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# Aerospace Medicine and Human Performance

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# Aerospace Medicine and Human Performance

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**This journal, representing the members of the Aerospace Medical Association, is published for those interested in aerospace medicine and human performance. It is devoted to serving and supporting all who explore, travel, work, or live in hazardous environments ranging from beneath the sea to the outermost reaches of space.**



**AEROSPACE MEDICAL ASSOCIATION** is an organization devoted to charitable, educational, and scientific purposes. The Association was founded when the rapid expansion of aviation made evident the need for physicians with specialized knowledge of the flight environment. Since then, physicians have been joined in this Association by professionals from many fields and from many countries, all linked by a common interest in the health and safety of those who venture into challenging environments.

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# Aerospace Medicine and Human Performance

OCTOBER 2023 VOLUME 94 NUMBER 10

# 94th AsMA Annual Scientific Meeting: "Honoring the Past…Preparing for the Future"

## Hyatt Regency Hotel, Chicago, IL, USA
## May 5 – 9, 2024

# Call for Abstracts

# Deadline: November 1, 2023
# *No Exceptions!*

The Aerospace Medical Association's 2024 Annual Scientific Meeting will be held in Chicago, IL, USA. The year's theme is **"Honoring the Past ... Preparing for the Future."** Since announcement of the Artemis program, efforts are underway to return humans to the Moon after more than 50 years. Throughout the Apollo, Skylab, ISS, and now Commercial Crew program, substantial advances in technology have arisen, and a greater understanding of human physiology and performance have progressed through longer duration spaceflight. Lunar missions will entail combined governmental, commercial, and International Partner collaboration. Extravehicular activity, environmental, and habitation challenges will be substantial. General, civil, and military aviation have also seen significant advances over past decades. Human factors, safety, mental health, and environmental aspects merit continued vigilance. Expansion of unpiloted aerial vehicles and eventual transorbital flight provide unique challenges. Advanced telescopes are re-writing the Astronomy textbooks with the search for planetary locales potentially harboring the building blocks of life. Developing the next generation of scientists, engineers, researchers, and clinicians for the exciting years ahead requires our collective energies.

The Annual Scientific Meeting is the premier international forum to learn and discuss evolving trends and multidisciplinary best practices in research, clinical applications, human performance, and flight safety. The 94th Annual Scientific Meeting welcomes abstracts in the many areas related to Aerospace Medicine. For a complete list see the box on p. 2 of this form.

**ASMA ABSTRACT SUBMISSION PROCESS**

**LIMIT: 350 words/2500 characters including spaces; NO Tables or Figures or References should be included in the abstract. All abstracts must be submitted via the electronic submission system linked on the association's web site: *https://www.asma.org*.**

**ATTENTION:** You **MUST** use personal email addresses when entering your abstracts and those of your co-authors.

**ABSTRACT TYPES AND CATEGORIES**

The Annual Scientific Meeting highlights several types of presentation formats. Posters are on display for two full conference days, each in its assigned space. Authors will be asked to present their poster for a single designated 120-min period on one of these days. PowerPoint presentations will be organized by topic area and presented during 90-minute blocks of time, 6 periods of 15 minutes each. **Individual PowerPoint presentations are limited to 15 minutes,** including 3 to 5 minutes for questions and discussion. Panels also have 90 minutes: ideally 5 presentations of 15 minutes each, followed by a 15-minute discussion period.

There are four **TYPES** of submissions:

**1. Poster:** Standalone Digital Poster presentation that will be integrated into a session, grouped by topic. The presntation must be submitted as a PowerPoint with up to 10 slides. Video and audio clips can be embedded. They will be displayed digially.

**2. PowerPoint:** Standalone 15-minute slide presentation with questions/discussion that will be integrated into a session, grouped by topic.

**3. Individual Invited Panel:** Invited Presentation that will link to support a Panel Overview containing five (non-case study) or six (case study) abstracts presented as a cohesive whole.

**4. Individual Invited Workshop:** Invited Presentation that will link to and support a Workshop Overview.

**CATEGORIES**

There are two categories based on the topic to be presented. Templates and examples (examples available on the submission site) are provided for each type and will be available at the abstract submission website. Authors will be required to enter abstract text under the headings as described below.

**1. Original Research:** Material that is original in nature and has not been previously presented. Original analysis of a hypothesis involving data collection and analysis. Headings include Introduction, Methods, Results and Discussion.

**2. Education:** Typically, a discussion of information that is already available.

***a. Program / Process Review:*** Description of a program or process that is used to solve a problem or accomplish a task. Headings include Background, Description, and Discussion.

***b. Tutorial /Review:*** An educational session intended as a review of established material. Headings include Introduction, Topic, and Application.

***c. Case Study:*** A single clinical or human performance event. Headings include Introduction, Case Description, and Discussion.

**PANEL GUIDANCE**

**Panels** must be composed of a coordinated sequence of 4-5 abstracts that flow logically from one to another supporting the central theme. Panels must contain abstracts that allow 15 minutes of structured discussion at the end of the session.

**Case Study Panels:** Case Study Panels can have 6 abstracts, and are intended to highlight a particular institution, community or aeromedical issue, usually presented from the same institution or aeromedical community.

***It is the responsibility of the Panel Chairs to ensure that the abstract authors describe in each abstract how it relates to the Panel theme.*** If the Panel theme is not clearly identified and/or the abstracts do not support a central theme, the Scientific Programming Committee may unbundle individual abstracts and evaluate them as separate slide or poster abstracts. Unrelated abstracts from a laboratory or organization do not constitute a Panel (unless they are Case Studies). Panel Chairs are also responsible for preparing questions and discussion points to facilitate a moderated discussion with the audience during the sixth period. Each Panel speaker should cite or link directly to the Panel theme, and at the end of their talk should provide a logical segue to the next abstract.

**WORKSHOPS**

Rules for workshops and the review process are similar to those for Panels (above). Overview abstracts should reflect the material to be presented in this long format for up to 8 hours of CME credit. Individual abstracts must be entered for each invited presenter and all necessary information must be entered in the same

manner as all other abstracts, including conflict of interest statements. Course materials should be made available for registrants. A separate fee is charged for Workshops registration. For additional information contact Jeff Sventek, Executive Director, at jsventek@asma.org.

**AsMA ABSTRACT SUBMISSION PROCESS**

All abstracts must be submitted via the electronic submission system linked to the association's web site: https://www.asma.org. Click on the link to the abstract submission site--available on the AsMA home page and Meetings page on or about September 1, 2023. Authors with questions regarding the abstract submission process should contact AsMA directly at (703) 739-2240, x 101 (Ms. Rachel Trigg); or e-mail rtrigg@asma.org.

The following information is required during the submission process: Abstract title, presenting author information (including complete mailing and e-mail addresses and telephone numbers), topic area (from list provided on back of form), contributing authors and their e-mails and institutions, abstract **(LIMIT: 350 words/2500 characters including spaces)**, **at least 2 Learning Objectives** (the Accreditation Council for Continuing Medical Education-ACCME requires brief statements on the speaker's learning objectives for the audience). In addition, three (3) multiple choice questions and answers will be required for each Slide and Panel presentation for Enduring Materials for CME credit. Read instructions online for further details. Poster presenters are required to upload their poster as a PowerPoint in advance of the meeting.

**PLEASE NOTE:** Presenters (including panelists) are required to register for the meeting. There is a discounted fee for non-member presenters. *Registration limited to the day of presentation will be available onsite.*

**Financial Disclosure/Conflict of Interest/Ethics**

Abstracts will not be accepted without a financial disclosure/conflict of interest form. The form is included in the website submission process. The presenting author must agree to comply. Scientific presentations at AsMA-sponsored events will adhere to the highest standards of scientific ethics, including appropriate acknowledgment or reference to scientific and/or financial sources. *Presenters must avoid the endorsement of commercial products in their abstracts and during their presentations. There must be no advertisements on Posters, AV, or handout materials.*

**Presentation Retention Policy**

AsMA will use live capture to make presentations from the Meeting available to members / attendees after the meeting. Authors are required to provide permission for live capture and a nonexclusive license to repurpose the content. An electronic copy of the presentation suitable for release at the time of the presentation must be provided. Electronic copies of Poster presentations must be uploaded to a submission site when directed.

**Permissions and Clearances**

It is the author's responsibility to obtain all necessary permissions and clearances prior to submission of the abstract. AsMA assumes no liability or responsibility for the publication of any submitted material.

**Acceptance Process**

Abstracts will be reviewed by a minimum of three members of the AsMA Scientific Program Committee. Acceptance will be based on the abstract's originality, relevance, scientific quality, and adherence to the guidelines provided. Criteria for non-acceptance include, but are not limited to: insufficient, inconsistent, or ambiguous data; commercialism; or reviews of previously published literature. **Abstracts must be 100% complete upon submission, including all final data and results.** How well authors abide by submission and format guidelines will also be one of the criteria used to determine acceptance of abstracts.

Presenters are limited to **one** senior-authored presentation, unless given specific prior permission by the Scientific Program Committee Chair, Dr. Eilis Boudreau, at: sciprog@asma.org. Following review by the Scientific Program Committee in November, all contributors will receive a notification of acceptance or non-acceptance by e-mail. Accepted abstracts will be published in *Aerospace Medicine and Human Performance*.

While the Scientific Program Committee strives to honor the presenter's desired presentation format, for reasons such as space limitations or dissimilar content, an abstract may be changed to an alternative presentation format. Assignment of an abstract to either a poster or a slide presentation will be recommended by the Scientific Program Committee, but the final decision will be made by the Program Chair.

**Abstract Withdrawal**

Withdrawing abstracts is strongly discouraged. However, if necessary, a request to withdraw an abstract should be sent to Dr. Eilis Boudreau, the Scientific Program Chair, at sciprog@asma.org; and Rachel Trigg at rtrigg@asma.org. The request for withdrawal must include the abstract title, authors, ID number, and reason for withdrawal. Due to publishing deadlines, withdrawal notification should be received by January 15, 2023. As abstracts are published in *Aerospace Medicine and Human Performance* prior to the scientific meeting, a list of abstracts withdrawn or not presented will be printed in the journal following the annual meeting.

**MENTORSHIP**

**Optional review / feedback for student and resident presenters at AsMA 2024**

AsMA is continuing its mentorship initiative for student and resident authors for the 2024 Scientific Meeting. You have the option to submit a draft of your abstract to a group of senior AsMA members for review and feedback. If you have questions about this opportunity, please e-mail sciprog@asma.org. E-mail your abstract to sciprog@asma.org no later than 1 October 2023. The Program Mentor Group will review provide feedback via e-mail by 20 October 2023. The abstract will still need to be finalized in the submission system.

**TOPIC AREAS:** (These will be listed on a drop-down menu on the submission site. They are used to organize the abstracts into sessions.)

**1: Human Performance**
1.1 Personnel Selection
1.2 Training
1.3 Hypobaric & Hyperbaric Physiology
1.4 Thermal Physiology
1.5 Acceleration / Vibration/ Impact
1.6 Fatigue
1.7 Neurophysiology & Sensory (inc. Vision, Auditory, Vestibular, Spatial Disorientation)
1.8 Aerospace Human Factors & Psychology
1.9 Aerospace Human Systems Integration

**2: Clinical Medicine**
2.1 Aviation Medicine
2.2 Health Promotion and Wellness Programs
2.3 Medical Standards / Aircrew Health
2.4 Occupational / Environmental Medicine
2.5 Operational Medicine
2.6 Hyperbaric Medicine

**3: Travel and Transport Medicine**
3.1 Travel Medicine
3.2 Aeromedical Transport / Air Evacuation
3.3 Air Transport Medicine
3.4 Commercial
3.5 Pandemic Preparedness

**4: Space Medicine**
4.1 Space Medicine
4.2 Space Operations

**5: Safety and Survivability**
5.1. Escape / Survival
5.2. Flight Safety/Accident Investigation

**6: Other**
6.1 History of Aerospace Medicine
6.2 Ethics

Follow the link to the abstract submission site on our home page: **https://*www.asma.org***
**Deadline is November 1, 2023 (NO EXCEPTIONS!!!!!!!)**

## Authors!

The cost of color printing has dropped significantly. Please consider printing your Figures and Images in full color for the next issue of *Aerospace Medicine and Human Performance*. If interested, download the Agreement to Pay Extra Charges form from Editorial Manager for your next submission.

**$60**
per figure

...down from $900 for only the first page!

### 2023 FAA Seminars

| | | |
|---|---|---|
| Oct. 5-7 | Omaha, NE | CAMA |
| Oct. 23-27 | Oklahoma City, OK | Basic |
| Nov.17-19 | Jacksonville, FL | Refresher |

See http://www.faa.gov/other_visit/aviation_industry/designees_delegations/designee_types/ame/seminar_schedule/ for more information.

### Read Current News Online!

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## CALL FOR AUTHORS

- Join a project spearheaded by the History and Archives Committee as we prepare for the upcoming **100th anniversary** of AsMA in 2029!
- The Committee is seeking **authors interested in contributing a chapter to a book** that examines the ways in which the fields of aviation and aerospace medicine have changed over the past century, as reflected by our Society through the years.
- Help us celebrate the history of our organization and the innovations in our field that many of you have not only witnessed but engineered.
- Please **contact Committee Chair Walt Dalitsch at walt3@dalitsch.com** if interested.


## The Need For SPEED

*Farther, faster, together.*

The Aerospace Medical Association Foundation is working to accelerate its efforts by empowering the next generation of Aerospace Medicine scientists who will take humans to deep space. In order to achieve these objectives, they are setting a goal in "The Need for Speed" campaign of $5 million by AsMA's 100th Anniversary! Donations can be in cash or in stock and can be made by credit card or PayPal through the AsMAFoundation.org website. AsMA Members: consider joining the Heritage Society and include the Foundation in your estate planning.

**Support the Foundation!**

# Congratulations, American Board of Preventive Medicine: 75th Anniversary!

Joseph Dervay, M.D., M.P.H., MMS, FACEP, FAsMA, FUHM

The American Board of Preventive Medicine (ABPM) has reached the 75th year Anniversary milestone! Many congratulations.

As shared by ABPM Chair, Dr. Wendy Braund, "ABPM has expanded from a small group of certified Public Health physicians to a diverse community of well over 10,000 physicians certified in three specialties and four subspecialities. ABPM owes its growth to the talented, thoughtful, and hard-working physician leadership it has enjoyed through its history."

ABPM has striven with a collective commitment and goal to create a healthier world through the practice of Preventive Medicine. The three specialties are: Aerospace Medicine, Occupational and Environmental Medicine, and Public Health & General Preventive Medicine. The four subspecialties include: Addiction Medicine, Clinical Informatics, Medical Toxicology, and Undersea & Hyperbaric Medicine. The breadth of care indeed ranges from the sea to space.

Our Executive Director Jeff Sventek and I were invited by ABPM to represent the Aerospace Medical Association (AsMA) at the 75th Anniversary Gala in Chicago this August. We were also joined by five AsMA Past Presidents who were able to attend the celebration and who previously served on the ABPM Board of Directors: Drs. George Anderson, Jeffrey Davis, Richard Jennings, Glenn Mitchell, and Joe Ortega. Three of those attending, Drs. Anderson, Davis, and Ortega, also served as a prior Chair of ABPM. Current AsMA members serving on the ABPM Board of Directors include Drs. Brent Klein, Tim LaVan, Joanna Nelms, David Miller, and recent Vice Chair for Aerospace Medicine Dr. Cheryl Lowery. *We thank and honor all prior AsMA members who served ABPM over the years for their dedicated service and contributions. You have all truly made a difference for our specialty!*

Dr. Duncan Hughes, AsMA member and Virgin Galactic Chief Medical Officer, served as the keynote speaker. He shared insights into the Virgin Galactic flight program and highlighted some very recent flight activity. As Dr. Braund noted, "Dr. Hughes provides a unique perspective, not only on the value of ABPM Certification, but also on the exciting work of Space Medicine that so many of our Diplomats engage in today."

I had the opportunity to share some remarks at the celebration, on behalf of AsMA, and thanked ABPM for their longstanding professionalism, collegiality, and hard work over the years supporting the specialty of Aerospace Medicine. As a token of gratitude and congratulation from AsMA upon their anniversary, Jeff Sventek and I presented ABPM with a lovely, engraved crystal glass gift for display at their Home Office. The gift and acknowledgement from AsMA were very much appreciated by ABPM.

Preceding the ABPM event, the AsMA Executive Committee (EXCOM) held its summer meeting at the Hyatt Regency in Chicago, the site of the 2024 Annual Scientific Meeting. Over a two-and-a-half-day period, several activities were on the docket. A site visit was completed of the Hyatt and the various spaces that will be used for events ranging from registration, opening ceremonies, presentation rooms of various sizes, locales for luncheons and committee meetings, and exhibits. The hotel liaison and staff were very gracious and addressed our questions and concerns. Leadership from the AsMA Wing joined the site visit and explored various Wing activities in Chicago which they may incorporate into their schedule for next May. The streets of downtown Chicago were indeed bustling with people, shops, and a wide range of restaurants. We felt totally safe walking about and enjoyed the views of the Chicago River, Lake Michigan, shoreline, and a wide range of activities at the Navy Pier complex.

The ensuing formal EXCOM meeting over a day and a half was excellent and covered a wide range of agenda items regarding AsMA. Many thanks to the EXCOM members for their focus and collegial nature throughout our proceedings. I will share more information about our discussions and initiatives during a future correspondence.

There is no doubt we are in store for a wonderful Annual Scientific Meeting and venue in Chicago in May 2024.

*Keep 'em flying…and Full Steam Ahead!*


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**Email:** President@asma.org • **Web site:** www.asma.org • **Facebook:** Aerospace Medical Association • **Twitter:** @Aero_Med

# Early Human Pathophysiological Responses to Exertional Hypobaric Decompression Stress

Desmond M. Connolly; Leigh A. Madden; Victoria C. Edwards; Timothy J. D'Oyly; Stephen D. R. Harridge; Thomas G. Smith; Vivienne M. Lee

**INTRODUCTION:** Consistent blood biomarkers of hypobaric (altitude) decompression stress remain elusive. Recent laboratory investigation of decompression sickness risk at 25,000 ft (7620 m) enabled evaluation of early pathophysiological responses to exertional decompression stress.

**METHODS:** In this study, 15 healthy men, aged 20–50 yr, undertook 2 consecutive (same-day) ascents to 25,000 ft (7620 m) for 60 and 90 min, breathing 100% oxygen, each following 1 h of prior denitrogenation. Venous blood was sampled at baseline (T0), immediately after the second ascent (T8), and next morning (T24). Analyses encompassed whole blood hematology, endothelial microparticles, and soluble markers of cytokine response, endothelial function, inflammation, coagulopathy, oxidative stress, and brain insult, plus cortisol and creatine kinase.

**RESULTS:** Acute hematological effects on neutrophils (mean 72% increase), eosinophils (40% decrease), monocytes (37% increase), and platelets (7% increase) normalized by T24. Consistent elevation (mean five-fold) of the cytokine interleukin-6 (IL-6) at T8 was proinflammatory and associated with venous gas emboli (microbubble) load. Levels of C-reactive protein and complement peptide C5a were persistently elevated at T24, the former by 100% over baseline. Additionally, glial fibrillary acidic protein, a sensitive marker of traumatic brain injury, increased by a mean 10% at T24.

**CONCLUSIONS:** This complex composite environmental stress, comprising the triad of hyperoxia, decompression, and moderate exertion at altitude, provoked pathophysiological changes consistent with an IL-6 cytokine-mediated inflammatory response. Multiple persistent biomarker disturbances at T24 imply incomplete recovery the day after exposure. The elevation of glial fibrillary acidic protein similarly implies incomplete resolution following recent neurological insult.

**KEYWORDS:** decompression stress, hyperoxia, exertion, oxidative stress, venous gas emboli (VGE), biomarkers.

**Connolly DM, Madden LA, Edwards VC, D'Oyly TJ, Harridge SDR, Smith TG, Lee VM. *Early human pathophysiological responses to exertional hypobaric decompression stress*. Aerosp Med Hum Perform. 2023; 94(10):738–749.**

The pathophysiological basis of hypobaric (altitude) decompression stress is poorly defined, while consistent blood biomarkers of hyperbaric (diving) decompression sickness (DCS) remain elusive. A recent altitude chamber study evaluated risk of DCS in physically active men exposed twice in quick succession to a pressure altitude of 25,000 ft (7620 m), enabling investigation of pathophysiological responses to hypobaric stress using selected biomarkers.[6]

The primary mechanism of physical and cellular injury in DCS is the generation of bubbles in tissues supersaturated with inert gas. Besides mechanical obstruction of vessels, intravascular bubbles initiate procoagulant and proinflammatory responses through "foreign body" surface interaction with blood proteins, cellular components, and vascular endothelium, triggering platelet aggregation, leukocyte activation, cytokine and chemokine release, and activation of complement, kinin, and coagulation systems.[3] Gaseous microemboli damage endothelial cells and surface glycocalyx, compromising vascular integrity, increasing permeability, and facilitating further bubble adhesion.[18] The release of

From QinetiQ PLC, Farnborough, Hampshire, UK.

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microparticles (MPs) reflects endothelial dysfunction secondary to oxidative stress and interaction with bubbles, influencing the systemic response to decompression.[13] MPs may be aggravated by circulating bubbles and act as foci for further bubble formation.[23,27] Local initiation of inflammatory and coagulant cascades can result in persistent effects even after the bubbles have passed, e.g., small gas emboli in the cerebral microcirculation promoting endothelial disruption with focal ischemia, inflammation, and edema.

The goal of the current study was to evaluate the pathophysiological basis of nonhypoxic altitude exposure by investigating human biomarker responses to severe hypobaric decompression stress. Inconsistent outcomes from previous research leave a broad range of potential biomarkers of interest. Specific targets selected for analysis crossed six areas, comprising cytokine responses, inflammatory markers, coagulation markers, endothelial function, oxidative stress, and brain insult/stress. **Table I** lists the final choice of target proteins.

Potent inflammatory cytokines, implicated in previous studies of decompression stress, include interleukin-1β (IL-1β), IL-6, IL-8, and interferon gamma (IFN-γ).[2,8] Chosen inflammatory markers include C-reactive protein (CRP), an acute phase protein that increases oxidative stress and correlates inversely with endothelial function, and neutrophil gelatinase-associated lipocalin (NGAL), a marker of neutrophil activation that is elevated by regular daily diving. Additionally, complement peptide C5a is elevated upon complement activation by the alternative pathway, the potential mechanism of action of intravascular bubbles, and may promote oxidative injury to pulmonary endothelium.[26] Markers of coagulopathy are coagulation factor III, also known tissue factor (CFIII/TF) or thromboplastin, primary initiator of the extrinsic coagulation pathway, and Platelet Factor 4 (PF4), a marker of platelet activation that is elevated in animal studies of hyperbaric DCS.

The hallmark of endothelial activation is cell surface expression of adhesion molecules, triggering leukocyte interaction

**Table I.** Assay Test Kit Manufacturers/Suppliers.

| BIOMARKER GROUP | TARGET | ASSAY TYPE | SUPPLIER | WEB REFERENCE |
|---|---|---|---|---|
| Cytokines | IL-1β | ELISA | R&D Systems | human-il-1-beta-il-1f2-quantikine-hs-elisa-kit_hslb00d |
| | IL-6 | ELISA | R&D Systems | human-il-6-quantikine-hs-elisa-kit_hs600c |
| | IL-8 | ELISA | R&D Systems | human-il-8-cxcl8-quantikine-elisa-kit_d8000c |
| | IL-10 | ELISA | R&D Systems | human-il-10-quantikine-hs-elisa-kit_hs100c |
| | IFNγ | ELISA | R&D Systems | human-ifn-gamma-quantikine-elisa-kit_dif50c |
| Inflammation | CRP | ELISA | Enzo Life Sciences | ENZ-KIT102/crp-human-elisa-kit/ |
| | C5a | ELISA | Thermo Fisher Scientific | Complement-C5a-Human-ELISA-Kit/BMS2088 |
| | NGAL | ELISA | Thermo Fisher Scientific | NGAL-Human-ELISA-Kit/KIT036 |
| Coagulopathy | CFIII/TF | ELISA | R&D Systems | human-coagulation-factor-iii-tissue-factor-quantikine-elisa_dcf300 |
| | PF4 | ELISA | Abcam | human-pf4-elisa-kit-cxcl4-ab100628 |
| Endothelial function | ICAM-1 | ELISA | Thermo Fisher Scientific | ICAM-1-Soluble-Human-ELISA-Kit/BMS201 |
| | Endoglin | ELISA | Thermo Fisher Scientific | Endoglin-CD105-Human-ELISA-Kit/EHENG |
| | VCAM-1 | ELISA | Thermo Fisher Scientific | VCAM-1-Soluble-Human-ELISA-Kit/BMS232 |
| | eNOS | ELISA | Thermo Fisher Scientific | elisa/product/EH169RB.html |
| | VEGF | ELISA | R&D Systems | human-vegf-quantikine-elisa-kit_dve00 |
| Endothelial microparticles | CD54 | Flow cytometry | Bio Rad | monoclonal/human-cd54-antibody-84h10-mca532 |
| | CD105 | Flow cytometry | Bio Rad | monoclonal/human-cd105-antibody-sn6-mca1557 |
| | CD106 | Flow cytometry | Bio Rad | monoclonal/human-cd106-antibody-1-g11b1-mca907 |
| Oxidative stress | SOD | Colorimetry | Cambridge Bioscience | product~97,995 |
| | GSH | Colorimetry | Thermo Fisher Scientific | product/EIAGSHC |
| | TBARS | Colorimetry | Cambridge Bioscience | product~91,969 |
| Brain injury | S100B | ELISA | Abcam | s100b-elisa-kit-ab234573 |
| | NSE | ELISA | Abcam | human-neuron-specific-enolase-elisa-kit-ab217778 |
| | GFAP | ELISA | Abcam | human-gfap-elisa-kit-ab223867 |
| | Glu | Colorimetry | Abcam | glutamate-assay-kit-ab83389 |
| Others | Cortisol | ELISA | Enzo Life Sciences | ADI-900-071/cortisol-elisa-kit/ |
| | CK-M | ELISA | Abcam | human-ckm-elisa-kit-ab264617 |

ELISA = enzyme-linked immunosorbent assay; IL = interleukin; IFN = interferon; CRP = C-reactive protein; C5a = Complement peptide 5a; NGAL = neutrophil gelatinase-associated lipocalin; CFIII/TF = coagulation factor III/tissue factor; PF = platelet factor; ICAM = intercellular adhesion molecule; VCAM = vascular cell adhesion molecule; eNOS = endothelial nitric oxide synthase; VEGF = vascular endothelial growth factor; CD = cluster of differentiation (microparticles); SOD = superoxide dismutase; GSH = glutathione; TBARS = thiobarbituric acid reactive substances; S100B = S100 calcium-binding protein B; NSE = neuron specific enolase; GFAP = glial fibrillary acidic protein; Glu = glutamate; CK-M = muscle creatine kinase.

within minutes. Endothelium activated or damaged by decompression stress sheds endothelial microparticles (EMPs) that express these adhesion molecules and may themselves mediate further endothelial injury.[28] Another membrane glycoprotein, endoglin, is important for endothelial integrity. EMPs expressing endoglin are associated with oxidative stress and impaired endothelial function post-dive.[4,14] We have targeted soluble and microparticle-associated forms of adhesion molecules and endoglin. Endothelial nitric oxide synthase (eNOS) is specifically included as its induction may mitigate bubble formation upon decompression. We have included enzymatic (superoxide dismutase, SOD), nonenzymatic (glutathione, GSH) and lipid peroxidation (thiobarbituric acid reactive substances, TBARS) markers of oxidative stress.

The nature of the association between nonhypoxic decompression stress and brain white matter hyperintensities (WMH) remains unclear. Modest, brief, infrequent hypobaric decompressions appear harmless, but exposures sufficient to present significant risk of DCS, whether hypoxic or nonhypoxic, influence cerebral physiology.[16,17] The nonhypoxic decompression stress imposed in the current study afforded an opportunity to target biomarkers of potential brain insult implicated in diving decompression stress, including serum calcium-binding protein S100β and neuron-specific enolase (NSE). We included astrocyte-derived glial fibrillary acidic protein (GFAP), a sensitive marker of brain insult,[1] and serum glutamate (Glu). Glu is the main excitatory neurotransmitter in the central nervous system (CNS). Extracellular homeostasis is maintained by endothelial cells of the blood–brain barrier, which actively transport Glu into the blood. After CNS injury, permeability increases to avoid local neurotoxicity, elevating Glu efflux for peripheral redistribution and metabolism.[25]

Finally, samples for hematology evaluated cellular responses and enabled correction of whole blood and plasma volumes with respect to hydration status. Serum cortisol was included to assess the corticosteroid stress response, as well as muscle creatine kinase (CK-M) to evaluate any influence of muscle damage secondary to repeated bouts of asymmetric exercise (squats).

## METHODS

### Subjects

The underpinning study design, methodology, and nonbiomarker outcomes are described in detail elsewhere.[6] Relevant details are summarized here for convenience. The study adhered to the principles of the Declaration of Helsinki. The research was funded by the UK Ministry of Defence (MOD) and the experimental protocol was approved in advance by the MOD Research Ethics Committee, an independent body constituted and operated in accordance with national and international guidelines.

Study participants were 15 healthy, nonsmoker men, ages 20–50 yr, mean (± SD) age 38 ± 11 yr. By chance, they comprised 5 men aged under 30 yr (mean 24 yr, range 20–28) and 10 men aged over 40 yr (mean 46 yr, range 41–50). Their mean (± SD) height was 1.82 ± 0.07 m, mean weight was 82.2 ± 8.4 kg, and mean body mass index was 24.9 ± 2.4 kg · $m^{-2}$. Following medical examination, healthy volunteers were screened using bubble contrast echocardiography, at a clinical center of excellence, to exclude underlying right-to-left vascular shunts, either intracardiac (patent foramen ovale) or pulmonary. Those who passed were then screened using high-resolution brain magnetic resonance imaging (fluid-attenuated inversion recovery sequences), at an academic research unit, to exclude excess prior white matter hyperintensities. A maximum of five discrete punctate subcortical lesions were allowed for study entry, consistent with previous reports.

Subjects avoided hypobaric or hyperbaric environments (i.e., flying, diving, parachuting, or mountaineering) in the 72 h prior to decompression and for 24 h afterwards. They also avoided alcohol and strenuous physical exertion for 48 h prior to decompression and 24 h afterwards. Otherwise, they ate and drank normally and ensured a good night's rest before and after their experiment. This comprised two consecutive ascents to an equivalent pressure altitude of 25,000 ft (7620 m) for 60 and then 90 min, each preceded by 1 h of denitrogenation, breathing 100% oxygen throughout. Exposures were separated by 1 h of breathing air normally at ground level. Subjects drank freely before and between ascents to maintain normal hydration and had a snack lunch between exposures. They simulated the duties of parachutist dispatchers, including short spells of load carriage at ground level prior to each ascent. Predominantly ambulatory activities at altitude included numerous squats, notably at 25,000 ft, where sessions of 16 squats over about 4 min were undertaken every 15 min, simulating equipment checks prior to parachutist dispatch. The response to decompression stress was evaluated using precordial "2D + Doppler" echocardiography conducted every 15 min at altitude. Apical four-chamber views enabled grading of venous gas emboli (VGE) "bubble" loads passing through the right side of the heart, consistent with an Expanded Eftedal-Brubakk scale, by an experienced investigator.[6]

### Equipment

Biomarkers were analyzed at the University of Hull using bespoke high-sensitivity test kits, mostly enzyme-linked immunosorbent assays (ELISAs) with some colorimetric assays. EMP counts were analyzed by flow cytometry as previously described.[11] The specific assays used are detailed in Table I. Test kit suppliers were Abcam (Cambridge, UK), Bio-Rad AbD Serotec GmbH (Puchheim, Germany), Bio-Techne (Abingdon, UK, for R&D Systems), Cambridge Bioscience (Cambridge, UK), Enzo Life Sciences (UK) Ltd (Exeter, UK), and Life Technologies Ltd. (Paisley, UK, for Thermo Fisher Scientific). ELISAs were performed following the relevant manufacturers' instructions and plates were read at the appropriate wavelength using a BioTek Synergy HT Microplate Reader running Gen5 software (BioTek now Agilent, Santa Clara, CA, USA). For fluorescence-activated cell-sorting

(FACS), plasma samples (25 µL) were incubated with appropriate antibodies (5 µL) for 30 min in the dark at room temperature. Phosphate-buffered saline (0.2 µm filtered, 150 µL) and AccuCheck counting beads (25 µL, PCB100, Invitrogen, Waltham, MA, USA) were added prior to MP quantification by flow cytometry. Samples were analyzed using a BD FACSCalibur flow cytometer running CELLQuest Pro software (BD Biosciences, San Jose, CA, USA). The MP gate was set as described previously, using Megamix SSc beads (Biocytex, Marseille, France).[5]

### Procedure

Decompressions were conducted in the high-performance hypobaric chamber of the Altitude Research Facility at MOD Boscombe Down, Wiltshire, UK. Subjects arrived at 08:00 and, following confirmation of fitness to proceed, a 20 mL pre-exposure baseline (T0) venous blood sample was obtained by antecubital venipuncture before 08:30. The first ascent typically commenced between 09:30–10:00 such that the second ascent was completed around 16:00. A second post-exposure (T8) venous sample was collected immediately after completion of the second ascent. Subjects returned the following day for a third midmorning sample, approximately 24 h after the start of their first ascent (recovery, T24). Experiments were curtailed by three instances of limb bend DCS, including two on the first ascent after 29 and 37 min at 25,000 ft (7620 m), and one after 60 min on the second ascent. All resolved with recompression and were treated with 100% oxygen for 1 h at ground level. These subjects' T8 samples were collected immediately upon completion of oxygen breathing.

Each 20 mL venous collection was divided to provide one 3.0 mL ethylene diamine tetra-acetic acid (EDTA) sample for whole blood hematology; two 2.5 mL citrated samples for plasma; and two 6.0 mL silica-coated "clotted" samples for serum. The plasma samples were immediately double-centrifuged, first at 1500 × g for 10 min and then, after careful pipetting to leave a generous residue, at 5000 × g for 20 min. A total of 2.0 mL of platelet-free plasma was derived and aliquoted into four 0.5 mL vials. After standing for 45 min, the clotted samples were centrifuged for 15 min at 2000 × g to obtain a total 6.0 mL of serum, aliquoted into four 1.5 mL vials. All samples were packaged and transported immediately to the Clinical Laboratory at Defence Science and Technology Laboratory (DSTL) Porton Down, Wiltshire, UK. A complete blood count and differential was performed on the EDTA sample. The vials of plasma and serum were labeled and frozen at -80°C, within 2 h of sampling, for later batch transfer on dry ice to the University of Hull, Hull, UK.

### Analysis

Post-exposure (T8, T24) values for whole blood cellular biomarkers were corrected for minor fluctuations in circulating blood volume with respect to hemoglobin (Hb) levels, in accordance with Eq. 1, since total Hb was not expected to fluctuate significantly between samples.[15] Post-exposure plasma and serum markers were corrected for fluctuations in plasma volume to adjust for hydration state, in accordance with convention, Eq. 2.[7]

$$\Delta TBM = \frac{BM_{post}}{BM_{pre}} \text{ X } \frac{Hb_{pre}}{Hb_{post}} - 1 \qquad \text{Eq. 1}$$

Eq. 1 shows calculation of post-exposure correction for changes in total blood volume applied to whole blood cellular markers. $\Delta TBM$ = change in total blood marker; BM = blood marker value pre- or post-exposure; Hb = hemoglobin.

$$\Delta PV = \frac{PV_{post} - PV_{pre}}{PV_{pre}} = \frac{Hb_{pre}\ x\ (1 - Hct_{post})}{Hb_{post}\ x\ (1 - Hct_{pre})} - 1 \qquad \text{Eq. 2}$$

Eq. 2 shows calculation of post-exposure correction for changes in plasma volume applied to plasma/serum markers. $\Delta PV$ = change in plasma volume; Hct = hematocrit.

Occasional extreme values in a minority of datasets were attributed to test kit technical errors. To ensure consistent analyses for all biomarkers, outliers were defined as any extreme values greater than three SD from the cohort mean at any sample time; all of that subject's data were then removed from the dataset for that marker. No more than one subject's data required removal from any dataset and no subject had their data removed from more than one dataset, supporting the impression that these were random outliers. Most datasets remained intact (45 data points). Each T0 dataset was examined for a normal distribution using the Shapiro-Wilk test ($\alpha = 0.05$). Data transforms ($\log_{10}$, square root or inverse) were applied, if necessary, to achieve this, and then extended to the entire dataset for that marker. Inferential analysis employed one-way repeated measures analysis of variance (rmANOVA) for the factor "Sample Time" (T0, T8, T24). The assumption of sphericity was checked with Mauchly's test and, if violated, Greenhouse-Geisser correction was applied automatically. If rmANOVA achieved statistical significance ($P < 0.05$), paired *t*-tests isolated pairwise differences, with Sidak correction for multiple comparisons. If the normality assumption was not satisfied, corresponding nonparametric analyses were performed. Possible associations were evaluated using simple scatter plots with linear or polynomial regression.

