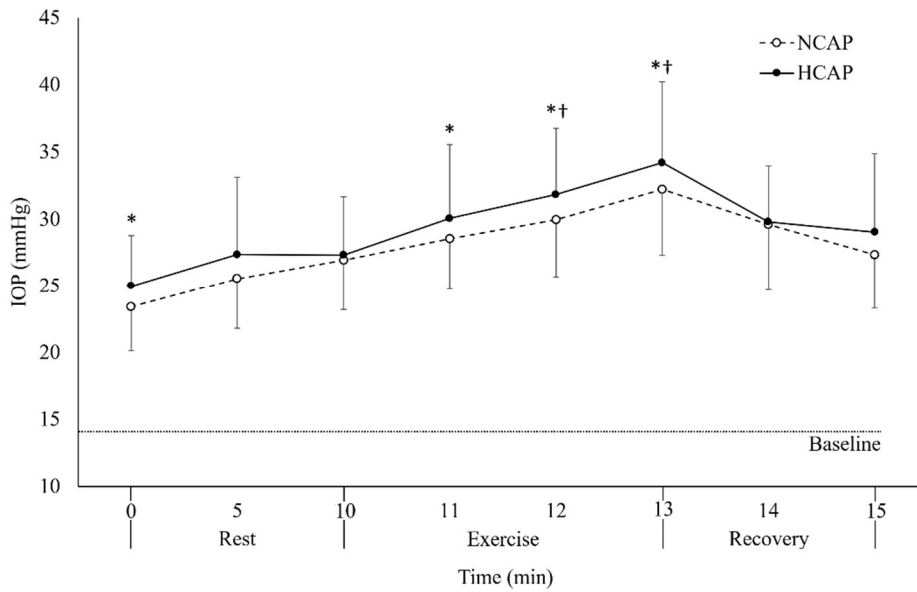


Figure 4.2: Intraocular pressure (IOP) during prone 6° head-down tilt in the normocapnic (NCAP) and hypercapnic (HCAP) trials, each comprising a 10 min rest period, followed by a 3 min period of exercise during which the participant performed an isometric handgrip exercise at 30% of maximum, and a 2 min recovery period. The dotted line represents the average IOP of the group (14.1 ± 2.9 mmHg) obtained in the upright seated position while breathing room air (*HCAP trial significantly different from NCAP trial; †significantly different response during exercise compared to R10; $p \leq 0.05$).

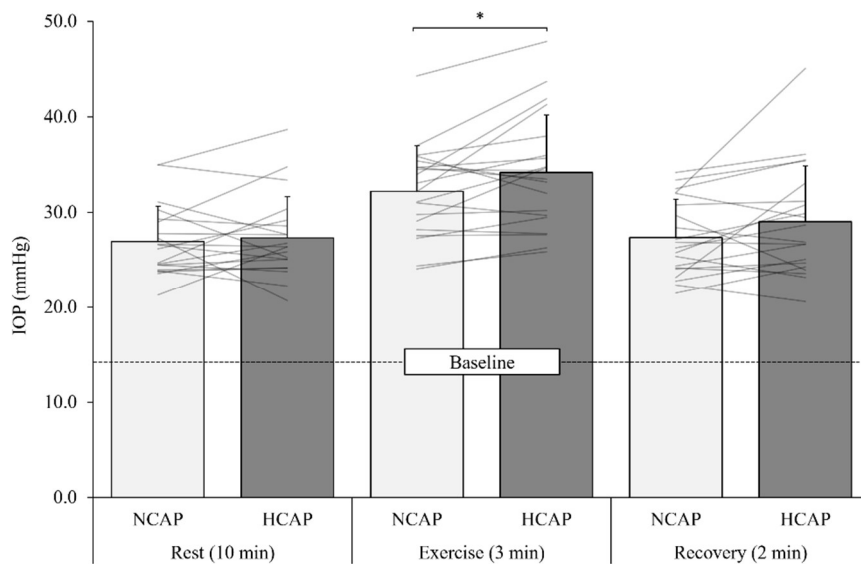


Figure 4.3: Mean and individual response of intraocular pressure (IOP) attained in the last minute of the 10 min rest, 3 min exercise and 2 min recovery, during the normocapnic (NCAP; open bars) and hypercapnic (HCAP; filled bars) trials. The Baseline indicates the average IOP obtained during the upright seated position while breathing (normocapnic normoxic) room air (*HCAP trial significantly different from NCAP trial; $p \leq 0.05$).

Males from the present study were significantly older compared to younger males from Chapter 3 ($t(18) = 17.386, p < 0.001, d = 7.8$). The two groups did not differ significantly in height ($t(18) = -0.582, p = 0.284$), weight ($t(18) = 0.465, p = 0.324$), strength ($t(18) = -1.597, p = 0.064$) and IOP_{Control} ($t(18) = 0.866, p = 0.199$). Moreover, no significant correlation was found between participants' IOP when measured in a seated position (IOP_{Control}) and age ($r = 0.152, p = 0.523$).

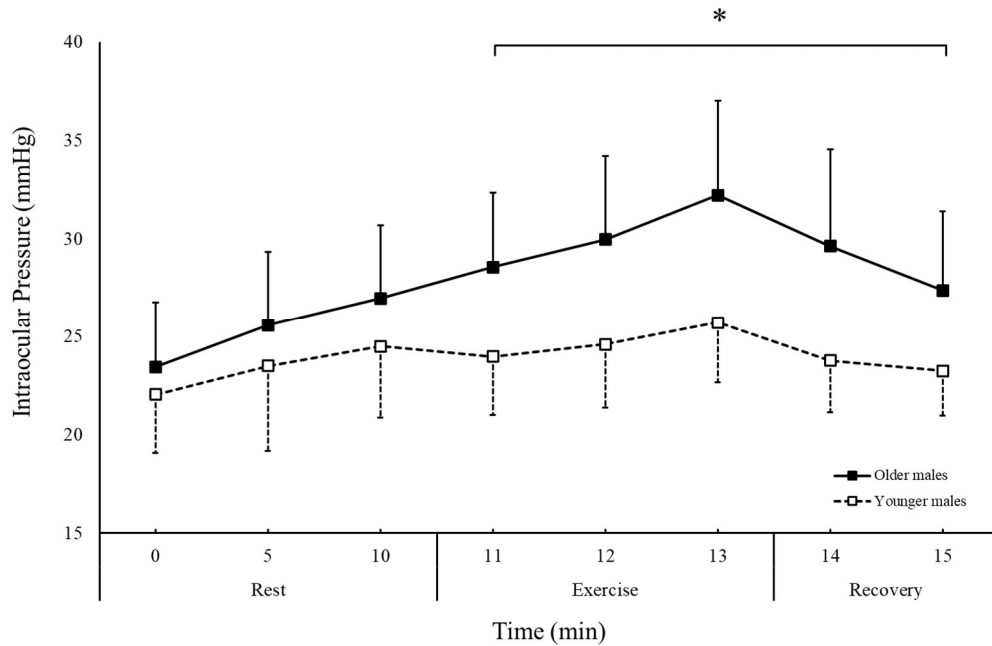


Figure 4.4: Intraocular pressure response of males from the present study compared to younger males from Chapter 3 during rest and exercise in prone 6°HDT position and in a normocapnic condition (Note: *significantly different between the two groups; $p < 0.05$).

The results of mixed model ANOVA showed a significant main effect of time ($F_{(3.945, 110.446)} = 19.227, p < 0.001$), and time*age interaction ($F_{(3.945, 110.446)} = 4.880, p = 0.001$) on IOP. *Post hoc* pairwise comparison analysis revealed that IOP was significantly greater in older males compared to younger males throughout the 3-min exercise and 2-min recovery period (T1: $p = 0.002, d = 1.3$; T2: $p = 0.002, d = 1.4$; T3: $p < 0.001, d = 1.6$; P1: $p = 0.002, d = 1.5$; P2: $p = 0.006, d = 1.3$), as seen in Figure 4.4.

4.3 Discussion

The principal finding of the present study is the substantial elevation of IOP in older males in the prone 6°HDT position compared to the seated upright position, which was further augmented by 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 than was observed in the supine position. Whereas Laurie et al. (2017) observed a significant effect of CO₂ during resting conditions (supine 6°HDT), I did not (prone 6°HDT). Rather, I observed the hypercapnic augmentation of IOP during the static exercise.

The baseline (seated upright) value of IOP observed in our participants (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 the Goldmann Applanation Tonometer (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 trial, 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 long-term values above this are considered indicative of ocular hypertension (The Royal College of Ophthalmologists, 2016) 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 (Figure 4.2).

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 Figures 4.2 and 4.3 that their IOP may be consistently elevated above the threshold for ocular hypertension, and recurrently even further elevated during the exercise conducted on the Advanced Resistive Exercise Device (ARED).

Despite the CO₂ potentiation of the exercise-induced increase in IOP in older participants, 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 is affected by CO₂, and this effect may contribute to the elevated IOP. The mechanisms for these responses and their potential contribution to SANS are discussed below.

4.3.1 Intracranial pressure

Increased ICP has been postulated to contribute to the aetiology of SANS (Alexander et al., 2012), due to its direct effect on IOP (Lashutka et al., 2004; Salman, 1997). 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 (MacDougall et al., 1985) most likely causes a substantial increase in ICP and consequently IOP.

4.3.2 Gravitational vector

The source of the changes in hydrostatic pressures within the eye (IOP) and brain (ICP) is the headward shift of body fluids resulting from the changes in the gravitational vector. 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 (1912) (for a 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 bed rest, 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 participants to be in bed rest continuously, and in the supine position. Another crucial aspect of Levinsohn's study is that during the 10°HDT, 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.

4.3.3 Posture: supine vs. 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 et al., 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. Zhang & Hargens, 2018).

Based on the work of Levinsohn, it would appear that the supine horizontal or 6°HDT bed rest may not be the best experimental model for studying SANS. The supine 6°HDT model may be suitable for studying the effects of microgravity on brain activity, and cardiovascular and musculoskeletal systems. However, it would appear that this position is not appropriate to result in any significant gravity-induced changes in the eyes' axial length due to their anatomical position within the orbit. 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 (Anderson et al., 2016; Eklund et al., 2016). 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.

4.3.4 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 CO₂ 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 CO₂ removal system on the ISS is at maximum capacity, it is not capable of maintaining the CO₂ concentration in the space habitat at the same level as on Earth. CO₂ is vasoactive and has a marked effect on brain blood flow (Sato et al., 2012; Willie 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).

Previously, hypercapnia was demonstrated to have no effect on IOP responses in younger males (Mlinar et al., 2021; Chapter 3), whereas, in the present study, the IOP responses to static handgrip exercise in prone 6°HDT position were significantly elevated in hypercapnic compared to normocapnic conditions. Participants in both studies that observed elevations in IOP when exposed to acute hypercapnia were older (Laurie et al.

(2017): 25 to 49 years; present study: 48 to 65 years) than the participants included in our previous study (Mlinar et al., (2021): 19 to 29 years). The discrepancy in the results could also be attributed to the diminished ventilatory response to hypercapnia in the elderly (Kronenberg & Drage, 1973; Peterson et al., 1981). Interestingly, Kronenberg and Drage (1973) observed a 40% lower hypercapnic ventilatory drive in elderly (64 to 73 years old) compared to younger (22 to 30 years old) men.

4.3.5 Resistive exercise

Exercise countermeasures on both Mir and ISS, for preventing significant musculoskeletal and cardiovascular deconditioning, 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 $2.5 \text{ h} \cdot \text{day}^{-1}$ 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 Jr et al., 2014). However, during the 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, respectively, measured during weightlifting (MacDougall et al., 1985). These arterial pressure responses during weightlifting have been implicated in a variety of pathological responses (see Dickerman et al., 1999), particularly in the brain (i.e., cerebral haemorrhage) and eye (i.e., subarachnoid haemorrhage, retinal haemorrhage and detachment, foveal haemorrhage). Dickerman et al. (1999) reported that during a maximal isometric contraction of the leg muscles in the seated position, with participants performing a concomitant Valsalva manoeuvre, IOP increased to 28 ± 9.3 mmHg, with one participant generating an IOP of 46 mmHg. The IOP elevations during prone 6° HDT observed in the present study during a submaximal (30% maximum) handgrip 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 (Marshall-Bowman et al., 2013), particularly considering that any effects of exercise would be superimposed on the gravitational effects discussed earlier.

4.3.6 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 & Van de Voorde, 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 brain circulation.

In a recent review, Zhang & Hargens (2018) emphasized 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.

4.3.7 Translaminar cribrosa 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 translaminar 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 (McMonnies, 2016). 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 (Friberg & Weinreb, 1985), and static exercise (O'Connor & 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.

4.3.8 Age

Ageing leads to changes in all the structures of the eye, the most common of which are presbyopia, age-related macular degeneration, and cataracts. The resistance to the outflow of aqueous humour also increases with age, which can precipitate the development of glaucoma (Salvi et al., 2006). The age of the active flying astronaut population is increasing, with most aged 40 to 60 years (Marshall-Bowman et al., 2013). Mader et al. (2011) noted that hyperopic shifts were more commonly observed in older astronauts and are potentially exacerbated by age-related presbyopia.

A positive relationship between IOP and age in the European population has been observed in most (Bonomi et al., 1998; Hollows & Graham, 1966), but not all (Perkins, 1973) studies. The European Eye Epidemiology consortium, a collaborative network of 38 population-based studies across Europe which involved 43,500 adults, reported an inverted U-shaped association of IOP with age (Khawaja et al., 2016). IOP was observed to increase up to the age of 60 years and decrease in participants older than 70 years. In the present thesis, IOP measured in a resting seated position was independent of participants' age. However, in the prone 6°HDT position, significantly higher IOP was observed in older males compared to younger males from Study I (Chapter 3), throughout the 3-min isometric handgrip exercise, and the subsequent 2-min recovery period, potentially due to better vascular and mechanical compliance in the lamina cribrosa region in a younger population (Kohn et al., 2015), which could counteract exercise-induced increases in IOP.

It is important to note that the dynamometer used in the present study was not the same as the one used in Chapter 3 (Mlinar et al., 2021), therefore definitive conclusions regarding the effect of age on the IOP responses to rest and exercise in a prone 6°HDT position cannot be made.

4.3.9 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 weightlifting, 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. Most exercise studies to date have been performed in the seated position, and thus the results are different from those of the present study in which the static exercise was performed in the prone 6°HDT position. Whereas CO₂ has been considered a potential contributing factor (Risner et al., 2009), this is only the second study (the first being Mlinar et al., 2021), to our knowledge, investigating its action on IOP in the prone 6°HDT position. The present study on older participants 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 vessels; consequently, the resistive exercise-induced elevation in IOP is enhanced. The known age-related changes in the structure of the lamina cribrosa may cause a strain on the optic nerve head, leading to ocular changes observed in SANS. 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.

4.3.10 Limitations

The main limitation of this study is the reliance on non-contact pneumotometry rather than GAT. However, the reliability of the adopted tonometer was established in preliminary experiments. The duration of the experiments was short and only included older participants. Future experiments should 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 sexes. The IOP responses to hypercapnic static exercise in the prone position reported for male participants should be compared to the responses observed in young and older females in a further study.

Chapter 5

Effect of Sex on Intraocular Pressure During Static Handgrip Exercise in Astronaut-Aged Individuals

A plethora of differences in adaptation to space has been found between male and female astronauts (Mark et al., 2014), including in eyes (Platts et al., 2014). It has been reported that female astronauts are less likely to develop ophthalmic and neurologic symptoms of Spaceflight Associated Neuro-ocular Syndrome (SANS) (Mader et al., 2011; Marshall-Bowman et al., 2013; Nelson et al., 2014). The precise aetiology of SANS and the differences that arise between sexes are yet unknown. A few possible factors that could potentially contribute to the latter are body weight, vascular compliance, and age (Buckey et al., 2018; Marshall-Bowman et al., 2013). It is not yet known whether these variances occur due to physiological sex differences or if they are just a consequence of a small sample size. As of 2020, only 64 (11.4 %) out of 562 astronauts ever in space were female (M. G. Smith et al., 2020).

The precise aetiology of SANS is yet unclear, however, a number of factors have been identified to have the potential to contribute to the development of SANS (Alperin & Bagci, 2018; Mader et al., 2011; Mader et al., 2013; Marshall-Bowman et al., 2013).

In order to investigate intraocular pressure (IOP) during rest and exercise in acute simulated microgravity, a series of experiments was carried out. In our previous study (Mlinar et al., 2021; Chapter 3), IOP was measured in three positions (seated, supine 6°head-down tilt (HDT), and prone 6°HDT). Based on the results of this study, we concluded that a prone 6°HDT position might be a more appropriate ground-based simulation of acute microgravity than a supine 6°HDT position. Additionally, we previously showed that IOP in older (45 to 65 years of age) males significantly increased with isometric handgrip exercise in the prone 6°HDT position (Mekjavic et al., 2020; Chapter 4). This increase was further exacerbated by levels of hypercapnia ($F_i\text{CO}_2 = 0.01$) that are similar to those experienced by astronauts on the International Space Station (ISS) (Alexander et al., 2012; Law et al., 2014). Conversely, no such increases were observed in younger (19 to 29 years of age) males (Mlinar et al., 2021).

The age of the astronauts on their first expedition has increased, and many of them participate in more than one long-term space mission (Smith et al., 2020). Since the beginning of space missions on the ISS in 2000, the mean astronaut age increased from 38 years to 46.7 years in 2011 (Marshall-Bowman et al., 2013). Therefore, to further investigate the response of IOP during rest and isometric exercise in acute exposure to simulated microgravity as mimicked by prone 6°HDT position, participants between 45

and 65 years of age were recruited. Additionally, to investigate whether the variances in ocular changes in astronauts are determined by sex, both male and female participants were recruited. Similar to our previous studies (Mekjavic et al., 2020; Mlinar et al., 2021; Chapters 4 and 3, respectively), our goal in the present study was not to simulate the length and type of daily exercise that astronauts undertake on the Advanced Resistive Exercise Device (ARED) while staying on the ISS and its effects on fitness, but rather to observe general effects of isometric exercise on IOP. Submaximal isometric handgrip exercise was chosen as a preferred mode of exercise as it has been previously shown to significantly elevate mean arterial pressure and IOP (Bakke et al., 2009). Additionally, such form of exercise offers the easiest measurement of IOP compared to others, when measured during the exercise bout.

5.1 Methods

5.1.1 Participants

Male ($N = 12$) and female ($N = 12$) healthy, non-smoking volunteers, between 45 and 65 years of age, gave their signed informed consent to participate in the study. Participants' anthropometric characteristics are presented in Table 5.1.

Volunteers were excluded from participation if they were younger than 45 or older than 65 years of age, were hypotensive, suffered from any acute or chronic ophthalmic disorders, or had any conditions which would prevent them from conducting isometric handgrip exercise in a prone 6°HDT position. The study conformed to the Declaration of Helsinki and was approved by the National Medical Ethics Committee (approval no. 0120-31/2020/9) at the Ministry of Health, Republic of Slovenia.

