Aus dem Institut für Physiologie
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DISSERTATION

„Anthropometrische Einflussfaktoren auf die vaskuläre und kardiale Anpassung des Menschen an Hypergravitation in der Kurzarmzentrifuge“.

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Introduction

Manned exploration of space leads to encounters with alterations in gravitational conditions, which triggers a cascade of cardiovascular adaptations. The sum of these adaptations is known as “cardiovascular deconditioning” (CD), which can lead to orthostatic intolerance (OI) upon return to planetary gravitational environments and entails an overall reduction in cardiac contractility force, and downregulation of systemic vascular resistance. The development of a counter-measure system (CMS), via the use of artifical gravity (AG) is essential in order to counter CD. This project thus investigated anthropometric factors, microvascular activity, and gender specific cardiovascular reactions during an AG-CMS protocol using a short arm human centrifuge (SAHC).

Methods

28 women and men were recruited for this study. Prior to AG exposure, an anthropometric analysis was undertaken using air displacement plethysmography (ADP), which measured body mass (BM), volume (BV), surface area (BSA), fat free mass (FFM) and fat mass (FM). During AG exposure, non-invasive cardiovascular parameters such as heart rate (HR), diastolic blood pressure (DBP) and systemic vascular resistance (SVR) were recorded. Additionally, micro-vascular activity was observed using laser doppler and near-infrared spectroscopy (NIRS). The AG protocol consisted of a G-force interval training (GIT) protocol. Anthropometric analysis between finisher/non-finisher subjects was undertaken, as well as microvascular activity for all finisher subjects, and a comparison between female and male hemodynamic patters during AG exposition were performed.

Results

Upon completion of the study, anthropometric factors such as BM, BV, and BSA were correlated with a higher tolerance to increased gravitational vectors. Concerning microvascular activity, it could be shown that microvascular resistance can withstand a +2 G gravitational vector, and that there exist a biphasic “fast” and “slow” microvascular filling compartments during AG. Lastly, gender specific cardiovascular response patterns were observed, in which cardiovascular factors in women (HR, DBP, and SVR) increased in a uniform fashion during AG, whereas men showed higher variations in these factors.

Discussion

In conclusion, greater body dimensions (BM, BV, and BSA), contribute to OI resiliency during AG. Also, the data indicates that lower body resistance vessels withstand the unphysiologically high transmural pressure induced by AG. The fast component of reflects microvascular blood pooling while the slow component may be due to hemoconcentration or capillary recruitment. Lastly, this GIT protocol stimulated the cardiovascular systems involved in orthostatic integrity amongst women, whereas in men, these findings were not replicated. This GIT protocol may be more useful in women as an OI CMS, whereas men may require higher +Gz gradients.

Abstract, Deutsch

Einführung

Die bemannte Erkundung des Weltraums führt auf Grund veränderter Gravitationsbedingungen zu adaptiven Veränderungen im Herz-Kreislauf-System. Die Summe dieser Anpassungen wird als "kardiovaskuläre Dekonditionierung" (CD) bezeichnet, die bei Rückkehr in die planetarische Gravitation zu orthostatischer Intoleranz (OI) führen kann. Diese geht mit einer Verringerung der kardialen Kontraktionskraft sowie einer Herunterregulierung des systemischen Gefäßwiderstandes einher. Die Entwicklung eines wirksamen Gegenmaßnahmensystems (CMS) unter Einsatz der künstlichen Schwerkraft (AG) ist unerlässlich, um der CD entgegenzuwirken. Daher wurden in diesem Projekt im Rahmen eines AG-CMS-Protokolls anthropometrische Faktoren, Veränderungen der mikrovaskulären Aktivität und geschlechtsspezifische kardiovaskuläre Reaktionen unter Verwendung einer Kurzarm-Humanzentrifuge (SAHC) untersucht.

Methodik

Für diese Studie wurden 28 Frauen und Männer (16F/12M) rekrutiert. Vor der AG-Exposition wurde eine anthropometrische Analyse mittels Luftverdrängungsplethysmographie (ADP) durchgeführt und die Körpermasse (BM), Volumen (BV), Oberfläche (BSA), fettfreie Masse (FFM) und Fettmasse (FM) gemessen. Während der AG-Exposition wurden nicht-invasive kardiovaskuläre Parameter wie Herzfrequenz (HR), diastolischer Blutdruck (DBP) und systemischer Gefäßwiderstand (SVR) erfasst. Zusätzlich wurde die mikrovaskuläre Aktivität mit Hilfe von Laserdoppler und Nahinfrarotspektroskopie (NIRS) ermittelt. Das AG-Protokoll bestand aus einem G-force Intervalltraining (GIT). Anthropometrische Analysen zwischen Probanden, die das Training absolvieren konnten (Finisher) und Nicht-Finisher wurden durchgeführt, ebenso wie die Bestimmung der mikrovaskulären Aktivität für alle Finisher sowie ein Vergleich zwischen weiblichen und männlichen hämodynamischen Mustern während der AG-Exposition wurde durchgeführt.

Ergebnisse

Nach Abschluss der Studie korrelierten die anthropometrischen Faktoren wie BM, BV und BSA mit einer höheren Gravitationstoleranz. Hinsichtlich der mikrovaskulären Aktivität konnte gezeigt werden, dass der mikrovaskuläre Widerstand einem Gravitationsvektor von +2 G standhalten kann. Während der AG zeigte sich eine zweiphasige "schnelle" und "langsame" mikrovaskuläre Füllkammer. Zusätzlich unterschieden sich die kardiovaskulären Reaktionsmuster in Bezug auf das Geschlecht. Die kardiovaskulären Faktoren bei Frauen (HR, DBP und SVR) nahmen während der AG einheitlich zu, während Männer höhere Variationen dieser Faktoren aufwiesen.

Diskussion

Zusammenfassend, eine insgesamt größere Körpermaße (BM, BV und BSA) und nicht Körperabteilungen (FFM und FM) zur OI-Resilienz während der AG beitragen. Außerdem deuten die Daten darauf hin, dass Gefäße mit niedrigem Körperwiderstand dem AG-induzierten unphysiologisch hohen transmuralen Druck standhalten. Die schnelle Füllkomponente reflektiert die mikrovaskuläre Blutansammlung, wohingegen die langsame Füllung auf eine Hämokonzentration oder Kapillarrekrutierung zurückzuführen sein könnte. Durch dieses GIT-Protokoll konnte gezeigt werden, dass des Kardiovaskuläresystems bei Frauen stimuliert wurden. Bei Männern konnten

diese Ergebnisse hingegen nicht reproduziert werden. Daher könnte dieses GIT-Protokoll bei Frauen als Gegenmaßnahme bei OI nützlich sein, während Männer möglicherweise höhere +Gz-Gradienten benötigen.

1. Introduction

Human exploration of the cosmos inherently leads to short or long-term micro-gravity (micro-g) exposure, which induces changes in human physiology. This sum of physiological adaptations that occur during exposure to micro-g is known as cardiovascular deconditioning [1]. Changes that occur comprise of cephalic fluid shifts, which leads to increases in cardiac output and myocardial atrophy [2] with resulting decreases in blood volume [3]. Further changes that have been recorded are reductions in cardiac diastolic function [4], heart rate [5], and drastic reductions in systemic vascular resistance [2]. All of these contribute to 50% reductions in baroreflex function [6,7]. As the gravitational force increases (+G), such as during escape velocity from and re-entry into planetary atmospheres, a cranial to caudal shunting of blood volume occurs, in which perfusion to the vital organ systems becomes compromised [8], thereby leading to orthostatic intolerance (OI) [9]. Orthostatic stability during +G exposure relies upon an efficient autonomic cardiovascular response system to maintain mean arterial pressure (MAP, mmHg), in order to maintain vital organ perfusion [10]. The counter-regulatory physiological response which maintains blood pressure is mediated by the baroreceptor reflex via heart rate increase due to withdrawal of parasympathetic and increase in sympathetic activity and adrenergic α-receptor mediated sympathetic constriction of resistance vessels and of venous capacitance vessels. Despite these effective physiological countermeasures, orthostatic cardiovascular dysfunction occurs in astronauts after prolonged exposure to microgravity [18].

Additional, evidence suggests that women astronauts undergo profound endothelial dysregulation during gravitational unloading [13], which may contribute to a higher incidence of OI in women astronauts upon returning to Earth [14,15]. Furthermore, Fong et al. determined that females are more prone to orthostatic instability during exposure to +G and that in anthropometric factors could be the cause for this trend [16]. An OI event could have drastic consequences for a manned crew and the mission as a whole, such as the inability to perform high-cognition tasks or ambulate out of a ship upon re-entry to a planetary atmosphere.

With the advent of commercial space ventures, as well as the planning of longer-term low-earth orbit, lunar, and mars missions, a comprehensive understanding of the anatomical and physiological factors involved in maintaining orthostatic stability when exposed to an increase in +G is vital for the success of these missions [17]. A standardised countermeasure system which can effectively augment the endogenous cardiovascular resilience to OI will be needed for manned space crews in the 21st century. One such system that has shown great potential in Earth based analogues is the implementation of artificial gravity (AG) exposure via short arm human centrifuge (SAHC), in order to counter microgravity associated cardiovascular deconditioning [18].

Repetitive exposure to hyper-gravity, as in the case of fighter pilots, during simulated air combat maneuvers, or centrifuge exposition seems to stimulate adaptive responses which enhance the tolerance of the cardiovascular system during gravitational stress [19–22]. To date this adaptation is attributed to an increased baroreceptor reflex sensitivity[22]. Whether an increased vasoconstrictor capacity of lower body microvasculature may contribute to this increased tolerance to hyper-gravity has not been investigated. The only AG experiment to be flown in space revealed that astronauts undergoing intermittent +1Gz training per day did not experience OI upon returning to Earth [18]. Several Earth-based studies have provided evidence that AG training can improve tolerance against OI, and that benefits of short intermittent AG exposure surpasses that of continuous static AG exposure [23–28]. There is also evidence to suggest that short intermittent AG exposure improves tissue oxygenation [29] in addition to its cardiovascular benefits. It is important to note however that these studies primarily involved research in men, and focused on the maintenance of orthostatic stability upon re-introduction of 1G conditions, and not so much on AG training itself.

Regarding anthropometric factors, under standard terrestrial conditions (1G), the percentage of fat mass in relation to total body weight (FM%) and fat free mass in relation to total body weight (FFM%), have a significant influence upon hemodynamic functions [30,31]. Furthermore, a greater FM% can negatively affect autonomic nervous system functioning [32–34]. On the other hand, greater FFM% has been shown to augment the cardiovascular function [35,36]. Epperson et al, determined that the degree of muscle mass, calculated via circumference and skin fold measurements, is a protective factor against OI in young men undergoing +G centrifugation [37]. Heaps et al, found that the absolute amount of FFM, using skin fold thickness, and anaerobic power output, was positively correlated with the duration of orthostatic stability under high +G strain in female fighter pilots, regardless of menstrual cycle [38]. Webb et al, observed, however, that +G tolerance is inversely proportional to height, and that overall weight does play somewhat of a protective factor [39]. They, however, concluded that no single anthropometric factor is decisive, rather the combination of anthropometric factors, as well as an effective hemodynamic response system play a role in +G tolerance. Furthermore, FFM% decreases during micro-gravitational exposure, which is hypothesized to play a role in the orthostatic intolerance experienced by astronauts upon return to standard gravity conditions [40].

Secondly, despite the contribution of resistance vessels to the maintenance of blood pressure during gravitational challenges, detailed knowledge about altered micro-vascular responsiveness after cardiovascular deconditioning and possible limits of their ability to withstand the unphysiologically high transmural pressures during exposure to hyper-gravity is lacking. The second aim of this study, therefore, was to comprehensively characterise the acute microvascular responses to gravity and hyper-gravity induced by a short arm human centrifuge (SAHC) in healthy young human subjects. We combined different non-invasive techniques, namely laser-Doppler imaging combined with iontophoretic drug application to assess responses of normal and dilated resistance vessels, near-infrared spectroscopy (NIRS) to monitor blood pooling in the skeletal muscle microvasculature, and measurement of skin temperature to assess the effects of vasoconstriction and blood pooling on thermoregulation.

