npj | microgravity

Article

Published in cooperation with the Biodesign Institute at Arizona State University, with the support of NASA

https://doi.org/10.1038/s41526-025-00516-6

Implications of sex differences in orthostatic tolerance during exposure to acute artificial gravity

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Development of countermeasures to minimise spaceflight deconditioning is of paramount importance, such as the short-arm human centrifuge (SAHC); however, sex differences in cardiovascular responses should be considered. 20 participants (female = 10, male = 10) conducted an identical centrifugation protocol of 10-min phases of standing (NG), 1Gz centrifugation (1GRF), and 2Gz centrifugation (2GRF). Separated by 10-min each in hot (29.1 ± 0.8 °C) conditions, and either normoxia (PO_2 = 133 mmHg) or hypoxia (PO_2 = 92 mmHg). Haemodynamics, microvascular blood flow (arm and leg) and temperatures (skin and gastrointestinal) were continuously recorded. At 2GRF, females lasted 2.7 ± 1.6 min less than males. Arm blood flow significantly decreased, and leg blood flow increased, in 2GRF ($p > 0.001$); with higher female leg blood flow in 2GRF ($p = 0.002$). 2GRF altered all haemodynamic variables and females exhibited significantly higher heart rate, and lower stroke volume index ($p < 0.001$). Female participants exhibited greater cardiovascular strain, and encountered pre-syncopal symptoms earlier, during 2GRF. Future research should consider individualised and tolerable gravitational loads.

As a consequence of the endeavours of Space Life Sciences, we have established a permanent presence in Earth's lower orbit with humans continuously inhabiting the International Space Station (ISS) in this century. Another milestone scheduled for this century is the interplanetary mission to Mars and potentially establishing a permanent presence on the Moon. This development has heightened the interest in the hazardous effects of prolonged exposure to weightlessness, as would be experienced by astronauts during such deep space missions. More importantly, there is an urgent need to develop efficient countermeasures to minimise, or possibly eliminate, the well-known micro-gravity induced deconditioning. Such adaptation of physiological systems includes fluid changes, muscle atrophy and force reduction, hormonal changes, bone demineralisation, cardiovascular deconditioning, and autonomic deconditioning, to name but a few[1–3]. One of the key indicators of cardiovascular deconditioning upon return to earth is the orthostatic tolerance (OT) test, which assesses an individual's ability to withstand the influence of gravity on their body and avoid syncope. Previous research has identified that differences in OT exist between females and males, both in terrestrial settings[4,5] and upon astronaut's return-to-earth[6,7]. On earth, pre-syncope during tilt-tests occurs in 69% of females and 31% of males[8], and the time to pre-syncope is ~23% lower in females[4]. Upon return from space it was found that 100% of females and 20% of males experienced pre-syncope during a 10-min OT test after 5–16 days in space[7]; however, this study was impacted by a severe sex imbalance of 30 males and 5 females. Clearly, the historical lack of female-specific research and astronaut selection is impacting our knowledge in this area; as of March 2023, only 72 out of 622 (11.5%) people who have been to space were female[9,10]. Additionally, the duration of spaceflight appears to be correlated with a reduction in OT, with 25% of astronauts experiencing pre-syncope after short flights (8–16 days) and 83% after long flights (129–190 days); though no sex comparisons were reported[6]. Possible reasons for sex differences include anatomical and anthropometric factors, differences in sympathetic activity and vascular responses, hormonal autonomic control and haemodynamic differences[4,5,11]. Therefore, without appropriate countermeasures the return to Earth will have serious health consequences for astronauts, but considerations should be made for the differences in response to these countermeasures between sexes.

One such countermeasure is exposing astronauts to periods of artificial gravity (AG) equivalent to at least that of Earth within space vehicles and future habitats. This can be achieved with a short-arm human centrifuge (SAHC). Several studies have assessed the acute cardiovascular responses to centrifugation on a SAHC, each presenting differing gravitational loading and exposure times; yet they each observe similar responses[12–16]. These studies, despite recruiting female participants, contain no analyses of sex differences. Watenpaugh et al.[12] reported 4/7 females were disqualified due

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to low tolerance, and Goswami et al.[13] reported unacceptable blood pressure and cerebral blood flow values leading to exclusion of 4/10 males and 6/10 females. To our knowledge, four studies have assessed sex differences in the cardiovascular response to passive centrifugation on a SAHC; however, one used an onboard cycle ergometer to power the centrifuge at low exercise intensities[17], and another reported no other cardiovascular response besides protocol completion[18]. During a graded Gz profile (1/2/1 Gz) using 4-min stages, cardiovascular parameters were recorded in 12 males and 16 females[19]. Five females and zero males experienced an orthostatic event, and a significant increase in heart rate and blood pressure in both sexes; however, they concluded that the SAHC stimulated cardiovascular systems to a greater extent in females. Kourtidou-Papadeli et al.[17] did not utilise purely passive centrifugation, however they observed significantly higher cardiac output, cardiac power, and mean arterial pressure in males vs female participants; during 5-min increments in Gz up to 2.0 Gz. Studies assessing the effects of 90-min passive centrifugation below a predetermined presyncopal limit, in hypovolemic males and females, has been reported in reference to cardiovascular responses[20] and autonomic indices[21]. These studies found that single bouts of AG improved OT in both males (30%) and females (22%), and that females had overall higher heart rate, and lower stroke volume, and arterial pressures than males. In addition, the single bout of AG improved male sympathetic response to orthostatic stress, but not in females. Finally, Fong et al.[18] conducted a 60-min AG test with 1 Gz at the heart level, in six males and five females. It was found that five males and one female were able to complete the session successfully, yet no reasoning was provided for these differences.