Table 5.1: Participants' physical characteristics, their maximum isometric grip strength and intraocular pressure (IOP) measured in a clinical seated position (IOP_{Control}). (Note: BMI – body mass index).

		Range			
		Mean	SD	Min	Max
Males	Age (years)	57.7	5.2	50	65
	Height (cm)	177.3	7.3	162.6	190.7
	Weight (kg)	78.6	8.6	64.3	96.3
	BMI ($\text{kg} \cdot \text{m}^{-2}$)	24.9	1.5	22.6	27.7
	Max grip (kg)	36.9	5.9	28.6	45.4
	IOP_{Control} (mmHg)	14.0	2.8	10.0	19.9
Females	Age (years)	53.4	5.1	46	62
	Height (cm)	166.9	5.3	159.2	177.3
	Weight (kg)	67.1	8.3	53.5	79.5
	BMI ($\text{kg} \cdot \text{m}^{-2}$)	24.0	2.4	19.1	27.4
	Max grip (kg)	27.4	4.2	18.9	33.8
	IOP_{Control} (mmHg)	14.5	1.9	10.8	18.4

5.1.2 Experimental protocol

All the testing was conducted at the Jožef Stefan Institute, Ljubljana, Slovenia (altitude: ~295 m). Participants visited the laboratory on one occasion. On arrival, their weight (TPT 5N, Libela Elsi, Slovenia) and height (Vita, Libela Elsi, Slovenia) were recorded, followed by an IOP measurement (Pulsair IntelliPuff Tonometer, Keeler, Windsor, United Kingdom) in a clinical seated position. Three measurements were obtained from a right eye and an average of those measurements was taken as the participant's control IOP (IOP_{Control}).

Following the initial IOP measurement, participants' maximal isometric handgrip strength was evaluated. Participants assumed a prone 6°HDT position, with their right elbow maintained at a 90° angle. On instruction, they conducted a maximal handgrip twice for 5 s with 60 s between the two exertions. The maximum value obtained during the two exertions was taken as their maximum isometric handgrip strength.

After measurement of maximal strength, participants were instrumented with the Physioflow Q-Link device (Manatec Biomedical, Paris, France) for the measurement of heart rate (HR, min^{-1}), and a blood pressure cuff (Withings model BP-800, Issy-les-Moulineaux, France) was fitted on their left arm for the measurement of diastolic and systolic arterial pressure (DAP and SAP, respectively) from which mean arterial pressure (MAP) was calculated using Equation 3.1.

Following the instrumentation, participants assumed a prone 6°HDT position. The trial was 23-min long and consisted of a 10-min rest, 3-min isometric handgrip exercise (at 30% of the participant's maximal strength), and a 10-min post-exercise recovery period. IOP, SAP, DAP, and HR were measured as soon as participants assumed the position (R0), towards the 5th and 10th min of the initial rest (R5 and R10, respectively), each minute of the 3-min exercise (E1, E2, and E3), and towards the end of the 1st, 2nd, 5th, 7th, and 10th min of the post-exercise recovery period (P1, P2, P5, P7 and P10, respectively).

5.1.3 Statistical analyses

All data were assessed for normality using the Shapiro-Wilk and Kolmogorov-Smirnov test of normality. Sex-related differences in participants' anthropometric characteristics (age, height, weight, strength, and IOP_{Control}) were assessed using an independent samples *t*-test.

A mixed model ANOVA was implemented to compare the main effect of time, and sex*time interaction on all measured variables (HR, MAP, and IOP), with the Bonferroni correction on subsequent *post hoc* analysis where the main effects were significant. The relationship between weight, height, and body mass index (BMI) and IOP measured in a clinical seated position (IOP_{Control}), and the relationship between weight, height, and BMI and maximal IOP measured during the trials were investigated using Pearson's correlation analysis in GraphPad Prism (v.8.4, GraphPad Software, Inc., San Diego, USA). Correlation coefficients were applied as recommended by Cohen (2013): strong: $0.60 \leq r$; moderate: $0.40 \leq r < 0.59$; weak: $0.20 \leq r < 0.39$.

Descriptive statistics were expressed as means and standard deviations, and the significance level for all statistical tests in this study was set at $p < 0.05$, *a priori*. All statistical analyses were performed using SPSS (v.25, IBM, NY, USA) software. Cohen's standardized-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 an extremely large effect when $d \leq 4.0$ (Hopkins et al., 2009).

5.2 Results

5.2.1 Participants

All participants successfully completed both trials. Age, body mass index BMI and IOP_{Control} did not differ significantly between the two groups ($t(22) = 2.021$, $p = 0.056$; $t(22) = 1.130$, $p = 0.270$, and $t(22) = -0.489$, $p = 0.315$, respectively). Males were significantly taller ($t(22) = 3.975$, $p < 0.001$, $d = 1.6$), heavier ($t(22) = 3.340$, $p = 0.001$, $d = 1.4$) and stronger ($t(22) = 4.558$, $p < 0.001$, $d = 1.9$) than females.

5.2.2 Heart rate

A significant main effect of time ($F_{(2.638, 58.025)} = 23.023$, $p < 0.001$) and sex ($F_{(1, 22)} = 9.181$, $p = 0.006$), and a non-significant sex*time interaction ($F_{(2.638, 58.025)} = 1.316$, $p = 0.278$) on HR was observed.

In males, HR was elevated significantly throughout the exercise compared to the initial rest (E1: $p = 0.010$, $d = 0.7$; E2: $p < 0.001$, $d = 1.0$; E3: $p = 0.004$, $d = 1.2$), as seen in Table 5.2. In females, HR increased with exercise only in the 2nd minute (E2: $p = 0.004$, $d = 0.7$). Upon cessation of exercise (P1 compared to E3), HR in males decreased immediately ($p = 0.009$, $d = 1.2$).

Table 5.2: Participants' heart rate and mean arterial pressure responses to rest and exercise in a prone 6°HDT position (Note: [!]significant exercise-induced increase (compared to R10); *significant post-exercise decrease (compared to E3); $p < 0.05$).

		Heart Rate (min ⁻¹)										
		R0	R5	R10	E1	E2	E3	P1	P2	P5	P7	P10
Males		55	55	56	60	62	66	55	54	54	54	55
		(7)	(6)	(5)	(7) [!]	(7) [!]	(11) [!]	(7)*	(7)*	(7)*	(7)*	(8)*
Females		65	64	64	66	69	71	64	64	64	64	64
		(9)	(8)	(7)	(8)	(8) [!]	(8)	(8)	(8)	(7)	(8)	(6)

		Mean Arterial Pressure (mmHg)										
		R0	R5	R10	E1	E2	E3	P1	P2	P5	P7	P10
Males		97	97	98	103	109	115	98	96	98	97	99
		(9)	(9)	(7)	(10)	(13) [!]	(16) [!]	(9)*	(9)*	(10)*	(9)*	(9)*
Females		91	90	91	96	98	103	91	91	91	91	91
		(9)	(7)	(6)	(7)	(7)	(9) [!]	(7)*	(7)*	(7)*	(8)*	(7)*

5.2.3 Mean arterial pressure

Similar to HR, a significant main effect of time ($F_{(3.330, 73.259)} = 36.853$, $p < 0.001$) and sex ($F_{(1, 22)} = 5.134$, $p = 0.034$), and a non-significant sex*time interaction ($F_{(3.330, 73.259)} = 1.473$, $p = 0.226$) on MAP was observed.

Post hoc pairwise comparison analysis revealed that compared to the initial rest (R10), a significant increase in MAP in males was observed in the 2nd and 3rd minute of exercise ($p < 0.001$, $d = 1.1$ and $p < 0.001$, $d = 1.4$, respectively), as seen in Table 5.2. In females, an exercise-induced increase in MAP was observed only in the last minute of exercise ($p =$

0.013, $d = 1.6$). Both in males and females, MAP decreased immediately upon cessation of exercise.

5.2.4 Intraocular pressure

Similar to HR and MAP, there was no significant sex*time interaction ($F_{(4.048, 89.067)} = 1.659$, $p = 0.166$) on IOP. The main effect of time ($F_{(4.048, 89.067)} = 6.333$, $p < 0.001$) on IOP was present, however, no significant increases in IOP with exercise were observed in either males or females, as seen in Figure 5.1. Additionally, no main effect of sex on IOP was observed ($F_{(1, 22)} = 0.360$, $p = 0.555$).

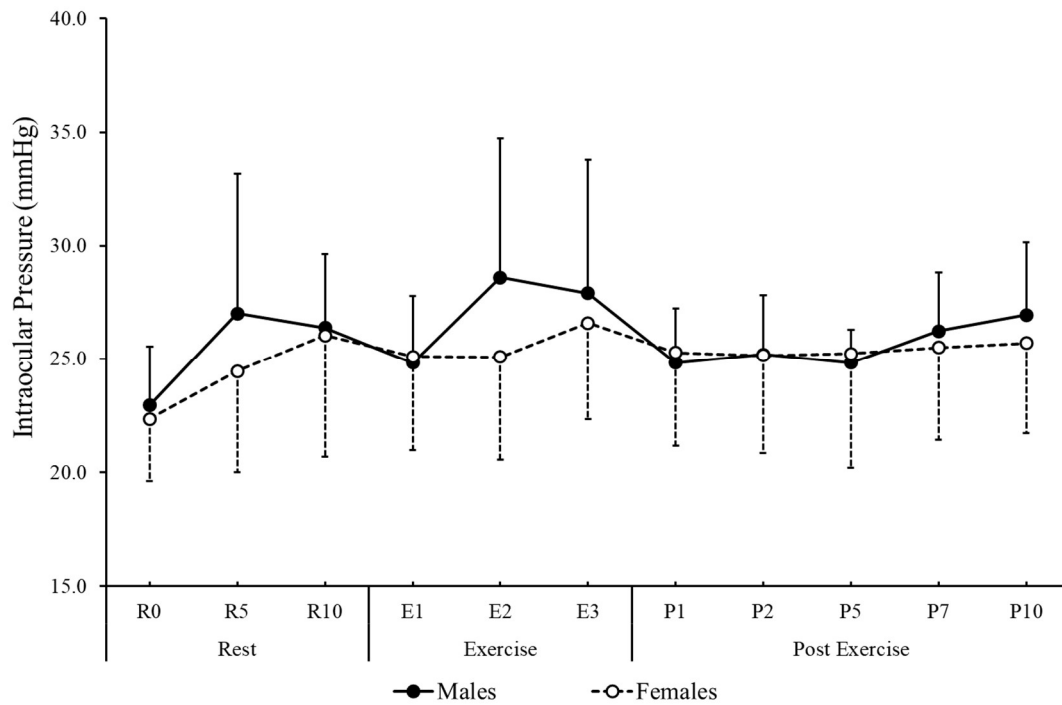


Figure 5.1: Intraocular pressure response to rest and isometric handgrip exercise in a prone 6°HDT position.

IOP measured in a clinical, seated position ($IOP_{Control}$) was not correlated with weight ($r = -0.175$; $p = 0.414$), height ($r = -0.149$; $p = 0.487$), or BMI ($r = -0.135$; $p = 0.529$) of the participants, as seen in Figure 5.2. Similarly, the highest IOP obtained during the trial in a prone 6°HDT position was not correlated to the participant's weight ($r = 0.101$, $p = 0.640$), height ($r = 0.148$, $p = 0.489$), or BMI ($r = -0.003$, $p = 0.989$).

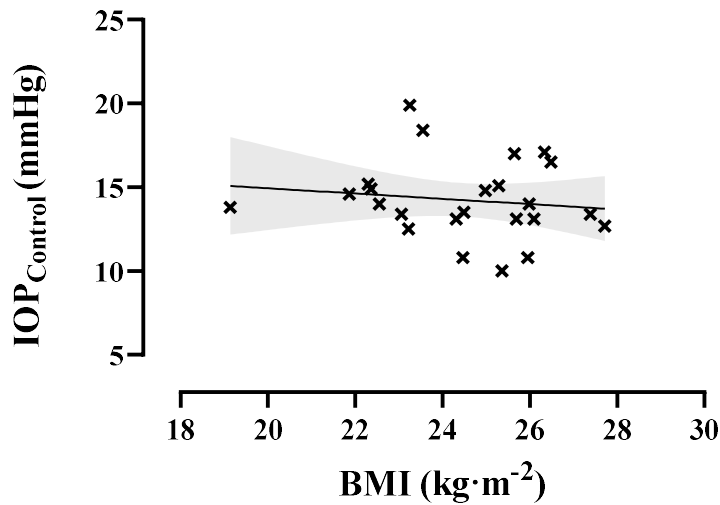


Figure 5.2: Relationships between seated IOP (IOP_{Control}) and BMI of the participants in the present study, associated with the regression line (Note: BMI – body mass index).

5.3 Discussion

The main finding of the present study is that IOP during rest and static handgrip exercise in a prone 6°HDT position was not significantly different between males and females of astronaut age. Similar to the results obtained in our previous studies (Mekjavic et al., 2020; Mlinar et al., 2021; Chapters 4 and 3, respectively), males' and females' IOP was constantly elevated above the normal upper level of 21 mmHg (The Royal College of Ophthalmologists, 2016).

5.3.1 Ground-based results

Results of previous European studies investigating the relationship between sex and IOP are inconsistent (Bonomi et al., 1998; Bulpitt et al., 1975; Foster et al., 2011; Hoehn et al., 2013; Hollows & Graham, 1966; Khawaja et al., 2016; Perkins, 1973). In the present study, IOP measured in a standard seated clinical position (IOP_{Control}) was in line with the normative values for a given European population (Khawaja et al., 2016) and did not differ between the two groups. Sex-related ocular variations have previously been suggested to result from differences in oestrogen levels (Zetterberg, 2016). It has been demonstrated that IOP decreases during pregnancy (i.e., when oestrogen levels are elevated), and increases after menopause (i.e., when oestrogen levels are reduced), signifying a role for oestrogen in IOP regulation (Ebeigbe et al., 2012; Qureshi, 1997). Oestrogen-influenced alterations in aqueous humour production and outflow are regulated by oestrogen receptors located in the ciliary epithelium (Ogueta et al., 1999). In the present study we did not control for the presence of menopause in female participants, however, considering the age range of the female participants (46 – 62 years), it is likely that some of them had already entered menopause, which might contribute to non-significant differences in IOP_{Control} between the two groups.

The BMI of male and female participants ($24.9 \pm 1.5 \text{ kg} \cdot \text{m}^{-2}$ and $24.0 \pm 2.4 \text{ kg} \cdot \text{m}^{-2}$, respectively) was not significantly different. Based on the BMI, 11 out of 24 (45.8%) participants were overweight ($\text{BMI} \geq 25 \text{ kg} \cdot \text{m}^{-2}$, as defined by the World Health

Organization). High BMI has been associated with increases in IOP previously (Khan et al., 2021; Mori et al., 2000), but not in the present study. In contrast to BMI, body height has been observed to correlate negatively with IOP (Khawaja et al., 2016; Zheng et al., 2010). The precise mechanism by which IOP is lowered in taller individuals is not known. It has been suggested that it is related to a larger vertical distance between the eye and the heart, and consequent reductions in ciliary body perfusion and aqueous production (Khawaja et al., 2016). This is in line with the results from our previous study where a higher IOP was observed in a supine 6°HDT compared to a seated position (Chapter 3; Mlinar et al., 2021). In the present study, male participants were significantly taller than females (177.3 ± 7.3 cm and 166.9 ± 5.3 cm, respectively), however no correlation between height and IOP_{Control} was observed.

Several studies have investigated the effect of sex on MAP and HR responses during isometric exercise, and the results are inconsistent (Ettinger et al., 1996; Samora, Incognito, et al., 2019; Samora, Teixeira, et al., 2019; Sanchez et al., 1980). In the present study, a 3-min isometric handgrip exercise at 30% of the participant's maximum resulted in significantly increased HR in males but not in females, and significantly increased MAP in both males and females. No significant differences were observed in HR or MAP at the 3rd minute of exercise between the two groups, which is in line with the observations of Sanchez et al. (1980).

In contrast to the results of our previous study where IOP in older males was observed to increase with submaximal isometric handgrip exercise (Chapter 4; Mekjavic et al., 2020), no such exercise-induced increase was observed in the present study in either males or females. Additionally, during the exercise, IOP between the two groups was not statistically different, which is in agreement with the rest of the literature (Vera, Jiménez, et al., 2019; Vera, Raimundo, et al., 2019; Vera et al., 2020).

5.3.2 Microgravity

Early reports on the presence of SANS in astronauts noted that the clinical symptoms were more frequent and/or severe in male astronauts (Mader et al., 2011; Marshall-Bowman et al., 2013; Platts et al., 2014). In a study of 17 male and 8 female ISS astronauts, neuro-ocular changes were observed in 82.3% and 62.5% of evaluated males and females, respectively. This difference was not statistically significant but the symptoms were milder in females than in males (Platts et al., 2014).

Exposure to microgravity results in slight increases in ICP, due to the absence of diurnal fluctuations driven by postural changes as experienced on Earth. This increase, although not pathological, results in a continuous decrease in the translaminal pressure gradient posterior of the eye that may lead to optic remodelling (Lawley et al., 2017). The difference between the ICP in microgravity and the estimated 24-hour average on Earth (calculated as one-third supine and two-thirds seated), and the consequent decrease in the pressure gradient, was significantly larger in males than females, meaning that female participants might be better protected against the stimuli causing globe flattening. In contrast, a retrospective analysis of pre- and post-flight eye examination data of 45 long-duration flight astronauts revealed that the extent of refraction changes was not correlated to the sex of the astronauts, but rather their BMI. None of the female astronauts included in the analysis developed optic disc oedema or choroidal folds. Among the male astronauts, the individuals who developed ocular changes weighed significantly more than the members of the flight crew in whom such ocular changes were not observed. The fact that the refractive changes, choroidal folds, and optic disc oedema were positively correlated with BMI, and that on average female astronauts weighed significantly less than their male counterparts, might in part explain why ocular changes are less common in females (Buckey et al., 2018). It is

important to mention that similar to other studies investigating sex-related changes in response to microgravity, the underrepresentation of female astronauts compared to males was substantial (9 females, 36 males) also in the study by Buckey et al. (2018).

To our knowledge, the present study is the first to measure females' IOP in a prone 6°HDT position. Usually, the effects of microgravity in ground-based studies are simulated by a supine horizontal or 6°HDT position. In the present study, IOP was not significantly different between males and females neither during rest nor during a 3-min submaximal isometric handgrip exercise and subsequent recovery period. Similar results were observed during rest and with the addition of artificial gravity in a horizontal supine position (Anderson et al., 2018). Another ocular tissue impacted by fluid shifts, for example in microgravity, is the optic nerve sheath (ONS). The ONS biomechanical properties are important in several sight-influencing pathologies, and ONS stiffness has previously been shown to aggravate high optic nerve head (ONH) strains, namely prelaminar and lamina cribrosa strains, during eye movements (Shin et al., 2017; X. Wang et al., 2017). ONS was observed to be significantly stiffer in males than in females in a ground-based spaceflight analogue. ONS stiffness exhibited a positive relationship with BMI when both sex groups were combined, however, considering this correlation was not present when it was investigated in each sex group separately, and that BMI was significantly higher in male than in female participants, the authors speculated that the observed positive correlation might have been primarily driven by participants' sex (C. Lee et al., 2020). These sex-related changes in ONS stiffness have been suggested to arise due to the differences in the sex hormone oestrogen, which has been shown to increase compliance of ocular tissues (Spoerl et al., 2007; Wei et al., 2012).

