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Hemolysis contributes to anemia during long-duration space flight

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Anemia in astronauts has been noted since the first space missions, but the mechanisms contributing to anemia in space flight have remained unclear. Here, we show that space flight is associated with persistently increased levels of products of hemoglobin degradation, carbon monoxide in alveolar air and iron in serum, in 14 astronauts throughout their 6-month missions onboard the International Space Station. One year after landing, erythrocytic effects persisted, including increased levels of hemolysis, reticulocytosis and hemoglobin. These findings suggest that the destruction of red blood cells, termed hemolysis, is a primary effect of microgravity in space flight and support the hypothesis that the anemia associated with space flight is a hemolytic condition that should be considered in the screening and monitoring of both astronauts and space tourists.

As humankind plans extraterrestrial travel, understanding the health implications of living in space will be critical to planning safe journeys. Space anemia was previously documented and characterized by a 10–12% decrease in red blood cell (RBC) mass happening in the first 10 days in space¹. Current understanding of space anemia is that the decrease in RBCs constitutes an acute adaptation to major hemodynamic events of cephalad fluid shifts, hemoconcentration and low erythropoietin (EPO) levels upon entering microgravity^{1,2}. Thereafter, beyond 10 days in space, when the hemoglobin concentration returns to near-earthly values, erythrocytic regulation would proceed normally, but this has not been measured precisely². Recently, astronauts were found to remain mildly hemoconcentrated throughout long-duration mission³, and epidemiological data showed that the severity, time to recovery and longitudinal effects of postflight anemia were proportional to the time spent in space⁴. These reports challenged the current understanding of space anemia. Longer missions to the moon and Mars, as well as space tourism and commercialization, require a better understanding of space-induced anemia. Because astronaut orthostatism, exercise tolerance and fatigue are key functions affected by anemia, RBC management will be vital for human missions landing on extraterrestrial worlds without medical supervision.

While a variety of hypothetical causes (e.g., RBC dysfunction, decreased production, sequestration or increased destruction) have been proposed for space anemia, the physiologic mechanisms are not fully established⁵, and studying these mechanisms in space is challenging. Hemolysis releases hemoglobin, and heme rings are broken down by heme oxygenases⁶. Each heme molecule produces one ferrous iron, one carbon monoxide (CO) and one biliverdin molecule. In basal conditions, approximately 85% of endogenously produced CO arises from hemoglobin⁶. The quantification of CO

molecules eliminated is therefore a direct measure of hemolysis. Recently developed methods to precisely quantify endogenous CO now permit the measurement of hemolysis in space⁷. Using these methods, 20 participants showed increased hemolysis (by an average of 23%) throughout 60 days of the antiorthostatic bed-rest microgravity analogue⁸. These findings suggested that increased hemolysis may be an important primary effect of the microgravity analogue, a hypothesis never tested in space. We therefore measured hemolysis markers in breath and blood samples from astronauts preflight, four times inflight and up to 1 year after their 6-month missions to the International Space Station (ISS).

A total of 14 astronauts were recruited (11 men and 3 women; 45 ± 7 years) between 2015 and 2020 (Fig. 1a). The astronauts flew ISS missions of 167 ± 31 days duration. Each astronaut collected alveolar and ambient air samples as well as blood samples according to a prespecified schedule (Fig. 1b,c). The space samples were downmassed using automated reentry vehicles.

Figure 2a shows that mean baseline CO elimination was 1,662 ppb (95% confidence interval (CI), 1,433–1,891). After an average of 5 ± 1 days on the ISS, CO elimination was 2,627 ppb (95% CI, 2,342–2,913); the mean increase from preflight was 56% (95% CI, 36–76; Supplementary Table 1). Increased hemolysis may have started earlier than day 5 (the first sampling time). The finding of increased CO elimination was corroborated by the observation that serum iron and iron carrier proteins transferrin and ferritin also increased (Fig. 2b–d). Large elevations in heme degradation products both in alveolar air and blood samples constitute the first direct demonstration of upregulated hemolysis in space and support the hypothesis that space-related anemia is a hemolytic condition.

The next question was whether the hemolysis stopped when the hemoglobin concentration reached near-earthly values after 10 days in space. CO elimination in space over 6 months (average of 46 measures) was increased 54% (95% CI, 39–70) compared to preflight (Fig. 2a; Supplementary Tables 1 and 2). Figure 2b–d shows that serum iron, transferrin saturation and ferritin levels in space (average of 55 measures each) were also higher compared to preflight throughout the 6-month missions (Supplementary Tables 1 and 2). Persistently increased CO elimination in space showed that hemolysis was not an acute adaptation to hemodynamic alterations upon entering microgravity. Increased hemolysis rather constituted a primary effect of exposure to space. This conclusion is strengthened by the postflight data. The astronaut first postflight measure (taken 4 days after landing, separated by only 14 days from the last inflight measure) showed sharp decreases in hemolysis markers coinciding with the return to Earth's surface gravity (Fig. 2a–d). The pattern of hemolysis in the three phases of the study (on Earth, in