While AG alone appears to be a severe cardiovascular stressor, the conditions in which this AG is conducted must also be considered. Previous research (thermo/baro/chemo interaction)[22,23] has identified that when multiple homoeostatic response mechanisms are activated at once, interaction/competition for cardiovascular regulation must occur. Increases in core temperature may occur during an exercise countermeasure as a result of metabolic heat production [24] and/or high ambient temperatures (T_A); the international space station regularly experiences fluctuations in temperature from 18 to 23 °C[25]. In addition, EVA/EHAs will be conducted much more frequently during planetary explorations, in contrast to ISS missions, so in order to minimise preparation time it is envisaged that future planetary habitats will be hypobaric with an enriched oxygen ambient; similar to that at an altitude in excess of 2500 m[26–28]. A lower blood volume and associated oxygen-carrying capacity in females, even when adjusted for anthropometrics, has been shown to impact OT[8]. Therefore, the addition of hypoxia may further exacerbate this difference in OT between sexes. Even though it is well known that humans can acclimatise to the levels of hypoxia anticipated in future habitats[29], the manner in which hypoxia and T_A might affect the physiological responses to AG countermeasures is not.

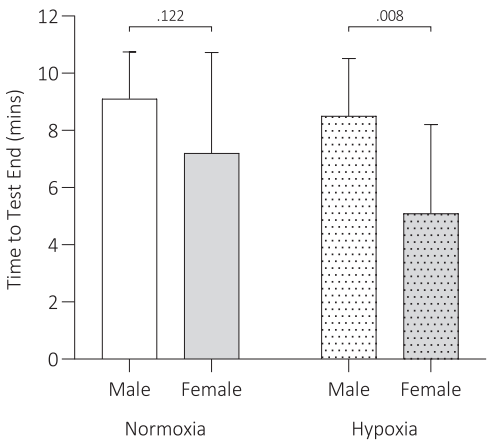

Fig. 1 | Time to test cessation at 2GRF in males and females, in normoxia and hypoxia. Significance of male vs. female difference detailed in each OXYGEN condition.

Therefore, the aim of the present study was to assess the sex differences in haemodynamic and blood flow responses to AG in the head-to-foot direction on a SAHC with and without reduced oxygen availability. This is likely to illicit interaction of homoeostatic mechanisms towards either heat removal or OT. It is hypothesised that (1) higher levels of gravitational loading will increase the magnitude of orthostatic intolerance, or reduce time to pre-syncope, in females compared to males, (2) reduced oxygen availability will further exacerbate orthostatic intolerance in both males and females, with a greater effect observed in females.

Results

All female and male participants completed the 10-min NG and 1GRF protocol phases successfully, in both conditions. Pre-syncopal symptoms were exhibited by several participants during the 2GRF centrifugation condition, resulting in premature termination on the condition. No participant experienced full syncope under any test condition. The time to test cessation at 2GRF was significantly lower in females than in males ($p = 0.004$, F [1,36] = 9.746) irrespective of condition. Multiple comparison analyses identified that hypoxia alone produced a significant difference between sexes ($p = 0.008$). However, both males and females responded similarly to the different OXYGEN conditions, with regards to test duration. In normoxia, females lasted 7.2 ± 3.5 min and males lasted 9.1 ± 1.6 min; in hypoxia, females lasted 5.0 ± 3.3 min and males lasted 8.5 ± 2.0 min (Fig. 1).

In addition, participants were thermally comfortable throughout NG (females: 0.0 ± 0.0, males: 0.3 ± 0.3), 1GRF (females: 0.0 ± 0.0, males: 0.3 ± 0.1) and 2GRF (females: 0.0 ± 0.0, males: 1.0 ± 0.0). Females also perceived a lower thermal sensation than males in NG (females: 0.1 ± 0.0, males: 1.0 ± 0.0), 1GRF (females: 0.0 ± 0.0, males: 0.5 ± 0.2) and 2GRF (females: 0.0 ± 0.0, males: 1.0 ± 0.4). Finally, ratings of perceived exertion also increased with increasing G-load; NG (females: 6.3 ± 0.4, males: 6.5 ± 0.2) compared to 1GRF (females: 6.7 ± 0.2, males: 7.3 ± 0.3) and 2GRF (females: 8.5 ± 0.1, males: 8.9 ± 0.4).

Microvascular blood flow (M_{BF})

Analyses of the arm M_{BF} only identified a significant effect of GRAV ($p = 0.036$, F [2,36] = 3.665), and no effect of either SEX or OXYGEN ($p > 0.05$). Further analyses identified male M_{BF} in the arm was significantly reduced by the increase in GRAV (NG = 21.8 ± 8.5 %max, 1GRF = 20.4 ± 8.6 %max, 2GRF = 18.7 ± 10.7 %max; $p < 0.001$), whereas the female arm M_{BF} did not vary significantly (NG = 27.4 ± 15.8 %max, 1GRF = 21.4 ± 8.5 %max, 2GRF = 21.2 ± 11.6 %max); which may be due to higher individual variability in the female response.