Research on AG is relatively new and evidence concerning potential standardised AG training protocols are lacking. Issues needing to be addressed involve the intensity and the duration of the +Gz level applied. Also of utmost importance is an AG protocol that would provide benefits to both men and women, as there is evidence to suggest that there are gender differences regarding cardiovascular functioning [41–43]. Much like the benefits high-intensity interval training provides for maintaining aerobic fitness [44,45], AG training should serve similarly to maintain orthostatic tolerance via short duration and intense training intervals. Therefore, this study was performed in order to implement a graded +Gz interval training protocol (GIT) via SAHC in order to establish whether the cardiovascular systems involved in maintaining orthostatic integrity could be effectively stimulated and to reveal any apparent gender differences regarding cardiovascular reactions.

In summary, this compilation of 3 major hypothesis sets out to address the integrative physiology between anthropometric factors, microvascular networks, and gender specific cardiovascular responses during a novel +Gz interval training program, with the goal of being able to add critical information needed in order to design an implement effective counter-measure systems in order to enable human health during space voyages.

2. Methods

In total, 28 healthy, Caucasian civilian subjects gave their informed consent to partake freely in this experiment, which took place at the German Aerospace Institute (DLR), at the European Space Agency (ESA) short arm human centrifuge (SAHC) test facility in Cologne, Germany. All subjects gave their informed consent to partake freely in this investigation. The subject pool consisted of 16 women and 12 men who were matched for age (28.4 ±5.3 years). No subject had any history of cardiovascular, metabolic, or neurological abnormalities nor were any subjects commercial or military pilots, professional or elite athletes. This study was performed according to the Declaration of Helsinki and was approved by the Ethics Committee of Aerztekammer Nordrhein (Düsseldorf, Germany) [46].

2.1 SAHC protocol

The SAHC (SAHC-TN-007-VE, QinetiQ Company, Antwerp, Belgium) used for this study has a +Gz range of +0.1 to +5.0, an acceleration rate of maximal +0.4Gz/s, a maximum radius of 2.82 m and a maximum RPM of 40. The ESA-SAHC has been extensively used for +Gz training and various studies in the field of gravitational physiology [47,48].

The +Gz profile consisted of an initial 15 minutes of baseline at +0Gz followed by two identical +1/2/1Gz graded acceleration/deceleration phases, which were separated by a +0Gz phase. Except for phase 1 (P1, 15 minutes) and P5 (5 minutes), each phase lasted 4 minutes. Acceleration/deceleration between each phase lasted 15 seconds. The test subjects assumed a supine position with the gravitational vector along the head-to-foot (z-axis) of the body. In the case that any subject exhibited signs of orthostatic intolerance (OI) during the centrifuge runs, such as perfuse sweating, confusion, dizziness, nausea, a narrowing of the visual field or if the flight physician observed any one of the hemodynamic decompensation criteria, such as new-onset ECG abnormalities, an abrupt drop in MAP of >20 mmHg or a critical narrowing of the pulse pressure, the +Gz level was reduced or the protocol was terminated completely. Additionally, each subject could press a "panic button" if they wished to abort the test. Direct verbal and visual contact was maintained between the flight physician and the test subject via camera and microphone.

2.2 Anthropometric analysis

Prior to SAHC testing, the first 20 subjects underwent a body composition analysis, via ADP (BOD POD®, Life Measurement Inc. Concord, CA). The following parameters were measured: body mass (BM, kg), body volume (BV, L), body surface (BSA, m^2), normalised fat free mass (FFM%) and normalised fat mass (FM %). During ADP measurement, it was ensured that each subject maintained proper posture (90° upright sitting position) as well as a resting respiratory effort whilst undergoing analysis. Male subjects wore only tight fitting underwear and a head cap and female subjects wore a two piece undergarment set, as well as a head cap during measurement.

2.3 Cardiovascular recording

Mean arterial blood pressure (MAP, mmHg) and pulse pressure waves were monitored continuously and non-invasively via finger plethysmography (Portapres®, Finapres Medical Systems BV monitoring, Amsterdam, The Netherlands). The collected blood pressure waves were analysed with Beat-scope® software. Systolic and diastolic blood pressure (SBP and DBP respectively) as well as stroke volume (SV, ml) were extracted from the MAP. Heart rate (HR, bpm) was recorded via a 3-lead ECG. Cardiac output (CO, L/min) and systemic vascular resistance (SVR, mmHg·min/l) were calculated via the Wesseling formula [49]. To adjust for gender differences in anthropometrics, CO, SV, and SVR were converted to cardiac index (CI L/min/m^2), stroke volume index (SVI ml/m^2) and systemic vascular resistance index (SVRI, mmHg·min/l/m^2) using by dividing CO, SV, and SVR with BSA

2.4 Near-Infrared Spectroscopy (NIRS)

Microvascular blood pooling was determined in the right grastrocnemic muscle by near-infrared spectroscopy (NIRO 200, Hamamatsu Instruments, Hamamatsu, Japan). To this end a pair of optodes (emitter and detector) was fixed on the lateral head of gastrocnemius muscle by a black rubber scaffold which ensured a constant distance of 4 cm between both optodes by means of a double sided adhesive disc. The differential path length factor was set at 4.5 resulting in an assumed path-length of 18 cm. Concentration changes of oxygenated (O2Hb) and deoxygenated haemoglobin (deoxyHb) as well as regional oxygen saturation (rSO_2) were continuously recorded at a sampling rate of 1 Hz. Changes of total haemoglobin concentration were calculated (O2Hb + deoxyHb) offline. A second pair of optodes was placed on the distal biceps muscle, close to the plane of orthostatic indifference, and served as control.

2.5 Laser Doppler Imaging and Iontophoresis

Pretibial skin perfusion approximately 25 cm proximal to the ankle was measured by laser Doppler imaging (Periscan PIM3, Perimed, Uppsala, Sweden). The observation area was shaved and defatted with alcoholic disinfectant. The scanner was mounted to the centrifuge platform to ensure optimal stability. Mean distance from the skin was 18.8 ± 0.2 cm. The scanned area was 10.3 ± 0.1 cm long and 1.9 ± 0.1 cm wide. Average duration of a complete scan was 12.7 ± 0.7 s resulting in a temporal resolution of 4.9 ± 0.3 scans per min. In the center of the area an iontophoresis chamber was fixed with a double sided adhesive disc and filled with 1% solution of freshly dissolved sodium nitroprusside (Nipruss, Schwarz Pharma, Monheim, Germany). An adhesive reference eletrode was placed about 20 cm distally to the chamber. Iontophoresis with 100 µA was started 20 min prior to centrifugation and continued throughout the duration of the protocol. For data analysis, three regions of interest (ROI) were defined. One within the iontophoresis chamber and one each at the distal and proximal ends of the scanned area. Since centrifugation induced a distal movement of the legs, the three ROIs were moved simultaneously such that the central ROI always covered the whole iontophoretic chamber area. Mean laser Doppler flux from each of the ROIs was averaged over the last 4 to 5 scans (about 1 min) during each experimental phase.

2.6 Data analysis and statistics

After completion of the study, subjects were either classified into either a finisher or non-non-finisher group. Finisher subjects completed the entire +G exposure plan without abort or +G reduction. Non-finisher subjects had their +G exposure aborted or reduced by the flight physician. Anthropometric data from these two groups was statistically compared upon study completion using a Mann-Whitney U testData is reported as median and range. To assess the relationship between anthropometrics and cardiovascular variables, a linear regression analysis was performed and a Pearson's correlation coefficient was calculated. The critical significance value was set at 0.05, and all statistical analysis was performed using Data Graph version 4β (Visual data tools Inc.) and SOFA statistical software version 1.4.3 (Paton-Simpson & Associates Ltd).

Data for NIRS and laser Doppler imaging were averaged over the last 30 seconds of each acceleration phase unless stated otherwise. Statistical analyses were performed with SigmaStat (Systat Software, Inc., San Jose, CA). Data are presented as mean ± standard error. Data was tested for normality prior to statistical compar isons and analysed by one-way repeated measures ANOVA followed by Holm-Sidak post-hoc test. In order to assess differences in temperature changes between the calf and the upper arm, two way repeated measures ANOVA was applied.

For gender specific cardiovascular analysis, last 60 seconds from each phase were averaged for all cardiovascular parameters and used for the statistical analysis. Assumption checks were made using a Mauchly's test of Sphericity followed by Greenhouse-Geiser corrections. Test for equality of variance between men and women, was performed using a Levene's test. Statistical comparison for cardiovascular reactions were performed via repeated measures ANOVA measures using phase comparisons from baseline as a within subject factor and gender as a between subject factor. Statistically significant differences were further analysed using t-tests with Holm-Sidak post hoc correction. This was done for the whole group as well as independently for women and men. All data is presented as mean and ± standard error of the mean (SEM). The level of statistical significance was defined at alpha=0.05. Cardiovascular parameter syncing and creation of averages was performed using "R" statistical environment version 3.2.5 (R Core Team 2017). Inferential statistics were performed using JASP Version 0.8.2 for Mac OS (JASP Team 2017). Graphics were created using Data Graph 4.2 software for Mac OS (Visual Data Tools, Inc. 2017).

3. Results

From the total pool of 28 test subjects recruited for the study, 23 subjects (11 females, 12 males) completed the +G exposure plan without interruption. Microvascular data was used for 12 of the participants from the first part of the study, while ADP analysis was performed for the first 19 participants. Gender specific cardiovascular data was utilised from all test subjects that completed the GIT from both parts of the study. From the 7 remaining test subjects (5 female and 1 male) 3 females, and 1 male subject had their respective +G exposure aborted by the flight physician for a decrease in MAP >20 mmHg during the first +G round. During the second run, 1 female exhibited a decrease in MAP of >20 mmHg and had her run terminated. One female reported feeling nausea, and therefore had her +G level reduced. This female subject went on to finish the protocol, however at a lower +G acceleration than the study protocol mandated.

3.1 Anthropometric and baseline hemodynamic analysis

Female subjects had significantly higher FM%, whereas all other parameters were significantly higher for male subjects. Male subjects exhibited significantly higher baseline SV and n.s higher CO, while females exhibited higher median MAP and TPR. The NALOC group (n=13) was composed of 62% males (n=8) and 38% females (n=5). The ALOC group (n=6) was made up of 84% female (n=5) and 16% male (n=1) test subjects. The NALOC test subjects exhibited significantly ($p<0.05$) higher BM, BV, and BSA than the ALOC group. All other anthropometric factors were higher in the NALOC group, except for FM%, in which the median value was higher in the ALOC subjects. Regarding baseline hemodynamic parameters, ALOC subjects exhibited a n.s higher baseline TPR and MAP than the NALOC group whereas NALOC subjects exhibited a n.s higher CO and SV at baseline. Median HR was slightly higher in the NALOC group. Significantly positive correlations ($p<0.05$) were found between BM, BSA, BV, height, and TGV with baseline CO and SV. No significant correlations were found between baseline MAP, HR, TPR, and anthropometric parameters. When grouped according to gender, female ALOC subjects had a significantly higher baseline TPR ($p=0.03$), and n.s higher MAP than NALOC female subjects. Baseline CO and SV were n.s higher for the NALOC females, while no differences were found con cerning heart rate. The one ALOC male demonstrated higher values in all hemodynamic parameters in comparison with his NALOC male counterparts.

3.2 Near-Infrared Spectroscopy (NIRS)

In the control area in the biceps, centrifugation induced a dose dependent increase in deoxygenated Hb (deoxy Hb) concentration mirrored by a similar decrease in the concentration of oxygenated Hb (O2Hb). Total Hb (tHb) concentration was only minimally affected while regional oxygen saturation (rSO_2) decreased dose dependently. After return to +0Gz all values returned to baseline. In the gastrocnemius, O2Hb only increased slightly at +2Gz. Deoxy Hb increased moderately at +1Gz by 7.4 ± 1.0 µM and more pronounced to 23.4 ± 1.7 µM at +2Gz. After return to +1Gz deoxy Hb decreased only slightly to 18.9 ± 1.4 µM but returned to baseline after 5 min of rest at +0Gz. Accordingly tHb increased by 7.5 ± 1.4 µM at +1Gz, further to 26.6 µM at +2Gz and decreased only moderately after return to +1Gz to 18.8 ± 1.8 µM. Gastrocnemius rSO_2 decreased only minimally at +1Gz but markedly at +2Gz and remained low after return to +1Gz. After onset of +1Gz, a rapid accumulation of tHb in muscle tissue within the first 30 s was observed. This was followed by a slow incremental increase which did not reach steady state until the end of the 4 min centrifugation phase. After acceleration to +2Gz, a steady but more pronounced increase of tHb can be observed, which again did not reach steady state within the 4 min time interval. Reduction of acceleration from +2Gz to +1 Gz induces a quick drop in muscle tHb, which plateaus at more than twice the value of the initial +1Gz phase. After return to +0Gz, tHb quickly drops to near baseline values.