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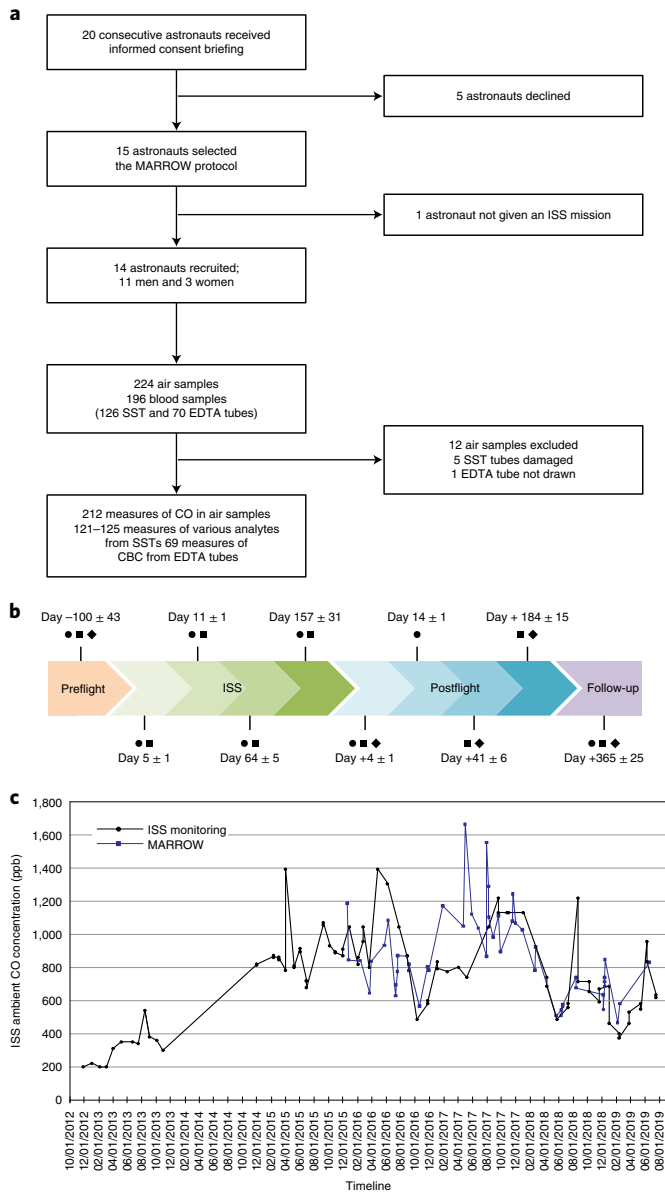


Fig. 1 | Bone Marrow Adipose Reaction: Red Or White (MARROW) study design and outcomes. **a**, Flow chart showing recruitment and samples harvested. SST, serum separator tube. **b**, Schematic showing the study design and planned sample collection. Astronauts recruited to the MARROW study performed serial measures at prespecified time points preflight, onboard the ISS and up to 1 year postflight. The average number of days (\pm s.d.) of sampling in relation to takeoff and landing are indicated. The interval between the last CO sampling in space and the first sampling on Earth after landing was 14 ± 5 days. Circles, CO measurements; squares, serum analytes (iron, transferrin percent saturation, ferritin, haptoglobin, bilirubin, C-reactive protein and EPO); diamonds, complete blood counts (CBCs) and reticulocytes. **c**, Time course of ambient air CO concentration onboard the ISS.

space and back on Earth) confirmed that increased hemolysis was tightly linked to the space environment.

Increased hemolysis as a primary effect of exposure to space constitutes a paradigm shift in our understanding of space anemia⁵. Major pathophysiological implications include that space anemia may be dependent on the duration of exposure to space and independent of fluid shifts, EPO levels and the RBC production

environment on Earth versus in space and that it may trigger a compensatory increase in RBC production and enhance nutritional needs. Persistent hemolysis during space missions suggests that the longer the exposure, the worse the anemia. A similar conclusion was reached after analyzing data from 711 missions from American and Canadian astronauts between 1968 and 2015 (ref. ⁴). Increased hemolysis was measured both during fluid shifts (inflight days 5 and 11) and long after fluid shifts were completed (inflight days 64 and 157), dissociating it from a causation effect. Increased hemolysis appeared EPO independent. In the current trial, increased hemolysis was sustained under both suppressed (inflight days 5 and 11) and corrected EPO levels (inflight days 64 and 157; Fig. 2e). In the bone marrow, colony-forming unit erythroid cells depend on EPO for survival and differentiation into erythroblasts, with only the latter synthesizing hemoglobin⁹. Reticulocytes and mature RBCs lack EPO receptors and cease responding to EPO⁹. Colony-forming unit erythroid cell suppression under low-EPO conditions should not enhance heme degradation, whereas degradation of erythroblasts, reticulocytes and mature RBCs would. Therefore, our data support increased hemolysis of EPO-independent erythroblasts and/or more differentiated RBCs. Upon entering space, all astronaut RBCs are terrestrial born. Based on normative RBC lifespan, after 120 days in space, all RBCs are space born. In this study, both RBC populations hemolyzed at higher rates while in space. At postflight day 4, the hemolysis of space-born RBCs sharply decreased. These results suggest that intrinsic characteristics of Earth-born or space-born RBCs do not influence hemolysis. Space hemolysis attenuates the initial hemoconcentration caused by fluid shifts obviating the need for compensatory RBC production. Later in the mission, a continuing 54% increased hemolysis risks causing severe anemia. That astronauts remain mildly hemoconcentrated throughout long-duration space missions³ implies a compensatory increased RBC production. Enhanced RBC production in space was not directly measured in this study, but robust EPO levels at inflight days 64 and 157 and increased reticulocytes at postflight day 4 supported stimulated RBC production (Fig. 2e,f and Supplementary Table 2). Hemolysis with compensatory RBC production will expand bone marrow erythropoietic activity, the higher energy requirements of which must be factored in astronaut nutritional planning. C-reactive protein levels remained unchanged throughout ISS missions, arguing against an inflammatory component to space anemia from background inflammation in space or free hemoglobin as a potent inflammatory inducer (Supplementary Tables 1–3).

