Changes in cerebral oxygen saturation and cerebral blood flow velocity under mild +Gz hypergravity

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INTRODUCTION

Humans are exposed to hypergravity in various situations, such as during launchings/landings of spacecraft and while performing aerial maneuvers in high-performance aircraft, riding roller coasters, and participating in motorsports. The physiological impacts of hypergravity depend on various characteristics, such as direction, magnitude, onset rate, and/or sustained time (33). On the other hand, it has been proposed that intermittent exposure to mild +Gz (head-to-foot) hypergravity in a human centrifuge could help prevent or mitigate spaceflight-induced physiological deconditioning (7, 10). Understanding the physiological changes experienced by humans during exposure to mild +Gz hypergravity is therefore important for understanding various situations, as well as for the future implementation of human centrifuges (10). We previously reported that cerebral blood flow (CBF) as measured by transcranial Doppler ultrasonography (TCD) was significantly decreased under even mild +Gz hypergravity, and did not lead to any changes in arterial blood pressure (ABP) at heart level (15, 20, 24).

Cerebral oxygenation as measured by near-infrared spectroscopy (NIRS) has been clinically used to monitor cerebral ischemia (3, 14, 19). For example, decreases in cerebral oxygenation indexes are thought to reflect reduced CBF, hemoglobin concentration, or arterial oxygen saturation (SaO2). Thus it was hypothesized that cerebral oxygenation would decrease in association with reduced CBF under mild +Gz hypergravity. However, to our knowledge, no study has been conducted to evaluate simultaneously changes in CBF and cerebral oxygenation under +Gz hypergravity. Therefore, to test this hypothesis, we evaluated changes in regional cerebral oxygen saturation as measured by NIRS (C-rSO2) during 21 min of +1.5 Gz centrifugation and compared the results with those in CBF velocity as measured by TCD.

METHODS

Participants. The entire study protocol was approved by the Institutional Review Board of Nihon University School of Medicine (No. 29–2–0; 4 July 2017) and registered in the University Hospital Medical Information Network (UMIN) clinical trial registry (ID: UMIN000028466). The study procedures adhered to the tenets of the Declaration of Helsinki. In total, 17 healthy male volunteers who had no prior experience with a human centrifuge participated in the study (age 24 ± 1 yr; height 172.6 ± 6.6 cm; weight 69.2 ± 7.7 kg; mean ± SD). All participants provided written informed consent and were screened based on their medical history and a physical exami-
nation, including an electrocardiogram and ABP measurement. In addition, in all participants it was confirmed that CBF velocity signals in the middle cerebral artery (MCA) could be obtained by TCD. All participants fasted for ≥2 h before the experiments, and refrained from engaging in heavy exercise or consuming caffeinated or alcoholic beverages for at least 12 h before the experiments.

Equipment. The short-arm human centrifuge (Daiichi Medical, Tokyo) at Nihon University was used in the present study. The experimental room where the centrifuge is located was environmentally controlled at an ambient temperature of 23–25°C. A gimbaled cabin was attached to the end of a rotating arm with a radius of 1.7 m. The participants were seated facing outside the cabin and instructed to minimize their head movement. The top of the cabin reclined toward the center during centrifugation. The resultant force, which was a combination of the Earth’s gravitational force and the centrifugal force, was directed along the participant’s longitudinal (head-to-foot) axis. To limit the visual stimuli and prevent nausea during centrifugation, the cabin door was closed so that the participant could not see outside.

A three-lead electrocardiogram and SaO2 by pulse oximetry (SpO2) were monitored (Life Scope PT, BSM-1763; Nihon Kohden, Tokyo). Regional oxygen saturation (rSO2) was measured using two probes on an NIRS module (INVOS SPS; Coviden, Mansfield, MA), and the data were sent to a Life Scope TR monitor (BSM-6301, Nihon Kohden). One probe was placed on the right side of the forehead to measure C-rSO2 and the other was placed on the left upper arm at heart level to measure peripheral rSO2 (P-rSO2). Partial pressure of expiratory carbon dioxide (CO2) was monitored by an infrared CO2 sensor (OLG-2800; Nihon Kohden). Continuous ABP in the left middle finger was measured, and brachial ABP at heart level was obtained by subtracting the hydrostatic pressure between the finger and the heart using a height sensor (Finometer MIDI; Finapres Medical Systems, Amsterdam, The Netherlands). Continuous CBF velocity in the MCA was measured by TCD with a 2-MHz probe placed over the right temporal window (EZ-Dop; Compumedics Germany, Singen, Germany). The probe was fixed at a constant angle with a probe holder that was individually customized to fit the facial bones and ear structures of each participant just before data measurement.

Commercial software (Notocord-hem 4.3.0.74; Notocord, Paris, France) was used to record waveforms of the electrocardiogram, ABP, CBF velocity, and expiratory CO2 with a 1-kHz sampling rate. The SpO2, C-rSO2, and P-rSO2 data were recorded using the Life Scope BSM-1763 with a 0.3-Hz sampling rate. Dedicated software (BSM PC-Viewer; Nihon Kohden) was used to extract these data.

