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Blood coagulability changes in females exposed to dry immersion: examining a mechanism for the development of venous thromboembolism in microgravity

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ABSTRACT

The recent report of a blood clot in the internal jugular vein (IJV) of an astronaut aboard the International Space Station (ISS) has prompted the need of the international scientific community to investigate the potential underlying pathophysiology. Current published research on this topic is male-centered, despite known clotting sex-differences and heightened risk for females on Earth. In this study, we assessed the hemostatic responses of female participants exposed to dry immersion simulated microgravity. Based on previous evidence from studies of males in simulated microgravity (head-down tilt bedrest), we hypothesized an initial increase in pro-coagulation activity along with increased anticoagulation activity. Eighteen healthy female participants (28.2 ± 4.1 years, 59.4 ± 6.4 kg, 64.7 ± 6.0 cm) took part in the ESA-sponsored VIVALDI I five-day dry immersion study. Coagulation risk was assessed with rotational thromboelastometry and thrombin-antithrombin tests. The results showed a significant increase from baseline for coagulation and maximum clot firmness measurements, and a decrease in clot formation time by the last day of dry immersion. Thrombin-antithrombin levels were unchanged in response to dry immersion. These data present evidence of significant changes in hemostatic responses for females exposed to five days of dry immersion and suggest a possibility for increased clotting risks during flight.

1. Introduction

The recent report of a blood clot in the internal jugular vein (IJV) of a female astronaut aboard the International Space Station (ISS) has prompted the international scientific community to identify possible underlying mechanisms for this event occurrence [1]. Although Virchow's triad describes three contributing factors to venous thromboembolism—blood stasis, endothelial injury/dysfunction, and hypercoagulability—many consider a hypercoagulable state the primary risk factor, indicating a shift in the hemostatic system toward pathogenic

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blood clotting [2,3]. Hemostasis refers to the ability of a blood vessel to stop blood loss in the presence of an injury, which occurs through a combination of vasoconstriction, platelet activation, and activation of the coagulation cascade [4,5]. Primary hemostasis refers to the activation of platelets and their subsequent adhesion to the injury site, while secondary hemostasis is the reinforcement of the platelet plug by a fibrin clot [6]. Several coagulation tests assess an individual's clotting status by measuring the activation of secondary hemostasis, such as prothrombin time, which reflects clotting time attributable to the coagulation cascade [7]. The different tests provide information on real-time changes in the activity of the coagulation cascade at a time of interest. Alternatively, real-time coagulation testing technology, like rotational thromboelastometry (ROTEM), can evaluate hemostatic shifts and can be used with coagulation biomarkers, such as thrombin-antithrombin (TAT) complexes.

ROTEM provides a real-time visual assessment of blood clotting from the initiation phase to the stabilization of the fibrin clot, followed by its eventual breakdown through fibrinolysis [8]. Its measurements are referred to as coagulation time (CT) (the initiation phase of coagulation), clot formation time (CFT) (the time required for a stable clot to form), maximum clot firmness (MCF) (the strength and stability of the clot), and alpha angle (the speed of clot formation and relates to the kinetics of clot development) (Fig. 1). While traditional coagulation tests assess individual aspects of the coagulation cascade, ROTEM offers the additional benefit of allowing visualization of the entire cascade [9]. Deviations in these measurements can suggest heightened or impaired clotting. For instance, a shortened CT can reflect an increase in coagulation activity and a potential shift towards hypercoagulation.

Biomarkers, such as the TAT complex, also play a relevant role in investigating coagulation. TAT is a marker of a hypercoagulable state as it increases with thrombin production [10]. Plasma concentrations of TAT can help determine disruptions in hemostasis, particularly increased thrombin formation, especially when combined with other coagulation tests such as ROTEM.

On Earth, thromboelastometry (TEM) and ROTEM responses are modulated by sex, with females showing earlier onset of coagulation, a faster rate of clot development, and broader clot amplitudes with wider alpha angles compared to males [11–13]. Hemostatic responses in true spaceflight have not been reported, while findings from head-down tilt bed rest (HDTBR), a ground-based analogue of space, remain unclear [14]. Published research on hemostatic responses to microgravity alterations has focused solely on males exposed to bed rest models, yet it offers valuable insights.

One study examining the changes in hematological parameters of healthy males during and after exposure to 60-day 6° HDTBR found that while CFT, alpha angle, and MCF changed over the time course of the study, there was no evidence that these parameters reached a point that showed hyper- or hypo-coagulation [15]. A similar 21-day 6° HDTBR study examining 11 healthy males used calibrated automated thrombography (CAT) and tissue factor-triggered TEM, revealing a prolonged CT and a reduced alpha angle. However, no clinically significant hypercoagulability was caused by the exposure to HDTBR [16]. Rosenfeld et al. also reported no increase in hypercoagulable states in males after 36 h of non-tilt bed rest,but a potential increase in fibrinolytic activity was observed [17]. However, some research reported a tendency toward hypercoagulability upon re-ambulation [15,16]. Overall, these studies suggest that bed rest does not increase the risk of coagulation in healthy males; however, whether the same applies to females remains to be determined.

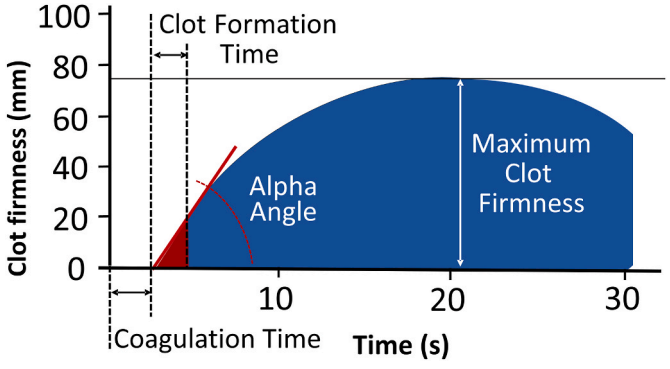

Fig. 1. Illustration of ROTEM measurements for a visual description of coagulation time (CT), clot formation time (CFT), maximum clot firmness (MCF), and alpha angle.