The return to Earth's surface also causes major hemodynamic changes, as fluid shift reversal increases plasma volume and dilutes RBCs^{1,5}. Our astronaut cohort had an 8.8% (95% CI, 8.1–9.5) decrease in hemoglobin concentration 4 days after landing compared to preflight (Fig. 2g and Supplementary Table 2) with 5 of 13 astronauts (38.5%) reaching clinical levels of anemia¹⁰. Low hemoglobin concentrations postflight indicate that astronauts' first landing measure, just like inflight measures past 10 days in space, originated from fewer RBCs¹. These predicted proportionately lower CO elimination, iron and iron carrier protein concentrations. The implication is that the hemolysis figures we report compared to preflight data, as high as they are, are likely underestimating the actual rate of hemolysis at these time points.

The next question was how reversible these effects were up to 1 year after long-duration missions. Erythrocytic effects of space include increased hemolysis (herein reported), correspondingly elevated RBC production that is assumed and previously reported hemoglobin reset at higher concentrations³. Previous spaceflight data suggested persistent erythrocytic effects with higher hemoglobin concentrations (0.27 g dl^{-1}) in 58 astronauts 1 year after missions averaging 145 days⁴. In the current trial, 1 year after landing, CO elimination was 30% (95% CI, 2–57) higher, reticulocytes were 16% (95% CI, 7–24) higher and hemoglobin concentration