Protocol. Pre-hypergravity (+1.0 Gz) data were collected from the participants before centrifugation for 6 min after ≥15 min of quiet rest in an upright sitting position in the cabin of centrifuge. The participants were then exposed to mild hypergravity (+1.5 Gz) generated by the centrifuge. The centrifugation was kept at 24.3 rpm for 21 min to generate +1.5 Gz at heart level. The onset and offset rates were +0.5 G/min and −0.1 G/min, respectively. Mild hypergravity (+1.5 Gz) data were collected for 21 min of +1.5 Gz centrifugation. Waveforms of the electrocardiogram, ABP, CBF velocity, and expiratory CO2 were continuously monitored by the doctors during centrifugation. In addition, a charge-coupled device camera was installed in the cabin to monitor the conditions of both the on-board participant and the inside of the cabin. Moreover, the on-board participant and the doctor who was in charge of operating the centrifuge could have a conversation via an intercom. However, participants who completed the scheduled protocol did not have conversations during centrifugation. If any signs and/or symptoms of suspected presyncope, such as nausea, sweating, gray-out, Bradycardia, or hypotension, were observed, the centrifugation was terminated. In the present study, 15 participants completed the scheduled centrifuge protocol, but 2 could not because of the development of strong nausea accompanied by abnormal vital signs.

Data analysis. Mean CBF velocity in the MCA (MCBFV_{MCA}) and mean ABP at heart level (MAP_{heart}) were obtained from each continuous waveform of CBF velocity and ABP on a beat-by-beat basis. The distance between the heart and the position where the TCD probe was placed was measured to calculate the hydrostatic pressure between heart and MCA level. Hydrostatic pressure was estimated as the measured distance (in cm) multiplied by 0.78 mmHg at +1.0 Gz or 1.17 mmHg at +1.5 Gz, assuming that the specific gravity of mercury at 37°C (density 13.5 kg/m³) referenced to 37°C water (density 1000 kg/m³) is 13.6, and the specific gravity of whole blood at 37°C referenced to 37°C water is 1.06 (35). Mean ABP at the MCA level (MAP_{MCA}) was then estimated by subtracting hydrostatic pressure from MAP_{heart}. Heart rate (HR) was calculated on a beat-by-beat basis from the R–R interval obtained from the electrocardiogram continuous waveform. End-tidal CO2 (ET_{CO2}) was obtained from the expiratory CO2 continuous waveform. The SpO2, C-rSO2, and P-rSO2 data were extracted with a 1-Hz sampling rate.

To evaluate the time course of changes in the measured variables, the initial 5 min of 6-min pre-hypergravity (+1.0 Gz) data were used as a pre-hypergravity data segment. Mild hypergravity (+1.5 Gz) data during the 21-min centrifugation period were divided into the following four data segments by 5-min intervals from the point at which centrifugation reached 24.3 rpm (+1.5 Gz at heart level): 0–5 min, 5–10 min, 10–15 min, and 15–20 min. A total of five data segments (a pre-hypergravity data segment and 4 hypergravity data segments) were used for the analysis. Five-minute averages for MCBFV_{MCA}, C-rSO2, P-rSO2, MAP_{heart}, MAP_{MCA}, HR, SpO2, and ET_{CO2} were obtained by averaging data during each 5-min data segment.

Statistical analysis. All statistical analyses were performed using R (The R Foundation for Statistical Computing, Vienna, Austria). Data are shown as means ± SD. Values of P < 0.05 were considered statistically significant. Normality was evaluated by the Kolmogorov-Smirnov test. For the variables with a normal distribution, one-way repeated-measures analysis of variance (ANOVA) was performed with data segment (pre-hypergravity, 0–5 min, 5–10 min, 10–15 min, and 15–20 min) as a factor, followed by Holm’s post hoc test (paired t-test with the P value adjusted by Holm’s method) for multiple comparisons. If the sphericity assumption was violated by Mauchly’s test in the ANOVA, the Greenhouse-Geisser correction was used to adjust the degrees of freedom. Therefore, for the variables for which sphericity were violated, the degrees of freedom (df) was not an integral number. For the variables that were not normally distributed, Friedman tests were performed with data segment as a factor, followed by Holm’s post hoc test (Wilcoxon signed-rank test with the P value adjusted by Holm’s method) for multiple comparisons. These statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan; https://cran.r-project.org/web/packages/RcmdrPlugIn/EZR/), which is a graphical user interface for R (16). To evaluate the relationship between C-rSO2 and MCBFV_{MCA}, the repeated-measures correlation analysis first introduced by Bland and Altman (5) was performed using the rmcorr R package developed by Bakdash and Marusich (https://cran.r-project.org/web/packages/rmcorr/) (4).

