Reduced regional cerebral blood flow measured by 99mTc-HMPAO SPECT in microgravity simulated by 5-day dry immersion

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Research Article

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Abstract

Purpose Neuro-ophthalmological changes defined as spaceflight-associated neuro-ocular syndrome have been reported after long duration space flights. The pathophysiology of this syndrome remains unclear, with the possible involvement of elevated intracranial pressure. Changes in blood flow in the brain, evaluated indirectly by Doppler, have been reported in flight. However, the effects of microgravity on regional cerebral blood flow (rCBF) are not known. We therefore investigated changes in rCBF in a 5-day dry immersion (DI) model. Moreover, we tested thigh cuffs as a countermeasure to prevent potential microgravity-induced modifications in rCBF.

Methods 18 healthy male participants underwent 5-day DI with or without a thigh cuffs countermeasure. They were randomly allocated to a control ($n = 9$) or cuffs ($n = 9$) group. rCBF was measured 4 days before DI (Pre-DI) and at the end of the fifth day of DI (Post-DI), using single-photon emission computed tomography (SPECT) with radiopharmaceutical ^{99m}Tc-hexamethyl propylene amine oxime (HMPAO). SPECT images were processed using statistical parametric mapping (SPM12) software.

Results At DI5, we observed a significant decrease in rCBF in 32 cortical and subcortical patterns, with greater hypoperfusion in the occipital region (occipital peak level: $z = 4.51$, $p_{uncorr} < 0.001$) and basal ganglia (putamen peak level: $z = 4.71$, $p_{uncorr} < 0.001$; caudate nuclei peak level: $z = 3.80$, $p_{uncorr} < 0.001$). No significant difference was found between the control and cuffs groups on variations in rCBF at DI5.

Conclusion 5-day DI induces a relative decrease in rCBF in cortical and subcortical regions. Nevertheless, the consequences of this decrease for brain function and mechanisms need further investigation.

Introduction

Exposure to microgravity has detrimental effects on human physiology, such as muscle atrophy, bone demineralization, sensorimotor and cardiovascular deconditioning, and immune, hormonal and metabolic changes [1, 2]. Body fluid redistribution begins in the first hours of space flight. This so-called cephalad fluid shift is responsible for cephalic venous stasis, characterized by dilation of the jugular vein and facial oedema. This phenomenon is mainly due to loss of the cranial-to-caudal flow gradient induced by weightlessness [3, 4]. Neuro-ocular symptoms have been observed in astronauts on their return from long-duration spaceflights, such as hyperopia, papillary oedema, choroidal folds, cotton wool spots, and posterior globe flattening. These symptoms were recently defined as spaceflight associated neuro-ocular syndrome [5], and some have also been reported in intracranial idiopathic hypertension [6]. During long-duration spaceflights, the cephalad fluid shift observed in astronauts may increase intracranial pressure (ICP), as suggested by the assessment of optic nerve sheath diameter (ONSD) by ultrasound and MRI [7, 8]. However, these mechanisms are not fully understood.

Indirect assessment of cerebral blood flow (CBF) by transcranial Doppler ultrasound of the middle cerebral artery, has revealed a decrease in cerebral vascular resistance (CVR) and an increase in CBF during the first days of space flight, after which these parameters normalize [3]. Cerebral autoregulation is

the mechanism that maintains CBF relatively constant, despite variations in cerebral perfusion pressure (CPP). Short-term studies have shown that cerebral autoregulation is preserved or even improved in microgravity, whereas long-term studies have found that it is impaired [9]. Nevertheless, the mechanisms behind modifications in CBF, CVR and cerebral autoregulation after exposure to weightlessness have not yet been clearly elucidated. Few studies have measured regional (r) CBF in humans after exposure to simulated microgravity. In a study in head-down bed rest (HDBR), rCBF measured by ^{133}Xe inhalation method had increased at 6 hours, but returned to the baseline state at 72 hours [10]. No study has so far measured rCBF during both spaceflight and microgravity analogues such as dry immersion (DI).