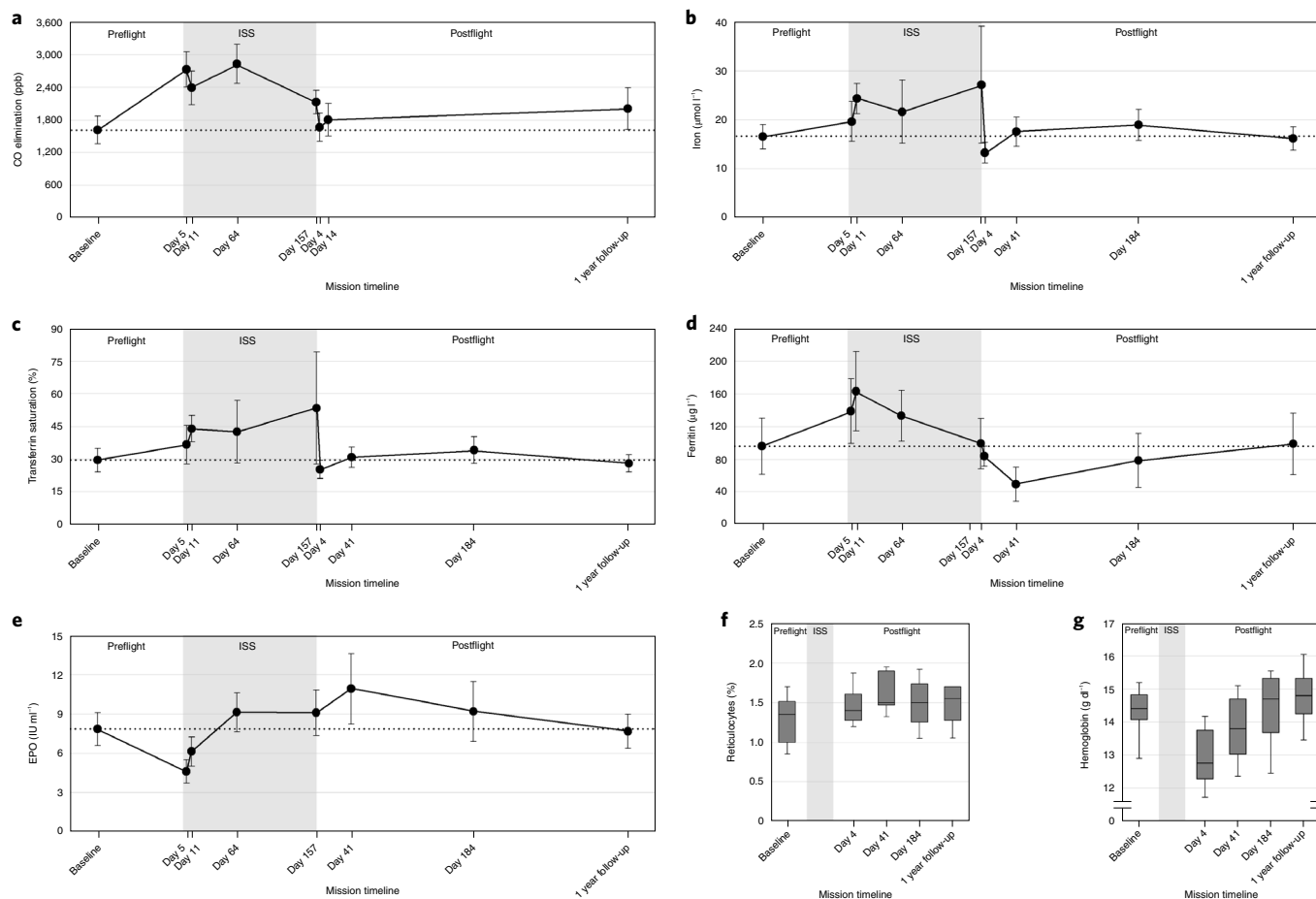


Fig. 2 | Hemolysis and erythropoietic markers of astronauts preflight, onboard the ISS and postflight. **a**, CO elimination. **b–e**, Serum iron concentrations (**b**), transferrin saturation (**c**), ferritin concentrations (**d**) and EPO concentrations (**e**). Data points and error bars represent mean values with $\pm 95\%$ CIs. Shaded areas represent data from ISS samples. **f, g**, Percentage of reticulocytes (**f**) and hemoglobin concentrations (**g**). The center line in the box plots represents the median, the upper limit of the box plots represents the third quartile (75th percentile), the lower limit of the box plots represents the first quartile (25th percentile), the upper whisker is the maximum value of the data that are within 1.5 times the interquartile range over the 75th percentile and the lower whisker is the minimum value of the data that are within 1.5 times the interquartile range under the 25th percentile. $N=14$ astronauts who provided individual samples at five, eight or nine independent time points according to the outcome.

was 3.5% (95% CI, 1.5–5.4) (0.50 g dl^{-1}) higher than preflight levels (Fig. 2a,f,g and Supplementary Tables 1 and 2). These data support increased hemolysis, RBC production and hemoglobin concentration 1 year after long-duration space exposure. These erythrocytic space effects may not be permanent, as longitudinal astronaut data over 20 years predicted lower lifelong hemoglobin concentrations (-0.001 and -0.002 g dl^{-1} for male and female astronauts, respectively) for every day in microgravity⁴. Erythrocytic effects 1 year postflight support long-term monitoring and consideration on the optimal interval between repeated space missions.

The anatomic site and mechanism of space hemolysis remain unknown. Hemolysis can be intravascular or extravascular in the spleen, liver or bone marrow. Increased heme degradation without haptoglobin depletion in this trial argues against intravascular hemolysis (Supplementary Tables 1 and 2). Erythroblast and reticulocyte destruction in the bone marrow before their egress into the circulation (sometimes called inefficient erythropoiesis) could arise from marrow adipose tissue accumulation from the lack of bone stimulation in space and dyserythropoiesis¹¹. Altered size and shape of erythrocytes or the spleen in space may increase extravascular hemolysis¹². Mature RBC lifespan may be shortened by mitochondrial stress and dysregulation¹³. Gene modifiers (e.g., BCL11A and

KLF1) modulate the expression of globin genes¹⁴. The regulation of gene expression of these globin modifiers involves the methylation state of the DNA regions impacting their expression¹⁵. DNA hypomethylation in the β -globin gene cluster was linked to ineffective erythropoiesis in thalassemia¹⁵. Interestingly, altered methylation levels in CD4 and CD8 lymphocytes were reported after 1 year in space². Space or radiation effects on DNA methylation of regulatory regions controlling the expression of globin and gene modifiers constitute potential mechanisms of space hemolysis. Elucidating the site and mechanism of space hemolysis is crucial to developing successful mitigation strategies. The specific exercise and nutritional countermeasures of modern space travel did not prevent hemolysis and postflight anemia in our cohort, and both could have health consequences. Increased CO levels from space hemolysis can have second-messenger modulatory effects on intracellular processes of the cardiac, vascular, ocular, bone, nutrition, circadian cycle, orthostatic hypotension, brain and muscle systems of astronauts^{16,17}.

Space tourism will considerably expand the number of space travelers¹⁸. Medical screening of future astronauts and space tourists might benefit from a preflight profiling of globin gene and modifiers. Postlanding monitoring should cover conditions affected by anemia and hemolysis. Monitoring individual astronaut's levels of

hemolysis during mission may be indicated to reduce health risks¹⁸. Our results support adding space travel to the list of etiologies for hemolytic anemia.

Erythrokinetic response to microgravity may have critical relevance to understand erythroid physiology and pathophysiology in contexts other than space flight. Data from the mid-1990s focusing on the acute hemoconcentration entering space led to hypotheses on normal erythroid mass control, prevention from becoming excessive, hypoxic environments, polycythemia and EPO withdrawal⁵. Preferential hemolysis of younger erythrocytes after descent from altitude is currently debated^{19,20}. Our findings of continuous hemolysis in space carry broad implications for populations on Earth with increased hemolysis or high prevalence of unexplained anemia, including patients receiving critical care, patients with chronic anemia, older patients, sedentary patients and bedridden patients^{21–23}. Like astronauts, the environment of these individuals is characterized by a marked decrease in skeleton stimulation and decreased standing time. These individuals experience cardiovascular deconditioning, muscle atrophy and osteopenia. Potential alterations in bone marrow and spleen functions in space may be relevant to pathophysiological changes in their erythrocytic control. Our findings support investigating whether increased hemolysis contributes to anemia on Earth and could be targeted by specific physical rehabilitation strategies.