Leg M_{BF} was also significantly affected by the increase in GRAV ($p < 0.001$, F [2,36] = 27.43), displaying increases from NG up to 2GRF. Further analyses identified that the increase in GRAV significantly increased M_{BF} in both males (NG = 18.3 ± 6.6 %max, 1GRF = 23.8 ± 13.3 %max, 2GRF = 29.2 ± 13.3 %max; $p < 0.001$) and females (NG = 19.0 ± 7.8 %max, 1GRF = 22.0 ± 10.1 %max, 2GRF = 47.3 ± 22.6 %max; $p < 0.001$). In addition, the leg M_{BF} was also significantly altered by the interactions of GRAV + SEX ($p = 0.002$, F [2,32] = 7.470) and GRAV + OXYGEN ($p = 0.001$, F [2,32] = 3.777). Multiple comparisons analyses have identified the hypoxia in 2GRF condition as cause of this variation, as the leg M_{BF} in females was significantly higher (+30 %max, $p < 0.001$). Figure 2 displays mean ± SD M_{BF} under differing ambient conditions and gravitational loads.

Deep body and skin temperatures

A significant effect of SEX was observed between females and males ($p < 0.001$); however no other interaction effect was observed. In the NG condition, the male T_{gi} was 37.1 ± 0.2 °C and female was 37.5 ± 0.3 °C. In 1GRF the male temperature was 37.2 ± 0.2 °C and female was 37.6 ± 0.2 °C. Finally, in 2GRF the male temperature was 37.2 ± 0.2 °C and female was 37.5 ± 0.3 °C. These values are provided as an average of both conditions in each sex, due to the lack of significant difference as a result of OXYGEN.

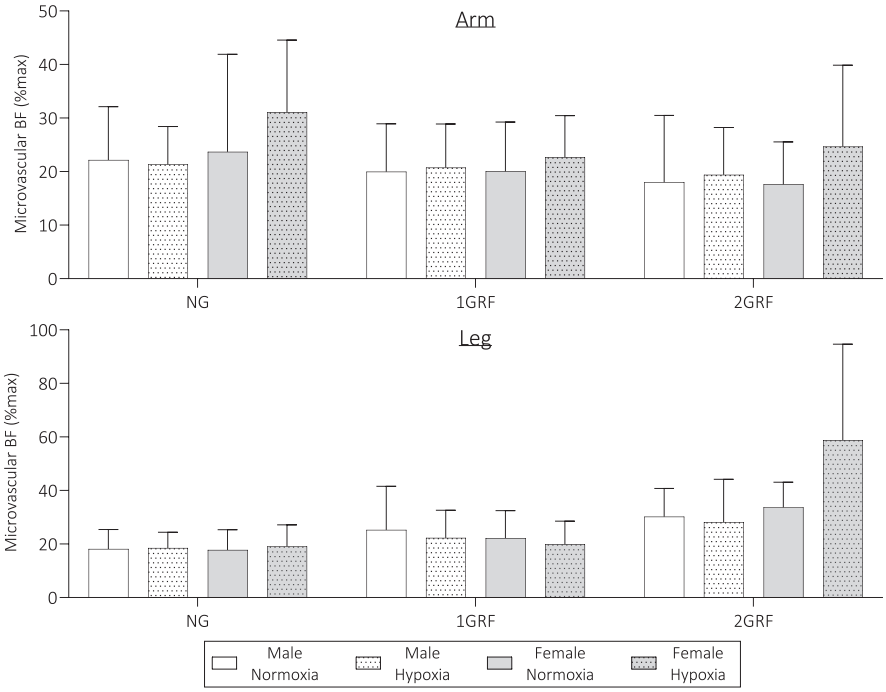

Fig. 2 | Microvascular blood flow under differing ambient conditions and gravitational loads.

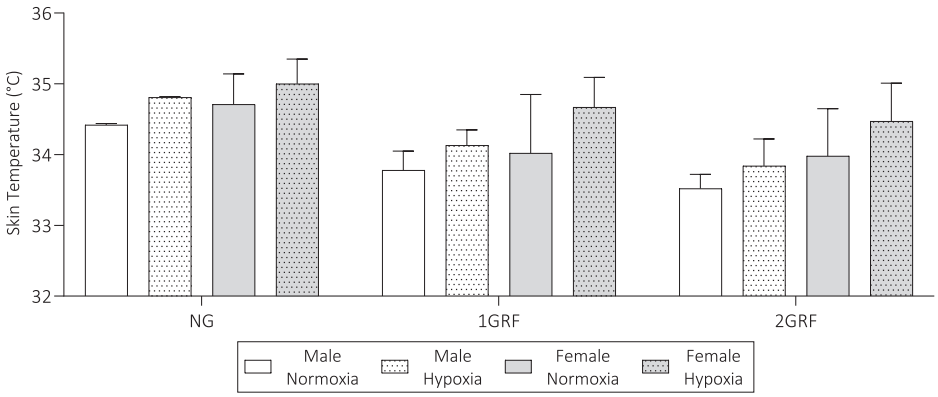

Fig. 3 | Skin temperatures under differing ambient conditions and gravitational loads.