The aim of this analysis was to assess the hemostatic responses of female participants exposed to simulated microgravity using dry immersion (DI) to determine if hypercoagulation may contribute to the development of VTE. The current investigation was a secondary use analysis of ROTEM and TAT data from the VIVALDI I dry immersion campaign, which involved female volunteers participating in the DI experiment. Dry immersion involves submersion in water for extended periods of time while remaining dry, with participants placed in specially designed water baths and surrounded by a waterproof sheet throughout. The adaptation of participants' bodies when submerged in this manner causes changes to their neurological, cardiovascular, and metabolic systems that resemble changes in microgravity [18,19]. Based on evidence from previous studies of males in ground-based simulated microgravity, it was hypothesized that exposure to the DI environment would initially increase pro-coagulation activity, which would then reduce. It is further hypothesized that there will also be increases in anticoagulation activity in response to DI. Upon re-ambulation; it is hypothesized that there may be increases in hypercoagulation activity.

2. Materials & methods

2.1. Participants, dry immersion set-up & blood sample collection

A comprehensive description of this study, including inclusion and exclusion criteria, has been previously published [20]. Briefly, eighteen healthy female participants (28.2 ± 4.1 years old; range: 22–39 years) who completed the dry immersion study weighed 59.4 ± 6.4 kg and were 164.7 ± 6.0 cm tall (body mass index of 21.9 ± 1.8 kg/m^2), with an average VO_2peak of 38.4 ± 6.5 ml/kg/min (values are reported as means ± standard error). The VIVALDI I study (ClinicalTrials.gov Identifier: NCT05043974) was approved in France by the National Ethic Committee and French Health Authorities. All participants voluntarily gave their written informed consent. The ethics for secondary use investigation were approved by Simon Fraser University Research Ethics. All participants were medically screened to be cleared of any conditions that would interfere with coagulation activity.

Although the participants' menstrual cycles were not controlled for, analysis of blood progesterone and estradiol levels in these participants by Robin et al. [20] found no effects menstrual cycle on their responses to dry immersion.

The dry immersion tank comprised a specially designed bath with thermoneutral (32–34.5 °C) tap water and a highly elastic waterproof sheet on top to prevent the participant from contacting the water. The participant lay on a cotton fabric that was placed on top of the waterproof sheet. The tub was equipped with an elevating platform for lowering and raising the participant in and out of the tank during the study. Submerged in the tank up to the level of the neck/chest, the participants could use their arms for daily activities. The participants had access to a designated medical crew (doctors, nurses, etc.) that provided 24-h medical supervision throughout the investigation.

When participants left their tank for showering and bathroom activities, they remained in a 6° head-down tilt position on a stretcher and passively transferred to avoid weight-bearing and reversal of fluid shifts. An external catheter system was used to collect urine throughout the rest of the day while in the tank. Partial confirmation of the cephalic fluid

shift was examined using MRI imaging of the skin and muscle volume of the lower legs of the participants, which showed a reduction in fluid volume [20]. Corresponding data from upper body vessels have yet to be published, although previous studies of the IJV during DI show that there is an increase in IJV volume [21,22].

The study began with a four-day ambulatory control period, when baseline data collection (BDC) took place. This was followed by five days of exposure to the DI environment and two days post-DI for assessment and recovery (R). Blood samples totalling 34 mL were collected at BDC-1, DI2, DI5, and R+1 for coagulation analysis for each female participant (Fig. 2). Thus, a total volume of 373 mL of blood was collected from each participant for this research during the study. Blood was collected in the morning before breakfast and this plasma and serum samples were analyzed for blood count, hemostasis, markers, blood chemistry, metabolic state markers, vascular markers, bone markers, hormones (volume-regulating, sex, thyroid) [20]. Blood parameters from these assays, reported by Robin et al. [20], were used in this study to assess interaction with coagulation processes.

2.2. Rotational thromboelastometry analysis (ROTEM)

ROTEM analysis was performed by the primary research team using the TEM coagulation analyzer (ROTEM®05, Matel Medizintechnik, Graz, Austria) yielding four parameters: CT, CFT, MCF and alpha angle. CT is the time taken from adding the trigger to the initial fibrin formation; CFT is defined as the time it took for the clot to reach 20 mm in firmness; MCF represents clot stability; and the alpha angle indicates the speed of fibrin build-up and cross-linking. Since the standard ROTEM assays do not provide the high sensitivity that is needed to detect small changes in hemostasis, the researchers prepared a low tissue factor solution described by Sørensen et al. which creates a type of assay that assesses the extrinsic pathway of the coagulation cascade [12,23]. This solution, referred to as the trigger solution, contained 0.35 pmol/L recombinant human tissue factor thromboplastin (Innovin®) and 3 mmol/L $CaCl_2$ (Dade Behring Marburg GmbH, Marburg, Germany). The lyophilized product was dissolved in 4 mL of distilled water and then diluted at a ratio of 1:250 in 0.9 % sodium chloride solution (Fresenius Kabi, Austria, GmbH; tissue factor-stock solution). Clot formation was started with the addition of 40 μL of the trigger solution to 300 μL of citrated plasma.

2.3. Thrombin-antithrombin complex (TAT) analysis

TAT analysis was performed on the plasma with the EnzygnostR TAT micro test kit (Siemens Healthcare Diagnostics Products GmbH in Marburg, Germany). Prior to analysis, plasma samples underwent a 1:2 dilution with 0.9 % saline solution, followed by determination of TAT concentrations in accordance with the manufacturer's guidelines.