Limitations of this study include potential confounding factors associated with exposure to space, including fluid shifts, ambient CO concentration, altered circadian cycle and muscle atrophy. These factors were addressed with ambient CO sampling with each alveolar sample, chronostandardization and serial measures throughout ISS missions (Fig. 1c). Hemolysis with longer space exposure, such as 1-year ISS or Moon missions or journeys to Mars, and cumulative exposure constitute knowledge gaps. Recruitment was limited to astronauts, which threatens the generalization of these findings to space tourists. The unequal sex distribution precluded a robust examination of potential physiological differences (Supplementary Tables 3 and 4).

RBC regulation demonstrated major disruptions in space compared to on Earth. Increased hemolysis by 54% was a primary effect of exposure to space in astronauts that persisted throughout their long-duration missions and may constitute the leading mechanism of space-related anemia. Space hemolysis should be considered in the screening, monitoring and follow-up of astronauts launching on space exploration missions, as well as space tourists.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41591-021-01637-7>.

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Methods

Design and exposure. Between 2015 and 2020, astronauts were consecutively interviewed approximately 1 year ahead of their ISS missions. The participating astronauts collected air and blood samples 3 months before Soyuz takeoff, four times onboard the ISS and serially after landing (total of 224 air and 196 blood samples; Fig. 1a,b). Each individual astronaut could have elected to participate in a complement of different experiments, some of which might have included additional venipunctures, causing blood and iron losses. To our knowledge, astronauts in this cohort were not screened for or confirmed to not be carrying any hemoglobinopathy or one of 1,000 known β -globin variants, some of which may affect hemoglobin levels in vivo (<https://globin.bx.psu.edu/hbvar/>)^{24,25}. In this report, preflight measures served as the comparator to test the effects of exposure to space and determine whether one or more individual astronauts unknowingly had a β -globin variant (<https://globin.bx.psu.edu/hbvar/>).

Outcomes. The primary outcome was CO elimination quantified as the concentration of CO gas molecules [CO] in parts per billion eliminated through pulmonary ventilation. Increased CO elimination can arise from RBC destruction (intravascular or extravascular) in the spleen, liver or bone marrow. Clinically, whereas the first three conditions are called hemolysis, the latter is sometimes referred to as intramedullary hemolysis or ineffective erythropoiesis. In this paper, we defined, measured and discussed hemolysis as heme degradation products of the heme oxygenase enzymes arising from all sites. Secondary outcomes included other direct (iron) and indirect (bilirubin, transferrin percent saturation and ferritin) markers of heme degradation as well as haptoglobin, EPO, CBC and C-reactive protein^{26,27}. CBC, reticulocytes and erythrocyte sedimentation rate (ESR) were not measured during flight, as they require analyses on fresh whole blood. In addition, measuring CBC and reticulocytes requires a hemocytometer, and measuring ESR requires gravity, both of which are unavailable on the ISS. In this trial, we did not measure the age of erythrocytes to comment on the subpopulation of RBCs present in space.

Laboratory analyses. Various markers of increased erythrocyte destruction intravascular or extravascular are used clinically, including the presence of schistocytes on a blood smear, free hemoglobin in blood, decreased haptoglobin for its high affinity with free hemoglobin, increased bilirubin (indirect or direct), increased ferritin, increased LDH, d-dimer and hemosideruria²⁸. However, for different reasons, these markers are not precise, quantitative or direct markers of hemolysis²⁹. Iron and transferrin saturation may rise with many types of anemia, including hypoproliferation/hypoplasia when iron is not cleared from the plasma. Similarly, serum ferritin can increase with any cause of anemia other than iron deficiency and/or bleeding. The iron in circulating RBCs is transferred to reticuloendothelial stores, which release a proportional amount of ferritin. For these reasons, we chose endogenous CO elimination as a precise, direct and quantitative marker of hemolysis as the primary outcome measure of the study. We measured CO elimination following the methods of Shahin et al.⁷. Briefly, upon waking up, after a 20-s breath hold, astronauts exhaled through a mouthpiece connected to a one-way valve sequentially discarding 400 ml of air from airways and filling a 750-ml collection bag with alveolar air (Supplementary Video 1). The alveolar air was immediately transferred to a vacuumed 200-ml canister. Concurrently, the astronauts filled a second canister with ambient air. Canisters sampled on the ISS were stowed and boarded the next Earth-bound reentry vehicle. These methods accommodated for changes in pressure, temperature and vibration and maintained stable [CO] for at least 11 months⁷. Both air samples were extracted from the canisters and run through a gas chromatograph with a reduction gas detector. Subtracting the ambient air [CO] from the alveolar air [CO] provided the CO elimination.