## RESULTS

There were three occurrences of limb bend DCS that curtailed exposures, two in the first ascent and one in the second. The remaining 12 subjects completed both decompression profiles. There were 11 subjects who generated heavy and persistent loads of VGE, especially during the first ascent, with older subjects (>40 yr) consistently generating earlier and heavier bubble loads.[6] This influence of age was not reflected in any biomarker dataset. For the results that follow, outcomes of rmANOVA or Friedman's analysis are shown in the relevant figures. Results of post hoc pairwise comparisons are detailed in the text.

**Table II.** Total Hemoglobin and Hematocrit.

| HEMOGLOBIN/HEMATOCRIT* | BASELINE (T0) (MEAN ± SD) | POST-EXPOSURE (T8) (MEAN ± SD) | RECOVERY (T24) (MEAN ± SD) |
|---|---|---|---|
| Total Hemoglobin (Hb), $g \cdot dL^{-1}$ | 15.5 ± 0.7 | 15.3 ± 1.2 | 15.4 ± 0.8 |
| Hematocrit (Hct), % | 0.465 ± 0.03 | 0.458 ± 0.04 | 0.463 ± 0.02 |

*$N = 15$.

Hemoglobin and hematocrit data are summarized in **Table II**. Hematocrit data are also represented in **Fig. 1A**, omitting a single aberrant (impossibly low) T8 value, attributed to technical error during measurement, which lowered the cohort mean T8 value in Table II. Fig. 1A illustrates that hematocrits were consistent throughout, reflecting well-maintained normal hydration states across the cohort.

Hematological responses were highly consistent between subjects (Fig. 1). **Fig. 1B** illustrates a consistent (mean 40%) increase in circulating leukocyte count at T8 that recovered to baseline by T24. Only one subject, whose first ascent was curtailed by DCS after only 29 min at 25,000 ft (7620 m), failed to show an elevated white cell count at T8. There was a mean 70% increase in circulating neutrophils at T8 (**Fig. 1C**), with every subject exhibiting an increase relative to T0. Total lymphocyte counts (mean ± SD) were: T0, 1.69 ± 0.41; T8, 1.75 ± 0.3; T24, $1.59 \pm 0.3 \times 10^9 \cdot L^{-1}$. The slight variation is consistent with diurnal variation in healthy young men.[21] A modest but statistically

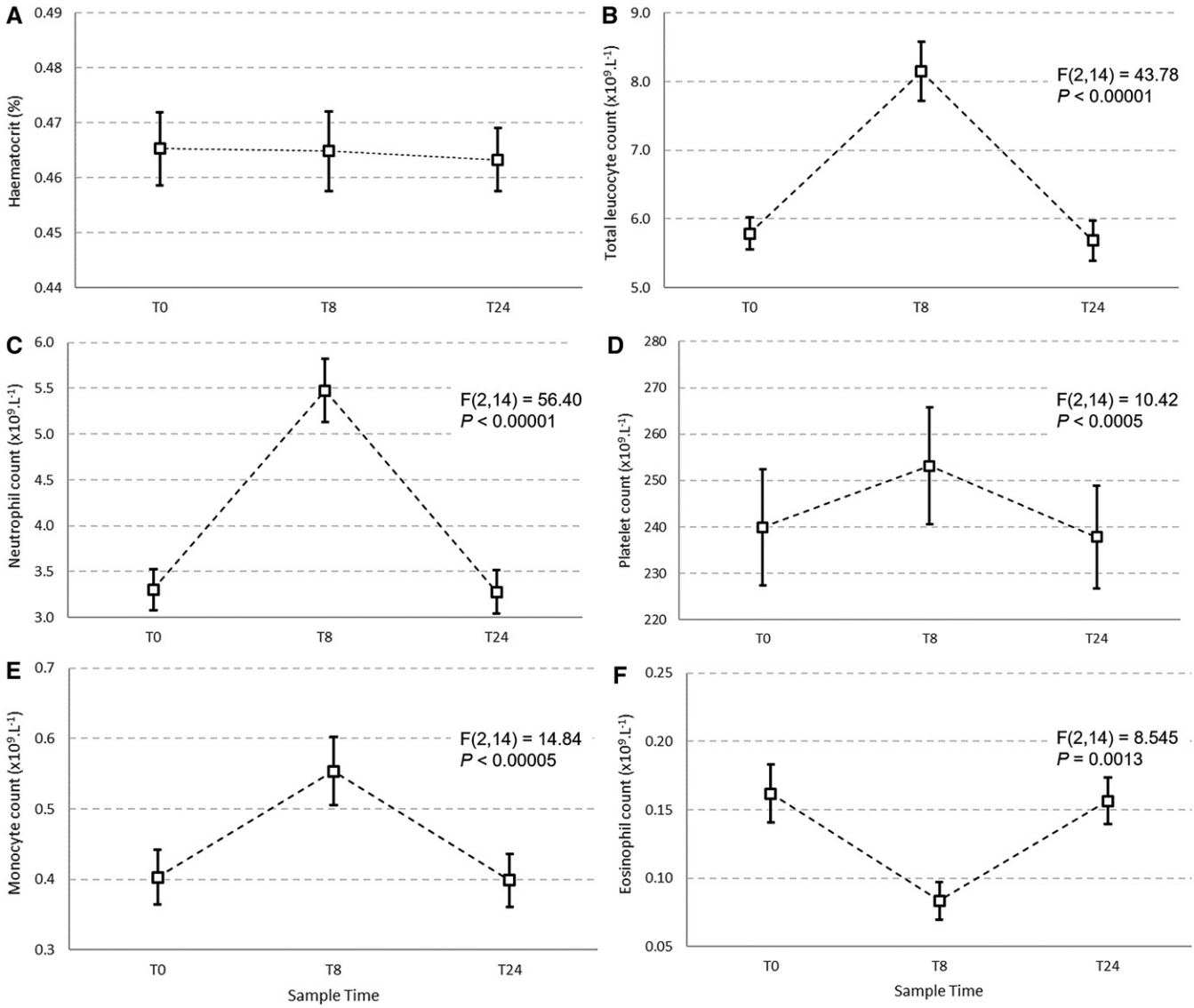


**Fig. 1.** Hematological parameters (mean ± SE). The statistically significant cell count changes at T8, recovering to baseline by T24, reflect highly consistent responses across the cohort.

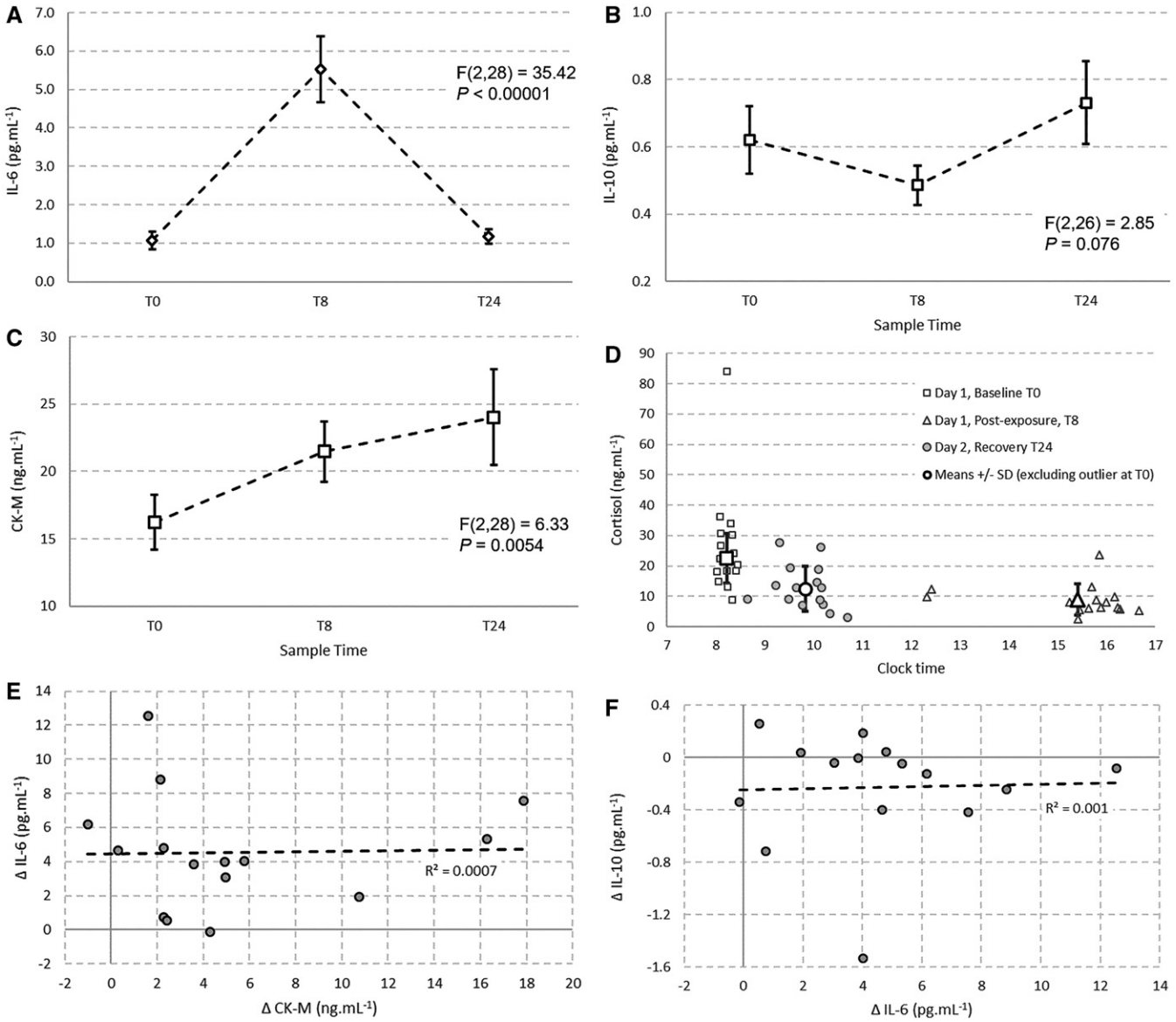


**Fig. 2.** Cytokine, creatine kinase, and cortisol responses. A) Mean (± SE) IL-6; B) mean (± SE) IL-10; C) mean (± SE) CK-M; D) mean (± SD) cortisol; E) absence of association between CK-M and IL-6 responses at T8; and F) absence of association between IL-6 and IL-10 responses at T8. For abbreviations, see caption under Table I.

significant (mean 6%) increase in circulating platelets at T8 (**Fig. 1D**) reflected an increase over baseline in 14 subjects (range 1–14%), with just 1 decreasing by 10%. This slight elevation is also consistent with diurnal variation.[21] At T8, there was also a mean 37% increase in circulating monocytes (**Fig. 1E**) and a 40% reduction in eosinophils (**Fig. 1F**), both normalizing by T24.

There was a substantial, highly statistically significant, mean fivefold elevation of IL-6 immediately post-exposure at T8, relative to the expected normal background levels of ~1.0 pg · $mL^{-1}$ seen at T0 and T24 (**Fig. 2A**). IL-6 may be generated as an anti-inflammatory myokine in working skeletal muscle, upregulating the anti-inflammatory IL-10, so IL-10 was also evaluated.[22] Modest fluctuations of IL-10 were not statistically significant (**Fig. 2B**). IL-1β, IL-8, and IFN-γ were undetectable throughout, remaining below the highly sensitive detection thresholds of the respective test kits (IL-1β < 0.1 pg · $mL^{-1}$; IL-8 < 31.2 pg · $mL^{-1}$; IFN-γ < 15.6 pg · $mL^{-1}$). Modest post-exposure elevation of CK-M was statistically significant at T8 ($P < 0.0005$) but not at T24 ($P = 0.039$; Sidak $\alpha = 0.017$) (**Fig. 2C**). Baseline (T0) cortisol samples were taken within the 30 min preceding diurnal acrophase (0830); subsequent post-exposure (T8) and recovery (T24) samples were proportionately lower and consistent with expectation for diurnal variation (**Fig. 2D**). Scatter plots of T8 increments over T0 for CK-M relative to IL-6 (**Fig. 2E**) and for IL-6 with respect to IL-10 (**Fig. 2F**) do not suggest any association.

After post hoc Sidak correction ($\alpha = 0.017$), complement peptide C5a was statistically significantly elevated over baseline values at T24 ($P = 0.008$) but not at T8 ($P = 0.04$) (**Fig. 3A**). After removing one outlier with an exceptionally high T0 baseline value, CRP was significantly elevated at T24 relative to both

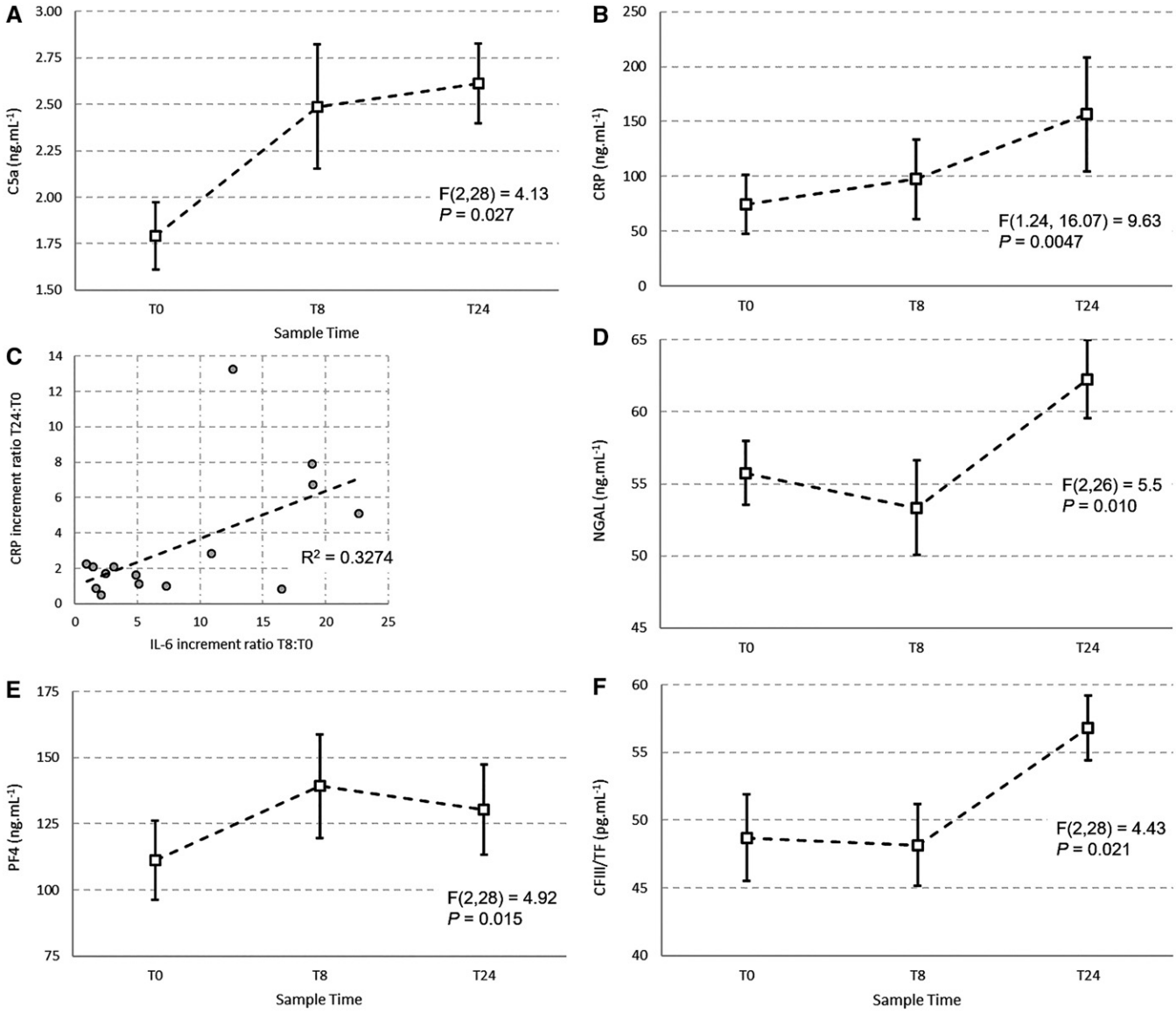


**Fig. 3.** Mean (± SE) biomarker data for: A) complement activation (peptide C5a); B) acute phase response (CRP); D) neutrophil activation (NGAL); E) platelet activation (PF4); and F) circulating tissue factor (CFIII/TF). C) Relationship between IL-6 increment at T8 and CRP response at T24. For abbreviations, see caption under Table I.

T0 ($P = 0.006$) and T8 ($P = 0.008$) (**Fig. 3B**). The IL-6 increment ratio at T8, relative to baseline, may have influenced the magnitude of the CRP response at T24 (**Fig. 3C**). NGAL levels (**Fig. 3D**) increased significantly at T24 relative to a slight post-exposure dip ($P < 0.005$). Relative to T0, PF4 was elevated at T8 ($P = 0.010$) but not at T24 ($P = 0.079$) (**Fig. 3E**). CFIII/TF was elevated at T24 relative to T8 ($P = 0.015$) but not with respect to T0 after Sidak correction ($P = 0.04$) (**Fig. 3F**).

The data for all endothelial biomarkers are summarized in **Table III**. Within-subject responses of all three EMP types were highly consistent, exemplified when comparing the T24:T0 increment ratios for CD54 (intercellular adhesion molecule-1, ICAM-1) and CD105 (endoglin), shown in **Fig. 4A**. CD106 (vascular cell adhesion molecule-1, VCAM-1) responded similarly. Accordingly, rather than differentiate between their responses, we have considered EMPs collectively by evaluating subject total EMP counts; these are represented in **Fig. 4B** omitting one subject with a grossly elevated total EMP count at T8 that is disproportionate to the rest of the cohort. The collective responses of the remaining subjects are better characterized by the boxplots of EMP counts in **Fig. 4C**, suggesting elevated levels at T24 driven by around half the subjects. On this basis, nonparametric comparison ($N = 15$) supports an increase at T24 relative to T0 (one-tailed Wilcoxon $Z = 1.851$, $P = 0.032$). Of the soluble endothelial markers listed in Table III, only ICAM-1 exhibited a notable response (**Fig. 4D**), with T24 levels significantly lower than T8 ($P = 0.002$).

The data for oxidative stress biomarkers are included in Table III. The response pattern for SOD suggested initial upregulation at T8, followed by downregulation at T24, but did not

**Table III.** Selected Biomarker Data.

| BIOMARKER | T0 (MEAN ± SE) | T8 (MEAN ± SE) | T24 (MEAN ± SE) | NOTES* |
|---|---|---|---|---|
| Endothelial Markers | | | | |
| EMP counts | | | | |
| CD54 (ICAM-1), $\mu L^{-1}$ | 635 ± 108 | 990 ± 416 | 1004 ± 231 | |
| CD105 (Endoglin), $\mu L^{-1}$ | 470 ± 72 | 764 ± 222 | 850 ± 211 | |
| CD106 (VCAM-1), $\mu L^{-1}$ | 536 ± 116 | 736 ± 243 | 689 ± 157 | |
| Total EMPs, $\mu L^{-1}$ | 1641 ± 247 | 2491 ± 864 | 2543 ± 585 | |
| Soluble membrane glycoproteins | | | | |
| ICAM-1, ng · $mL^{-1}$ | 408 ± 15 | 424 ± 19 | 374 ± 16 | |
| Endoglin, ng · $mL^{-1}$ | 10.99 ± 1.8 | 9.85 ± 1.5 | 10.41 ± 2.1 | |
| VCAM-1, ng · $mL^{-1}$ | 800 ± 52 | 784 ± 34 | 868 ± 87 | |
| Endothelial function | | | | |
| eNOS, ng · $mL^{-1}$ | 268 ± 57 | 240 ± 59 | 245 ± 62 | $N = 12^{\dagger}$ |
| VEGF, pg · $mL^{-1}$ | 448 ± 73 | 389 ± 73 | 464 ± 69 | |
| Oxidative Stress Markers | | | | |
| SOD, U.$mL^{-1}$ | 71 ± 5 | 78 ± 4 | 65 ± 4 | $N = 14$ |
| Total GSH, μM | 1.96 ± 0.25 | 1.61 ± 0.15 | 3.98 ± 0.72 | |
| TBARS (MDA), μM | 4.98 ± 0.5 | 5.81 ± 0.8 | 6.36 ± 1.0 | $N = 14$ |
| Brain Insult Markers | | | | |
| S100B, pg · $mL^{-1}$ | – | – | – | Undetectable (<139 pg · $mL^{-1}$) |
| NSE, ng · $mL^{-1}$ | 2.35 ± 0.29 | 2.42 ± 0.34 | 2.49 ± 0.36 | |
| GFAP, ng · $mL^{-1}$ | 7.9 ± 3.9 | 8.2 ± 4.1 | 9.2 ± 4.9 | |
| Glu, nmol | 8.9 ± 0.4 | 10.1 ± 0.7 | 8.9 ± 0.7 | |

MDA = malondialdehyde activity; SE = standard error.
*$N = 15$ except where stated.
†Sample size decreased due to undetectable levels throughout in some subjects.

achieve statistical significance after sphericity and Sidak correction. Total glutathione data could not be normalized. Friedman analysis supports a significant increase in total glutathione at T24 ($\chi^2_r$ [2, $N = 15$] = 15.6, $P < 0.0005$). Due to technical difficulty, oxidized glutathione was not available. After removal of one outlier, TBARS data indicate no effect on malondialdehyde activity (lipid peroxidation).

Brain biomarker outcomes are also summarized in Table III. S100β was not detected in any sample. Minor fluctuations of NSE were inconsistent between individuals, with no clear response pattern across the cohort. In contrast, despite widely varying baseline values between individuals, across two orders of magnitude, there was a consistent mean 10% (SE ± 3.7%) increase in GFAP at T24 relative to baseline (Friedman's $\chi^2_r$ [2, $N = 15$] = 8.93, $P = 0.015$), with no change at T8 (0 ± 2%). A suggestive but statistically nonsignificant increase in Glu at T8 recovered to baseline at T24 ($F[2,14] = 2.69$, $P = 0.085$).

## DISCUSSION

Approximately 90% of the increase in total leukocyte count at T8 is attributable to neutrophils, with the remainder attributable to monocytes, suggesting an innate immune response (**Fig. 5**). Two instances of limb pain DCS curtailed initial ascents, thereby probably limiting their neutrophil responses (Fig. 5A).

Neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), and Systemic Immune-inflammation Index (SII) are detailed in **Table IV**. The cohort NLR and SII are elevated at T8, recovering to baseline by T24. The normal mean NLR in healthy young adults is 1.65, range (± 1.96 SD) is 0.78–3.53.[9] Subjects' NLR at T8 ranged from 2.07–4.41, with 5 exceeding the upper limit of the normal range. PLR remained steady throughout, with minor fluctuations in platelets and lymphocytes consistent with diurnal variation. The elevated SII suggests a proinflammatory response or possibly a corticosteroid-mediated stress response. However, lymphocyte counts were not suppressed and cortisol data do not support a stress response, with T8 values proportionate for diurnal variation relative to acrophase (Fig. 2D). The fall in eosinophils at T8 is consistent with an acute phase response and contrasts with the expected morning nadir at cortisol acrophase and elevation toward evening (Fig. 1F). Overall, hematological data suggest an acute phase response rather than a stress response.

The headline finding is mean fivefold elevation in IL-6 at T8 that recovers to baseline by T24 (Fig. 2A). This was highly consistent between subjects (Fig. 5B) and greatly exceeded normal diurnal variation.[20] In contrast, IL-1β, IL-8, and IFN-γ were undetectable. IL-6 is a proinflammatory cytokine generated by neutrophils and endothelial cells, but it can also be produced as an anti-inflammatory myokine in working skeletal muscle. A proinflammatory cytokine response is most likely in the current study. The low intensity exertion during decompression promoted modest elevation of CK-M (Fig. 2C), unrelated to IL-6 increments (Fig. 2E), whereas myokine responses are associated with high intensity exercise to volitional fatigue or endurance activities (e.g., marathon running). Further, the half-life of circulating IL-6 myokine is short (only a few minutes), so grossly disproportionate myokine release would be necessary to generate the observed IL-6 peaks at T8, around 20–25 min following

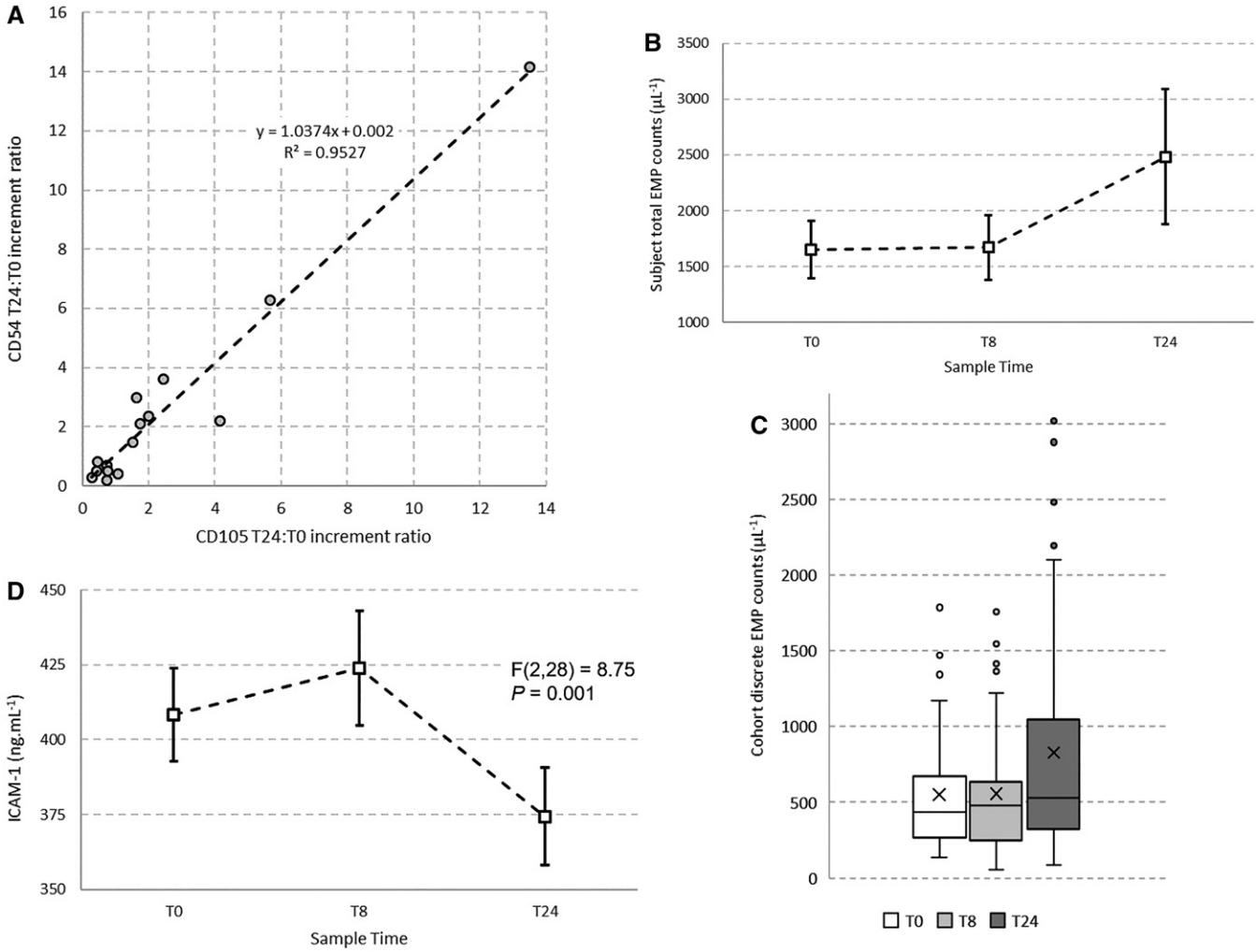


**Fig. 4.** Endothelial microparticle and adhesion molecule responses. A) Example of within-subjects correlation of all three measured EMP responses, here showing CD54 with respect to CD105; B) mean (± SE) individual subject total EMP counts at each sample time ($N = 14$, excluding one gross outlier with almost 10-fold elevation of EMP counts at T8 and which would misrepresent the T8 data of the cohort as a whole); C) box plot of all EMP counts, by quartiles with outliers shown, representing 42 measures at each sample time, mean represented by X ($N = 14$ subjects, excluding the same outlier as B); and D) soluble (circulating) ICAM-1 ($N = 15$). For abbreviations, see caption under Table I.

the last activity at 25,000 ft (7620 m).[24] Also, myokine responses elevate IL-10 and cortisol, whereas cohort (Fig. 2B) and individual (Fig. 2F) IL-10 responses were unrelated to IL-6 increments, and cortisol levels were unaffected.[22] Finally, IL-6 responses may be associated with severity of VGE loading (Fig. 5C).[6]

Increased C5a and CRP at T24 (Figs. 3A and 3B) provide additional indirect evidence of an inflammatory response that does not resolve fully by the following day. In sum, a proinflammatory IL-6 cytokine peak appears related to severity of decompression stress as measured by VGE bubble load, in turn influencing the magnitude of the acute phase response, as reflected by CRP levels (Fig. 3C).

The modest elevation of PF4 at T8 is attributable to diurnal variation in platelet levels and does not suggest activation.[10] However, increased NGAL (Fig. 3D) and CFIII/TF (Fig. 3F) at T24 provide indirect evidence, respectively, of persistent neutrophil activation and prior vascular endothelial disruption with exposure of subendothelial TF to the circulation. The latter may indicate a tendency toward coagulopathy, although TF expression is upregulated by CRP, C5a, and cytokines, among others. Post-exposure elevation of total EMPs, continuing in over half the subjects at T24 (Fig. 4C), indicate prior endothelial dysfunction and altered vascular responsivity, the latter implied also by elevation of CRP.

The consistent between-subjects mean 10% elevation of GFAP at T24 (Fig. 5E) is disconcerting as this is a sensitive biomarker that strongly implies specific CNS insult.[1] In this context, it is arguably inappropriate to dismiss lightly the elevation of serum Glu at T8 that reverts to baseline by T24 (Fig. 5D), but without quite achieving statistical significance. This change was the basis for including Glu as a target and it may be that a study with greater power would demonstrate a significant effect. Notably, recent reports link inflammatory indices (specifically the SII) with WMH burden in the context of cerebral small vessel disease.[12,19] Future work should explore these and additional brain biomarker responses to decompression stress.

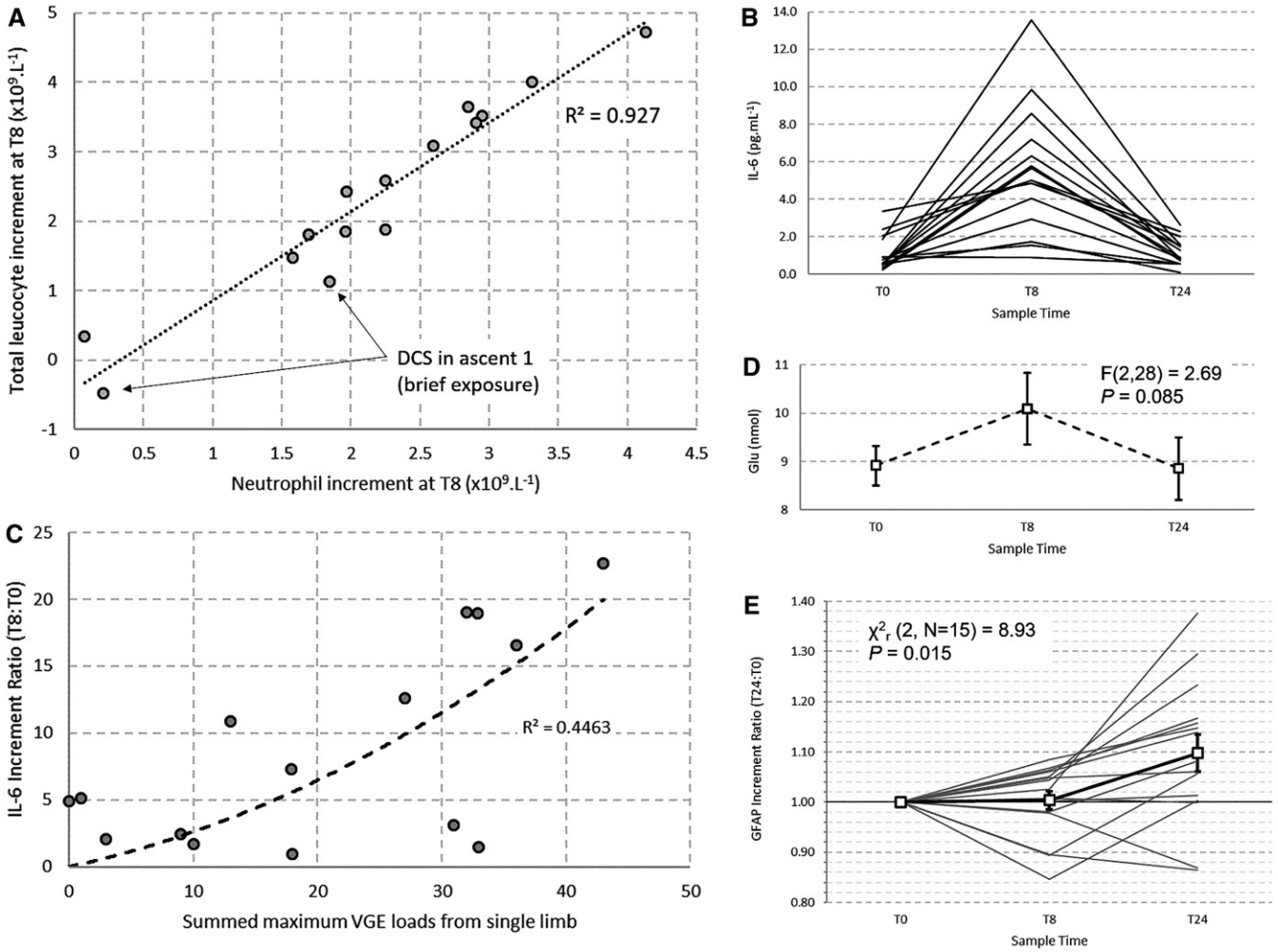


**Fig. 5.** Detailed blood, cytokine, and neurometabolite responses. A) Relationship between total leukocyte increment and neutrophil increment at T8 relative to baseline (T0); B) individual IL-6 responses ($N = 15$); C) relationship of IL-6 response at T8 to cumulative maximum load of venous gas emboli (VGE) from a single limb, summed over all test epochs;[6] D) cohort Glu data ($N = 15$); and E) individual and cohort GFAP responses relative to baseline at T0 ($N = 15$). For abbreviations, see caption under Table I.

Other results reported here may be summarized briefly as suggestive of endothelial dysfunction and oxidative stress. Overall, the data have implications for recovery time following hypobaric stress that imposes risk of DCS. Although hematological and cytokine responses normalized by T24, numerous markers remained elevated, including C5a, CRP, NGAL, TF, total EMPs, and GFAP, indicating ongoing inflammatory response, endothelial dysfunction, and incomplete recovery. Additional decompression under these conditions might be associated with exaggerated responses, possibly increasing risk of DCS, and warrants further investigation. Evaluation of responses to hypobaric decompression on consecutive days would be illuminating and should encompass additional indicators of neurological insult.

The main limitation of this study is the inability to disambiguate the relative influences of the three stressors that comprise the composite environmental stress, specifically hyperoxia, decompression (microbubble generation), and moderate physical activity at altitude. Six sets of control exposures would be required to isolate fully the individual and pairwise responses

**Table IV.** Hematological Inflammatory Indices.

| INDEX | BASELINE (T0) (MEAN ± SD) | POST-EXPOSURE (T8) (MEAN ± SD) | RECOVERY (T24) (MEAN ± SD) | rmANOVA |
|---|---|---|---|---|
| NLR | 2.13 ± 0.87 | 3.16 ± 0.75 | 2.13 ± 0.72 | $F(2, 28) = 42.3$, $P < 0.00001$ |
| PLR | 152 ± 55 | 148 ± 35 | 153 ± 35 | $F(1.22, 17.04) = 0.29$, $P = 0.644$ |
| SII | 513 ± 247 | 804 ± 260 | 511 ± 202 | $F(2, 28) = 44.01$, $P < 0.00001$ |

$N = 15$. The SII is the product of the neutrophil and platelet counts divided by the lymphocyte count. The normality assumption was met for all repeated measures analysis of variance (rmANOVA). Sphericity correction was applied for PLR analysis.

to these three stressors, plus a seventh "zero exposure" control for diurnal influences, far beyond the original scope of the work.[6] Nonetheless, the composite stress is representative of occupational exposure profiles, with direct relevance to military parachutist dispatchers working at high altitude. Additional limitations relate to analytical power and range. Subject numbers, biomarker targets, and sample times were all constrained by available resources. A study with greater power may have identified additional statistically significant effects, particularly for SOD and Glu. Additionally, three exposures were unavoidably inconsistent, curtailed prematurely due to limb bend DCS, and followed by 1 h of hyperoxia at ground level prior to taking T8 venous samples. While no age-related effects were apparent in the biomarker data, two-thirds of our test cohort were over 40 yr of age and all were men, almost exclusively Caucasian. A cohort with a different age–sex distribution and/or ethnic composition might behave quite differently.

In summary, the early pathophysiological response to exertional, hyperoxic, hypobaric decompression stress, in a cohort of predominantly middle-aged men, is proinflammatory, generating an acute phase response whereby mean CRP level remains elevated by 100% above baseline the day after exposure. Further work is required to validate and extend these observations, and to explore the disconcerting possibility of unexplained brain insult suggested by GFAP elevation the day after decompression.