2.4. Statistical analysis

A mixed-model statistical analysis tested for differences between the means of the ROTEM and TAT values obtained at BDC-1, DI2, DI5, and R+1. The repeated measures approach accounted for the interdependencies between measurements for the same subject and included model fitting, diagnostic checks, and the analysis of differences in mean scores between time points for each of the coagulation tests. Where indicated, the Tukey HSD post-hoc test was used to determine differences in mean scores among the time points to identify specific statistically significant differences. The model fit was checked for normality, with additional confirmation that the residuals predicted plot exhibited a mean centered on 0 with constant variance and examined for any potential outliers. Those with skewed residual distribution plots were natural log-transformed to meet model assumptions. All statistical tests were performed using JMP® (Version 17, SAS Institute Inc., Cary, NC). Results are reported as means with standard error (SE). There were data losses due to sample damage for one participant, bringing the total number of data sets to 17 for CT, MCF, and alpha angle. Further complications at the DI facility resulted in the loss of eight participants' CFT tests, leaving only 10 data sets for CFT.

2.5. Correlations between TAT and ROTEM to blood parameters

To investigate the relationship between changes in the TAT and ROTEM measures and blood parameters [20] in the participants, data were converted from absolute BDC-1 and DI5 values to the change (Δ) from pre-to post-DI. To account for multiple comparisons, the false discovery rate (FDR) was used to estimate the significance of the correlations [24] using the robust Huber M-estimation method [25]. Significance was set at a positive false discovery rate of $p < 0.05$ [26]. Statistical tests were performed using JMP® (Version 17. SAS Institute Inc., Cary, NC).

3. Results

3.1. Rotational thromboelastometry

3.1.1. Coagulation time (CT)

The fixed effects analysis investigated changes across the measurement days ($n = 17$, $F = 7.9570$, $p = 0.0002$) on CT. There was a significant increase in CT from baseline (BDC-1: 70.8 s) to DI5 (83.2 s), showing a 12.4 s rise in CT (Fig. 3A). Conversely, a significant decrease in CT was observed from DI5 (83.2 s) to R+1 (75.7 s), reflecting a 7.5 s reduction in CT (Fig. 3A). No statistically significant differences were detected between any other days (Fig. 3A). The CT standard error was 4.4 s with 83.2 % of the variability attributed to individual differences among participants, highlighting substantial inter-subject variability.

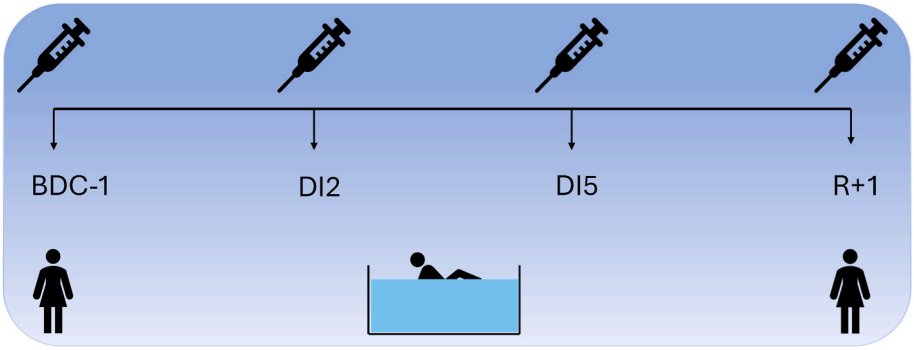

Fig. 2. Illustration demonstrating the time course of the dry immersion(DI) study marked by days where blood samples were taken including BDC-1 (one day before DI and during the baseline data collection period), DI2 (second day of DI), DI5 (fifth day of DI), and R+1 (first day of recovery following DI).

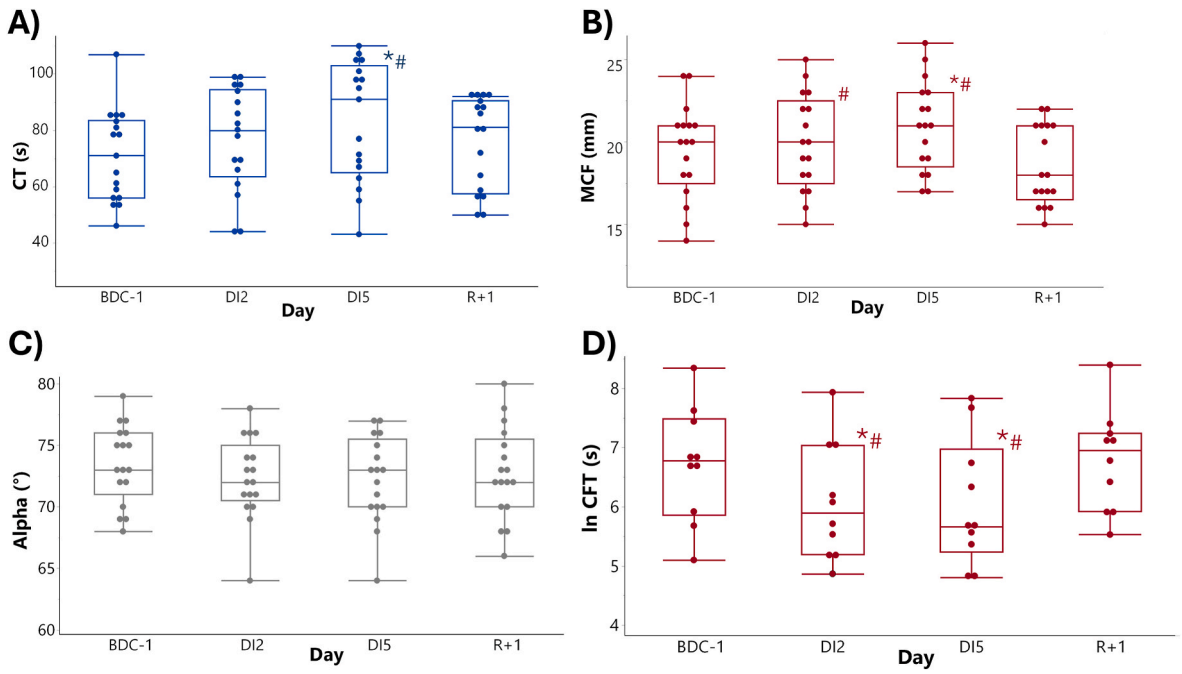

Fig. 3. Mean ROTEM coagulation parameters A) CT, B) MCF, C) alpha angle, and D) ln CFT for measurement days BDC-1 (one day before DI and during the baseline data collection period), DI2 (second day of DI), DI5 (fifth day of DI), and R+1 (first day of recovery following DI).for 17 female participants exposed to dry immersion for CT, MCF, and alpha angle, and 10 females for lnCFT. Graphs in grey had no significant changes. Graphs in blue show significant changes in the anti-coagulation direction, while red shows significant changes in the pro-coagulation direction. The symbol * indicates a significant difference from BDC-1 and # indicates a significant difference from R+1. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.1.2. Maximum clot firmness (MCF)