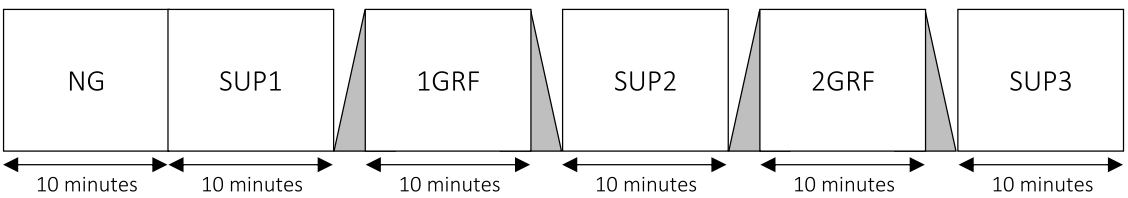

Fig. 4 | Session protocol displaying the order of conditions, identical both sessions.

T_{sk} (Fig. 3) was significantly affected by SEX ($p < 0.001$, F [2,108] = 24.88), as well as GRAV ($p < 0.001$, F [1,108] = 35.81) and OXYGEN ($p < 0.001$, F [1,108] = 27.94). Multiple comparisons analysis identified a significant difference from NG in both 1GRF and 2GRF conditions. In the NG condition, the male T_{sk} was 34.6 ± 0.2 °C and female was 34.8 ± 0.2 °C. In 1GRF the male T_{sk} was 34.0 ± 0.3 °C and female was 34.4 ± 0.3 °C. Finally, in 2GRF the male T_{sk} was 33.7 ± 0.3 °C and female was 34.2 ± 0.3 °C. As above, these values are provided as an average of both conditions in each sex, due to the lack of significant difference as a result of OXYGEN (Fig. 4).

Haemodynamic responses

Mean ± SD of haemodynamic variables in both sexes, and all GRAV conditions are displayed in Table 1. In response to SEX, HR was higher in females than in males (+15.0 b·min^{-1}; $p < 0.001$, F [1,108] = 27.55), SVI was higher in males than females (+6.7 mL; $p < 0.001$, F [1,108] = 23.07), RPP was higher in females compared to males (+2478.8 mmHg·min^{-1}; $p < 0.001$, F [1,108] = 22.66), and SVR was higher in females compared to males (+0.2 mmHg·min·mL^{-1}; $p < 0.001$, F [1,108] = 19.29).

The main effect of GRAV caused a significant effect in many variables, however multiple comparisons analyses identified that no differences

Table 1 | Mean ± SD haemodynamic values at each gravitational stimulus, in both males and females

Haemodynamic Parameter	Sig.	GRAV condition	Male		Female	
			Normoxia	Hypoxia	Normoxia	Hypoxia
Heart Rate ($b \cdot min^{-1}$)	a b c	NG	80.7 ± 7.2	87.9 ± 10.8	93.9 ± 14.3	97.2 ± 18.5
		1GRF	76.5 ± 7.1	80.6 ± 11.2	85.9 ± 14.2	94.9 ± 19.7
		2GRF	107.1 ± 21.0	97.5 ± 22.6	111.5 ± 15.7	127.4 ± 17.9
Stroke Volume Index (mL)	a b	NG	37.0 ± 4.3	36.1 ± 6.7	28.0 ± 8.9	29.0 ± 7.9
		1GRF	36.3 ± 5.6	33.5 ± 6.3	30.2 ± 11.1	30.9 ± 8.4
		2GRF	30.8 ± 6.0	31.0 ± 8.9	23.3 ± 6.8	23.0 ± 8.5
Cardiac Index ($L \cdot min^{-1}$)		NG	3.0 ± 0.4	3.1 ± 0.4	2.6 ± 0.7	2.8 ± 0.6
		1GRF	2.8 ± 0.3	2.7 ± 0.3	2.6 ± 0.8	2.8 ± 0.6
		2GRF	3.4 ± 1.0	3.2 ± 0.8	2.7 ± 1.1	2.7 ± 0.8
Systemic Vascular Resistance ($mmHg \cdot min \cdot mL^{-1}$)	a b c	NG	1.0 ± 0.2	0.9 ± 0.2	1.4 ± 0.5	1.3 ± 0.4
		1GRF	1.1 ± 0.3	1.1 ± 0.3	1.5 ± 0.5	1.3 ± 0.4
		2GRF	1.1 ± 0.3	1.1 ± 0.4	1.6 ± 0.6	1.5 ± 0.8
Systolic Arterial Pressure (mmHg)	a	NG	131.3 ± 10.8	128.6 ± 17.3	125.7 ± 13.1	129.1 ± 13.7
		1GRF	133.5 ± 14.9	135.7 ± 14.9	132.3 ± 15.0	134.3 ± 11.7
		2GRF	150.2 ± 17.7	143.0 ± 15.0	140.9 ± 15.7	138.9 ± 17.1
Diastolic Arterial Pressure (mmHg)	a	NG	85.2 ± 10.2	86.7 ± 16.8	89.1 ± 10.2	91.3 ± 10.6
		1GRF	87.7 ± 13.3	92.8 ± 13.1	91.3 ± 13.2	93.5 ± 10.0
		2GRF	106.4 ± 15.5	103.5 ± 15.5	107.0 ± 14.6	105.2 ± 14.4
Mean Arterial Pressure (mmHg)	a	NG	103.0 ± 12.6	102.7 ± 18.8	104.5 ± 18.3	106.4 ± 16.9
		1GRF	105.7 ± 17.3	109.5 ± 19.2	107.9 ± 17.1	110.1 ± 15.4
		2GRF	123.3 ± 21.4	118.6 ± 16.1	120.7 ± 17.6	117.5 ± 23.6
SpO_2 (%)	c	NG	95.5 ± 1.2	85.2 ± 2.2	97.3 ± 0.9	86.6 ± 3.7
		1GRF	95.9 ± 1.0	84.9 ± 2.3	96.9 ± 1.1	83.8 ± 3.8
		2GRF	96.3 ± 1.4	84.8 ± 3.3	97.0 ± 0.9	85.3 ± 2.4

a = significant effect of gravity, b = significant effect of sex, c = significant effect of oxygen, $p < 0.05$.