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# Daily Caffeine Intake and the Effect of Caffeine on Pilots' Performance After Extended Wakefulness

Yara Q. Wingelaar-Jagt; Thijs T. Wingelaar; Lotte de Vrijer; Willem J. Riedel; Johannes G. Ramaekers

**INTRODUCTION:** Fatigue is a major contributor to aviation accidents. Sufficient sleep may be difficult to achieve under operational conditions in military aviation. Countermeasures include caffeine, however, studies evaluating its effects often do not represent daily practice with regular caffeine consumption. This study aims to establish the effect of caffeine on psychomotor performance in a realistic scenario (i.e., after a limited period of extended wakefulness).

**METHODS:** This randomized, double-blind, crossover, placebo-controlled trial included 30 aeromedically fit subjects. On trial days, subjects followed their normal routine till 17:00, after which caffeine intake was stopped. At midnight, subjects were given 300 mg of caffeine or placebo and performed the Psychomotor Vigilance Test, Vigilance and Tracking Test, and the Stanford Sleepiness Scale hourly up to 04:00 and again at 06:00 and 08:00. Four blood samples were collected. Statistical analyses included repeated-measures ANOVA or Friedman tests, marginal models, and Wilcoxon Signed Rank tests.

**RESULTS:** Median time awake at midnight was 17 h (IQR 16.5–17.5 h). Performance decreased significantly less during the night in the caffeine condition versus placebo. Neither habitual intake nor daytime caffeine consumption affected this. No statistically significant correlation was identified between blood concentrations of caffeine and performance.

**DISCUSSION:** A single dose of 300 mg of caffeine has beneficial effects on performance during the night in a realistic scenario for military aviation. Daytime caffeine consumption does not affect the effects of caffeine at night. These findings could be relevant for all industries in which optimal performance is required during nighttime after a limited period of extended wakefulness.

**KEYWORDS:** aviation, fatigue, shift work, sleep, wakefulness-promoting agents, performance enhancement, caffeine.

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In 2008, Air Traffic Control lost contact with Mesa Airlines Canadair Flight YV-1002, which flew 26 mi past the destination airport. Luckily, communications with the flight crew were restored and the airplane landed safely at the designated airport. According to the National Transportation Safety Board, the probable cause of this incident was the captain and first officer inadvertently falling asleep during the flight.[30] The captain's undiagnosed obstructive sleep apnea and the flight crew's recent work schedules (with consecutive days of early-morning start times) were reported to be contributing factors in this incident.

This incident might have easily become an accident if there had been a shortage of fuel or if the pilots had remained asleep longer. Fatigue contributed to 21–24% of major aviation accidents in the past two decades, but the significance of fatigue in aviation is probably even more paramount because, like in this incident, not all occurrences of fatigue lead to accidents.[30,44]

As stated in the International Civil Aviation Organization's definition of fatigue, fatigue can impair one's performance: "a physiological state of reduced mental or physical performance capability resulting from sleep loss, extended wakefulness,

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circadian phase, and/or workload (mental and/or physical activity) that can impair a person's alertness and ability to perform safety related operational duties."[19]

This definition identifies several possible causes of fatigue and potential areas for prevention or mitigation. The best way to prevent fatigue is to get sufficient sleep. However, as illustrated by the incident with flight YV-1002, this is often difficult to achieve because of flight crews' demanding work schedules.[30,44] It is even more difficult during military deployments as it may be tactically necessary to perform military operations at night, thereby disrupting the normal sleep pattern. This, combined with poorer sleep quality at deployment, may lead to irregular sleep during deployment, which may cause fatigue.[21] Also, when performing nighttime operations, pilots might be forced to fly during circadian phases dedicated for sleep, like the Window of Circadian Low (WOCL), when levels of attention are at their lowest, additionally increasing the chance of incidents.[39]

One possible option for counteracting the effects of fatigue is to prescribe stimulants, i.e., medications that increase vigilance and reduce fatigue. Caffeine is a widely available and well-known stimulant that has shown its beneficial effects on vigilance in different populations, such as students, but also military aviators.[12,26,37] It is a nonprescription substance that stimulates the central nervous system by blocking adenosine receptors.[6] Absorption of caffeine through the small intestine (i.e., after drinking a cup of coffee or energy drink or taking caffeine pills) is quick (15–40 min) and its effects are noticeable within 15–20 min.[4] When using caffeine chewing gum, the absorption rate is higher, with effects observed after 3–5 min.[44] Its half-life is 4–6 h, and it has beneficial effects in vigilance tasks for as long as 8 h after administration which can increase after repeated administration.[23,36] Coffee is one of the most widely used stimulants worldwide, and its consumption has been promoted as an optimal method to temporarily sustain the alertness of personnel with a limited level of medical oversight.[3] A survey of naval aviation candidates found that 86% drank coffee daily, consistent with the percentage of the general population.[33] Side-effects are dose- and user-dependent, and include agitation, irritability, tremor, dysrhythmia, and gastrointestinal complaints.[6] Consumption of caffeine at low dosages (<200 mg, equal to approximately two cups of coffee) is generally regarded as safe, with few or no side-effects reported.[27] Higher dosages may lead to side-effects such as nausea, jitteriness, and nervousness.[24] Additionally, individuals consuming higher daily quantities may experience withdrawal symptoms, such as headaches and muscle tremors, when caffeine intake is halted. Relative contraindications for caffeine use are hypertension, hyperthyroidism, epilepsy, mania, schizophrenia, and gastric and duodenal ulcers.[6]

The Royal Netherlands Air Force (RNLAF) allows the use of 300 mg caffeine tablets as an in-flight fatigue countermeasure.[28] Unfortunately, some aircrew members report that caffeine tablets are not sufficiently effective for reducing fatigue. This may be due to individual differences in caffeine metabolism or tolerance development. The metabolism of caffeine is primarily based on the action of CYP1A2.[31] CYP1A2 activity may vary by 5–6-fold between individuals due to environmental and (epi)genetic factors.[17] For example, 23% of the Caucasian population has a genetic CYP1A2 variant that increases tolerance to caffeine.[45] Additionally, caffeine clearance is increased by smoking and decreased by oral contraceptives.[17]

Tolerance development can be explained in two ways. Firstly, chronic intake of caffeine upregulates adenosine receptors in the brain. Over time, a larger amount of caffeine is required to attain the same stimulation as before.[38] Secondly, chronic intake of caffeine can induce CYP1A2, which increases the clearance rate and thus reduces and shortens the stimulatory effect of caffeine.[5,7] However, the influence of tolerance on the behavioral effects of caffeine is disputed and probably varies between individuals.[15,31,41]

Even so, differences in CYP1A2 variants and tolerance development may influence the effect of caffeine administration on performance. Additionally, genetic determinants may influence one's susceptibility to sleep deprivation and caffeine intake.[9,11] However, most research studying the efficacy of caffeine administration only included low-to-moderate caffeine users and/or instructed subjects to abstain from caffeine consumption for 48 h or longer prior to the start of the study.[8,24,42] This is impossible and/or impracticable in operational conditions; therefore, it is necessary to know more about the effect of daily caffeine consumption on the effects of caffeine during periods of sleep deprivation. This knowledge may help to personalize stimulant use in pilots and increase flight safety.

This study is part of a larger randomized controlled trial, which investigated several aspects of implementation of modafinil and caffeine as countermeasures for fatigue in a scenario realistic to military aviation. In a previously published manuscript about this trial, we concluded that both modafinil and caffeine significantly decrease the effects of an extended period of continuous wakefulness on vigilance compared with placebo.[43] The present study intended to determine the influence of previous caffeine consumption on the effect of caffeine (300 mg) administration on performance during a limited period of sleep deprivation. The period of continuous wakefulness was 24 h, and caffeine consumption was monitored through journals. In addition, caffeine blood levels were determined. We expected higher previous caffeine consumption to negatively affect the efficacy of caffeine administration.

## METHODS

### Subjects

The randomized controlled trial that this study is part of was conducted at the Center for Man in Aviation, RNLAF (Soesterberg, Netherlands), and adhered to the principles of the Declaration of Helsinki, the International Conference on Harmonization, and the Good Clinical Practice guidelines. The protocol was approved by the Medical Ethical Committee Brabant (reference: NL62145.028.17/P1749) and the Surgeon

General of the Ministry of Defense. The trial was registered in the Dutch Trial Register (No. NTR6922) and EU Clinical Trials Register (No. 2017-002,288-16).

Healthy employees of the RNLAF aged between 18–60 yr were eligible for inclusion. Eligible subjects were fit to fly according to the RNLAF Military Aviation Regulations or European Aviation Regulations.[13,29] Exclusion criteria were mainly based on possible side-effects or any of the following: interactions of one or both medicines (e.g., pregnancy or breastfeeding); the use of medication that is metabolized through CYP3A4/5, CYP2C19, or CYP2C9; and/or a history of psychiatric illness, including sleep disorders.

After being informed, both verbally and in writing, about the aims, consequences, and constraints of the trial, subjects gave written consent. This informed consent was voluntary and could be retracted at any time without any consequences. According to international privacy regulations, no study data were included in the medical files of the subjects.

The trial included 32 subjects, 2 of whom only completed the placebo trial day due to operational reasons. Their test results were excluded from the analysis of the present study because analysis of treatment effects according to a cross-over design could not be performed. The 30 remaining subjects were aged between 25–59 yr (median: 30.4 yr, IQR: 28.8–34.2 yr). Of the 30 subjects, 5 (17%) were women and 21 (70%) were pilots. None of the subjects smoked during the trial days. There were three (10%) subjects who used oral contraceptives during the study. On the caffeine trial day, the median waking time of the subjects was 07:00, meaning that at caffeine administration, the subjects had a median period of wakefulness of 17 h (range: 15.5–19.25 h, IQR: 16.5–17.5 h). Similarly, on the placebo trial day, the median waking time was 07:00 and the median period of wakefulness was 17 h (range: 16–19.5 h, IQR: 16.9–17.9 h).

### Materials

On the trial days, several parameters were measured seven times: baseline measurement at 6 h (T-6) before administering the investigational product (T0) and at 1, 2, 3, 4, 6, and 8 h after T0 (T1, T2, T3, T4, T6, and T8, respectively). Between these measurements, subjects were free to choose what activity to take part in, except sleeping or napping.

The Vigilance and Tracking test (VigTrack) is a dual task that measures vigilance performance under the continuous load of a compensatory tracking task. The test has been used in various studies and is sensitive for measuring vigilance and alertness.[35,40] During the tracking task, subjects had to steer a blue dot, using a joystick, such that it remained below a red dot in the center of the display. The blue dot is programmed to move continuously from the center of the display. While tracking, subjects had to perform an additional vigilance task. Inside the red dot, a black square alternated with a diamond once per second. At random intervals, a hexagon was presented. When this occurred, subjects had to press an additional key on the joystick. The duration of this test was 10 min, and primary endpoints included root mean square tracking error, percentage omissions, and mean reaction time. At the start of every trial day, three familiarization sessions of 5 min of the VigTrack were scheduled for all subjects to avoid practice bias during the actual measurements.

The psychomotor vigilance task (PVT) measures the speed with which subjects respond to a red stimulus and is used to assess the vigilance of subjects.[1] The interstimulus interval, defined as the period between the last response and the appearance of the next stimulus, varies randomly from 2–10 s. The duration of this test was 10 min, and primary endpoints included 1/mean reaction time and lapses. Lapses (errors of omission) were defined as reaction times ≥500 ms. At the start of every trial day, a familiarization session of 5 min of the PVT was scheduled for all subjects to avoid practice bias during the actual measurements.

The Stanford Sleepiness Scale (SSS) was used to subjectively assess the degree of sleepiness in subjects during the trial days.[18] This subjective rating scale is sensitive to any significant increase in sleepiness or fatigue and is highly correlated with flying performance and the threshold of information-processing speed during periods of intense fatigue.[32]

Blood samples were taken four times throughout the night to determine caffeine blood levels (at T0, T3, T6, and T8). These samples were taken by qualified medical personnel in concordance with Dutch quality and safety standards and were analyzed by an external, qualified diagnostic laboratory.

### Design

This study was part of a larger, randomized, double-blind, crossover, active- and placebo-controlled clinical trial, in which the effects of modafinil and caffeine administration on vigilance were compared with those of placebo.[43] This trial had a within-subjects 3×7 design: treatment (modafinil, caffeine, placebo) x time (T-6, T0, T1, T2, T3, T4, T6, T8). It consisted of three nonconsecutive trial days for every participant during which modafinil, caffeine, or placebo capsules were each administered once just after midnight (see **Table I**). For the present study, only the results of the trial days on which caffeine and placebo were given were included, resulting in a 2×7 design. The dose of caffeine (300 mg) was the usual dose administered to RNLAF aircrew; it is considered a medium-range but effective dose, comparable to 3–4 cups of coffee.[4,25]

A wash-out period of at least 7 d was implemented to ensure that the investigational products were completely eliminated and would not interfere on subsequent trial days. The trial was double-blinded so that both the subjects and investigators were unaware of the treatment given on trial days. The order of the treatments for each individual subject (modafinil, placebo, or caffeine) was based on a computer-generated randomization schedule organized and monitored by an external statistician. Randomization was performed using six possible treatment sequences to ensure balance for carryover effects, i.e., improving skills or learning bias on the test battery. In the current study, even though the modafinil administration was excluded, the six possible treatment sequences were equally distributed across the population, maintaining a balanced cross-over design.

**Table I.** Overview of Study Design and Data Collection.*

| TIMING | ACTIVITY |
|---|---|
| The 3 d before every trial day | Sleep diary<br>Caffeine log |
| 16:30 | Vital parameters<br>Stanford Sleepiness Scale<br>Familiarization with PVT and VigTrack |
| 17:00 | Subject ceased caffeine consumption |
| 18:00 | Baseline block (T-6)<br>Stanford Sleepiness scale<br>Assessment of VigTrack and PVT |
| 00:00 | Second baseline block (T0)<br>Vital parameters<br>Stanford Sleepiness scale<br>Assessment of VigTrack and PVT<br>Blood samples<br>Investigational product administration |
| 01:00 | First test block (T1)<br>Stanford Sleepiness scale<br>Assessment of VigTrack and PVT |
| 02:00 | Second test block (T2)<br>Vital parameters<br>Stanford Sleepiness scale<br>Assessment of VigTrack and PVT |
| 03:00 | Third test block (T3)<br>Stanford Sleepiness scale<br>Assessment of VigTrack and PVT<br>Blood samples |
| 04:00 | Fourth test block (T4)<br>Stanford Sleepiness scale<br>Assessment of VigTrack and PVT |
| 06:00 | Fifth test block (T6)<br>Stanford Sleepiness scale<br>Assessment of VigTrack and PVT<br>Blood samples |
| 08:00 | Sixth test block (T8)<br>Vital parameters<br>Stanford Sleepiness scale<br>Assessment of VigTrack and PVT<br>Blood samples |
| Outtake | Sleep questionnaires |

*All trial days were identical, the only difference being the investigational product administered.

For every trial day, the researchers received a treatment kit from the pharmacist. The treatment kits were labeled with the subject number and the trial day and contained identical capsules.

### Procedure

For 1 wk prior to the start of the trial days, subjects remained within the time zone of the research center (GMT +1, daylight saving GMT +2) to prevent jetlag, which might confound the test results. During the trial days, no strenuous physical exercise (including sports) or sleeping was allowed, and subjects kept a log of their activities.

On three consecutive days before the trial day and on the trial day itself, the subjects recorded their caffeine intake in a journal. On the trial day, subjects were instructed to consume their normal amount of caffeine-based products until 17:00 and cease their consumption of caffeine products thereafter.

Vital signs (temperature, blood pressure, and pulse) were collected four times during each trial day, two times prior to investigational product administration, and 2 and 8 h after administration (see Table I). Additionally, on every trial day, female subjects were tested for pregnancy and all subjects were asked if they had taken any concomitant medication or unauthorized medications during the past 3 d. Subjects were asked about potential adverse events multiple times during the trial days. Any adverse events were recorded throughout the trial and at every visit after screening.

### Statistical Analysis

Sample size calculations were performed with G*Power.[14] The assumed means and standard deviations of VigTrack were used to obtain the effect size ($d$) for sample size analysis.[23] Two-way testing of treatment effect using a repeated-measures analysis of variance (ANOVA) within subjects, with $\alpha = 0.05$, $\beta = 0.8$, and the aforementioned effect size ($d$), required a minimum of $N = 18$ to show the effects of caffeine. However, to compensate for dropouts and sample failures, at least 30 subjects were included.

Statistical analyses were performed using SPSS Statistics for Windows (IBM Corp.; Armonk, NY, USA: 2020, version 27.0). A factorial repeated-measures ANOVA was conducted to analyze the main and interaction effects of time and treatment on the VigTrack and PVT parameters. SSS scores were analyzed by nonparametric tests (Friedman test for repeated measures). Mauchly's test was performed to test if the assumption of sphericity had been violated for the different parameters. If this was the case, the degrees of freedom were corrected using Huynh–Feldt or Greenhouse-Geisser estimates of sphericity where appropriate.[16] A $P$-value of <0.05 was considered statistically significant.

The relationship between caffeine intake and blood concentration of caffeine was analyzed using the Wilcoxon Signed Rank test. The relation between the aforementioned parameters and both caffeine intake and caffeine blood concentrations were analyzed using marginal models (generalized estimating equations), with time of measurement during the night as a cofactor. A $P$-value of <0.05 was considered statistically significant.

## RESULTS

The trial ended according to the protocol. No adverse events were encountered during the trial, and the subjects' vital signs were unaffected by drug administration. The results of the comparison between the effects of modafinil and caffeine with placebo on nighttime vigilance are published elsewhere.[43]

After checking for outliers in the data with boxplots, two subjects were removed from the analysis of the VigTrack parameters. These subjects showed extreme values for all the VigTrack parameters, likely because they may have not understood the task properly. No outliers were identified when analyzing other parameters.

### Caffeine vs. Placebo

The VigTrack and PVT parameters in the caffeine and placebo conditions were analyzed using a two-way repeated-measures ANOVA. In all instances, Mauchly's test indicated a violation of the sphericity assumption; therefore, Greenhouse-Geisser results were analyzed. The results of Mauchly's test and subsequent correction of the degrees of freedom are provided in **Table II**. SSS scores were analyzed using the Friedman test. For all indices, performance degraded significantly less in the caffeine than in the placebo condition across the night. Test results for all primary endpoints are displayed in **Fig. 1** and Table II.

### Caffeine Intake

Median caffeine intake on the caffeine trial day was 260.0 mg (range: 0.0–765.0 mg, IQR: 172.5–347.5 mg), which was nearly identical to the median habitual caffeine intake of 260.0 mg (range: 0–770 mg, IQR: 173.1–340.0 mg). The results of statistical analyses of habitual caffeine consumption were similar to those of statistical analyses of caffeine intake on the trial day and were therefore not included in this study. Median caffeine intake on the placebo trial day was slightly lower at 247.2 mg (range: 0.0–632.0 mg, IQR: 102.3–340.0 mg).

**Table III** shows the results of the marginal model for caffeine intake on the caffeine trial day. For all primary endpoints (PVT, SSS and VigTrack parameters), marginal models did not show a statistically significant effect of the amount of caffeine intake on the trial day. Time of assessment was associated with a statistically significant lower performance on all parameters, with the exception of the VigTrack mean tracking error ($P = 0.083$). **Fig. 2** displays these results visually, in which the trendlines show the relation between caffeine intake on the trial day and the performance at 00:00, 03:00, 06:00, and 08:00, respectively. An analysis of the subjects with the upper and lower 25% of caffeine intake revealed no statistically significant difference compared to the other subjects.

### Caffeine Blood Concentrations

The mean caffeine concentration in blood at 00:00 on the caffeine trial day was 1.4 $\mu g \cdot ml^{-1}$ (range: < 0.1–12.5 $\mu g \cdot ml^{-1}$, IQR: 0.6–3.7 $\mu g \cdot ml^{-1}$). This was similar to the mean caffeine concentration in blood at 00:00 on the placebo trial day, which was 1.3 $\mu g \cdot ml^{-1}$ (range: <0.1–11.0 $\mu g \cdot ml^{-1}$, IQR: 0.6–2.5 $\mu g \cdot ml^{-1}$). After 00:00, the caffeine concentrations in blood showed different patterns in the two conditions (**Fig. 3**). Mauchly's test indicated that sphericity was met [$\chi^2(5) = 4.820$, $P = 0.438$]. In the marginal model, the caffeine concentration was significantly higher in the caffeine condition than in the placebo condition [$F(3, 87) = 56.662$, $P < 0.0001$] and peaked at 3 h after administering caffeine tablets.

The Wilcoxon Signed Rank test showed that caffeine consumption during the trial days had no statistically significant effect on the blood concentration at 00:00 ($P < 0.001$). This was also the case for habitual caffeine consumption and blood caffeine concentrations ($P < 0.001$).

**Table II.** Results of the Statistical Tests of the Main Effects Per Parameter.*

| TEST | PVT–1/MEAN REACTION TIME | PVT–NUMBER OF LAPSES | SSS | VIGTRACK–MEAN TRACKING ERROR | VIGTRACK–MEAN PERCENTAGE OMISSIONS | VIGTRACK–MEAN REACTION TIME |
|---|---|---|---|---|---|---|
| Mauchly's test Correction | $\chi^2(27) = 57.020$, $P = 0.001$ $\varepsilon = 0.632$ (GG) | $\chi^2(27) = 67.234$, $P < 0.001$ $\varepsilon = 0.616$ (GG) | NA | $\chi^2(27) = 224.153$, $P < 0.001$ $\varepsilon = 0.143$ (GG) | $\chi^2(27) = 274.794$, $P < 0.001$ $\varepsilon = 0.143$ (GG) | $\chi^2(27) = 89.600$, $P < 0.001$ $\varepsilon = 0.445$ (GG) |
| ANOVA caffeine group | $F(3.593, 104.199) = 19.438$, $P < 0.001$, $\eta^2 = 0.401$ | $F(3.387, 98.230) = 15.022$, $P = 0.001$, $\eta^2 = 0.341$ | NA | $F(1.454, 42.179) = 2.025$, $P = 0.156$, $\eta^2 = 0.065$ | $F(1.385, 40.154) = 1.643$, $P = 0.210$, $\eta^2 = 0.054$ | $F(2.309, 61.903) = 3.913$, $P = 0.023$, $\eta^2 = 0.119$ |
| ANOVA placebo group | $F(3.914, 121.347) = 49.705$, $P < 0.001$, $\eta^2 = 0.616$ | $F(4.060, 125.845) = 31.597$, $P < 0.001$, $\eta^2 = 0.505$ | NA | $F(2.371, 73.509) = 10.539$, $P < 0.001$, $\eta^2 = 0.254$ | $F(1.640, 50.852) = 13.609$, $P = 0.001$, $\eta^2 = 0.305$ | $F(3.739, 115.919) = 36.159$, $P < 0.001$, $\eta^2 = 0.538$ |
| Friedman test | NA | NA | $\chi^2(1) = 148.324$, $P < 0.001$ | NA | NA | NA |
| Interpretation | Performance after caffeine administration degraded less across the night than after placebo administration | Performance after caffeine administration degraded less across the night than after placebo administration | Subjective sleepiness across the night is less affected after caffeine administration than after placebo administration | Performance after caffeine administration degraded less across the night than after placebo administration | Performance after caffeine administration degraded less across the night than after placebo administration | Performance after caffeine administration degraded less across than after placebo administration |

*After Mauchly's test indicated a violation of the sphericity assumption, the degrees of freedom were corrected using Greenhouse-Geisser (GG) estimates. Afterwards, separate ANOVAs were conducted to test the effects of the test condition on each parameter. *P*-values lower than 0.05 indicated statistically significant results. For the Stanford Sleepiness Scale (SSS) a Friedman test was conducted due to the nonparametric nature of the data. PVT: Psychomotor Vigilance Test; SSS: Stanford Sleepiness Scale; VigTrack: Vigilance and Tracking Test.

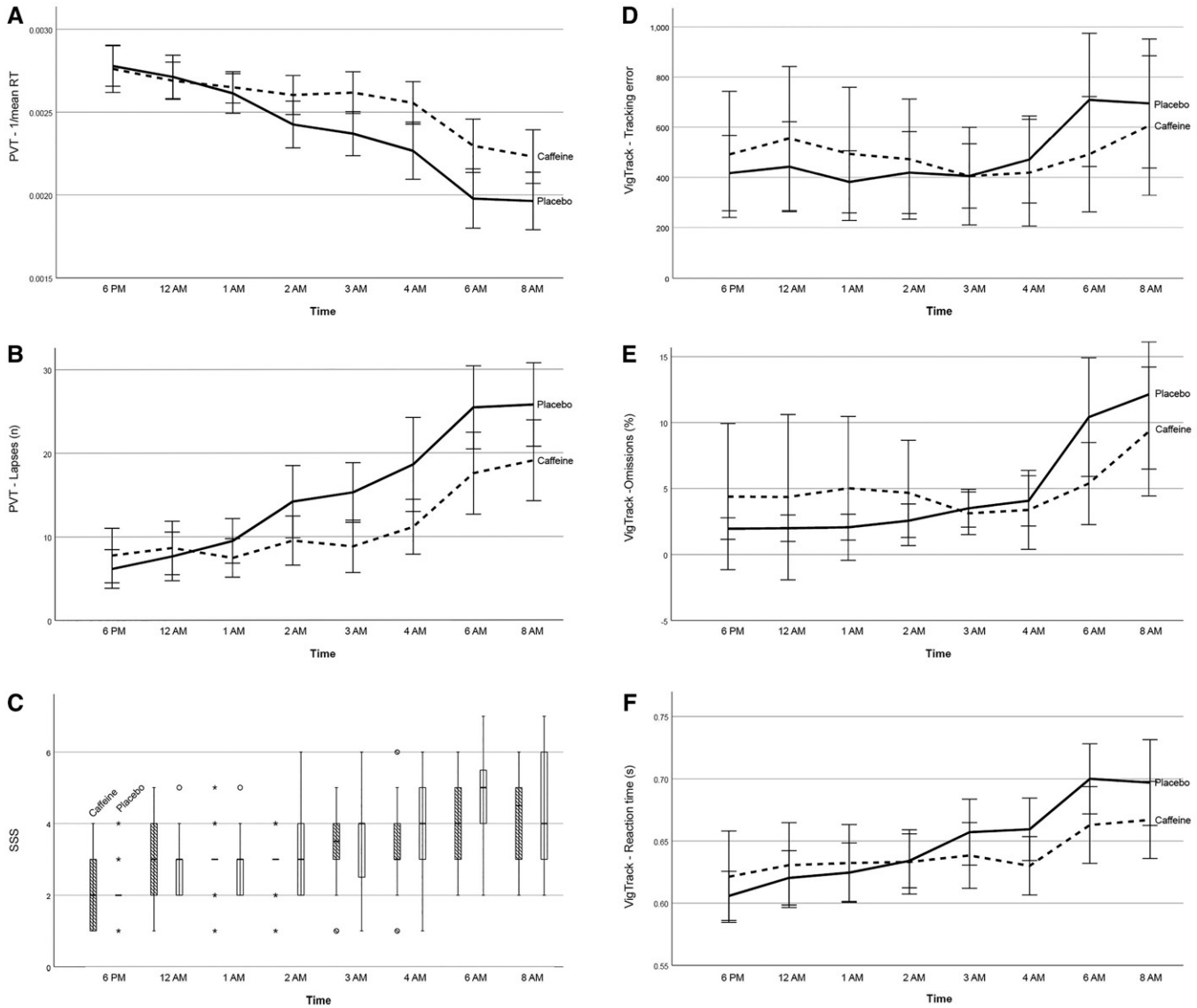


**Fig. 1.** Performance on caffeine trial day vs. placebo trial day. A) PVT–1/mean reaction time; B) PVT–number of lapses; C) SSS; D) VigTrack–mean tracking error; E) VigTrack–mean percentage omissions; and F) VigTrack–mean reaction time. Dashed line = caffeine, solid line = placebo. A lower score is a lower performance, except for PVT—1/mean reaction time.

Similar to caffeine intake, no statistically significant relationship was found between caffeine blood concentrations and performance on any primary endpoints (PVT, SSS, and VigTrack parameters), using a marginal model. **Table IV** shows the results of this marginal model. Time of assessment was associated with a statistically significantly lower performance on all parameters. **Fig. 4** displays these results visually, and the trendlines show the relationship between caffeine blood concentration on the trial days and the performance at 00:00, 03:00, 06:00 and 08:00, respectively.

**Table III.** Results of the Marginal Model; Performance vs. Trial Day Caffeine Intake.

| COVARIATE | PVT–1/MEAN REACTION TIME | PVT–NUMBER OF LAPSES | SSS | VIGTRACK–MEAN TRACKING ERROR | VIGTRACK–MEAN PERCENTAGE OMISSIONS | VIGTRACK–MEAN REACTION TIME |
|---|---|---|---|---|---|---|
| Intercept | 0.003 | 4.817 | 2.601 | 317.867 | −1.364 | 0.598 |
| *P*-value | <0.001* | 0.011* | <0.001* | <0.001* | 0.472 | <0.001* |
| Assessment | $-7.07.10^{-5}$ | 1.874 | 0.213 | 18.373 | 1.133 | 0.008 |
| *P*-value | <0.001* | <0.001* | <0.001* | 0.083 | 0.003* | 0.001* |
| Caffeine | $-6.39.10^{-8}$ | 0.001 | $-7.24.10^{-5}$ | −0.069 | 0.002 | $2.82.10^{-5}$ |
| *P*-value | 0.742 | 0.873 | 0.933 | 0.750 | 0.763 | 0.767 |

*Statistically significant results ($P < 0.05$) from the Wald Chi-squared test. PVT: Psychomotor Vigilance Test; SSS: Stanford Sleepiness Scale; VigTrack: Vigilance and Tracking Test.

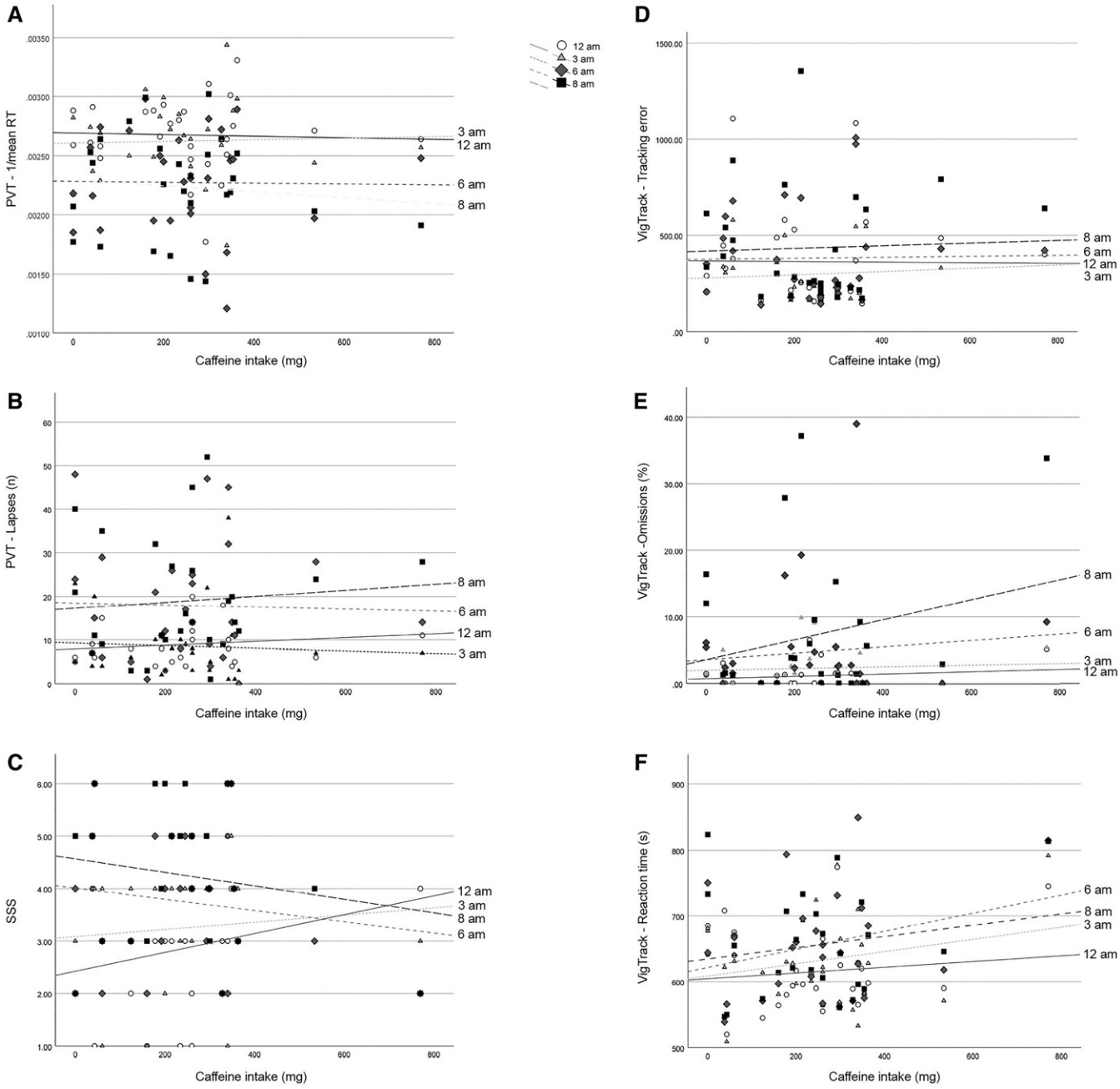


**Fig. 2.** Performance vs. trial day caffeine intake. A) PVT–1/mean reaction time; B) PVT–number of lapses; C) SSS; D) VigTrack–mean tracking error; E) VigTrack–mean percentage omissions; and F) VigTrack–mean reaction time. A lower score is a lower performance, except for PVT–1/mean reaction time.

## DISCUSSION

This study demonstrates that previous caffeine consumption does not interfere with the effect of caffeine administration on performance during an extended period of continuous wakefulness (median 17 h). Additionally, although administration of caffeine improved performance compared with placebo, this study revealed no statistically significant relationship between the height of the caffeine blood concentration and the effect on performance. In addition, there was no statistically significant relationship between the blood caffeine concentration at midnight and caffeine intake on the trial day or habitual caffeine consumption.

Studies have reported mixed and inconclusive results regarding the effect of caffeine administration on vigilance during continuous wakefulness.[10] Several studies found no clear evidence of objective benefit, while pilots receiving caffeine tended to perceive their performance too optimistically, which might cause safety problems.[22,25] Other studies found that although caffeine does not improve subjectively assessed sleepiness, it increases vigilance and performance of sleep-deprived individuals, sometimes beyond baseline levels.[8,20] These conflicting

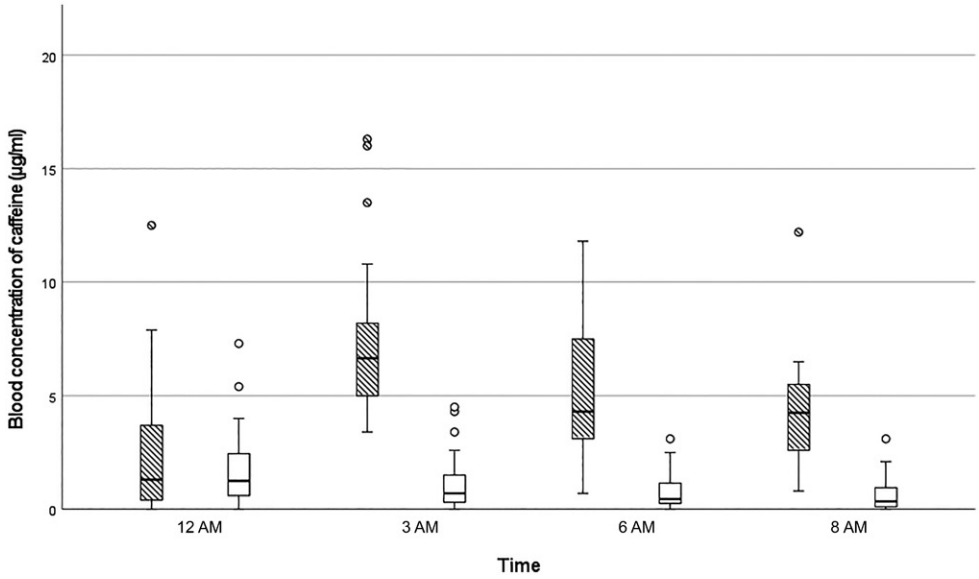


**Fig. 3.** Concentration-time curve of caffeine on the caffeine and placebo trial day. Striped = caffeine, blank = placebo.

results may be due to differences in subject characteristics, study procedures, sample sizes, and statistical power. In the previously published manuscript about this trial, it was concluded that both modafinil and caffeine significantly decrease the effects of an extended period of continuous wakefulness on vigilance compared with placebo.[43] In the present study, caffeine did not fully counteract the negative effect of extended wakefulness on performance because parameters were negatively affected in both conditions during the night. However, all performance parameters were affected less after caffeine administration than after placebo administration. Therefore, this study confirms that caffeine administration led to less impaired vigilance during an extended period of continuous wakefulness.