There were significant effects over measurement days on MCF (n = 17, F = 9.3970, p = 0.0001). Specifically, DI5 (20.9 mm) was statistically significant from baseline (BDC-1: 19.5 mm), with a 1.4 mm increase in MCF from baseline (Fig. 3B). MCF showed a significant decrease from DI2 and DI5 to R+1 (19.9 and 18.5 mm, respectively) (Fig. 3B). The MCF standard error was 0.7 mm, with 75.6 % of the variance in MCF values attributed to variability between individual participants.

3.1.3. Alpha angle

There was no significant change in alpha angle across measurement days (n = 17, F = 1.6272, p = 0.1954) (Fig. 3C). The alpha angle standard error was 0.8° with 72.6 % of the variability in alpha angle attributable to inter-subject differences.

3.1.4. Clot formation time (CFT)

CFT had a skewed residual distribution plot and was natural log-transformed to meet model assumptions. There was a significant effect of measurement days on the natural log-transformed CFT (n = 10, F = 6.4785, p = 0.0019) with a significant decrease in average formation time from baseline (BDC-1: 6.7s) to DI2 (6.1s) and DI5 (6.1s), indicating a faster response time in dry immersion (Fig. 3D). A significant increase in average CFT was observed in dry immersion compared to recovery (R+1: 6.8s) (Fig. 3D). The ln CFT standard error indicated that 74.6 % of the variability in CFT was related to inter-individual differences.

3.1.5. TAT plasma levels

There was no significant change in TAT levels over the days of measurement (n = 17, F = 0.9880, p = 0.4064) (Fig. 4).

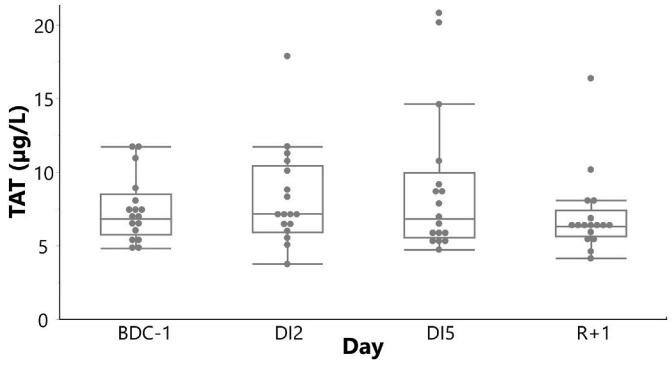

Fig. 4. Mean TAT responses for measurement days BDC-1, DI2, DI5, and R+1 for 17 female participants exposed to dry immersion. The graph in grey depicts no significant changes.

3.1.6. Effect of the menstrual cycle on coagulation responses to DI

The individual progesterone and estradiol levels for these participants along with their cycle phases respective to the DI protocol are presented by Robin et al. [20] in their supplementary documentation. Based on the cycle phase analysis criteria used by Robin et al. [20] (follicular phase: maximal blood progesterone threshold of >2 µg·L−1 during 5-day DI; luteal phase: maximal blood estrogen ≥200 ng·L−1 during 5-day DI), we identified seven participants in the follicular and five in the luteal phase during the five days of DI. A two-way repeated measures analysis of variance across phase and DI was performed. Tukey HSD was used for *post hoc* comparisons between conditions. There was no significant impact of the menstrual cycle on the coagulation factors with DI (all p > 0.05). Similarly, a mixed model with day as the repeated measure and progesterone or estradiol as the covariate, and using all participants, showed no significant effects of menstrual hormones on the coagulation measures during DI.

3.1.7. False discovery rate analysis (FDR)

Correlation and FDR analysis comparing TAT and ROTEM parameters with blood values from female participants between BDC-1 and DI5 showed significant associations of TAT, CT, alpha angle, and MCF with

mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), basophils, partial thromboplastin time (PTT), and prothrombin time (PT) ratio (Fig. 5). TAT, CT, and alpha angle also displayed a significant association with red blood cells (RBC), hemoglobin, hematocrit, eosinophils, prothrombin, and international normalized ratio (INR) (Fig. 5). CT also had a significant relationship with white blood cells (WBC), neutrophils, monocytes, platelets, and fibrinogen (Fig. 5). TAT was also found to have a significant correlation with lymphocytes and fibrinogen (Fig. 5). Lastly, alpha angle was shown to have a significant relationship with both lymphocytes and platelets (Fig. 5). Correlation coefficients and robust FDR p-values for these relationships can be found in the Supplementary Materials.