existed between NG and 1GRF in any variables; significant differences were produced via the differences in NG vs. 2GRF and 1GRF vs. 2GRF. The increase in GRAV condition caused elevation in HR ($p < 0.001$, F [2108] = 25.78), RPP ($p < 0.001$, F [2108] = 23.80), SVR ($p = 0.027$, F [2108] = 3.752), SBP ($p = 0.009$, F [2108] = 4.865), DBP ($p < 0.001$, F [2108] = 15.19) and MAP ($p = 0.007$, F [2108] = 5.263). The increase in GRAV caused a decrease in SVI ($p = 0.001$, F [2108] = 7.178).

The main effect of OXYGEN caused an increase in HR ($p = 0.03$, F [1108] = 4.807) during the hypoxic trial, whilst a decrease in SRV ($p = 0.045$, F [1108] = 4.122) and SpO_2 ($p < 0.001$, F [1108] = 763.0) were observed.

Discussion

Known differences exist in the response of sexes to an orthostatic stress; largely indicating a lowered tolerance in females compared to males. In the present study, female participants experienced pre-syncopal symptoms during hyper-gravity (2GRF) condition significantly earlier than males (hypothesis 1). In addition, exposure to a hypoxic stressor similar to those proposed for future space habitats[26–28] further influenced time to pre-syncope in both males and females, with a significantly reduced time to presyncope observed in females (hypothesis 2). These incidences of pre-syncope match those of previous research, however the present study intends to elucidate reasons for these differences via assessments of haemodynamic and vascular responses to AG.

In those returning from space, the prevalence of orthostatic intolerance is higher in females than in males. Following space shuttle missions lasting between 5 and 16 days, pre-syncope during postflight OT tests was reported in 70–100% of females and 0–5% of males[7,30,31]. In a review of 165 astronauts during short duration spaceflight, 28% of female astronauts and 7% of male astronauts experienced pre-syncopal symptoms after 10 min in an upright posture[32]. Fewer studies have assessed OT during AG exposure in males and females, however, those that have, calculated that 58% of females and 96% of males can successfully withstand 1 Gz centrifugation with varying session durations[17–19,33]. Factors that differ between sexes, including relative blood volumes, haemodynamic responses, vascular control mechanisms and Hb carrying capacities, may all contribute to these differences[4,8]. In the present study, which utilised up to 2 Gz AG, presyncope was reached by both males and females; yet females still experienced a reduction in the time to pre-syncope compared to males. This calls into question the suitability of a standardised centrifugation programme as a countermeasure for spaceflight-induced cardiovascular deconditioning; the use of an individualised approach seems to be more appropriate. By utilising centrifugation individualisation techniques for AG training, as described by Goswami et al. [34], it may be possible to isolate sex differences in cardiovascular deconditioning, and response to training, during exposure to microgravity or one of its analogues (bedrest, dry immersion, etc.). Indeed, Stenger et al.[33] revealed that three weeks of 1–2.5 Gz (foot level) centrifugation for 35 min/day increased OT by 17.3% in males and 9.3% in females; as measured by a tilt-test with lower-body negative pressure (LBNP). It is unclear why the improvement was smaller in females, with the authors citing methodological issues in the location of the LBNP chamber affecting the splanchnic region more in females rather than sex differences; yet it seems possible that in fact individualised training may have reduced the differences between sexes. To individualise AG training[34], proposed using a 'pre-syncopal development test' which increased the AG load by 0.1 Gz every three mins until pre-syncopal symptoms were reached; the training level was then defined as one level below pre-syncopal level. Using this method, Goswami et al.[34] noted increased OT after 45-min of training in 53% of females and 23% of males, and overall the tolerability of AG training improved; all participants completed the full 90-min AG protocol. It is also worthy of note that the participants in the present study were relatively young and fit. Advanced age and reduced fitness have been identified as potential exacerbators of orthostatic intolerance as a result of a diminished HR response[35], yet the research is not completely equivocal[36]. Yet, with an average astronaut female age of 43.2 years and male age of 45.9 years[37], which is expected to increase as spaceflight becomes safer and easier to operate in, it is possible that the results of the present study may be exacerbated. In addition, it is known that elderly males tend to exhibit less tolerance to orthostatic stress than elderly females[38]. Therefore, more research may be needed to understand the effects of AG in an older population, and to see how the differences between sexes may alter.