Although this, to our knowledge, is the first randomized, placebo-controlled trial to allow the use of caffeine products within 48 h of caffeine administration, we still imposed a 7 h caffeine-free period before caffeine administration. We introduced this period to mimic operational situations in which aircrews are advised to observe a similar period in order to maximize caffeine's beneficial effects. Caffeine's half-life is 4–6 h; therefore, caffeine blood concentrations would have decreased by 50–75% after 7 h.[23] This is congruent with the low median blood caffeine concentrations at 00:00 on the caffeine and placebo trial days of 1.35 and 1.30 $\mu g \cdot ml^{-1}$, respectively, which are regarded as low and harmless levels.[34] Allowing caffeine consumption until closer to investigational product administration likely would have increased caffeine blood concentrations at 00:00 and throughout the night. In the current study, caffeine blood concentrations did not exceed 20 $\mu g \cdot ml^{-1}$, which is considered an elevated, but nontoxic, concentration.[34] Additionally, shortening the caffeine-free period might have revealed a relationship between the amount of previous caffeine consumption and the effects of caffeine administration on performance because caffeine's beneficial effects can last up to 8 h.[23] Despite using several tests for statistical analyses, no statistically significant relationship was found between the height of blood caffeine concentrations and the effect on performance. This might be attributable to the limited number and timing of blood samples taken during the night. Samples were taken at 3, 6, and 8 h after caffeine administration. Given that caffeine's half-life is 4–6 h, caffeine concentrations would already have decreased significantly at 3 h after administration. For this reason, a statistical comparison between the area under the curve and performance parameters was not deemed advantageous. Although the sample size was sufficient to reject the null hypothesis in this study, increasing the number of blood samples would have allowed us to better establish caffeine blood concentration curves during the night and to correlate these

**Table IV.** Results of the Marginal Model; Performance vs. Caffeine Blood Concentrations.

| COVARIATE | PVT–1/MEAN REACTION TIME | PVT–NUMBER OF LAPSES | SSS | VIGTRACK–MEAN TRACKING ERROR | VIGTRACK–MEAN PERCENTAGE OMISSIONS | VIGTRACK–MEAN REACTION TIME |
|---|---|---|---|---|---|---|
| Intercept | 0.003 | 5.358 | 2.526 | 306.986 | −0.0.870 | 0.616 |
| *P*-value | <0.001* | 0.029* | <0.001* | <0.001* | 0.481 | <0.001* |
| Assessment | $-8.08.10^{-5}$ | 1.901 | 0.207 | 19.037 | 1.136 | 0.009 |
| *P*-value | <0.001* | <0.001* | <0.001* | 0.035* | 0.002* | <0.001* |
| Caffeine | $-2.96.10^{-7}$ | −0.077 | 0.017 | −2.064 | −0.011 | −0.003 |
| *P*-value | 0.986 | 0.873 | 0.622 | 0.842 | 0.960 | 0.297 |

*Statistically significant results ($P < 0.05$) from the Wald Chi-squared test. PVT: Psychomotor Vigilance Test; SSS: Stanford Sleepiness Scale; VigTrack: Vigilance and Tracking Test.

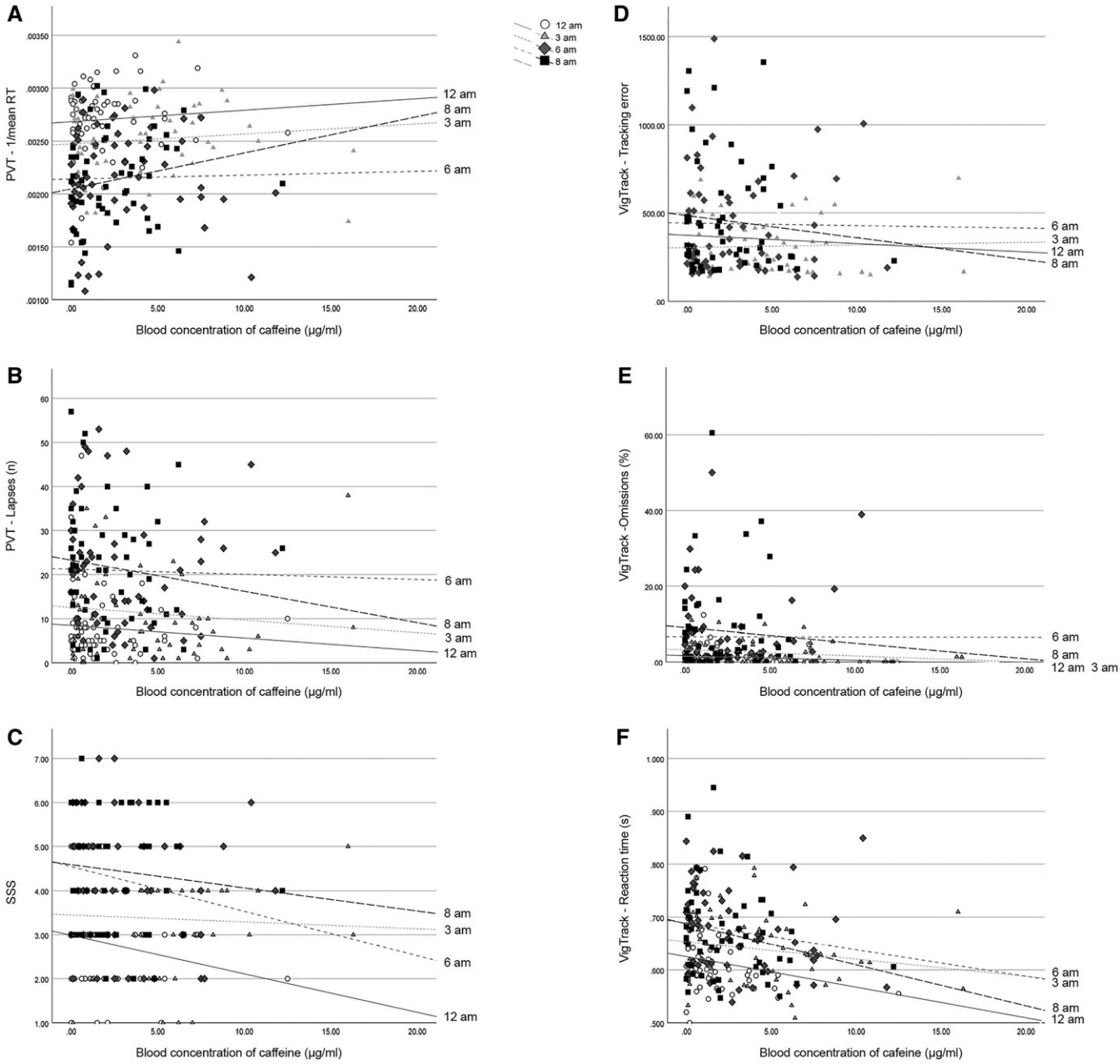


**Fig. 4.** Performance vs. caffeine blood concentrations. A) PVT–1/mean reaction time; B) PVT–number of lapses; C) SSS; D) VigTrack–mean tracking error; E) VigTrack–mean percentage omissions; and F) VigTrack–mean reaction time. A lower score is a lower performance, except for PVT–1/mean reaction time.

with the performance parameters. Moreover, the lack of a statistically significant relationship between blood caffeine levels and results of the psychomotor parameters, despite a sufficiently powered study, could mean there is a higher than earlier assumed interindividual response to the effects of caffeine. Alternatively, the conclusion might be that there is no clear relation between response and concentration in blood, as was suggested in previous literature.[2] Further studies are required to elucidate this.

Another possible limitation of our study is the reliability of the caffeine intake equations. The amount of caffeine in various caffeinated drinks varies and is not always reproduceable. It was impossible to account for this in the current study and therefore average amounts were used. Despite this possible incongruency, we do not believe this significantly influenced the results because the same equations were used for all subjects, and subjects consumed caffeine at the same site, meaning all subjects were affected similarly. Furthermore, calculated caffeine intake on the trial days was very similar to habitual caffeine consumption. This suggests that subjects did not change their caffeine consumption during the trial days. Both nicotine and oral contraceptives influence caffeine clearance.[17] None of the subjects smoked during the study, but 3 (10%) of the 30 subjects who completed the caffeine trial day took oral contraceptives.

Oral contraceptives decrease caffeine clearance; therefore, the caffeine blood concentrations of these subjects may have been higher than those of the other subjects. However, there was no statistically significant relationship between the caffeine blood concentration and performance; therefore, we believe the effects were limited. Additionally, 23% of the Caucasian population has a CYP1A2 variant that increases tolerance to caffeine.[45] There was no statistically significant relationship between the caffeine blood concentration and performance; therefore, we do not advise that the CYP1A2 variant be analyzed in aircrew members.

This trial was designed to resemble realistic operational situations (i.e., the period of wakefulness was limited to approximately 17 h). Additionally, to best reflect circumstances of operational military aviation, the subjects were not given specific bedtimes or waking times. Therefore, the time since the last sleeping period and the duration of that sleeping period differed between subjects. These differences may have caused variation in performance during the trial periods. However, due to its crossover design and the similar waking times of the subjects on the placebo and caffeine trial days, we do not believe this affected the results of our study. Additionally, we allowed all types of caffeine consumers to participate in this trial because the RNLAF aircrew comprises low-to-high caffeine users. Our study shows that previous caffeine consumption does not interfere with the effect of caffeine administration on performance. Additionally, we checked whether subjects with the upper and lower 25% of caffeine intake scored differently than the other subjects, but we did not detect a statistically significant difference. Thus, we conclude that aircrew can continue their habitual caffeine consumption as long as they abstain from caffeine for 7 h before caffeine administration. Future research should investigate whether a shorter caffeine-free period is sufficient to benefit from the effects of caffeine administration.

In conclusion, although administration of caffeine (300 mg) improved performance compared with placebo, neither previous caffeine consumption nor the caffeine blood concentration interfered with the effect of caffeine administration on performance during an extended period of continuous wakefulness (median 17 h). Stimulants may play an important role in military aviation, especially in situations where pilots are already fatigued but operational necessity requires them to continue their mission. Therefore, it is paramount to be able to properly advise aircrew about what to use and when. This study shows that a 7 h caffeine-free period seems to be sufficient to negate any interfering effects of previous caffeine consumption. Additionally, there was no difference between subjects with high caffeine intake and those with low caffeine intake, allowing aircrew to continue their habitual caffeine consumption. Future research should investigate whether a shorter caffeine-free period is sufficient to benefit from the effects of caffeine administration. Furthermore, future studies could include more blood samples to better establish blood caffeine concentration curves during the night and to correlate these with the performance parameters. The results of this study are not just relevant for military aviation, in which the use of caffeine tablets is already allowed, but for all industries in which peak performance is demanded during nighttime or after periods of continuous wakefulness (like civil aviation, healthcare, and logistics). Caffeine is widely available and therefore these findings can be used to determine when to have a cup of coffee, caffeine chewing gum, or any other caffeine-containing product, not just caffeine tablets.

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# Neuro-Cardiovascular Responses to Sympathetic Stimulation in Fighter Pilots

Marcus Vinicius dos Santos Rangel; Grace Barros de Sá; Paulo Farinatti; Juliana Pereira Borges

**INTRODUCTION:** The chronic effects of regular exposure to high acceleration levels (G-force) on the neuro-cardiovascular system are unclear. We compared the mean arterial pressure (MAP) and cardiac autonomic modulation between nonpilots (NP) vs. military fighter (FP) and transport (TP) pilots. Additionally, we correlated the cardiac autonomic indices with the cardiorespiratory fitness and flight experience of FP.

**METHODS:** A total of 21 FP, 8 TP, and 20 NP performed a tilt test (TT), during which beat-to-beat blood pressure and heart rate were recorded.

**RESULTS:** No difference was detected between groups for changes in MAP and heart rate variability indices during the TT. However, the analysis of areas under the curves showed a greater increase in MAP in FP vs. TP and NP. Conversely, there was a greater decrease in indices reflecting vagal modulation in TP vs. FP and NP (rMSSD, pNN50, and SDNN), and a greater increase in heart rate and sympathovagal balance in TP vs. other groups (LF/HF). The maximal oxygen uptake was strongly correlated with the vagal reserve in FP ($r = -0.74$). Moreover, the total flying hours of FP were positively correlated with resting HFnu ($r = 0.47$) and inversely correlated with resting LFnu ($r = -0.55$) and LF/HF ($r = -0.46$).

**CONCLUSION:** FP had a higher pressor response to TT than TP and NP. Vagal withdrawal and sympathovagal increase induced by TT in FP were similar vs. NP and attenuated vs. TP. Greater cardiorespiratory fitness and accumulated flying hours in FP seemed to favor lower sympathetic and greater vagal modulation at rest.

**KEYWORDS:** $+G_z$, military aviation, aviation physiology, autonomic nervous system.

**dos Santos Rangel MV, de Sá GB, Farinatti P, Borges JP. *Neuro-cardiovascular responses to sympathetic stimulation in fighter pilots*. Aerosp Med Hum Perform. 2023; 94(10):761–769.**

Fighter pilots are military-trained aviators responsible for defending their country's airspace through air-to-air or air-to-ground combat. Their job is challenging due to the mechanical, chemical, and biological stressors they face continuously and concomitantly,[11] including high acceleration in the $+G_z$ axis while performing maneuvers in high-performance aircraft.[1,30]

Despite countermeasures like anti-G suits, positive pressure breathing, cockpits designed with reclining seats,[22] and the anti-G straining maneuver, during which pilots perform the Valsalva maneuver associated with repeated isometric contractions of the lower limbs to increase venous return,[1] fighter pilots still experience cardiovascular problems when dealing with the effects of high acceleration. G-induced symptoms recurrently occur, such as gray out, blackout, almost loss of consciousness, and loss of consciousness, which result from reduced cerebral perfusion.[24] The $+G_z$ forces acting in the cephalad-to-foot direction induce cerebral hypotension due to the redistribution of blood flow to lower limbs, impairing cerebral perfusion. In response, compensatory neural mechanisms that regulate the cardiovascular system are activated, such as baroreflex resetting.[1,32] Thus, the ability to make rapid cardiovascular adjustments is essential for mission success.[1]

Repeated exposure to elevated $+G_z$ can lead to alterations in the neuro-cardiovascular axis.[20] However, most of the literature

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on the physiological challenges faced by fighter pilots concerns mechanical loading during flights, resulting in neck and spine injuries.[23] Very little is known about how long-term exposure to high G-force affects the neuro-cardiovascular axis of fighter pilots. Some authors report favorable adaptations,[17,18] including increased baroreflex sensitivity,[6] while others associate regular exposure to acceleration forces with compromised autonomic responses[3,24,27] such as a higher predominance of the sympathetic nervous system.[33] Research on this subject is important because it can contribute to the development of prophylactic or therapeutic strategies to preserve pilots' health.

Several factors seem to modulate the orthostatic and G-force tolerance.[7] Although some controversy exists,[10,16] aerobic exercise training[31] and the pilots' flight experience[17,27] have been shown to increase tolerance to orthostatic stress or $+G_z$ exposure, possibly due to changes in cardiovascular autonomic regulation. Thus, investigating the relationship between cardiac autonomic modulation with fighter pilots' physical fitness and flight experience would provide insights into the determinants of an increased orthostatic and $+G_z$ tolerance.

To address these gaps, we aimed to compare the blood pressure and cardiac autonomic modulation of fighter pilots with nonpilots and transport pilots of the Brazilian Air Force at rest and during an orthostatic challenge (tilt test). We also correlated total flying hours and physical fitness levels with the cardiac autonomic indices of fighter pilots. We hypothesized that fighter pilots would have similar neuro-cardiovascular responses at rest compared to the other groups, but greater blood pressure and vagal withdrawal, as well as lower sympathetic modulation, in response to the orthostatic challenge. Additionally, we hypothesized that the cardiac autonomic indices of fighter pilots would be related to their physical fitness and flight experience.

## METHODS

### Subjects

Subjects were Brazilian Air Force officers, ages 20–40 yr, allocated into 3 groups according to their occupation: nonpilots (NP; $N = 20$); fighter pilots (FP; $N = 21$); and transport pilots (TP; $N = 8$). Pilots were active in flight and had an experience of more than 1500 total flying hours, while the NP group was composed of officers who operated only on the ground. Exclusion criteria consisted of: 1) smoking; 2) diagnosis of diabetes mellitus; 3) resting blood pressure ≥140/90 mmHg; and 4) body mass index ≥30 $kg \cdot m^{-2}$. All volunteers provided informed written consent before participation in the study, which complied with the recommendations established by the Declaration of Helsinki and gained approval from the Ethics Review Board of the Pedro Ernesto University Hospital (Rio de Janeiro, Brazil, CAAE 76,680,416.7.0000.5259).

### Study Design

After the group allocation, subjects underwent evaluations within 2 d interspersed with 24- to 48-h intervals, always on a weekday morning (08:00–11:00) to minimize circadian effects on outcomes, in a quiet temperature-controlled environment (21–22°C). All evaluations were performed by trained technicians blinded for the study purposes and group allocation.

On the morning scheduled for the tests, volunteers presented in a 2-h fasting condition. Additionally, they were instructed to avoid physical exercise 48 h prior or ingestion of caffeine or alcohol in the 12 h before the experiment. On the first visit, after a general medical examination and anthropometric measurements, the subjects answered the International Physical Activity Questionnaire, which is a valid 27-item self-reported instrument that quantifies the level of daily physical activity.[15] Subsequently, they remained at rest in a supine position for 15 min before performing a tilt test (TT) protocol, during which beat-to-beat heart rate and blood pressure were assessed. On the next visit, the volunteers performed a maximal cardiopulmonary exercise test.

### Procedures

The TT was conducted on an electric tilt table to test the cardiac autonomic control upon sympathetic stimulation induced by orthostatic stress. The subjects were instructed to remain in the supine position on the tilt table and not to perform any voluntary muscle contraction of the lower limbs and to avoid any type of movement. The TT lasted approximately 21 min and consisted of three 1-min stimuli with a passive tilt at 70° (Tilt 1, 2, and 3), followed by a 5-min recovery in a supine position after each stimulus (Rec 1, 2, and 3). The time to change the participant's position (upright and supine) was approximately 33 ± 3 s, varying with their body mass.

A treadmill cardiopulmonary exercise test was performed to assess the maximal oxygen uptake ($\dot{V}O_{2max}$), using a ramp protocol designed to elicit maximal volitional effort within 8–12 min. Respiratory gas analysis was made using breath-by-breath analysis of $O_2$ and $CO_2$ using a calibrated, computer-based exercise system (VO2000, Medical Graphics™, Saint Louis, MO, USA). The incremental test was interrupted when patients reported any discomfort preventing exercise continuity. Tests were considered as maximal in the presence of at least three of the five following criteria[12]: 1) maximum voluntary exhaustion; 2) ≥95% predicted maximal heart rate (HR; 220 – age) or presence of HR plateau (ΔHR between two consecutive work rates ≤4 bpm); 3) presence of $\dot{V}O_2$ plateau ($\Delta \dot{V}O_2$ between two consecutive work rates <2.1 $mL \cdot kg^{-1} \cdot min^{-1}$); 4) respiratory exchange ratio >1.1; or 5) a score of 10 on the Borg CR-10 scale.

Body mass and height were measured using a calibrated electronic scale (Filizola™; São Paulo, Brazil) and wall stadiometer (Sanny™; São Paulo, Brazil), respectively. Body mass index was calculated ($kg \cdot m^{-2}$).

Resting blood pressure was reported through the average of three successive measurements interspersed with 3-min pauses using an oscillometric device (Omron™ HEM 7200, Matsusaka, Japan). During TT, beat-to-beat blood pressure was continuously measured using photoplethysmography (Finometer™, Finapress Medical System BV, Enschede, The Netherlands). Beat-to-beat HR was continuously recorded at rest and during

TT through a cardiotachometer (Polar® S810i, Polar Electro OY, Kempele, Finland), and signals were transferred to the Polar Precision Performance Software (Polar Electro™). After replacing the nonsinus beats with interpolated data derived from adjacent normal RR intervals, times series data were exported to a heart rate variability (HRV) analysis software (Kubios™ HRV software, version 3.2, Biosignal Analysis and Medical Imaging Group, University of Kuopio, Kuopio, Finland). HR recording and HRV analysis in time and frequency domains were performed according to the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology.[10]

In the present study, the following indices in the time domain were assessed: standard deviation of the NN intervals (SDNN), the square root of the mean squared successive differences from adjacent RR intervals (rMSSD), and the percent number of pairs of adjacent RR intervals differing by more than 50 ms (pNN50). The SDNN reflects total variability, while rMSSD and pNN50 are estimates of short-term components of HRV reflecting parasympathetic modulation.[10] Spectral analysis was obtained and spectral power was calculated by integrating the power spectrum density function in the high- (HF; 0.15–0.4 Hz) and low-frequency bands (LF; 0.04–0.15 Hz).[10] HF is considered to reflect the parasympathetic modulation of HR, whereas LF is influenced by both sympathetic and parasympathetic activity.[10] The ratio between LF and HF was also calculated, which is acknowledged as an estimate of overall HRV and indicates the balance between sympathetic and parasympathetic influence.

### Statistical Analysis

The sample size was calculated a priori by GPower™ 3.1.9.4 (Kiel University, Kiel, Germany) based on SDNN as a primary outcome, considering 80% power, 5% significance level, and an effect size of 0.25 determined by an eta squared ($\eta^2$) of medium effect size (0.06).[5] A total of 10 individuals in each group was estimated as necessary. Data normality was verified by the Shapiro-Wilk test. Data are presented as mean ± SD, or median and interquartile range if nonnormally distributed.

In all cases, a time window of 1 min in recordings was averaged and used for analysis, as follows: 1) the last minute of rest; 2) during each 1-min tilt; and 3) the first minute after each tilt. Sample characteristics, hemodynamic, and autonomic variables at rest were compared between groups by one-way ANOVA, while SDNN, rMSSD, LF/HF, and flying hours per day were compared by Kruskal-Wallis and Mann-Whitney tests, respectively. Changes from baseline in autonomic indices during TT were compared within and between groups using two-way ANOVA for repeated measures followed by Tukey post hoc multiple comparison tests in the event of significant *F* ratios for parametric data. The Friedman test was adopted for comparing nonparametric data.

Due to the probable insufficient statistical power of the two-way model, the intergroup analysis was complemented by comparing the areas under the curves (AUC) during TT using one-way ANOVA for the parametric indices and the Kruskal-Wallis test for the nonparametric indices. The associations between total flying hours vs. maximum aerobic capacity and autonomic indices were calculated using Pearson correlations for parametric indices, and Spearman correlations for nonparametric data. All calculations were performed using GraphPad™ software (Version 8.0.1, La Jolla, CA, USA) and the statistical significance level was set at $P \leq 0.05$.

## RESULTS

As shown in **Table I**, there were no differences between groups for sample characteristics except for the number of days per week of flight and flying minutes per day, which were respectively higher and lower in FP compared to TP. Resting hemodynamic and autonomic outcomes are depicted in **Table II**. No difference was found between groups for HR, blood pressure, or HRV indices in the time and frequency domains.

**Fig. 1** shows the changes (Δ) from baseline in HR and mean arterial pressure (MAP) for each tilt and recovery stimulus and the AUCs calculated from those responses. No main effect or interaction was found between group × time factors for HR [Fig. 1A; $F(10,215) = 0.71$; $P = 0.70$] or MAP [Fig. 1C; $F(10,215) = 0.38$; $P = 0.95$]. Comparisons of AUCs revealed that TP showed greater increases in HR (Fig. 1B) than NP ($P < 0.001$) and FP ($P < 0.001$), with no difference between NP and FP ($P = 0.78$). On the other hand, the MAP increase was greater in FP (Fig. 1D) vs. TP ($P = 0.01$) and NP ($P = 0.03$). No significant

**Table I.** General Characteristics of Non-Pilots, Fighter Pilots, and Transport Pilots.

| CHARACTERISTIC | NP (*N* = 20) | FP (*N* = 21) | TP (*N* = 8) | *P*-VALUE |
|---|---|---|---|---|
| Age (yr) | 33.2 (3.8) | 31.3 (2.0) | 31.0 (3.3) | 0.15 |
| Height (cm) | 175.1 (6.3) | 177.1 (5.6) | 176.4 (6.1) | 0.51 |
| Body mass (kg) | 80.2 (9.8) | 82.4 (7.9) | 81.1 (6.5) | 0.53 |
| Body mass index ($kg \cdot m^{-2}$) | 26.1 (2.1) | 26.2 (1.7) | 26.1 (1.6) | 0.84 |
| Habitual physical activity (IPAQ) | 1.8 (0.6) | 1.5 (0.5) | 1.9 (1.0) | 0.28 |
| $\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$) | 40.9 (8.8) | 40.8 (8.8) | 39.7 (3.3) | 0.97 |
| Total flying hours | - | 1425.0 (176.8) | 1377.5 (979.8) | 0.79 |
| Number of d/wk of flight | - | *3.0 [1.0]* | *1.5 [1.0]* | 0.001 |
| Flying minutes per day | - | 57.1 (4.8) | 168.7 (86.2) | 0.008 |

NP: non-pilots; FP: fighter pilots; TP: transport pilots; IPAQ: International Physical Activity Questionnaire. Continuous variables with normal distribution are presented as mean (SD); nonnormal variables are reported in italics as median [interquartile range].

**Table II.** Hemodynamic and Autonomic Variables at Rest of Non-Pilots, Fighter Pilots, and Transport Pilots.

| VARIABLE | NP (*N* = 20) | FP (*N* = 21) | TP (*N* = 8) | *P*-VALUE |
|---|---|---|---|---|
| Hemodynamic variables | | | | |
| HR (bpm) | 59.8 (9.8) | 56.4 (8.5) | 61.7 (10.5) | 0.45 |
| SBP (mmHg) | 117.9 (7.8) | 121.2 (12.6) | 122.7 (13.4) | 0.51 |
| DBP (mmHg) | 63.3 (7.0) | 63.0 (8.1) | 65.7 (5.9) | 0.73 |
| MAP (mmHg) | 81.5 (6.3) | 82.4 (8.8) | 84.7 (7.2) | 0.64 |
| Heart rate variability indices | | | | |
| SDNN (ms) | *40.7 [29.2]* | *47.3 [47.7]* | 57.0 (24.8) | 0.31 |
| rMSSD (ms) | 47.0 (26.5) | *49.5 [51.1]* | 63.2 (34.8) | 0.46 |
| pNN50 (%) | 23.8 (19.6) | 29.5 (22.6) | 37.3 (25.4) | 0.33 |
| LF ($ms^2$) | 1206.7 (1859.1) | 1982.5 (2219.9) | 1788.4 (1218.2) | 0.10 |
| HF ($ms^2$) | 827.7 (703.0) | 1283.0 (1678.6) | 1948.0 (1785.3) | 0.34 |
| LF (nu) | 55.9 (18.6) | 60.7 (22.7) | 55.7 (17.9) | 0.77 |
| HF (nu) | 44.1 (18.6) | 36.9 (21.7) | 44.2 (17.9) | 0.55 |
| LF/HF | *1.2 [1.8]* | *1.7 [3.4]* | 1.5 (0.9) | 0.67 |

Continuous variables with normal distribution are presented as mean (SD); nonnormal variables were reported in italics as median [interquartile range]. NP: non-pilots; FP: fighter pilots; TP: transport pilots; HR: heart rate; DBP = diastolic blood pressure; MAP = mean arterial pressure; nu = normalized units; SBP = systolic blood pressure; SDNN: standard deviation of normal-to-normal intervals; rMSSD: root mean square of successive differences; pNN50: percent number of pairs of adjacent RR intervals differing by more than 50 ms; LF: low frequency; HF: high frequency.

difference between TP and NP was found for AUC of the MAP (Fig. 1D; $P = 0.57$).

**Fig. 2** and **Fig. 3** show the changes (Δ) from baseline in autonomic indices for each tilt and recovery stimulus and the AUCs calculated from those responses. No main effect or interaction was found between group × time factors for any HRV indices for the time domain {Fig. 2; A [$F(10,230) = 0.52$, $P = 0.87$], C [$F(10,230) = 1.36$, $P = 0.19$], and E [$F(10,230) = 2.1$, $P = 0.10$]} or frequency domain {Fig. 3; A [$F(10,230) = 0.88$, $P = 0.55$], C [$F(10,230) = 0.92$, $P = 0.51$], and E [$F(10,229) = 1.42$, $P = 0.16$]}. On the other hand, comparisons of AUCs (Fig. 2B) revealed that the reduction in SDNN was greater in TP vs. FP and NP ($P \leq 0.001$), and in FP vs. NP ($P = 0.01$). As for rMSSD and pNN50 (Fig. 2D and 2F, respectively), TP showed greater reductions than NP ($P < 0.001$) and FP ($P < 0.001$), while no difference between NP and FP was found (rMSDD: $P = 0.43$; pNN50: $P = 0.93$).

The increase in LFnu and decrease in HFnu (Fig. 3B and 3D, respectively) were greater in NP vs. FP ($P < 0.001$) and TP ($P = 0.04$), with no difference between FP and TP ($P > 0.8$). Regarding LF/HF (Fig. 3F), TP presented a greater increase than FP ($P = 0.02$) and NP ($P < 0.01$).

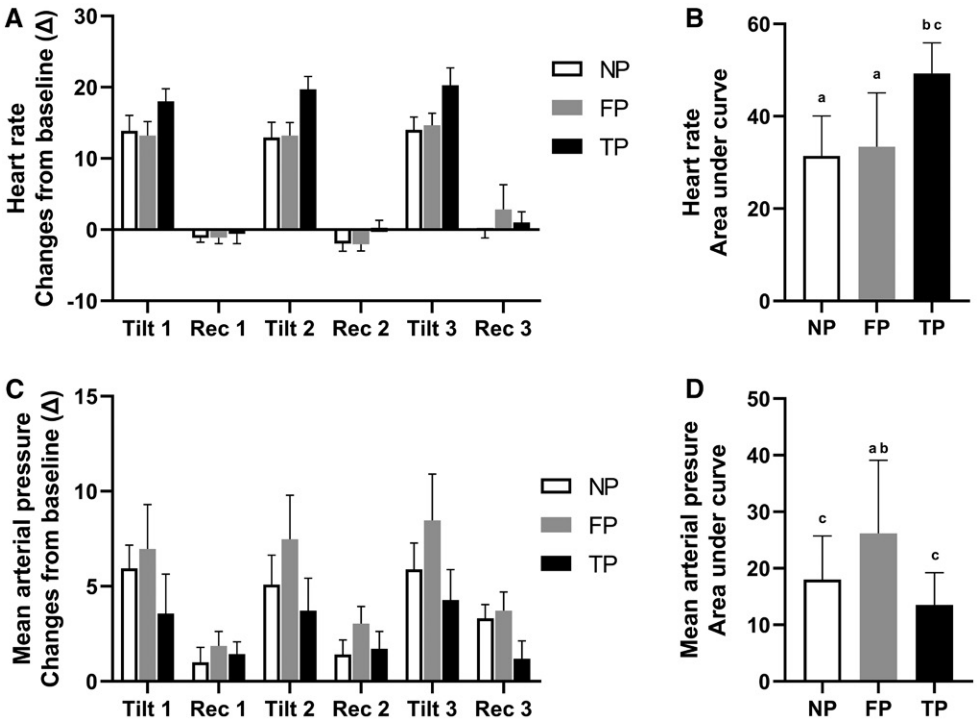


**Fig. 1.** Changes from baseline (A and C) and area under the curve (B and D) during tilt (1, 2, and 3) and recovery (Rec 1, 2, and 3) for HR and MAP in nonpilots, fighter pilots, and transport pilots. [a]$P < 0.05$ vs. TP; [b]$P < 0.05$ vs. NP; [c]$P < 0.05$ vs. FP.

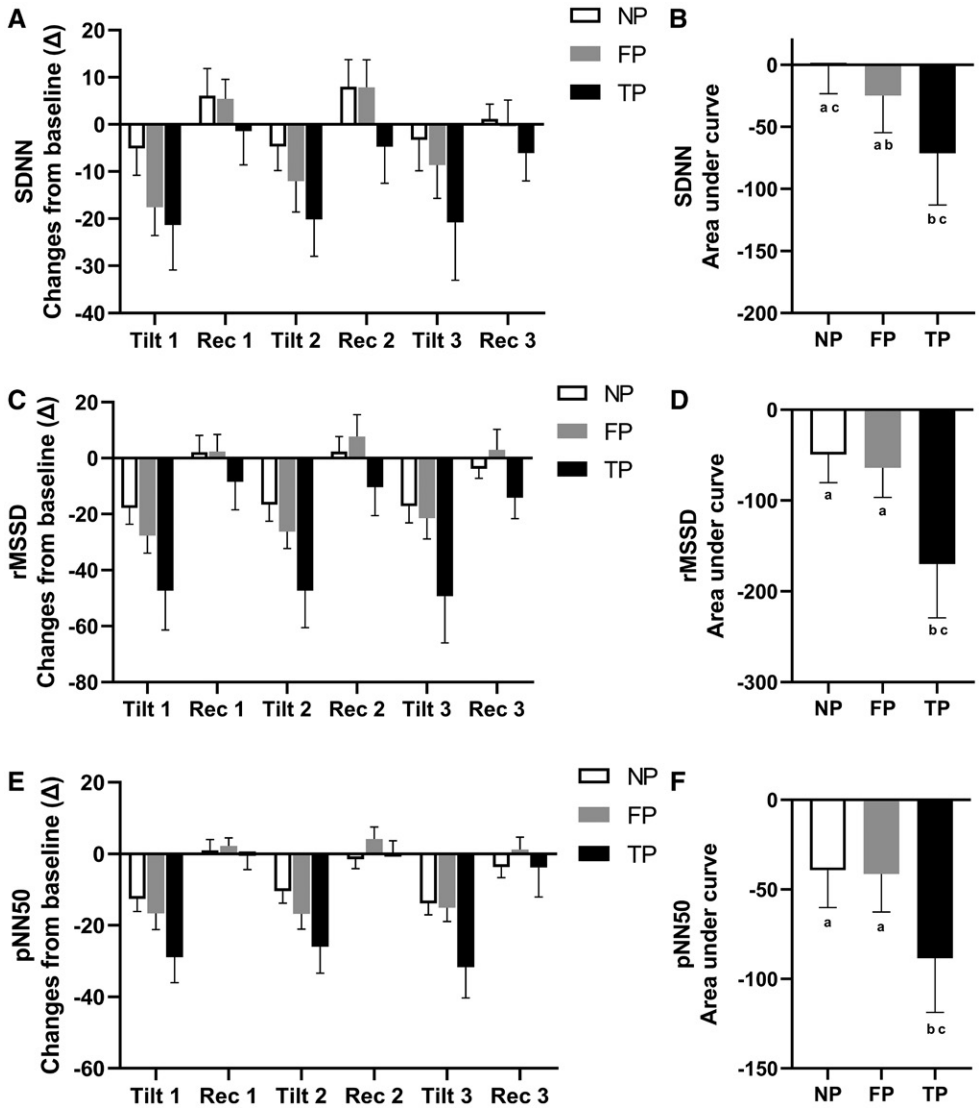


**Fig. 2.** Changes from baseline (A, C, and E) and area under the curve (B, D, and F) during tilt (1, 2, and 3) and recovery (Rec 1, 2, and 3) for time domain HRV indices in nonpilots, fighter pilots, and transport pilots. [a]$P < 0.05$ vs. TP; [b]$P < 0.05$ vs. NP; [c]$P < 0.05$ vs. FP.

**Fig. 4** exhibits the relationship between $\dot{V}O_{2max}$ and changes from baseline to the first tilt (vagal reserve) in rMSSD of FP. A strong inverse relationship was observed between $\dot{V}O_{2max}$ and vagal reserve (R = −0.74; $P = 0.01$). **Fig. 5** depicts the correlations between total flying hours and resting autonomic indices in FP. Total flying hours was inversely correlated with LFnu (R = −0.55; $P = 0.01$; Fig. 5D) and LF/HF (R = −0.46; $P = 0.03$; Fig. 5F), and directly correlated with HFnu (R = 0.47; $P = 0.02$; Fig. 5E).

## DISCUSSION

The main finding of the present study was that FP had a higher pressor response to TT than TP and NP. This response seems to rely on local vasoconstrictor mechanisms rather than cardiac autonomic modulation, as vagal withdrawal and sympathovagal balance responses to TT were attenuated in FP vs. TP, but similar vs. NP. In addition, the study expands current knowledge by originally showing that FP's experience, as expressed by the total flying hours, correlates inversely with sympathetic modulation and directly with vagal modulation assessed by frequency domain indices of HRV at rest. Furthermore, the cardiorespiratory fitness of FP is associated with changes in cardiac autonomic modulation during an orthostatic challenge.