5.3.3 Limitations

As in the previous studies, one of the limitations of the present study was the use of a non-contact tonometer instead of the Goldmann Applanation tonometer (GAT), which is considered the gold standard for the measurement of IOP. Again, the use of GAT was not possible due to the experimental setup, namely a prone 6°HDT position. It is unlikely that our choice influenced the results since an excellent agreement of measurements obtained by Pulsair IntelliPuff and GAT in normotensive and hypertensive individuals has been previously established (Hubanova et al., 2015).

Another limitation is that we did not control for the menstrual cycle or the presence of menopause in the female participants. As mentioned previously, oestrogen levels impact IOP regulation and ocular tissue compliance. By knowing our participants' menstrual status at the time of the trial, an investigation of the correlation between oestrogen and IOP responses during rest and exercise in a prone 6°HDT position would be possible. In order to elucidate the role of sex hormones and their potential contribution in the manifestation of SANS symptoms, future studies should involve female participants of various ages and in different stages of menstrual cycles or menopause.

Chapter 6

Effect of Age on Hypoxia-Induced Changes in Retinal Blood Vessel Diameter

Due to logistical and safety reasons, future habitats on Moon and Mars will likely be hypoxic (Bacal et al., 2008; Bodkin et al., 2006). In the event of the successful establishment of a permanent presence on either Moon or Mars, it is anticipated that habitats will be populated with individuals of all ages, including children.

The lower partial pressure of oxygen (PO_2) during exposure to hypoxia provokes increased ventilation and heart rate (HR), both at rest and during exercise (Bärtsch & Gibbs, 2007) in an attempt to maintain a higher alveolar PO_2 , and consequently maintain appropriate tissue oxygenation. Despite these cardiorespiratory adaptations, impaired aerobic performance has been observed in adults and children, both during submaximal and maximal exercise with the magnitude being similar between the two age groups (Kapus et al., 2017; Kriemler et al., 2016). However, comparisons between these age groups within other systems are sparse in the literature. Consequently, we set out to investigate the effect of hypoxia on retinal vessels, which share similar anatomical, physiological, and embryological characteristics with cerebral vessels (Baker et al., 2008), in adults and prepubertal children. Any changes observed in the retinal vasculature may therefore reflect similar processes occurring in the brain.

The retina is one of the most affected tissues in the eye during exposure to hypoxia. It is responsible for converting photon-induced stimulation of the rods and cones into neural-coded information regarding the image projected on its surface and is among the most metabolically active tissues in the body. Consequently, its oxygen demand is high and increases even further when in the dark (Eshaq et al., 2014). Exposure of adults to a simulated altitude (normobaric hypoxia) of 4,000 m for 10 days causes a significant increase in the thickness of the retinal neural fibre layer (Jaki Mekjavic et al., 2016) and the diameter of retinal vessels (Louwies et al., 2016). Supplementary to vasodilation of the vessels, other retinal changes such as cotton wool spots, haemorrhages, and papilledema, commonly known under the umbrella term high-altitude retinopathy (HAR), may be observed upon exposure to hypoxia (Morris et al., 2006). The formation of these symptoms may be further exacerbated by strenuous exercise (Honigman et al., 2001; Shults & Swan, 1975), and the examination of these features in prepubertal children is lacking.

Astronauts' ocular health in future long-duration missions on the International Space Station (ISS), as well as missions planned to the Moon and Mars, is of utmost importance for NASA and ESA. To ensure safe exposure to hypoxia encountered in future habitats, it

is essential to determine the effect of hypoxia on the visual system in different age groups. We compared the effect of a one-day exposure to hypoxia, equivalent to ~3,000 m, on the retinal blood vessels, specifically the arterioles and venules, between adults and children. It is currently predicted that the ambient PO_2 in the habitats will correspond to that at an altitude in excess of 2,000 m (Bacal et al., 2008; Bodkin et al., 2006), however, the present study was designed to exaggerate habitats' ambient conditions in order to maximise participants' responses.

6.1 Methods

6.1.1 Study design

Details of the study protocol have been outlined previously (Kapus et al., 2017). Briefly, each participating family (parents and children) visited the Olympic Sports Centre Planica (Rateče, Slovenia) on one occasion for three days. On Day 1, participants arrived at the facility in the afternoon and were familiarised with the researchers, laboratory, equipment, and experimental procedures. They also underwent a medical examination and spent the first (Day 1) and second day (Day 2) in normoxia (altitude of the facility: 940 m; $F_iO_2 = 20.9\%$; $P_iO_2 = 134.0 \pm 0.4$ mmHg). The participants entered the normobaric hypoxic environment at 20:00 on the second night where they remained throughout the night and Day 3 until the termination of the experimental procedure. By reducing the oxygen content of the air, the normobaric hypoxic environment simulated an equivalent altitude of 3,000 m ($F_iO_2 = 0.162 \pm 0.03$; $P_iO_2 = 105.0 \pm 0.6$ mmHg). Throughout Day 2 and Day 3 the participants took part in a series of physiological tests (for further details cf. Kapus et al., 2017). The experimental procedures on Day 2 (normoxia) and Day 3 (hypoxia) were conducted in the same order and at the same time of the day to avoid any diurnal fluctuations in the measured variables. The testing schedule (Figure 6.1) was designed in a way that the constraints for each test were met, and limited interaction was present between the tests. A paediatrician was present throughout the study.

The normobaric hypoxic conditions were established with a vacuum-pressure swing adsorption (VPSA) system, which delivered hypoxic gas to all the rooms and laboratory. The system sampled the gas in all rooms at 15-min intervals to ensure that the pre-set level of oxygen fraction was being maintained. In the event that the oxygen fraction decreased below the pre-set level in any of the rooms, the system would cease the delivery of hypoxic gas to that room. Should the oxygen fraction not return to the desired level within two sampling cycles, the system would automatically initiate a fan that would deliver external air to the room and also trigger an alarm. All rooms were equipped with portable clip-on type oxygen sensors programmed to initiate an alarm, should the oxygen fraction decrease below a pre-set threshold. There were no such untoward events during the course of the study.

Time	Test
07:30	LLS, morning SpO ₂ , and HR
08:00	Breakfast
08:30	Orthostatic tolerance test
09:00	Muscle strength assessment
09:30	
10:00	Morning snack
10:30	Spirometry
11:00	Submaximal graded cycle exercise test
11:30	
12:00	
12:30	Lunch
13:00	Posturography
13:30	Countermovement vertical jump test
14:00	Skiing simulator test
14:30	
15:00	Afternoon snack
15:30	Cognitive test/thermal sensitivity test
16:00	
16:30	Fatigue questionnaire
17:00	Retinal fundus examination

Figure 6.1: Testing schedule for Day 1 (normoxia) and Day 2 (hypoxia) (Note: LLS – Lake Louise score; S_pO₂ – peripheral oxygen saturation; HR – heart rate).

6.1.2 Participants

In total, 13 adults (7 males, 6 females) and 13 children (Tanner stage 1; 7 males, 6 females) took part in the KidSki project. Children’s participation in the study was subject to the consent of the parents, who also participated in the study, and upon the approval of the paediatrician, who conducted the medical examination. Due to the specifications of the Automated Retinal Image Analyser (ARIA; Peter Bankhead, Queen’s University Belfast) software, only retinal scans of 11 adults (7 males, 4 females) and 8 children (4 males, 4 females) were used in the final analysis. All participants were lowland residents with no hypoxic exposure in the 2 months prior to taking part in the study as specified in the inclusion criteria. Participants’ physical characteristics are presented in Table 6.1.

Exclusion criteria included smoking (adults only), asthma, hypertension, haematological or kidney disorders, exposure to altitude (>2,500 m) in the preceding 2 months, and any eye condition that could influence retinal vessels. Throughout the study, adult participants were requested not to consume any caffeine or alcohol.

The study conformed to the standards set by the Declaration of Helsinki, except for the registration in a database. Prior to taking part in the study, the participants were thoroughly informed about the aims, methodology, and potential risks of the study, after which the children assented and signed consent forms were obtained from the adults. The children’s consent forms were subsequently signed by the children and final consent was obtained from their parents/guardians, who were also participants in the study. The study was approved (approval no. 164/05/13) by the National Medical Ethics Committee at the Ministry of Health (Republic of Slovenia).

Table 6.1: Participants' physical characteristics (Abbreviations: M – males; F – females; BMI – body mass index; BF% - body fat percentage).

	Children	Adults
Number (M/F)	8 (4/4)	11 (7/4)
Age (years)	9.3 ± 1.3	40.1 ± 4.1
Height (cm)	141.2 ± 11.2	176.0 ± 8.5
Weight (kg)	31.2 ± 7.5	72.9 ± 12.1
BMI (kg · m²)	15.4 ± 1.6	23.4 ± 2.2
BF% (%)	10.8 ± 5.9	20.2 ± 9.2

6.1.3 General adaptation to hypoxia

The participants' resting morning HR and oxygen saturation (S_pO_2) were measured in normoxia (Day 2) and hypoxia (Day 3) upon waking using a finger pulse oximeter (Nellcor, BCI 3301, Boulder, USA). Additionally, to assess for the potential presence of acute mountain sickness (AMS), participants completed the self-assessment section of the Lake Louise mountain sickness questionnaire (Roach et al., 1993) to obtain the Lake Louise score (LLS; 0–15). The collection of children's LLS was adapted following the recommendations of the International Federation for Climbing and Mountaineering Medical Commission guidelines (Meijer & Jean, 2012).

6.1.4 Retinal fundus examination

Digital high-resolution retinal colour fundus photographs of the left and right eyes centred on the optic disc were taken according to procedures described elsewhere (De Boever et al., 2014). Measurements were performed by an ophthalmologist using a 45° 6.3 megapixel digital nonmydriatic camera (Canon, Hospithera, Brussels, Belgium). During the fundus photography, patients were seated on a chair in a darkened room with their chin resting on a chin support. The presence of HAR was determined with direct fundus ophthalmoscopy.

6.1.5 Data processing and analysis

Retinal vessels were analysed so that left and right eye central retinal arteriolar (CRAE) and venular equivalent (CRVE) in hypoxia and normoxia were determined using the ARIA. The calibration was set at 8.077 μm per pixel. Only retinal scans, where the same three venules and three arterioles within the region between 0.5- and 1.0-disc diameters away from the disc margin were able to be identified in both normoxia and hypoxia, were used in the statistical analysis. In the event that an obtained retinal scan was of poor quality, the scan was repeated. Upon completion of the study, all scans were analysed with ARIA. The image analysis software deemed some of the scans of insufficient quality to be used in the analysis. Since the aim of the study was a comparison of images obtained before and after the normoxic and hypoxic confinements, a poor-quality scan obtained either pre- or post-exposure for a given participant would render the results of this participant unusable by the software, requiring the exclusion of the participant from the analysis. As a consequence, only retinal scans of 8 children (4 females) and 11 adults (4 females) were of a quality that could be properly analysed by ARIA. Due to the high inter-eye correlation reported previously (Leung et al., 2003), the average value of the left and right eyes was used in the statistical analysis. The eye examinations were performed by an

ophthalmologist, thus any changes of clinical concern would have been identified immediately.

All of the statistical analyses were performed using SPSS (v.25, IBM, NY, USA) software. The data are presented as mean \pm standard deviation (SD) unless indicated otherwise. The significance level for all statistical tests in this study was set at $p < 0.05$, *a priori*. Unbiased effect sizes were estimated using Hedges' g test and defined as small when $g \leq 0.2$, moderate when $g \leq 0.5$, and large when $g \leq 0.8$ (Hedges, 1981). All data were assessed for normality using the Shapiro-Wilk test of normality. A paired-samples t -test was used to assess whether the effect of hypoxia (hypoxia vs. normoxia) was present in the morning HR and S_pO_2 measurements, and Wilcoxon signed-rank test to assess the effect of hypoxia in LLS. Potential hypoxia-induced CRAE and CRVE differences between both age groups were investigated using a mixed model ANOVA (group [children, adults]*condition [hypoxia, normoxia]) and potential changes between CRAE and CRVE within each age group were assessed using a two-way repeated measures ANOVA (vessel [CRAE, CRVE]*condition [hypoxia, normoxia]). Where appropriate, a Bonferroni *post-hoc* test was applied to investigate interaction effects in greater detail.

6.2 Results

No negative events related to the hypoxic environment were reported by the participants, nor were they observed by the attending paediatrician.

6.2.1 General adaptation to hypoxia

Morning resting HR was significantly higher in hypoxia compared to normoxia in children ($t(7) = -3.108$, $g = 0.74$, $p = 0.017$) but not in adults ($t(10) = -0.359$, $p = 0.727$), as seen in Table 6.2.

Morning resting S_pO_2 decreased significantly in hypoxia in both age groups (children: $t(7) = 6.840$, $g = 3.42$, $p < 0.001$; adults: $t(10) = 8.061$, $g = 3.07$, $p < 0.001$). There was no statistical difference in S_pO_2 between the two groups.

Hypoxia had no significant effect on children's ($Z = -1.897$, $p = 0.058$) or adults' ($Z = -1.549$, $p = 0.121$) LLS. LLS values ≥ 3 were observed in two adults and two children following a 12-hour night-time exposure to hypoxia. The median (range) LLS values in hypoxia were comparable between the two age groups. No other signs of AMS were observed by the attending paediatrician.

Table 6.2: Adults' and children's morning HR, S_pO_2 and LLS following a 12-hour night-time hypoxia exposure (Note: M – males; F – females; S_pO_2 – peripheral oxygen saturation; LLS – Lake Louise score; * significantly different than in normoxia; $p \leq 0.05$).

Condition	Adults		Children	
	Normoxia	Hypoxia	Normoxia	Hypoxia
Heart rate (min^{-1})	80.1 \pm 15.4	92.5 \pm 16.1*	64.2 \pm 14.8	65.5 \pm 13.0
S_pO_2 (%)	98.0 \pm 1.4	92.4 \pm 1.7*	96.5 \pm 0.8	90.1 \pm 2.7*
LLS (median (range))	0 (0 – 2)	1.5 (0 – 4)	0 (0 – 2)	1 (0 – 4)

6.2.2 Retinal fundus

6.2.2.1 High altitude retinopathy

Based on direct fundus ophthalmoscopy, the ophthalmologist confirmed that no signs of HAR were present.

6.2.2.2 Central retinal arteriolar equivalent (CRAE)

CRAE increased with hypoxia in both children (normoxia: $105.32 \pm 7.72 \mu\text{m}$, hypoxia: $110.13 \pm 7.16 \mu\text{m}$) and adults (normoxia: $105.35 \pm 10.67 \mu\text{m}$, hypoxia: $110.77 \pm 8.36 \mu\text{m}$), as seen in Figure 6.2. No main effect of group ($p = 0.933$) or group*condition interaction ($p = 0.785$) was present. In contrast, the main effect of the condition on CRAE was observed (normoxia: $105.34 \pm 9.30 \mu\text{m}$, hypoxia: $110.50 \pm 7.67 \mu\text{m}$, $p < 0.001$, $g = 0.59$).

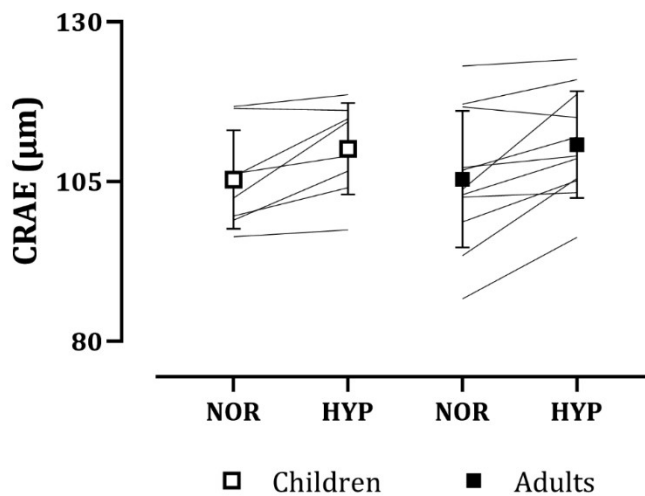


Figure 6.2: Adults' and children's central retinal arteriolar equivalents (CRAE) in normoxia (NOR) and hypoxia (HYP).

6.2.2.3 Central retinal venular equivalent (CRVE)

Similarly to CRAE, CRVE increased with hypoxia in both children (normoxia: $123.39 \pm 8.34 \mu\text{m}$, hypoxia: $130.11 \pm 8.54 \mu\text{m}$) and adults (normoxia: $126.89 \pm 7.24 \mu\text{m}$, hypoxia: $132.03 \pm 9.72 \mu\text{m}$), as seen in Figure 6.3. Again, no main effect of group ($p = 0.488$) or group*condition interaction ($p = 0.446$) was present. The main effect of condition on CRVE was observed (normoxia: $125.41 \pm 7.70 \mu\text{m}$, hypoxia: $131.22 \pm 9.05 \mu\text{m}$, $p < 0.001$, $g = 0.68$).

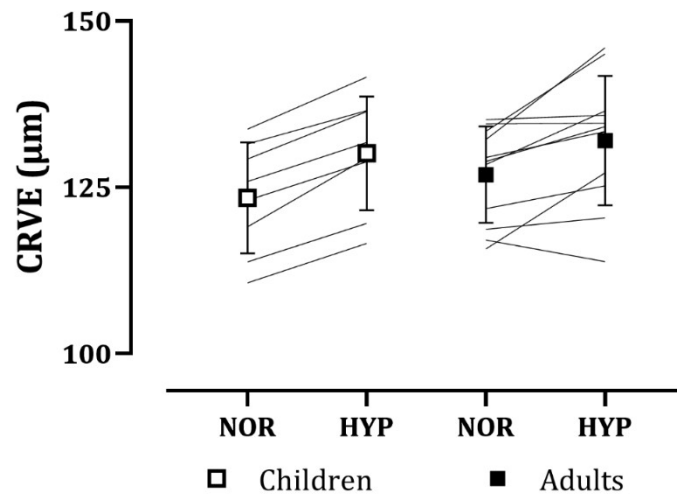


Figure 6.3: Adults' and children's central retinal venular equivalents (CRVE) in normoxia (NOR) and hypoxia (HYP).

6.2.2.4 Central retinal arteriolar vs venular equivalent

The main effect for vessels was observed both in children (CRAE: $107.73 \pm 7.61 \mu\text{m}$, CRVE: $126.75 \pm 8.86 \mu\text{m}$, $p = 0.001$, $g = 2.25$) and adults (CRAE: $108.06 \pm 9.76 \mu\text{m}$, CRVE: $129.46 \pm 8.77 \mu\text{m}$, $p < 0.001$, $g = 2.27$). However, no main effect of vessel*condition interaction was observed in either age group (children: $p = 0.218$; adults: $p = 0.878$).