As observed previously[13–15], significant differences in haemodynamic parameters were not observed between the NG and 1GRF conditions, in either sex. The increase in GRAV (i.e. NG/1GRF vs 2GRF), therefore, induced a strain on the human body that required a response from the cardiovascular system for maintenance of stable oxygenated blood delivery under severe environmental and gravitational stress. Despite increases in cardiovascular strain resulting from the 2GRF condition occurring in both sexes, there were also significant differences in haemodynamic variables between females and males. The increase in HR may be an autonomic response mechanism to the higher GRAV condition, to counteract the loss in circulating blood volume and lowered SV via blood pooling[4]. It has been previously observed that females exhibit a greater sympathetic response under the same level of LBNP[39–41], whereas males tend to exhibit a greater and more coordinated vascular response[42,43]. Indeed, the difference in SVR between NG and 2GRF is larger in males (0.2 mmHg·min·mL^{-1}). The changes in HR and SVI, and differences between sexes, may be a positive response to counteract the GRAV conditions; changes in RPP and SVI are more likely to be negative consequences of the overall decrease in circulating blood volume and consequent drop in blood pressure[44,45]. Another indicator of these detriments in haemodynamic function is observed in the microvascular blood flow. While no significant sex differences occurred, the interaction of GRAV + SEX was significant; largely due to the considerably higher M_{BF} in the leg in females (+30 %max). It is possible that these exaggerated leg M_{BF} values are either a general lack of vascular resistance to 2GRF in females or, more likely perhaps, it is the visualisation of blood pooling impacting venous/capillary flow[46]. When considering the whole cardiovascular system contributing to the maintenance of OT, the results of the present study indicate that a lack of vascular resistance in the lower limbs caused a cascade of haemodynamic responses which were ultimately unable to prevent pre-syncopal symptoms in female participants. In males, the known vascular resistance differences may have alleviated some of this haemodynamic strain, lengthening the time to pre-syncope.

It was anticipated in the present study that the combination of high T_A and hypoxia would elicit interaction/conflict between cardiovascular regulatory mechanisms. Indeed, a lower OT was observed in hypoxic conditions, yet it is unclear whether a conflict between regulatory mechanisms was responsible for this reduction in OT. However, it is more likely that a reduction in circulating blood volume due to pooling of blood in 2GRF, combined with a lower SpO_2, resulted in a lack of oxygenated blood transport to vital organs and subsequent pre-syncopal symptoms[47,48]. While an interaction/conflict between multiple mechanisms may have impacted the OT of males and females in the present study, it is clear that differing thermoregulatory responses had a clear impact. Differences between sexes in deep body temperatures were considerable, albeit expected. During the chosen phase of the menstrual cycle (i.e. luteal), there is a known increase in resting deep body temperature of 0.3–0.7 °C[49]; clearly observed in the present study. Skin temperature decreased from NG through 1GRF and 2GRF, which is likely due to the air flow produced via rotation of the SAHC; at higher speeds, the SAHC spins at ~40 kph, producing wind speeds as high as 4.2 m/s. The differences due to sex may be due to factors such as smaller body mass and surface area, lower metabolically active tissue, higher body fat and greater insulation during vasoconstriction[50,51]. Despite the variations in deep body temperature and skin temperature, it is also reported that increases in the autonomic thresholds for thermoregulation also occur from the early follicular vs the mid-luteal phases[52]. This may have resulted in a protective thermoregulatory/cardiovascular response to the combined high ambient temperatures and centrifugation whereby vasodilation required for thermoregulation in hot ambient temperatures occurs later. Centrifugation during earlier stages of the menstrual cycle may result in even earlier

development of pre-syncopal symptoms due to greater levels of vasodilation, and thus must be considered in SAHC training prescription.

We recognise two limitations within this study. Firstly, the use of high ambient temperature alone is not reflective of SAHC training conditions, as the high occurrence of pre-syncopal symptoms observed would be detrimental to short- and long-term training performance. Due to technical limitations, stable measurement of haemodynamic signals in cold conditions in females were unobtainable; constituting an insurmountable safety risk as pre-syncope was not determinable. Nonetheless, the authors reaffirm the exacerbation of cardiovascular strain via heating, hypoxia and hypergravity provides valuable data regarding the limits to sex differences in SAHC utilisation. Secondly, female participants were recruited during the luteal phase of the menstrual cycle only. With the anticipated use of a SAHC as a full-time exercise countermeasure during future space missions, a consideration to the cardiovascular responses during a complete menstrual cycle must be considered. Previous research assessing the effects of the menstrual cycle on orthostasis provide a clear consensus; differing phases have no effect of OT[53,54]. However, when central hypovolemia is produced via LBNP, significant variations in cardiovascular response are displayed between follicular and luteal phases[55]; proposed to be related to changes in oestrogen levels and sympathetic activity. Similar central hypovolemia occurs during centrifugation, therefore the role of the menstrual cycle during SAHC activity must be assessed.

Known sex differences in tolerance to a gravitational stimulus are also reflected in hypergravity, particularly when the ambient conditions exacerbate the cardiovascular burden. Female participants exhibited greater haemodynamic and microvascular strain, and encountered pre-syncopal symptoms earlier than male participants, during 2GRF centrifugation. For future research regarding the use of a SAHC as an exercise countermeasure, these differences must be considered and perhaps mitigated via individualised and tolerable gravitational loads.

Methods

A total of 10 female and 10 male participants were recruited for an experimental randomised cross- design study, their mean ± SD anthropometrics are displayed in Table 2. The protocol was approved by the National Committee for Medical Ethics at the Ministry of Health of the Republic of Slovenia (approval no. 0120-180/2023/7) and conformed to the guidelines of the Declaration of Helsinki.