3.3 Skin Perfusion by Laser Doppler Imaging and Iontophoresis

Iontophoresis of sodium nitroprusside induced a 4.4 fold increase in skin perfusion from 38 ± 2 to 168 ± 11 perfusion units (PU), while perfusion in the untreated control areas remained essentially unchanged. Application of +1Gz increased perfusion in the dilated area further to 208 ± 14 PU. In contrast, control area perfusion decreased by 19% from 58 ± 5 to 47 ± 3 PU. Acceleration to +2Gz further increased perfusion in the dilated area to 226 ± 16 PU while no further change occurred in the control areas. Return to +1Gz reduced perfusion in the dilated area to the level of the initial +1Gz phase while again no change occurred in the control areas. After termination of +Gz acceleration all perfusion values essentially returned to the initial +0Gz levels.

3.4 Cardiovascular reactions amongst women and men

Statistical analysis amongst women showed that HR was significantly higher than baseline throughout all +Gz phases, with significantly higher HR levels during +2Gz phases than +1Gz phases. SVRI was also significantly higher than baseline in all +Gz phases, with no significance between +2Gz and +1Gz. SVI was significantly lower during all +Gz phases than baseline, and also significantly lower in +2Gz than +1Gz. DBP was significantly elevated throughout the first round, however during the second round significantly elevated only in P7. MAP was significantly increased over baseline in P3 and P4. SBP and CI showed no significant changes from baseline. Furthermore, no significant differences were found when comparing the 2 rounds of GIT.

3.5 Cardiovascular reactions amongst men

Final statistical analysis in men revealed significant HR elevations over baseline during P2, P3, and P7. DBP was significantly elevated over baseline during P3, P4, and throughout the second round, with MAP expressing significant increases in only P6 and P8. SVI was the only parameter that exhibited consistently significant decreases from baseline throughout both rounds (P2-P4 and P6-P8), with an additional increase during P5 compared to baseline. CI demonstrated significant decreases from baseline during P2, P4, and throughout the second round. SBP did not exhibit significant deviations from P1. Finally, SVRI, although elevated during +Gz, was not significantly different from baseline. No significant changes were seen for SBP. Lastly, no significant differences were found when comparing the 2 rounds of GIT.

3.6 Gender comparisons

Women exhibited non-significant HR increases over men throughout all phases of GIT (p=0.093). Women also tended to have a higher CI over men throughout the GIT. SVI trends were identical in both genders. Men tended to have higher MAP, DBP, SBP, and SVRI than women, however no significant differences were found. Conversely, MAP, DBP, and SVRI displayed a lesser degree of variability in women than men during all phases. For both genders, MAP, DBP, HR, and SVRI increased with higher +Gz levels, whereas SVI and CI displayed an inverse relationship with the +Gz level.

4. Discussion

The results of this study are that an overall lower body dimension, lower SV or CO, and a higher TPR at baseline, were predisposing factors associated with low +G tolerance (ALOC). The NALOC group exhibited higher overall body dimensions (BM, BV, BSA), CO, and SV prior to +G onset, than those subjects exhibiting lower +G tolerance (ALOC). The ALOC group exhibited lower overall body dimensions, and higher MAP and TPR at baseline in comparison to the NALOC's. The mechanisms of orthostatic stability in healthy young subjects are thus dependent upon anthropometric characteristics, cardiovascular morphology and autonomic control.

Concerning the investigations regarding microvascular responses during hypergravity using a SAHC, the following conclusions could be drawn; constriction of resistance vessels in response to gravity can withstand the high transmural pressures even at +2Gz for 4 minutes and thus contributes to the maintenance of mean arterial pressure during exposure to moderate hypergravity and that microvascular blood pooling in the lower body can be quantified with NIRS measurement of total tissue haemoglobin concentration and its dynamics suggest that in addition to quick venular filling, an additional slow component contributes to blood pooling in the lower body.

Lastly, the tolerability and effectiveness of a novel GIT protocol as a potential countermeasure against micro-g associated cardiovascular deconditioning was determined to have met its primary goals, which were to induce significant elevations in the cardiovascular parameters responsible for maintaining orthostatic integrity (SVRI, DBP, and HR) amongst men and women. It could be determined that women showed significant increases in DBP, HR, and SVRI throughout the GIT, whereas men exhibited significant increases in DBP and HR mainly in the +2Gz phases, with no significant changes in SVRI. The lack of significant SVRI findings could be attributed to a high inter-group variability amongst the men involved in this study. Thus, these results indicate that this particular GIT protocol may be more in beneficial for women as a countermeasure against micro-g induced cardiovascular deconditioning.

Furthermore, the findings from this study show that a majority of the test subjects (82% in total) endured the GIT protocol without experiencing OI, (100% of men, 70% of women), while 30% of the women involved experienced an OI event during the GIT. This supports evidence that women tend to be more prone to OI during gravitational stress.

4. Discussion

4.1 Baseline anthropometrics, hemodynamics, and +G tolerance

The original hypothesis proposed, that FFM% would be a protective anthropometric factor associated with high +G tolerance, was not supported by the analysis of the data. FFM% was hypothesized to be a protective factor, as it is reflective of metabolically active tissue mass, which plays a role in augmenting overall autonomic function [36]. According to previous studies [37,38], FFM% has been shown to have a correlation with +G tolerance, in fighter pilots previously exposed to +G. The anthropometric factors significantly associated with higher +G tolerance, were in fact total BM BV, and BSA. The only study that reported similar results observed BM in astronauts. This study concluded that a reduction in overall BM is associated with decreased orthostatic stability upon re-exposure to Earth's gravity, after spending an extended time period in a micro-gravitational environment [50]. The results of that study, as well as this investigation, provide further evidence that an overall higher BM, along with BV and BSA, are decisive factors in maintaining orthostatic stability during +G. The key role in maintaining orthostatic tolerance therefore, is the degree of SV, which is reflective of total venous return [51]. An augmented cardiac pre-load would lead to greater SV, despite +G induced lower body venous pooling [52]. The degree of SV, contributes to an overall greater CO, thus enabling adequate central organ perfusion/oxygenation [51,53].

The findings in this study did in fact show, that especially in female candidates, an increased baseline TPR was associated with an occurrence of an orthostatic event. Furthermore, TPR in males has been shown to be more pronounced than in females [54]. Furthermore, this study demonstrated that hemodynamic trends prior to +G onset may yield information regarding orthostatic stability during +G, however, more studies of this nature would have to take place in order to see if these trends can be reproduced.

4.2 Anthropometric and hemodynamic gender differences

Gender differences were apparent in the study, as males completed the +G testing without abort or interruption, whereas only 50% of female test subjects exhibited high +G tolerance. Male subjects had an advantage over female subjects during +G exposure, which is primarily due to having an overall greater body dimension. Males, compared to females, also have a larger cardiac structure and possess larger great blood vessel diameters, which improves overall hemodynamic functioning during orthostatic challenges [54]. The results of this study support the findings of previous studies examining gender differences during +G exposure, which have demonstrated that females are more prone to orthostatic instability than their male counter-parts. The cause for this trend is hypothesized to be due to hormonal influences on endothelial function, alterations in catecholamine activity, hemodynamic function, and body composition [16,38,55]. Furthermore, orthostatic instability in female astronauts has been attributed to a lower center of gravity in women, which contributes to a greater risk of orthostatic instability [56]. Based on the results of this study, female +G intolerance is also due to having an overall smaller body composition, thereby affecting hemodynamic functioning. Once gender specific critical anthropometric characteristics have been determined, physical fitness and nutritional programs can be implemented in order to maintain this threshold.

When separating the groups for gender, ALOC females had significantly higher TPR at baseline than NALOC subjects. Exhibiting a higher vascular resistance would invariably lead to decreases in venous return, thereby decreasing the degree of SV and CO [57]. Once an increase in gravitational force occurs, the volume in the central circuit is sub-optimal to withstand an increase in gravitational challenge. Inevitably, this increases the likelihood of an orthostatic event. An increased TPR at baseline could be due to an exaggerated pre-mature activation of alpha adrenergic receptors [58]. Lastly, TPR was not significantly correlated with any baseline anthropometric parameter in this study, meaning that vasomotor activity is independent of anthropometric characteristics.

4.3 Microvascular reactions and adaptations

Numerous previous studies have elucidated the hemodynamic responses to gravity and, fewer, to hypergravity. It is well understood that gravitational forces along the body axis lead to venous blood pooling in the lower body which reduces cardiac preload and, thus stroke volume. Baroreceptor reflex activity counteracts the resulting decrease in arterial pressure by increasing heart rate and total peripheral resistance to maintain arterial pressure. These responses were also present in the subjects of our study, however much more pronounced during +2Gz as compared to +1Gz.

The focus of this study was on microvascular responses, namely in the lower body, in which blood vessels are exposed to dramatically increased transmural pressures as a result of the effect of gravity on the intravascular blood volume. It has been suggested that resistance vessels would respond by vasoconstriction resulting from the combined effects of two mechanisms: local myogenic response to increased transmural pressure [59] and a systemic α-adrenergic receptor mediated constriction due to baroreceptor reflex induced sympathetic activation [60]. Such constriction should result in parallel decrease of perfusion because the arterial to venous pressure gradient is only minimally affected since exposure to gravity increases arterial and venous pressures similarly. Accordingly, during +1Gz, we detected a decrease of pretibial skin perfusion by 19%, indicating substantial resistance vessel constriction. This is considerably less than the almost 40% decrease observed by Watenpaugh et al. under similar conditions [61].

Increasing acceleration to +2Gz induced no further change in skin perfusion, indicating that resistance vessels were able to maintain diameters against the increased transmural pressure but did not constrict further despite the fact that both constrictive stimuli, that is transmural pressure and sympathetic activation, as evident from the increase in heart rate, increased markedly. However, at +2Gz, with a mean arterial pressure of 100 mmH at heart level, local arterial pressure at pretibial skin well exceeded 200 mmHg [61] and was thus above the upper limit of myogenic auto-regulation until which renal or cerebral perfusion are maintained constant [62,63]. Obviously, increased sympathetic vasoconstriction was able to maintain flow resistance beyond this auto-regulatory pressure range and it would be interesting to examine until which G-load resistance vessel smooth muscle can withstand the increasing transmural pressure.

In order to better appreciate the ability of resistance vessels to withstand increasing transmural pressure, skin perfusion was also measured in areas treated with iontophoretic application of the potent vasodilator sodium nitroprusside. As expected, the gravitational induced rise of transmural pressure in resistance vessels increased skin perfusion by 24% at +1Gz and by an additional 9% at +2Gz versus +1Gz indicating passive distension of vessels. The striking difference between these relative increases during identical increments of transmural pressure may be explained by the nonlinear pressure-diameter relationship of dilated resistance vessels.

NIRS measurement of haemoglobin concentration has been used previously during tilt table testing to determine the pressure-volume relationship of calf muscle microvasculature [64]. For a reference control area, a second probe was placed on the distal aspect of the biceps muscle that is roughly at the hydrostatic indifference level and should thus not be exposed to local pressure changes during acceleration. Accordingly, total haemoglobin concentration as a marker of total intravascular blood volume in the microcirculation was not markedly affected by exposure to +1 or +2Gz. However, deoxy Hb concentration increased with rising acceleration while O2Hb concentration and rSO_2 decreased in a similar manner. This is best explained by increased oxygen extraction due to a reduction of perfusion via sympathetic vasoconstriction of skeletal muscle resistance vessels. In contrast to the biceps muscle, the gastrocnemius muscle total Hb concentration increased markedly by 7.5 and 26.6 µmol/L of muscle tissue at, respectively during +1 and +2Gz, thereby reflecting microvascular blood pooling. Obviously, the bulk of blood pooling occurs in large veins and the microvascular increase of haemoglobin concentration by 26.6 µmol/L at +2Gz equalises to only 11.4 ml of blood pooling within a volume of 1 L muscle tissue when assuming a mean blood haemoglobin concentration of 15 g/100mL [64]. The differential changes in deoxy Hb, O_2Hb and rSO_2 are consistent with the notion that microvascular blood pooling occurs mostly in venules.