The aim of the present study was to investigate possible changes in rCBF using DI as a microgravity simulation model. A second objective was to test whether thigh cuffs can serve as a countermeasure, limiting any changes in rCBF, by restricting the cephalad fluid shift and potential increase in ICP.

Materials And Methods

Participants

Twenty healthy men were recruited. Two of them withdrew before the 4 days of baseline data collection for reasons unrelated to the protocol. A total of 18 participants were therefore included in the study and randomly allocated to either a control or a cuffs group (9/9 split). All participants were informed about the experimental procedures and gave their written consent. The experimental protocol was conducted in accordance with the standards set by the Declaration of Helsinki and approved by the local ethics committee (CPP Est III: 2 October 2018, no. ID RCB 2018-A01470-55) and French health authorities (ANSM: 13 August 2018). ClinicalTrials.gov identifier: NCT03915457.

General protocol

The present study was part of DI5-CUFFS, an experiment carried out at the MEDES Space Clinic in Toulouse (France) from 19/11/2018 to 23/03/2019. The experimental protocol consisted of 4 days of ambulatory baseline data collection before DI (Pre-DI), 5 days (120 hours) of DI (DI1 to DI5), and 2 days of ambulatory recovery (post DI).

A week before the beginning of the protocol, participants went to MEDES for a Pre-DI thigh muscle biopsy and resting metabolic rate measurement.

Participants randomized to the cuffs group wore the thigh cuffs throughout the 5 days of DI, from 10 am to 6 pm on DI1, and from 8 am to 6 pm on DI2-DI5. Thigh cuffs are elastic strips that are designed to have the same effects on lower-limb distensibility as a counterpressure of about 30 mmHg [11] (**Fig. 1**). Calf plethysmography, performed in the supine position at Pre-DI, was undertaken to adjust the cuffs to each participant. At DI1, thigh cuffs were put on immediately prior to DI onset at 10 am.

The general protocol for DI was implemented according to the methodology described elsewhere [12] (**Fig. 2**). Two participants, one control and one cuffs, underwent DI simultaneously in the same room, in two separate baths (except for two participants, one cuffs and one control, who were each alone in the room). Thermoneutral water temperature was continuously maintained (32.5–33.5 °C). Lights were switched off from 11 pm to 7 am. Daily hygiene, weighing and some specific measurements required exit from the bath. During these out-of-bath periods, participants maintained the -6° head-down position. Total out-of-bath supine time for the 120 h of immersion was 9.7 ± 1.3 hr. On DI1-DI4, out-of-bath time was 1.1 ± 0.6 hr/day. On DI5, out-of-bath time was 5.3 ± 1.1 hr, owing to a muscle biopsy in the right thigh and encephalic and spinal MRI. Otherwise, during DI, participants remained immersed in a half-seated position for all activities and were continuously subjected to video monitoring. Bodyweight, blood pressure, heart rate and tympanic body temperature were measured daily. Water intake was fixed at 35-60 ml/kg/day. Within these limits, water intake throughout the protocol was ad libitum and quantified. The menu for each experimental day was identical for all participants, and dietary intake was individually tailored and controlled during the study. Measurements of heart rate and arterial blood pressure were performed with an automatic device twice a day (morning and evening).

SPECT acquisitions

^{99m}Tc-hexamethyl propylene amine oxime (^{99m}Tc-HMPAO) is a lipophilic radiopharmaceutical used for measuring rCBF. The radio-labelled compound was prepared from a commercial kit (Cerestab^{TM}; GE Healthcare, Norway), mixed with sodium-(^{99m}Tc)-pertechnetate and diluted in a saline solution (0.9% sodium chloride). Four days before DI (Pre-DI), 261 ± 8 MBq of ^{99m}Tc-HMPAO were intravenously administered, within 3 hours of preparation. Before and after the injection, participants were isolated from sensory stimulations. More specifically, for 10 minutes before and after the injection, they lay in a dark and quiet room, wearing earplugs and a sleep mask. The ^{99m}Tc-HMPAO injection at Pre-DI was conducted in a half-seated position, so that participants were in a similar position to that at Post-DI when, just before the end of DI, 263 ± 10 MBq were injected while participants were immersed in the bath. Both injections took place in the morning.