4. Discussion

For the first time, we show females exposed to dry immersion display significant changes in hemostatic responses, adding to past investigations of only male participants. When looking at the results of the ROTEM tests, there were several significant results found within the coagulation tests. First, there was a significant increase from baseline (BDC-1) to DI5 for the clot formation time, indicating that the participants, on average, were taking longer to clot when compared to their pre-exposure response. There was a significant decrease in coagulation time from DI5 to R+1, as it returned toward baseline upon re-ambulation following removal from the DI environment. This is like

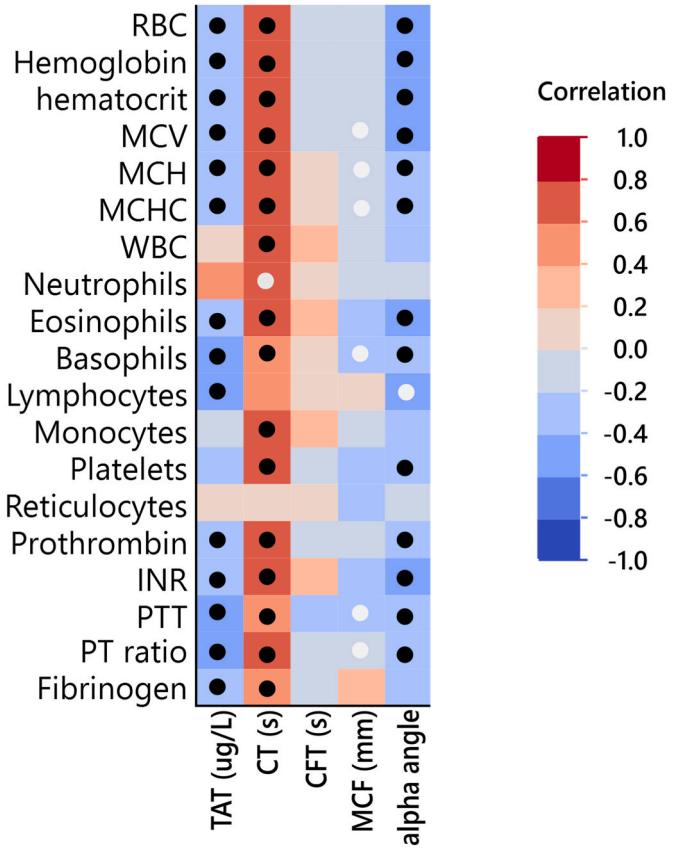

Fig. 5. Colour map of correlations for difference of means from BDC-1 and DI5 between TAT and ROTEM tests for this dry immersion investigation against blood parameter measures from the VIVALDI I campaign. Dots placed on top of correlation boxes represent significant relationships identified through robust FDR analysis, where the black dots represent significant relationships with $p < 0.01$ and white dots represent significant relationships $0.01 < p < 0.05$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

past HDTBR studies in males, which also showed a prolonged CT in response to HDTBR, followed by CT returning to normal upon re-ambulation [16]. Additionally, the lengthened CT may indicate an impaired ability to clot, similar to previous findings from Rosenfeld et al. regarding their observation of increased hypocoagulation activity in males in non-tilt bed rest [17].

Coagulation time is influenced by activities of clotting factors found within the coagulation cascade, such as Factor V. Two studies, where one investigated a decrease in Factor V activity and the other in Factor V deficiency, showed that reduced Factor V can result in prolonged CT in plasma [27,28]. Furthermore, CT was found to be prolonged in mice with Factor VIII and IX knockouts as well [29]. Shorter CT times, on the other hand, are seen in conditions of accelerated fibrin polymerization with the presence of medically provided fibrinogen and thrombin in trauma patients [30]. However, results reported from VIVALDI I by Robin et al. reported that fibrinogen levels were increased in these same participants [20], yet a prolonged CT was observed in the presented analysis here. Alpha angle, which measures the fibrin polymerization rate, was unchanged in this DI experiment, further contraindicating that CT would have been increased.

This likely means that there are more components leading to the prolongation of CT other than fibrinogen—perhaps activity of the previously mentioned coagulation factors. Looking at the expression levels of these proteins or their genes would allow us to assess potential influences of these factors. Also of note, heparin can increase bleeding time, resulting in prolonged clotting by acting together with antithrombin to prevent fibrin clot formation [31]. ROTEM CT tests are a tool used in the management of heparin therapy, which warrants additional research to see if heparin played a role here [32].

On the opposite end of hemostatic balance, dry immersion shortened clot formation time and increased clot stability in healthy females. The analysis of MCF and CFT revealed significant findings that potentially highlight an increase in procoagulant activity. This is slightly similar to the findings from an HDBR study performed with males, which showed trends of shortened CFT and increased MCF in these participants, which ultimately reached significance upon re-ambulation [15]. However, the results from this female DI study did not show any difference in CFT or MCF upon re-ambulation compared to the baseline measurements, which contrasts the male HDBR [15].

Increasing clot stability can be associated with a healthy hemostatic system, but evidence suggests that a notably shortened CFT and increased MCF are indicators of hypercoagulability across populations [33,34]. One study found that lung cancer patients with MCF near or above the upper limit of normal ranges developed a deep vein thrombosis (DVT) within a 12-month follow-up period [35]. With malignancy being a risk factor for thrombosis, another ROTEM study examined the coagulation states of specific cancer patients and found shortened CFT and high MCF values on all assay types when compared to their healthy counterparts [33]. Further, a study assessing 313 patients who underwent major surgery and developed postoperative thrombotic complications had significantly lowered CFT and elevated MCF ROTEM values [33]. The association of increased MCF and shortened CFT with clotting risk is concerning when considering these results with female dry immersion participants.

Maximum clot firmness is affected by fibrin, fibrinogen and/or thrombin concentration, platelets, factor XIII, as well as hematocrit [33,36]. A study of cancer patients with hypercoagulable states also showed that fibrinogen and platelets had a significant association with MCF and CFT [37]. A previous publication from the VIVALDI I study showed significant increases in fibrinogen, platelets, and hematocrit from these same participants on days DI3 and DI5, though these parameters remained within normal reference ranges [20]. This suggests that these specific increased blood parameters reported by Robin et al. [20] may be linked to the observed increase in MCF and shortened CFT observed in this investigation. Based on the correlational and FDR analysis of the ROTEM results to blood parameters in this DI study, changes in MCF

were associated with blood parameters related to hemoconcentration, such as MCH, although clinical relevance is difficult to confer since the blood parameters associated with these tests remained within clinically normal ranges [20].