6.3 Discussion

The main finding of the present study is that a 20-hour hypoxic exposure equivalent to an altitude of $\sim 3,000$ m caused significant increases in the diameter of retinal venules and arterioles in adults and children. Furthermore, hypoxia-induced increases in CRAE and CRVE were not significantly different between the two age groups. This confirms that the vasomotor sensitivity of the retinal vessels to acute hypoxia is comparable between adults and prepubertal children.

6.3.1 The effect of hypoxia on retinal vessel diameters

Despite the high blood flow, the total blood volume of the retinal vessels is relatively low due to their small diameter and sparse distribution (Wolf et al., 1991). As mentioned previously, the retina is one of the most metabolically active tissues in the body, with a high arteriovenous PO_2 difference and poor capacity to tolerate periods of low perfusion (Funk, 1997). Both the central retinal artery, which supplies the majority of blood to the retina (Kur et al., 2012), and the vein lack neural innervation which could provide regulation of the vascular tone. Therefore, blood flow in the retina is maintained by autoregulation that is mainly dependent on local myogenic and metabolic factors, including arterial blood gases, pH, and lactate (Garhöfer et al., 2003; Venkataraman et al., 2006). In case of reduced oxygen availability, as reflected by the reduced S_pO_2 , adequate retinal blood flow is achieved through vasodilation to match the augmented oxygen demand (Morris et al., 2006; Neumann et al., 2016). In the present study, morning S_pO_2 following a 12-hour overnight hypoxic exposure decreased from $97.2 \pm 1.2\%$ in normoxia (Day 2) to $91.3 \pm$

2.4% (both age groups combined), initiating vasodilation in both retinal arterioles and venules. The reactivity of adult retinal vessels to various levels of PO_2 was first reported by Cusick et al. (1942). Their findings have since been replicated and confirmed during exposure to both acute and chronic hypoxia (Louwies et al., 2016; Neumann et al., 2016), including in the present study. Additionally, retinal changes, including changes in retinal vessel diameter, have previously been observed in both normobaric and hypobaric hypoxia. The effect on the retinal vessel diameter was not dependent on the ambient pressure (i.e., hypobaric *versus* normobaric), but solely on the PO_2 (Neumann et al., 2016). No such research has previously been conducted in children.

Arteriolar walls consist of smooth muscle cells, whereas the venular walls are thinner and consist of a single layer of endothelial cells and a few smooth muscle cells (Kur et al., 2012). It has been proposed that venular walls are therefore more compliant and potentially exhibit larger autoregulatory responses (Louwies et al., 2016). The results of several studies investigating the diameters of retinal vessels while breathing hyperoxic or hypoxic gas mixtures are equivocal. While some authors reported greater reactivity of venules than arterioles (Cusick et al., 1942; Louwies et al., 2016), other studies observed no differences in reactivity (Fallon et al., 1985; Neumann et al., 2016), including the present study where the diameter of retinal arterioles increased by $5.16 \pm 4.67 \mu\text{m}$ in hypoxia, and similarly, the diameter of venules increased by $5.81 \pm 4.28 \mu\text{m}$ (both age groups combined).

6.3.2 Adults vs. children

Retinal vessel diameters decrease with age, independent of blood pressure and other factors (Leung et al., 2003; Wong et al., 2003). In contrast, neither CRAE nor CRVE of the children and adults participating in the present study were significantly different. Most likely, the adults in the present study were not old enough (40.1 ± 4.1 years) for the age-related retinal changes to be manifest. For example, participants in studies conducted by Leung et al. (2003) and Wong et al. (2003) were aged >49 and >43 years, respectively, whereas the age range of adult participants in our study varied from 32 to 47 years (median: 41 years). Due to the low sample size, correlation analysis of age and CRAE and CRVE was not conducted.

Normoxic retinal vessel diameters measured in children and adults participating in the present study are smaller than the values reported in the literature (Li et al., 2011; Mitchell et al., 2007; Neumann et al., 2016). A similar phenomenon was observed in adults' CRAE and CRVE measurements during exposure to hypoxia (Neumann et al., 2016). This can be attributed primarily to the differences in the analysis technique. In the present study, only three venules and three arterioles per retinal scan were included in the analysis. Due to the limited region of interest (between 0.5- and 1.0-disc diameters away from the optic disc margin), overlapping of vessels, and their branching, it was not always possible to select the six widest vessels.

Hypoxia-induced increases in retinal vessel diameters observed in both adults and children were not significantly different between the two age groups. To our knowledge, this is the first study to investigate children's retinal vessel diameter changes during exposure to hypoxia. As discussed previously, hypoxia-induced vasodilation in retinal vessels in adults observed in the present study is in line with the previous research (Cusick et al., 1942; Louwies et al., 2016; Neumann et al., 2016). When looking at adults' responses, considerable individual variability can be noted, especially in the adult's CRVE, compared to the children's. This is also reflected in the standard deviation of the responses ($\pm 1.70 \mu\text{m}$ in children and $\pm 5.46 \mu\text{m}$ in adults). Perhaps, such a range in individual responses of CRVE to hypoxia could be partially attributed to a considerable age range of adult participants.

6.3.3 High-altitude headache, high-altitude retinopathy, and acute mountain sickness

HAR and AMS encompass a spectrum of physiological and pathological changes commonly occurring in unacclimatised individuals exposed to hypoxia. High-altitude headache (HAH), a type of headache occurring during ascents to altitudes above 2,500 m and resolving spontaneously within 24 hours after descent, can appear along with other signs and symptoms which constitute AMS (Bian et al., 2013; Burtcher et al., 2011) or as an isolated symptom.

HAR has been noted to occur more frequently in individuals undergoing strenuous activity during hypoxic exposure, especially when Valsalva manoeuvres are involved (Arora et al., 2011). Meanwhile, symptoms of AMS are more commonly observed in adults than in children (Kriemler et al., 2014; Rexhaj et al., 2011). Other risk factors for the development of AMS, HAR and/or HAH include the level of hypoxic exposure, the rate and length of ascent, low arterial oxygen saturation, and an individual's susceptibility (Bian et al., 2013; McFadden et al., 1981; Schneider et al., 2002).

Arteriolar and venular retinal vessel diameter has previously been correlated with the development of AMS (Bosch et al., 2009). Additionally, HAH burden was found to correlate strongly with retinal venous vasodilatation (Wilson et al., 2013).

In the present study, the presence of HAR, AMS, and/or HAH was not observed or reported by any of the participants. This is likely the result of the following two factors: i) the level of hypoxia was low, and ii) the duration of the exposure was short.

6.3.4 Clinical implications

The retinal and cerebral macro- and microvasculature share many morphological and physiological properties, including similar vascular regulatory processes (Delaey & Van de Voorde, 2000). Due to these similarities, changes in cerebral microvasculature, resulting from diseases such as vascular dementia (Varma et al., 2002) and stroke (Powers & Zazulia, 2003) are often reflected in changes in retinal microvasculature. Furthermore, hypoxia-induced cerebral venous vasodilation has been observed to correlate strongly with the venous vasodilation observed in the retina (Wilson et al., 2013). It has been proposed that the small retinal vessel leakage that occurs in individuals exposed to high altitudes could also be mimicked in the vessels in the brain, contributing to the brain volume increase of a few millilitres observed following hypoxic exposures (Kallenberg et al., 2007; Willmann et al., 2013).

Exposure to hypoxia can have a significant effect on human vision, manifesting as changes in colour discrimination (Connolly et al., 2008), reduction in dark adaptation (Kobrick & Appleton, 1971), and loss of contrast sensitivity (Pescosolido et al., 2015), with the changes being more evident in low light environments (Connolly et al., 2008). In contrast, hypoxia has no effect on visual acuity (Bosch et al., 2009). In the event of blood leakage into the vitreous humour or when HAR-related haemorrhages manifest on the macula, the result can be an acute severe visual impairment. Most commonly, these hypoxia-induced vision changes are not clinically important and are reversed within weeks upon return to normoxia. Similarly, vasodilation of retinal vessels is reversible when adequate oxygen availability is restored (Bosch et al., 2009; Jaki Mekjavic et al., 2021). Visual function tests were not performed in the present study, however, no apparent hypoxia-related effects on vision were observed or reported by the participants, most likely due to low levels of hypoxia.

6.3.5 Limitations

A major limitation of the present study is a small sample size, especially in the younger group, mainly due to measurement errors when obtaining fundus photographs. Altogether retinal scans from 7 participants were discarded and not used in the final analysis because of blurriness, inappropriate lighting and/or composition (e.g., images not centred on the optic disc, mainly due to the inability of younger children to fix their gaze during the measurement). In future studies, this problem can be minimised or eliminated with more thorough familiarisation of the participants with the experimental equipment and procedures, improved measuring techniques, and taking duplicates or triplicates of each retinal scan.

Another limitation of the present study is that mean arterial pressure (MAP) was not measured during the retinal scans. It has previously been reported that MAP is inversely related to retinal vessel diameter (Kaushik et al., 2007; Wong et al., 2003), thus any hypoxia-induced increment in MAP would have caused a decrease in the retinal vessel diameter. In the present study, exposure to hypoxia resulted in retinal vessel vasodilation in both children and adults. Therefore, it may be speculated that retinal vascular regulation is more strongly affected by hypoxia than by the changes in MAP.

Chapter 7

Conclusions and Future Work

7.1 Conclusions

The present thesis investigated the effects of several spaceflight-related factors, namely age, hypercapnia, hypoxia, resistive exercise, sex and posture, on ocular structures, primarily intraocular pressure (IOP). On the basis of the results of the four studies, the following conclusions can be drawn:

Study I (Mlinar et al., 2021; Chapter 3) demonstrated that posture affects IOP, mean arterial pressure (MAP) and heart rate (HR) during rest and 3-min static handgrip exercise. IOP was significantly elevated in the prone 6°head-down tilt (HDT) position compared to seated and supine 6°HDT positions. As a consequence, H_01 was rejected and the alternative hypothesis H_{A1} was accepted. Neither exercise nor hypercapnia had any significant effect on IOP, therefore hypotheses H_02 , H_03 , and H_04 are confirmed. Additionally, IOP values obtained in the prone 6°HDT position were similar to IOP values obtained shortly after entering microgravity. It would therefore appear that the prone, rather than supine 6°HDT position is more suitable for the simulation of ocular changes that occur in acute microgravity.

Study II (Mekjavic et al., 2020; Chapter 4) demonstrated that resistive exercise, mimicking that conducted by astronauts to minimise the musculoskeletal atrophy resulting from prolonged exposures to microgravity, significantly increases IOP in older, astronaut-aged males. Exercise-induced increases in IOP were further augmented by hypercapnia similar to the carbon dioxide (CO_2) levels observed on the International Space Station (ISS). Therefore, hypotheses H_05 , H_06 , and H_07 are rejected, and alternative hypotheses H_{A5} , H_{A6} , and H_{A7} are accepted. Whereas mild hypercapnia did not elevate IOP in younger males (Study I, Chapter 3), it did so in older males indicating the potential presence of age-related effects of hypercapnia on IOP. Perhaps, hypercapnia is an important contributing factor in the development of Spaceflight-Associated Neuro-ocular Syndrome (SANS) in older male astronauts, but not in younger ones.

Study III (Mlinar et al., 2023, submitted; Chapter 5) showed that males and females of astronaut age have similar IOP responses to rest and exercise in a prone 6°HDT position. Therefore, hypothesis H_08 is accepted. Whether this difference between sexes occurs in astronauts during spaceflight and helps protect against the stimuli causing globe flattening is unknown and this hypothesis requires further investigation.

Study IV (Mlinar et al., 2023; Chapter 6) determined that one-day exposure to hypoxia equivalent to ~3,000 m significantly increases retinal vessel diameters in adults and prepubertal children, therefore hypothesis H_09 is rejected, and alternative hypothesis H_{A9} is accepted. The level of hypoxia-induced vasodilation did not differ between the two age groups, so hypothesis H_010 is accepted. Adults and children appear to be similarly sensitive to changes in ambient partial pressure of oxygen (PO_2), hence during exposures to hypoxia,

children should adhere to the same precautions as adults. No acute hypoxia-related effects on the ocular system were observed in any of the participants. Since retinal vessel vasodilation on its own does not have any clinical consequences, I conclude that exposure to hypoxia equivalent to altitudes up to 3,000 m is, from an ophthalmological perspective, safe for both adults and children.

7.2 Future Work

All of the studies conducted within the scope of the present thesis focused on the effects of short-term exposure to factors associated with current and/or future spaceflight. Based on the results of Study I (Mlinar et al., 2021; Chapter 3), it would appear that a prone 6°HDT position is more appropriate for simulating the effects of microgravity on the ocular system than a supine 6°HDT position. To assess the suitability of a prone 6°HDT position as a new ground-based model, future studies should be of longer duration. Since prolonged bedrest in the prone 6°HDT position is not possible, it might be interesting to use the experimental model developed in the present thesis, namely prone static hand grip exercise, at regular intervals during a bed rest campaign. Furthermore, additional measurements should be employed, such as optical coherence tomography (OCT), magnetic resonance imaging (MRI), and intracranial pressure (ICP) measurement in conjunction with IOP to observe fluctuations in translaminal cribriform pressure difference (TLCPD) and its role in the development of SANS. Due to slight differences in the measurement equipment, the results of Study I and Study II (Mekjavic et al., 2020; Chapter 4) are not fully comparable, however, they do indicate the possible presence of age-related effects of hypercapnia on IOP which should be further investigated.

The results of Study III (Mlinar et al., 2023, submitted; Chapter 5) showed that sex does not affect IOP responses in simulated microgravity in an older population. However, considering SANS appears to be more common and severe in male than female astronauts, further investigation implementing additional measuring techniques and participants of both sexes and varying ages is needed.

Lastly, Study IV (Mlinar et al., 2023; Chapter 6) investigated the effects of one-day exposure to hypoxia on retinal blood vessels in adults and children. Without sufficient technological advancements, life support systems in future habitats will likely not be capable of maintaining CO₂ concentration at the same level as on Earth. Therefore, since retinal vessels are vasoactive, and both hypoxia and hypercapnia have vasodilatory properties, future studies should consider investigating the effect of hypoxia in combination with hypercapnia. Future studies should therefore aim to replicate the methodology and concentration of gas mixtures used in Chapters 3, 4, 5, and 6, to allow for comparison of individual and combined effects across the studies. Additionally, future studies should be of longer duration to better mimic everyday life in future habitats.

As mentioned before, SANS threatens the success of all future long-duration missions to space. The discovery of its precise aetiology and the finding of appropriate countermeasures to prevent its occurrence is one of the top priorities of NASA and ESA. The findings of the present thesis have positively contributed to this research field and clearly demonstrated the effect of several spaceflight-related factors on IOP and retinal blood vessels. Furthermore, new research questions have been proposed that will need to be addressed in future studies.

References

- Albon, J., Purslow, P. P., Karwatowski, W. S., & Easty, D. L. (2000). Age related compliance of the lamina cribrosa in human eyes. *British Journal of Ophthalmology*, *84*(3), 318-323. doi:10.1136/bjo.84.3.318
- Alexander, D., Gibson, C., Hamilton, D., Lee, S., Mader, T., Otto, C., . . . Scott, J. (2012). Risk of spaceflight-induced intracranial hypertension and vision alterations. *Evidence Report, Human Research Program, Human Health Countermeasures Element, version, 1*, 12.
- Alperin, N., & Bagci, A. M. (2018). Spaceflight-induced visual impairment and globe deformations in astronauts are linked to orbital cerebrospinal fluid volume increase. In *Intracranial Pressure & Neuromonitoring XVI* (pp. 215-219): Springer.
- Alperin, N., Lee, S. H., Loth, F., Raksin, P. B., & Lichtor, T. (2000). MR-Intracranial pressure (ICP): a method to measure intracranial elastance and pressure noninvasively by means of MR imaging: baboon and human study. *Radiology*, *217*(3), 877-885.
- Anderson, A. P., Babu, G., Swan, J. G., Phillips, S. D., Knaus, D. A., Toutain-Kidd, C. M., . . . Buckley, J. C. (2017). Ocular changes over 60 min in supine and prone postures. *Journal of Applied Physiology*, *123*(2), 415-423. doi:10.1152/jappphysiol.00687.2016
- Anderson, A. P., Butterfield, J. S., Subramanian, P. S., & Clark, T. K. (2018). Intraocular pressure and cardiovascular alterations investigated in artificial gravity as a countermeasure to spaceflight associated neuro-ocular syndrome. *Journal of Applied Physiology*, *125*(2), 567-576. doi:10.1152/jappphysiol.00082.2018
- Anderson, A. P., Swan, J. G., Phillips, S. D., Knaus, D. A., Kattamis, N. T., Toutain-Kidd, C. M., . . . Buckley, J. C. (2016). Acute effects of changes to the gravitational vector on the eye. *Journal of Applied Physiology*, *120*(8), 939-946. doi:10.1152/jappphysiol.00730.2015
- Arora, R., Jha, K., & Sathian, B. (2011). Retinal changes in various altitude illnesses. *Singapore Medical Journal*, *52*(9), 685.
- Avunduk, A. M., Yilmaz, B., Sahin, N., Kapicioglu, Z., & Dayanir, V. (1999). The comparison of intraocular pressure reductions after isometric and isokinetic exercises in normal individuals. *Ophthalmologica*, *213*(5), 290-294. doi:10.1159/000027441

- Awad, H., Santilli, S., Ohr, M., Roth, A., Yan, W., Fernandez, S., . . . Patel, V. (2009). The Effects of Steep Trendelenburg Positioning on Intraocular Pressure During Robotic Radical Prostatectomy. *Anesthesia and Analgesia*, *109*(2), 473-478. doi:10.1213/ane.0b013e3181a9098f
- Bacal, K., Beck, G., & Barratt, M. R. (2008). Hypoxia, hypercarbia, and atmospheric control. In M. Barrat & S. Pool (Eds.), *Principles of clinical medicine for space flight* (pp. 445-473). New York, NY: Springer.
- Baker, M. L., Hand, P. J., Wang, J. J., & Wong, T. Y. (2008). Retinal signs and stroke: revisiting the link between the eye and brain. *Stroke*, *39*(4), 1371-1379. doi:10.1161/STROKEAHA.107.496091
- Bakke, E. F., Hisdal, J., & Semb, S. O. (2009). Intraocular pressure increases in parallel with systemic blood pressure during isometric exercise. *Investigative Ophthalmology and Visual Science*, *50*(2), 760-764. doi:10.1167/iovs.08-2508
- Barisano, G., Sepehrband, F., Collins, H. R., Jillings, S., Jeurissen, B., Taylor, J. A., . . . Nosikova, I. (2022). The effect of prolonged spaceflight on cerebrospinal fluid and perivascular spaces of astronauts and cosmonauts. *Proceedings of the National Academy of Sciences*, *119*(17), e2120439119.
- Bärtsch, P., & Gibbs, J. S. R. (2007). Effect of altitude on the heart and the lungs. *Circulation*, *116*(19), 2191-2202. doi:10.1161/CIRCULATIONAHA.106.650796
- Baumgartner, R. W., Mathis, J., Sturzenegger, M., & Mattle, H. P. (1994). A validation study on the intraobserver reproducibility of transcranial color-coded duplex sonography velocity measurements. *Ultrasound in Medicine and Biology*, *20*(3), 233-237. doi:[https://doi.org/10.1016/0301-5629\(94\)90063-9](https://doi.org/10.1016/0301-5629(94)90063-9)
- Bellner, J., Romner, B., Reinstrup, P., Kristiansson, K.-A., Ryding, E., & Brandt, L. (2004). Transcranial Doppler sonography pulsatility index (PI) reflects intracranial pressure (ICP). *Surgical Neurology*, *62*(1), 45-51.
- Berdahl, J. P., Fautsch, M. P., Stinnett, S. S., & Allingham, R. R. (2008). Intracranial pressure in primary open angle glaucoma, normal tension glaucoma, and ocular hypertension: a case-control study. *Investigative Ophthalmology and Visual Science*, *49*(12), 5412-5418. doi:10.1167/iovs.08-2228
- Berdahl, J. P., Yu, D. Y., & Morgan, W. H. (2012). The translaminar pressure gradient in sustained zero gravity, idiopathic intracranial hypertension, and glaucoma. *Medical Hypotheses*, *79*(6), 719-724. doi:10.1016/j.mehy.2012.08.009
- Bhan, A., Browning, A. C., Shah, S., Hamilton, R., Dave, D., & Dua, H. S. (2002). Effect of corneal thickness on intraocular pressure measurements with the pneumotonometer, Goldmann applanation tonometer, and Tono-Pen. *Investigative Ophthalmology and Visual Science*, *43*(5), 1389-1392.
- Bian, S.-Z., Zhang, J.-H., Gao, X.-B., Li, M., Yu, J., Liu, X., . . . Huang, L. (2013). Risk factors for high-altitude headache upon acute high-altitude exposure at 3700 m in young Chinese men: a cohort study. *The journal of headache and pain*, *14*(1), 1-7. doi:10.1186/1129-2377-14-35
- Bodkin, D., Escalera, P., & Bocam, K. (2006). A human lunar surface base and infrastructure solution. In *Space 2006* (pp. 7336).