SPECT-CT acquisitions were performed on a dual-head hybrid camera (SymbiaT6; Siemens Healthcare, Erlangen, Germany) equipped with a low-energy high-resolution collimator. The energy window was 140 keV ± 7.5% (with additional low energy window for scatter correction). Acquisition parameters for SPECT were: 60 projections over 180°, with 30 seconds per projection (matrix: 128 × 128, zoom 1.78). To perform attenuation correction, a brain CT was also acquired with the following parameters: 110 kV, 50 mAs, collimation 6 × 2 mm. Iterative reconstruction was performed with a flash3D algorithm (12 iterations, 8 subsets, 8-mm Gaussian filter). Images with scatter and CT-attenuation corrections were then generated. Any decrease in radioactivity was corrected during analysis with statistical parametric mapping (SPM12) software, by applying a weighting factor depending on the radioactivity period of ^{99m}Tc for each acquisition.

Statistical analysis

SPECT images were processed using SPM12 software [13], implemented in MATLAB (MathWorks, Sherborn, MA). SPM (statistical parametric mapping) combines the general linear model and theoretical Gaussian fields to make statistical inferences about regional effects. All SPECT images were realigned and normalized to a standard template in MNI space (Montreal neurological institute) using SPM12 [14], then smoothed with a Gaussian kernel filter of 8 mm at full width and half maximum. We compared rCBF at Pre-DI and at Post-DI for each group using a paired *t* test. We also compared the variations in rCBF during DI between the cuffs and control groups, using a two-sample *t* test. We tested the null hypothesis that the voxel to voxel contrast is zero. We chose an uncorrected threshold $p_{uncorr} < 0.001$ with an extended threshold of 100 voxels. General haemodynamic parameters (heart rate, systolic, diastolic and mean arterial blood pressure) were expressed as mean ± *SD*. A paired Student *t* test was used for comparisons of data between Pre-DI and Post-DI for each group. An unpaired Student *t* test was performed to compare data between groups (cuffs vs. control). Differences were considered statistically significant when $p < 0.05$.

Results

Baseline group characteristics are detailed in **Table 1**. There were no significant differences between the two groups (cuffs and control) at baseline.

rCBF was significantly reduced in cortical and subcortical regions at Post-DI, compared with Pre-DI, with a significance threshold of $p_{uncorr} < 0.001$ and an extended threshold of 100 voxels. 32 cortical and subcortical patterns that were significantly less perfused at Post-DI than at Pre-DI were individualized, the decrease in rCBF being greater in bilateral occipital regions (occipital peak level: $z = 4.51$, $p_{uncorr} < 0.001$) and basal ganglia (putamen peak level: $z = 4.71$, $p_{uncorr} < 0.001$; caudate nuclei peak level: $z = 3.80$, $p_{uncorr} < 0.001$) (**Table 2, Fig. 3**).

There was no significant difference in the variation in rCBF at Post-DI compared with Pre-DI between the cuffs and control groups ($p_{uncorr} < 0.001$ and extended threshold of 100 voxels).

At post-DI, two participants presented a frank hypersignal on the SPECT images, located in the left thalamus. This hypersignal was not present in the images at pre-DI. Analysis of the images of the 18 participants using SPM12 software did not reveal any increase in thalamic blood flow at post-DI compared with pre-DI, for $p_{uncorr} < 0.001$ and an extended threshold of 100 voxels (**Fig. 4**).

There was no significant difference in blood pressure and heart rate between the cuffs and control groups at Post-DI compared with Pre-DI. Moreover, there was no significant variation in these parameters between the measurements made at Pre-DI and at Post-DI for each group (**Table 3**).