RESULTS

For the group averages, data from 15 participants who completed 21 min of exposure (age 24 ± 1 yr; height 172.5 ± 6.8 cm; weight 69.7 ± 7.7 kg; mean ± SD) were used. Table 1 shows the 5-min averages of measured variables in each of the five data segments: one pre-hypergravity and four data segments during +1.5 Gz centrifugation (0–5 min, 5–10 min, 10–15 min, and 15–20 min). A significant main effect of data segment was found in MCBFV_{MCA} [F(1.83, 25.73) = 15.18, P < 0.001 (ANOVA)]. MCBFV_{MCA} tended to decrease from the beginning of centrifugation, but

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MCBFV_{MCA} at 0–5 min did not reach statistical significance compared with pre-hypergravity (−1.2%). Then, MCBFV_{MCA} significantly decreased at 5–10 min (−4.8%), 10–15 min (−6.7%), and 15–20 min (−7.4%). However, no significant difference was found between 10–15 min and 15–20 min. Figure 1 shows the changes in MCBFV_{MCA} for all individual participants who completed the scheduled centrifugation. C-rSO₂ [F(1,82,25.49) = 1.98, P = 0.160 (ANOVA)] showed almost no change (−1.0% at 15–20 min). P-rSO₂ [F(2,27,31.89) = 0.50, P = 0.632 (ANOVA)] did not change significantly throughout centrifugation. Figure 2 shows the repeated-measures correlation between C-rSO₂ and MCBFV_{MCA}. The correlation coefficient (rₐ) value was 0.208 (df = 59, 95% confidence interval [−0.05,0.44], P = 0.106).

MAP_{pa} significantly increased throughout centrifugation compared with pre-hypergravity [χ² = 24.765, df = 4, P < 0.001 (Friedman)]. On the other hand, MAP_{MCA} during centrifugation was lower than that during pre-hypergravity (−9.2% at 0–5 min, −8.6% at 5–10 min, −7.8% at 10–15 min, and −5.7% at 15–20 min), and statistical significance was found at 0–5 min and 5–10 min [χ² = 18.268, df = 4, P = 0.001 (Friedman)]. HR significantly increased throughout centrifugation compared with pre-hypergravity [F(2,20,30.87) = 26.777, P < 0.001 (ANOVA)]. SpO₂ slightly but significantly increased compared with pre-hypergravity at 0–5 min, and returned to pre-hypergravity levels after 5–10 min [F(2.58,36.25) = 10.04, P < 0.001 (ANOVA)]. ETCO₂ significantly decreased compared with pre-hypergravity throughout centrifugation [F(4,56) = 77.48, P < 0.001 (ANOVA)].

Two participants could not complete 21 min of exposure because of strong nausea accompanied by abnormal vital signs. In one case, MAP_{MCA} rapidly decreased without decreases in HR (Fig. 3, case 1). Both MCBFV_{MCA} and C-rSO₂ decreased simultaneously after ~18 min of exposure to +1.5 Gz hypergravity. These decreases (percent change from pre-hypergravity data) during the last 3 min of +1.5 Gz hypergravity were −16.2% and −7.5%, respectively (dotted box in Fig. 3), while those during the last 1 min were −24.3% and −11.2%, respectively. HR remained increased, but showed a sudden drop just before the deceleration (termination of +1.5 Gz exposure). In the other case, although both HR and MAP_{MCA} tended to increase, both MCBFV_{MCA} and ETCO₂ rapidly decreased after ~12 min of exposure to +1.5 Gz hypergravity (Fig. 3, case 2). No obvious change in C-rSO₂ was observed during this period. These cases were carefully monitored, and both recovered a short time after stopping the centrifuge. Neither of the participants had any past medical history or any significantly different background characteristics compared with the other participants.

**DISCUSSION**

The aim of the present study was to test our hypothesis that C-rSO₂ would decrease in association with a reduction in CBF during mild +Gz centrifugation by evaluating simultaneously changes in CBF and cerebral oxygenation. The results showed that MCBFV_{MCA} gradually decreased from the beginning of the +1.5 Gz centrifugation and reached statistical significance after a 5–10 min data segment. On the other hand, no significant change in C-rSO₂ was detected throughout centrifugation. No significant correlation was found between MCBFV_{MCA} and C-rSO₂. Contrary to our hypothesis, the results of the present study suggest that the changes in C-rSO₂ did not precisely reflect the reduction in CBF under mild +Gz hypergravity in the participants who completed the exposure without any symptoms.

Monitoring of cerebral oxygenation as measured by NIRS has been widely used to detect cerebral ischemia, especially during cardiovascular surgery (3, 14, 19). Several assumptions, such as unaltered arteriovenous volume ratio, hemoglobin

### Table 1. Five-minute averages of measured variables before and during +1.5 Gz centrifugation

| +1.0 Gz | +1.5 Gz | P value |
|--------|--------|---------|
|        | 0–5 min| 5–10 min| 10–15 min| 15–20 min|         |
| MCBFV_{MCA}, cm/s | Pre-hypergravity | 51.5 ± 12.5 | 50.3 ± 10.7 | 48.5 ± 10.4**†† | 47.5 ± 9.9***‡‡‡ | <0.001 (A) |
| C-rSO₂, % | 75.9 ± 6.0 | 75.3 ± 5.8 | 74.8 ± 5.2 | 74.8 ± 5.3 | 75.0 ± 5.2 | 0.160 (A) |
| P-rSO₂, % | 79.5 ± 4.6 | 79.6 ± 4.8 | 79.0 ± 4.9 | 79.6 ± 5.2 | 79.6 ± 5.3 | 0.632 (A) |
| MAP_{pa}, mmHg | 79.9 ± 13.9 | 86.3 ± 11.4** | 86.4 ± 10.8* | 86.8 ± 10.4** | 88.0 ± 10.8*** | <0.001 (F) |
| MAP_{MCA}, mmHg | 56.1 ± 14.3 | 50.6 ± 12.1** | 50.8 ± 11.7* | 51.1 ± 11.3 | 52.3 ± 11.6 | 0.001 (F) |
| HR, beats/min | 65.0 ± 9.0 | 71.8 ± 8.6*** | 72.1 ± 9.6*** | 72.6 ± 9.4*** | 73.4 ± 10.0*** | <0.001 (A) |
| SpO₂, % | 98.0 ± 1.0 | 98.7 ± 0.8* | 98.1 ± 0.9†† | 98.0 ± 0.9†† | 98.0 ± 0.9†† | <0.001 (A) |
| ETCO₂, Torr | 39.0 ± 2.5 | 35.4 ± 2.9*** | 34.8 ± 2.8*** | 34.8 ± 2.9*** | 34.4 ± 2.7***† | <0.001 (A) |