To expand further on how blood parameters may lead to the ROTEM values observed here, hematocrit is another important factor to consider. Increased RBC within the increased hematocrit can also contribute to greater clot firmness because of the availability of RBC to contribute to the clot [38,39]. It is difficult to assume that the shortened CFT and increased MCF were related to changes in hemoconcentration as the samples came from platelet-poor plasma. It is possible that the changes in CFT and MCF are because of the heightened sensitivity of the trigger solution to detect very small shifts in hemostasis, including changes that occur before the cross-linking speed of fibrin increases—thus no corresponding change in alpha angle.

Changes in hematocrit can affect ROTEM measurements [40], which it did during DI [20]. Changes in hematocrit, such as hemodilution where hematocrit decreases, can cause lower coagulation factor concentrations and result in falsely prolonged coagulation time measurements—however, a hematocrit above 60 % can also prolong CT measurements [41]. However, this investigation showed prolonged CT with increased hematocrit levels never rising above 60 %, making this likely not the case [20].

It is also worth noting that TAT showed no significant changes in females in response to DI in this study. This differs from past HDTBR studies with males, who showed decreased TAT in response to simulated microgravity [16]. Similarly, alpha angle showed no changes in females during DI. However, this contrasts with findings in males, who showed increasing trends in alpha angle—reaching significance during re-ambulation in one HDTBR study—and significant decreases in another HDTBR study [15,16]. Further studies of ROTEM responses in males and females are likely needed to tease out these coagulation differences, including the discrepancy between the evidence in males [14].

The contrasting results of this study from previous investigations with male participants suggest that there may be sex differences within hemostatic control and, therefore, VTE risk astronauts may face when compared to their opposite-sex counterparts. Although the occlusive thrombus and suspected partially occlusive thrombus observed in the incidental finding on the ISS occurred in both a female and male crewmember, would suggest that all may be at risk [1]. However, only the fully occlusive thrombus was observed in real time in a female astronaut, with the partial thrombus only being observed retrospectively and is, as such, suspected rather than confirmed [1,42].

Future research that includes study designs that allow for comparison of the female and male hemostatic responses should provide evidence on specific preventive measures or treatments required based on the physiology of the astronaut heading to space. This differentiation would also be important for those earthbound patients subjected to bed rest for medical reasons and can help reduce the sex-related gap in female response data. Similarly, collection of more data on hemostatic responses to microgravity would improve safety around space venous-thrombosis risk.

The present study took place under simulated microgravity conditions for only five days. A short experimental time in simulated space which resulted in statistically, though not clinically, significant changes in hemostasis. This could be of concern and warrants further investigation since the average spaceflight mission to the ISS spans approximately six months, with rare cases of stays over one year. If five days in simulated space is enough to cause statistically significant shifts in hemostasis, one must wonder what coagulation activity is taking place 50, or even 90, days into a mission.

Future directions need to include a comprehensive assessment of the risk of VTE in space travellers if humanity hopes to extend long duration missions to reach a target like Mars. The potentially fatal complications of VTE development warrant better knowledge regarding when astronauts are at the highest risk of developing clots and what preventative measures must be taken to lower the risk of loss of human life on spaceflight missions. Therefore, collecting data throughout missions aboard the ISS can aid in understanding if, and when, coagulation parameters reach peaks to determine when the crew is at their highest risk of VTE.

5. Conclusions

The findings of this investigation provide evidence that female hemostatic equilibrium is sub-clinically disrupted by exposure to the dry immersion environment for five days and add to the evidence that hypercoagulability may be a key player in the development of VTE observed in space. Future investigations of longer duration could expose clinical shifts in coagulation, and further research into sex differences could shed more light on whether this is a sex-linked risk only, or whether spaceflight provides the unique conditions necessary for any spacefarers to develop this disease. Closer to home, this line of investigation may also provide further insight into whether there are sex-related risks to immobility for reasonably healthy females experiencing longer-term bedrest, from illness or injury, as current data only comes from males.

CRediT authorship contribution statement

T.E. Stead: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis. **G. Cvirn:** Writing – review & editing, Validation, Investigation, Data curation. **P. De Boever:** Writing – review & editing, Validation, Methodology, Investigation, Conceptualization. **A. Bergauer:** Writing – review & editing, Investigation. **D.A. Green:** Writing – review & editing, Validation, Investigation, Conceptualization. **O. White:** Writing – review & editing, Conceptualization. **H.E. Arron:** Writing – review & editing. **A.P. Blaber:** Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **N. Goswami:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.actaastro.2025.11.065.

References

[1] K. Marshall-Goebel, S.S. Laurie, I.V. Alferova, P. Arbeille, S.M. Auñón-Chancellor, D.J. Ebert, S.M.C. Lee, B.R. MacIas, D.S. Martin, J.M. Pattarini, R. Ploutz-Snyder, L. C. Ribeiro, W.J. Tarver, S.A. Dulchavsky, A.R. Hargens, M.B. Stenger, Assessment of jugular venous blood flow stasis and thrombosis during spaceflight, JAMA Netw. Open 2 (2019), https://doi.org/10.1001/JAMANETWORKOPEN.2019.15011.

[2] S. McRae, Hypercoagulable States, Mechanisms of Vascular Disease: A Reference Book for Vascular Specialists. https://doi.org/10.1017/UPO9781922064004.011, 2011.

[3] R.L.K. Virchow, Thrombosis and Emboli, Canton Science History Publications, 1998.