Discussion

After 5 days of DI, we observed a significant decrease in rCBF in 32 cortical and subcortical patterns. No previous study had previously measured rCBF in humans after microgravity simulation by DI. A study in HDBR measuring rCBF with the ^{133}Xe inhalation method found an initial increase at 6 hours, but no difference at 72 hours [10]. Some studies measuring rCBF have been performed in animals. In a 2-week head-down tail suspension study performed in rats, Wilkerson et al. demonstrated a decrease in rCBF in 21 cortical and subcortical patterns, measured with [^{14}C]-IPIA autoradiography, the decrease being more intense in the basal ganglia [15]. In our study, we found a greater alteration in CBF in the basal ganglia and occipital cortex. The basal ganglia interact with the cortex in a system of cortico-subcortical loops, in order to integrate cortical information and relay it to the cortex via the thalamus and brainstem [16]. As they form the hub of information processing in the brain, these regions may be more intensely affected by variations in CBF. Another explanation concerns the potential modification in neurotransmitter metabolism. Until now, to the best of our knowledge, there has not been any research on neurotransmitter metabolism in humans in microgravity. In a study with rats, a change in the binding of neurotransmitters to their receptors was noted after 7 days on board Spacelab 3; 5-HT1 receptors were more numerous, and binding of dopamine D-2 in the striatum was decreased [17]. We measured an alteration in rCBF in some regions involved in the control of movements and equilibrium, in sensorimotor, vegetative, cognitive, and limbic functions. These functions have also been found to be impaired during microgravity exposure [18-21]. However, because of the semi-quantitative measurement of rCBF with ^{99m}Tc-HMPAO, it is not possible to determine whether the decrease in rCBF was sufficient to induce or be a consequence of brain function impairments. Further studies are needed to explore modifications in CBF and their relation to these impairments.

As the cranial box is rigid and inextensible, and intracranial content is not compressible, ICP depends on three parameters: craniospinal elastance, resistance to cerebrospinal fluid flow, and brain blood volume [22]. Although ICP has never been directly measured during long exposure to microgravity in humans, indirect evaluation methods (measurement of ONSD) suggest an increase in ICP favoured by the cephalad fluid shift [8, 23]. However, the magnitude of a possible increase in ICP during space flights and its precise underlying mechanisms remain unclear.

CPP is the result of mean arterial pressure (MAP) and ICP, according to the equation CPP = MAP - ICP [24]. Studies have shown that MAP does not seem to vary significantly in studies in HDBR [3] and after 3-day DI [25], consistent with our finding that blood pressure remained unchanged.

According to Poiseuille's law, CVR depends on cerebral vessel diameter. CBF depends on CPP and CVR, according to the equation CBF = CPP / CVR [22]. Cerebral autoregulation is the process of maintaining CBF relatively constant for CPP ranging from 50 to 150 mmHg. Above these limits, CBF varies proportionally to CPP [22]. Cerebral autoregulation is mainly mediated by small arteries that modify their diameter according to the variation in CPP, in order to maintain constant CBF [26]. There is no direct measurement of ICP in microgravity in humans. However, direct measurements performed in animals [27] and indirect measurements in humans [8, 28] argue in favour of a moderate elevation of ICP. During 3-day DI, Kermorgant et al. showed an increase in ONSD of about 30%, as measured with ultrasound (Pre-DI:

4.64 ± 0.40 mm; DI3: 6.01 ± 0.49 mm; $p < 0.001$) [28]. These ONSD values are equivalent to an elevation of ICP around 20 mmHg, the normal range being between 7 and 15 mmHg [29]. It therefore seems unlikely that a moderate elevation in ICP during DI would exceed the adjustment capacities of CPP. Indeed, cerebral autoregulation has been shown to be preserved or even improved in short-term studies [9]. Nevertheless, according studies in rats, an increase in ICP may increase CVR through compression of the cerebral blood vessels [30].