Values are means ± SD. Pre-hypergravity: average of pre-hypergravity 5-min sections (+1.0 Gz); 0–5 min, 5–10 min, 10–15 min, and 15–20 min: 5-min averages of the 0–5-min, 5–10-min, 10–15-min, and 15–20-min data segments during +1.5 Gz centrifugation. MCBFV_{MCA}: mean cerebral blood flow velocity in the middle cerebral artery; C-rSO₂: regional cerebral oxygen saturation; P-rSO₂: regional oxygen saturation at heart level (upper arm); MAP_{pa}: mean arterial pressure at heart level; MAP_{MCA}: mean arterial pressure at the middle cerebral artery level; HR: heart rate; SpO₂: peripheral arterial oxygen saturation; ETCO₂: partial pressure of end-tidal carbon dioxide. P values are expressed as one-way repeated-measures analysis of variance with data segment as a factor (A), or Friedman tests with data segment as a factor (F). *P < 0.05, **P < 0.01, ***P < 0.001 (P value of Holm’s post hoc test compared with the +1.0 Gz hypergravity data segment); †P < 0.05, ††P < 0.01, †††P < 0.001 (P value of Holm’s post hoc test compared with the 0–5-min data segment).
concentration, extracranial blood flow, and brain activity (6, 19, 28, 34, 38), need to be satisfied to monitor cerebral ischemia by cerebral oxygenation. Prior to the present experiment, we had assumed that the arteriovenous volume ratio at brain level would not change considerably during centrifugation. The internal jugular vein, as the main venous outflow from the brain, might collapse in the upright sitting position in most study participants (13); therefore, an increase in the gravitational force by \(1.5 \text{ Gz} \) might change venous blood volume minimally in the brain. Also, we had assumed that hemoglobin concentration would not change during centrifugation. We had assumed that the impact of extracranial blood flow would be negligible because the NIRS-based oximetry used in the present study measures oxygen saturation using a fixed arteriovenous volume ratio of 75% venous and 25% arterial (34). Second, hemoglobin concentration might increase because of plasma volume extravasation by the gravitational force (22) under hypergravity. Third, the NIRS-based oximetry used in the present study has been reported to be affected by extracranial contamination (17), so extracranial blood flow might change and affect the C-rSO\(_2\) value during centrifugation. Finally, brain activity might increase under even mild \(1.5 \text{ Gz} \) hypergravity, inducing increases in regional blood flow by vascular dilation to compensate for the increased oxygen demand (37). In fact, Smith et al. (31) reported that in participants who showed symptoms of presyncope, prefrontal cortex activity was increased by psychological stress during centrifugation.

However, contrary to our hypothesis, no significant changes in C-rSO\(_2\) were detected throughout centrifugation, although MCBFV\(_{\text{MCA}}\) decreased significantly. There are several possible mechanisms for explaining the present results of unchanged C-rSO\(_2\) despite the reduced CBF. First, the arteriovenous volume ratio at brain level might change during centrifugation; this may be induced by decreases in cerebral venous blood volume due to the gravitational force during centrifugation. In some participants, an additional 0.5 gravitational force might drain venous blood through the noncollapsed internal jugular vein or the secondary veins of the vertebral, epidural, and deep cervical veins in the sitting position (2, 9) during hypergravity. In addition, the cerebral autoregulation system should dilate arterioles as resistance vessels to maintain CBF, mitigating the reduced arterial pressure in the MCA during some periods of hypergravity, as will be mentioned later. It is possible that the dilation of arterioles leads to a relative increase in the percentage of arterial blood volume despite decreases in CBF. The changes in the arteriovenous ratio should reduce the accuracy of C-rSO\(_2\) monitoring because the NIRS-based oximetry used in the present study measures oxygen saturation using a fixed arteriovenous volume ratio of 75% venous and 25% arterial (34). Second, hemoglobin concentration might increase because of plasma volume extravasation by the gravitational force (22) under hypergravity. Third, the NIRS-based oximetry used in the present study has been reported to be affected by extracranial contamination (17), so extracranial blood flow might change and affect the C-rSO\(_2\) value during centrifugation. Finally, brain activity might increase under even mild \(1.5 \text{ Gz} \) hypergravity, inducing increases in regional blood flow by vascular dilation to compensate for the increased oxygen demand (37). In fact, Smith et al. (31) reported that in participants who showed symptoms of presyncope, prefrontal cortex activity was increased by psychological stress during centrifugation.