In contrast to our results, previous research has found reduced vagal or increased sympathetic modulation at rest, during, and after stressful stimuli in FP compared to NP.[9,13,19] This suggests that regular exposure to the fighter pilot environment may lead to autonomic adaptations. However, most studies included pilots with little experience, with a wide range of flying hours (e.g.; between 400 and 1700), or did not control for this factor.[13,18,33]

Interestingly, there is evidence that the career stage of FP influences the autonomic adaptations to +$G_z$ exposure.[17,27] Studies conducted by Sukhoterin and Pashchenko[26,27] have

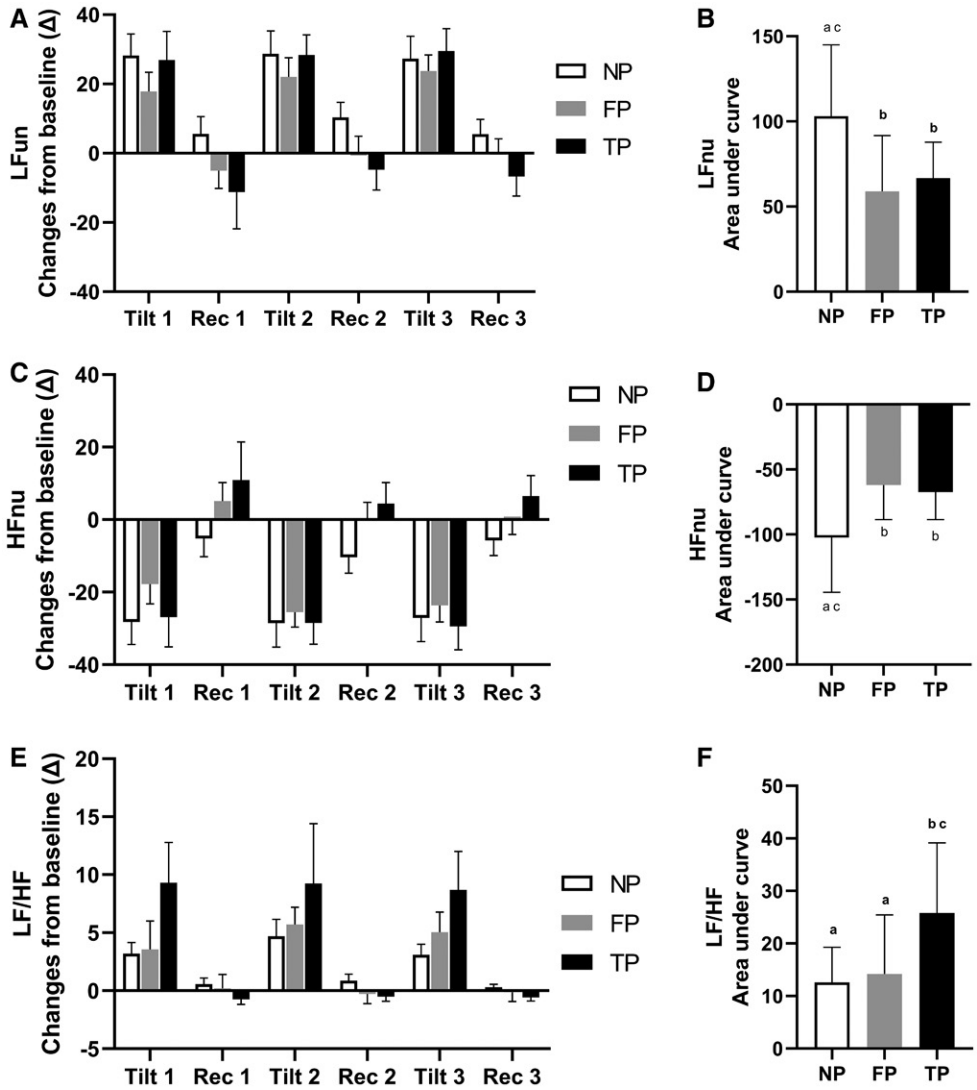


**Fig. 3.** Changes from baseline (A, C, and E) and area under the curve (B, D, and F) during tilt (1, 2, and 3) and recovery (Rec 1, 2, and 3) for frequency domain HRV indices in nonpilots, fighter pilots, and transport pilots. $^{a}P < 0.05$ vs. TP; $^{b}P < 0.05$ vs. NP; $^{c}P < 0.05$ vs. FP.

demonstrated that vagotonia at rest would be greater in more experienced vs. novice FP. Another trial[26] demonstrated that subjecting rats to acute gravitational loads resulted in reactive changes in the central autonomic nuclei. With repeated exposure, these changes became progressively destructive, resulting in the depletion of nucleate chromatin, a reduction in electron density within the mitochondrial matrix, and homogenization of mitochondrial cristae. Therefore, it is feasible to speculate that changes in sympathovagal modulation along the FP career might result from a depletion of sympathetic reserves that are constantly activated due to high accelerations, leading to chronic damage of the involved structures. Our results reinforce this hypothesis, as the total flying hours among FP correlated with indices of resting autonomic modulation, especially in the frequency domain. In other words, within-group correlation analysis demonstrated that FP with longer experience expressed by the accumulated flying hours tended to exhibit higher vagal and lower sympathetic modulation at rest.

This relationship between exposure to high accelerations and cardiac autonomic control may help to explain the attenuated autonomic responses to TT in FP vs. TP. As the average total flying hours of FP were generally higher than those presented in previous studies,[16,33] this factor may have influenced our results. However, it must be considered that the comparison between groups admitted the average behavior of FP, which had pilots with different levels of experience and +$G_z$ tolerance. The higher SD values for the resting autonomic control in FP vs. TP and NP reinforce that the interindividual variability of pilots' experience may have contributed to the attenuated autonomic response to TT and the lack of difference between groups at rest.

Another possible explanation for the lower cardiac autonomic responses of FP vs. TP to TT could be a greater dependence on local regulations of blood flow, such as myogenic activation of vascular smooth muscle, to increase blood pressure levels rather than cardiac autonomic changes. Our MAP

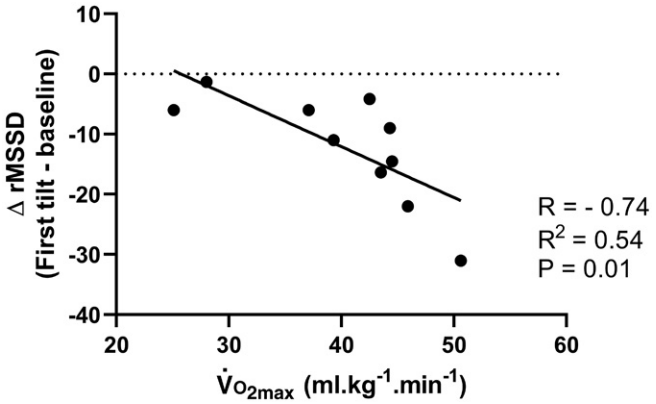


**Fig. 4.** Correlation between maximal aerobic capacity ($\dot{V}O_{2max}$) with changes in rMSSD from rest to 1st tilt in fighter pilots ($N = 10$).

and HR data reinforce this hypothesis. Consistent with our findings, previous research[16,18] reported similar stroke volume and HR, but greater blood pressure gains and peripheral vascular resistance (PVR) in FP than NP during TT. Another trial[28] demonstrated that individuals with high vs. low tolerance to +$G_z$ had greater arterial stiffness and PVR during sympathetic activation, potentially due to increased myogenic responsiveness in precapillary vessels. The blood pressure increases under stress conditions in individuals with high tolerance to +$G_z$ would result from a greater local vasoconstrictor reserve, while for those with low tolerance to +$G_z$ this response would mainly rely on central mechanisms (cardiac output and stroke volume). However, the lack of trials investigating the hemodynamic responses to TT in FP hinders further insights into the contribution of central and intrinsic vascular factors to blood pressure changes during the orthostatic challenge in this particular population.

We observed a direct association between the pilots' cardiorespiratory fitness and vagal withdrawal after tilt stimulus. A recent review[21] reinforced the assumption that aerobic conditioning provokes autonomic adaptations, increasing parasympathetic and decreasing sympathetic activity at rest, which also seems to occur during orthostatic challenge in nonpilot militaries.[31] Prior evidence indicates that aerobic training also increases the postexercise vagal reentry even without changes in vagal tone at rest, especially in individuals exhibiting lower resting vagal tone at baseline.[8] However, most studies failed to demonstrate adaptations resulting from exercise training on +$G_z$ tolerance among FP.[14,25] Slungaard et al.[25] investigated the effect of 12-wk physical training on G-force tolerance in a human centrifuge and found no differences in blood pressure between trained and control groups. Similarly, in a cross-sectional design, Kölegård and Mekjavic[14] found no differences when comparing +$G_z$ tolerance and pressor response to exercise in sedentary, endurance-, and strength-trained individuals. Interestingly, those authors hypothesized that during +$G_z$ exposure individuals performed anti-G straining maneuvers, activating muscle mechano- and metaboreflex to maintain blood pressure levels. However, we could not find studies on the effects of physical training on autonomic control or muscle metaboreflex sensitivity in association with +$G_z$ tolerance.

Unexpectedly, when compared to NP and FP, TP showed greater vagal withdrawal and sympathetic modulation expressed by the LF/HF ratio in response to TT. Exceptions were the $LF_{nu}$ and $HF_{nu}$ indices, which may have been influenced by slower recovery from TT. Since there were no differences between the groups for cardiorespiratory fitness, habitual physical activity, and autonomic control at rest, we believe that these differences may be due to specific adaptations resulting from the occupational activities in TP, such as lower flight frequency and longer missions in comparison to FP. Regarding flight duration, Dussault et al.[9] evaluated the sympathovagal balance before and after 2 h of long vs. short combat flights and showed that the autonomic response could differ depending on the mission characteristics. After shorter flights, the sympathovagal balance, as expressed by LF/HF increased, while it decreased after longer flights. Similar results were found by Jouanin et al.,[13] who identified that short flights (30 min) induced an increase in LF/HF up to 2 h after the mission. However, it should be noted that these findings are limited since there was no comparison with a control group exposed to the same stressor (+$G_z$).

Few studies have investigated the autonomic behavior of pilots during flight or in sympathetic stimulation protocols. Among those that used TT, prolonged stress-mediated orthostatic changes were the most commonly adopted protocols,[2,9,13] consistent with research conducted in other populations.[4,29] In the present study, we chose a protocol that involved successive short passive tilts to better mimic the incremental and irregular blood flow redirection stimuli experienced during flights. In this case, analyzing the areas under the curves seemed ideal, as it allows for insight into both the response to stimuli and recovery.

This study has both strengths and limitations. One limitation is the relatively small sample size in the TP group ($N = 8$), which was inferior to the estimated sample size. The difficulty in recruiting this group due to flight schedules resulted in a discrepancy in the number of individuals included in the FP ($N = 21$) and NP ($N = 20$) groups. On the other hand, the FP sample was much greater than in most available research, which is undoubtedly a strength of our study. Moreover, FP with different experience levels were compared not only to NP but also to military pilots who typically perform significant flight volumes (TP) but do not undergo intense +$G_z$ stress. Secondly, respiratory rate was not assessed during the assessment of RR intervals. However, subjects were instructed to maintain relaxed normal breathing—typically around 12–20 breaths/min. This may have lessened the potential effects of respiratory rate on our HRV data, although we cannot completely rule out this possibility. Another major limitation was the duration of TT. Previous studies adopted protocols in which the time to change the tilt table position was approximately 4 s,[16,17] while the present experiment applied a much longer duration (around 33 s). This may have influenced our findings since faster stimuli would be more similar to hemodynamic stress in flight conditions.[17] Finally, the lack of a more robust cardiovascular and

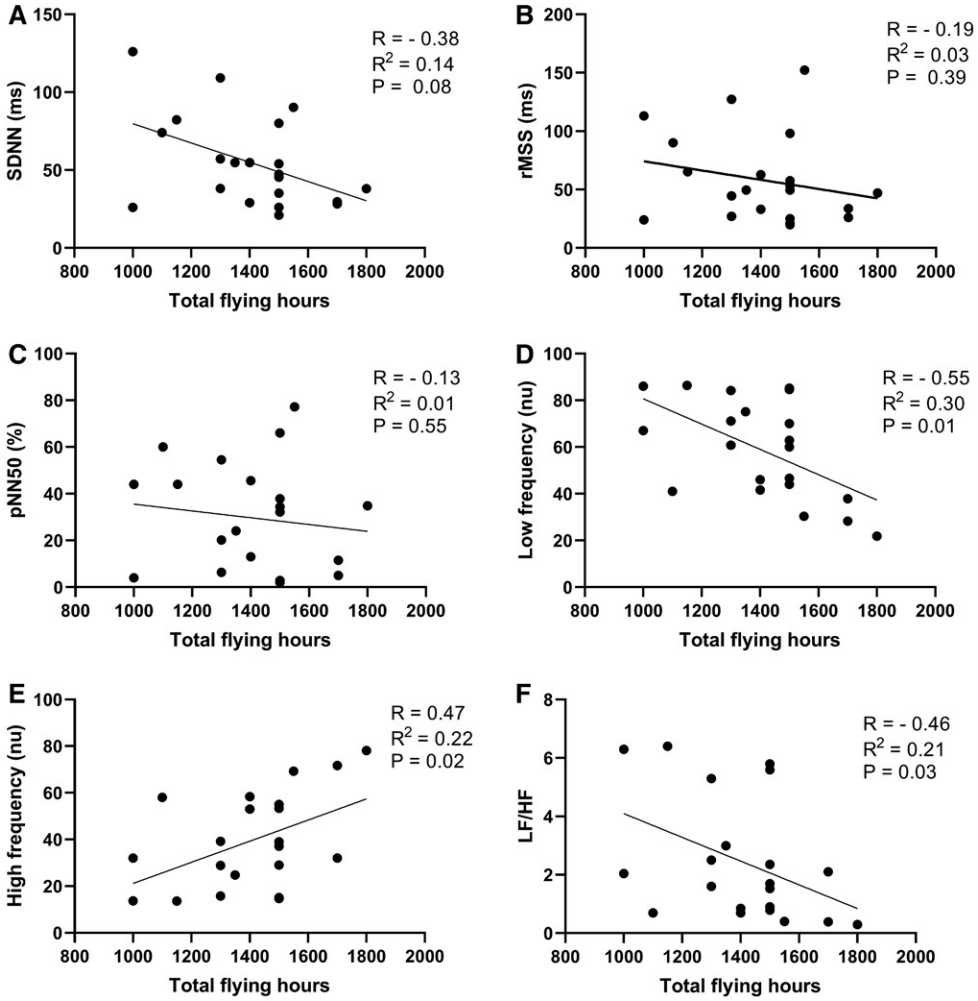


**Fig. 5.** Correlation between total flying hours with indices of resting HRV for the time domain SDNN, rMSSD, and pNN50 (Panels A, B, and C) and frequency domain LFnu, HFnu, and LF/HF (Panels D, E, and F) in fighter pilots ($N = 21$).

sympathetic activity assessment should be mentioned. Data on the cardiac baroreflex sensitivity, cardiac output, PVR, and muscle sympathetic nerve activity during TT could have given a better understanding of the neuro-cardiovascular responses. Although HRV has the advantage of being a simple, noninvasive method capable of assessing dynamic changes in the autonomic control of heart rate,[10] more objective measurements of sympathetic outflow, such as microneurography, would provide more specific and direct information on sympathetic activity at the periphery.

In conclusion, FP with an average of 1425 flying hours showed a higher pressor response, but a modest response to autonomic activation when subjected to TT. This was reflected by lower vagal withdrawal and sympathetic gain compared to TP. On the other hand, the autonomic responses in FP were generally similar to those in NP. Additionally, the autonomic modulation at rest among FP appeared to be influenced by cardiorespiratory fitness and accumulated flying hours. Greater aerobic capacity and flight experience were strongly associated with higher vagal and lower sympathetic modulation in this group. These findings are relevant because they can contribute to interventions and decision-making regarding the operational training and health preservation of military pilots. However, our data should be considered preliminary and must be confirmed by further research, including larger samples and a longitudinal approach.

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# Additive Sensory Noise Effects on Operator Performance in a Lunar Landing Simulation

Sage O. Sherman; Young-Young Shen; Daniel Gutierrez-Mendoza; Michael Schlittenhart; Cody Watson; Torin K. Clark; Allison P. Anderson

**INTRODUCTION:** Adding noise to a system to improve a weak signal's detectability is known as stochastic resonance (SR). SR has been shown to improve sensory perception and cognitive performance in certain individuals, but it is unknown whether this performance improvement can translate to meaningful macrocognitive enhancements in performance for complex, operational tasks.

**OBJECTIVE:** We investigated human operator performance in a lunar landing simulation while applying auditory white noise and/or noisy galvanic vestibular stimulation.

**METHODS:** We measured performance ($N = 16$ subjects) while completing simulation trials in our Aerospace Research Simulator. Trials were completed with and without the influence of auditory white noise, noisy galvanic vestibular stimulation, and both simultaneously in a multimodal fashion. Performance was observed holistically and across subdimensions of the task, which included flight skill and perception. Subjective mental workload was collected after completing four trials in each treatment.

**RESULTS:** We did not find broad operator improvement under the influence of noise, but a significant interaction was identified between subject and noise treatment, indicating that some subjects were impacted by additive noise. We also found significant interactions between subject and noise treatment in performance subdimensions of flight skill and perception. We found no significant main effects on mental workload.

**CONCLUSIONS:** This study investigated the utility of using additive sensory noise to induce SR for complex tasks. While SR has been shown to improve aspects of performance, our results suggest additive noise does not yield operational performance changes for a broad population, but specific individuals may be affected.

**KEYWORDS:** stochastic resonance, auditory white noise, noisy galvanic vestibular stimulation, aerospace research simulator.

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Spaceflight frequently requires astronauts to complete a variety of complex tasks across a mission's duration, necessitating peak human performance. However, living in the spaceflight environment for an extended period of time poses physiological and psychological hazards that impact crew mental health and, thus, human performance.[10] Long-duration deep space missions will also lead to greater morphological and radiative destructive changes in the central nervous system, which may lead to large cognitive and behavioral declines.[14] As such, NASA's Human Factors and Behavioral Performance group identified that on a long duration deep space or planetary mission the risk of adverse cognitive or behavioral conditions on operations requires mitigation.[18] Thus, there stands a need to develop safe, effective, and standalone countermeasures that the crew could use to offset these human performance decrements when performing spaceflight mission tasks. One such countermeasure could leverage the mechanism of stochastic resonance (SR).

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SR is a phenomenon where additive noise can improve the detectability of a signal in nonlinear systems.[9] Human experimentation shows that SR can improve perception performance within and across sensory modalities.[2,9,21] While SR has traditionally been explored for perception purposes, Hidaka et al. have shown that noise enhanced sensory information could be used within the central nervous system, suggesting that SR may also affect higher order information processing.[4] This implies that SR could enable a safer, noninvasive form of neuromodulation as it involves stimulating sensory systems instead of directly stimulating the brain, as is the case with alternative neuromodulation techniques, such as transcranial direct current stimulation (tDCS). This notion is supported in the literature through a few human subject experiments that improved cognitive task performance by inducing single modality SR (i.e., applying noise to only one sensory system). Background auditory white noise (AWN) (~78 dB SPL) improved verbal recall, visuo-spatial working memory, and motor response in inattentive school children.[3,19] Noisy galvanic vestibular stimulation (nGVS) was found to reduce visual memory recall speed in healthy adults.[24] In an evaluation of comprehensive cognition, SR cognitive performance enhancement was not found in the broad population, but it was present in subjects who preferred working in noisy environments.[17] These studies, though, focus on specific and separate microcognitive functions, such as working memory, failing to provide insight on the utility of SR in real-world contexts.

Macrocognition refers to cognitive functioning in natural environments.[6] Completing complex mental tasks involves synthesizing many microcognitive skills to execute a relevant task, such as driving a car or landing a spacecraft. To our knowledge, the benefits of SR have not been assessed for macrocognition, as it is potentially difficult to analyze the results of complex tasks with a great degree of sensitivity. Usher and Feingold found SR improved speed of memory retrieval for multiplication.[20] Multiplication may be more analytical and complex than other cognitive domain assessment tasks, but this task is not operationally relevant and a weak indicator for overall performance enhancement. Beyond SR, the literature has explored macrocognitive benefits for traditional forms of neuromodulation. Choe investigated tDCS effects for an nBack task and a flight simulator, finding improved performance and learning in both tasks, implying benefits in the microcognitive and macrocognitive domains.[1] Further, Scheldrup et al. found improvements in multitasking while utilizing tDCS, suggesting improved macrocognitive performance.[16] In addition to direct performance enhancement, tDCS has been shown to reduce perceived temporal workload in surgical simulations.[23] High levels of mental workload can lead to stress and performance decrements in operators[22]; thus, neuromodulation techniques that influence mental workload may indirectly impact operator performance. Given the success of other neuromodulation techniques to improve operationally relevant performance, the absence of research on SR macrocognitive effects presents a substantial literature gap that needs to be addressed.

We aimed to fill this gap in the literature by assessing the potential for enhancing operator performance using sensory noise. We hypothesized that single modality noise (either AWN or nGVS) would enhance performance in human subjects when compared to performance without noise (sham); additionally, we hypothesized that stimulating both modalities simultaneously to induce multimodal SR (MMSR) would have additive benefits and enhance performance to a greater degree than using a single modality alone. To assess this, subjects performed a series of lunar lander simulation tasks under sensory noise and a no noise sham. This task loads several perceptual, cognitive, and motor coordination domains, such that the results provide comprehensive insight into the influence of noise for operational tasks.

Additionally, SR perception studies imply that perception performance enhancement is greater for at-threshold perceptual stimuli, but suprathreshold enhancement is possible.[8,15] Thus, we believed that "at-threshold" operational enhancement could be a factor in noise benefit effectiveness. We hypothesized that the extent of SR performance enhancement may vary as a result of task challenge. Our task design allowed us to modulate task difficulty and assess whether improvements are related to task difficulty.

Finally, SR studies have suggested that some individuals are susceptible to SR improvements, while others are not.[2,12] This suggests that performance enhancement may be seen in some subjects as a result of noise but not others. Thus, we also hypothesized that only some subjects would see SR benefits. Building upon a previous study our lab conducted where we saw individual SR sensitivity within cognitive performance,[17] we evaluated whether there was a positive correlation between operator performance and preference enhancement for working in a noisy environment to help identify individuals who receive benefits from noise.

## METHODS

### Subjects

A total of 16 subjects (9 women/7 men), ages 29 ± 7 yr (range = 20–41 yr) completed testing in the Bioastronautics Lab at the University of Colorado Boulder. An a priori power analysis based on the results of Scheldrup et al. suggested that we needed 16 subjects for our study design to find an effect size greater than 0.3, as Scheldrup et al. found for tDCS.[16] This research was approved by the University of Colorado-Boulder's Institutional Review Board (protocol #20-0347) and written informed consent was obtained prior to participation. Subjects were prescreened and excluded if they reported a history of health issues that could impact cognitive abilities, such as severe head trauma or disorders associated with thinking impairment. They were also excluded if they reported health issues that could impact auditory or vestibular processing, such as language impairment or vestibular dysfunction. Additionally, subjects underwent auditory screening to verify healthy and unobstructed ear

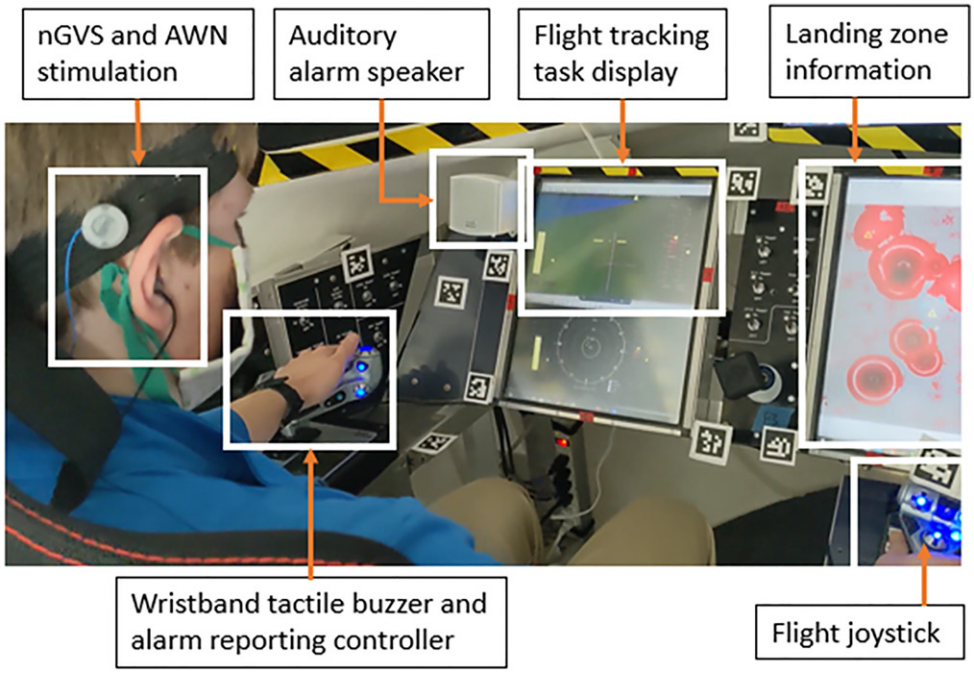


**Fig. 1.** Over-the-shoulder view of the AReS lunar lander simulation used in this experimental paradigm. Relevant hardware and displays are highlighted in overlaid white boxes. Subjects sit stationary directly in front of the flight tracking task display with their right hand on the flight joystick which they use for the tracking task. To the right of the flight display is a map display indicating lunar topography and possible landing zone locations (i.e., landing zone information). To the left of the flight display is an external auditory speaker that intermittently presents the auditory alarm. A tactile buzzer is attached to the subject's left wrist to present the tactile alarm. The subject rests their left hand on the throttle, which they use to signal when they notice that the auditory or tactile alarm is occurring. nGVS electrodes and AWN earbuds are fixed to the subject's head in all trials, including sham where no noise was administered.

canals (via otoscopy), normal tympanometry, and normal hearing (audiometric thresholds ≤25 dB HL up to 8 kHz).

### Equipment and Materials

The task used in this study aimed to be a representative analog for a macrocognitive task that individuals in an operational environment may face. The simulation task was completed using our Aerospace Research Simulator (AReS), shown in **Fig. 1** and **Fig. 2**. AReS is a demonstrated, macrocognitive landing task that incorporates several cognitive processes at once.[11,25] Fig. 1 illustrates the hardware and interface of the AReS fixed-base flight simulation, while the software provides a realistic replication of lunar landing vehicle dynamics, piloting control responses, and fuel consumption. In the AReS lunar lander simulation task, subjects were presented with six landing points scattered across a 2D contour map of the lunar surface. They attempted to choose the optimal landing point, considering its distance with respect to three scientific points of interest (i.e., nearest the centroid) and its potential presence within hazardous areas, such as steep slopes (Fig. 2B). The lander descended at a constant rate, continuously consuming fuel. To navigate to their designated landing zone, subjects were required to complete a tracking task on their primary flight display using a joystick by aligning the spacecraft's pitch and roll attitude (the yellow reticle) to the flight guidance cue (the magenta cue) (Fig. 2A). At a lander altitude of 250 ft (76 m; roughly 40 s into the task), a simulated lidar gave the subject a new topography map which presented additional hazard information not visible initially, such as rock fields where the subject would not be able to land (bottom right panel of Fig. 2B). At this point, the subject could continue to fly toward their original landing zone choice or, by pressing buttons on the joystick, redesignate a new landing site or abort the landing [allowable between 200 and 50 ft (61 and 15 m) of altitude].

Novel to previous work done with AReS,[11,25] we embedded two perception tasks. One was a tactile vibration presented to the wrist and the other was an auditory alarm presented to the cockpit via speakers. Both alarms indicated that a simulated thruster was stuck and consuming more fuel than usual. The magnitude of these perception alarms were initially low, beginning subthreshold and gradually increasing to a suprathreshold level. Subjects pressed a button on the throttle as soon as they identified either alarm to effect a "reset" that solved their fuel leakage problem. The fuel decreased at a faster rate than usual while the alarm was active to incentivize the subjects to attend to the perception task. Each perception task occurred twice during a trial and occurred at random intervals. The timing of the four perception alarms was randomly assigned to four set times (10, 30, 50, and 80 s into the task) with a random time amount (between 1–10 s) added to each of those four set times. Input was only accepted when the alarms were present; unsolicited presses of the button were not registered.

This task was designed to load the operational subdimensions of flight skill, decision-making, and perception. The task's dependent variables were performance metrics that make up the task subdimensions found in **Table I**. Each metric quantified an aspect of performance that we hypothesized may be sensitive to SR

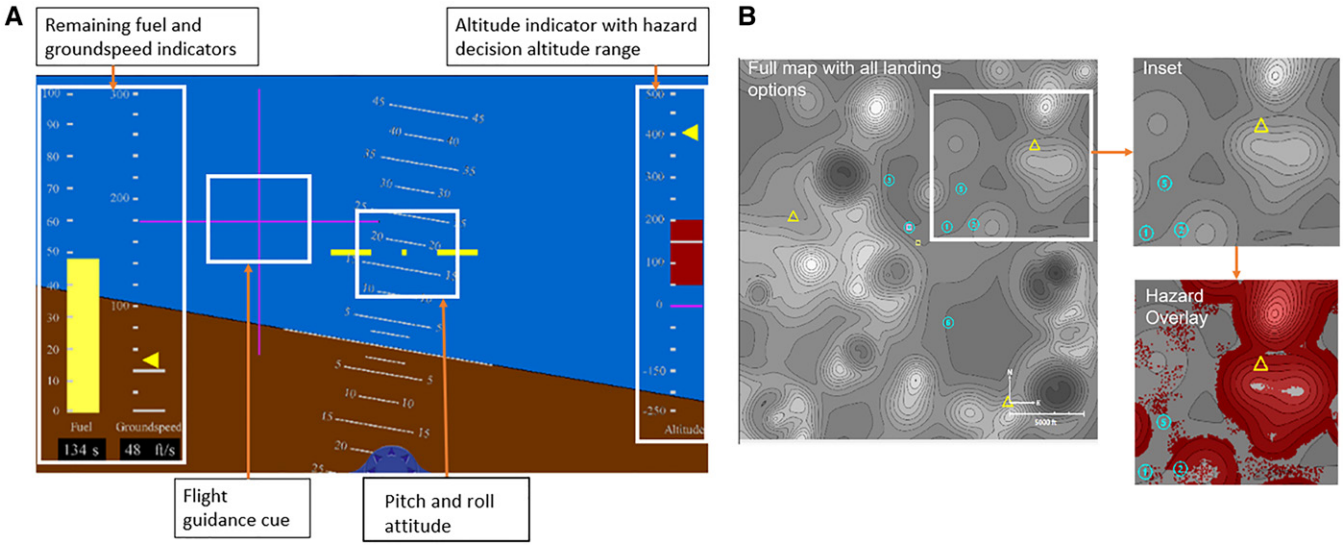


**Fig. 2.** A) Visual information presented to the subject in the primary flight display. The panel displayed the spacecraft's pitch and roll attitude and altitude (and depiction of the hazard decision range in red), groundspeed, and fuel. The subject tracked the magenta flight guidance cue to align with the spacecraft's pitch and roll attitude as represented by the yellow reticle. B) Topography maps made available to subjects. The left was displayed to the subject at the start of the task. The yellow triangles depicted three scientific points of interest and blue circles were landing zones that subjects chose from. The top right panel is a zoomed-in inset of the map for legibility, the bottom right panel is the same display with hazard data from a simulated lidar sensor overlay that appears once the spacecraft reaches 250 ft (76 m) of altitude.

performance improvement. A description of how each metric relates to performance is also given in Table I. Combining these subdimensions yields a comprehensive performance measure to capture overall operator changes caused by SR.

As hypothesized, we also investigated whether operational enhancement due to SR may be dependent upon task difficulty. For example, performance on an easy task may be insensitive to adding sensory noise. Thus, we tested three levels of task difficulty (easy, medium, or hard) as determined by the layout of hazards, points of scientific interest, and potential landing zones on the landing maps, a description of which is found in **Appendix A** (found online at https://doi.org/10.3357/amhp.6251sd.2023).

The independent variable of this research was the four treatments of sensory noise administered. Broadband AWN (20–20,000 Hz) was administered to subjects through ear buds (Essential Earphones HD; Essential Products, Inc., Palo Alto, CA, USA) and a Samsung Tablet A; the auditory profiles were developed and calibrated by Creare LLC (Hanover, NH, USA).

**Table I.** A Description of Each Performance Metric in the Lunar Lander Simulation.

| SUBDIMENSION & PERFORMANCE METRIC | METRIC DESCRIPTION | METRIC JUSTIFICATION |
|---|---|---|
| Flight | | |
| Root mean square distance (RMS; degrees) | The RMS distance error of the yellow reticle from the magenta cue over the simulation duration. | The ability of subjects to track the lander's attitude with the guidance system. Better performance corresponds to a reduction in RMS error. |
| Joystick input (stick) | The percentage of time the subject spent giving an input to (i.e., deflecting) the joystick during the simulation. | A measure of efficiency, the simulated lander has an attitude hold. If subjects overuse the joystick when it is not necessary, they are spending more fuel. |
| Smooth flying (smooth) | The number of times the subject crosses over the magenta cue in pitch and roll as they track with the reticle. | A measure of excessive control. A flyer who overshoots the magenta cue spends more fuel correcting for their mistake; better flying results in less overcorrecting. |
| Decision-Making | | |
| Landing zone (LZ) | A ranked score based on the combination of initial and posthazard display landing zone choices. | Some landing zones are better choices than others in terms of their distance to scientific points and the presence of hazards. Reselecting a better landing zone based upon lidar-updated hazard information was rewarded. |
| Crash, abort, or land (CAL) | A ranked score based on whether the subject landed, crashed, or aborted when it was or was not possible to land. | Based on their landing zone selections and flight performance, subjects may need to make trade-offs for safety or landing success. |
| Perception Identification | | |
| Tactile (seconds) | The time it takes for subjects to detect and report each of the two tactile alarms. | A quicker reaction time to press the alarm button results in less fuel loss, suggesting enhanced perceptual performance. |
| Auditory (seconds) | The time it takes for subjects to detect and report each of the two auditory alarms. | A quicker reaction time to press the alarm button results in less fuel loss, suggesting enhanced perceptual performance. |

Broadband, unipolar, zero-mean white noise (0–100,000 Hz) was bilaterally administered to subject mastoids through the Galvanic Vestibular Oscillating Stimulator (model 0810, Soterix Medical, Woodbridge, NJ, USA) using electrodes with a contact area of 2 square cm.[21] The third sensory noise treatment consisted of using both AWN and nGVS administered simultaneously in a multimodal fashion. A sham treatment where no sensory noise was administered, but with electrodes and earbuds applied, served as the baseline.

### Procedure

A within-subject experimental design was implemented. After enrollment, subjects watched a 15-min tutorial video to orient them to the lunar landing task. They then completed a minimum of nine practice trials of the task, or until they felt comfortable with the controls, displays, and goals. This was done to ensure they had fully learned how to operate the simulation and understood all dimensions of the task. Further, a test operator assessed the subject's basic competency level with the task before proceeding.

On a separate test day (within 1 wk of their initial visit), subjects completed 34 trials of the task. Each trial contained a unique map with differing terrain (and thus hazards) and landing points from the other trials. There were two phases to the experimental trials on the test day. The first phase identified the subject-specific optimal noise levels in AWN and nGVS for testing in the second phase, as will be described in the next paragraph. In the second phase, we investigated our main hypotheses for task performance and subjective workload using our four sensory noise treatments (AWN, nGVS, MMSR, and sham).

There is an optimal level of noise to induce SR that depends on subject, task, and sensory system.[9] This has been demonstrated in studies evaluating noise enhancement of sensory perception within and across modalities.[2,12,21] We believed this would be the case for cognitive performance enhancement; thus, an initial suite of three nGVS levels (0.2, 0.5, and 0.8 mA) and three AWN levels (40, 55, and 70 dB SPL) were tested in a randomized order, as has been done in our prior work.[17] Subjects completed three trials for each level, resulting in 18 total trials in this first phase to identify the subject-specific best noise level. Raw performance in each metric was fractionally ranked across the 18 trials and assessed. In order to identify each subject's best noise level, broad task performance was quantified (Eq. 1) from this initial set of trials. This metric is the sum of each individual metric captured and equally weighted among the three subdimensions of flight performance, decision-making, and perception.

$$P_{Total} = \frac{1}{3} * \left(P_{RMS} + P_{Stick} + P_{Smooth}\right) + \frac{1}{2} * \left(P_{LZ} + P_{CAL}\right) + \frac{1}{2} * \left(P_{Tactile} + P_{Auditory}\right) \quad \text{Eq. 1}$$

The SR noise level that yielded the best performance described by Eq. 1 was selected as the subject-specific best (experimentally close to optimal) AWN and nGVS level. The performance value calculated in Eq. 1 was not used for any further analysis beyond identifying these best noise levels.

Once the subject-specific best SR levels were obtained in the first phase, subject-specific best level of AWN, nGVS, and MMSR were tested across 16 additional unique trials (4 trials per treatment) in the second phase. Within each treatment, four trials were administered based on the map difficulty (one easy, two medium, and one hard map) in a randomized order. After each treatment was tested, mental workload was captured using a modified Bedford workload scale.[5,13] This allowed us to assess average subjective workload independent of map difficulty. All sensory noise treatments were presented in a randomized order. Data from these 16 trials were retained for analysis.

After completing all trials, subjects completed a subjective five-point Likert scale questionnaire that asked how well they could maintain focus in quiet and noisy environments. Their noisy environment preference score was defined as the difference in their ranking between quiet and noisy environments (i.e., a negative score means the subject prefers working in quiet places and a positive score means they prefer working in noisy places).[17] This survey can be found in **Appendix B** (found online at https://doi.org/10.3357/amhp.6251sd.2023).