A surprising observation was the marked difference between total Hb concentration at +1Gz before and after exposure to +2Gz. We assumed that following increases in Gz, a steady state would not be reached within the 4 minutes observation period. Therefore, the dynamics of total Hb concentration changes was analysed at 30 s intervals using normalised Hb values. Indeed total Hb concentrations were still rising at the ends of the +1Gz and +2Gz exposure periods of 4 minutes but had reached a plateau about 2 minutes after deceleration from +2Gz to +1Gz. Since the shape of these curves suggests the presence of a fast and a slow filling compartment, a biexponential fit was applied to the data. This is analogous to the well established technique of venous occlusion plethysmography where the fast component represents limb volume increase by venous filling and the slow component volume increase by transvascular fluid filtration. Accordingly, the fast component of haemoglobin concentration increase in muscle tissue is easily explained by venous filling. The origin of the slow component is less clear. It may represent either increasing blood volume in muscle or increasing haemoglobin concentration in muscle microvascular blood volume. At rest only 32 to 43% of capillaries in skeletal muscle are perfused [65]. While capillaries are partly protected from gravity induced pressure rise by concomitant precapillary vasoconstriction [66], increased pressure in post-capillary venules directly affects the venular end of capillaries. Thus, progressive retrograde blood filling of previously non-perfused capillaries from the venular end might explain the prolonged slow increase of haemoglobin concentration in calf muscle. This would support the alternative explanation of a slow increase in muscle microvascular haemoglobin concentration resulting from increased capillary fluid filtration which would tend to increase blood haemoglobin concentration at the venular end of capillaries. At low venular blood flow velocity due to the combined effects of precapillary vasoconstriction and venular pressure induced distension, blood in the venules would be slowly replaced by capillary outflow with higher haemoglobin concentration. Indeed, hemo-concentration of venous blood from the foot has been reported as a response to gravitational pressure increase [67] and even the time course of hematocrit increasing from 41 to 49% during the first 20 minutes and further to 51% during another 20 minutes is consistent with the slow component's time to 90% of maximum of 25.3 minutes in our study. Of course, a combination of both effects is also possible. Simultaneous measurement of limb volume by plethysmography and muscle haemoglobin concentration by NIRS might allow for further insights into these events. This may also allow to investigate the relations of the envisaged mechanism, to previous measurements of fluid filtration in response to gravitational pressure increase, which attributed the slow component of limb volume increase exclusively to increased interstitial fluid volume [68].

The +Gz dependent decrease of upper arm skin temperature is consistent with +Gz dependent vasoconstriction of skin resistance vessels [69], which was also evident for biceps muscle resistance vessels from the NIRS data. Interestingly, on pretibial skin temperature decreased only minimally despite the observed decrease of skin perfusion. Here, pooling of blood in skin venules might increase the heat capacitance of skin and thus counteract the effect of vasoconstriction on skin cooling.

4.4 Cardiovascular reactions during GIT: female specific reactions

Cardiovascular responses amongst women during SAHC exposure has not been thoroughly examined, however there is scant evidence to suggest that orthostatic tolerance in women is improved after undergoing AG exposure via SAHC [26,27]. In this study, women that completed the GIT exhibited significant upsurges in the cardiovascular mechanisms responsible for maintaining orthostatic integrity. These significant increases in HR and SVRI could be attributed to greater baroreflex sensitivity in women [70], and less plasma volume compared to men, which would lead to a heightened baroreflex sensitivity during SVI decreases. Another contributing factor could be strongly linked to plasma epinephrine, renin, and vasopressin levels, which have been recorded to be more elevated in women than men during gravitational challenges [71]. Although not measured in this study, increased plasma concentrations of the aforementioned neuroendocrine components could have led to the augmented SVRI, as well as HR activity. It is important to note here again, that significant changes in SVRI amongst women may also be due low inter-individual variability exhibited by women as a group. The major takeaway thus is that cardiovascular reactions in women during this particular GIT were uniform, which could prove beneficial when implementing a GIT protocol for multiple crew-members.

4.5 Cardiovascular reactions during GIT: male specific reactions

During GIT, men did not exhibit the same significant changes from baseline as women did. During the GIT, DBP, and not SVRI, was significantly increased over baseline during the +2Gz phases along with HR. In men, a minimum of +2Gz seems to be needed in order to induce significant HR increases, a response which has also been recorded by Goswami et al., during SAHC training [72], Ueda et al., [73] and Polese et al., [74]. A possible explanation to why men require a stronger gravitational stimulus in order to trigger increases in HR may be due to higher resilience to venous pooling and central volume loss due to a greater amount of available plasma volume. Therefore, during +1Gz phases, while the decrease in functional blood volume is enough to trigger the beta sympathetic response in women, men may require a higher degree of SVI loss until HR increases become apparent. Surprisingly, SVRI in men showed no significant changes from baseline. However, as previously mentioned, this is more likely due to high inter-individual variability observed amongst men rather than low absolute values recorded. Possible explanations for these findings have been proposed by some study groups, which include polymorphic genetic differences in vascular resistance regulation [75], or elevated respiratory rate during GIT inducing a higher amount of venous return to the central circuit [76]. Although these factors may offer reasons for varying intensities of SVRI regulation, they do not offer insight to the difference in variability observed between genders, as these studies included exclusively men. Amongst men, this protocol of mild/moderate GIT was not enough to induce significant changes in SVRI, however HR was significantly stimulated at +2Gz levels. Also, DBP was significantly elevated in all but one +Gz phase, meaning that this GIT is useful in augmenting diastolic cardiac function in men. In order to train both active vasomotor and cardiac mechanisms in men, a higher +Gz-level gradient may be needed.

4.6 Cardiovascular activity during GIT: gender differences

Although no significant gender differences in cardiovascular reactions were recorded, women tended to respond with heightened HR responses over men subjects during the GIT. Other working groups have recorded similar HR responses in women over men during gravitational stress, however these studies involved lower body negative pressure (LBNP) [77,78], and thus may not be directly comparable to SAHC studies as the gravitational stimulus differs [61]. The heightened HR response in women can be attributed to gender differences in the autonomic circuitry, myocardial structure, as well as hormonal status. Compared with men, women show increased parasympathetic withdrawal to the cardiac circuit during orthostatic stress thereby leading to an increase in HR [41,54]. Also, the hormone estradiol may augment epinephrine sensitivity during orthostatic stress and thus be a contributing factor[14,79]. Further reasons for an increased HR in women could be differences in trans-mitral filling velocity and faster myocardial velocities [80]. These factors would equate to a higher cardiac filling rate and faster myocardial contraction than men, thus offering an account for differences in HR.

Regarding SVRI, no significant difference was recorded between genders, although men exhibited higher overall SVRI than women. This was also reflected via higher MAP, DBP, and SBP in recorded amongst men. While some groups have also observed significantly increased vasoconstrictor response in men over women during orthostatic challenges [78,81,82], there is more evidence to suggest that peripheral vasoconstrictor activity does not differ between genders during orthostatic stress [77,83–88]. Certain authors have shown that during beta and muscarinic blockade, there is a predominant vascular regulation in men compared to dominant parasympathetic influence on heart rate regulation in women [41,54]. Those studies used different modalities to provoke orthostatic stress such as LBNP, head-up tilt (HUT), as well as pharmacological means. Previous studies that have recorded increases in vasomotor activity in men over women [81] have attributed these differences to an overall higher magnitude of sympathetic nerve activity to the periphery in men [42,89]. Since micro-g induced cardiovascular conditioning in women leads to a high degree of vascular dysregulation upon reintroduction to standard gravity [15], potential countermeasures should ensure a specific protocol which stimulates SVRI and DBP responses, that is of short duration and is tolerable for a majority of crew-members. This SAHC GIT would seem to fulfil these desired criteria mainly for women, with higher +Gz gradients needed for men.

5. Limitations, future perspectives and conclusions

This study is the first of its kind to implement a novel GIT as a potential counter-measure for use in manned-space flight. Three distinct investigations took place which examined baseline factors that contribute to orthostatic stability during GIT, microvascular adaptations during GIT, and overall cardiovascular reactions during GIT. This study was also unique in that a relatively equal number of men and women were examined. The significant correlations between anthropometric factors (BM, BV, and BSA) and CO indicate that form (body composition) does influence function (autonomic hemodynamic function), and thus orthostatic stability during +G.

The limitations regarding microvascular activity were that a small sample size was investigated, although in comparison with similar studies, relatively good. Secondly, only a sub-set of test subjects were investigated for the microvascular part. This was mainly due to the cumbersome equipment involved and delays in shipping and reception of the equipment. Given the small sample size involved, no gender specific analysis could be made. In order to further strengthen the results concerning micro-vascular activity, more studies of this nature would have to be performed utilising the exact same GIT protocol. The combination of different techniques, namely laser-Doppler imaging combined with iontophoretic drug application, NIRS and skin temperature measurements, made possible a comprehensive assessment of microvascular adaptations to gravity and hypergravity induced on the SAHC. Lower body resistance vessel smooth muscle tone increased during exposure to gravity and was found to withstand the high transmural pressures during moderate hyper-gravitation of +2Gz. The skin perfusion increase of dilated resistance vessels in response to gravitationally increased transmural pressure reflects the expected diameter increase according to literature data on pressure-diameter relationship of resistance vessels. Microvascular blood pooling in calf muscle was found to be biphasic with a fast component due to venular filling and a slow component possibly reflecting either capillary recruitment or enhanced fluid filtration and pooling of blood with higher haemoglobin concentration. In the skin of the lower limb the cooling effect of skin vessel constriction is markedly alleviated by venular blood pooling. We propose that by assessing putative alterations of these acute microvascular adaptations to gravity might allow for new insights into the mechanisms of orthostatic dysfunction or thermoregulatory discomfort of astronauts undertaking prolonged spaceflights.

Finally, while this study is the first that directly tested gender differences during GIT via SAHC, the findings should be interpreted with caution. Firstly, the small sample size, makes drawing larger conclusions for the average population difficult. Also, the gender differences observed in this study have to be compared with data from other hypergravity analogues, as there is a lack of data concerning gender specific differences during SAHC exposure. While these methods produce an adequate orthostatic stimulus, there is a contrast to the gravitational vector deployed as well as cardiovascular reactions observed in subjects [61,90]. Moreover, this study did not set out to induce maximal +Gz limits in the human test subjects nor was the +Gz gradient tailored for each individual test subject. Had maximal +Gz profiles been deployed, or individual +Gz profiles been used, the results may have been different. Lastly, serum catecholamine, testosterone, estrogen, progesterone, and hemoglobin levels were not measured in this study. Thus, their role in the gender specific cardiovascular findings of this study can only be speculated. Additionally, no restrictions were placed on women with regard to menstrual cycle phases nor oral-contraceptive use upon partaking in this study. Certain authors have suggested these factors may have an effect upon autonomic functioning in women during gravitational stress 14. Women exhibited non-significant heightened HR response over men during all phases of +Gz. HR and SVRI were consistently increased over baseline in women during the entire protocol, whereas in men, only HR was increased during +2Gz. DBP in men was significantly increased and CI significantly decreased for nearly all of the GIT. SVI was decreased throughout the GIT, with no gender differences observed. These findings add to the evidence that gender specific reactions are apparent during +Gz, and that women are more prone to an OI event than men. Furthermore, this study showcased a potential GIT protocol for use onboard future manned space programs, if an SAHC is provided. To expand upon these findings, the +Gz level could be adjusted according to gender, with a potential +2/3/2 Gz profile for males. Lastly, this GIT protocol could be integrated into bed-rest, HDT bed-rest, isolation, and real microgravity environments, in order to determine its effectiveness as well as feasibility as a countermeasure against microgravity-induced cardiovascular deconditioning.

This study addresses a few critical point according to the NASA human research roadmap [91]. The study was able to observe a small sample of men & women, matched by number, age and level of fitness, who underwent the same standardised protocol of body composition assessment and centrifuge profile on a SAHC. Each test subject that volunteered for this study however, expressed an enthusiasm for space research, and if given the chance, would enter into a commercial or government manned space mission training program. Therefore the results of this study are relevant for the target population being studied, i.e current and future space explorers.