[4] D.W. Schwertz, S. Penckofer, Sex differences and the effects of sex hormones on hemostasis and vascular reactivity, Heart Lung 30 (2001) 401–428, https://doi.org/10.1067/MHL.2001.118764.
[5] S.A. Smith, R.J. Travers, J.H. Morrissey, How it all starts: initiation of the clotting cascade, Crit. Rev. Biochem. Mol. Biol. 50 (2015) 326–336, https://doi.org/10.3109/10409238.2015.1050550.
[6] A.J. Gale, Continuing education course #2: current understanding of hemostasis, Toxicol. Pathol. 39 (2011) 273–280, https://doi.org/10.1177/0192623310389474.
[7] J.H. Levy, F. Szlam, A.S. Wolberg, A. Winkler, Clinical use of the activated partial thromboplastin time and prothrombin time for screening: a review of the literature and current guidelines for testing, Clin. Lab. Med. 34 (2014) 453–477, https://doi.org/10.1016/J.CLL.2014.06.005.
[8] D. Whiting, J.A. Dinardo, TEG and ROTEM: technology and clinical applications, Am. J. Hematol. 89 (2014) 228–232, https://doi.org/10.1002/AJH.23599.
[9] S.V. Mallett, D.J.a. Cox, Thrombelastography, Br. J. Anaesth. 69 (1992) 307–313, https://doi.org/10.1093/BJA/69.3.307.
[10] K. Rimpo, A. Tanaka, M. Ukai, Y. Ishikawa, M. Hirabayashi, T. Shoyama, Thrombin-antithrombin complex measurement using a point-of-care testing device for diagnosis of disseminated intravascular coagulation in dogs, PLoS One 13 (2018), https://doi.org/10.1371/JOURNAL.PONE.0205511.
[11] G. Cvirn, J.E. Waha, B. Brix, A. Rössler, A. Jantscher, A. Schlagenhauf, M. Koestenberger, W. Wonisch, T. Wagner, N. Goswami, Coagulation changes induced by lower-body negative pressure in men and women, J. Appl. Physiol. 126 (1985) (2019) 1214–1222, https://doi.org/10.1152/JAPPLPHYSIOL.00940.2018.
[12] B. Sørensen, P. Johansen, K. Christiansen, M. Woelke, J. Ingerslev, Whole blood coagulation thrombelastographic profiles employing minimal tissue factor activation, J. Thromb. Haemostasis 1 (2003) 551–558, https://doi.org/10.1046/J.1538-7836.2003.00075.X.
[13] C. Sucker, K. Tharra, J. Litmathe, R.E. Scharfv, R.B. Zotz, Rotation thromboelastography (ROTEM) parameters are influenced by age, gender, and oral contraception, Perfusion 26 (2011) 334–340, https://doi.org/10.1177/0267659111407695.
[14] D.S. Kim, S. Vaquer, L. Mazzolai, L.N. Roberts, J. Pavela, M. Watanabe, G. Weerts, D.A. Green, The effect of microgravity on the human venous system and blood coagulation: a systematic review, Exp. Physiol. 106 (2021) 1149–1158, https://doi.org/10.1113/EP089409.
[15] T. Haider, H.C. Gunga, R. Matteucci-Gothe, E. Sottara, A. Griesmacher, D.L. Belavý, D. Felsenberg, A. Werner, W. Schobersberger, Effects of long-term head-down-tilt bed rest and different training regimes on the coagulation system of healthy men, Phys. Rep. 1 (2013) 1–13, https://doi.org/10.1002/PHY2.135.
[16] G. Cvirn, J.E. Waha, G. Ledinski, A. Schlagenhauf, B. Leschnik, M. Koestenberger, E. Tafeit, H. Hinghofer-Szalkay, N. Goswami, Bed rest does not induce hypercoagulability, Eur. J. Clin. Invest. 45 (2015) 63–69, https://doi.org/10.1111/ECI.12383.
[17] B.A. Rosenfeld, N. Faraday, D. Campbell, N. Sakima, W. Bell, The effects of bedrest on circadian changes in hemostasis, Thromb. Haemost. 72 (1994) 281–284.
[18] N.M. Navasiolava, M.A. Custaud, E.S. Tomilovskaya, I.M. Larina, T. Mano, G. Gauquelin-Koch, C. Gharib, I.B. Kozlovskaya, Long-term dry immersion: review and prospects, Eur. J. Appl. Physiol. 111 (2011) 1235–1260, https://doi.org/10.1007/S00421-010-1750-X.
[19] E. Tomilovskaya, T. Shigueva, D. Sayenko, I. Rukavishnikov, I. Kozlovskaya, Dry immersion as a ground-based model of microgravity physiological effects, Front. Physiol. 10 (2019), https://doi.org/10.3389/FPHYS.2019.00284.
[20] A. Robin, A. Van Ombergen, C. Laurens, A. Bergouignan, L. Vico, M.T. Linossier, A. Pavy-Le Traon, M. Kermorgant, A. Chopard, G. Py, D.A. Green, M. Tipton, A. Choukér, P. Denise, H. Normand, S. Blanc, C. Simon, E. Rosnet, F. Larcher, P. Fernandez, I. de Glisezinski, D. Larrouy, I. Harant-Farrugia, I. Antunes, G. Gauquelin-Koch, M.P. Bareille, R. Billette De Villemeur, M.A. Custaud, N. Navasiolava, Comprehensive assessment of physiological responses in women during the ESA dry immersion VIVALDI microgravity simulation, Nat. Commun. 14 (2023), https://doi.org/10.1038/S41467-023-41990-4.
[21] P. Arbeille, P. Avan, L. Treffel, K. Zuj, H. Normand, P. Denise, Jugular and portal vein volume, middle cerebral vein velocity, and intracranial pressure in dry immersion, Aerosp. Med. Hum. Perform. 88 (2017) 457–462, https://doi.org/10.3357/AMHP.4762.2017.
[22] P. Arbeille, D. Greaves, L. Guillon, S. Besnard, Thigh cuff effects on venous flow redistribution during 4 days in dry immersion, Aerosp. Med. Hum. Perform. 91 (2020) 697–702, https://doi.org/10.3357/AMHP.5524.2020.