During HDBR studies, Doppler measurements show an increase in CVR and a decrease in CBF during the first week, after which these parameters returned to baseline values [3, 31, 32]. After 3-day DI, Ogoh et al. failed to observe any variation in CBF as measured by Doppler ultrasound. However, they did observe an increase in CVR [25]. Compared with the literature, our results showing a decrease in CBF after 5-day DI are consistent with the increase in CVR measured during the first week in simulated microgravity. According to studies performed in head-down tail suspension (HDT) in rats, the increase in CVR could be a consequence of prolonged vasoconstriction in the first days, in response to the increased blood flow in the brain, due to the HDT position. After several days, the chronic vasoconstriction induces hypertrophy and modifications in the wall of cerebral arteries [30]. Researchers have found hypertrophy in the media layer, an increase in thickness, an increase in spontaneous tone, and myogenic vasoconstriction of brain arteries mediated by altered secretion of endothelial NO [15, 33, 34]. According to the authors, the prolonged vasoconstriction and these histological changes could be responsible for an increase in CVR, thus contributing to the decrease in CBF [15]. During DI5-CUFFS, Robin et al. observed a decrease in plasma volume across the 5 days [35]. Likewise, during 3-day DI, Ogoh et al. demonstrated a correlation between the decrease in plasma volume and the decrease in blood flow velocity and conductance in the internal carotid artery [25], suggesting that the loss of plasma volume also contributes to the vasoconstriction of cerebral arteries. In our study, we hypothesized that the decrease in rCBF after 5-day DI is the consequence of three mechanisms that all contribute to the increase in CVR: vasoconstriction of cerebral arteries in response to increased CBF induced by the cephalad fluid shift; the decrease in plasma volume; and a moderate increase in ICP, which may contribute to the increase in CVR through compression of cerebral blood vessels.

We did not find any significant variation in rCBF after 5-day DI between the cuffs and control groups. We hypothesized that, by limiting the cephalad fluid shift and its consequences, thigh cuffs limit the increase in CVR. During 5-day DI, Arbeille et al. [36] found a significantly attenuated increase in volume in the right jugular vein (measured with ultrasound) at 2 hours post-immersion in the cuffs groups. However, at DI4, there was no longer any significant difference between the control and cuffs group. Moreover, the right jugular vein was less dilated than it had been 2 hours post-immersion. Therefore, thigh cuffs seem to be effective in limiting the dilatation of the jugular vein in the first few hours of DI, but their effectiveness seems to diminish after a few days of DI. Studies suggest that thigh cuffs have an effect on the cephalad fluid shift and its consequences only when they are worn, and that there is no significant memory effect when they are removed at night [37]. It is worth nothing that rCBF was measured in the morning, after a night without thigh cuffs. Therefore, the absence of a significant effect of thigh cuffs on the modification of rCBF in our study has many possible explanations, including a lack of statistical power, the fact that

thigh cuffs appear to have little effect on the cephalad fluid shift after 5-day DI, and the absence of a memory effect on rCBF after a night without thigh cuffs.

We observed a frank hypersignal of the left thalamus in two participants at post-DI that had not been present at pre-DI. During the protocol, participants underwent a muscle biopsy in the right thigh about a week before DI, and a second biopsy at DI5. Although the biopsy was performed under local anaesthesia, pain is often experienced after biopsy, its intensity varying according to the individual [38]. The protocol did not include an assessment of post-biopsy pain, but no specific events were reported during the muscle biopsies. The questionnaires on back pain and general discomfort only elicited significantly higher scores than in the pre-immersion period during the first two days of immersion for both groups [35]. We believe that the most likely explanation for the increased CBF in the left thalamus at post-DI is the sensory stimulus generated by the muscle biopsy in the right thigh, the day before the brain SPECT.

^{99m}Tc-HMPAO is a tried and tested technique for measuring rCBF [39], but it is a SPECT tracer, which has lower spatial resolution than a PET tracer (e.g., ^{18}F-FDG, $O^{15}H_2$). The choice of this tracer was ideal for our study, as ^{99m}Tc-HMPAO reaches its binding peak 2-3 minutes after being injected. This allowed us, by injecting participants at the end of DI in the bath, to image rCBF while they were still immersed. After the end of DI, participants underwent a lower-body negative pressure test at the MEDES clinic after the injection, and then went to the nuclear medicine department for the SPECT scans. On account of the study design, the interval between the injection and scans was different at Pre-DI than at Post-DI: scans began 20 minutes after injection at Pre-DI, and after 90 min at Post-DI. Because of the irreversible brain binding of ^{99m}Tc-HMPAO after its injection, we were able to make the acquisitions comparable, by correcting for the radioactive decay of ^{99m}Tc by applying a weighting factor for each image in the analysis with SPM12 software.