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7. Eidesstattliche Versicherung

„Ich, Michael Nordine, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema: Anthropometrische Einflussfaktoren auf die vaskuläre und kardiale Anpassung des Menschen an Hypergravitation in der Kurzarmzentrifuge, selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe.
Alle Stellen, die wörtlich oder dem Sinne nach auf Publikationen oder Vorträgen anderer Autoren beruhen, sind als solche in korrekter Zitierung kenntlich gemacht. Die Abschnitte zu Methodik (insbesondere praktische Arbeiten, Laborbestimmungen, statistische Aufarbeitung) und Resultaten (insbesondere Abbildungen, Graphiken und Tabellen) werden von mir verantwortet.
Meine Anteile an etwaigen Publikationen zu dieser Dissertation entsprechen denen, die in der untenstehenden gemeinsamen Erklärung mit dem/der Betreuer/in, angegeben sind. Für sämtliche im Rahmen der Dissertation entstandenen Publikationen wurden die Richtlinien des ICMJE (International Committee of Medical Journal Editors; www.icmje.og) zur Autorenschaft eingehalten. Ich erkläre ferner, dass mir die Satzung der Charité – Universitätsmedizin Berlin zur Sicherung Guter Wissenschaftlicher Praxis bekannt ist und ich mich zur Einhaltung dieser Satzung verpflichte.
Die Bedeutung dieser eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unwahren eidesstattlichen Versicherung (§§156, 161 des Strafgesetzbuches) sind mir bekannt und bewusst.“

____
26.11.2019 Unterschrift

8. Anteilserklärung

Michael Nordine hatte folgenden Anteil an den folgenden Publikationen:

Publikation 1: Form influences function: Anthropometry and orthostatic stability during sustained acceleration in a short arm Human centrifuge. Michael Nordine, Martina Anna Maggioni, Alexander Stahn, Stefan Mendt, Katharina Brauns, Hanns-Christian Gunga, Helmut Habazettl, Andrea Nitsche, Oliver Opatz. May 2015 Acta Astronautica 115C:138–146 DOI:10.1016/j.actaastro.2015.05.025
Beitrag im Einzelnen:
Primäre Hypothesenformulierung, Zusammenstellung des gesamten Manuskripts, statistische Analyse, Grafikerstellung, Proofreading, Bearbeitung von Reviewer-Kommentaren sowie endgültige Einreichung.

Publikation 2: Microvascular responses to (hyper-)gravitational stress by short-arm human centrifuge: arteriolar vasoconstriction and venous pooling. Helmut Habazettl, Alexander Stahn, Andrea Nitsche, Michael Nordine, Axel Pries, Hanns-Christian Gunga, Oliver Opatz.

August 2015 European Journal of Applied Physiology 116(1) DOI: 10.1007/s00421-015-3241-6

Beitrag im Einzelnen:

Recherche und Zusammensetzung des Abschnitts "Methods", insbesondere des Unterabschnitts "Short-Arm Human centrifuge" und "Hemodynamics". Statistische Analyse der "Hemodynamics" in den Ergebnissen sowie Zusammensetzung der Tabelle 1. Korrekturlesen sowie Unterstützung bei der Beantwortung von Kommentaren und Vorschlägen der Reviewers.

Publikation 3: Gender-Specific Cardiovascular Reactions to +Gz Interval Training on a Short Arm Human Centrifuge. Zeynep Masatli, Michael Nordine, Martina Anna Maggioni, Stefan Mendt, Ben Hilmer, Katharina Brauns, Anika Werner, Anton Schwarz, Helmut Habazettl, Hanns-Christian Gunga, Oliver Opatz. July 2018 Frontiers in Physiology 9 DOI: 10.3389/fphys.2018.01028

Beitrag im Einzelnen:

Mitwirkung bei der Formulierung der Hypothese, Zusammensetzung des Abschnitts "Methods" und "Results", Zusammensetzung der Grafiken, die geschlechtsspezifische kardiovaskuläre Reaktionen darstellen, Korrekturlesen sowie Unterstützung bei der Beantwortung von Kommentaren und Vorschlägen der Reviewers.

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Unterschrift, Datum und Stempel des betreuenden Hochschullehrers, Prof. Hanns-Christian Gunga

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Unterschrift des Doktoranden/der Doktorandin, Michael Nordine

9. Form influences function: Anthropometry and orthostatic stability during sustained acceleration in a short arm human centrifuge.

Source reference:

Michael Nordine, Martina Anna Maggioni, Alexander Stahn, Stefan Mendt, Katharina Brauns, Hanns-Christian Gunga, Helmut Habazettl, Andrea Nitsche, Oliver Opatz

Form influences function: Anthropometry and orthostatic stability during sustained acceleration in a short arm human centrifuge,

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10. Microvascular responses to hyper-gravitational stress by short-arm human centrifuge: arteriolar vasoconstriction and venous pooling.

Source reference:

Habazettl H, Stahn A, Nitsche A, Nordine M, Pries AR, Gunga HC, Opatz O.

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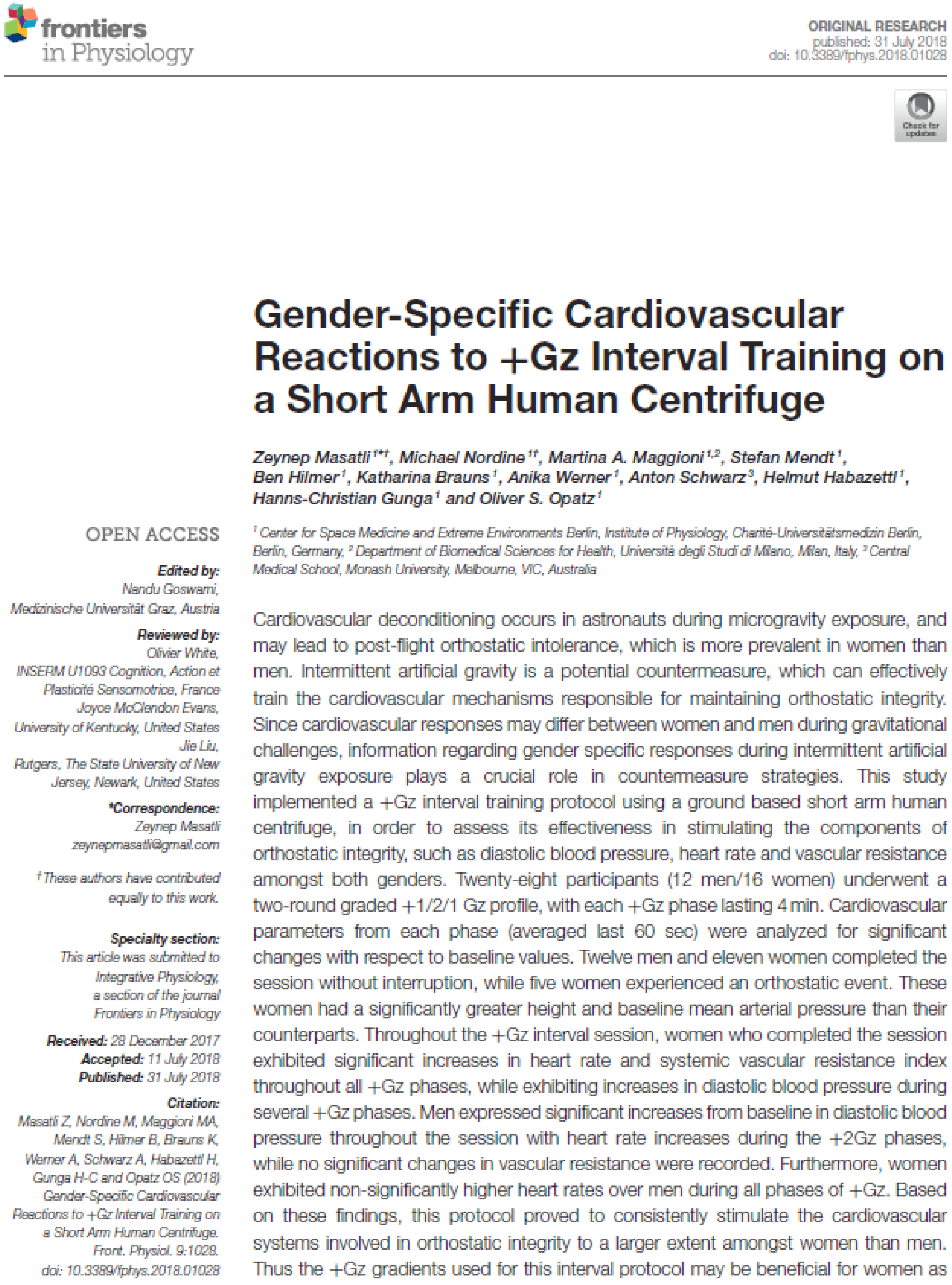
11. Gender specific cardiovascular reactions to +Gz Interval training on a short arm human centrifuge.



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ORIGINAL RESEARCH
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Gender-Specific Cardiovascular Reactions to +Gz Interval Training on a Short Arm Human Centrifuge

Zeynep Masatli[1*†], **Michael Nordine**[1†], **Martina A. Maggioni**[1,2], **Stefan Mendt**[1], **Ben Hilmer**[1], **Katharina Brauns**[1], **Anika Werner**[1], **Anton Schwarz**[3], **Helmut Habazettl**[1], **Hanns-Christian Gunga**[1] and **Oliver S. Opatz**[1]

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Cardiovascular deconditioning occurs in astronauts during microgravity exposure, and may lead to post-flight orthostatic intolerance, which is more prevalent in women than men. Intermittent artificial gravity is a potential countermeasure, which can effectively train the cardiovascular mechanisms responsible for maintaining orthostatic integrity. Since cardiovascular responses may differ between women and men during gravitational challenges, information regarding gender specific responses during intermittent artificial gravity exposure plays a crucial role in countermeasure strategies. This study implemented a +Gz interval training protocol using a ground based short arm human centrifuge, in order to assess its effectiveness in stimulating the components of orthostatic integrity, such as diastolic blood pressure, heart rate and vascular resistance amongst both genders. Twenty-eight participants (12 men/16 women) underwent a two-round graded +1/2/1 Gz profile, with each +Gz phase lasting 4 min. Cardiovascular parameters from each phase (averaged last 60 sec) were analyzed for significant changes with respect to baseline values. Twelve men and eleven women completed the session without interruption, while five women experienced an orthostatic event. These women had a significantly greater height and baseline mean arterial pressure than their counterparts. Throughout the +Gz interval session, women who completed the session exhibited significant increases in heart rate and systemic vascular resistance index throughout all +Gz phases, while exhibiting increases in diastolic blood pressure during several +Gz phases. Men expressed significant increases from baseline in diastolic blood pressure throughout the session with heart rate increases during the +2Gz phases, while no significant changes in vascular resistance were recorded. Furthermore, women exhibited non-significantly higher heart rates over men during all phases of +Gz. Based on these findings, this protocol proved to consistently stimulate the cardiovascular systems involved in orthostatic integrity to a larger extent amongst women than men. Thus the +Gz gradients used for this interval protocol may be beneficial for women as

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a countermeasure against microgravity induced cardiovascular deconditioning, whereas men may require higher +Gz gradients. Lastly, this study indicates that gender specific cardiovascular reactions are apparent during graded +Gz exposure while no significant differences regarding cardiovascular responses were found between women and men during intermittent artificial gravity training.

Keywords: artificial gravity, gender, short arm human centrifuge, cardiovascular deconditioning, artificial gravity training, countermeasure

INTRODUCTION

Human space exploration inherently leads to micro-gravity (micro-g) exposure, which induces changes in cardiovascular functioning. The sum of physiological adaptations that occur during exposure to micro-g is known as cardiovascular deconditioning (Komorowski et al., 2016). Changes that occur comprise of cephalic fluid shifts, which lead to increases in cardiac output and myocardial atrophy (Norsk et al., 2015) with resulting decreases in blood volume (Agnew et al., 2004). Further changes that have been recorded are reductions in cardiac diastolic function (Convertino and Cooke, 2005), heart rate (Verheyden et al., 2009), and drastic reductions in systemic vascular resistance (Norsk et al., 2015). All of these adaptations contribute to a 50% reduction in baroreflex functioning (Antonutto and di Prampero, 2003; Beckers et al., 2009), which can lead to orthostatic intolerance (OI) upon return to Earth or any other gravitational environment (Lee et al., 2015). This is characterized by the inability of the neurohumoral reflex, namely increases in heart rate and vasoconstriction, to maintain adequate mean arterial pressure. Additional evidence also suggests that women astronauts undergo profound endothelial dysregulation during gravitational unloading (Demiot et al., 2007), which may contribute to the higher incidence of OI in women astronauts upon returning to Earth (Waters et al., 2002; Wenner et al., 2013).