[23] L. Sunnersjö, H. Lindström, U. Schött, N. Törnquist, T. Kander, The precision of ROTEM EXTEM is decreased in hypocoagulable blood: a prospective observational study, Thromb. J. 21 (2023), https://doi.org/10.1186/S12959-023-00468-5.
[24] Y. Benjamini, Y. Hochberg, Controlling the false discovery rate: a practical and powerful approach to multiple testing, J. Roy. Stat. Soc. B 57 (1995) 289–300, https://doi.org/10.1111/J.2517-6161.1995.TB02031.X.
[25] P.J. Huber, E.M. Ronchetti, Robust statistics. Robust Statistics, 2009, pp. 1–354, https://doi.org/10.1002/9780470434697. Second Edition.
[26] K. Korthauer, P.K. Kimes, C. Duvallet, A. Reyes, A. Subramanian, M. Teng, C. Shukla, E.J. Alm, S.C. Hicks, A practical guide to methods controlling false discoveries in computational biology, Genome Biol. 20 (2019), https://doi.org/10.1186/S13059-019-1716-1.
[27] T. Haas, M.M. Cushing, L.M. Asmis, Influence of factor V deficiency on ROTEM® clotting time, Blood 124 (2014) 5039, https://doi.org/10.1182/BLOOD.V124.21.5039.5039.
[28] L. Spiezia, C. Radu, E. Campello, C. Bulato, D. Bertini, G. Barillari, V. De Angelis, P. Pradella, E. Zanon, P. Simioni, Whole blood rotation thromboelastometry (ROTEM(®)) in nine severe factor V deficient patients and evaluation of the role of intraplatelets factor V, Haemophilia 18 (2012) 463–468, https://doi.org/10.1111/J.1365-2516.2011.02710.X.
[29] J.A. Schroeder, E.A. Kuether, J. Fang, W. Jing, H. Weiler, D.A. Wilcox, R. R. Montgomery, Q. Shi, Thromboelastometry assessment of hemostatic properties in various murine models with coagulopathy and the effect of factor VIII therapeutics, J. Thromb. Haemostasis 19 (2021) 2417–2427, https://doi.org/10.1111/JTH.15456.
[30] M. Ponschab, W. Voelckel, M. Pavelka, C.J. Schlimp, H. Schöchl, Effect of coagulation factor concentrate administration on ROTEM® parameters in major trauma, Scand. J. Trauma Resuscitation Emerg. Med. 23 (2015), https://doi.org/10.1186/S13049-015-0165-4.
[31] D.L. Rabenstein, Heparin and heparan sulfate: structure and function, Nat. Prod. Rep. 19 (2002) 312–331, https://doi.org/10.1039/B100916H.
[32] M. Mittermayr, J. Margreiter, C. Velik-Salchner, A. Klingler, W. Streif, D. Fries, P. Innerhofer, Effects of protamine and heparin can be detected and easily differentiated by modified thrombelastography (Rotem): an in vitro study, Br. J. Anaesth. 95 (2005) 310–316, https://doi.org/10.1093/BJA/AEI197.
[33] O.M. Akay, The double hazard of bleeding and thrombosis in hemostasis from a clinical point of view: a global assessment by rotational thromboelastometry (ROTEM), Clin. Appl. Thromb. Hemost. 24 (2018) 850–858, https://doi.org/10.1177/1076029618772336.
[34] N. van Veenendaal, T.W.L. Scheeren, K. Meijer, P.H.J. van der Voort, Rotational thromboelastometry to assess hypercoagulability in COVID-19 patients, Thromb. Res. 196 (2020) 379–381, https://doi.org/10.1016/J.THROMRES.2020.08.046.
[35] N.A. Davies, N.K. Harrison, A. Sabra, M.J. Lawrence, S. Noble, S.J. Davidson, V. J. Evans, R.H.K. Morris, K. Hawkins, P.R. Williams, P.A. Evans, Application of ROTEM to assess hypercoagulability in patients with lung cancer, Thromb. Res. 135 (2015) 1075–1080, https://doi.org/10.1016/J.THROMRES.2015.03.021.
[36] O.M. Theusinger, W. Baulig, L.M. Asmis, B. Seifert, D.R. Spahn, In vitro factor XIII supplementation increases clot firmness in rotation thromboelastometry (ROTEM), Thromb. Haemost. 104 (2010) 385–391, https://doi.org/10.1160/TH09-12-0858.
[37] O.M. Akay, Z. Ustuner, Z. Canturk, F.S. Mutlu, Z. Gulbas, Laboratory investigation of hypercoagulability in cancer patients using rotation thrombelastography, Med. Oncol. 26 (2009) 358–364, https://doi.org/10.1007/S12032-008-9129-0.
[38] K.C. Gersh, C. Nagaswami, J.W. Weisel, Fibrin network structure and clot mechanical properties are altered by incorporation of erythrocytes, Thromb. Haemost. 102 (2009) 1169–1175, https://doi.org/10.1160/TH09-03-0199.
[39] S. Kattula, J.R. Byrnes, S.M. Martin, L.A. Holle, B.C. Cooley, M.J. Flick, A. S. Wolberg, Factor XIII in plasma, but not in platelets, mediates red blood cell retention in clots and venous thrombus size in mice, Blood Adv. 2 (2018) 25–35, https://doi.org/10.1182/BLOODADVANCES.2017011890.
[40] S.A. Smith, M.A. McMichael, S. Gilor, A.J. Galligan, C.M. Hoh, Correlation of hematocrit, platelet concentration, and plasma coagulation factors with results of thromboelastometry in canine whole blood samples, Am. J. Vet. Res. 73 (2012) 789–798, https://doi.org/10.2460/AJVR.73.6.789.
[41] S.M. Bates, J.I. Weitz, Coagulation assays, Circulation 112 (2005), https://doi.org/10.1161/CIRCULATIONAHA.104.478222.
[42] M.M. Elahi, A.N. Witt, E.L.G. Pryzdial, P.B. McBeth, Thrombotic triad in microgravity, Thromb. Res. 233 (2024) 82–87, https://doi.org/10.1016/J.THROMRES.2023.11.020.