Fig. 1. Time course of changes in mean cerebral blood flow velocity in the middle cerebral artery before and during \(1.5 \text{ Gz} \) centrifugation for all participants who completed the study protocol. Pre-hypergravity: average of pre-hypergravity 5-min sections (+1.0 Gz); 0–5 min, 5–10 min, 10–15 min, and 15–20 min: 5-min averages of the 0–5-min, 5–10-min, 10–15-min, and 15–20-min data segments during +1.5 Gz centrifugation. Lines with markers represent the changes in mean cerebral blood flow velocity in the middle cerebral artery during each data segment for each participant (\(n = 15\)). White bars with error bars represent the average values and SDs of the 15 participants.

Fig. 2. Repeated-measures correlation between regional cerebral oxygen saturation (C-rSO\(_2\)) and mean cerebral blood flow velocity in the middle cerebral artery (MCBFV\(_{\text{MCA}}\)). Each marker represents the measured values of each participant, and the total of 75 data points (5 data segments \(\times 15\) participants who completed the task) are shown. Solid lines represent the regression line of each participant. The value of the repeated measures correlation coefficient \(r_{\text{rm}}\) is shown with the \(P\) value.
Thus the assumptions needed to use cerebral oxygenation as measured by NIRS for detecting cerebral ischemia cannot always be satisfied during mild +Gz hypergravity. In fact, some studies have evaluated the changes in CBF by TCD and NIRS simultaneously. For example, good correlations between CBF velocity by TCD and NIRS-derived variables during cardiovascular surgery have been reported (14, 19); however, the response to head-up tilt has been

Fig. 3. Time courses of changes in measured variables for 2 participants whose centrifugation was terminated. Left-side charts show time courses of changes in measured variables for case 1, and right-side charts show those for case 2. From the top, each column shows the time courses of changes in heart rate (HR), mean arterial pressure at the middle cerebral artery level (MAPMCA), mean cerebral blood flow in the middle cerebral artery (MCBFV_MCA), regional cerebral oxygen saturation (C-rSO2), regional peripheral (upper arm at heart level) oxygen saturation (P-rSO2), partial pressure of end-tidal carbon dioxide (ETCO2), and peripheral arterial oxygen saturation (SpO2). Values for HR, MAPMCA, and MCBFV_MCA were plotted on a beat-by-beat basis. Values for C-rSO2, ETCO2, P-rSO2, and SpO2 were plotted with a 1-Hz sampling rate. The dotted box shows the last 3 min of +1.5 Gz hypergravity for case 1. In case 1, the elapsed time of +1.5 Gz centrifugation reached nearly 21 min, but was not fully completed.
controversial. Krakow et al. (21) reported the good followability of oxyhemoglobin and C-rSO2 to MCBFV_{MCA}, but Canova et al. (8) reported finding no correlation between tissue hemoglobin index and MCBFV_{MCA}. Thus the relationship between TCD and NIRS has not always been constant in head-up tilt studies (8, 21), which have used different durations, head-up tilt angles, and participant populations. It is therefore important to consider the details of the study protocol and conditions that minimize the violation of necessary assumptions of NIRS.

Although intermittent and repeated exposure to artificial hypergravity via a human centrifuge has been proposed as a countermeasure against spaceflight-induced physiological deconditioning (7, 10), in the present study, MCBFV_{MCA} was significantly decreased under even mild +Gz hypergravity, which was consistent with our previous reports (15, 20, 24). Hence, the decreases in CBF were thought to be one of the adverse effects during even mild centrifugation, suggesting that careful monitoring of CBF is needed during the exposure. However, several challenges remain for using TCD during centrifugation in a practical rather than an experimental setting, such as the difficulty in the fixation of the TCD probe at a constant angle without some specific methods, or the possibility of an inadequate temporal acoustic window in some individuals (23, 25). We had believed that alternative means would be needed to monitor CBF during centrifugation, and we had expected that the monitoring of cerebral oxygenation as measured by NIRS would be easily utilizable and applicable to everyone. To our knowledge, there have been no reports that reduced CBF during centrifugation can be detected by NIRS during A-LOC by nitric oxide (NO) accompanying the release of acetylcholine in post hoc tests in the present study compared with previous studies. Although both of the incomplete cases showed rapid decreases in MCBFV_{MCA} during the last few minutes before the termination of the study protocol, the mechanisms inducing the decreases in CBF seemed to differ. In one case (Fig. 3, case 1), which was similar to an orthostatic hypotension patient, MAP_{MCA} remarkably decreased during the last few minutes of centrifugation; in association, MCBFV_{MCA} also decreased. Furthermore, HR suddenly dropped just before the termination of exposure to +1.5 Gz, suggesting that the vasovagal reflex was induced. It was thought that this participant almost fell into syncope and the decreases in MCBFV_{MCA} indicated obvious cerebral hypoperfusion. Thus exposure to even mild +Gz hypergravity occasionally involves presyncope with cerebral hypoperfusion. In the other case (Fig. 3, case 2), both MCBFV_{MCA} and \( \text{ET}_{CO2} \) decreased remarkably during the last few minutes of +1.5 Gz centrifugation, suggesting that hyperventilation was induced. It was considered that the nausea and hyperventilation in this participant were induced by vestibular and/or other stimuli; however, we cannot clearly state that these decreases in MCBFV_{MCA} with hyperventilation hypocapnia have similar physiological meaning or significance to the obvious cerebral hypoperfusion with hypotension, such as presyncope. No simultaneous change in C-rSO2 was observed during this period in this participant; thus the relationship between C-rSO2 and CBF velocity under hyperventilated hypocapnia remains unclear.