### Statistical Analysis

A within-subjects analysis was completed to evaluate operator performance differences due to sensory noise treatments. Each of the performance metrics described in Table I have different measurement units, making them difficult to combine into a composite performance score. Thus, ranking was used. For each metric, the raw performance values in each of the 16 trials were fractionally ranked for each subject [e.g., when assessing performance for root mean square (RMS) distance, each of the subject's 16 trials were ordered and ranked from best to worst]. This allowed us to compile ranked data across metrics to assess overall operator performance and per subdimension by isolating the subdimension metrics of flight, decision-making, and perception.

Upon visualization, the subdimension of decision-making yielded substantial violations of normality assumptions for residuals. This is due to the skewed nature of the nominal and ordinal data collected for the decision-making metrics. Specifically, across all subjects, 89% of trials were landed successfully and in 66% of trials subjects identified the optimal landing zone (LZ) in their first selection (this increased to 70% by their second selection). Therefore, we determined that this subdimension was not sensitive enough to observe deviations in performance based upon nonparametric rankings, so data related to this subdimension was removed from our overall operator performance analysis. Thus, we conducted a separate Chi-squared goodness of fit analysis to observe differences between treatments in each separate decision-making subdimension metric, including crash, abort, or land (CAL) and LZ selection. Subdimensions of flight and perception were analyzed as no observable assumption violations were present. This suggests that a parametric statistical analysis for this data was appropriate and retained for overall operator performance analysis.

For overall operator performance analysis, and the subdimensions of flight and perception, a repeated measures analysis of variance (RM ANOVA) was conducted between noise treatments on the fractional ranked values. Fixed main effects included in the model were noise treatment, from which our main hypothesis was investigated, and map difficulty. Additionally, the interaction of noise treatment with subject was included since only some subjects may exhibit performance changes with SR.[17] An interaction between noise treatment and map difficulty was included to test whether the effect of sensory noise on performance was influenced by task difficulty. Assumptions for homogeneity and residual normality were tested to ensure that parametric statistics were appropriate. If the *F*-test results from the RM ANOVAs were significant, Tukey HSD multiple pairwise comparisons were used to identify which treatments were different from one another.

A nonparametric Friedman test was used to assess mental workload from our Bedford scale data, as the data is ordinal in nature. We also applied an RM ANOVA to the Bedford scale data for completeness, using the same factors as described for the fractionally ranked performance data.

Additionally, we aimed to see whether a subject's noisy environment preference indicated operator performance sensitivity to the noise treatments. Within individual metrics, performance was averaged across the four maps, resulting in one value per noise treatment per subject. From there, performance in the sham treatment was subtracted from their performance in each sensory noise treatment. This data resulted in 240 outcomes (16 subjects × 3 baseline-adjusted sensory noise conditions × 5 metrics in Table I = 240 outcomes). Linear regression models were fit to this entire performance dataset against subjects' noisy environment preference scores to identify if subjects with a preference for noisy environments benefited more from SR.

## RESULTS

The AWN and nGVS noise levels presented in these results are the subject best noise levels which were derived from pretrial performance evaluation across all metrics (Eq. 1). Visualizations of this pretrial performance data for each subject are given in **Appendix C** (found online at https://doi.org/10.3357/amhp.6251sd.2023). **Table II** displays the RM ANOVA results that correspond to overall operator performance (flight and perception subdimensions combined). Contrary to our hypothesis, no significant differences were found for our noise treatment alone. However, consistent with our hypothesis, a significant interaction between subject and noise treatment was identified. A main effect of map difficulty was also identified for this compiled dataset. A multiple comparisons analysis for the main effects of map difficulty on overall performance found that performance in "easy" maps [mean (M) = 0.10 ± SD 0.26] was significantly better than "medium" maps (M = −0.01 ± 0.23) and "hard" maps (M = −0.05 ± 0.24) and performance in "medium" maps was significantly better than "hard" maps. Contrary to our hypothesis, no significant interaction effects were identified between noise treatment and map difficulty. These results are visualized in **Fig. 3A**.

**Table II.** RM ANOVA Results for Overall Operator Performance.

| FACTOR | *F* (DOF) | *P*-VALUE | $\eta_p^2$ |
|---|---|---|---|
| Noise Treatment | 0.069 (3, 1223) | 0.61 | 0.003 |
| Map Difficulty | 35.28 (2, 1223) | <0.005* | 0.055 |
| Noise Treatment × Subject | 2.13 (45, 1223) | <0.005* | 0.073 |
| Noise Treatment × Map Difficulty | 1.29 (6, 1223) | 0.26 | 0.006 |

*Factors that met a statistical significance below 0.05.

**Table III** displays the RM ANOVA results that correspond to the subdimensions of flight and perception. For the flight subdimension, we found a significant main effect of map difficulty, as well as a significant interaction between noise treatment and subject. A multiple comparisons analysis for the main effects of map difficulty on flight data found that performance in "easy" maps (M = 0.17 ± 0.3) was significantly better than "medium" maps (M = −0.02 ± 0.25) and "hard" maps (M = −0.09 ± 0.25) and performance in "medium" maps was significantly better than "hard" maps. Contrary to our hypothesis, no significant effects were identified for the noise treatments or the interaction of noise treatment and map difficulty. These results are visualized in **Fig. 3B**.

For the perception subdimension, we found a significant main effect of noise treatment and a significant interaction between treatment and subject. A multiple comparisons analysis for the main effects of treatment on the perception data found that performance in the AWN treatment (M = −0.04 ± 0.22) was significantly lower (i.e., worse) than in the sham treatment (M = 0.04 ± 0.17). No other significantly different comparisons were identified. As might be expected, map difficulty had no effect on the perception task. These results are visualized in **Fig. 3C**. Note that no significant interactions between noise treatment and map difficulty were identified for the subdimension (Table III) performance evaluations.

For the subdimension of decision-making, a separate analytical approach was applied. The frequency of the nominal outcomes is presented in **Table IV**. A Chi-squared goodness of fit test was applied to each decision-making metric presented in Table IV. When assessing the CAL metric, due to the low frequency of aborts or crashes, these outcomes had to be combined to meet the assumption for sufficiently sized expected frequencies. Thus, the statistical test was applied to the outcomes of "land" and "not-land". For the CAL metric, the resulting test statistic was $\chi^2(3) = 4.17$. For the landing zone selection metric, the resulting test statistics were $\chi^2(3) = 0.63$ for the choice before hazards were displayed and $\chi^2(3) = 1.84$ for the choice after hazards were displayed. Thus, contrary to our hypothesis, no significant effects were identified between the noise treatments when it came to our decision-making metrics.

A nonparametric Friedman analysis was used to assess the Bedford workload scale data. Contrary to our hypothesis, our results showed no significant main effects of noise treatment [$\chi^2(3) = 4.49$, $P = 0.21$]. For completeness, an RM ANOVA test was also performed since it may have had more power, but the ordinal data technically violated the model's assumptions; however, it yielded the same conclusion [$F(3,45) = 1.45$, $P = 0.24$].

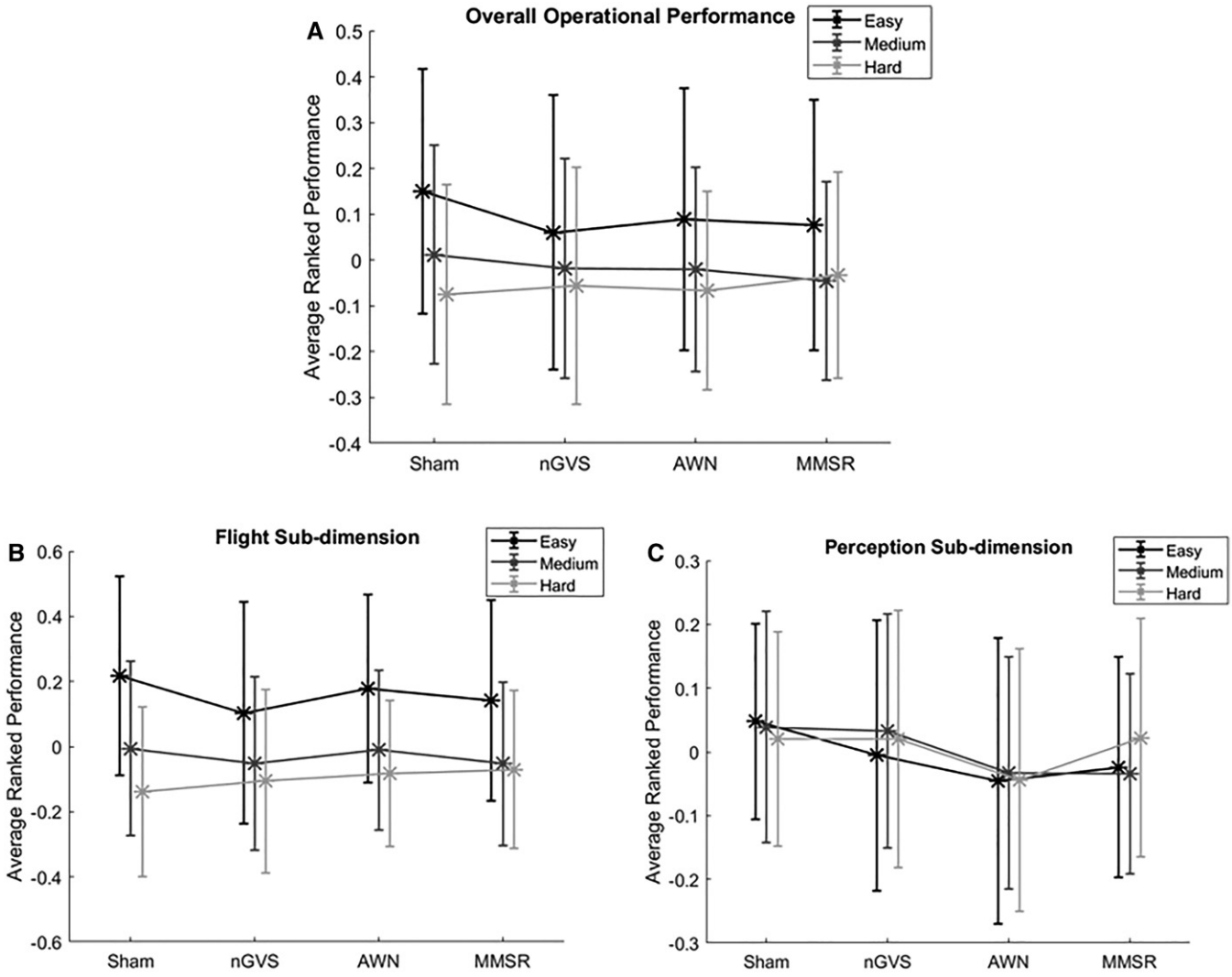


**Fig. 3.** A) Main effects plot of noise treatments and map difficulty for the overall performance aggregated dataset. The three sensory noise treatments were applied at subject-specific best levels determined in the first phase of testing. Higher ranks correspond to better performance. Error bars represent the standard deviation. B) Main effects plot of noise treatment and map difficulty for the flight subdimension. Error bars represent the standard deviation. C) Main effects plot of noise treatment and map difficulty for the perception subdimension. Error bars represent the standard deviation.

Additionally, a linear regression was fit between subject performance difference for the aggregated dataset across all noise treatments and the subject's noisy environment preference. Contrary to our hypothesis, we did not find a significant correlation between noisy environment preference and operator performance relative to sham (slope = −0.46, $P = 0.57$).

## DISCUSSION

This research aimed to understand the utility of using additive sensory noise to improve operator performance. To our knowledge, this is the first assessment of SR for macrocognitive tasks. This was done by having subjects complete a complex lunar landing task, requiring participants to make decisions, actively

**Table III.** RM ANOVA Results for the Flight and Perception Subdimensions.

| | FLIGHT | | | PERCEPTION | | |
|---|---|---|---|---|---|---|
| **FACTOR** | ***F* (DOF)** | ***P*-VALUE** | $\eta_p^2$ | **F (DOF)** | ***P*-VALUE** | $\eta_p^2$ |
| Noise Treatment | 0.53 (3, 711) | 0.67 | 0.005 | 2.75 (3455) | 0.049* | 0.026 |
| Map Difficulty | 43.3 (2, 711) | <0.005* | 0.130 | 0.14 (2455) | 0.87 | 0.001 |
| Noise Treatment x Subject | 2.49 (45, 711) | <0.005* | 0.136 | 1.62 (45,455) | 0.008* | 0.138 |
| Noise Treatment x Map Difficulty | 0.97 (6, 711) | 0.44 | 0.008 | 0.58 (6455) | 0.75 | 0.008 |

*Factors that met a statistical significance below 0.05.
DOF: degrees of freedom.

**Table IV.** Outcome Frequency Table for the Decision-Making Metrics.

| OUTCOME | SHAM | nGVS | AWN | MMSR |
|---|---|---|---|---|
| Crash – Abort – Land Metric | | | | |
| Land | 53 | 57 | 60 | 58 |
| Abort | 5 | 5 | 3 | 6 |
| Crash | 6 | 2 | 1 | 0 |
| Optimal landing zone selection (prior to hazard appearance) | | | | |
| Selects Optimal LZ | 43 | 43 | 44 | 40 |
| Fails to select Optimal LZ | 21 | 21 | 20 | 24 |
| Optimal landing zone selection (after hazard appearance) | | | | |
| Selects Optimal LZ | 41 | 47 | 47 | 44 |
| Fails to select Optimal LZ | 23 | 17 | 17 | 20 |

nGVS: noisy galvanic vestibular stimulation; AWN: auditory white noise; MMSR: multimodal stochastic resonance; LZ: landing zone.

track moving stimuli, and vigilantly identify perceptual alarms under sensory noise aimed to induce SR.

By observing performance across subdimensions of flight and perception, we intended to identify what attributes of operations that sensory noise may influence. We found no main effect of noise treatment on performance in the flight task, but noise had a significant main effect in the perceptual task; however, based upon our pairwise comparison, we found this significant difference results from AWN masking the auditory alarm, reducing auditory detection relative to the sham treatment. While certain levels of additive AWN are shown to reduce auditory thresholds and enhance perception,[9] these intensity levels are often low, in contrast to higher levels inducing masking behavior.[12] However, the auditory noise levels needed to induce SR across sensory modalities (e.g., in visual perception) and enhance cognitive functions are sufficiently suprathreshold.[7,19] This is a relevant concern when it comes to implementing auditory white noise treatments in operational environments (i.e., the high level of AWN necessary to induce cross-modal SR may produce decrements in auditory perception via masking).

Interestingly though, the interaction results reflect findings in other perceptual and cognitive SR literature. Previous perception studies found that only some individuals exhibit SR benefits where noise can lower perception thresholds.[2,12] This has also been shown in microcognitive task performance. Söderlund et al. reported that AWN improves cognitive performance in inattentive school children, whereas attentive school children did not exhibit benefits from AWN.[19] Previous work that we conducted found that applying AWN or nGVS had no effect on overall cognition for the broad population. However, subjects who self-reported preferring to work in noisy environments received cognitive enhancement from additive sensory noise.[17] Building upon this work, the noisy environment preference questionnaire was included in this study to further investigate this notion. While we found a significant interaction between subject and noise treatment, no correlations were identified between noise preference and performance changes. Our results suggest that individual differences may be a dominant factor in whether SR improves operator performance, but our noise preference questionnaire may not be a useful indicator in this context.

Map difficulty was identified as a main effect in influencing flight skill, but not perception. This may be expected, as map difficulty modifies the simulation's optimal flight pattern and trajectory without changing aspects of the perception task (auditory and tactile detection response times). We hypothesized that there may be an interaction between sensory noise treatment and map difficulty, as SR effects might be more pronounced at certain levels of task difficulty. Our results did not find a significant interaction between treatment and difficulty

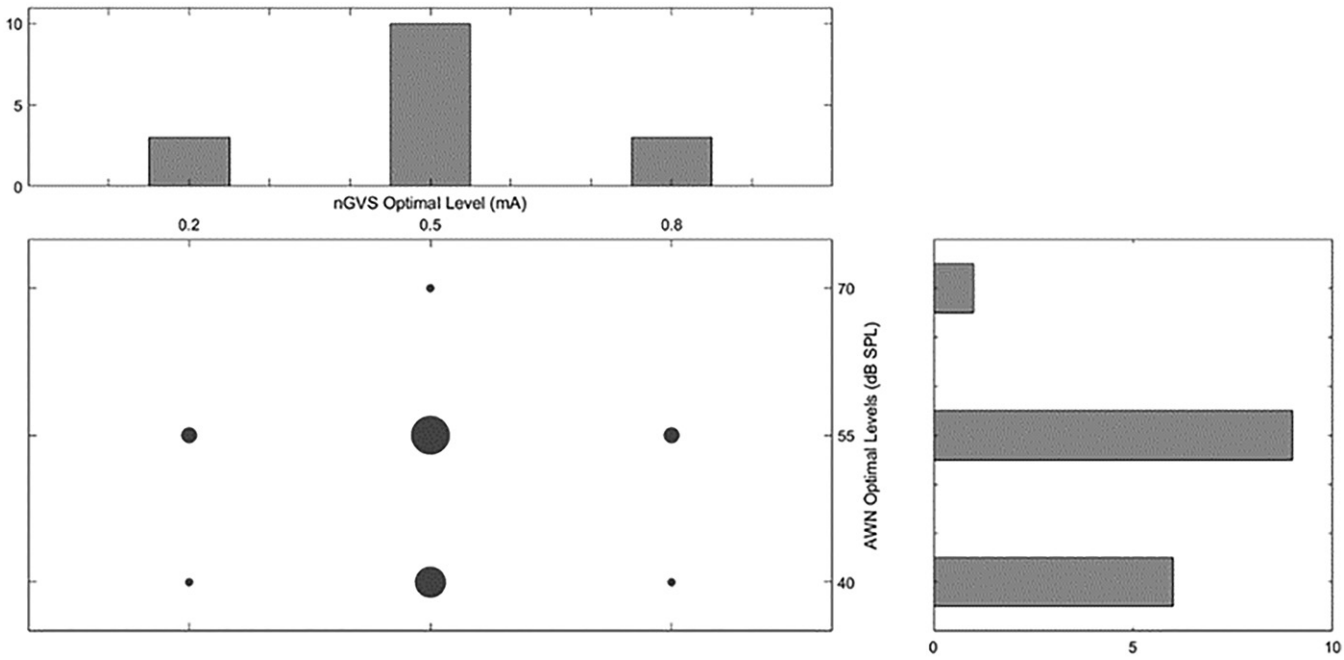


**Fig. 4.** Scatter bubble plot with marginal histograms showing the frequency of best levels identified for nGVS (x-axis) and AWN (y-axis) across our subject pool. Larger bubbles indicate a higher frequency of that combination. Note that the best levels in each sensory modality were the central levels tested.

in the overall or subdimension performance analyses to support this hypothesis. While SR has been shown to improve suprathreshold performance in sensory systems,[7,15] it is classically believed to modulate threshold, or at-limit, capabilities; therefore, by varying task difficulty we could capture whether improvements are only observed near subject limits. It is possible that our task was not challenging enough for our subjects to achieve this at-limit improvement, as subjects, on average, successfully landed 89% of the trials (95% for hard maps, 86.7% for medium maps, and 87.5% for easy maps). This appears consistent with our average subjectively reported mental workload, as the reported average was 3.2 with a 1.1 standard deviation, which suggests the task was always "satisfactory" or "tolerable."

While a null finding cannot prove there is no effect, this is the first evidence supporting that both nGVS and AWN do not enhance multiple aspects of operational performance. Like any study, it could be that there is an effect and our study was just not sufficiently well powered to identify it. First, this investigation consisted of 16 subjects as guided by our a priori power analysis. While we mention that our task may not have been sensitive enough to find performance differences, it is entirely possible that the effect size is small enough such that a greater number of subjects is needed to increase power and identify significant changes. Small effect sizes can result from large measurement variability. It was noted that older subjects had greater challenges adjusting to pitch inversion, finding the task more challenging than younger subjects, which could result in larger measurement variability. Originally, to avoid this, some subjects were given a longer training session than others. An exploratory analysis found that age had no significant effect on operational performance despite these reported challenges ($P > 0.9$), so age may not be a result of variability. Note that we report effect sizes to enable future meta-analyses. Second, it could be that our specific lunar landing task is not susceptible to SR effects, while other operational tasks may be. This is a first investigation and motivates future work. However, the lack of evidence across multiple subdomains of the complex task does not support benefits in other complex tasks. Third, other SR work has concluded that different levels of sensory noise are optimal for different individuals and tasks, but many cognition-based SR studies investigate a single noise level across all participants. To try to address this we rigorously conducted an initial suite of tests at three different sensory noise levels (0.2, 0.5, and 0.8 mA for nGVS and 40, 55, and 70 dB SPL for AWN) to identify the subject-specific best levels. The frequency of best levels identified are shown in **Fig. 4**; further visualization of subject performance in each metric for each noise level is provided in Appendix C (found online at https://doi.org/10.3357/amhp.6251sd.2023). It is possible that this procedure was inadequate at identifying a level of sensory noise that was beneficial for each individual, either because our suite was not inclusive of the optimal levels for most subjects (e.g., a subject's optimal nGVS level was 1 mA and our suite only extended up to 0.8 mA) or because the suite was not fine enough (e.g., optimal was 0.65 mA and we only tested at 0.5 and 0.8 mA, neither of which yielded much benefit). However, our suite was selected based upon levels at which SR benefits had previously been observed,[2,3] have been used by us previously,[17,21] and reasonably traded off the time required to do the initial suite (and associated subject learning/fatigue/boredom). Fig. 4 shows that levels with increased sensitivity around 0.5 mA nGVS and 55 dB SPL AWN levels should be further explored. Additionally, the results found in Appendix C (found online at https://doi.org/10.3357/amhp.6251sd.2023) show it is possible that some noise levels may be more appropriate for specific subdimensions within subjects (e.g., 40 dB SPL may be best for perception detection, but 70 dB is best for flight). This can pose operational challenges in identifying noise levels that are comprehensive in performance improvement.

While the literature shows that SR may help enhance perception and aspects of cognition, we did not find that it has a substantial influence on operator performance for the broad population. Our work, however, is the most comprehensive assessment of sensory noise effects on operator performance in a complex task to date. As such, for complex aerospace applications like the one investigated in this study, it may not be a critical operationally relevant countermeasure. Nonetheless, since neither nGVS or AWN seemed to affect some individuals, but was not related to an individual's noise preference, future work should explore these individual differences in SR susceptibility for operator performance.

This investigation evaluated the utility of applying sensory noise to improve performance in a macrocognitive task. We conclude that applying additive noise to auditory and vestibular modalities will not result in improved operator performance or reduced perceived mental workload for the broad population. However, similar to other SR investigations, we find that specific individuals may be affected by additive noise. We had subjects report their preference for working in noisy environments to build upon previous work on whether preference is a useful indicator for individual SR performance effects. We found no correlation between noisy environment preference and performance under noise influence.

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# $+G_z$ Standards for the Indian Air Force

Ajay Kumar

**INTRODUCTION:** $+G_z$ tolerance is an important aspect for the success of fighter aircrew as it reflects the ability of the neuro-cardiovascular response to compensate and prevent adverse manifestations such as gray-out, black-out, and G-induced loss of consciousness (G-LOC) under high-G stress.

**METHODS:** The data for aircrew taking the Operational Training in Aerospace Medicine for Fighters course at the Institute of Aerospace Medicine Indian Air Force (IAF) from January 2017 to December 2020 were analyzed to assess the effectiveness of the existing training goal to recommend a G-tolerance standard for fighter aircrew.

**RESULTS:** During the study period, 334 aircrew took the Operational Training in Aerospace Medicine for Fighter course. Only three aircrew failed to achieve the training goal of the course (failure rate <1%). There was a significant difference in the relaxed gradual onset rate tolerance of aircrew experiencing G-LOC and not experiencing G-LOC during the training. The odds of experiencing G-LOC at 9 G after clearing the 7-G and 8-G profiles were 4.4 and 4.7, respectively.

**DISCUSSION:** It is generally accepted that aircrew having higher G tolerance have less chance of G-LOC in the air. There is a need to have an operational definition of G tolerance for fighter aircrew that aligns with the operational training goal of the organization. The G tolerance of IAF aircrew is as per the institutional definition of the IAF Institute of Aerospace Medicine. The high-G training has stood the test of time and has served well for the IAF.

**KEYWORDS:** G tolerance, high sustained G, fighter aircrew, Operational Training in Aerospace Medicine, Indian Air Force.

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Fighter aircrew get exposed to $+G_z$ acceleration frequently during aerobatics and combat. The effect of $+G_z$ acceleration is known to manifest most commonly in the form of visual symptoms like tunnelling of vision or gray-out (also known as peripheral light loss or PLL) and blackout or central light loss (CLL) and not so commonly in the form of neurological symptoms such as G-induced loss of consciousness (G-LOC). These manifestations depend on the tolerance of an individual and have been reported to be 4.47 G ± 0.69 for a relaxed Indian aircrew for gray-out at a gradual onset rate (GOR).[16] Tolerance is defined as the G level or duration at G by which a specific body system starts manifesting signs of failure (e.g., PLL or CLL for vision and G-LOC for the central nervous system). Tolerance measurement of the high-G environment should, therefore, measure both this component G level as well as G duration. Tolerance criteria for component G level usually involve the ability of a subject to maintain vision or consciousness, which is classically determined on subjects who are "relaxed" and is considered an individual's basic G tolerance, which measures the cardiovascular response to an increased G exposure. For G duration, the usual tolerance criterion is a subjective fatigue endpoint that can be validated with blood lactate level.[1] The G-tolerance parameter is required for the selection and training of aircrew as well as the evaluation of aircrew and G-protective equipment. The most used parameter is G-level tolerance under GOR with PLL as the endpoint.

The Institute of Aerospace Medicine, Indian Air Force (IAM IAF), has conducted high-G training of Indian fighter aircrew and aircrew from 12 friendly foreign countries since 1991. The Institute is also responsible for evaluating aircrew (with suspected low G tolerance) and G-protective equipment (e.g., anti-G suit or AGS). Traditionally, relaxed GOR tolerance has been used for such evaluation. However, the nonavailability of a

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well-defined G-tolerance standard has led to wide variation in such an approach. This is also the fact that the values of GOR tolerances vary among various centers with different centrifuges as well as the target population. These variations could be due to different arm lengths of the centrifuges, different protocols (PLL to 52–56° of the lightbar, 100% PLL, 50% CLL, 100% CLL, etc.), and different onset rates ($0.1\,G \cdot s^{-1}$, $1/15\,G \cdot s^{-1}$, $0.25\,G \cdot s^{-1}$, etc.). Hence, it is important for every center to develop its own G-tolerance standard.

The reported relaxed G-tolerance values for Indian aircrew appear to be significantly lower than their U.S. Air Force counterparts.[16] Despite this, they have been successfully meeting the high-G training requirements of 9 G for 5 s during Operational Training in Aerospace Medicine for Fighters (OPTRAM-F). This is because relaxed G tolerance does not correlate well with G-level tolerance, especially when it is determined while using an AGS and/or an anti-G straining maneuver (AGSM).[1] The traditional way of identifying low G tolerance by means of GOR ($0.1\,G \cdot s^{-1}$) or rapid onset rate (ROR; $1\,G \cdot s^{-1}$) may not be reliable as these parameters fail to predict performance in a high sustained G environment. Parkhurst et al. demonstrated that individuals with normal G tolerances can be trained to endure 9 G for up to 45 s or more safely.[19] The aim of this paper was to study the G tolerance of the pilots reporting to IAM IAF for high-G training and recommend optimal standards for the high-G training based on the data available from the training experience.

## METHODS

### Subjects

The training data available in the Department of Acceleration Physiology and Spatial Orientation from January 2017 to December 2020 was used for the purpose of the study. Approval was received from the Institute Ethical Committee for this retrospective analysis of the G tolerance of fighter pilots who underwent training during this period in the department.

### Equipment

The high-G exposure was given to all aircrew using an 8-m arm, high-performance human centrifuge with three degrees of freedom. The specification and technical details are available in the article published earlier by this author.[16]

### High G Training Profiles

During high-G training in the OPTRAM-F, aircrew are gradually exposed to 4 $G_z$ for 60 s, 6 $G_z$ for 30 s, 7 $G_z$ for 15 s, 8 $G_z$ for 10 s, and 9 $G_z$ for 5 s in the target tracking mode after initially checking their relaxed GOR tolerance at $0.1\,G \cdot s^{-1}$ with the endpoint of PLL of 52–56° on the lightbar over the period of 3 d. Optionally, they are also subjected to a simulated aerial combat maneuver of 4 $G_z$ for 10 s and 8 $G_z$ for 10 s with onset/offset rates of $6\,G \cdot s^{-1}$, with a maximum of six such exposures in the pilot out-of-loop profile. Additionally, the "push-pull effect" is demonstrated where relaxed GOR tolerance at $0.1\,G \cdot s^{-1}$ with the AGS not inflated is checked before and after exposure to −1.5 $G_z$. The reduction in the relaxed GOR tolerance after exposure to −1.5 $G_z$ is explained as the result of the "push-pull effect".[18] All aircrew sign a consent for the use of their training data for research and academic purposes.

The training goal of OPTRAM-F is 9 G for 5 s and exposure to a simulated aerial combat (SACM) profile of 4 G for 10 s and 8 G for 10 s of six loops is an optional profile. The training data for all aircrew who underwent training up to 9 G and their relaxed GOR tolerance data available in the system were included in the study. The incidence of almost loss of consciousness has been included as G-LOC as it is considered part of the G-LOC syndrome rather than a separate entity.[15] The success of training at any G level was analyzed based on G-LOC episodes experienced during the subsequent exposures.

### Statistical Analysis

The data obtained were tabulated and descriptive analysis, *t*-test for relaxed GOR tolerance of aircrew experiencing G-LOC and not experiencing G-LOC during the training, and odds ratio for G-LOC during various G profiles were calculated using SPSS 26.

## RESULTS

During the study period, a total of 334 pilots underwent high-G training. However, not all of them were exposed to the full OPTRAM-F profiles of up to 9 G for 5 s. Hence, these aircrew were not included in the study. Further, three aircrew experienced G-LOC during the relaxed GOR tolerance assessment. Although they successfully completed the remaining portions of the OPTRAM-F profile, their relaxed GOR tolerance data for gray-out was unavailable. Consequently, these three aircrew were excluded from the study.

The remaining dataset comprised the training data of 302 pilots, which was deemed suitable for further analysis. However, one pilot's relaxed GOR data at 7.9 G was identified as an outlier and subsequently removed from the study. Interestingly, this

**Table I.** The Descriptive Parameters for the Pilots Included in the Study.

| PARAMETERS | *N* | MINIMUM | MAXIMUM | MEAN | STANDARD DEVIATION |
|---|---|---|---|---|---|
| Age (yr) | 300 | 19.22 | 44.23 | 26.91 | 4.75 |
| Height (cm) | 300 | 162 | 205 | 174.51 | 5.62 |
| Weight (kg) | 301 | 53 | 94 | 72.11 | 7.54 |
| Flying hours | 301 | 15 | 2900 | 638.38 | 598.94 |
| GOR tolerance | 301 | 2.9 | 7.02 | 4.56 | 0.71 |

*N* = number of pilots and GOR = relaxed gradual onset rate tolerance at $0.1\,G \cdot s^{-1}$ with PLL of 52–56° as endpoint.

**Table II.** The Descriptive Parameters for the Pilots Experiencing G-LOC at Various G Levels.

| G LEVEL | *N* | AGE (YR) (MEAN ± SD) | HEIGHT (cm) (MEAN ± SD) | WT (kg) (MEAN ± SD) | FLYING HOURS (MEAN ± SD) | GOR (MEAN ± SD) |
|---|---|---|---|---|---|---|
| 6 G TT | 7 | 24.86 ± 3.60 | 174 ± 5.41 | 68.43 ± 10.49 | 412 ± 429 | 4.31 ± 0.68 |
| 7 G TT | 21 | 25.26 ± 4.25 | 177.48 ± 4.99 | 72.24 ± 8.20 | 537.86 ± 642 | 4.33 ± 0.68 |
| 8 G TT | 39 | 25.54 ± 3.20 | 175.03 ± 5.34 | 71.26 ± 7.58 | 524.69 ± 484 | 4.40 ± 0.69 |
| 9 G TT | 36 | 26.11 ± 4.47 | 174.97 ± 5.21 | 72 ± 7.43 | 530.31 ± 434 | 4.46 ± 0.55 |
| SACM | 11 | 23.93 ± 2.26 | 175.64 ± 5.56 | 70.45 ± 5.50 | 317.72 ± 224 | 4.35 ± 0.74 |
| Mean | 80 | 25.68 ± 3.98 | 174.98 ± 5.08 | 70.65 ± 6.74 | 505.2 ± 491 | 4.34 ± 0.60 |

*N* = number of pilots; GOR = relaxed gradual onset rate tolerance at 0.1 G · $s^{-1}$ with PLL of 52–56° as endpoint; TT = target tracking; SACM = simulated aerial combat maneuver.

particular pilot experienced G-LOC at 9 G. Therefore, the final dataset analyzed for the study consisted of the training data of 301 pilots.

The mean age, height, weight, flying hours, and GOR tolerance of the pilots in the study experiencing G-LOC at various G levels and not experiencing G-LOC are found in **Table I**, **Table II**, and **Table III**, respectively.

A total of 80 pilots in the study experienced G-LOC at various G levels during the training. None of the pilots experienced G-LOC during the 4.5 G target tracking run. The GOR tolerance of pilots not experiencing G-LOC (Table III) was significantly higher than the pilot experiencing G-LOC ($P < 0.001$). However, there was no significant difference in GOR tolerance among pilots experiencing G-LOC at various G-levels (Table II). As reported in our previous study,[16] it appears from the data (Table II and Table III) that older pilots and those with more flying hours may have a lower frequency of experiencing G-LOC. However, no statistical analysis was conducted to confirm these findings, as it was beyond the scope of the study.

**Fig. 1** shows the number of pilots experiencing G-LOC during various high-G training profiles. The number of pilots experiencing G-LOC tripled from 6 G (2.3%) to 7 G (7%) and doubled from 7 G to 8 G (12.9%). It remained similar at 8 G and 9 G (12.3%). Of the pilots undergoing training, 88% (267) volunteered for the SACM profile, and 4.1% of those pilots suffered G-LOC during the training. The mean G-duration tolerance of successful aircrew was 44.8 s. The success rate for the SACM profile was 95.9%.

**Fig. 2** shows the distribution of relaxed GOR tolerance among pilots experiencing G-LOC and not experiencing G-LOC during the high-G training. As expected, G-LOC incidences are higher for people with lower relaxed GOR tolerance which stagnates at 4.5 G and beyond.

Of the pilots who experienced G-LOC at 6 G, 43% (3 out of 7) also suffered G-LOC at 7 G. A similar percentage of pilots (43%, i.e., 9 out of 21) suffering G-LOC at 7 G also suffered G-LOC at 8 G, whereas approximately 29% of pilots experiencing G-LOC at 7 G and 8 G also suffered G-LOC at 9 G. The odds of experiencing G-LOC at 9 G for these pilots were 4.4 and 4.7, respectively. At the same time, 28% of aircrew experiencing G-LOC during the course did not experience G-LOC at 7 G and below, but experienced G-LOC at 9 G. Only three aircrew failed to achieve the training goal of the course (failure rate <1%), implying failure in the course to be <1%.

## DISCUSSION

Defining G tolerance is important for the selection of aircrew for high performance fighter aircraft, comparing G protection provided by an AGS or other G-protective methods, and disposal of cases of low G tolerance. Japan, Korea, and many Warsaw Pact air forces (Serbia, Denmark, Netherlands, and Germany, etc.) have used centrifuges for the evaluation of G tolerance as a part of their selection process for high performance fighter aircraft.[10] GOR tolerance with PLL as an endpoint has been most widely used for these purposes, with the premise that this defines the best G protection available to a subject due to the baroreceptor response. This is also due to the simplicity of assessment and availability of continuous data that allows the use of the parametric test. However, Ludwig and Krock caution that a single determination has a very high standard error (±0.78 G, 95% confidence interval, range 1.5 G) and low reliability, making it unacceptable for most scientific and clinical applications.[17] Relaxed G tolerance in ROR is assessed using an epoch pattern which is ordinal data, resulting in nonparametric tests for any statistical analysis, which has lower strength than parametric tests. Other than this and the concern raised by Ludwig and Krock, it is also observed that a high relaxed G-level tolerance is not necessary for tolerating high G levels when customary AGS and AGSM are used, as only 16% of the high-G straining tolerance is dependent upon a person's relaxed ROR tolerance.[9] The G tolerance at higher rapid onset

**Table III.** The Descriptive Parameters for the Pilots Not Experiencing G-LOC During High-G Training.

| PARAMETERS | *N* | MINIMUM | MAXIMUM | MEAN | STANDARD DEVIATION |
|---|---|---|---|---|---|
| Age (yr) | 220 | 20.51 | 44.23 | 27.35 | 4.93 |
| Height (cm) | 220 | 162 | 205 | 174.35 | 5.80 |
| Weight (kg) | 221 | 53 | 94 | 72.64 | 7.76 |
| Flying hours | 221 | 15 | 2900 | 686.59 | 627.49 |
| GOR tolerance | 221 | 2.9 | 7.02 | 4.63 | 0.73 |

*N* = number of pilots and GOR = relaxed gradual onset rate tolerance at 0.1 G · $s^{-1}$ with PLL of 52–56° as endpoint.