- Bonomi, L., Marchini, G., Marraffa, M., Bernardi, P., De Franco, I., Perfetti, S., . . . Tenna, V. (1998). Prevalence of glaucoma and intraocular pressure distribution in a defined population: The Egna-Neumarkt study. *Ophthalmology*, *105*(2), 209-215. doi:[https://doi.org/10.1016/S0161-6420\(98\)92665-3](https://doi.org/10.1016/S0161-6420(98)92665-3)
- Bosch, M. M., Merz, T. M., Barthelmes, D., Petrig, B. L., Truffer, F., Bloch, K. E., . . . Schoch, O. D. (2009). New insights into ocular blood flow at very high altitudes. *Journal of Applied Physiology*, *106*(2), 454-460. doi:10.1152/jappphysiol.90904.2008
- Buckey, J. C., Phillips, S. D., Anderson, A. P., Chepko, A. B., Archambault-Leger, V., Gui, J., & Fellows, A. M. (2018). Microgravity-induced ocular changes are related to body weight. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *315*(3), R496-R499.
- Büki, B., Avan, P., Lemaire, J., Dordain, M., Chazal, J., & Ribari, O. (1996). Otoacoustic emissions: a new tool for monitoring intracranial pressure changes through stapes displacements. *Hearing Research*, *94*(1-2), 125-139.
- Büki, B., Giraudet, F., & Avan, P. (2009). Non-invasive measurements of intralabyrinthine pressure changes by electrocochleography and otoacoustic emissions. *Hearing Research*, *251*(1-2), 51-59.
- Bulpitt, C., Hodes, C., & Everitt, M. (1975). Intraocular pressure and systemic blood pressure in the elderly. *British Journal of Ophthalmology*, *59*(12), 717-720.
- Burtscher, M., Mairer, K., Wille, M., & Broessner, G. (2011). Risk factors for high-altitude headache in mountaineers. *Cephalalgia*, *31*(6), 706-711. doi:10.1177/0333102410394678
- Carlson, K. H., McLaren, J. W., Topper, J. E., & Brubaker, R. F. (1987). Effect of body position on intraocular pressure and aqueous flow. *Investigative Ophthalmology and Visual Science*, *28*(8), 1346-1352.
- Cheng, M. A., Todorov, A., Tempelhoff, R., McHugh, T., Crowder, C. M., & Laurysen, C. (2001). The effect of prone positioning on intraocular pressure in anesthetized patients. *The Journal of the American Society of Anesthesiologists*, *95*(6), 1351-1355. doi:10.1097/00000542-200112000-00012
- Chiquet, C., Custaud, M.-A., Le Traon, A. P., Millet, C., Gharib, C., & Denis, P. (2003). Changes in intraocular pressure during prolonged (7-day) head-down tilt bedrest. *Journal of Glaucoma*, *12*(3), 204-208. doi:10.1097/00061198-200306000-00004
- Chung, K.-y., Woo, S. J., Yi, S., Choi, G.-h., Ahn, C.-h., Hur, G.-c., . . . Kim, T.-W. (2011). Diurnal pattern of intraocular pressure is affected by microgravity when measured in space with the pressure phosphene tonometer (PPT). *Journal of Glaucoma*, *20*(8), 488-491. doi:10.1097/IJG.0b013e3181f464d2
- Clayton, J. A., & Davis, A. F. (2015). Sex/Gender Disparities and Women's Eye Health. *Current Eye Research*, *40*(2), 102-109. doi:10.3109/02713683.2014.986333
- Cohen, J. (2013). *Statistical power analysis for the behavioral sciences*: Routledge.
- Conkin, J., Norcross, J. R., & Abercromby, A. F. J. (2016). *NASA Human Research Programme evidence report: Risk of decompression sickness (DCS)*. Retrieved from Houston, Texas: <https://humanresearchroadmap.nasa.gov/evidence/reports/DCS.pdf>

- Connolly, D. M., Barbur, J. L., Hosking, S. L., & Moorhead, I. R. (2008). Mild hypoxia impairs chromatic sensitivity in the mesopic range. *Investigative Ophthalmology and Visual Science*, *49*(2), 820-827. doi:10.1167/iovs.07-1004
- Craven, E. R. (2008). Raised episcleral venous pressure. *Ophthalmology*.
- Cusick, P., Benson, O., & Boothby, W. (1942). Effect of anoxia and of high concentrations of oxygen on the retinal vessels: Preliminary report. *Anesthesia and Analgesia*, *21*(1), 18-19.
- Dane, Ş., Aslankurt, M., Yazici, A. T., & Gümüştekin, K. (2003). Sex-related difference in intraocular pressure in healthy young subjects. *Perceptual and motor skills*, *96*(3_suppl), 1314-1316.
- De Boever, P., Louwies, T., Provost, E., Panis, L. I., & Nawrot, T. S. (2014). Fundus photography as a convenient tool to study microvascular responses to cardiovascular disease risk factors in epidemiological studies. *JoVE (Journal of Visualized Experiments)*(92), e51904. doi:10.3791/51904
- De Moraes, C. G. V., Prata, T. S., Liebmann, J., & Ritch, R. (2008). Modalities of tonometry and their accuracy with respect to corneal thickness and irregularities. *Journal of Optometry*, *1*(2), 43-49.
- Delaey, C., & Van de Voorde, J. (2000). Regulatory mechanisms in the retinal and choroidal circulation. *Ophthalmic Research*, *32*(6), 249-256. doi:10.1159/000055622
- Dickerman, R. D., Smith, G. H., Langham-Roof, L., McConathy, W. J., East, J. W., & Smith, A. B. (1999). Intra-ocular pressure changes during maximal isometric contraction: does this reflect intra-cranial pressure or retinal venous pressure? *Neurological Research*, *21*(3), 243-246. doi:10.1080/01616412.1999.11740925
- Dielemans, I., Vingerling, J. R., Hofman, A., Grobbee, D. E., & de Jong, P. T. (1994). Reliability of intraocular pressure measurement with the Goldmann applanation tonometer in epidemiological studies. *Graefe's archive for clinical and experimental ophthalmology*, *232*, 141-144.
- Dorrell, M. I., Aguilar, E., & Friedlander, M. (2002). Retinal vascular development is mediated by endothelial filopodia, a preexisting astrocytic template and specific R-cadherin adhesion. *Investigative Ophthalmology and Visual Science*, *43*(11), 3500-3510.
- Draeger, J., Schwartz, R., Groenhoff, S., & Stern, C. (1993). Self-tonometry under microgravity conditions. *The Clinical Investigator*, *71*(9), 700-703. doi:10.1007/BF00209723.
- Draeger, J., Schwartz, R., Groenhoff, S., & Stern, C. (1995). Self-tonometry under microgravity conditions. *Aviation, space, and environmental medicine*, *66*(6), 568-570.
- Draeger, J., Schwartz, R., Stern, C., Groenhoff, S., & Hechler, B. (1997). Intraocular Pressure in Microgravity: Automatic Self-Tonometry During the Spacelab D2 Mission. *ESA SP*, *1*, 191.
- Drozдова, N. T., & Nesterenko, O. P. (1970). State of the visual analyzer during hypodynamia. In A. M. Genin & P. A. Sorokin (Eds.), *Problems of space biology*

- *Prolonged Limitation of Mobility and Its Influence on the Human Organism* (Vol. 13, pp. 192-195). Washington D. C.: NASA.
- Ebeigbe, J., Ebeigbe, P., & Ighoroje, A. (2012). Ocular changes in pregnant Nigerian women. *Nigerian Journal of Clinical Practice*, *15*(3), 298-301.
- Eklund, A., Jóhannesson, G., Johansson, E., Holmlund, P., Qvarlander, S., Ambarki, K., . . . Malm, J. (2016). The pressure difference between eye and brain changes with posture. *Annals of Neurology*, *80*(2), 269-276. doi:10.1002/ana.24713
- Eshaq, R. S., Wright, W. S., & Harris, N. R. (2014). Oxygen delivery, consumption, and conversion to reactive oxygen species in experimental models of diabetic retinopathy. *Redox biology*, *2*, 661-666. doi:10.1016/j.redox.2014.04.006
- Ettinger, S. M., Silber, D. H., Collins, B. G., Gray, K. S., Sutliff, G., Whisler, S. K., . . . Sinoway, L. I. (1996). Influences of gender on sympathetic nerve responses to static exercise. *Journal of Applied Physiology*, *80*(1), 245-251. doi:10.1152/jappl.1996.80.1.245
- Evensen, K. B., & Eide, P. K. (2020). Measuring intracranial pressure by invasive, less invasive or non-invasive means: limitations and avenues for improvement. *Fluids and Barriers of the CNS*, *17*(1), 34. doi:10.1186/s12987-020-00195-3
- Fallon, T., Maxwell, D., & Kohner, E. (1985). Retinal vascular autoregulation in conditions of hyperoxia and hypoxia using the blue field entoptic phenomenon. *Ophthalmology*, *92*(5), 701-705. doi:10.1016/S0161-6420(85)33978-7
- Faraci, F. M., Breese, K. R., & Heistad, D. D. (1994). Cerebral vasodilation during hypercapnia. Role of glibenclamide-sensitive potassium channels and nitric oxide. *Stroke*, *25*(8), 1679-1683. doi:10.1161/01.STR.25.8.1679
- Faul, F., Erdfelder, E., Lang, A.-G., & Buchner, A. (2007). G* Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods*, *39*(2), 175-191. doi:10.3758/BF03193146
- Fernandes, P., Díaz-Rey, J. A., Queiros, A., Gonzalez-Meijome, J. M., & Jorge, J. (2005). Comparison of the ICare® rebound tonometer with the Goldmann tonometer in a normal population. *Ophthalmic and Physiological Optics*, *25*(5), 436-440.
- Ficarrotta, K. R., & Passaglia, C. L. (2020). Intracranial pressure modulates aqueous humour dynamics of the eye. *The Journal of Physiology*, *598*(2), 403-413. doi:10.1113/JP278768
- Fischman, J. J., Cowen, R., Petersen, L., Healey, R., & Hargens, A. (2018). The Effects of Resistance Exercise on Intracranial Pressure. *The FASEB Journal*, *32*(1_supplement), 587-588. doi:10.1096/fasebj.2018.32.1_supplement.587.8
- Fleischman, D., & Allingham, R. R. (2013). The role of cerebrospinal fluid pressure in glaucoma and other ophthalmic diseases: A review. *Saudi Journal of Ophthalmology*, *27*(2), 97-106. doi:10.1016/j.sjopt.2013.03.002
- Foster, P. J., Broadway, D. C., Garway-Heath, D. F., Yip, J. L. Y., Luben, R., Hayat, S., . . . Khaw, K.-T. (2011). Intraocular Pressure and Corneal Biomechanics in an Adult British Population: The EPIC-Norfolk Eye Study. *Investigative Ophthalmology and Visual Science*, *52*(11), 8179-8185. doi:10.1167/iovs.11-7853

- Frenkel, R. E. P., Hong, Y. J., & Shin, D. H. (1988). Comparison of the Tono-Pen to the Goldmann Applanation Tonometer. *Archives of Ophthalmology*, *106*(6), 750-753. doi:10.1001/archophth.1988.01060130820030
- Frey, M., Mader, T., Bagian, J., Charles, J., & Meehan, R. (1993). Cerebral blood velocity and other cardiovascular responses to 2 days of head-down tilt. *Journal of Applied Physiology*, *74*(1), 319-325. doi:10.1152/jappl.1993.74.1.319
- Friberg, T. R., Sanborn, G., & Weinreb, R. N. (1987). Intraocular and episcleral venous pressure increase during inverted posture. *American Journal of Ophthalmology*, *103*(4), 523-526. doi:10.1016/s0002-9394(14)74275-8
- Friberg, T. R., & Weinreb, R. N. (1985). Ocular manifestations of gravity inversion. *JAMA*, *253*(12), 1755-1757. doi:10.1001/jama.1985.03350360081023
- Frisén, L. (1982). Swelling of the optic nerve head: a staging scheme. *Journal of Neurology, Neurosurgery and Psychiatry*, *45*(1), 13-18.
- Funk, R. (1997). Blood supply of the retina. *Ophthalmic Research*, *29*(5), 320-325. doi:10.1159/000268030
- Garhöfer, G., Zawinka, C., Resch, H., Menke, M., Schmetterer, L., & Dorner, G. T. (2003). Effect of intravenous administration of sodium-lactate on retinal blood flow in healthy subjects. *Investigative Ophthalmology and Visual Science*, *44*(9), 3972-3976. doi:10.1167/iovs.02-1272
- Goldmann, H., & Schmidt, T. (1957). Uber Applanationstonometrie [Applanation tonometry]. *Ophthalmologica*, *134*(4), 221-242. doi:10.1159/000303213
- Greenwald, S. H., Macias, B. R., Lee, S. M., Marshall-Goebel, K., Ebert, D. J., Liu, J. H., . . . Hargens, A. R. (2021). Intraocular pressure and choroidal thickness respond differently to lower body negative pressure during spaceflight. *Journal of Applied Physiology*, *131*(2), 613-620.
- Hardy, P., Varma, D. R., & Chemtob, S. (1997). Control of cerebral and ocular blood flow autoregulation in neonates. *Pediatric Clinics of North America*, *44*(1), 137-152. doi:10.1016/S0031-3955(05)70467-3
- Hashemi, H., Kashi, A., Fotouhi, A., & Mohammad, K. (2005). Distribution of intraocular pressure in healthy Iranian individuals: the Tehran Eye Study. *British Journal of Ophthalmology*, *89*(6), 652-657.
- Hayes, J., Thornton, W., Williams, M., Lee, S., MacNeill, K., & Moore Jr, A. (2013). Developing Countermeasure Systems for Optimizing Astronaut Performance in Space. In D. Risin & P. Stepaniak (Eds.), *Biomedical Results of the Space Shuttle Program* (pp. 289-313). Washington, DC: NASA.
- Hedges, L. V. (1981). Distribution theory for Glass's estimator of effect size and related estimators. *Journal of Educational Statistics*, *6*(2), 107-128. doi:10.3102/1076998600600210
- Hoehn, R., Mirshahi, A., Hoffmann, E. M., Kottler, U. B., Wild, P. S., Laubert-Reh, D., & Pfeiffer, N. (2013). Distribution of intraocular pressure and its association with ocular features and cardiovascular risk factors: the Gutenberg Health Study. *Ophthalmology*, *120*(5), 961-968.