Our study had several limitations; there was the small sample size (N = 18), which may have weakened the statistical power of our results. This could explain why we did not find a significant result by applying an alpha risk adjustment with the familywise error rate or false discovery rate. However, we did adjust the alpha risk and applied a good extent threshold that made our results more robust. For radioprotection reasons, we injected our volunteers with a less active radiotracer (261 ± 8 MBq), but increased the acquisition time to 30 minutes to limit the deterioration of the signal-to-noise ratio. Moreover, DI immersion is a model of microgravity with a particular environment for the subject, such as physical immobility, that could also influence the decrease in cerebral perfusion.

Conclusion

Our experiment showed that a 5-day DI induces a decrease in rCBF in cortical and subcortical regions. Prolonged vasoconstriction of cerebral arteries in response to increased CBF resulting from the cephalad fluid shift and a moderate increase in ICP may contribute to the increase in CVR, thus inducing a decrease in rCBF. As possible consequences for brain function were not investigated in our study, further studies are needed to better understand the effects and consequences of microgravity on CBF.

Abbreviations

CBF: cerebral blood flow

rCBF: regional cerebral blood flow

CPP: cerebral perfusion pressure

CVR: cerebral vascular resistance

DI: dry immersion

HDBR: head-down bed rest

HDT: head-down tail suspension

HMPAO: hexamethyl propylene amine oxime

ICP: intracranial pressure

MAP: mean arterial pressure

MNI: Montreal neurological institute

ONSD: optic nerve sheath diameter

SPM: statistical parametric mapping

Declarations

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Conflicts of interest/Competing interests: none.

Availability of data and material : on demand if needed.

Code availability: version 9.3 (R2017b) of the MATLAB Runtime and SPM12.

Authors' contributions : Bareille, Beck, Pavy-Le Traon and Payoux conceived and designed the study. Guillon, Bareille, Cassol, Beck, Beaurain, Lotterie, Pavy-Le Traon and Payoux took part at the experimentation. Guillon analysed the data. Guillon, Kermorgant, Cassol, Pavy-Le Traon and Payoux drafted the manuscript. All authors interpreted results and revised the manuscript.

Ethics approval: the experimental protocol was conducted in accordance with the standards set by the Declaration of Helsinki and approved by the local ethics committee (CPP Est III: 2 October 2018, no. ID

RCB 2018-A01470-55) and French health authorities (ANSM: 13 August 2018).

Consent to participate: all participants were informed about the experimental procedures and gave their written consent.

Consent for publication: all authors gave the consent for publication.

Clinical Trial Registration: ClinicalTrials.gov Identifier: NCT03915457. Registered 16 April 2019, retrospectively registered.

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Tables

	All (*N*=18)	Control (*n*=9)	Cuffs (*n*=9)	Unpaired *t* test Control vs. Cuffs
Pre-DI Age (years)	34.0±5.5	33.9±7.1	34.1±3.7	*p*=0.93
Right-handed	16	8	8	*p*=1.00
Height at selection (cm)	178±6	176±6	180±4	*p*=0.08
Pre-DI Weight (kg)	74.1±8.0	73.9±7.5	74.3±8.8	*p*=0.91
Pre-DI BMI (kg/m^2)	23.3±1.8	23.9±1.7	22.7±1.8	*p*=0.16
Pre-DI VO_2 max (ml/min/kg)	46.7±6.9	46.5±8.1	46.9±5.8	p=0.91
Pre-DI morning T (°C)	36.4±0.4	36.4±0.3	36.4±0.5	p=0.71