Methods for secondary outcomes. The astronauts serially drew blood in tubes with double-polymer gel (serum separator tube) (Fig. 1). Blood was centrifuged at 1,515g for 10 min, and the tubes were frozen at -80°C . For reentry, they were loaded into the -80°C freezer aboard the Dragon capsule and processed using commercial clinical analyzers for iron, transferrin percent saturation, ferritin, haptoglobin, bilirubin, EPO and C-reactive protein (Supplementary Table 5). Blood was also drawn pre- and postflight in EDTA tubes for CBC and reticulocytes at Johnson Space Center (JSC) clinical laboratory.

Blinding and anonymization. Each participant was assigned a random study number. The gas chromatograph operator was blinded to the subject identity and sampling time but, for calibration purposes, knew whether samples were ambient or alveolar. Aggregate data are presented instead of specific age, date or flight duration to prevent attributability.

Standard of care and ethics. Ethics approval was obtained from the National Aeronautics and Space Administration (NASA) Human Research Multilateral Review Board (#Pro1283) and the Ottawa Health Sciences Research Ethics Board (#2009646-01H). The trial was registered at NASA's Life Sciences Data Archives (<https://lsda.jsc.nasa.gov/Experiment/exper/13399>). All participants signed an informed consent form.

Assumptions and control for confounding. Preflight measures ensured that astronauts had no baseline anemia or hemolysis. Ambient [CO] varies widely. Given the higher and fluctuating ambient [CO] on the ISS, determining hemolysis based only on alveolar [CO] would have grossly overestimated CO elimination in space (Fig. 1c). In this trial, ambient air samples in the immediate environment of the astronaut were collected with each alveolar air sample. While altered pulmonary ventilation in space constitutes a potential unmeasured confounder, previous experiments reported uniform ventilation and similar ventilation/perfusion ratio in space as on Earth²⁹. Disrupted circadian cycle in orbit (16 sunrises and sunsets per 24 h) may affect hemolysis³⁰. We controlled for this potential confounder by chronostandardizing the sampling according to the astronaut schedule set on Greenwich meridian time. Nonhemoglobin heme proteins (cytochromes and myoglobin) are catalyzed by heme oxygenases to generate 15% of the human CO², but this has never been measured in space. No liver or muscle necrosis was reported in space³¹, and muscle atrophy in space was addressed through exercise countermeasures. Earth-based data on muscle atrophy showed negligible changes in muscle myoglobin content^{32,33}. Finally, myoglobin content is approximately eightfold less than hemoglobin content, decreasing the likelihood that nonhemoglobin heme degradation biased the interpretation of CO elimination data³². Entering space triggers a 10–12% blood volume contraction due to fluid shifts, which leads to hemoconcentration^{1,2,5,34}. We did not apply a 10–12% correction to the initial ISS data, because serum analytes have short half-lives. In addition, Haldane's equation determining O₂ and CO binding to hemoglobin is unaffected by hemoconcentration. Importantly, ISS ambient pressure reproduces atmospheric pressure, validating Henry's law governing CO elimination according to partial pressure gradients.

Statistical analyses. For this exposure study, summary statistics are limited to point estimates with unadjusted 95% CI widths. No *P* values are provided. We also present mean percent increases from baseline. Because a few samples were damaged, the ratio of estimates at individual time points may differ slightly from the percent increase estimates based on paired observations. Paired observations were necessary to calculate CIs on percent increase.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Aggregated data to understand and assess the conclusions of this research are available in the figures and supplementary tables. Individual astronaut source data have been deposited in NASA's Life Sciences Data Archives. Investigators can request access to the astronaut data at <https://lsda.jsc.nasa.gov/>.

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Author contributions

G.T. was responsible for the conception and direction of the study. G.T. and H.L. established methods and supervised measures. G.T., N.S. and T.R. were responsible for data treatment and analysis. G.T., H.L., N.S., O.L. and T.R. contributed to data interpretation and manuscript writing.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41591-021-01637-7>.

Correspondence and requests for materials should be addressed to Guy Trudel.

Peer review information *Nature Medicine* thanks Lawrence Rice, Hanns-Christian Gunga, Joseph Borg and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Michael Basson was the primary editor on this article and managed its editorial process and peer review in collaboration with the rest of the editorial team.

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Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a Confirmed

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection

No software was used for data collection

Data analysis

Data analysis was carried out using Microsoft Excel Version 2102.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Aggregated data to understand and assess the conclusions of this research are available in the Figures and Supplementary Tables. Astronaut source data are restricted for confidentiality. Individual astronaut data are freely available by request to the NASA's Life Sciences Data Archives (LSDA) at <https://lsda.jsc.nasa.gov/>. Access to human data will be granted following a review and JSC IRB approval.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