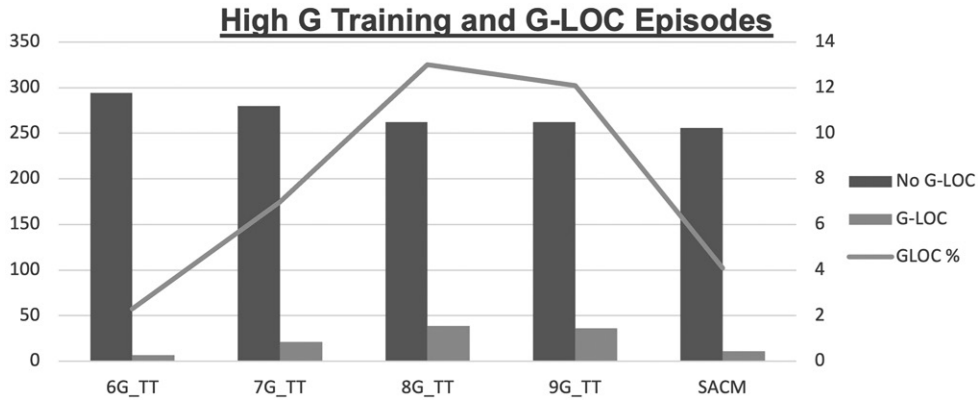


**Fig. 1.** G-LOC experienced by pilots during high-G training profiles.

rates such as high rapid onset rates (defined as $>3\,G \cdot s^{-1}$) and very high rapid onset rates (defined as $>6\,G \cdot s^{-1}$) reduces further.[1] Since the G tolerance estimated through these means may not accurately predict pilot performance at higher G levels, it is prudent to test aircrew for the G level and G duration in which the pilot is required to operate. G-level tolerance of IAF aircrew with fully operational anti-G trousers and AGSM was 9 G for 5 s and G-duration tolerance using the SACM profile was 45 s, which meets the institutional definition of G tolerance. The success rate was 99% for G-level tolerance and 96% for G-duration tolerance as per the institutional definition. G-duration tolerance may be higher as most of the trainees did not continue for more than two loops, which was the optional minimum requirement during the course.

Analysis of G-LOC data at various G levels revealed that the incidence of G-LOC was lowest at 6 G (2.3%), tripled at 7 G (7%), was 5.6 times at 8 G (12.9%), and was 5.3 times at 9 G (12.3%). Further, a significant number of aircrew (28%) who did not experience G-LOC at 6 G or 7 G experienced G-LOC at 9 G. Hence, it is amply clear that performance at 6 G and 7 G did not predict performance at higher G levels. This has a significant implication in setting high G training goals for fighter aircrew. This study suggests that it should never be less than the capability of the aircraft being flown (the G level which is likely to be encountered in a worst case scenario), even if that G level is not routinely encountered, as the basic aim of the high G training is to make an aircrew aware of his/her G tolerance and allow the practice of good AGSM. Assuming 100% effort is taken to perform a good AGSM at 9 G, one cannot realize this potential without getting exposed to it.[1]

Since 1977 the U.S. Air Force School of Aerospace Medicine adopted an informal "G-tolerance standard" of +7.0 $G_z$, applied at a rate of $1\,G \cdot s^{-1}$ or greater and sustained for 15 s, for subjects seated in an upright seat (13° seatback angle), wearing a functioning AGS, and performing an AGSM. The rationale for this G-tolerance criterion was based on analyses of G-tolerance distribution data available in the U.S. Air Force School of Aerospace Medicine Acceleration Stress Data Repository in 1977 and upon reports of subsequent G-LOC in flight occurring in pilots not tested to the 7-G, 15-s tolerance level.[10,12] In 1981, a NATO Standardization Agreement (STANAG 3827) adopted this as the definition of "low G tolerance."[5] However, Gillingham observed that this would be an "extremely lenient standard" for an actively flying aircrew.[11] Currently, STANAG 3827 has dropped the definition of low G tolerance and instead recommends high-G training of aircrew which should be commensurate to the aircraft being flown by them.[3,4]

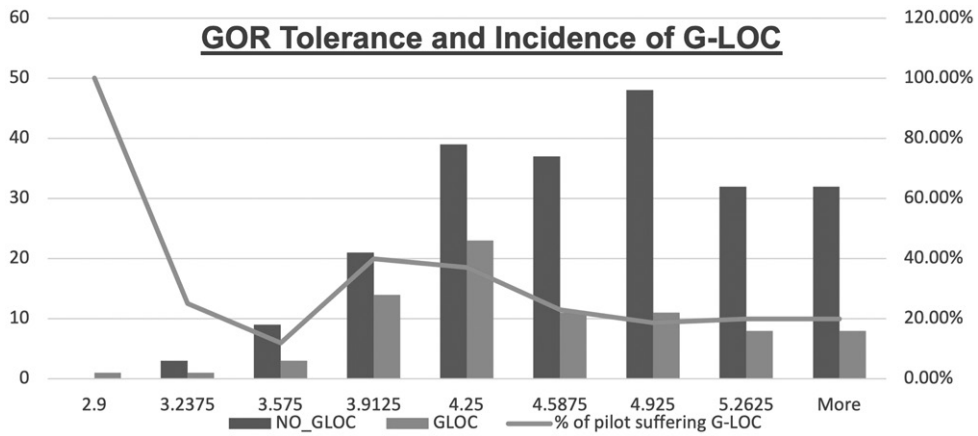


**Fig. 2.** Distribution of GOR tolerance among pilots experiencing G-LOC and not experiencing G-LOC during the high-G training at IAM.

The Indian Air Force does not use any G-tolerance standard for the intake of candidates for fighter flying. Only those pilots or cadets who experience repeated episodes of G-LOC are evaluated for low G tolerance at the IAM IAF. In 2018, IAM IAF defined the G-tolerance standard as an attempt to standardize institutional protocol, where the definition of low G tolerance was adopted as "failure to maintain consciousness at 9 G for 5 s while wearing functional AGS and performing AGSM; low G duration tolerance as failure to complete two peaks of SACM (4 G × 10 s and 8 G × 10 s) while wearing AGS and performing AGSM." The rationale for this G-tolerance criterion was based on the requirement of the IAF for all fighter aircrew to complete OPTRAM-F, where these were the qualifying criteria for passing the course as well as our experience with actively flying aircrew, where 99% of aircrew could meet these requirements (failure rate ≤1%). In the last 5 yr, 12 hypertensive fighter aircrew on medication have also been evaluated using these criteria. All of them could successfully clear the profiles. One case of syncope was upgraded based on these criteria in the last 2 yr and continues to fly fighters without any in-flight episodes of G-LOC. One case of a trainee pilot (after full medical evaluation) who failed to meet this requirement was declared unfit for fighter flying as a case of low G tolerance.[20] There has been no report of in-flight G-LOC among trained aircrew in the last 3 yr. Hence, it can be safely assumed that these criteria have served us well.

Gillingham recommended that eventually a higher G-tolerance standard, designed to optimize the match between the G load-generating capability of a particular aircraft and the G tolerance of the pilot selected to fly that aircraft, will probably be indicated.[11] Though our definition of G tolerance is serving the purpose at present, this may be considered lenient, as tolerating 9 G for 5 s is within "the physiological reserve (5 s)" of an individual and may give a false sense of confidence of tolerating 9 G. It is also evident from this study that, contrary to our expectation, there was a lower incidence of G-LOC at 9 G in comparison to 8 G, which is a 10-s profile (Fig. 1). The odds of experiencing G-LOC at 9 G for pilots clearing 7 G and 8 G training profiles were similar (4.4 and 4.7, respectively). The incidence of G-LOC is 1.5 times more at 9 G than at 7 G in centrifuge training (Fig. 1). This suggests that the G-tolerance standard of 7 G for 15 s of the 1981 STANAG 3827 is inadequate to predict performance at 9 G.[5] Our adversaries and contemporaries are training for 9 G for 15 s, which makes IAF aircrew flying 4$^{th}$/4.5 generation aircraft inadequately prepared in comparison.[12,22] The Advisory Group of Aerospace Research and Development also recommends that the high-G training goal for pilots of high performance aircraft should be 9 G for 15 s.[1] The incidence of G-LOC during SACM is even less than that at 7 G and 96% of pilots could meet the current requirement of this profile without experiencing G-LOC. As IAF aircrew are flying highly agile and super maneuverable platforms of 4$^{th}$ and 4.5 generations, where variable sustained G during aerial combat is a routine requirement, the SACM profile may be made a mandatory training goal rather than an optional profile during the high-G training as it prepares and tests aircrew for G-duration tolerance rather than the G-level tolerance. The Royal Air Force (UK) uses a dynamic flight simulator as an actual flight simulator where a pilot undergoes high-G training while wearing full aircrew flying clothing ensembles (AGS, helmet, and mask).[21] With the upgrade of high performance human centrifuges to dynamic flight simulators, the possibility of imparting high-G training in a more realistic manner like the Royal Air Force may be explored.

Traditionally, aircraft designers have placed a rigid or fixed G limit on various types of aircraft based on dynamic load requirements and depending on how the aircraft was to be used, e.g., fighter, bomber, trainer, aerobatic, transport, etc. The traditional 7.33-G design load limit which applied to some of the earlier century series fighters has been raised under recent design criteria to as high as 8.7 G for some modern fighter aircraft.[6] Pilots have long known that the design load limit can be exceeded by 150% to the ultimate load limit and have used this safety factor while under the stress of combat. Short duration loads of 10 G and higher have been reported in actual aerial engagements during and since World War II.[14] The current fighter aircraft in the IAF inventory has been designed for modern-day requirements. Hence, they can sustain 9 G and beyond, even though their operational role is limited to lower G levels during peacetime. Parkhurst et al. demonstrated that a normal human being can be safely trained to tolerate 9 G for up to 45 s and even higher.[19] Burns et al. demonstrated that G protection is available up to +12 $G_z$ using existing G-protection methods.[8]

The super maneuverable aircraft with thrust vectoring in IAF inventory may expose aircrew simultaneously to multiaxial G stress which can either enhance or reduce relaxed +$G_z$ tolerance (simultaneous $G_y$ and $G_z$ enhances, whereas simultaneous $G_x$ and $G_z$ reduces). However, these differences have been estimated to be too small to be operationally relevant.[7] These super maneuverable aircraft with thrust vectoring do not expose pilots to greater +$G_z$ than current legacy aircraft and the air combat techniques rely more on beyond visual range techniques rather than the erstwhile dogfight. High linear and angular velocities and accelerations will be needed to avoid adverse weapons during beyond visual range combat. In close combat situations (within visual range), vectored thrust gives high maneuverability at low speed and these pilots will thereby have better possibilities to win and survive.[2] However, this comes with additional challenges of its own. Since these aircraft have the capability to generate sustained variable +$G_z$ without any limit for the duration (theoretically endless), it exposes pilots to a unique variable G environment as acceleration, in this case, means sustained high $G_z$, other G-vectors, and push-pull effects. All these acceleration stresses combined with a lot of vestibular peculiarities may result in loss of situational awareness, spatial disorientation, and motion sickness. Besides the acceleration effects on the cardiovascular system, the spine, assisting muscles, and joints are heavily stressed.[2] This cocktail of aeromedical stressors is likely to compromise the performance of an aircrew more if he/she is not confident in handling this G stress. The push-pull effect has been implicated as an

important cause of G-LOC, ranging from 29–31% in different studies.[13,18] IAF aircrew is exposed to a push-pull profile during the OPTRAM-F course where +$G_z$ GOR tolerance is measured before and after exposure to −1.5 $G_z$, demonstrating a reduction in +$G_z$ GOR tolerance and explaining its significance. It is reasonable to conclude that there is no operational justification for at least reducing the current standard of high-G training in the IAF.

This study reaffirms that the search for a G-tolerance standard to predict performance at higher G levels remains elusive. The data indicates that no performance at lower G levels can reliably predict performance at higher G levels or duration. Hence, aircrew should be exposed to the G level that he/she is likely to experience in a worst-case scenario. The IAF's decision to use 9 G as a standard for all aircrew appears reasonable as it allows every aircrew to experience and test their AGSM skill in the most demanding conditions. This also allows flexibility in using the aircrew across all fleets of fighter aircraft without the need for a refresher in high-G training. However, 9 G for 5 s as a standard may not be better than 8 G for 10 s as the incidence of G-LOC was similar or somewhat higher in the 8-G profile (Fig. 1), possibly due to the higher exposure time in the 8-G profile. It would be prudent to replace the 9 G for 5 s profile with a 9 G for 15 s profile, which would allow the practice of at least two AGSM cycles beyond the physiological reserve (physiological reserve of 6 s and each AGSM cycle of 4 s), thus allowing assessment of the effectiveness of AGSM performed at its peak. Considering the performance of IAF aircrew at 8 G for 10 s, 9 G for 5 s, and SACM profiles, it is reasonable to assume that they should be able to train for 9 G for 15 s as well without much difficulty.

In conclusion, the G tolerance of IAF aircrew is as per the institutional definition and meets the current operational goal of the IAF. The current high-G training profile has stood the test of time and appears to serve well.

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# X-Ray Imaging in the Simulated Microgravity Environment of Parabolic Flight

David Lerner; Michael Pohlen; Adam Wang; Jeanne Walter; Michael Cairnie; Sheyna Gifford

**INTRODUCTION:** The advancement of human spaceflight has made urgent the need to develop medical imaging technology to ensure a high level of in-flight care. To date, only ultrasound has been used in spaceflight. Radiography has multiple advantages over ultrasound, including lower operator dependence, more rapid acquisition, typically higher spatial resolution, and characterization of tissue with acoustic impedance precluding ultrasound. This proof-of-concept work demonstrates for the first time the feasibility of performing human radiographs in microgravity.

**METHODS:** Radiographs of a phantom and human subject's hand, knee, chest, cervical spine, and pelvis were obtained aboard a parabolic flight in microgravity and simulated lunar gravity with various subject and operator positions. Control radiographs were acquired with the same system on the ground. These radiographs were performed with a Food and Drug Administration-approved ultra-portable, wireless, battery-powered, digital x-ray system.

**RESULTS:** The radiographs of the phantom acquired in reduced gravity were qualitatively and quantitatively compared to the ground controls and found to exhibit similar diagnostic adequacy. There was no statistically significant difference in contrast resolution or spatial resolution with a spatial resolution across all imaging environments up to the Nyquist frequency of 3.6 line-pairs/mm and an average contrast-to-noise ratio of 2.44.

**DISCUSSION:** As mass, power, and volume limitations lessen over the coming decades and the miniaturization of imaging equipment continues, in-flight implementation of nonsonographic modalities will become practical. Given the demonstrated ease of use and satisfactory image quality, portable radiography is ready to be the new frontier of space medical imaging.

**KEYWORDS:** radiography, space radiology, x-ray, diagnostic imaging.

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Human spaceflight has advanced rapidly in scope and frequency, with the successful completion of the first Artemis mission, the continued success of the Commercial Crew Program, and the advent of private spaceflights from multiple providers. Both national and commercial spaceflight programs promise additional milestones over the next two decades, including the development of space stations in both Earth and lunar orbits, a permanent return to the Moon, and eventual landings on Mars. As humankind begins to venture further into space and the population living in microgravity or on extraterrestrial surfaces increases, the risk of medical and surgical emergencies also increases. Because crew injury and illness can threaten the success of the mission and the life of the individual, accurate and timely diagnosis and treatment of medical events is an area of significant concern for NASA and its government and private partners. However, there are significant limitations to the equipment and skill set available in spaceflight for the performance of these tasks due to constraints in space, power, mass, and training time, among others, necessitating optimization of the available crew healthcare delivery system.[16]

As in the terrestrial environment, imaging is central to medical diagnostics in spaceflight. Diagnostic ultrasound was first

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used 40 yr ago aboard Soviet spacecraft and has continued to act as the workhorse for in-flight medical imaging, with two iterations of multiprobe ultrasound systems flown aboard the International Space Station (ISS) and, more recently, a hand-held single-probe whole-body unit aboard both the ISS and a commercial flight.[2,16] These units have been used both autonomously and with remote guidance and have been used in the evaluation of nearly every major organ system, including lung, cardiac, vascular, bowel, renal, bladder, spinal, and ocular ultrasound, among others.[4,5,13] Ultrasound has been used for research and diagnostic purposes but has also been proposed for future use in interventional guidance.[8–10] However, other than optical coherence tomography, no additional medical imaging modalities have been used for research or clinical purposes in spaceflight.

While ultrasound is versatile, portable, and free of ionizing radiation, it is not without limitations, including its operator dependence and limited acoustic windows. As a result, other advanced imaging modalities have been proposed for future use in spaceflight.[3,7] Foremost among these modalities is radiography, which possesses numerous advantages, including relatively low radiation dose, numerous potential applications, low power consumption, small size, and increasing portability.[11] There are multiple medical conditions of concern in spaceflight for which diagnostic radiography would prove superior or complementary to ultrasound, including but not limited to dental disease, spine/musculoskeletal trauma, inhalational injury, pneumothorax, and arthritis. However, no urgent use case for radiography in low Earth orbit has yet necessitated a technical demonstration of this modality in spaceflight. As a first step toward developing this capability, this article describes the methodology and results of the first-ever radiographs of a human subject in microgravity via the use of ultra-portable diagnostic x-ray imaging.

## METHODS

Selection of a commercial off-the-shelf, Food and Drug Administration-approved, ultra-portable, wireless digital x-ray system was performed (Impact Wireless, Complete Battery-Powered Portable Digital Radiography System, MinXray, Inc., Northbrook, IL, United States). The selection was based on availability, portability, prior Food and Drug Administration approval, off-the-shelf capability, and having already undergone systems impact testing, including vibrational, temperature, altitude simulation, shock, external short circuit, and overcharge testing. The battery-powered x-ray generator (TR90BH) used by the selected system measures 21.9 × 19 × 44.0 cm and has a mass of 7.7 kg. The battery that powers this TR90BH generator is a custom-built rechargeable Li-ion battery M910BL with specifications of 57.6V, 1700 mAh, 97.92 Wh. It is charged using a custom pin block charger with an AC adaptor. Approximately 100 to 400 exposures per charge can be obtained with this unit depending on the output technique used. The CsI wireless image receptor measures 38.4 × 46.0 × 1.5 cm with a mass of 3.7 kg, an active area of 35.6 × 42.7 cm, and a pixel size of 0.140 × 0.140 mm. During on-the-ground testing and protocol development, a torso-harness system was created for securing the x-ray generator to the operator to stabilize the TR90BH during flight while maximizing targeting capability. The harness features a metal plate that is mounted to the back of the generator, allowing the generator to be secured to a heavy-duty, hinged arm designed for videography equipment.

Prior to flight, the two technicians who acted as x-ray generator operator and receiver operator/anatomic target rehearsed the protocol for 48 h. During this preflight testing phase, the TR90BH generator, harness system, and positioning relative to the hand-held receptor were tested, along with the associated laptop computer and imaging software. For this testing, an x-ray line phantom was secured with medical tape directly onto the surface of the imaging receptor. Radiographs of the line phantom were then obtained to act as terrestrial gravity (1 G) control images.

Next, this imaging system was flown on a parabolic research flight aboard a modified Boeing 727 operated by ZeroG Incorporated (Exploration Park, FL, United States). The parabolic research flight consisted of six sets of parabolas with five parabolas in each for a total of 30 parabolas. In terms of the variable gravity fields experienced during the flight, lunar gravity (1/6 G) was created during the first three parabolas. The remaining 27 parabolas were microgravity (0 G) exposures between 20 and 30 s in length. Only images of the line phantom were obtained during the limited lunar gravity parabolas (**Fig. 1**).

The technician and equipment positioning for the parabolas were as follows. For the first set of six parabolas, both the technician operating the generator (hereafter technician 1) and the technician holding the receiver and acting as the anatomic target (hereafter technician 2) were seated and wearing seatbelts. The seatbelts were standard commercial airline lap belts. They were worn for safety while the technicians became accustomed to the altered gravity fields and tested the equipment in the parabolic flight environment. During the second set of six parabolas, technician 2 unbelted and placed their foot in a foothold to reduce target motion while technician 1 remained seated. During the third set of six parabolas, both technicians were unbelted. Both had one foot in a foothold during image x-ray generation and image creation. During the fourth set of parabolas, technician 2 was floated to the ceiling by the ZeroG flight crew. Technician 2 then hugged the detector against their chest and pelvis to reduce motion of the target relative to the receptor, while technician 1 lay on the floor of the craft, aiming the x-ray beam up at the anatomic targets. During parabola set five, technician 1 floated without the aid of a foothold while technician 2 stabilized themselves with a foothold and braced an iPad (iPad Mini 5$^{th}$ Generation, Apple Inc., Cupertino, CA, United States) against the detector. Parabola set six was reserved to repeat any unsuccessful imaging attempts. No repetitions were necessary.

During the first set of six parabolic intervals, technician 1 discovered that during microgravity the springs in the arm of the torso harness system used for securing the generator forced the generator away from their body. To compensate for this, between parabola sets 1 and 2, technician 1 disconnected the generator's mounting plate from the arm and safely stowed the


**Fig. 1.** A) Two of the authors pose in microgravity aboard a parabolic flight with the ultra-portable x-ray generator and image receptor. B, C, D) Example image acquisitions of the two technicians in various positions. Photo credit: Steve Boxall, Zero Gravity Corp., 2022.

arm. Thereafter, the generator's angle and distance from their body were allowed to be freely determined by technician 1 as they acquired images. Throughout the flight, the x-ray generator remained tethered to technician 1 by a nylon safety cable during all phases of flight.

The imaging target protocol proceeded as follows. During parabola set one, where the first three parabolas were lunar gravity and the final two were microgravity, the line pair phantom was imaged. After the generator was removed from the harness during the straight and level flight between parabola sets one and two, the phantom was again imaged during parabolas six and seven. The phantom was then removed from the receptor surface and secured. During parabolas 8–10, technician 2 placed their hand directly on the image receptor surface and the first human radiographs were obtained in microgravity. During the third through fifth sets of parabolas, technician 2 moved themselves and the detector into various positions relative to one another and to the generator to allow for imaging of multiple body parts, including the hand, knee, chest, cervical spine, and pelvis (**Fig. 2**). By the start of parabola set six (parabola 26), 21 images had been acquired, including 6 phantom images and 15 images of human anatomy. All images were acquired at 90 kVp and 1.65 to 4 mA, with a source-to-image distance ranging from approximately 1.0 to 1.7 m. Images were wirelessly transferred from the imaging receptor to a secured laptop computer in flight after each acquisition using MinXray imaging software. Each image was inspected visually between parabolas to ensure a minimum quality before moving to the next image in the protocol.

After landing, further quantitative and qualitative assessment of the acquired images was performed. Nonblinded qualitative assessment was performed by three board-certified fellowship-trained radiologists employed at an academic training program (two with body imaging fellowship training and one with musculoskeletal imaging fellowship training) using a Barco Nio (MDNC-3421; Barco, Poperinge, Belgium) PACS monitor. Quantitative assessment was performed by a radiology imaging physicist. The presampling modulation transfer function was measured by fitting an error function to the supersampled edge of the line phantom (**Fig. 3**). Contrast-to-noise ratio was measured for a low-contrast target with respect to the background.

## RESULTS

The phantom and human radiographs obtained on the ground, in simulated lunar gravity, and in microgravity environments

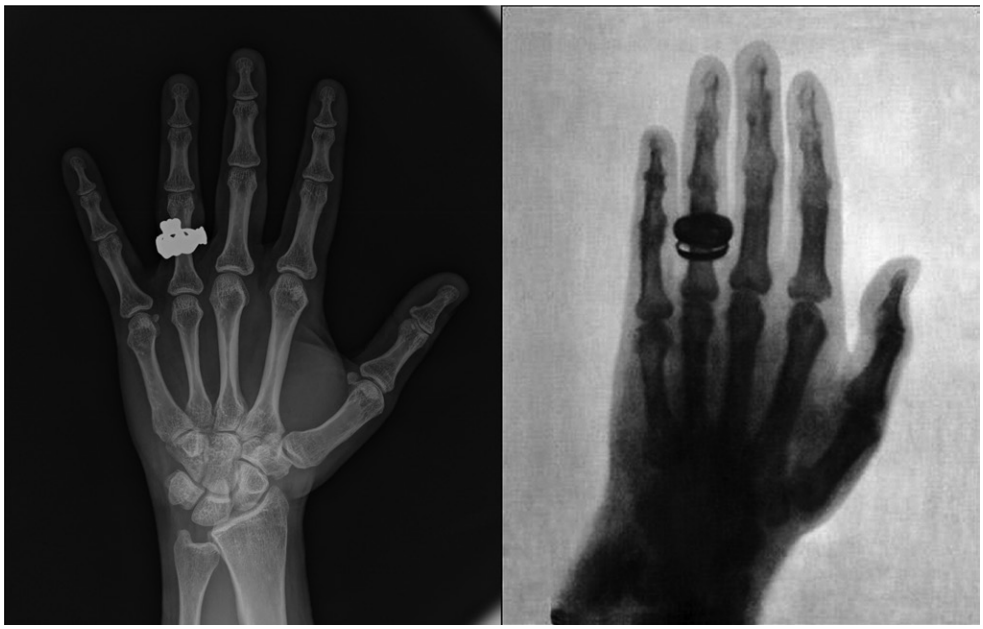

**Fig. 2.** The first radiograph of a human acquired in microgravity (left) with one of the earliest radiographs acquired by Wilhelm Roentgen (right).

were deemed to be qualitatively diagnostic by the radiologists for the pathology expected to be evaluated by radiography. Quantitatively, the phantom radiographs had no statistically significant difference in spatial resolution or contrast resolution. For all imaging conditions, the spatial resolution appeared comparably sharp. Line pairs up to the Nyquist frequency of 3.6 line-pairs/mm were clearly visible for all imaging environments, and the presampling modulation transfer functions were comparable (**Fig. 4**). Average contrast-to-noise ratio for the low-contrast target was 2.44 and consistent across imaging conditions, with no statistically significant differences (Fig. 4).

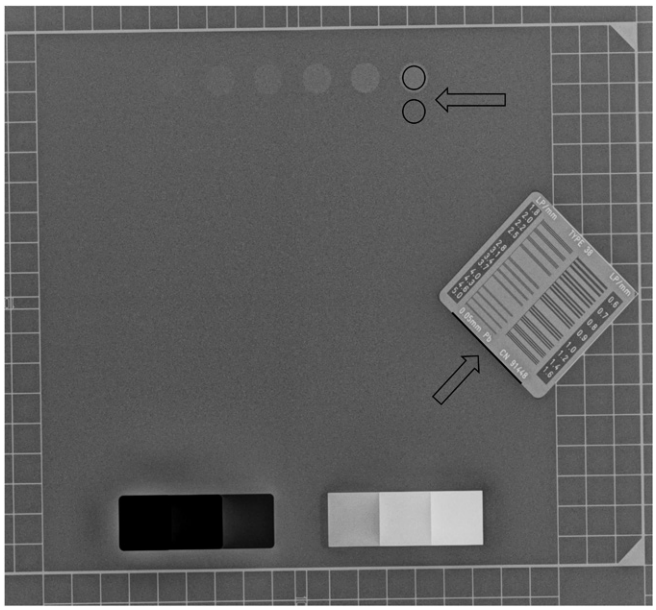

**Fig. 3.** The presampling modulation transfer function was measured by fitting an error function to the supersampled edge of the line-pair phantom (black line indicated by arrow). The contrast-to-noise ratio was measured for a low contrast target with respect to the background (black circles indicated by arrow).

## DISCUSSION

This pathfinding study demonstrates a feasible approach to the performance of multiple radiographic exams in microgravity and reduced gravity environments. Both limited qualitative and basic quantitative assessments of contrast and spatial resolution relative to ground controls suggest diagnostic quality exams can be performed in flight with this commercial off-the-shelf equipment. The preflight training for successful utilization of this ultra-portable unit was accomplished within a few days before flight and images were wirelessly transferred and available for instant interpretation after acquisition. This radiography system and methodology produced images with diagnostic adequacy while minimizing mass, space, power, training, and dose relative to previously proposed radiography units for spaceflight.

Human iatrogenic radiation exposure is an important consideration for medical equipment on exploration class missions, particularly as astronauts traveling beyond the Van Allen belts will be exposed to higher ambient radiation doses. For example, a Mars mission may expose the crew to up to 1 Sv (1000 mSv) of radiation, while exposures range on average from 0.153 to 0.231 $mSv \cdot d^{-1}$ on the ISS and 0.3 $mSv \cdot d^{-1}$ on the lunar surface, depending on available shielding.[14,18] When compared to the daily background dose, the dose associated with in-flight radiography would be relatively small and carry a lower risk profile in comparison. For example, the typical effective dose to a patient for a chest radiograph is 0.1 mSv and 0.001 mSv for a hand radiograph. There would briefly be even lower doses of scattered radiation immediately surrounding the imaged patient,

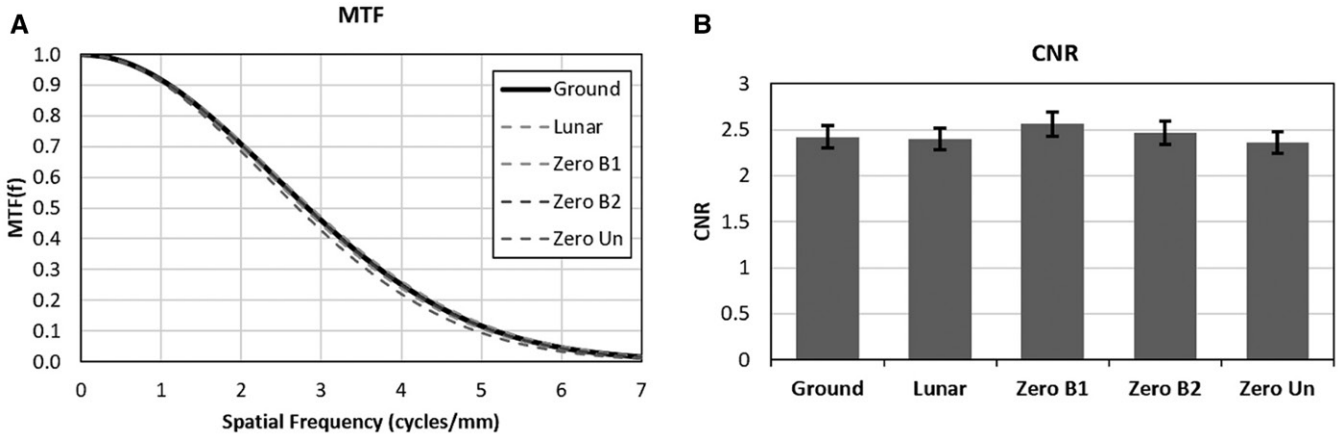


**Fig. 4.** A) Modulation transfer function (MTF) and B) contrast-to-noise ratio (CNR) at 1 G and at varying levels of reduced gravity.

so the area should be cleared of other crew during acquisition. While not in operation, the x-ray generator unit does not produce radiation and, therefore, there is no unexpected exposure. The generator unit itself is embedded with shielding so that, during acquisition, radiation exposure to the surrounding area not within the beam is minimal. This shielding is included in the off-the-shelf measurements of size and mass. This shielding would also provide partial protection from galactic cosmic radiation for the unit electronics, which is a known concern in flight. Additionally, the electronics could be radiation hardened further as necessary, as is sometimes performed for other sensitive equipment. While lithium-ion batteries such as that in the generator are already used aboard the ISS, a safety and compatibility evaluation of the particular battery specifications used to power this or any other x-ray generator would be needed before any orbital flight.

This study is not without limitations. Parabolic flight is an imperfect simulation of spaceflight in low Earth orbit or in cis- and translunar space. The ambient radiation environment, vibration load, cabin volume, equipment available for subject positioning, and vehicular sources of electromagnetic interference could all potentially contribute to degradations or limitations in image quality in spaceflight relative to parabolic flight. However, the time limitations for positioning and exposure imposed by the short periods of microgravity in parabolic flight would be removed, facilitating these exams. Second, this proof-of-concept work used a single healthy human technician as an anatomic target, and limited exam types were performed. Appropriate patient positioning in altered gravity may be particularly difficult in a scenario where the subject is acutely ill. Furthermore, it is expected that reduced or absent gravity may limit x-ray sensitivity and specificity for pathology with some gravity-dependent findings, such as pneumothorax and small bowel obstruction.[16] We observe the need to perform additional suborbital and low Earth orbit flights to replicate our findings and ensure that diagnostic quality images are consistently reproducible with different operators and subjects.

Opportunities to perform further testing of x-ray imaging in altered gravity environments should be pursued. Private and state-sponsored exploration class missions returning to the lunar surface and beyond to Mars are rapidly shifting from the realm of science fiction to science fact. As mission length and crew complement increase, so too does the risk of a medical or surgical emergency. NASA has developed a series of risk assessment tools to evaluate and help mitigate these potential conditions through optimization of the mission medical kit.[15] Depending on the constraints of the kit, medical imaging equipment will likely be included on long-duration missions on the lunar and Martian surfaces. Dozens of the conditions of highest concern require imaging for confident diagnosis and/or definitive management.[1,17] While handheld ultrasound demonstrates utility for a majority, radiography would allow for superior or simplified evaluation for a subset of these, including musculoskeletal trauma, dental and oromaxillofacial disease, and thoracic pathology, some of which have already occurred during spaceflight.[12] While ultrasound has been favored historically due to its lack of ionizing radiation, versatility, small size, low mass, and interventional utility, providing a terrestrial standard of care on another planetary surface will necessitate additional modalities, especially those which can enhance or complement the capabilities of handheld ultrasound. In addition to clinical use, modifications of terrestrial radiography techniques, such as dual-energy x-ray absorptiometry, may assist in answering critical research questions in flight, such as changes in bone mineral density during long-duration missions. Other benefits include the ability to perform in-flight nondestructive testing and evaluation of equipment, including but not limited to malfunctioning solar panels or potential hull damage, such as occurred on Expedition 56/57.[6]

Development and testing of portable radiography equipment for spaceflight have long been proposed, though no urgent use case has yet necessitated implementation, particularly given the successful extensive adaptations of in-flight ultrasound and the ability to rapidly evacuate from low Earth orbit. However, since first suggested in the 1980s for the never-constructed Space Station Freedom, the radiation dose, mass, size, and power requirements of portable digital radiography have decreased substantially, altering the calculus for its

inclusion in future technical demonstrations in low Earth orbit and for potential permanent use in large off-world habitats. To this end, this work paves the foundational steps of acquiring radiographs with ultra-portable units in a reduced gravity environment. Our results suggest that widely available commercial off-the-shelf units are sufficient for this task and ready for trial in suborbital or orbital demonstration flights. The authors hope that this is a small but concrete first step toward a bright future for space radiology.

## ACKNOWLEDGMENTS

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# Burnt by His Cellphone During a Parachute Jump

Vincent des Robert; Luc Saint-Jean; Simon-Pierre Corcostegui; Emeric Romary; Clément Derkenne

**BACKGROUND:** Many current cell phone (mobile phone, smartphone) batteries are lithium-ion. These batteries can overheat and catch fire under certain conditions. If it happens during a flight or air activity, this might compromise aviation safety. We report a case of a man whose phone caught fire during a parachute jump.

**CASE REPORT:** The individual, a member of Police Special Forces, is required to regularly perform parachute jumps. During the incident flight, the man had a cell phone in a pocket that ignited during the jump. He was able to land and then extract the phone with burns requiring acute medical care and later a skin graft.

**DISCUSSION:** This is a cautionary tale of lithium-ion batteries in flight. Many other situations could also occur with these batteries. There is little medical documentation of the risk of fire with lithium-ion batteries causing injuries during flight operations. To reduce the risk of fire, the devices should be powered down and phones should not be worn directly touching the skin. Damaged devices are more prone to overheating.

**KEYWORDS:** aerospace, battery fire, lithium-ion battery, skin burn injury, phone, smartphone.

**des Robert V, Saint-Jean L, Corcostegui S-P, Romary E, and Derkenne C. *Burnt by his cellphone during a parachute jump*. Aerosp Med Hum Perform. 2023; 94(10):792–795.**

Lithium-ion batteries from a cell phone can be dangerous and ignite under certain circumstances. However, very little research is available regarding the consequences and dangers such as skin burns that may be caused by cell phone batteries.[5] The fire hazards and chemical dangers of lithium-ion batteries remain unexplored.[2] In July 2019, a mobile power bank with a lithium battery (portable charger) belonging to a Virgin Atlantic passenger caught fire in a seat on a flight from New York to London. The crew managed to put the fire out quickly and the pilot diverted to Boston's Logan International Airport, where all 217 passengers were safely evacuated from the plane.[8,9]

An overheated cell in a battery can ignite and cause a rapid conduction fire that can quickly reach intense temperatures of up to 900°C.[9] A battery in a phone consists of lithium-ion cells. The cells are connected in a series and separated by insulation sheets that provide insulation between the cells and the aluminum casing. Although the material used to construct the lithium-ion battery contains nonflammable materials, the electrolytes are flammable and the coating on the anode and cathode contains chemically reactive components.[2] For different reasons, the cells can be damaged, causing the electrodes to short-circuit and overheat. This increase in temperature can cause a chemical reaction between the highly flammable electrolytes and electrodes, leading to a thermal runaway.[2,7] This paper discusses a case of a 41-yr-old man whose phone caught fire during a parachute jump.