Table 1. Mean (standard deviation) baseline group characteristics at Pre-DI

	z_{score} peak level	t_{score} peak level	Number of voxels in cluster
Basal ganglia			
Left caudate	3.39	4.13	680
Left putamen	4.09	5.46	680
Right caudate	3.80	4.88	3213
Right putamen	4,71	6.92	3213
Brainstem			
Left midbrain	4.16	5.61	1006
Right midbrain	*ns*		
Cerebellum			
Left cerebellum	3.78	4.82	120
Right cerebellum	3.93	5.12	551
Cortex			
Cingulate			
Left middle cingulate gyrus	*ns*		
Left anterior cingulate gyrus	*ns*		
Left posterior cingulate gyrus	*ns*		
Right middle cingulate gyrus	3.55	4.40	105
Right anterior cingulate gyrus	3.47	4.27	269
Right posterior cingulate gyrus	3.97	5.20	130
Frontal			
Left superior frontal gyrus	*ns*		
Left medial orbital gyrus	3.45	4.24	144
Left middle frontal gyrus	3,72	4,72	100
Left posterior orbital gyrus	3.54	4.40	144
Left superior frontal gyrus medial	*ns*		
Right superior frontal gyrus	3.57	4.44	269
Right medial orbital gyrus	3.42	4.20	3213

Right middle frontal gyrus	*ns*		
Right posterior orbital gyrus	3.92	5.09	3213
Right superior frontal gyrus medial	3.57	4.44	269
Insula			
Left insula	4.09	5.46	680
Right insula	4.10	5.48	3213
Occipital			
Left inferior occipital gyrus	4.35	6.02	6570
Left middle occipital gyrus	4.35	6.02	6570
Left superior occipital gyrus	4.51	6.42	6570
Right inferior occipital gyrus	4.38	6.09	6570
Right middle occipital gyrus	4.38	6.09	6570
Right superior occipital gyrus	4.38	6.09	6570
Parietal			
Left angular gyrus	*ns*		
Left postcentral gyrus	*ns*		
Right angular gyrus	3.17	3.78	157
Right postcentral gyrus	3.74	4.75	157
Temporal			
Left fusiform gyrus	3.61	4.52	121
Left inferior temporal gyrus	4.06	5.40	201
Right fusiform gyrus	*ns*		
Right inferior temporal gyrus	4.07	5.41	308
Thalamus			
Left thalamus	4.46	6.30	1006
Right thalamus	3.67	4.62	1006

Table 2. Negative variation in regional cerebral perfusion at Post-DI compared with Pre-DI in the 18 participants, maximum z_{score}, maximum t_{score}, number of significant voxels per cluster; $p_{uncor} < 0.001$ and extent threshold > 100 voxels.

	All (*N* = 18)	Control (*n* = 9)	Cuffs (*n* = 9)	Unpaired *t* test Control vs. Cuffs
Pre-DI HR (bpm)	58±7	57±6	58±8	*p*=0.60
Pre-DI SBP (mmHg)	116±10	115±11	117±10	*p*=0.29
Pre-DI DBP (mmHg)	68±7	68±5	68±9	*p*=0.92
Pre-DI MBP (mmHg)	86±7	86±5	86±9	*p*=0.89
Post-DI HR (bpm)	58±8	60±7	57±9	*p*=0.38
Post-DI SBP (mmHg)	117±10	120±8	114±10	*p*=0.13
Post-DI DBP (mmHg)	67±8	68±9	66±7	*p*=0.55
Post-DI MBP (mmHg)	85±7	87±7	83±7	*p*=0.20

Table 3. Measurements for heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP) systolic blood pressure (SBP) and mean blood pressure (MBP), at baseline (Pre-DI) and the end of dry immersion (Post-DI). All measurements were performed in the morning. Mean ± standard deviation.

Significance coefficient between the Cuffs and Control groups with unpaired Student *t* test, significance threshold $p < 0.05$. No significant difference was found between control vs. cuffs and between measurements at Pre-DI vs. Post-DI for each group.