Research in effective cardiovascular deconditioning countermeasures for both genders is imperative and is as a goal of the Human Research Roadmap put forth by NASA, as well as the EU (Aubert et al., 2016; Vernikos et al., 2016). A countermeasure system that can potentially offset micro-g induced cardiovascular deconditioning is the implementation of artificial gravity (AG) exposure via short arm human centrifuge (SAHC). AG via SAHC creates a gravitational vector along the z-axis of the body thereby stimulating the baroreflex system to induce upsurges in cardiac and vascular resistance activity (Moore et al., 2005). Several Earth-based studies have provided evidence that AG training can improve tolerance against OI, and that benefits of short intermittent AG exposure surpass those of continuous static AG exposure (Stenger et al., 2007, 2012; Young and Paloski, 2007; Goswami et al., 2015a; Clément et al., 2016; Zhang et al., 2017). Additionally, there is also evidence to suggest that short intermittent AG exposure improves tissue oxygenation (Marijke et al., 2017). Much like the benefits high-intensity interval training provides for maintaining aerobic fitness (Gibala et al., 2012; Milanović et al., 2015), AG training could prove to be an effective counter-measure against OI via short duration and intense training intervals. However, more research must be conducted using Earth-based AG protocols prior to testing in microgravity environments.

The limited number of AG studies performed to date, have primarily involved research in men, and have focused on orthostatic stability upon re-introduction of 1G conditions, and not so much on cardiovascular responses during the AG training itself. The cardiovascular responses in women during SAHC AG exposure have not been thoroughly documented and no studies have specifically have recorded and analyzed gender specific cardiovascular reactions active during an intermittent AG exposure. It has yet to be determined whether women and men exhibit similar or diverging cardiovascular responses during an identical AG protocol. A thorough comparison of gender specific cardiovascular responses during AG is important in order to verify its effectiveness on eliciting the cardiovascular responses needed to overcome an OI event. There is also substantial evidence indicating that cardiovascular functioning differs between men and women (Evans et al., 2001; Hart et al., 2009; Hart and Charkoudian, 2014), particularly cardiac and vascular resistance activity, with women tending to exhibit greater cardiac activity, whereas men tend to respond with heightened vascular resistance activity during orthostatic stress (Shoemaker et al., 2001). In addition, women have exhibited a greater gravity-dependent baroreflex sensitivity than men during orthostatic stress, which leads to profound differences in cardiovascular responses during exposure to an equal level of orthostatic stimulus (Drudi and Grenon, 2014). Prior to deployment of any AG protocol for manned space crews, it must be determined what +Gz gradient is required in order to elicit significant increases in cardiac and vascular resistance in both women and men, while minimizing any OI event during the exposure. Therefore, this study implemented a graded +Gz interval training (GIT) via SAHC in order to establish whether the cardiovascular systems involved in maintaining orthostatic integrity can effectively be stimulated amongst men and women, and to examine if any gender specific cardiovascular responses became apparent.

METHODS

Subjects

Twenty-eight healthy, Caucasian civilian subjects gave their written informed consent to participate freely in this experiment, which took place at the German Aerospace Institute (DLR), at the European Space Agency (ESA) short arm human centrifuge (SAHC) test facility in Cologne, Germany. The first 13 subjects were tested in the fall of 2012, while the remaining 15 were studied in summer of 2015. The subject pool consisted of 16 women and 12 men who were matched for age (28.4 ± 5.3 years). Screening comprised of a medical questionnaire and a physical examination performed by an independent general physician who was not involved in the study. This screening examination included a resting ECG, a Schellong test to screen for orthostatic susceptibility, and cycle ergometry to determine baseline cardiovascular fitness. Upon completion of the screening, no subjects were excluded, nor had any history of cardiovascular, metabolic, or neurological diseases. Furthermore, it was ensured that none were commercial or military pilots, professional or elite athletes. This study was carried out in accordance with the recommendations of the Medical Ethics Committee of Nordrhein-Westfalen, Germany (Aerztekammer Nordrhein, Düsseldorf, Germany) in accordance with the Declaration of Helsinki. The protocol was approved by the Medical Ethics Committee of Nordrhein-Westfalen, Germany (Aerztekammer Nordrhein, Düsseldorf, Germany).

Study Protocol

The SAHC (SAHC-TN-007-VE, QinetiQ Company, Antwerp, Belgium) used for this study has a +Gz range of +0.1 to +5.0, an acceleration rate of maximal +0.4Gz/s, a maximum radius of 2.82 m and a maximum RPM of 40. The ESA-SAHC has been extensively used for +Gz training and various studies in the field of gravitational physiology (Zander et al., 2013; Frett et al., 2015).

The +Gz profile (see **Figure 1**) consisted of an initial baseline for 15 min followed by two identical +1/2/1Gz graded acceleration/deceleration rounds, composed of 3 phases each. These 2 rounds were separated by a +0Gz phase. Except for phase 1 (P1, 15 min) and P5 (5 min), each phase lasted 4 min. Acceleration/deceleration between each phase lasted 15 s. For the first 5 min of P1, the SAHC was not rotated. This was done to ensure good signal quality. For the next 10 min, the SAHC was slowly rotated at 5 RPM. During +1Gz phases (P2, P4, P6 and P8), the gravitational vector along the body was as follows: head +0.3Gz, mediastinum +0.5Gz, and feet +1Gz with an approximate RPM of 16. During +2Gz phases (P3 and P7), the gravitational vector along the body was +0.6Gz for the head, +0.9Gz for the mediastinum and +2.0Gz for the feet with an approximate RPM of 26.4. During P5, the SAHC was rotated at 5 RPM.

The purpose of implementing a graded +Gz protocol was to reduce the occurrence of OI due to rapid inductions of +2Gz. Therefore, the first +1Gz phase (mild hyper-gravity) was used to reduce any sudden occurrence of an OI event and to prime the baroreflex system for a moderate gravitational stress stimulus. The +2Gz phases induced moderate +Gz and the desired cardiovascular reactions. A reduction back to +1Gz allowed for mild +Gz exposure, with the addition of absorbing any carry-over effects of the +2Gz phase. P5 allowed for a return to baseline conditions before starting the second round. The two rounds of +Gz were set up in order to ascertain whether these reactions could be replicated, adding to the principle of interval training.

In case of signs of orthostatic intolerance (OI) during the GIT, the +Gz level was reduced or the protocol was terminated completely. These included perfuse sweating, confusion, dizziness, nausea, a narrowing of the visual field or the presence of hemodynamic decompensation criteria, such as new-onset ECG abnormalities, an abrupt drop in MAP of >20 mmHg or a critical narrowing of the pulse pressure. Additionally, each subject could press a "panic button" if they wished to abort the test. Direct verbal and visual contact was maintained between the flight physician and the test subject via camera and microphone.

Cardiovascular Recording

Mean arterial blood pressure (MAP, mmHg) and pulse pressure waves were monitored continuously and non-invasively via finger plethysmography (Portapres®, Finapres Medical Systems BV monitoring, Amsterdam, the Netherlands). The collected blood pressure waves were analyzed with Beat-scope® software. Systolic and diastolic blood pressure (SBP, mmHg and DBP, mmHg respectively) as well as stroke volume (SV, ml) were extracted from the continuous blood pressure waveform. Heart rate (HR, bpm) was recorded via a 3-lead ECG. Cardiac output (CO, L/min) and systemic vascular resistance (SVR, mmHg·min/l) were calculated via the Wesseling formula (Veerman et al., 1995). To control for significant baseline anthropometric gender differences, CO, SV, and SVR were converted to cardiac index (CI L/min/m^2), stroke volume index (SVI ml/m^2) and systemic vascular resistance index (SVRI, mmHg·min/l/m^2).

Data and Statistical Analysis

All recorded data was synced according to phase and grouped according to gender. The last 60 s from each phase were averaged and used for the statistical analysis. This time section was chosen primarily to provide continuity with a previous study based on the first part of the same experiment, (Habazettl et al., 2016). In that study, microvascular and cardiovascular responses from the last 60 s of each phase were analyzed in order to highlight the maximal response. Furthermore, cardiovascular parameters in most subjects showed oscillation patterns during the first 2–3 min of each phase, thereby exhibiting high variability thus not allowing for an accurate assessment of cardiovascular reactions. Secondly, the last 60 s were used with the goal of reflecting maximal cardiovascular response as well as steady state without the interference of acute changes in +Gz transitions. Diaz Artiles et al. (2016) recorded the above-mentioned cardiovascular trends during an SAHC study, where it was shown that the participants reached maximal response or steady state during the last 60 s of SAHC exposure and the variability during this period was at a minimum.

Statistical analysis of cardiovascular reactions during the GIT were done via repeated measures ANOVA using phase comparisons from baseline as a within and gender as a between

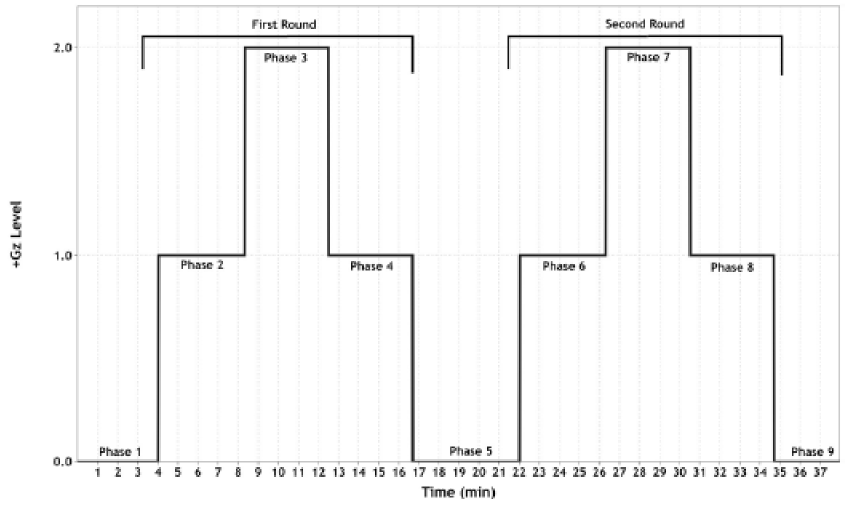

FIGURE 1 | Schematic of the +Gz interval training (GIT) protocol. Each phase (1–9) is labeled accordingly, indicating the corresponding +Gz level over time in minutes.

subject factor. To test for equality of variance between men and women the Levene's test was employed. Assumption checks were made using a Mauchly's test of Sphericity followed by Greenhouse-Geiser corrections. Effect size per cardiovascular parameter was calculated via partial Eta squared test. This was done for the whole group as well as independently for women and men. Changes from baseline were further analyzed using t-tests with Holm-Sidak *post-hoc* correction. To assess for differences in baseline, a student's t-test was used. All data is presented as mean and ± standard error of the mean (SEM). The level of statistical significance was defined at alpha = 0.05. Cardiovascular parameter synchronizing and averaging was performed using "R" statistical environment version 3.2.5 (R Core Team, 2013). Inferential statistics were performed using SPSS Statistics 23 (IBM, New York, USA) and JASP Version 0.8.2 for Mac OS (JASP Team, 2018). Graphics were created using Data Graph 4.2 software for Mac OS (Visual Data Tools, Inc., 2013).

RESULTS

From the 28 subjects, 23 (11 women and 12 men) completed the GIT without any interruption. All five non-finishers (NF) were women and will be referred to as womenNF. One woman exhibited an OI event in both +2Gz phases, however she endured all +1Gz phases. Three other women experienced an OI event during P3, two of which requested to terminate their GIT exposure at that point. One went on to complete P4 before terminating her exposure. Another woman experienced an OI event during P3, however she completed all subsequent phases, and P7 at a reduced +Gz level (1.5+Gz instead of +2Gz).

Baseline

Anthropometric data are represented in **Table 1**. Height, weight and BSA were all significantly greater amongst men than women who finished the GIT ($p < 0.05$). The womenNF were significantly taller than finisher women ($p < 0.05$). Baseline cardiovascular values were obtained from the last 60 s of P1 and are represented in **Table 2**. The womenNF exhibited significantly greater MAP, DBP, and SVRI ($p < 0.05$), and non-significantly greater SBP ($p = 0.054$) than women who finished the GIT. There were no significant differences found between men and women who finished the GIT regarding baseline cardiovascular parameters. However, men had slightly higher DBP and SVRI at baseline, whereas women exhibited a higher HR.