Life sciences Behavioural & social sciences Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	20 astronauts were presented with the MARROW protocol. 15 astronauts agreed to participate. 1 astronaut was not provided a mission during the duration of MARROW study. 11 male and 3 female astronauts completed the trial. Considering a baseline endogenous CO of 1600ppb with sd of 400ppb, 14 astronauts allowed detecting a 300ppb difference between Earth and space CO at an alpha level of 0.05 with 80% power (https://clincalc.com/stats/samplesize.aspx)
Data exclusions	12 air samples were excluded due collection, transfer or logistical errors. 5 blood samples in serum separation tubes (SST) were damaged at re-entry and yielded invalid result for one or more analytes. 1 EDTA tube for CBC was not collected. Therefore 212 measures of CO, 121 to 125 measures of specific serum analytes and 69 measures of CBC populated the dataset.
Replication	The air samples were measured for CO concentration in quintuplicates or until signal stabilization as per Shahin, N., Louati, H. & Trudel, G. Measuring Human Hemolysis Clinically and in Extreme Environments Using Endogenous Carbon Monoxide Elimination. Ann Biomed Eng. 48, 1540-50 (2020). The blood samples were not sufficiently large to run duplicates.
Randomization	<i>Randomization is not applicable, Astronauts recruited to the MARROW protocol performed serial measures at prespecified time points preflight, onboard the International Space Station (ISS) and postflight up to 1 year after their space missions.</i>
Blinding	<i>Each participant was assigned a random study number. The gas chromatograph operator was blinded to the subject identity and sampling time but, for calibration purposes, knew whether samples were ambient or alveolar. Aggregate data are presented instead of specific age, date or flight duration to prevent attributability.</i>

Behavioural & social sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	<i>Briefly describe the study type including whether data are quantitative, qualitative, or mixed-methods (e.g. qualitative cross-sectional, quantitative experimental, mixed-methods case study).</i>
Research sample	<i>State the research sample (e.g. Harvard university undergraduates, villagers in rural India) and provide relevant demographic information (e.g. age, sex) and indicate whether the sample is representative. Provide a rationale for the study sample chosen. For studies involving existing datasets, please describe the dataset and source.</i>
Sampling strategy	<i>Describe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient. For qualitative data, please indicate whether data saturation was considered, and what criteria were used to decide that no further sampling was needed.</i>
Data collection	<i>Provide details about the data collection procedure, including the instruments or devices used to record the data (e.g. pen and paper, computer, eye tracker, video or audio equipment) whether anyone was present besides the participant(s) and the researcher, and whether the researcher was blind to experimental condition and/or the study hypothesis during data collection.</i>
Timing	<i>Indicate the start and stop dates of data collection. If there is a gap between collection periods, state the dates for each sample cohort.</i>
Data exclusions	<i>If no data were excluded from the analyses, state so OR if data were excluded, provide the exact number of exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.</i>
Non-participation	<i>State how many participants dropped out/declined participation and the reason(s) given OR provide response rate OR state that no participants dropped out/declined participation.</i>
Randomization	<i>If participants were not allocated into experimental groups, state so OR describe how participants were allocated to groups, and if allocation was not random, describe how covariates were controlled.</i>

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	Briefly describe the study. For quantitative data include treatment factors and interactions, design structure (e.g. factorial, nested, hierarchical), nature and number of experimental units and replicates.
Research sample	Describe the research sample (e.g. a group of tagged <i>Passer domesticus</i> , all <i>Stenocereus thurberi</i> within Organ Pipe Cactus National Monument), and provide a rationale for the sample choice. When relevant, describe the organism taxa, source, sex, age range and any manipulations. State what population the sample is meant to represent when applicable. For studies involving existing datasets, describe the data and its source.
Sampling strategy	Note the sampling procedure. Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.
Data collection	Describe the data collection procedure, including who recorded the data and how.
Timing and spatial scale	Indicate the start and stop dates of data collection, noting the frequency and periodicity of sampling and providing a rationale for these choices. If there is a gap between collection periods, state the dates for each sample cohort. Specify the spatial scale from which the data are taken
Data exclusions	If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.
Reproducibility	Describe the measures taken to verify the reproducibility of experimental findings. For each experiment, note whether any attempts to repeat the experiment failed OR state that all attempts to repeat the experiment were successful.
Randomization	Describe how samples/organisms/participants were allocated into groups. If allocation was not random, describe how covariates were controlled. If this is not relevant to your study, explain why.
Blinding	Describe the extent of blinding used during data acquisition and analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.
Did the study involve field work?	<input type="checkbox"/> Yes <input type="checkbox"/> No

Field work, collection and transport

Field conditions	Describe the study conditions for field work, providing relevant parameters (e.g. temperature, rainfall).
Location	State the location of the sampling or experiment, providing relevant parameters (e.g. latitude and longitude, elevation, water depth).
Access and import/export	Describe the efforts you have made to access habitats and to collect and import/export your samples in a responsible manner and in compliance with local, national and international laws, noting any permits that were obtained (give the name of the issuing authority, the date of issue, and any identifying information).
Disturbance	Describe any disturbance caused by the study and how it was minimized.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Included in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> Antibodies
<input checked="" type="checkbox"/>	<input type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology
<input checked="" type="checkbox"/>	<input type="checkbox"/> Animals and other organisms
<input type="checkbox"/>	<input checked="" type="checkbox"/> Human research participants
<input type="checkbox"/>	<input checked="" type="checkbox"/> Clinical data

Methods

n/a	Included in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input checked="" type="checkbox"/>	<input type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Antibodies

Antibodies used	<i>Describe all antibodies used in the study; as applicable, provide supplier name, catalog number, clone name, and lot number.</i>
Validation	<i>Describe the validation of each primary antibody for the species and application, noting any validation statements on the manufacturer's website, relevant citations, antibody profiles in online databases, or data provided in the manuscript.</i>