Participant information

A sample size calculation was conducted to assess the impact of environmental conditions of microvascular blood flow, utilising the results of a previous study[56]. During heating, a difference of 4.9 °C in proximal-distal temperature gradient ($\Delta Tsk_{p\text{-}d}$) was observed, producing an effect size (d) of 3.38 for the association between temperature and microvascular blood flow. Assuming an α of 0.05 and β of 0.99, eight participants provide sufficient power to detect a statistical difference in this association, and assuming a N2/N1 ratio of 1, the required sample size for sex analysis between two independent groups is 8 participants per group (G*Power Version 3.1.9.2). 10 male and 10 female participants were recruited and completed the full study. Participants were recruited using convenience sampling of advertising to the general public and student services. None of the participants met the following exclusion criteria: smokers, physically inactive, diabetic and/or a history of freezing or non-freezing cold injuries, cutaneous peripheral disease, high or low blood pressure, and/or hypoxic and g-tolerance acclimation. Female participants were tested during the luteal phase of their menstrual cycle (day 14–28). Prior to the start of the study, participants were familiarised with the study protocol and procedures, and gave their written consent for participation. Participants were asked to refrain from caffeine, alcohol, smoking, and intense physical exercise in the 24-h leading up the study, and from eating in the 2-h leading up to the testing session. In addition, they were requested to attend in a well-hydrated and rested state. Sessions were conducted at the same time of day for each participant.

Table 2 | Participant anthropometric data

		Sig.	Females	Males
N			10	10
Age (years)			28.8 ± 8.5	27.9 ± 6.3
Height (cm)		*	170.0 ± 7.8	179.8 ± 6.1
Weight (kg)		*	63.5 ± 9.2	78.2 ± 10.3
Body mass index (kg·m^2)			22.1 ± 3.5	24.2 ± 2.8
Body surface area (m^2)		*	1.7 ± 0.1	2.0 ± 0.2
Blood volume (L)		*	4.5 ± 0.4	5.3 ± 0.5
1GRF (%BW)			102.6 ± 3.4	99.9 ± 2.4
2GRF (%BW)			195.6 ± 6.4	187.0 ± 4.9
Distance from force platform	Head	*	158.2 ± 7.2	167.3 ± 5.7
	Heart	*	135.8 ± 6.2	143.6 ± 4.9
	CoM	*	103.8 ± 4.7	109.8 ± 3.7

Body surface area[63] and blood volume[64] were calculated using recorded body mass and body stature values. *CoM* Centre of Mass. * = Sig. difference between sex.

Experimental protocol

Female and male participants attended two testing sessions, where the T_A in the room was 29.1 ± 0.8 °C and ambient oxygen concentration was either normoxic (PO_2 = 133 mmHg) or hypoxic (PO_2 = 92 mmHg). Relative humidity (RH) remained constant at 49.5 ± 8.1%. During this session, the participants experienced a protocol conducted on a SAHC located the Jožef Stefan Institute's Gravitational Physiology Laboratory (Planica, Slovenia), at an altitude of 940 m. Despite T_A only reaching ~23 °C on the ISS, a higher T_A was chosen in the present study to further exacerbate thermoregulatory/baroreflex/chemoreflex conflict, such as may be seen during exercise during centrifugation.

The participants initially rested in thermoneutral conditions for a 10-min, before entering the SAHC chamber and being fully instrumented with all sensors; maximal cutaneous blood flow responses were also obtained (described in section 'Haemodynamic Responses'). Figure 4 displays the protocol used in the present study, which involved collecting resting measurements in three postures across six conditions. During centrifugation, the definition of gravitational stimuli experienced by the participants was defined by ground reaction force (GRF) applied to a force platform the participants were 'stood' on. Measuring GRF rather than Gz is required for the production of a consistent acceleration profile between participants of differing body anthropometrics; due to the non-uniform acceleration produced by the SAHC. Table 2 displays mean ± SD % bodyweight, associated with 1GRF and 2GRF, and the distance the head, heart and CoM from the force platform. The six conditions are as follows; standing in normal gravity (NG), stationary supine on centrifuge (SUP1), 1GRF centrifugation (1GRF), stationary supine on centrifuge (SUP2), 2 GRF centrifugation (2GRF), stationary supine on centrifuge (SUP3). Participants were asked to lie or stand calmly with no movement in their right (instrumented) arm and leg. They were monitored with video cameras, and were in constant audio contact with the SAHC controller. The test would have been stopped if any of the following indicators of pre-syncope were observed: persistent decrease in systolic blood pressure >35 mmHg, or a decrease in heart rate > 15 b·min^{-1}.