In contrast to previous studies (15, 20, 24), we selected laymen who had no experience in a centrifuge as participants in the present study to detect physiological changes under mild hypergravity more clearly. As expected, many more data segments and indexes showed statistically significant differences in post hoc tests in the present study compared with previous studies. Although the standard deviation of MCBFV_{MCA} was relatively large, the individual trends of changes in MCBFV_{MCA} for each participant were similar (Fig. 1). The decreasing rates of MCBFV_{MCA} for the 0–5-min, 5–10-min, and 10–15-min data segments were smaller than those of MAP_{MCA}. On the other hand, MAP_{MCA} tended to be restored in the latter half of +1.5 Gz centrifugation, but MCBFV_{MCA} was still decreasing, resulting in the greater decreasing rate of MCBFV_{MCA} \((-7.4\%)\) compared with MAP_{MCA} \((-5.7\%)\) for the 15–20-min data segment. This result suggested that cerebral autoregulation functioned during the early stages of mild +Gz hypergravity, but declined in the last stages of a 21-min centrifugation session in the laymen. However, it was thought that these small decreases in CBF did not lead to any symptoms, including cognitive deficits, in the present study. Moreover, in the present study, two of the participants could not accomplish the scheduled centrifugation protocol. Therefore, the physiological impacts of hypergravity for laymen seemed to be stronger than those for experienced participants, suggesting that the duration of centrifugation and more careful monitoring should be considered for individuals who do not have much experience with centrifuges, even if the magnitude of hypergravity is small.

There were some limitations in the present study. The possibility of changes in MCA diameter is a common limitation for studies using TCD. The changes in CBF were estimated by the changes in CBF velocity in the MCA based on the assumption that the MCA diameter does not change (1, 29). Recent studies have shown both dilation and constriction of the
MCA by high-resolution MRI during higher levels of hypercapnia and hypocapnia, respectively (11, 12, 36). Therefore, the possibility of the constriction of the MCA due to significant decreases in ETCO₂ during centrifugation in the present study could not be ruled out. However, the constriction of the MCA would cause the recorded CBF velocity to underestimate the actual CBF decrease. Thus it was thought that the possibility of changes in MCA diameter would not affect the present results of significant decreases in CBF, at least from the viewpoint of arterial blood gas; however, whether hypergravity would affect the MCA diameter remains unclear. In fact, the possibility that the MCA diameter would dilate under mild +Gz hypergravity also cannot be ruled out. If this dilation occurred, it could lead to normal or even elevated volume flow despite decreases in CBF velocity. In addition, if an increase in local blood volume just under the NIRS electrode occurred, it could also lead to the present result that C-rSO₂ did not change despite decreases in CBF velocity. Moreover, since the relationship between CBF velocity and C-rSO₂ under hyperventilated hypocapnia remains unclear, further study to evaluate this relationship using controlled breathing would be necessary. Another study evaluating the sensibility of C-rSO₂ against changes in true hemoglobin saturation using mild hypoxic gas inhalation would also be useful. Furthermore, the participants in the present study had no experience with centrifuges before the experiment, and were much younger (24 ± 1 yr) than the astronauts recently participating in long-duration spaceflight (48.6 ± 4.7 yr) (26). Therefore, the findings in the present study might not be applicable to recent astronauts and future space travelers.

In conclusion, to test our hypothesis that C-rSO₂ would decrease in association with a reduction in CBF, we evaluated simultaneously C-rSO₂ and MCBFV MCA during +1.5 Gz centrifugation. Contrary to our hypothesis, C-rSO₂ did not change throughout the centrifugation, whereas MCBFV MCA decreased significantly. In addition, no significant correlation was found between MCBFV MCA and C-rSO₂. The present results suggest that the necessary assumptions to monitor cerebral ischemia by cerebral oxygenation may not always be applicable, and cerebral oxygenation as measured by NIRS may not reflect decreases in CBF precisely under mild +Gz hypergravity. Thus measuring changes in CBF by NIRS may not be appropriate for the research setting. On the other hand, if the extent of decreases in CBF preponderate the impact of the violated necessary assumptions for NIRS, cerebral oxygenation might be able to detect decreases in CBF before the development of presyncope under even mild +Gz hypergravity, and therefore, might be useful for monitoring decreased CBF in clinical practice, such as in the field of aerospace medicine.