- Hollows, F. C., & Graham, P. A. (1966). Intra-ocular pressure, glaucoma, and glaucoma suspects in a defined population. *British Journal of Ophthalmology*, *50*(10), 570-586. doi:10.1136/bjo.50.10.570
- Honigman, B., Noordewier, E., Kleinman, D., & Yaron, M. (2001). Case report: high altitude retinal hemorrhages in a colorado skier. *High Altitude Medicine & Biology*, *2*(4), 539-544. doi:10.1089/152702901753397117
- Hopkins, W. G., Marshall, S. W., Batterham, A. M., & Hanin, J. (2009). Progressive statistics for studies in sports medicine and exercise science. *Medicine and Science in Sports and Exercise*, *1*(41), 3-13. doi:10.1249/MSS.0b013e31818cb278
- Hubanova, R., Aptel, F., Zhou, T., Arnol, N., Romanet, J.-P., & Chiquet, C. (2015). Comparison of intraocular pressure measurements with the Reichert Pt100, the Keeler Pulsair Intellipuff portable noncontact tonometers, and Goldmann applanation tonometry. *Journal of Glaucoma*, *24*(5), 356-363. doi:10.1097/01.jg.0000435776.99193.41
- Hvidberg, A., Kessing, S. V., & Fernandes, A. (1981). Effect of changes in PCO₂ and body positions on intraocular pressure during general anaesthesia. *Acta Ophthalmologica*, *59*(4), 465-475. doi:10.1111/j.1755-3768.1981.tb08331.x
- Jablonski, K. S., Rosentreter, A., Gaki, S., Lappas, A., & Dietlein, T. S. (2013). Clinical use of a new position-independent rebound tonometer. *Journal of Glaucoma*, *22*(9), 763-767.
- Jaki Mekjavic, P., Eiken, O., & Mekjavic, I. B. (2002). Visual function after prolonged bed rest. *Journal of Gravitational Physiology*, *9*(1), P31-32.
- Jaki Mekjavic, P., Lenassi, E., Eiken, O., & Mekjavic, I. B. (2016). Effect of acute hypercapnia during 10-day hypoxic bed rest on posterior eye structures. *Journal of Applied Physiology*, *120*(10), 1241-1248. doi:10.1152/jappphysiol.00930.2015
- Jaki Mekjavic, P., Tipton, M. J., & Mekjavic, I. B. (2021). The eye in extreme environments. *Experimental Physiology*, *106*(1), 52-64. doi:10.1113/EP088594
- James, J. (2008). Carbon Dioxide. In *Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants* (Vol. 5, pp. 112-124): National Academy Press.
- Jonas, J. B., & Wang, N. (2012). Association between arterial blood pressure, cerebrospinal fluid pressure and intraocular pressure in the pathophysiology of optic nerve head diseases. *Clinical & Experimental Ophthalmology*, *40*(4), e233-e234. doi:10.1111/j.1442-9071.2011.02625.x
- Jorge, J. M. M., Gonzalez-Mejome, J. M., Queiros, A., Fernandes, P., & Parafita, M. A. (2008). Correlations between corneal biomechanical properties measured with the ocular response analyzer and ICare rebound tonometry. *Journal of Glaucoma*, *17*(6), 442-448.
- Jost, P. D. (2008). Simulating human space physiology with bed rest. *Hippokratia*, *12*(Suppl 1), 37.
- Kallenberg, K., Bailey, D. M., Christ, S., Mohr, A., Roukens, R., Menold, E., . . . Knauth, M. (2007). Magnetic resonance imaging evidence of cytotoxic cerebral edema in acute mountain sickness. *Journal of Cerebral Blood Flow and Metabolism*, *27*(5), 1064-1071. doi:10.1038/sj.jcbfm.9600404
- KFORCE. (2017). KAPA-INVENT (Version 4.4.2) [Mobile application software]

- Kapus, J., Mekjavic, I. B., McDonnell, A. C., Ušaj, A., Vodičar, J., Najdenov, P., . . . Debevec, T. (2017). Cardiorespiratory responses of adults and children during normoxic and hypoxic exercise. *International Journal of Sports Medicine*, *38*(08), 627-636. doi:10.1055/s-0043-109376
- Karadag, R., Sen, A., Golemez, H., Basmak, H., Yildirim, N., Karadurmus, N., . . . Akin, A. (2008). The effect of short-term hypobaric hypoxic exposure on intraocular pressure. *Current Eye Research*, *33*(10), 864-867. doi:10.1080/02713680802416696
- Kaushik, S., Kifley, A., Mitchell, P., & Wang, J. J. (2007). Age, blood pressure, and retinal vessel diameter: separate effects and interaction of blood pressure and age. *Investigative Ophthalmology and Visual Science*, *48*(2), 557-561.
- Kemp, D. T. (1978). Stimulated acoustic emissions from within the human auditory system. *The Journal of the Acoustical Society of America*, *64*(5), 1386-1391.
- Khan, S., Kirubarajan, A., Lee, M., Pitha, I., & Buckley, J. C. (2021). The correlation between body weight and intraocular pressure. *Aerospace Medicine and Human Performance*, *92*(11), 886-897.
- Khawaja, A. P., Springelkamp, H., Creuzot-Garcher, C., Delcourt, C., Hofman, A., Höhn, R., . . . On behalf of the European Eye Epidemiology, C. (2016). Associations with intraocular pressure across Europe: The European Eye Epidemiology (E3) Consortium. *European Journal of Epidemiology*, *31*(11), 1101-1111. doi:10.1007/s10654-016-0191-1
- Kiss, B., Dallinger, S., Polak, K., Findl, O., Eichler, H.-G., & Schmetterer, L. (2001). Ocular hemodynamics during isometric exercise. *Microvascular Research*, *61*(1), 1-13. doi:10.1006/mvre.2000.226910.1006/mvre.2000.2269
- Kiuchi, Y., Mishima, H. K., Hotehama, Y., Furumoto, A., Hirota, A., & Onari, K. (1994). Exercise intensity determines the magnitude of IOP decrease after running. *Japanese Journal of Ophthalmology*, *38*(2), 191-195. Retrieved from <http://europepmc.org/abstract/MED/7967212>
- Klein, B. E., Klein, R., & Linton, K. L. (1992). Intraocular pressure in an American community. The Beaver Dam Eye Study. *Investigative Ophthalmology and Visual Science*, *33*(7), 2224-2228.
- Klingelhöfer, J., Conrad, B., Benecke, R., & Sander, D. (1987). Intracranial flow patterns at increasing intracranial pressure. *Klinische Wochenschrift*, *65*(12), 542-545.
- Kobrick, J. N. L., & Appleton, B. (1971). Effects of extended hypoxia on visual performance and retinal vascular state. *Journal of Applied Physiology*, *31*(3), 357-362. doi:10.1152/jappl.1971.31.3.357
- Kohn, J. C., Lampi, M. C., & Reinhart-King, C. A. (2015). Age-related vascular stiffening: causes and consequences. *Front Genet*, *6*, 112. doi:10.3389/fgene.2015.00112
- Korpole, N. R., Kurada, P., & Korpole, M. R. (2022). Gender Difference in Ocular Diseases, Risk Factors and Management with Specific Reference to Role of Sex Steroid Hormones. *J Midlife Health*, *13*(1), 20-25. doi:10.4103/jmh.jmh_28_22
- Koskinen, L.-O. D., Malm, J., Zakelis, R., Bartusis, L., Ragauskas, A., & Eklund, A. (2017). Can intracranial pressure be measured non-invasively bedside using a two-

- depth Doppler-technique? *Journal of Clinical Monitoring and Computing*, 31(2), 459-467.
- Kriemler, S., Bürgi, F., Wick, C., Wick, B., Keller, M., Wiget, U., . . . Bloch, K. (2014). Prevalence of acute mountain sickness at 3500 m within and between families: a prospective cohort study. *High Altitude Medicine & Biology*, 15(1), 28-38. doi:10.1089/ham.2013.1073
- Kriemler, S., Radtke, T., Bürgi, F., Lambrecht, J., Zehnder, M., & Brunner-La Rocca, H. (2016). Short-term cardiorespiratory adaptation to high altitude in children compared with adults. *Scandinavian Journal of Medicine and Science in Sports*, 26(2), 147-155. doi:10.1111/sms.12422
- Kristiansson, H., Nissborg, E., Bartek Jr, J., Andresen, M., Reinstrup, P., & Romner, B. (2013). Measuring elevated intracranial pressure through noninvasive methods: a review of the literature. *Journal of Neurosurgical Anesthesiology*, 25(4), 372-385.
- Kronenberg, R. S., & Drage, C. W. (1973). Attenuation of the ventilatory and heart rate responses to hypoxia and hypercapnia with aging in normal men. *The Journal of Clinical Investigation*, 52(8), 1812-1819. doi:10.1172/JCI107363
- Kur, J., Newman, E. A., & Chan-Ling, T. (2012). Cellular and physiological mechanisms underlying blood flow regulation in the retina and choroid in health and disease. *Progress in Retinal and Eye Research*, 31(5), 377-406. doi:10.1016/j.preteyeres.2012.04.004
- Lam, A., & Douthwaite, W. A. (1997). Does the change of anterior chamber depth or/and episcleral venous pressure cause intraocular pressure change in postural variation? *Optometry and Vision Science*, 74(8), 664-667. doi:10.1097/00006324-199708000-00028
- Lang, E. W., Paulat, K., Witte, C., Zolondz, J., & Mehdorn, H. M. (2003). Noninvasive intracranial compliance monitoring: technical note and clinical results. *Journal of Neurosurgery*, 98(1), 214-218.
- Lanigan, L. P., Clark, C. V., & Hill, D. W. (1989). Intraocular pressure responses to systemic autonomic stimulation. *Eye*, 3(4), 477-483. doi:10.1038/eye.1989.72
- Lashutka, M. K., Chandra, A., Murray, H. N., Phillips, G. S., & Hiestand, B. C. (2004). The relationship of intraocular pressure to intracranial pressure. *Annals of Emergency Medicine*, 43(5), 585-591. doi:10.1016/j.annemergmed.2003.12.006
- Laurie, S. S., Lee, S. M., Macias, B. R., Patel, N., Stern, C., Young, M., & Stenger, M. B. (2020). Optic disc edema and choroidal engorgement in astronauts during spaceflight and individuals exposed to bed rest. *JAMA Ophthalmology*, 138(2), 165-172.
- Laurie, S. S., Vizzeri, G., Taibbi, G., Ferguson, C. R., Hu, X., Lee, S. M., . . . Stenger, M. B. (2017). Effects of short-term mild hypercapnia during head-down tilt on intracranial pressure and ocular structures in healthy human subjects. *Physiological Reports*, 5(11), e13302. doi:10.14814/phy2.13302
- Law, J., Van Baalen, M., Foy, M., Mason, S. S., Mendez, C., Wear, M. L., . . . Alexander, D. (2014). Relationship between carbon dioxide levels and reported headaches on the international space station. *Journal of Occupational and Environmental Medicine*, 56(5), 477-483. doi:10.1097/JOM.0000000000000158

- Lawley, J. S., Petersen, L. G., Howden, E. J., Sarma, S., Cornwell, W. K., Zhang, R., . . . Levine, B. D. (2017). Effect of gravity and microgravity on intracranial pressure. *The Journal of Physiology*, *595*(6), 2115-2127. doi:10.1113/JP273557
- LeBlanc, A., Lin, C., Shackelford, L., Sinitsyn, V., Evans, H., Belichenko, O., . . . Bakulin, A. (2000). Muscle volume, MRI relaxation times (T2), and body composition after spaceflight. *Journal of Applied Physiology*, *89*(6), 2158-2164. doi:10.1152/jappl.2000.89.6.2158
- LeBlanc, A., Schneider, V., Shackelford, L., West, S., Oganov, V., Bakulin, A., & Voronin, L. (2000). Bone mineral and lean tissue loss after long duration space flight. *Journal of Musculoskeletal & Neuronal Interactions*, *1*(2), 157-160.
- Lee, A., Mader, T., Gibson, C., Brunstetter, T., & Tarver, W. (2018). Space flight-associated neuro-ocular syndrome (SANS). *Eye*, *32*(7), 1164-1167.
- Lee, C., Rohr, J., Sass, A., Sater, S., Zahid, A., Macias, B., . . . Oshinski, J. N. (2020). In vivo estimation of optic nerve sheath stiffness using noninvasive MRI measurements and finite element modeling. *Journal of the Mechanical Behavior of Biomedical Materials*, *110*, 103924.
- Lee, J. S., Lee, S. H., Oum, B. S., Chung, J. S., Cho, B. M., & Hong, J. W. (2002). Relationship between intraocular pressure and systemic health parameters in a Korean population. *Clinical & Experimental Ophthalmology*, *30*(4), 237-241. doi:<https://doi.org/10.1046/j.1442-9071.2002.00527.x>
- Lee, S., Moore, A. D., Everett, M. E., Stenger, M. B., & Platts, S. H. (2010). Aerobic exercise deconditioning and countermeasures during bed rest. *Aviation, space, and environmental medicine*, *81*(1), 52-63. doi:10.3357/ASEM.2474.2010
- Leung, H., Wang, J. J., Rochtchina, E., Tan, A. G., Wong, T. Y., Hubbard, L. D., . . . Mitchell, P. (2003). Computer-assisted retinal vessel measurement in an older population: correlation between right and left eyes. *Clinical & Experimental Ophthalmology*, *31*(4), 326-330. doi:10.1046/j.1442-9071.2003.00661.x
- Leung, H., Wang, J. J., Rochtchina, E., Tan, A. G., Wong, T. Y., Klein, R., . . . Mitchell, P. (2003). Relationships between age, blood pressure, and retinal vessel diameters in an older population. *Investigative Ophthalmology and Visual Science*, *44*(7), 2900-2904. doi:10.1167/iovs.02-1114
- Levine, B. D., Lane, L. D., Watenpaugh, D. E., Gaffney, F. A., Buckey, J. C., & Blomqvist, C. G. (1996). Maximal exercise performance after adaptation to microgravity. *Journal of Applied Physiology*, *81*(2), 686-694. doi:10.1152/jappl.1996.81.2.686
- Levinsohn, G. (1912). *Die Entstehung der Kurzsichtigkeit*. Karger.
- Li, L.-J., Cheung, C. Y.-L., Liu, Y., Chia, A., Selvaraj, P., Lin, X.-Y., . . . Wong, T.-Y. (2011). Influence of blood pressure on retinal vascular caliber in young children. *Ophthalmology*, *118*(7), 1459-1465. doi:10.1016/j.ophtha.2010.12.007
- Lind, A. R. (1970). Cardiovascular responses to static exercise (Isometrics, anyone?). *Circulation*, *41*(2), 173-176. doi:10.1161/01.cir.41.2.173
- Loehr, J. A., Lee, S., English, K. L., Sibonga, J., Smith, S. M., Spiering, B. A., & Hagan, R. D. (2011). Musculoskeletal adaptations to training with the advanced resistive

- exercise device. *Medicine and Science in Sports and Exercise*, 43(1), 146-156.
doi:10.1249/MSS.0b013e3181e4f161
- Louwies, T., Mekjavic, P. J., Cox, B., Eiken, O., Mekjavic, I. B., Kounalakis, S., & De Boever, P. (2016). Separate and combined effects of hypoxia and horizontal bed rest on retinal blood vessel diameters. *Investigative Ophthalmology and Visual Science*, 57(11), 4927-4932. doi:10.1167/iovs.16-19968
- MacDougall, J., Tuxen, D., Sale, D., Moroz, J., & Sutton, J. (1985). Arterial blood pressure response to heavy resistance exercise. *Journal of Applied Physiology*, 58(3), 785-790. doi:10.1152/jappl.1985.58.3.785
- Macias, B. R., Ferguson, C. R., Patel, N., Gibson, C., Samuels, B. C., Laurie, S. S., . . . Stenger, M. B. (2021). Changes in the Optic Nerve Head and Choroid Over 1 Year of Spaceflight. *JAMA Ophthalmol*, 139(6), 663-667.
doi:10.1001/jamaophthalmol.2021.0931
- Macias, B. R., Patel, N. B., Gibson, C. R., Samuels, B. C., Laurie, S. S., Otto, C., . . . Stenger, M. B. (2020). Association of Long-Duration Spaceflight With Anterior and Posterior Ocular Structure Changes in Astronauts and Their Recovery. *JAMA Ophthalmology*, 138(5), 553-559. doi:10.1001/jamaophthalmol.2020.0673
- Mader, T. (1991). Intraocular pressure in microgravity. *The Journal of Clinical Pharmacology*, 31(10), 947-950. doi:10.1002/j.1552-4604.1991.tb03654.x
- Mader, T., Gibson, C., Caputo, M., Hunter, N., Taylor, G., Charles, J., & Meehan, R. (1993). Intraocular pressure and retinal vascular changes during transient exposure to microgravity. *American Journal of Ophthalmology*, 115(3), 347-350. doi:10.1016/s0002-9394(14)73586-x
- Mader, T., Gibson, C., Pass, A., Kramer, L., Lee, A., Fogarty, J., . . . Sargsyan, A. (2011). Optic disc edema, globe flattening, choroidal folds, and hyperopic shifts observed in astronauts after long-duration space flight. *Ophthalmology*, 118(10), 2058-2069. doi:10.1016/j.ophtha.2011.06.021
- Mader, T., Gibson, C., Pass, A., Lee, A., Killer, H., Hansen, H.-C., . . . Sargsyan, A. (2013). Optic disc edema in an astronaut after repeat long-duration space flight. *Journal of Neuro-Ophthalmology*, 33(3), 249-255.
doi:10.1097/WNO.0b013e31829b41a6
- Mader, T., Taylor, G., Hunter, N., Caputo, M., & Meehan, R. (1990). Intraocular pressure, retinal vascular, and visual acuity changes during 48 hours of 10 degrees head-down tilt. *Aviation, Space and Environmental Medicine*, 61(9), 810-813.
- Marchbanks, R. (1989). Method and apparatus for measuring intracranial fluid pressure. In: Google Patents.
- Marchbanks, R. (1993). MMS-10 Tympanic Displacement Analyser in Research and Clinical Practice. *London: MMS Ltd.*
- Marcus, D., Edelhauser, H., Maksud, M., & Wiley, R. (1974). Effects of a sustained muscular contraction on human intraocular pressure. *Clinical Science and Molecular Medicine*, 47(3), 249-257. doi:10.1042/cs0470249
- Marcus, D., Krupin, T., Podos, S. M., & Becker, B. (1970). The effect of exercise on intraocular pressure: I. Human beings. *Investigative Ophthalmology and Visual Science*, 9(10), 749-752.

- Mark, S., Scott, G. B., Donoviel, D. B., Leveton, L. B., Mahoney, E., Charles, J. B., & Siegel, B. (2014). The impact of sex and gender on adaptation to space: executive summary. *Journal of Women's Health, 23*(11), 941-947.
doi:10.1089/jwh.2014.4914
- Marshall-Bowman, K., Barratt, M. R., & Gibson, C. R. (2013). Ophthalmic changes and increased intracranial pressure associated with long duration spaceflight: an emerging understanding. *Acta Astronautica, 87*, 77-87.
doi:10.1016/j.actaastro.2013.01.014
- Marshall-Goebel, K., Mulder, E., Donoviel, D., Strangman, G., Suarez, J. I., Venkatasubba Rao, C., . . . Bershada, E. M. (2017). An international collaboration studying the physiological and anatomical cerebral effects of carbon dioxide during head-down tilt bed rest: the SPACECOT study. *Journal of Applied Physiology, 122*(6), 1398-1405. doi:10.1152/jappphysiol.00885.2016
- Marshall, I., MacCormick, I., Sellar, R., & Whittle, I. (2008). Assessment of factors affecting MRI measurement of intracranial volume changes and elastance index. *British Journal of Neurosurgery, 22*(3), 389-397.
- Martin, X. (1992). Normal intraocular pressure in man. *Ophthalmologica, 205*(2), 57-63.
doi:10.1159/000310313
- McFadden, D. M., Houston, C. S., Sutton, J. R., Powles, A. P., Gray, G. W., & Roberts, R. S. (1981). High-altitude retinopathy. *JAMA, 245*(6), 581-586.
- McMahon, C. J., McDermott, P., Horsfall, D., Selvarajah, J. R., King, A. T., & Vail, A. (2007). The reproducibility of transcranial Doppler middle cerebral artery velocity measurements: Implications for clinical practice. *British Journal of Neurosurgery, 21*(1), 21-27. doi:10.1080/02688690701210539
- McMonnies, C. W. (2016). The interaction between intracranial pressure, intraocular pressure and lamina cribrosa compression in glaucoma. *Clinical and Experimental Optometry, 99*(3), 219-226. doi:10.1111/cxo.12333
- Meijer, H., & Jean, D. (2012). Children at Altitude. In. *Consensus statement of the UIAA medical commission*, 1-14.
- Mekjavic, I. B., Amoaku, W., Mlinar, T., & Jaki Mekjavic, P. (2020). Hypercapnia augments resistive exercise-induced elevations in intraocular pressure in older individuals. *Experimental Physiology*. doi:10.1113/EP088236
- Mitchell, P., Cheung, N., de Haseth, K., Taylor, B., Rochtchina, E., Islam, F. A., . . . Wong, T. Y. (2007). Blood pressure and retinal arteriolar narrowing in children. *Hypertension, 49*(5), 1156-1162. doi:10.1161/HYPERTENSIONAHA.106.085910
- Mlinar, T., Jaki Mekjavic, P., Royal, J. T., Valencic, T., & Mekjavic, I. B. (2021). Intraocular pressure during handgrip exercise: The effect of posture and hypercapnia in young males. *Physiological Reports, 9*(20), e15035.
doi:10.14814/phy2.15035.
- Mohan, S., Tiwari, S., Jain, A., Gupta, J., & Sachan, S. K. (2014). Clinical comparison of Pulsair non-contact tonometer and Goldmann applanation tonometer in Indian population. *Journal of Optometry, 7*(2), 86-90. doi:10.1016/j.optom.2013.06.002
- Mollan, S. P., Wolffsohn, J. S., Nessim, M., Laiquzzaman, M., Sivakumar, S., Hartley, S., & Shah, S. (2008). Accuracy of Goldmann, ocular response analyser, Pascal and