Cardiovascular Reactions

All cardiovascular parameters passed the test of normality ($p > 0.05$). The respective effect sizes, F-statistics and p-values for each parameter are summarized in **Table 3**. The cardiovascular responses for women and men during GIT are displayed as line plots in **Figures 2, 3**. Across the seven observed cardiovascular parameters, there was a significant main effect of +Gz ($p < 0.05$) across all phases for women and men, with the exception of SBP (**Table 3**). No significant differences were found amongst cardiovascular parameters between women and men.

TABLE 1 | Mean and SEM baseline anthropometric data for women and men.

	Men ($n = 12$)	Women ($n = 11$)	WomenNF ($n = 5$)
Age (years)	29.0 ± 1.46	28.3 ± 1.90	27.4 ± 0.84
Height (cm)	178.1 ± 1.76*	168.8 ± 1.63	176.1 ± 0.59#
Weight (kg)	76.0 ± 1.10*	65.5 ± 2.36	67.3 ± 0.79
BMI (kg/m^2)	24.0 ± 0.35	23.0 ± 0.66	21.6 ± 0.18
BSA (m^2)	1.90 ± 0.02*	1.70 ± 0.04	1.83 ± 0.01

**Denotes a significantly greater value in men compared to women ($p < 0.05$). #Denotes a significantly greater value in womenNF compared to the finisher women ($p < 0.05$).*

TABLE 2 | Mean and SEM baseline cardiovascular data for women and men.

	Men ($n = 12$)	Women ($n = 11$)	WomenNF ($n = 5$)
MAP (mmHg)	80.8 ± 4.05	77.7 ± 3.79	101.6 ± 8.05*
DBP (mmHg)	64.2 ± 3.80	58.1 ± 2.99	79.6 ± 6.20*
SBP (mmHg)	126.4 ± 4.17	126.1 ± 7.17	154.6 ± 12.55
SVRI (mmHg*min/l/m^2)	25.8 ± 2.26	21.9 ± 1.21	30.3 ± 1.41*
HR (bpm)	63.7 ± 3.75	68.5 ± 3.75	70.8 ± 6.67
CI (l/min/m^2)	3.30 ± 0.17	3.60 ± 0.19	3.30 ± 0.23
SVI (ml/m^2)	51.1 ± 2.27	52.0 ± 1.48	47.4 ± 3.70

**Denotes a significantly greater value in womenNF compared to the finisher women ($p < 0.05$). The p-value for SBP between womenNF and finisher women was nearly significant ($p = 0.053$).*

Central Blood Pressure Reactions

Figure 2 details the central blood pressure reactions (MAP, DBP, and SBP) recorded during GIT in women and men. MAP in women was increased over baseline in P3 and P4 ($p < 0.05$), with MAP in P3 being higher than in P2 and P4 ($p < 0.05$). Throughout P6-P8, MAP was non-significantly increased over baseline in women. In men, MAP expressed a significant increase only during P6 and P8 ($p < 0.01$), while remaining non-significantly above baseline in all other phases. DBP in women was elevated throughout the first round ($p < 0.01$), and was higher during +2Gz phases than +1Gz phases ($p < 0.001$). During the second round, DBP increased over baseline only during P7 ($p < 0.01$). DBP in this phase was also higher than P6 and P8 ($p < 0.01$). DBP in men was significantly elevated over P1 during P3, P4, and throughout P6-P8 ($p < 0.05$), while DBP was higher during P3 than +1Gz phases ($p < 0.05$). SBP showed no significant changes from baseline in both genders. Men tended to have overall higher MAP, DBP, and SBP than women, however there were no significant gender differences found. Women did show a lesser degree of variability compared with men for MAP and DBP, particularly during +2Gz phases.

Vascular Resistance and Cardiac Reactions

Figure 3 details SVRI, HR, CI, and SVI reactions apparent during GIT in both genders. SVRI in women was significantly higher than baseline throughout all +Gz phases ($p < 0.05$), with SVRI tending to be higher in the +2Gz phases. However, there was no statistical difference between +1Gz and +2Gz. SVRI amongst men, although elevated during +Gz, was not significantly different from baseline. SVRI was the parameter that showed the highest variability in men, and this variability was markedly greater in men over women.

Women showed a significantly higher HR over baseline throughout all +Gz phases ($p < 0.05$), with significantly higher HR levels during +2Gz phases than +1Gz phases ($p < 0.001$). Men expressed significant HR elevations over baseline during P2, P3, and P7 ($p < 0.05$, $p < 0.001$, and $p < 0.001$). HR was also significantly higher during the +2Gz phases than +1Gz phases ($p < 0.001$). Women displayed non-significantly higher HR values over men throughout all phases of GIT ($p = 0.094$). CI showed no significant decreases from baseline amongst women, while in men, CI demonstrated significant decreases from P1 during P2, P4, and throughout P6-P8 ($p < 0.05$). SVI in both genders was consistently lower than baseline during all +Gz phases than baseline ($p < 0.001$), and lower in +2Gz than +1Gz ($p < 0.001$). Only in men, SVI was significantly increased over baseline during P5 ($p < 0.05$). Finally, no significant differences were found when comparing the two rounds of GIT with each other. Despite the gender specific differences, there was an overall similarity in cardiovascular reactions for men and women. Both HR and SVRI increased with higher +Gz levels, while SVI and CI displayed an inverse relationship with the +Gz level.

Finisher Women vs. WomenNF

While a majority of women participating in this study (11/16) did not experience any orthostatic event during GIT, five experienced an orthostatic event and did not complete the full study protocol (womenNF). During the first minute of the initial +2Gz phase (P3), womenNF exhibited a significant decrease in MAP, SBP and SVI compared to baseline ($p < 0.05$), with no subsequent increases in HR and SVRI. Only two women went on to complete the protocol albeit at a reduced +Gz level (+1/1.5/1 Gz). A statistical comparison was performed between womenNF and finisher women during P2 and P3 ($n = 5$ vs. $n = 11$). Compared with finisher women, womenNF exhibited higher MAP, DBP, SBP, and SVRI during P2 ($p < 0.05$). During P3, the only difference between these groups was SVI, which was significantly lower in womenNF compared with finisher women ($p < 0.05$).

DISCUSSION

This study set out to determine gender specific cardiovascular reactions during intermittent +Gz interval protocol as a potential countermeasure against micro-g associated cardiovascular deconditioning. Upon conclusion of this study, it could be determined that women showed consistent significant increases in HR, and SVRI throughout the GIT, with significant DBP increases occurring during phases 2, 3, 4, and 7. On the other hand, men exhibited significant increases in DBP and HR only during the +2Gz phases with increases in DBP occurring in all but one +1Gz phase. No significant changes in SVRI for men were recorded. The lack of significant SVRI findings could be attributed to a high inter-group variability amongst the men involved in this study. SVRI in women did not show this extent of variation and responded in a uniform fashion. Amongst men,

TABLE 3 | Partial Eta-squared effect sizes, F-statistics, and *p*-values with Greenhouse-Geiser sphericity corrections per cardiovascular parameter for women, men, and all subjects for main effect of phase.

		Effect size partial Eta-squared per parameter	F-Statistics per parameter	*p*-value per parameter
MAP (mmHg)	Women	0.359	5.61	0.012
	Men	0.387	6.93	0.012
	All	0.368	12.22	<0.001
	Women vs. Men	0.04	0.804	0.380
DBP (mmHg)	Women	0.646	18.3	<0.001
	Men	0.553	13.6	0.001
	All	0.591	30.36	<0.001
	Women vs. Men	0.071	1.61	0.218
SBP (mmHg)	Women	0.037	0.39	0.748
	Men	0.092	1.11	0.343
	All	0.039	0.85	0.477
	Women vs. Men	0.013	0.273	0.607
SVRI (mmHg*min/l/m^2)	Women	0.600	15.02	<0.001
	Men	0.312	4.98	0.043
	All	0.352	11.39	0.001
	Women vs. Men	0.06	1.44	0.243
HR (bpm)	Women	0.873	68.8	<0.001
	Men	0.828	52.8	<0.001
	All	0.851	120.09	<0.001
	Women vs. Men	0.128	3.09	0.094
CI (L/m^2)	Women	0.450	8.17	<0.001
	Men	0.479	10.1	<0.001
	All	0.452	17.34	<0.001
	Women vs. Men	0.046	1.01	0.326
SVI (ml/m^2)	Women	0.874	69.5	<0.001
	Men	0.878	79.1	<0.001
	All	0.875	147.58	<0.001
	Women vs. Men	0.005	0.114	0.739

Partial Eta squared, F-statistics, and p-values comparing women and men with Type III sum of squares are reported in the last row for each parameter.

CI was significantly decreased throughout the GIT, while in women no significant changes were observed. As both genders had similar SVI reductions, this gender difference in CI is most likely due to the HR increases recorded amongst women over men. Finally, when comparing both rounds of the GIT, no significant differences were found. The cardiovascular reactions occurring in the first round were mirrored in the second round for both genders. This was the desired outcome of the interval protocol, and it can be assumed that a third round would have induced similar responses.

Cardiovascular Reactions During GIT: Female Specific Reactions

To date, only few of studies dealt specifically with the topic of gender related effects of AG exposure. Two studies concluded that a single exposure to a graded AG protocol (from +0.6Gz to submaximal +Gz) significantly improved orthostatic tolerance amongst normovolemic and hypovolemic women (Evans et al., 2015; Goswami et al., 2015a). A third study found that 3 weeks of daily 35-min intermittent +Gz exposure coupled with exercise improved orthostatic tolerance amongst ambulatory women (Stenger et al., 2007). Other AG studies have focused on the pre-frontal cortical activity, which was decreased in women undergoing AG exposure with respect to men (Smith et al., 2013; Schneider et al., 2014). In the present study we aimed to investigate gender related differences in cardiovascular reaction during exposure to graded +Gz levels, while testing for the occurrence of a training effect during the second run. We found that women undergoing GIT exhibited significant increases in DBP, HR, and SVRI, which can be attributed to greater baroreflex sensitivity with respect to men during gravitational challenges (Hogarth et al., 2007). Another contributing factor for these findings could be plasma epinephrine, renin, and vasopressin levels, which have been recorded to be higher in women than in men are during gravitational challenges (Geelen et al., 2002). Although not measured in this study, increased plasma concentrations of the aforementioned neuroendocrine components could have led to the augmented SVRI, as well as HR activity. The low inter-individual variability displayed by women as a group would

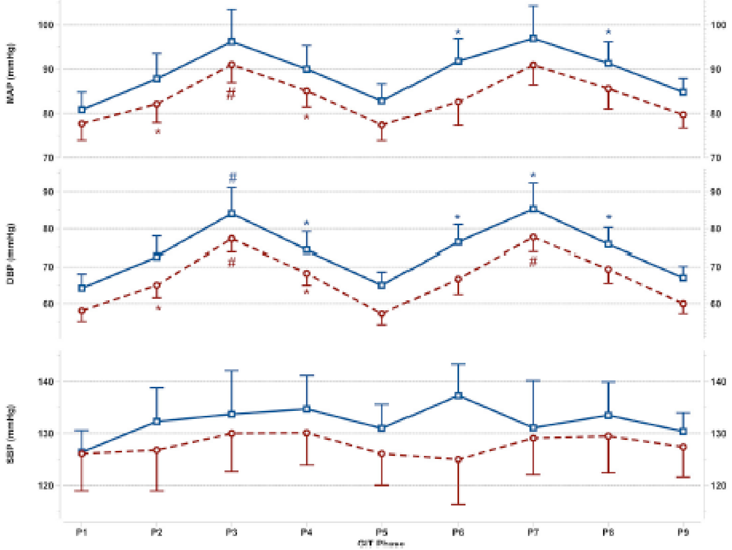

FIGURE 2 | Mean and SEM for central blood pressure reactions of the last 60 s per +Gz phase from top to bottom: MAP, mean arterial pressure DBP, diastolic blood pressure, and SBP systolic blood pressure during GIT for men (blue squares, solid lines $n = 12$) and women (red circles, spaced lines $n = 11$). *Denotes a significant change from baseline P1 ($p < 0.05$), and # indicates a significant change between +2Gz and +1Gz phases ($p < 0.05$). GIT phase nomenclature is indicated along the x-axis (P1-P9).

be advantageous when implementing future +Gz protocols, possibly negating the need to individualize a +Gz training profiles for women. A further reason for these reactions in women may be due to anthropometric factors, as a more compact body size in women may allow for increased sensitivity to gravitational challenges.