## CASE REPORT

A 41-yr-old paratrooper policeman made a 8202-ft (2500-m) routing parachute jump. As soon as he jumped, his Crosscal® Trekker-X4 (Langevin, France) mobile phone was engulfed in flames. The phone was in the right pocket of his flame-retardant combat pants (NFM Group®, Ski, Norway). During the free fall phase, he tried to remove the phone from his pocket with his right hand, but the phone was too hot and embedded in his pants. This caused a burn on his fingers. He made many 360°

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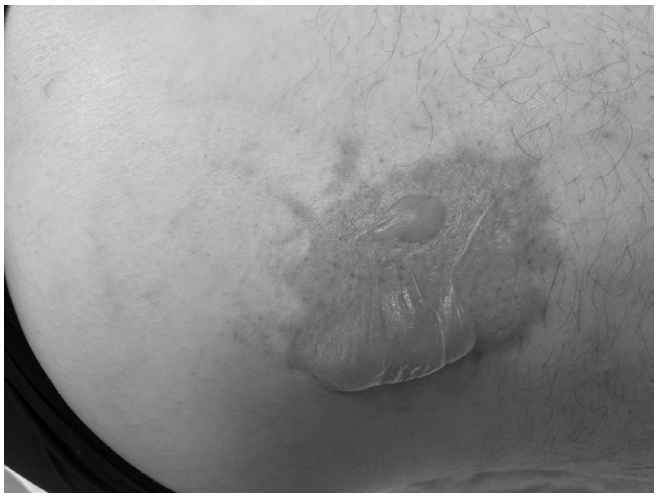

**Fig. 1.** Initial burn on the day of the accident.

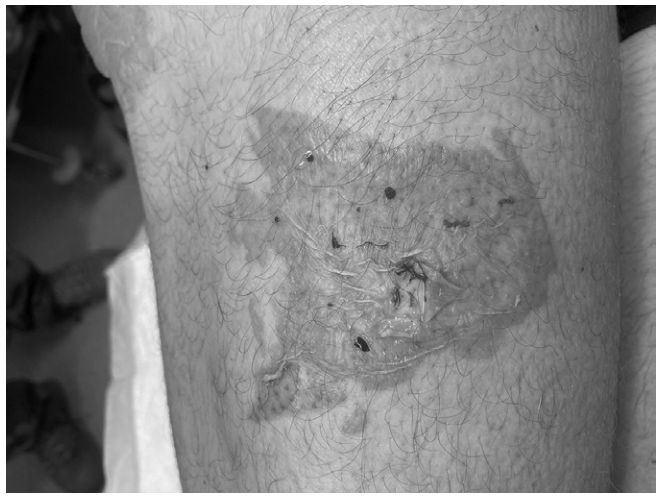

**Fig. 2.** Burn the day of the accident.

turns and opened his parachute tardily at the lowest altitude, attempting to reduce the time under canopy; in other words, to land as fast as possible. This jump was over an unrecognized area. After landing, he managed to get out of his trousers with the phone embedded in them. He was in contact with the burning phone for 3–4 min. Fortunately, he had insulated pants, which limited the contact from the flames to his skin. However, this did not protect the patient from the intense heat, which burned his skin. A military nurse who was present at the parachute club performed an advanced emergency protocol.[6] He cooled down the wound with a sterile hydrogel and gave the patient inhaled painkillers (Methoxyflurane). The nurse then took the patient to a military doctor near the parachute club. The total body surface area burned was estimated to be at 2%, with second- and third-degree burns (**Fig. 1**) on his thigh and second-degree burns on two fingers (index and thumb). The blisters were excised, the skin burn was washed with sterile water and cleaned with mild soap, and then covered with a large thickness of silver sulfadiazine cream and a bandage. The same bandage was redone every day for 1 wk, with water cleaning and silver sulfadiazine cream. The patient did not have any pain. The patient was seen by a burn specialist 7 d after the accident. Third-degree burns were confirmed on 1% of the skin with 1% second-degree burns (**Fig. 2**). After this consultation, the bandage consisted of dermal betadine with Vaseline-impregnated oil dressing. A skin graft was scheduled for the third-degree burn on his right thigh.

The patient's skin graft was performed 3 wk after the accident (**Fig. 3** and **Fig. 4**). The plastic surgeon removed buttock skin and transplanted it onto the wound on the thigh. The skin graft was a success. The wound care consisted of the nurse washing the area with sterile water and mild soap for a duration of 3 wk. The wound was covered by a fatty tulle grass bandage. The wounds healed very well and the patient was authorized for physical activity in his sports and returned to work 60 d after the accident. He had no complaints of any pain or disability.

## DISCUSSION

As a special forces police officer, this patient was well experienced in parachute jumping with more than 500 jumps, as they conduct drills for high jumps with oxygen. A special forces police officer is trained to be strong and a pain-resistant person. He is prepared to react to unusual situations under stress. This phone accident with an inexperienced skydiver could have been tragic, as the person might not have opened his parachute, he may have lost consciousness because of the pain, or not steered his parachute correctly with a risk of bad landing (i.e., in a tree, in a lake, etc.). Critical structures of the parachute could have also caught fire (such as the harness, line, or canopy). While the patient was focused on getting out his phone, he neglected to look at the altitude or at the other skydivers.

The flame-retardant combat pants worn by the patient limited the burning process. They are made of 93% meta-Aramid, 5% para-aramid, and 2% antistatic. These combat clothes are designed to protect an individual for 4 s, which is the average time to escape an intense fire in a building or a vehicle. Even though the patient experienced a faster than average parachute flight, the burning phone was on him for more than 4 s; it was on him between 3 to 4 min. When the patient landed on the ground the burns were quickly cooled by the nurse, which limited their damage. The ambient air temperature was 25°C on the ground and 16°C at 8202 ft (2500 m); however, this was likely not the cause of the fire.

Care of a lithium-ion battery skin burn is similar to the care for thermal burns. The objective is to achieve healing as quickly as possible. If the healing process takes longer than desired, eventual complications may arise. Skin grafting can be an integral part of the management for certain burns. Thin skin grafts accelerate and improve healing. Total skin grafts treat the skin sequelae and show less scarring. Thin skin grafts are necessary in case of deep burns, or after a directed healing phase in case of intermediate burns.[1] After a directed healing phase of 21 d, this

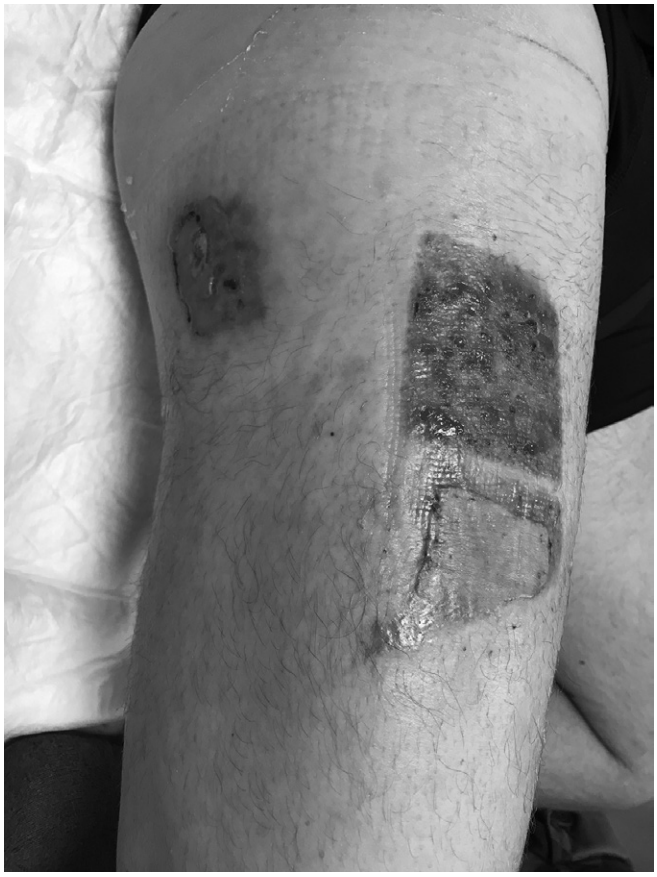

**Fig. 3.** Skin graft 3 wk after the accident.

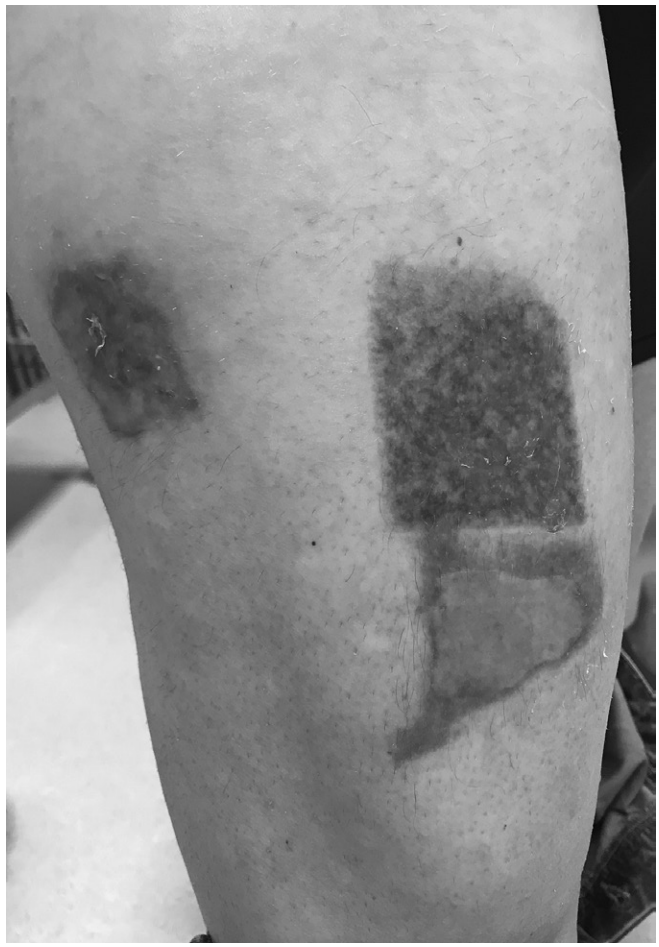

**Fig. 4.** Skin graft 3 wk after the accident.

patient had a thin skin autograft. His skin healed without burn scar contracture 3 mo after the accident.

The main reasons that a lithium battery catches fire are structural defects, technical defects, or improper use such as mechanical damage caused by dropping, damage to the protective casing caused by a pointed object, bending, thermal strain by external heating, overloading, or exposure to excessive temperature.[4] The contact with a metal-like key can also act as a conductor and initiate thermal runaway, with the destruction of the battery.[5,7] In this case, we did not find the cause of the explosion of the lithium battery. It was probably a short circuit from a structural or technical defect. Indeed, the patient did not handle the battery, he did not use the wrong charger, charge the battery too long, or drop his phone. We cannot explain why the phone ignited once the patient exited the plane and was in the air. It may have been caused by an increase in the pressure and oxygen during the free fall, which are two compounds that are favorable components for a combustion.

In this case, we could not have prevented this accident. To reduce the risk of lithium battery fire, it is recommended not to charge the phone to more than 99% (avoiding charging it all night) to limit overheating, not expose the battery to collision or fall, humidity, and heat sources, and to use the right phone charger. By using a fireproof phone or computer case composed of three layers of fiberglass, which can resist heats up to 1000°C, one can highly improve the chances of preventing a fire occurring in a plane or during parachute jumps.[3]

When a lithium-ion battery catches fire, it is best to not touch it and just let it burn. If it is necessary to put out the fire (in a house, in a plane, etc.), the best approach is to use an aerosol extinguishing system to cool the battery and avoid thermal runaway.

Military medics and doctors perform parachute jumps with lithium-ion battery devices such as a vital signs monitor, ultrasound machine, and syringe-pump. For flight safety, it is necessary to have an aeronautical certification or dedicated packaging for these devices. In conclusion, lithium-ion battery burns are rare but can be dangerous, especially in aeronautical activities.

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# Aerospace Medicine Clinic

This article was prepared by Maryrose D. Chuidian, D.O., M.P.H., and Kent-Andrew Boucher, M.D.



You are currently deployed as the flight surgeon at a location with only basic medical capabilities. One evening while manning the med bay, two airmen walk in assisting a third airman. This airman is a young female loadmaster you've flown with a few times during this deployment. As they get her to sit down, you observe she appears pale, disoriented, and extremely fatigued. Her friends inform you that they were all hanging out in their quarters when the loadmaster stood up, reached for a snack on the shelf, and passed out. One of her friends was able to catch her and assist her to the floor. The loadmaster appeared to be out for a "few minutes" prior to slowly regaining consciousness. She was able to sit up, although she required assistance to stand and walk to the med bay for further evaluation.

The loadmaster is a 24-yr-old woman with a past medical history significant for iron deficiency anemia (previously treated with oral iron supplements, but since discontinued), Hashimoto's thyroiditis (currently taking levothyroxine), and a gluten allergy with celiac disease ruled out. She reported worsening fatigue for approximately 1 mo, but in the past 3 d she developed increased dizziness and generalized weakness. She attributed her symptoms to being deployed, missing her family, and poor sleep hygiene, and thus did not seek medical attention. Additionally, she admitted to developing nausea, intermittent emesis, an episode of diarrhea, and decreased urine output over the last 48 h. She denies any dysuria, hematuria, or abdominal pain associated with the decreased urine output. She also denies any recent fevers, chills, myalgias, or contact with known COVID-19-positive individuals. Furthermore, she denies ever having lost consciousness before. Her initial vital signs were systolic blood pressure >100 mmHg, heart rate 140 bpm, and temperature 103°F. Her physical exam was positive for a pale and weak appearance, conjunctival pallor, tachycardia, and positive orthostatics. There were no concerns from medical personnel during her predeployment clearance regarding any of her medical history. Prior to deployment, thyroid-stimulating hormone (TSH) levels and complete blood counts (CBC) were within normal limits and were not a concern during medical out-processing.

1. What diagnoses are in your differential?
   A. Pernicious anemia.
   B. Undiagnosed alcohol use disorder.
   C. Hypothyroidism.
   D. Iron deficiency anemia.
   E. Infection.
   F. All of the above.

## ANSWER/DISCUSSION

**1. F**. All of the choices above are potential etiology of this patient's symptoms. Pernicious anemia is a rare disorder that results in vitamin B12 deficiency, which is important to the functionality of hematologic and neurological systems. Your patient's history of present illness encompasses symptoms that can be associated with pernicious anemia: fatigue, nausea, diarrhea, and pale appearance.[12] Given her history of Hashimoto's thyroiditis, there is increased likelihood of anemia, since anemia and hypothyroidism are commonly found together. Thyroid hormone levels affect erythrocyte precursors, so inappropriate levels of TSH can negatively influence red blood cell (RBC) production. Thus, her history of Hashimoto's thyroiditis could be associated with a form of anemia and explain her presentation.[11] Additionally, a history of one autoimmune disorder increases the likelihood of another. Pernicious anemia, an autoimmune disorder, results in impaired absorption of cobalamin, i.e., vitamin B12.[12] The patient already reported a history of iron deficiency anemia; thus, it should remain on the differential. Both pernicious anemia and iron deficiency anemia can present together, so an iron panel and CBC with a peripheral smear could be of value to assist with differentiating origin. Usually, iron deficiency anemia is associated with microcytic anemia, while pernicious anemia can be normocytic or macrocytic, as seen with 30% of patients.[9] Chronic use of alcohol has harmful effects on RBCs, white blood cells, blood


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cell precursors, and platelets. Excessive use can lead to suppression of bone marrow and inhibit the growth of mature blood cell lines. Thus, undiagnosed alcohol use is also a potential etiology of the patient's underlying symptoms.[2]

Upon further discussion with the patient, she admits that since arriving at your deployed location a few months ago, she has transitioned to a full vegan diet. She denies a history of ever using drugs or drinking alcohol. Initial laboratory workup was limited to CBC, prothrombin time/partial thromboplastin time, aspartate aminotransferase/alanine aminotransferase, lactate dehydrogenase, and urinalysis. Pertinent positives included hemoglobin (Hb)/hematocrit (Hct), white blood cell, and RBC values reported as "undetectable." Partial thromboplastin time was within normal limits, and lactate dehydrogenase was elevated at $1000\,U \cdot L^{-1}$.

2. Aside from adding an iron panel and TSH/free T4, what other labs would you consider ordering to assist in narrowing the differential diagnosis?
   A. Serum B12.
   B. Peripheral blood smear.
   C. Intrinsic factor antibody.
   D. Parietal cell antibody.
   E. Phosphatidylethanol (PEth).
   F. All of the above.

## ANSWER/DISCUSSION

**2. F**. All of the labs above can help narrow the differential diagnosis. Given the discovery that the patient has been a vegan for several months, there is a potential for vitamin B12 deficiency. Vitamin B12 is a critical component for producing blood cells and myelination of the nerves. Pernicious anemia results in impaired absorption of vitamin B12, leading to its deficiency, and can therefore result in profound anemia. There are no specific or "gold standard" tests for pernicious anemia. However, intrinsic factor antibody and parietal cell antibody are potential markers for pernicious anemia, although results are varied. Intrinsic factor antibody is highly specific (95–100%); however, it has a low sensitivity at 50–60%. As such, a positive test helps rule it in, although a negative test doesn't rule it out. Additionally, parietal cell antibody testing can be positive, but also has a mixed specificity between 50–100%.[7,12] The diagnosis is usually established after considering a combination of patient presentation, clinical evidence, laboratory testing of biomarkers, peripheral blood smear, and CBC.[7,12]

PEth is a lab test that is used to detect the presence of abnormal phospholipid formation secondary to the presence of alcohol. It has a half-life between 4–7 d and thus can be detectable in the blood of someone who has abstained from alcohol up to the 28th day.[10] Although the member has been in theater for a few months, the potential for alcohol use is still plausible. Thus, it would be useful to obtain this test if other diagnostic results are negative and the etiology is still in question.

In a deployed setting, you aren't likely to have the laboratory capabilities to obtain specialized tests such as PEth, parietal cell, or intrinsic factor antibodies. Potentially, you could do a peripheral blood smear yourself if you have a microscope in your small clinic. You should have the capability of ordering a CBC or Hb/Hct via an i-STAT device to check for anemia. That said, you likely will not be able to differentiate between pernicious or iron deficiency anemia, but you may be able to narrow your differential. A glucose check should be performed to evaluate for symptomatic hypoglycemia as a potential etiology for her loss of consciousness, dizziness, and fatigue. Lastly, you should perform a pregnancy test. An unknown pregnancy in a healthy young woman with a history of anemia, previously on iron supplements, could cause recurrence of anemia.

3. What is the pathophysiology of pernicious anemia?
   A. Poor dietary intake of vitamin B12.
   B. Poor dietary intake of folate.
   C. Infection of RBCs by a parasite leading to RBC lysis.
   D. Impaired absorption of dietary vitamin B12.
   E. Toxic accumulation of iron due to genetic disorder.

## ANSWER/DISCUSSION

**3. D**. Pernicious anemia is an autoimmune disorder that encompasses antigastric parietal cell antibodies and anti-intrinsic factor antibodies.[4,7,12] This leads to destruction of gastric parietal cells, resulting in an autoimmune gastritis.[4] Autoimmune gastritis and anti-intrinsic factor antibodies cause decreased intrinsic factor, which is critical for dietary vitamin B12 absorption.[4,7] Autoimmune gastritis also causes decreased gastric hydrochloric acid release, which decreases the amount of vitamin B12 released from dietary sources for absorption in the ileum.[1] The decreased release and decrease in absorption combined lead to worsening vitamin B12 deficiency.[4,7,12] Vitamin B12 deficiency causes impaired DNA synthesis and delayed cell division, resulting in the megaloblastic anemia that is associated with pernicious anemia.[7]

Autoimmune gastritis is a condition that can be present alongside pernicious anemia. Autoimmune gastritis results in a decrease of gastric hydrochloric acid release, leading to reduced iron absorption and iron deficiency anemia in addition to pernicious anemia.[4,7]

Poor dietary intake of vitamin B12 can lead to megaloblastic anemia, which can occur with a vegetarian or vegan diet. It can take up to 3 yr to fully deplete vitamin B12 and may take time to develop clinical symptoms.[1] However, coupled with the patient's other medical conditions, a mild deficiency may be an exacerbating factor of her presentation. Poor dietary intake of folate can also cause megaloblastic anemia, but this is not related to the pathophysiology of pernicious anemia. Malaria infects RBCs, causing lysis due to the parasite reproductive pattern, but this is associated with a microcytic anemia, unlike the megaloblastic anemia of pernicious anemia.[15]

Hereditary hemochromatosis is the genetic disorder that causes toxic accumulation of iron, which can cause anemia secondary to multiple organ damage caused by the toxic accumulation of iron.[6]

After you stabilize the patient, she is transferred to a higher level of care for additional treatment. Further workup reveals she is severely vitamin B12 deficient and thus diagnosed with pernicious anemia. She responds well to treatment with intramuscular (IM) vitamin B12. However, despite treatment, she continues to have intermittent episodes of fatigue and lightheadedness. Given her continued IM treatment and symptoms, she still requires routine appointments with a hematologist.

4. Which of the following would be most concerning regarding this aviator's ability to return to flying duties?
   A. Aviator requires continued vitamin B12 treatment.
   B. Aviator still being followed by a hematologist.
   C. Aviator continues to maintain a vegan lifestyle.
   D. Aviator continues to have intermittent episodes of fatigue and lightheadedness despite treatment of weekly IM vitamin B12.

## ANSWER/DISCUSSION

**4. D**. The aviator is still undergoing evaluation by hematology and immunology regarding the underlying cause of her pernicious anemia. The fact that she's still seeing specialists isn't in and of itself a grounding condition, unlike whether or not her condition is adequately being treated. Despite continued therapy with IM vitamin B12, the member remains symptomatic.

Each of the three military services has specific criteria regarding aeromedical waivers and the ability to return to flying duties with a history of anemia. The Air Force requires evaluation for a flying waiver if symptoms persist despite an appropriate treatment course and if the aviator requires more than an annual follow-up with a hematologist.[13] Additionally, the Air Force specifically requires a waiver for anemia if Hb measures below the lower limit of normal based on ethnicity and gender.[5] The Navy waiver guide requires a normal physical exam and normal Hct levels using an average of three levels drawn on different days.[8] Similar to the Air Force, the Army will grant a waiver for a flyer with a history of acquired anemia if it has been resolved without residual symptoms and need for continued specialty care.[14] In civil aviation, any case with a history of anemia must be deferred by an aviation medical examiner to the Federal Aviation Administration for any aeromedical disposition.[3] As the flight surgeon considering this patient for a waiver, it would be difficult to consider even recommending one at this time. Using the Air Force medical standards, your loadmaster meets the requirement for maintaining Hb levels above the specified lower limit of normal for a Caucasian female, $12.2\,g \cdot dL^{-1}$.[5] However, she continues to have symptoms despite continued care and appropriate treatment by a hematologist. As a flight surgeon, you have to evaluate the aviator's overall condition and determine if there would be an increased safety risk in allowing the aviator to return to flying duties. Your loadmaster continues to have intermittent episodes of lightheadedness and fatigue, which would impair any aviator from performing his or her duties, thus placing the entire crew and mission at risk.

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# Aerospace Medicine Clinic

This article was prepared by Jeffrey Harris, M.D., M.O.H.



You're the flight surgeon who receives a call from a 44-yr-old male U.S. Air Force pilot. He has recently moved into the area and is returning to an active flying job after a few years away. During the previous year he was started on testosterone therapy by a civilian medical provider. He did not follow up with the provider after initiating treatment. The patient reports improvement in his energy levels, mood, and libido since starting the therapy and is requesting a refill of the medication at the time of his flight physical.

You remember reading that testosterone replacement treatment (TRT) has become increasingly popular over the last few years, especially among military members, and that around 50% of military men on TRT did not meet the diagnostic criteria specified in consensus guidelines.[7,8] You explain to the pilot you will need the clinical notes from the provider who started him on the treatment and that he will require a flying waiver before he can be cleared to return to flying.[14] The Air Force Waiver Guide specifies that patients who do not meet the diagnostic criteria are "unlikely to receive a waiver"[9] for continued use of exogenous testosterone. The pilot agrees to send in the medical records from the endocrinologist for your review prior to his office visit.

1. Which is true regarding the initial diagnosis of hypogonadism (testosterone deficiency)?
   - A. Definitive results on the Androgen Deficiency in the Aging Male questionnaire, or another of the validated testosterone deficiency questionnaires, can be considered diagnostic.
   - B. Starting at age 40, men should undergo routine screening for low testosterone.
   - C. Free testosterone levels are a more reliable indicator of disease than total testosterone.
   - D. In a patient with symptoms, a single total testosterone level is sufficient to make the diagnosis if it is drawn in the morning and the result is more than 20% below the normal value.
   - E. A recent upper respiratory illness, alcohol use, and food intake will all lower testosterone levels.

## ANSWER/DISCUSSION

**1. E.** The American Urological Association (AUA) and the Endocrine Society each published consensus guidelines in 2018 regarding diagnosis and management of low testosterone levels.[1,10] Both guidelines agree that the diagnosis should be made only in a patient with symptoms of low testosterone and at least two morning total testosterone levels below 300 $ng \cdot dL^{-1}$. The labs should be drawn during the normal diurnal spike between 08:00 and 10:00 with the patient fasting. Symptoms that are considered more specific to low testosterone include loss of libido, erectile dysfunction, gynecomastia, and osteoporosis.[2] Many of the other symptoms are nonspecific, such as decreased energy, depression, poor concentration, and loss of muscle mass.[1]

There are no standardized questionnaires that are validated for use in making a definitive diagnosis.[10] Only men who have clinical symptoms suggesting deficiency should be tested; there is no reason for routine screening, regardless of age.[1]

Because the methods used to determine free testosterone may vary widely, its use is not routinely recommended in the AUA guidelines. The Endocrine Society does include a reliable, free testosterone value as a diagnostic criterion in certain clinical situations.[1] The majority of testosterone in the blood is bound to proteins, mainly albumin and sex hormone binding globulin (SHBG). In a patient with clinical symptoms of hypogonadism with borderline total testosterone levels, a physician may want to measure SHBG and free testosterone. Obesity, diabetes, use of glucocorticoids, and thyroid disorders are some of the more common reasons for abnormal SHBG levels.[1]

A single measurement should not be used to make the diagnosis because up to 50% of patients with total testosterone levels below 300 $ng \cdot dL^{-1}$ will have normal levels on repeat testing.[13] Several factors can temporarily lower hormone levels, including all those listed in response E. Individuals who


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have recently used opioids or glucocorticoids may have lower levels, as well as those who recently stopped using exogenous testosterone.[1]

The pilot contacts your clinic once he has received the notes and lab results from his endocrinologist. He asks if there are specific labs other than testosterone levels that you will need to see.

2. Besides total testosterone, what other screening/diagnostic tests should have been included in the pretreatment workup for this patient with hypogonadism?
   A. Hematocrit.
   B. Prostate specific antigen (PSA).
   C. Karyotype testing.
   D. Follicle stimulating hormone (FSH) and luteinizing hormone (LH).
   E. All of the above.

## ANSWER/DISCUSSION

**2. E.** Hematocrit levels should be checked prior to initiating therapy in all patients. PSA levels are recommended prior to initiating TRT for patients over 40 yr of age. Karyotype testing would be useful to evaluate for Klinefelter syndrome, which, along with history of trauma and orchitis, is a common cause of primary hypogonadism.[1] Studies estimate that only around 25% of men with Klinefelter syndrome are diagnosed, and the mean age of diagnosis is in the mid-30s.[6]

FSH and LH levels should be checked to help differentiate primary hypogonadism from secondary causes. Both levels are expected to be elevated in a patient with a functioning hypothalamic pituitary axis, and normal or low levels should prompt an evaluation for secondary hypogonadism.[2] Several of the causes of secondary disease may also impact a flyer's medical clearance and require further workup when FSH/LH levels are lower than expected.

3. Which of the following is not an aeromedically significant condition that should be considered as a possible cause of secondary hypogonadism?
   A. Pituitary tumor.
   B. Head trauma.
   C. Obstructive sleep apnea (OSA).
   D. Hemochromatosis.
   E. Hypothyroidism.

## ANSWER/DISCUSSION

**3. E.** Disease processes that affect the hypothalamic pituitary axis will result in secondary hypogonadism. Each of the answers A–D are potential causes and each can significantly affect safety of flight beyond the symptoms of testosterone deficiency. Patients with hypopituitarism will present with both hypothyroidism and hypogonadism. While they are sometimes associated, low testosterone is not caused by hypothyroidism; therefore, the correct answer is E.

When evaluating for secondary hypogonadism, a physician should consider potentially reversible causes of disease, such as nutritional deficiencies or obesity. A thorough history may reveal other potential causes, like significant head trauma or OSA. The provider should check prolactin levels and iron studies in all patients to exclude hyperprolactinemia and hemochromatosis. Patients with severe disease (total testosterone levels $<150\,\text{ng} \cdot \text{dL}^{-1}$) or any neurological symptoms should be evaluated with magnetic resonance imaging to look for a tumor or infiltrative disease of the hypothalamus or pituitary gland.[1]

The pilot brings in records from the endocrinologist documenting his pretreatment symptoms and lab results, which met diagnostic criteria in accordance with current guidelines. His FSH and LH levels were above normal, indicating primary hypogonadism. The exact cause of his testosterone deficiency is not known but is presumed to be a result of a viral orchitis he experienced several years previously. Your patient had no abnormalities in the screening labs from the previous provider. He has been using a topical testosterone preparation with good results in both symptom improvement and normalization of testosterone. You agree to continue the patient's current testosterone prescription.

Because he is using a testosterone gel, you counsel the patient to avoid transference of the medication from the gel to close contacts. You have a lengthy discussion with the patient explaining the significant risks and benefits of the medication.

4. Which of the following is an accurate statement about medication risks and benefits?
   A. Patients receiving TRT have a much higher risk of developing prostate cancer.
   B. Current evidence demonstrates TRT is associated with elevated risk of coronary artery disease.
   C. The most common adverse effect reported in trials of TRT is erythrocytosis.
   D. Properly dosed TRT improves OSA.
   E. Testosterone therapy increases sperm production.

## ANSWER/DISCUSSION

**4. C.** Erythrocytosis is a common adverse reaction and, for that reason, patients receiving TRT should have hematocrit monitored regularly.[1] Although the guidelines recommend checking a PSA prior to starting therapy in men over age 40, there is no evidence linking TRT to prostate cancer.[4] Similarly, available evidence does not show an increase in cardiovascular events in men on TRT.[12] Patients with untreated severe OSA may experience a worsening of their apnea if started on TRT.[1] Because many symptoms of hypogonadism can be shared with OSA, providers should be diligent about considering the diagnosis of OSA and controlling apnea symptoms prior to initiating TRT. It is well documented that exogenous testosterone inhibits

spermatogenesis and should not be used in men interested in conceiving.[10]

You check the medical standards references to determine what is needed to get this pilot approved to return to flying. For Air Force pilots, the diagnosis of hypogonadism and sustained use of hormone therapy are disqualifying.[14] The Navy and the Army* also consider hypogonadism as disqualifying for flying duty.[11] All three services allow for a waiver in adequately treated aviators.*,[9,11] In civilian aviation, the Federal Aviation Administration does not consider testosterone replacement therapy as disqualifying for medical certification.[5]

Your patient is granted a waiver and has safely returned to flying status. You check his testosterone levels and hematocrit at 6 mo and again prior to his 1-yr follow-up. During his appointment you notice increased acne on his face and back. His labs show supratherapeutic testosterone levels and polycythemia with a hematocrit of 56%.

5. Which statement is correct regarding erythrocytosis/polycythemia caused by exogenous testosterone therapy?
   A. When compared to other causes of polycythemia, TRT carries a greater risk of venous thromboembolic events (VTE).
   B. The patient should stop using testosterone until his labs normalize, then may be able to restart at a lower dose.
   C. The patient can continue on the same dose of medication if he regularly donates blood.
   D. The patient should be switched from the topical to the injectable form of the medication.

## ANSWER/DISCUSSION

**5. B**. As this patient has both elevated hematocrit and elevated testosterone levels, he should stop taking the testosterone until his hematocrit normalizes. If he needs to restart the medication, it should be at a lower dose.[1]

Polycythemia itself is a risk for VTE, but the role of TRT is not as clear. The Food and Drug Administration has required manufacturers to include a warning about VTE for TRT; however, studies looking at VTE have found conflicting results.[1] A recent study of U.S. veterans found current TRT patients did not have an increased risk of VTE.[12] The AUA recommends informing patients that there is not definitive evidence linking TRT with an increase in VTE.[10]

Using therapeutic phlebotomy could be an option for patients with polycythemia,[3] but in this case the patient's testosterone levels were elevated, so his dose must also be adjusted. Testosterone injections carry a higher incidence of polycythemia than topical forms.[1]

You advise the patient that he must discontinue the medication and bring him down off flight status until his symptoms and labs improve. You warn the patient that he is likely to experience a resurgence of testosterone deficiency symptoms, as he had grown accustomed to the exogenous steroid. You refer him to a local endocrinologist to assist in managing the withdrawal from the medication and determining when and how to restart TRT.

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## OCTOBER 1998

*Improved passenger crash position (University Hospital, Queens Medical Centre, Nottingham; RAF Institute of Aviation Medicine, Farnborough, Hampshire, UK):* "In 1989, a Boeing 737-400 aircraft crashed at Kegworth, near Nottingham, England. The survivors suffered a large number of pelvic and lower limb injuries, and approximately one-third of the passengers died. Subsequent research has suggested that the 'brace-for-impact' position that passengers are advised to adopt prior to a crash landing might be modified in order to reduce the incidence of such injuries. … Impact testing on forward-facing seats … mounted on a sled, were propelled down a track to impact at -16 $G_x$. … Four [test] dummy positions were investigated. … Impact testing revealed that the risk of a head injury … was greater in the upright position than in the braced forward position. … Flailing did not occur when the dummy was placed in a braced, legs-back position. … Such a recommendation should not obscure the fact that an occupant seated in a forward-facing aircraft seat, restrained only by a lap belt, is exposed to considerable forces during an impact … capable of producing injuries in the femur, pelvis, and lumbar spine."[1] See **Fig. 1**.

## OCTOBER 1973

*Rewarming cold water subjects (Webb Associates, Yellow Springs, OH):* "Rewarming was studied in three lightly clothed divers who had swum submerged in water of 5°, 10°, and 15°C for 45 to 60 minutes, reaching the limit of subjective tolerance to cold. Heat for rewarming the men after the dive came from warm water being circulated through a water cooling garment, plus their own metabolic heat. Both of these heat quantities were measured, and it was found that an average of 210 kcals (range 165-292 kcals) was needed to replace the heat lost during the dives. The completion of rewarming was signalled by: the release of body heat when previously it had been conserved by the cold subject; a rise in heart rate and the return of cutaneous vasomotor control of body heat loss; and a restoration of the normal balance between heat produced and heat lost. Over-warming led to sweating. None of the following body temperatures reliably indicated completion of rewarming: rectal, ear canal, esophageal, skin (mean or any of 8 sites), calf or chest subcutaneous temperature, or calculated mean body temperature."[3]

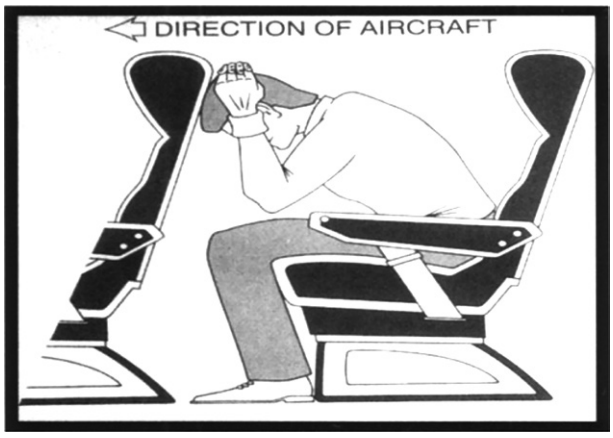


**Fig. 1.** Newly recommended improved brace position. Fig. 8 from Brownson et al.[1] with permission from the Aerospace Medical Association.

## OCTOBER 1948

*Air travel and disease spread (Colonel, Medical Corps, U.S. Army):* "One of the earliest recognized facts about communicable disease was that it followed lines of travel … [T]he plague epidemic which struck Egypt in 542 A.D … at its height it killed from 5,000 to 10,000 persons a day and for fifteen years it raged over all Europe and then lay dormant for 800 years. In 1348, just 600 years ago, it broke out again. … By Easter, 1,200,000 people gathered in Rome [for a Holy Year celebration] bringing plague with them, and over 1,000,000 of the travelers died of the disease.

"The centuries have not altered this property of epidemic diseases to move with people, and no later than February, 1947, we learn of smallpox coming to New York from Mexico City without the slightest trouble at the International Border. Last year, too, we saw the arrival of cholera in Egypt and witnessed the frantic efforts of nations, organizations, and individuals to contain this menace. At times, International Quarantine became international incidents with borders being closed and planes refused landings. …

"It is believed that the proposed World Health Organization is the proper agency for establishing control of epidemic disease in air travel and that this organization should stress sanitary airports of international travel and host factors in passengers."[2]

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This column is prepared each month by Walter Dalitsch III, M.D., M.P.H. Most of the articles mentioned here were printed over the years in the official journal of the Aerospace Medical Association. These and other articles are available for download from Mira LibrarySmart via https://submissions.miracd.com/asmaarchive/Login.aspx.



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