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	<i>State the source of each cell line used.</i>
Authentication	<i>Describe the authentication procedures for each cell line used OR declare that none of the cell lines used were authenticated.</i>
Mycoplasma contamination	<i>Confirm that all cell lines tested negative for mycoplasma contamination OR describe the results of the testing for mycoplasma contamination OR declare that the cell lines were not tested for mycoplasma contamination.</i>
Commonly misidentified lines (See ICLAC register)	<i>Name any commonly misidentified cell lines used in the study and provide a rationale for their use.</i>

Palaeontology

Specimen provenance	<i>Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information).</i>
Specimen deposition	<i>Indicate where the specimens have been deposited to permit free access by other researchers.</i>
Dating methods	<i>If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.</i>

Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals	<i>For laboratory animals, report species, strain, sex and age OR state that the study did not involve laboratory animals.</i>
Wild animals	<i>Provide details on animals observed in or captured in the field; report species, sex and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.</i>
Field-collected samples	<i>For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.</i>
Ethics oversight	<i>Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.</i>

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about [studies involving human research participants](#)

Population characteristics	<i>All astronauts participated in a planned 6 month excursion on board the International Space Station. 14 astronauts were recruited (11 men and 3 women; 45±7years)</i>
Recruitment	<i>Between 2015 and 2020, astronauts were consecutively interviewed approximately 1 year ahead of their ISS missions. 20 astronauts were presented with the MARROW protocol. 15 astronauts agreed to participate. 1 astronaut was not provided a mission during the duration of MARROW study. There are fewer woman than men in the astronaut corps. Our recruitment was representative with more male astronauts recruited.</i>
Ethics oversight	<i>The protocol was approved by both the NASA JSC Human Research Multilateral Review Board #Pro1283 and the Ottawa Health Sciences Research Ethics Board #2009646-01H</i>

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about [clinical studies](#)

All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration	<i>The trial was registered at NASA's Life Sciences Data Archives https://lsda.jsc.nasa.gov/Experiment/exper/13399.</i>
Study protocol	<i>The study protocol will be uploaded through the manuscript submission portal.</i>
Data collection	<i>The participating astronauts collected air and blood samples 3 months before Soyuz take off, 4 times onboard the ISS and serially after landing</i>
Outcomes	<i>The primary outcome was CO elimination quantified as the concentration of CO gas molecules [CO] in parts per billion (ppb) eliminated through pulmonary ventilation. Secondary outcomes included serum iron, bilirubin, transferrin % saturation, ferritin, haptoglobin, erythropoietin, C-reactive protein and complete blood count and were processed using commercial clinical analyzers (Supplementary Table S5).</i>

ChIP-seq

Data deposition

- Confirm that both raw and final processed data have been deposited in a public database such as [GEO](#).
- Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links <i>May remain private before publication.</i>	<i>For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.</i>
Files in database submission	<i>Provide a list of all files available in the database submission.</i>
Genome browser session (e.g. UCSC)	<i>Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.</i>

Methodology

Replicates	<i>Describe the experimental replicates, specifying number, type and replicate agreement.</i>
Sequencing depth	<i>Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.</i>
Antibodies	<i>Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.</i>
Peak calling parameters	<i>Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.</i>
Data quality	<i>Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.</i>
Software	<i>Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.</i>

Flow Cytometry

Plots

Confirm that:

- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	<i>Describe the sample preparation, detailing the biological source of the cells and any tissue processing steps used.</i>
Instrument	<i>Identify the instrument used for data collection, specifying make and model number.</i>

Software	<i>Describe the software used to collect and analyze the flow cytometry data. For custom code that has been deposited into a community repository, provide accession details.</i>
Cell population abundance	<i>Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.</i>
Gating strategy	<i>Describe the gating strategy used for all relevant experiments, specifying the preliminary FSC/SSC gates of the starting cell population, indicating where boundaries between "positive" and "negative" staining cell populations are defined.</i>

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Magnetic resonance imaging

Experimental design

Design type	<i>Indicate task or resting state; event-related or block design.</i>
Design specifications	<i>Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.</i>
Behavioral performance measures	<i>State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).</i>

Acquisition

Imaging type(s)	<i>Specify: functional, structural, diffusion, perfusion.</i>
Field strength	<i>Specify in Tesla</i>
Sequence & imaging parameters	<i>Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.</i>
Area of acquisition	<i>State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.</i>
Diffusion MRI	<input type="checkbox"/> Used <input type="checkbox"/> Not used

Preprocessing

Preprocessing software	<i>Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).</i>
Normalization	<i>If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.</i>
Normalization template	<i>Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.</i>
Noise and artifact removal	<i>Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).</i>
Volume censoring	<i>Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.</i>

Statistical modeling & inference

Model type and settings	<i>Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).</i>
Effect(s) tested	<i>Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.</i>
Specify type of analysis:	<input type="checkbox"/> Whole brain <input type="checkbox"/> ROI-based <input type="checkbox"/> Both
Statistic type for inference (See Eklund et al. 2016)	<i>Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.</i>
Correction	<i>Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).</i>

Models & analysis

n/a | Involved in the study

Functional and/or effective connectivity

Graph analysis

Multivariate modeling or predictive analysis

Functional and/or effective connectivity

Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).

Graph analysis

Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).

Multivariate modeling and predictive analysis

Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.