REFERENCES

1. Aaslid R, Markwalder TM, Nornes H. Noninvasive transcranial Doppler ultrasonic recording of flow velocity in basal cerebral arteries. J Neurosurg 57: 769–774, 1982. doi:10.3171/jns.1982.57.6.0769.
2. Alperin N, Lee SH, Shivaramakrishnan A, Hushek SG. Quantifying the effect of posture on intracranial physiology in humans by MRI flow studies. J Magn Reson Imaging 22: 591–596, 2005. doi:10.1002/jmri.20427.
3. Al-Rawi PG, Kirkpatrick PJ. Tissue oxygen index: thresholds for cerebral ischemia using near-infrared spectroscopy. Stroke 37: 2720–2725, 2006. doi:10.1161/01.STR.0000244807.99073.ac.
4. Baldass JZ, Marusich J.R. Repeated measures correlation. Front Psychol 8: 456, 2017. [Erratum in Front Psychol 10: 1201, 2019.] doi:10.3389/fpsyg.2017.00456.
5. Bland JM, Altman DG. Calculating correlation coefficients with repeated observations: Part I–Correlation within subjects. BMJ 310: 446, 1995. doi:10.1136/bmj.310.6977.446.
6. Brown R, Wright G, Royston D. A comparison of two systems for assessing cerebral venous oxymemoglobin saturation during cardiopulmonary bypass in humans. Anesthesia 43: 697–700, 1993. doi:10.1111/j.1365-2044.1993.tb0184.x.
7. Burton RR. A human-use centrifuge for space stations: proposed ground-based studies. Aviat Space Environ Med 59: 579–582, 1988.
8. Canova D, Roatta S, Bosone D, Micelli G. Inconsistent detection of changes in cerebral blood volume by near infrared spectroscopy in standard clinical tests. J Appl Physiol (1985) 110: 1646–1655, 2011. doi:10.1152/japplphysiol.00003.2011.
9. Ciuti G, Righi D, Forzoni L, Fabbri A, Pignone AM. Differences between internal jugular vein and vertebral vein flow examined in real time with the use of multigate ultrasonic color Doppler. AINR Am J Neurolradol 34: 2000–2004, 2013. doi:10.3174/ainr.A3557.
10. Clément GR, Bukley AP, Paloski WH. Artificial gravity as a countermeasure for mitigating physiological deconditioning during long-duration space missions. Front Syst Neurosci 9: 92, 2015. doi:10.3389/fnsys.2015.00092.
11. Coverdale NS, Gati JS, Opalevych O, Perrotta A, Shoemaker JK. Cerebral blood flow velocity underestimates cerebral blood flow during modest hypercapnia and hypocapnia. J Appl Physiol (1985) 117: 1090–1096, 2014. doi:10.1152/japplphysiol.00285.2014.
12. Coverdale NS, Lalande S, Perrotta A, Shoemaker JK. Noninvasive transcranial Dopp- ler ultrasonography and cerebral oximetry as indicators for shunting in carotid endarterectomy. Anesth Analg 91: 1339–1344, 2000. doi:10.1097/00000539-200012000-00006.
13. Dawson EA, Secher NH, Dalsgaard MK, Ogoh S, Yoshiga CC, González-Alonso J, Steensberg A, Raven PB. Standing up to the challenge of standing: a siphon does not support cerebral blood flow in humans. Am J Physiol Regul Integr Comp Physiol 287: R911–R914, 2004. doi:10.1152/japplphysiol.00196.2004.
14. Grubhofer G, Ploch W, Skolka M, Czerny M, Ehrlich M, Lassnigg A. Comparing Doppler ultrasonography and cerebral oximetry as indicators for shunting in carotid endarterectomy. Age 80: 1339–1344, 2009. doi:10.3389/fphysiol.2013.00456.
15. Kato S, Yoshitani K, Kubota Y, Inatomi Y, Ohnishi Y. Effect of posture and extracranial contamination on results of cerebral oximetry by near-infrared spectroscopy. J Anesth 31: 103–110, 2017. doi:10.1007/s00540-016-2275-1.
16. Kanda Y. Investigation of the freely available easy-to-use software ‘EZR’ for medical statistics. Bone Marrow Transplant 48: 452–458, 2013. doi:10.1038/bmt.2012.244.
17. Kato S, Yoshitani K, Kubota Y, Inatomi Y, Ohnishi Y. Effect of posture and extracranial contamination on results of cerebral oximetry by near-infrared spectroscopy. J Anesth 31: 103–110, 2017. doi:10.1007/s00540-016-2275-1.
18. Kawai Y, Puma SC, Hargens AR, Murthy G, Warkander D, Lundgren CE. Cerebral blood flow velocity and cranial fluid volume decrease during +Gz acceleration. J Gravit Physiol 4: 31–34, 1997.
19. Kirkpatrick PJ, Lam J, Al-Rawi P, Smielewski P, Czosnyka M. Defining thresholds for critical ischemia by using near-infrared spectroscopy in the adult brain. J Neurosurg 89: 389–394, 1998. doi:10.3171/jns.1998.89.3.0389.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

T. Konishi, Y.O., and K.I. conceived and designed research; T. Konishi, T. Kurazumi, T. Kato, C.T., Y.O., and K.I. performed experiments; T. Konishi and K.I. analyzed data; T. Konishi, T. Kurazumi, T. Kato, C.T., Y.O., and K.I. interpreted results of experiments; T. Konishi and K.I. prepared figures; T. Konishi and K.I. drafted manuscript; T. Konishi, T. Kurazumi, T. Kato, C.T., Y.O., and K.I. edited and revised manuscript; T. Konishi, T. Kurazumi, T. Kato, C.T., Y.O., and K.I. approved final version of manuscript.