- TonoPen XL tonometry in keratoconic and normal eyes. *British Journal of Ophthalmology*, *92*(12), 1661-1665.
- Moore, A. D., Lee, S. M., Stenger, M. B., & Platts, S. H. (2010). Cardiovascular exercise in the US space program: past, present and future. *Acta Astronautica*, *66*(7-8), 974-988. doi:10.1016/j.actaastro.2009.10.009
- Moore Jr, A. D., Downs, M. E., Lee, S. M., Feiveson, A. H., Knudsen, P., & Ploutz-Snyder, L. (2014). Peak exercise oxygen uptake during and following long-duration spaceflight. *Journal of Applied Physiology*, *117*(3), 231-238. doi:10.1152/jappphysiol.01251.2013
- Mori, K., Ando, F., Nomura, H., Sato, Y., & Shimokata, H. (2000). Relationship between intraocular pressure and obesity in Japan. *International Journal of Epidemiology*, *29*(4), 661-666. doi:10.1093/ije/29.4.661
- Morris, D. S., Somner, J., Donald, M. J., McCormick, I. J., Bourne, R. R., Huang, S. S., . . . Dhillon, B. (2006). The eye at altitude. In *Hypoxia and Exercise* (pp. 249-270): Springer.
- Moses, R. A. (1958). The Goldmann applanation tonometer. *American Journal of Ophthalmology*, *46*(6), 865-869.
- Murgatroyd, H., & Bembridge, J. (2008). Intraocular pressure. *Continuing Education in Anaesthesia, Critical Care & Pain*, *8*(3), 100-103. doi:10.1093/bjaceaccp/mkn015
- Nakamura, M., Darhad, U., Tatsumi, Y., Fujioka, M., Kusuhara, A., Maeda, H., & Negi, A. (2006). Agreement of rebound tonometer in measuring intraocular pressure with three types of applanation tonometers. *American Journal of Ophthalmology*, *142*(2), 332-334.
- Narloch, J. A., & Brandstater, M. E. (1995). Influence of breathing technique on arterial blood pressure during heavy weight lifting. *Archives of Physical Medicine and Rehabilitation*, *76*(5), 457-462. doi:10.1016/s0003-9993(95)80578-8
- Natsis, K., Asouhidou, I., Nousios, G., Chatzibalas, T., Vlasis, K., & Karabatakis, V. (2009). Aerobic exercise and intraocular pressure in normotensive and glaucoma patients. *BMC Ophthalmology*, *9*(1), 6. doi:10.1186/1471-2415-9-6
- Nelson, E. S., Mulugeta, L., & Myers, J. G. (2014). Microgravity-induced fluid shift and ophthalmic changes. *Life*, *4*(4), 621-665. doi:10.3390/life4040621
- Neumann, T., Baertschi, M., Vilser, W., Drinda, S., Franz, M., Brueckmann, A., . . . Jung, C. (2016). Retinal vessel regulation at high altitudes. *Clinical Hemorheology and Microcirculation*, *63*(3), 281-292. doi:10.3233/CH-162041
- Nicogossian, A. E., & Parker Jr., J. F. (1982). *Space Physiology and Medicine (NASA SP-447)*. Washington, D.C., USA: U.S. Government Printing Office.
- Nomura, H., Ando, F., Niino, N., Shimokata, H., & Miyake, Y. (2002). The relationship between age and intraocular pressure in a Japanese population: The influence of central corneal thickness. *Current Eye Research*, *24*(2), 81-85. doi:10.1076/ceyr.24.2.81.8161
- O'Connor, P. S., & Poirier, R. H. (1985). Ocular effects of gravity inversion. *JAMA*, *254*(6), 756-756. doi:10.1001/jama.1985.03360060053022

- Ogueta, S. B., Schwartz, S. D., Yamashita, C. K., & Farber, D. B. (1999). Estrogen Receptor in the Human Eye: Influence of Gender and Age on Gene Expression. *Investigative Ophthalmology and Visual Science*, *40*(9), 1906-1911.
- Organization, W. H. (2005). Surveillance of chronic disease risk factors: country level data and comparable estimates. Retrieved from https://apps.who.int/iris/bitstream/handle/10665/43190/9241593024_eng.pdf?sequence=1&isAllowed=y
- Pavy-Le Traon, A., Heer, M., Narici, M. V., Rittweger, J., & Vernikos, J. (2007). From space to Earth: advances in human physiology from 20 years of bed rest studies (1986–2006). *European Journal of Applied Physiology*, *101*(2), 143-194. doi:10.1007/s00421-007-0474-z
- Perkins, E. S. (1973). The Bedford glaucoma survey. II. Rescreening of normal population. *British Journal of Ophthalmology*, *57*(3), 186-192. doi:10.1136/bjo.57.3.186
- Pescosolido, N., Barbato, A., & Di Blasio, D. (2015). Hypobaric hypoxia: effects on contrast sensitivity in high altitude environments. *Aerospace Medicine and Human Performance*, *86*(2), 118-124. doi:10.3357/AMHP.3938.2015
- Peterson, D. D., Pack, A. I., Silage, D. A., & Fishman, A. P. (1981). Effects of aging on ventilatory and occlusion pressure responses to hypoxia and hypercapnia. *American Review of Respiratory Disease*, *124*(4), 387-391. doi:10.1164/arrd.1981.124.4.387
- Phillips, A., & Marchbanks, R. (1989). Effects of posture and age on tympanic membrane displacement measurements. *British Journal of Audiology*, *23*(4), 279-284.
- Platts, S. H., Bairey Merz, C. N., Barr, Y., Fu, Q., Gulati, M., Hughson, R., . . . Wenger, N. K. (2014). Effects of sex and gender on adaptation to space: cardiovascular alterations. *Journal of Women's Health*, *23*(11), 950-955.
- Ploutz-Snyder, L. L., Downs, M., Ryder, J., Hackney, K., Scott, J., Buxton, R., . . . Crowell, B. (2014). Integrated resistance and aerobic exercise protects fitness during bed rest. *Medicine and Science in Sports and Exercise*, *46*(2), 358-368. doi:10.1249/MSS.0b013e3182a62f85
- Popovic, D., Khoo, M., & Lee, S. (2009). Noninvasive monitoring of intracranial pressure. *Recent Patents on Biomedical Engineering (Discontinued)*, *2*(3), 165-179.
- Pournaras, C. J., Rungger-Brändle, E., Riva, C. E., Hardarson, S. H., & Stefansson, E. (2008). Regulation of retinal blood flow in health and disease. *Progress in Retinal and Eye Research*, *27*(3), 284-330. doi:10.1016/j.preteyeres.2008.02.002
- Powers, W. J., & Zazulia, A. R. (2003). The use of positron emission tomography in cerebrovascular disease. *Neuroimaging Clinics*, *13*(4), 741-758. doi:The use of positron emission tomography in cerebrovascular disease
- Qureshi, I. A. (1997). Measurements of intraocular pressure throughout the pregnancy in Pakistani women. *Chinese Medical Sciences Journal= Chung-kuo i Hsueh k'o Hsueh tsa Chih*, *12*(1), 53-56.
- Qureshi, I. A., Xi, X. R., Huang, Y. B., & Wu, X. D. (1996). Magnitude of decrease in intraocular pressure depends upon intensity of exercise. *Korean Journal of Ophthalmology*, *10*(2), 109-115. doi:10.3341/kjo.1996.10.2.109

- Raboel, P., Bartek, J., Andresen, M., Bellander, B., & Romner, B. (2012). Intracranial pressure monitoring: invasive versus non-invasive methods—a review. *Critical Care Research and Practice*, 2012.
- Ragauskas, A., Daubaris, G., Dziugys, A., Azelis, V., & Gedrimas, V. (2005). Innovative non-invasive method for absolute intracranial pressure measurement without calibration. In *Intracranial Pressure and Brain Monitoring XII* (pp. 357-361): Springer.
- Ragauskas, A., Matijosaitis, V., Zakelis, R., Kęstutis, P., Rastenyte, D., Piper, I., & Gediminas, D. (2012). Clinical assessment of noninvasive intracranial pressure absolute value measurement method. *Neurology*, 78(21), 1684-1691.
- Reid, A., Marchbanks, R., Bateman, D., Martin, A., Brightwell, A., & Pickard, J. (1989). Mean intracranial pressure monitoring by a non-invasive audiological technique: a pilot study. *Journal of Neurology, Neurosurgery and Psychiatry*, 52(5), 610-612.
- Reid, A., Marchbanks, R., Burge, D., Martin, A., Bateman, D., Pickard, J., & Brightwell, A. (1990). The relationship between intracranial pressure and tympanic membrane displacement. *British Journal of Audiology*, 24(2), 123-129.
- Rexhaj, E., Garcin, S., Rimoldi, S. F., Duplain, H., Stuber, T., Allemann, Y., . . . Scherrer, U. (2011). Reproducibility of acute mountain sickness in children and adults: a prospective study. *Pediatrics*, 127(6), e1445-e1448. doi:10.1542/peds.2010-3756
- Richard, R., Lonsdorfer-Wolf, E., Charloux, A., Doutreleau, S., Buchheit, M., Oswald-Mammosser, M., . . . Lonsdorfer, J. (2001). Non-invasive cardiac output evaluation during a maximal progressive exercise test, using a new impedance cardiograph device. *European Journal of Applied Physiology*, 85(3-4), 202-207. doi:10.1007/s004210100458
- Risau, W. (1997). Mechanisms of angiogenesis. *Nature*, 386(6626), 671-674. doi:10.1038/386671a0
- Risner, D., Ehrlich, R., Kheradiya, N. S., Siesky, B., McCranor, L., & Harris, A. (2009). Effects of exercise on intraocular pressure and ocular blood flow: a review. *Journal of Glaucoma*, 18(6), 429-436. doi:10.1097/IJG.0b013e31818fa5f3
- Roach, R., Bartsch, P., Hackett, P., Oelz, O., & Sutton, J. (1993). The Lake Louise AMS scoring consensus committee. *Hypoxia and molecular medicine*. Burlington, VT: Queen City Printers, 272-274.
- Robinson, F., Riva, C. E., Grunwald, J. E., Petrig, B. L., & Sinclair, S. H. (1986). Retinal blood flow autoregulation in response to an acute increase in blood pressure. *Investigative Ophthalmology and Visual Science*, 27(5), 722-726.
- Roddy, G., Curnier, D., & Elleberg, D. (2014). Reductions in Intraocular Pressure After Acute Aerobic Exercise: A Meta-Analysis. *Clinical Journal of Sport Medicine*, 24(5). Retrieved from https://journals.lww.com/cjsportsmed/Fulltext/2014/09000/Reductions_in_Intraocular_Pressure_After_Acute.2.aspx
- Rootman, D. S., Insler, M. S., Thompson, H. W., Parelman, J., Poland, D., & Unterman, S. R. (1988). Accuracy and precision of the Tono-Pen in measuring intraocular

- pressure after keratoplasty and epikeratophakia and in scarred corneas. *Archives of Ophthalmology*, 106(12), 1697-1700. doi:10.1001/archophth.1988.01060140869030
- Rowell, L. (1986). Regulation during physical stress. *Human circulation*. doi:10.1152/physrev.1974.54.1.75
- Rowell, L. B. (1993). *Human cardiovascular control*: Oxford University Press, USA.
- Salman, M. S. (1997). Can intracranial pressure be measured non-invasively? *The Lancet*, 350(9088), 1367. doi:10.1016/S0140-6736(05)65138-0
- Salvetat, M. L., Zeppieri, M., Tosoni, C., & Brusini, P. (2007). Comparisons between Pascal dynamic contour tonometry, the TonoPen, and Goldmann applanation tonometry in patients with glaucoma. *Acta Ophthalmologica Scandinavica*, 85(3), 272-279.
- Salvi, S. M., Akhtar, S., & Currie, Z. (2006). Ageing changes in the eye. *Postgraduate Medical Journal*, 82(971), 581-587. doi:10.1136/pgmj.2005.040857
- Samora, M., Incognito, A. V., & Vianna, L. C. (2019). Sex differences in blood pressure regulation during ischemic isometric exercise: the role of the β -adrenergic receptors. *Journal of Applied Physiology*, 127(2), 408-414. doi:10.1152/jappphysiol.00270.2019
- Samora, M., Teixeira, A. L., Sabino-Carvalho, J. L., & Vianna, L. C. (2019). Spontaneous cardiac baroreflex sensitivity is enhanced during post-exercise ischemia in men but not in women. *European Journal of Applied Physiology*, 119(1), 103-111. doi:10.1007/s00421-018-4004-y
- Sanchez, J., Pequignot, J. M., Peyrin, L., & Monod, H. (1980). Sex differences in the sympatho-adrenal response to isometric exercise. *European Journal of Applied Physiology and Occupational Physiology*, 45(2), 147-154. doi:10.1007/BF00421322
- Sato, K., Sadamoto, T., Hirasawa, A., Oue, A., Subudhi, A. W., Miyazawa, T., & Ogoh, S. (2012). Differential blood flow responses to CO₂ in human internal and external carotid and vertebral arteries. *The Journal of Physiology*, 590(14), 3277-3290. doi:10.1113/jphysiol.2012.230425
- Schmidt, B., Czosnyka, M., Raabe, A., Yahya, H., Schwarze, J. J. r., Sackere, D., . . . Klingelhöfer, J. r. (2003). Adaptive noninvasive assessment of intracranial pressure and cerebral autoregulation. *Stroke*, 34(1), 84-89.
- Schneider, M., Bernasch, D., Weymann, J., Holle, R., & Bartsch, P. (2002). Acute mountain sickness: influence of susceptibility, preexposure, and ascent rate. *Medicine and Science in Sports and Exercise*, 34(12), 1886-1891. doi:10.1097/00005768-200212000-00005
- Sechzer, P., Egbert, L. D., Linde, H., Cooper, D., Dripps, R., & Price, H. (1960). Effect of CO₂ inhalation on arterial pressure, ECG and plasma catecholamines and 17-OH corticosteroids in normal man. *Journal of Applied Physiology*, 15(3), 454-458. doi:10.1152/jappl.1960.15.3.454
- Selbach, J. M., Posielek, K., Steuhl, K.-P., & Kremmer, S. (2005). Episcleral venous pressure in untreated primary open-angle and normal-tension glaucoma. *Ophthalmologica*, 219(6), 357-361. doi:10.1159/000088378

- Shackelford, L., LeBlanc, A., Driscoll, T., Evans, H., Rianon, N., Smith, S., . . . Lai, D. (2004). Resistance exercise as a countermeasure to disuse-induced bone loss. *Journal of Applied Physiology*, *97*(1), 119-129.
- Shen, Q., Stuart, J., Venkatesh, B., Wallace, J., & Lipman, J. (1999). Inter Observer Variability of the Transcranial Doppler Ultrasound Technique: Impact of Lack of Practice on the Accuracy of Measurement. *Journal of Clinical Monitoring and Computing*, *15*(3), 179-184. doi:10.1023/A:1009925811965
- Shimbles, S., Dodd, C., Banister, K., Mendelow, A., & Chambers, I. (2005). Clinical comparison of tympanic membrane displacement with invasive intracranial pressure measurements. *Physiological Measurement*, *26*(6), 1085.
- Shin, A., Yoo, L., Park, J., & Demer, J. L. (2017). Finite element biomechanics of optic nerve sheath traction in adduction. *Journal of Biomechanical Engineering*, *139*(10).
- Shinojima, A., Iwasaki, K. I., Aoki, K., Ogawa, Y., Yanagida, R., & Yuzawa, M. (2012). Subfoveal choroidal thickness and foveal retinal thickness during head-down tilt. *Aviation, Space and Environmental Medicine*, *83*(4), 388-393. doi:10.3357/ASEM.3191.2012
- Shiose, Y. (1990). Intraocular pressure: New perspectives. *Survey of Ophthalmology*, *34*(6), 413-435. doi:[https://doi.org/10.1016/0039-6257\(90\)90122-C](https://doi.org/10.1016/0039-6257(90)90122-C)
- Shiose, Y., & Kawase, Y. (1986). A new approach to stratified normal intraocular pressure in a general population. *American Journal of Ophthalmology*, *101*(6), 714-721. doi:10.1016/0002-9394(86)90776-2
- Shults, W. T., & Swan, K. C. (1975). High altitude retinopathy in mountain climbers. *Archives of Ophthalmology*, *93*(6), 404-408. doi:10.1001/archophth.1975.01010020418003
- Sibony, P. A., Kupersmith, M. J., Feldon, S. E., Wang, J.-K., & Garvin, M. (2015). Retinal and choroidal folds in papilledema. *Investigative Ophthalmology and Visual Science*, *56*(10), 5670-5680. doi:10.1167/iovs.15-17459
- Siebenmann, C., Rasmussen, P., Sørensen, H., Zaar, M., Hvidtfeldt, M., Pichon, A., . . . Lundby, C. (2015). Cardiac output during exercise: a comparison of four methods. *Scandinavian Journal of Medicine and Science in Sports*, *25*(1), e20-e27. doi:10.1111/sms.12201
- Smith, M. G., Kelley, M., & Basner, M. (2020). A brief history of spaceflight from 1961 to 2020: An analysis of missions and astronaut demographics. *Acta Astronautica*, *175*, 290-299. doi:10.1016/j.actaastro.2020.06.004
- Smith, S. M., Heer, M. A., Shackelford, L. C., Sibonga, J. D., Ploutz-Snyder, L., & Zwart, S. R. (2012). Benefits for bone from resistance exercise and nutrition in long-duration spaceflight: Evidence from biochemistry and densitometry. *Journal of Bone and Mineral Research*, *27*(9), 1896-1906. doi:10.1002/jbmr.1647
- Speck, V., Staykov, D., Huttner, H. B., Sauer, R., Schwab, S., & Bardutzky, J. (2011). Lumbar catheter for monitoring of intracranial pressure in patients with post-hemorrhagic communicating hydrocephalus. *Neurocritical Care*, *14*(2), 208-215.