Although the majority of women (11/16) that participated in this study endured the GIT, five women did not follow suit and experienced an OI event. A predisposing factor for OI risk seems to be height and baseline central and systemic pressure, with taller women having higher MAP, DBP, SBP, and SVRI. This supports evidence that women have a higher propensity for OI during gravitational stress and the reasons for this have been discussed in prior research (Harm et al., 2001; Fu et al., 2004; Fong et al., 2007; Nordine et al., 2015) and indicate that anthropometric as well as physiological factors could play a role in this trend. It would appear that +2Gz exceeds the capacity for women with a certain height to maintain MAP, and a reduced +Gz profile would be beneficial. In order to ensure a 100% orthostatic tolerance rate amongst women, the +Gz limit for each person should be assessed prior to +Gz exposure, similar to the +Gz profile utilized by Goswami et al. (2015a). Alternatively, a modified +Gz profile could be used, as it appeared that 30% of women in this study could not tolerate full +2Gz for more than 60 s. These women may have benefitted from a starting +1Gz/+1.5Gz/+1Gz x2 profile.

Cardiovascular Reactions During GIT: Male Specific Reactions

During GIT, men did not exhibit the same significant changes from baseline as women did. HR and DBP were significantly increased over baseline during +2Gz, but not SVRI. In men, a minimum of +2Gz seems to be needed in order to induce significant HR increases, a response which has also been recorded by Goswami et al., during SAHC training (Goswami et al., 2015b), Ueda et al. (2015) and Polese et al. (1992). A possible explanation as to why men require a stronger gravitational stimulus in order to trigger increases in HR may be due to higher resilience to venous pooling and central volume loss due to a greater amount of available plasma volume. Therefore, during +1Gz phases, while the decrease in functional blood volume is enough to trigger the beta sympathetic response in women, men may require a higher degree of SVI loss until HR increases become apparent. Surprisingly, SVRI in men showed no significant changes from baseline. However, as previously mentioned, this is more likely due to high inter-individual variability observed amongst men rather than low absolute values recorded. Possible explanations for these findings have been proposed by some

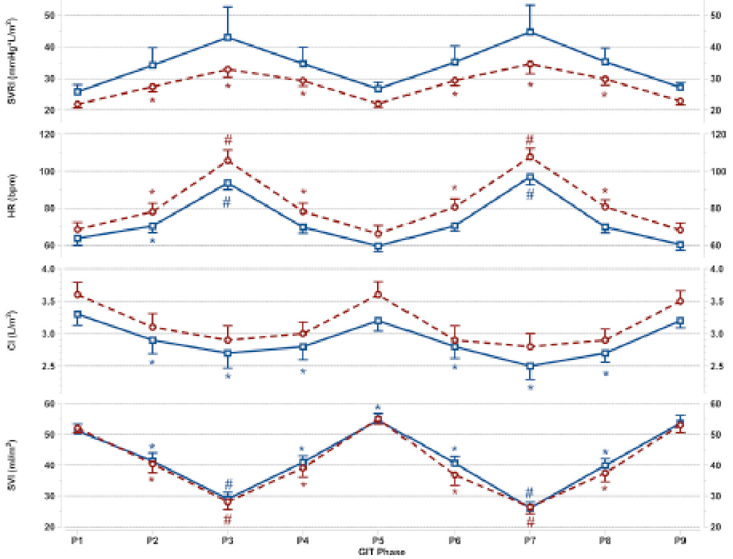

FIGURE 3 | Mean and SEM for vascular resistance and cardiac reactions of the last 60 s per +Gz phase from top to bottom: SVRI systemic vascular resistance, HR heart rate, CI cardiac index, and SVI stroke volume index for men (blue squares solid lines $n = 12$) and women (red circles spaced lines $n = 11$) during GIT. *Denotes a significant change from baseline P1 ($p < 0.05$), and # indicates a significant change between +2Gz and +1Gz phases ($p < 0.05$). GIT phase nomenclature is indicated along the x-axis (P1-P9).

study groups, which include polymorphic genetic differences in vascular resistance regulation (Seasholtz et al., 2006), or elevated respiratory effort during +Gz, which induces a higher amount of venous return to the central circuit (McKenzie, 1993). Although these factors may offer reasons for varying intensities of SVRI regulation, they do not offer insight to the difference in variability observed between genders, as these studies included exclusively men. In order to train both active vasomotor and cardiac mechanisms in men, a higher +Gz-level gradient may be required. For future GIT protocols in men, an individualized +Gz limit should be determined prior to training, as to maximize the effects on the cardiovascular system.

Cardiovascular Activity During GIT: Gender Differences

Although no significant gender differences in cardiovascular reactions were recorded, women tended to respond with heightened HR over men during the GIT. Other working groups have recorded similar HR responses in women compared to men during gravitational stress. These studies however used lower body negative pressure (LBNP) (Frey and Hoffler, 1988; Franke et al., 2000), and thus may not be directly comparable to SAHC studies. The gravitational vector applied by SAHC distributes a relatively equal transmural vascular pressure along the length of the body, whereas LBNP produces a strong transmural pressure directly below the LBNP seal (Dosel et al., 1998; Watenpaugh et al., 2004; Robertson, 2008).

The heightened HR response in women can be attributed to gender differences in the autonomic circuitry, myocardial structure, as well as hormonal status. Compared with men, women show increased parasympathetic withdrawal to the cardiac circuit during orthostatic stress thereby leading to an increase in HR (Evans et al., 2001; Huxley, 2007). In addition, the hormone estradiol may augment epinephrine sensitivity during orthostatic stress and thus be a contributing factor (Wenner et al., 2013; Gordon and Girdler, 2014). Further reasons for an increased HR in women could be differences in trans-mitral filling velocity and faster myocardial velocities (Nio et al., 2015). These factors would equate to a higher cardiac filling rate and faster myocardial contraction in women than in men, thus offering an account for differences in HR.

Regarding SVRI, no significant difference was found between genders, although men exhibited higher overall SVRI than women. This was reflected in higher MAP, DBP, and SBP recorded amongst men. Some groups have also observed significantly increased vasoconstrictor response in men over women during orthostatic challenges (Frey and Hoffler, 1988; Jarvis et al., 2010; Hachiya et al., 2012). However, more evidence suggests that peripheral vasoconstrictor activity does not differ between genders during orthostatic stress (Convertino, 1998; Franke et al., 2003; Kelly et al., 2004; Fu et al., 2005; Carter et al., 2015; Russomano et al., 2015; Patel et al., 2016). Certain authors have shown that during beta and muscarinic blockade, there is a predominant vascular regulation in men compared to dominant parasympathetic influence on heart rate regulation in women (Evans et al., 2001; Huxley, 2007). Those studies used different modalities to provoke orthostatic stress such as LBNP, head-up tilt (HUT), as well as pharmacological means. Previous studies that have recorded increases in vasomotor activity in men over women (Hachiya et al., 2012) have attributed these differences to an overall higher magnitude of sympathetic nerve activity to the periphery in men (Hart et al., 2009; Yang et al., 2012). It should be noted that SVRI is the total accumulation of vasoconstrictor activity in the circuit, which includes the extremities as well as the core (thorax, abdomen, and pelvis). Although not recorded in this study, studies have shown differences regarding vasomotor responses in different anatomical regions amongst men and women (Dart et al., 2002; Jarvis et al., 2010; Hachiya et al., 2012). Clearly, further AG studies are needed in order to critically examine gender differences regarding regional vascular activity during +Gz gradients via the use of Doppler sonography, laser Doppler, as well as near infrared spectroscopy.

LIMITATIONS

While this study directly compared gender specific cardiovascular reactions during a newly devised graded intermittent AG training protocol, the findings should be interpreted with caution. Firstly, the small sample size used for this study, although of good size for human AG studies, makes drawing larger conclusions for the average population difficult. Also, as there is a lack of data concerning gender specific differences during SAHC exposure, more studies of this nature should be performed in order to ascertain if these gender specific reactions can be replicated. Moreover, this study did not set out to induce maximal +Gz limits in the human test subjects nor was the +Gz gradient tailored for each individual test subject. Had maximal +Gz profiles been deployed, or individual +Gz profiles been used, the results may have been different. Lastly, serum catecholamine, testosterone, estrogen, progesterone, and hemoglobin levels were not measured in this study. Thus, their role in the gender specific cardiovascular findings of this study can only be speculated upon. Additionally, no restrictions were placed on women concerning menstrual cycle phase, nor oral-contraceptive use upon partaking in this study. Certain authors have suggested that these factors may have an effect on autonomic functioning in women during gravitational stress (Wenner et al., 2013). In order to strengthen the findings of this study, more studies involving gender responses during +Gz exposure via SAHC, particularly with a similar +Gz profile, are warranted.

CONCLUSIONS

This study showed that gender specific cardiovascular reaction patterns are indeed apparent during an intermittent AG session, while no significant differences between women and men were found. The gender specific cardiovascular differences in response to +Gz emphasize the importance of implementing AG countermeasure strategies specifically for women and men. Since micro-g induced cardiovascular deconditioning leads to vascular dysregulation upon reintroduction of standard gravity (Waters et al., 2002), a potential countermeasure protocol should ensure adequate vasomotor stimulation in both genders. This GIT, which lasted for 45 min, consistently stimulated vasomotor activity amongst women with SVRI increasing in all phases and DBP during the +2Gz phases. Men may require higher +Gz gradients in order to exhibit similar reactions. Also, only 70% of women finished the entire +Gz exposure. In order to ensure a 100% tolerability rate as well as ensuring the desired cardiovascular reactions needed to overcome orthostatic instability upon re-introduction of gravity modified or individualized +Gz profiles should be deployed, as was demonstrated by previous AG studies (Goswami et al., 2015a). Also, further testing in conjunction with bed-rest, immersion, and isolation studies are needed to see if this GIT training can mitigate cardiovascular deconditioning prior to manned space flight implementation.

AUTHOR CONTRIBUTIONS

ZM and MN wrote the manuscript and analyzed the data with the help of KB, BH, SM, and AW. KB, AW, BH, SM, and MN performed statistical analysis. AS and OO implemented the data collection. HH, H-CG and OO conceived and planned the study. MM, HH, H-CG, OO, AW, SM, and KB provided expertise, reviewed, and approved the final manuscript.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

12. Curriculumn Vitae

My curriculum vitae does not appear in the electronic version of my paper for reasons of data protection

13. Publication list

1. Form influences function: Anthropometry and orthostatic stability during sustained acceleration in a short arm Human centrifuge. Michael Nordine, Martina Anna Maggioni, Alexander Stahn, Stefan Mendt, Katharina Brauns, Hanns-Christian Gunga, Helmut Habazettl, Andrea Nitsche, Oliver Opatz. May 2015 Acta Astronautica 115C:138–146 DOI:10.1016/j.actaastro.2015.05.025

2. Microvascular responses to (hyper-)gravitational stress by short-arm human centrifuge: arteriolar vasoconstriction and venous pooling. Helmut Habazettl, Alexander Stahn, Andrea Nitsche, Michael Nordine, Axel Pries, Hanns-Christian Gunga, Oliver Opatz. August 2015 European Journal of Applied Physiology 116(1) DOI: 10.1007/s00421-015-3241-6

3. Gender-Specific Cardiovascular Reactions to +Gz Interval Training on a Short Arm Human Centrifuge. Zeynep Masatli, Michael Nordine, Martina Anna Maggioni, Stefan Mendt, Ben Hilmer, Katharina Brauns, Anika Werner, Anton Schwarz, Helmut Habazettl, Hanns-Christian Gunga, Oliver Opatz. July 2018 Frontiers in Physiology 9 DOI: 10.3389/fphys.2018.01028

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This work was undertaken in the firm belief that man will eventually become a multi-planetary species. I am fully aware that one may not live to see this day, however, I wanted to contribute to this critical step in human evolution. This work is thereby my contribution to providing current and future space explorers with valuable knowledge in order to safely traverse the solar system. This work would not have been possible without the guidance and academic stewardship of Professor Hanns-Christian Gunga, Professor Helmut Habazettl, and Dr. med. Oliver Opatz. Thank you all for providing me with the tools, resources, space, and wisdom in order to contribute to this project. Lastly, to my beautiful wife Zeynep, thank you for your having healthy curiosity about the cosmos and thank you providing me with love and stability.