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20. Konishi T, Kurazumi T, Kato T, Takko C, Ogawa Y, Iwasaki KI. Time-dependent changes in cerebral blood flow and arterial pressure during mild +Gz hypergravity. *Aerospace Med Hum Perform* 89: 787–791, 2018. doi:10.3357/AMHP.5106.2018.

21. Krakow K, Ries S, Daﬀertshofer M, Hennerici M. Simultaneous assessment of brain tissue oxygenation and cerebral perfusion during or thostatic stress. *Eur Neurol* 43: 39–46, 2000. doi:10.1159/000008127.

22. László Z, Rössler A, Hinghofer-Szalkay HG. Cardiovascular and hormonal changes with different angles of head-up tilt in men. *Physiol Res* 50: 71–82, 2001.

23. Marinoni M, Ginanneschi A, Forleo P, Amaducci L. Technical limits in transcranial Doppler recording: inadequate acoustic windows. *Ultrasound Med Biol* 23: 1275–1277, 1997. doi:10.1016/S0301-5629(97)00077-X.

24. Ogawa Y, Yanagida R, Ueda K, Aoki K, Iwasaki K. The relationship between widespread changes in gravity and cerebral blood flow. *Environ Health Prev Med* 21: 186–192, 2016. doi:10.1007/s12199-016-0513-7.

25. Postert T, Federlein J, Przuntek H, Bütter T. Insufficient and absent acoustic temporal bone window: potential and limitations of transcranial contrast-enhanced color-coded sonography and contrast-enhanced power-based sonography. *Ultrasound Med Biol* 23: 857–862, 1997. doi:10.1016/S0301-5629(97)00047-1.

26. Roberts DR, Albrecht MH, Collins HR, Aeseman D, Chatterjee AR, Spampinato MV, Zhu X, Chimowitz MI, Antonucci MU. Effects of Spaceﬂight on astronauts’ brain structure as indicated on MRI. *Neurology* 87: 1746–1753, 2017. doi:10.1212/WNL.0000000000004129.

27. Ryoo HC, Sun HH, Shender BS, Hrebien L. Consciousness monitoring using near-infrared spectroscopy (NIRS) during +Gz exposures. *Med Eng Phys* 26: 745–753, 2004. doi:10.1016/j.medengphy.2004.07.003.

28. Samra SK, Stanley JC, Zelenock GB, Dorje P. An assessment of contributions made by extracranial tissues during cerebral oximetry. *J Neurosurg Anesthesiol* 11: 1–5, 1999. doi:10.1097/00005806-199901000-00001.

29. Serrador JM, Picot PA, Rutt BK, Shoemaker JK, Bondar RL. MRI measures of middle cerebral artery diameter in conscious humans during simulated orthostasis. *Stroke* 31: 1672–1678, 2000. doi:10.1161/01.STR.31.7.1672.

30. Shender BS, Forster EM, Hrebien L, Ryoo HC, Cammarota JP Jr. Acceleration-induced near-loss of consciousness: the “A-LOC” syndrome. *Aviat Space Environ Med* 74: 1021–1028, 2003.

31. Smith C, Goswami N, Robinson R, von der Wiesche M, Schneider S. The relationship between brain cortical activity and brain oxygenation in the prefrontal cortex during hypergravity exposure. *J Appl Physiol* (1985) 114: 905–910, 2013. doi:10.1152/japplphysiol.01426.2012.

32. Steppan J, Hogue CW Jr. Cerebral and tissue oximetry. *Best Pract Res Clin Anaesthesiol* 28: 429–439, 2014. doi:10.1016/j.bpa.2014.09.002.

33. Stoll AM. Human tolerance to positive G as determined by the physiological end points. *J Aviat Med* 27: 356–367, 1956.

34. Thavasothy M, Broadhead M, Elwell C, Peters M, Smith M. A comparison of cerebral oxygenation as measured by the NIRS 5000 and the INVOS 5100 Near-Infrared Spectrophotometers. *Anaesthesia* 57: 999–1006, 2002. doi:10.1046/j.1365-2044.2002.02826.x.

35. Trudnowski RJ, Rico CR. Specific gravity of blood and plasma at 4 and 37 °C. *Clin Chem* 20: 615–616, 1974.

36. Verbree J, Bronzwaer A-SGT, Ghariq E, Versluis MJ, Daemen MJAP, van Buchem MA, Dahan A, van Lieshout JJ, van Osch MJP. Assessment of middle cerebral artery diameter during hypocapnia and hypercapnia in humans using ultra-high-field MRI. *J Appl Physiol* (1985) 117: 1084–1089, 2014. doi:10.1152/japplphysiol.00651.2014.

37. Villringer A, Planck J, Hock C, Schleinkofer L, Dirschl U. Near infrared spectroscopy (NIRS): a new tool to study hemodynamic changes during activation of brain function in human adults. *Neurosci Lett* 154: 101–104, 1993. doi:10.1016/0304-3940(93)90181-J.

38. Watzman HM, Kurth CD, Montenegro LM, Rome J, Steven JM, Nicolson SC. Arterial and venous contributions to near-infrared cerebral oximetry. *Anaesthesia* 93: 947–953, 2000. doi:10.1093/00000542-200010000-000012.

39. Wong BJ, Hollowed CG. Current concepts of active vasodilation in human skin. *Temperature (Austin)* 4: 41–59, 2016. doi:10.1080/23328940.2016.1200203.