- Spoerl, E., Zubaty, V., Raiskup-Wolf, F., & Pillunat, L. E. (2007). Oestrogen-induced changes in biomechanics in the cornea as a possible reason for keratectasia. *British Journal of Ophthalmology*, *91*(11), 1547-1550.
- Stamper, R. L. (2011). A history of intraocular pressure and its measurement. *Optometry and Vision Science*, *88*(1), E16-E28.
- Stenger, M., Tarver, W., Brunstetter, T., Gibson, C., Laurie, S., Lee, S., . . . Smith, S. (2017). Evidence report: Risk of spaceflight associated neuro-ocular syndrome (SANS).
- Taibbi, G., Cromwell, R. L., Zanello, S. B., Yarbough, P. O., Ploutz-Snyder, R. J., Godley, B. F., & Vizzeri, G. (2014). Ocular outcomes evaluation in a 14-day head-down bed rest study. *Aviation, Space and Environmental Medicine*, *85*(10), 983-992. doi:10.3357/ASEM.4055.2014
- Taibbi, G., Cromwell, R. L., Zanello, S. B., Yarbough, P. O., Ploutz-Snyder, R. J., Godley, B. F., & Vizzeri, G. (2016). Ocular outcomes comparison between 14-and 70-day head-down-tilt bed rest. *Investigative Ophthalmology and Visual Science*, *57*(2), 495-501. doi:10.1167/iovs.15-18530
- Taibbi, G., Kaplowitz, K., Cromwell, R. L., Godley, B. F., Zanello, S. B., & Vizzeri, G. (2013). Effects of 30-day head-down bed rest on ocular structures and visual function in a healthy subject. *Aviation, Space and Environmental Medicine*, *84*(2), 148-154. doi:10.3357/ASEM.3520.2013
- Taylor, C. R., Hanna, M., Behnke, B. J., Stabley, J. N., McCullough, D. J., Davis III, R. T., . . . Delp, M. D. (2013). Spaceflight-induced alterations in cerebral artery vasoconstrictor, mechanical, and structural properties: implications for elevated cerebral perfusion and intracranial pressure. *The FASEB Journal*, *27*(6), 2282-2292. doi:10.1096/fj.12-222687
- The Royal College of Ophthalmologists. (2016). Commissioning Guide: Glaucoma (Recommendations). Retrieved from <https://www.rcophth.ac.uk/wp-content/uploads/2016/06/Glaucoma-Commissioning-Guide-Recommendations-June-2016-Final.pdf>
- Thornton, W. E., Hoffer, G. W., & Rummel, J. A. (1977). Anthropometric changes and fluid shifts. *Biomedical results from Skylab*, *377*, 330.
- Topouchian, J., Agnoletti, D., Blacher, J., Youssef, A., Chahine, M. N., Ibanez, I., . . . Asmar, R. (2014). Validation of four devices: Omron M6 Comfort, Omron HEM-7420, Withings BP-800, and Polygreen KP-7670 for home blood pressure measurement according to the European Society of Hypertension International Protocol. *Vascular health and risk management*, *10*, 33. doi:10.2147/VHRM.S53968
- Trappe, S., Costill, D., Gallagher, P., Creer, A., Peters, J. R., Evans, H., . . . Fitts, R. H. (2009). Exercise in space: human skeletal muscle after 6 months aboard the International Space Station. *Journal of Applied Physiology*, *106*(4), 1159-1168. doi:10.1152/jappphysiol.91578.2008
- Trappe, T., Trappe, S., Lee, G., Widrick, J., Fitts, R., & Costill, D. (2006). Cardiorespiratory responses to physical work during and following 17 days of bed

- rest and spaceflight. *Journal of Applied Physiology*, *100*(3), 951-957.
doi:10.1152/jappphysiol.01083.2005
- Trappe, T. A., Burd, N. A., Louis, E. S., Lee, G. A., & Trappe, S. W. (2007). Influence of concurrent exercise or nutrition countermeasures on thigh and calf muscle size and function during 60 days of bed rest in women. *Acta Physiologica (Oxford, England)*, *191*(2), 147-159. doi:10.1111/j.1748-1716.2007.01728.x
- Tsivgoulis, G., Alexandrov, A. V., & Sloan, M. A. (2009). Advances in transcranial doppler ultrasonography. *Current Neurology and Neuroscience Reports*, *9*(1), 46-54. doi:10.1007/s11910-009-0008-7
- Uri, J. (2018). *Valentina Tereshkova and Sally Ride - Women Space Pioneers*. Retrieved from <https://ntrs.nasa.gov/api/citations/20180002182/downloads/20180002182.pdf>
- Van der Jagt, L. H., & Jansonius, N. M. (2005). Three portable tonometers, the TGDC-01, the ICARE and the Tonopen XL, compared with each other and with Goldmann applanation tonometry. *Ophthalmic and Physiological Optics*, *25*(5), 429-435.
- Varma, A., Adams, W., Lloyd, J., Carson, K., Snowden, J., Testa, H., . . . Neary, D. (2002). Diagnostic patterns of regional atrophy on MRI and regional cerebral blood flow change on SPECT in young onset patients with Alzheimer's disease, frontotemporal dementia and vascular dementia. *Acta Neurologica Scandinavica*, *105*(4), 261-269. doi:10.1034/j.1600-0404.2002.1o148.x
- Venkataraman, S. T., Hudson, C., Fisher, J. A., & Flanagan, J. G. (2006). Novel methodology to comprehensively assess retinal arteriolar vascular reactivity to hypercapnia. *Microvascular Research*, *72*(3), 101-107.
doi:10.1016/j.mvr.2006.06.002
- Vera, J., Jiménez, R., Redondo, B., Torrejón, A., Koulieris, G.-A., De Moraes, C. G., & García-Ramos, A. (2019). Investigating the Immediate and Cumulative Effects of Isometric Squat Exercise for Different Weight Loads on Intraocular Pressure: A Pilot Study. *Sports Health*, *11*(3), 247-253. doi:10.1177/1941738119834985
- Vera, J., Raimundo, J., García-Durán, B., Pérez-Castilla, A., Redondo, B., Delgado, G., . . . García-Ramos, A. (2019). Acute intraocular pressure changes during isometric exercise and recovery: The influence of exercise type and intensity, and participant's sex. *Journal of Sports Sciences*, *37*(19), 2213-2219.
- Vera, J., Redondo, B., Koulieris, G.-A., Torrejon, A., Jiménez, R., & Garcia-Ramos, A. (2020). Intraocular Pressure Responses to Four Different Isometric Exercises in Men and Women. *Optometry and Vision Science*, *97*(8), 648-653.
doi:10.1097/oxp.0000000000001545
- Vieira, G. M., Oliveira, H. B., de Andrade, D. T., Bottaro, M., & Ritch, R. (2006). Intraocular pressure variation during weight lifting. *Archives of Ophthalmology*, *124*(9), 1251-1254. doi:10.1001/archophth.124.9.1251
- Wåhlin, A., Holmlund, P., Fellows, A. M., Malm, J., Buckey, J. C., & Eklund, A. (2021). Optic Nerve Length before and after Spaceflight. *Ophthalmology*, *128*(2), 309-316.
doi:<https://doi.org/10.1016/j.ophtha.2020.07.007>

- Wang, B., Tran, H., Smith, M. A., Kostanyan, T., Schmitt, S. E., Bilonick, R. A., . . . Ishikawa, H. (2017). In-vivo effects of intraocular and intracranial pressures on the lamina cribrosa microstructure. *PLoS One*, *12*(11). doi:10.1371/journal.pone.0188302
- Wang, X., Fisher, L. K., Milea, D., Jonas, J. B., & Girard, M. J. A. (2017). Predictions of Optic Nerve Traction Forces and Peripapillary Tissue Stresses Following Horizontal Eye Movements. *Investigative Ophthalmology and Visual Science*, *58*(4), 2044-2053. doi:10.1167/iovs.16-21319
- Wang, Y. X., Xu, L., Wei, W. B., & Jonas, J. B. (2018). Intraocular pressure and its normal range adjusted for ocular and systemic parameters. The Beijing Eye Study 2011. *PLoS One*, *13*(5), e0196926. doi:10.1371/journal.pone.0196926
- Waters, W. W., Ziegler, M. G., & Meck, J. V. (2002). Postspaceflight orthostatic hypotension occurs mostly in women and is predicted by low vascular resistance. *Journal of Applied Physiology*, *92*(2), 586-594. doi:10.1152/jappphysiol.00544.2001
- Wei, X., Cai, S.-p., Zhang, X., Li, X., Chen, X., & Liu, X. (2012). Is low dose of estrogen beneficial for prevention of glaucoma? *Medical Hypotheses*, *79*(3), 377-380.
- Willie, C., Macleod, D., Shaw, A., Smith, K., Tzeng, Y.-C., Eves, N., . . . Day, T. (2012). Regional brain blood flow in man during acute changes in arterial blood gases. *The Journal of Physiology*, *590*(14), 3261-3275. doi:10.1113/jphysiol.2012.228551
- Willmann, G., Fischer, M. D., Schatz, A., Schommer, K., & Gekeler, F. (2013). Retinal vessel leakage at high altitude. *JAMA*, *309*(21), 2210-2212. doi:10.1001/jama.2013.5550
- Wilson, M. H., Davagnanam, I., Holland, G., Dattani, R. S., Tamm, A., Hirani, S. P., . . . Thornton, J. S. (2013). Cerebral venous system and anatomical predisposition to high-altitude headache. *Annals of Neurology*, *73*(3), 381-389. doi:10.1002/ana.23796
- Wolf, S., Arend, O., Toonen, H., Bertram, B., Jung, F., & Reim, M. (1991). Retinal capillary blood flow measurement with a scanning laser ophthalmoscope preliminary results. *Ophthalmology*, *98*(6), 996-1000. doi:10.1016/S0161-6420(91)32192-4
- Wong, T. Y., Klein, R., Klein, B. E., Meuer, S. M., & Hubbard, L. D. (2003). Retinal vessel diameters and their associations with age and blood pressure. *Investigative Ophthalmology and Visual Science*, *44*(11), 4644-4650. doi:10.1167/iovs.03-0079
- Young, F. A. (1964). The distribution of refractive errors in monkeys. *Experimental Eye Research*, *3*(3), 230-238. doi:10.1016/S0014-4835(64)80015-4
- Young, F. A., & Leary, G. A. (1973). Visual-optical characteristics of caged and semifree-ranging monkeys. *American Journal of Physical Anthropology*, *38*(2), 377-382. doi:10.1002/ajpa.1330380236
- Zebrowska, A., Gasior, Z., & Jastrzebski, D. (2013). Cardiovascular effects of the valsalva maneuver during static arm exercise in elite power lifting athletes. *Advances in Experimental Medicine and Biology*. doi:10.1007/978-94-007-4546-9_42
- Zetterberg, M. (2016). Age-related eye disease and gender. *Maturitas*, *83*, 19-26. doi:<https://doi.org/10.1016/j.maturitas.2015.10.005>

- Zhang, L.-F., & Hargens, A. R. (2018). Spaceflight-induced intracranial hypertension and visual impairment: pathophysiology and countermeasures. *Physiological Reviews*, *98*(1), 59-87. doi:10.1152/physrev.00017.2016
- Zhang, X., Medow, J. E., Iskandar, B. J., Wang, F., Shokouejinejad, M., Koueik, J., & Webster, J. G. (2017). Invasive and noninvasive means of measuring intracranial pressure: a review. *Physiological Measurement*, *38*(8), R143.
- Zheng, Y., Cheung, C. Y. L., Wong, T. Y., Mitchell, P., & Aung, T. (2010). Influence of Height, Weight, and Body Mass Index on Optic Disc Parameters. *Investigative Ophthalmology and Visual Science*, *51*(6), 2998-3002. doi:10.1167/iovs.09-4470

Bibliography

Publications Related to the Thesis

Journal Articles

- Mlinar, T.**, Debevec, T., Kapus, J., Najdenov, P., Ušaj, A., Mekjavic, I.B. and Jaki Mekjavic, P. (2023). Retinal blood vessel diameters in children and adults exposed to a simulated altitude of 3,000 m. *Frontiers in Physiology*, 14:1026987. doi: 10.3389/fphys.2023.1026987
- Mlinar, T.**, Jaki Mekjavic, P., Royal, J. T., Valencic, T., & Mekjavic, I. B. (2021). Intraocular pressure during handgrip exercise: The effect of posture and hypercapnia in young males. *Physiological Reports*, 9(20), e15035. doi: 10.14814/phy2.15035
- Mekjavic, I. B., Amoaku, W., **Mlinar, T.**, & Jaki Mekjavic, P. (2020). Hypercapnia augments resistive exercise-induced elevations in intraocular pressure in older individuals. *Experimental Physiology*, 105(4), 641-651. doi: 0.1113/EP088236
- Jaki Mekjavic, P., Amoaku, W., **Mlinar, T.**, & Mekjavic, I. B. (2020). The aetiology of Spaceflight-Associated Neuro-ocular Syndrome (SANS) might be explained by a neural mechanism regulating intraocular pressure. *The Journal of Physiology*, 598(8). doi: 10.1113/jp279699

Conference

- Jaki Mekjavic, P., **Mlinar, T.**, Amoaku, W. & Mekjavic, I. B (2020) The contribution of the hypercapnic exercise pressor response to the Space flight-associated neuro-ocular syndrome. *Annual Meeting of the American Academy of Ophthalmology*. Virtual meeting.
- Jaki Mekjavic, P., Debevec, T., Kapus, J., Ušaj, A., McDonnell, A., Najdenov, P., **Mlinar, T.**, Štular, M., & Mekjavic, I. B. (2018) Retinal blood vessel diameters in children and adults exposed to a simulated altitude of 3000 m: KidSki project. *Annual meeting of the European College of Sports Science*. Ireland, Dublin.

Other Publications

- Royal, J. T., Fisher, J. T., **Mlinar, T.**, Mekjavic, I. B., & McDonnell, A. C. (2022). Validity and reliability of capillary vs. Venous blood for the assessment of haemoglobin mass and intravascular volumes. *Frontiers in Physiology*, 2499. doi: 10.3389/fphys.2022.1021588
- Ciuha, U., Sotiridis, A., **Mlinar, T.**, Royal, J. T., Eiken, O., & Mekjavic, I. B. (2021). Heat acclimation enhances the cold-induced vasodilation response. *European Journal of Applied Physiology*, 121(11), 3005-3015. doi: 10.1007/s00421-021-04761-x

- Sotiridis, A., Debevec, T., Ciuha, U., McDonnell, A. C., **Mlinar, T.**, Royal, J. T., & Mekjavic, I. B. (2020). Aerobic but not thermoregulatory gains following a 10-day moderate-intensity training protocol are fitness level dependent: A cross-adaptation perspective. *Physiological Reports*, 8(3), e14355. doi: 10.14814/phy2.14355
- McDonnell, A. C., Cooper, D., **Mlinar, T.**, & Mekjavic, I. B. (2019). The effect of post-exercise application of either graduated or uniform compression socks on the mitigation of delayed onset muscle soreness. *Textile Research Journal*, 89(9), 1792-1806. doi: 10.1177/0040517518780002

Biography

Tinkara Mlinar is a PhD candidate at the Jožef Stefan International Postgraduate School and a member of the Environmental Physiology and Ergonomics Laboratory at the Jožef Stefan Institute, Ljubljana, Slovenia. Her research focuses on independent and interactive effects of intrinsic (i.e., age, sex) and extrinsic (i.e., hypercapnia, hypoxia, resistive exercise) spaceflight-associated factors on the ocular system, with particular reference to intraocular pressure responses. Her supervisor for her doctoral dissertation was Professor Igor B. Mekjavic (co-supervisor: Professor Polona Jaki Mekjavic, MD, PhD).

In 2018, Tinkara completed her MSc in Human and Applied Physiology with Distinction at the University of Portsmouth, Portsmouth, United Kingdom. The title of her dissertation, supervised by Dr. Heather Massey and Dr. Jim House, was “Repeated Upper Body and Face Cold Water Immersions Do Not Provoke Generic Autonomic Cross-Adaptation to Submaximal Exercise in Acute Hypoxia in Humans”.

In 2017, she completed her BSc (Hons) in Sports Science and Physics with First Class Honours at Loughborough University, Loughborough, United Kingdom. The title of her undergraduate thesis, supervised by Professor Feo Kusmartsev, was “Biomechanics of Human Motion”. She also received a secondary award Diploma in Industrial Sciences.

Appendix A

Ethics Approval



REPUBLIKA SLOVENIJA
MINISTRSTVO ZA ZDRAVJE

Komisija Republike Slovenije za medicinsko etiko

Podpisnik: Božidar Stefan Voljc
Izdajatelj: Republika Slovenija
Serijska številka: b4 b6 58 be 00 00 00 00 56 7b e7 4d
Datum podpisa: 13.59, 18.06.2020
Referenčna številka: 0120-31/2020/9

izr. prof. dr. Polona Jaki Mekjavič, dr. med.,
specialistka oftalmologije
Očesna klinika, UKC Ljubljana
Grablovičeva 46, 1000 Ljubljana

predstojnica Katedre za oftalmologijo
Medicinska fakulteta, Univerza v Ljubljani
Vrazov trg 2, 1000 Ljubljana

polona.jaki@guest.arnes.si

Številka: 0120-31/2020/9
Datum: 15. junij 2020

Zadeva: Ocena etičnosti predložene raziskave

Spoštovani,

Komisija Republike Slovenije za medicinsko etiko (v nadaljevanju KME RS) je dne 20. 1. 2020 (datirano z datumom 14. 1. 2020) od vas prejela vlogo za oceno etičnosti raziskave z naslovom "Vpliv hiperkapnije in izometrične vadbe na krvni in znotrajočesni tlak".

Gre za laboratorijsko raziskavo, katere odgovorna raziskovalca boste vi gospa izr. prof. dr. Polona Jaki Mekjavič in prof. dr. Igor Mekjavič, v raziskavi pa bo sodelovala še mlada raziskovalka Tinkara Mlinar.

KME RS je na seji 18. februarja 2020¹ obravnavala prejeto vlogo in ugotovila, da vloga ni popolna, ker niso bili jasno opredeljeni vključitveni in izključitveni kriteriji za sodelovanje v raziskavi, vlogi ni bil priložen vprašalnik. V pojasnilu sodelujočim osebam v raziskavi ni bilo navedeno, kakšne so morebitne posledice hiperkapnije oz. vdihavanja višjih koncentracij ogljikovega dioksida, zato vas je KME RS v dokumentu št. 0120-31/2020/4 pozvala k dopolnitvi vloge.

Dne 11. 5. 2020 ste na KME RS dostavili popravljeno vlogo.

KME RS je na seji dne 19. maja 2020² obravnavala dopolnjeno vlogo in ugotovila, da je vloga sedaj popolna ter raziskava etično sprejemljiva.

¹ Seznam članov KME, ki so odločali o vlogi, in izjava, da KME deluje v skladu z zadevnimi zakoni in priporočili, sta na voljo na spletni strani MZ (zavihek "O Ministrstvu – Komisija Republike Slovenije za medicinsko etiko", rubrika "Seje Komisije").

² Seznam članov KME, ki so odločali o vlogi, in izjava, da KME deluje v skladu z zadevnimi zakoni in priporočili, sta na voljo na spletni strani MZ (zavihek "O Ministrstvu – Komisija Republike Slovenije za medicinsko etiko", rubrika "Seje Komisije").

Appendix B

Publications Related to the Thesis