Data collection

Laser Doppler Flowmetry (LDF)—measured at the forearm (ArmBF) and calf (LegBF), on the right side of the body (Moors Instruments Laser doppler monitor, MoorVMS-LDF, Moor Instruments, UK). LDF is a non-invasive measure of capillary, arteriole and venule perfusion; which measures the Doppler shift caused by the movement of red blood cells within the illuminated tissue volume[57]. The device utilises probes producing a near-infrared laser with a power of 1.0 mW at a wavelength of 780 nm, sampling at

40 Hz. The device was calibrated using a fluid undergoing Brownian motion before each participant. LDF provides high temporal resolution, however, it is limited by poor spatial resolution and sensitivity to motion artifacts, and it provides only relative, not absolute, perfusion values[58]. Therefore, arbitrary units produced by the LDF (Laser doppler units: LDU) were converted into cutaneous vascular conductance as a ratio of LDU to mean arterial pressure. They were then normalised against maximum blood flow for both the arm and leg in normothermic conditions in order to produce a relative measure of percentage maximum blood flow (%maxBF). Maximum blood flow values were obtained by locally heating the limb and producing a post-occlusion reactive hyperaemia (PORH) via occlusion, then release the limb after 4 min.

Cardiovascular—Measurement of heart rate (HR, min^{-1}), stroke volume (SV, mL), cardiac output (CO, $L{\cdot}min^{-1}$), systemic vascular resistance (SVR, $mmHg{\cdot}min{\cdot}mL^{-1}$), rate pressure product (RPP, $mmHg{\cdot}min^{-1}$), systolic blood pressure (SBP, mmHg), diastolic blood pressure (DBP, mmHg), mean arterial pressure (MAP, mmHg), and oxygen saturation (SpO2, %) occurred continuously throughout the protocol (Finapres NOVA, Finapres Medical Systems B.V., Netherlands). Haemodynamic responses were calculated using the model flow algorithm[59] utilising the finger volume-clamp method and a 5-lead electrocardiogram. Reconstructed systolic and diastolic blood pressures were calculated via direct finger pressure measurements using waveform filtering and level correction[60], and normalised to the heart level via a height correction unit measuring the hydrostatic pressure difference. Additionally, participants held their arm in a sling with the measured finger at the heart level throughout all conditions.

Skin Temperature (T_{sk})—Continuous minute-by-minute skin temperatures was measured throughout the protocol using wireless iButton thermistors (type DS1921H, Maxim/Dallas Semiconductor Corp., USA) located at four sites (mid-belly of the bicep brachii, pectoralis major at mid-clavicular level, rectus femoris at femur midpoint, gastrocnemius on medial aspect) on the right side of the body. Weighed skin temperature (Tsk) was then determined using Ramanathan[61] equation.

Gastrointestinal Temperature (T_{gi})—Deep body temperature was measured via ingested telemetric pills measuring gastrointestinal temperature. The pills were ingested 60-min before start of the exercise protocol, and participants avoided drinking water after ingestion.

Subjective Measures—Participants were requested to verbally provide their thermal sensation, thermal comfort[62], and rating of perceived exertion (RPE) every 5-min. Temperature sensation is measured from -3 (cold) through to 3 (warm), thermal comfort is measured from 0 (comfortable) through to 3 (very uncomfortable), and RPE is measured from 6 (no exertion) to 20 (maximal exertion). Finally, for participant safety, motion sickness was recorded during centrifugation using a 6-point scale ranging from 0 (no symptoms) to 6 (vomiting).

Statistical analyses

Following conversion of raw LDF data into %maxBF data (described above), these data were averaged to produce minute-by-minute values throughout the full protocol. Cardiovascular data, measured beat-by-beat, was also averaged to produce minute-by-minute average. Finally, T_{sk} and T_{gi} were averaged to produce minute-by-minute values throughout the protocol. Subjective measures of thermal comfort, temperature sensation and RPE are reported as median and inter-quartile range. Three-way independent groups Analysis of Variance (ANOVA) tests compared the separate and combined effects of three dependant variables—level of artificial gravity (GRAV), participant sex (SEX) and oxygen concentration (OXYGEN)—on the independent variables (ArmBF, LegBF, HR, SVI, CI, SVR, RPP, SYS, DIA, MAP, T_{sk}, T_{gi}). If a significant main effect was established, pairwise multiple comparisons analyses would be conducted and corrected via a Tukey's Honestly Significant Difference test. Data was analysed using IBM SPSS statistics (Version 26, IL, USA) and Graphpad (Graphpad Prism 9, Version 9.1.2, USA); using an alpha value of $p < 0.05$.

Data availability

Data available upon request.

Received: 21 August 2024; Accepted: 28 July 2025;
Published online: 12 August 2025

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Acknowledgements
The authors are indebted to Miro Vrhovec, Jack Fortune, Victorien Faivre-Rampant, Dr. Leonidas Ioannou and Dr. Lydia Tsoutsoubi for their technical assistance, and project officer Dr. Jennifer Ngo Anh (European Space Agency) for her support during the project. The study was funded, in part, by the European Space Agency's Requesting Party Activity Grant (Grant No. 1-10992). Jason Fisher is recipient of a Slovene Research Agency Young Investigator Scholarship (PR-10488).

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Conceptualisation—J.T.F., U.C., I.B.M.; Methodology—J.T.F., U.C., I.B.M.; Formal Analysis—J.T.F.; Investigation—J.T.F., U.C.; Resources—J.T.F., U.C., I.B.M.; Data curation—J.T.F., U.C.; Writing (original draft)—J.T.F.; Writing (review and editing)—J.T.F., U.C., I.B.M.; Visualisation—J.T.F.; Supervision—U.C., I.B.M.; Project administration—J.T.F., U.C., I.B.M.; Funding acquisition—J.T.F., U.C., I.B.M.

Competing interests
The authors declare no competing interests.

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