Figures

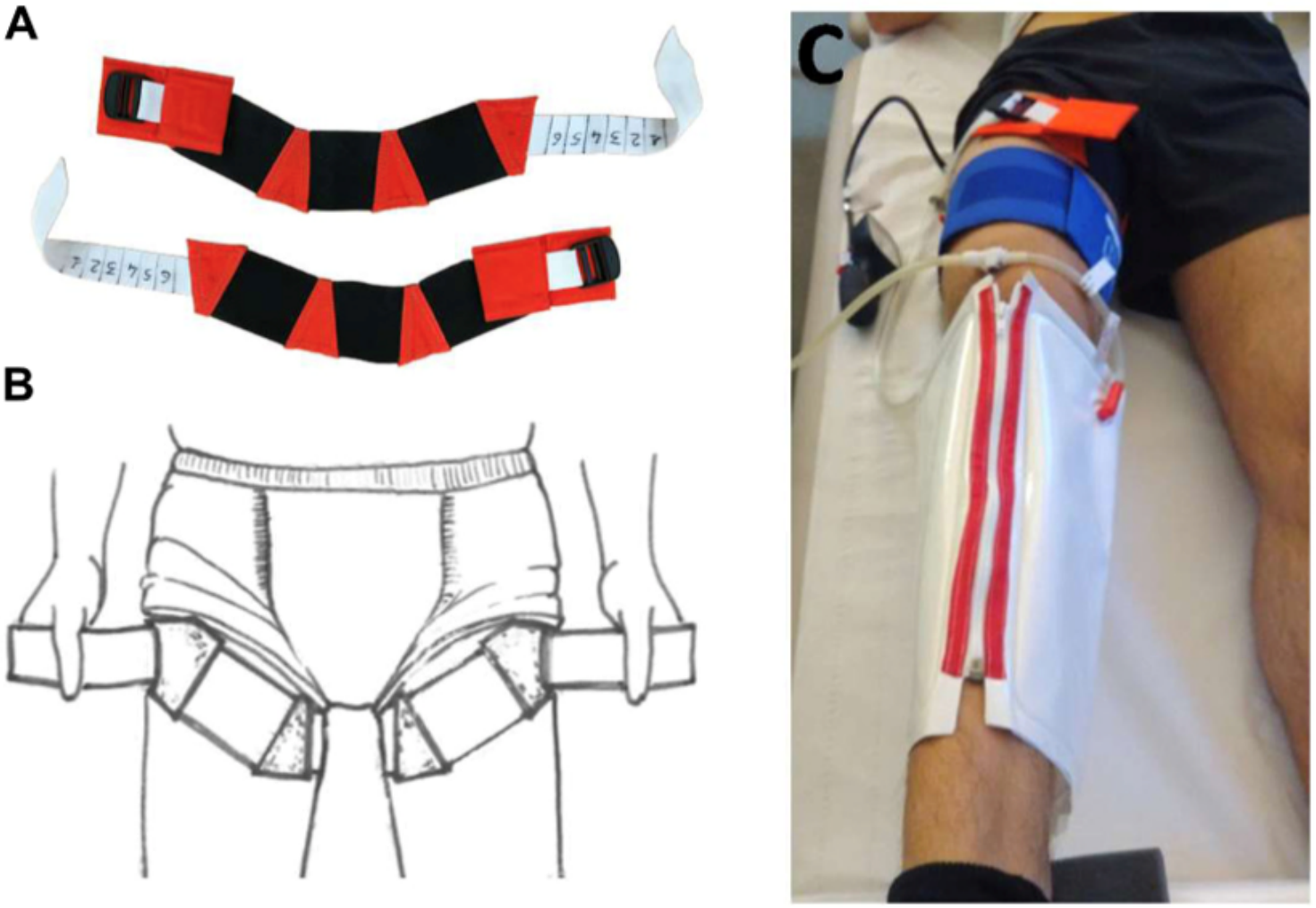

Figure 1

A) Thigh cuffs are elastic strips that can be adjusted to the size of the thigh with a clamping segment (white segment); B) Thigh cuffs are worn on the upper thigh. C) Individual adjustment of thigh cuffs with plethysmography to apply a 30-mmHg pressure on the upper thigh, performed at Pre-DI (Photo MEDES)

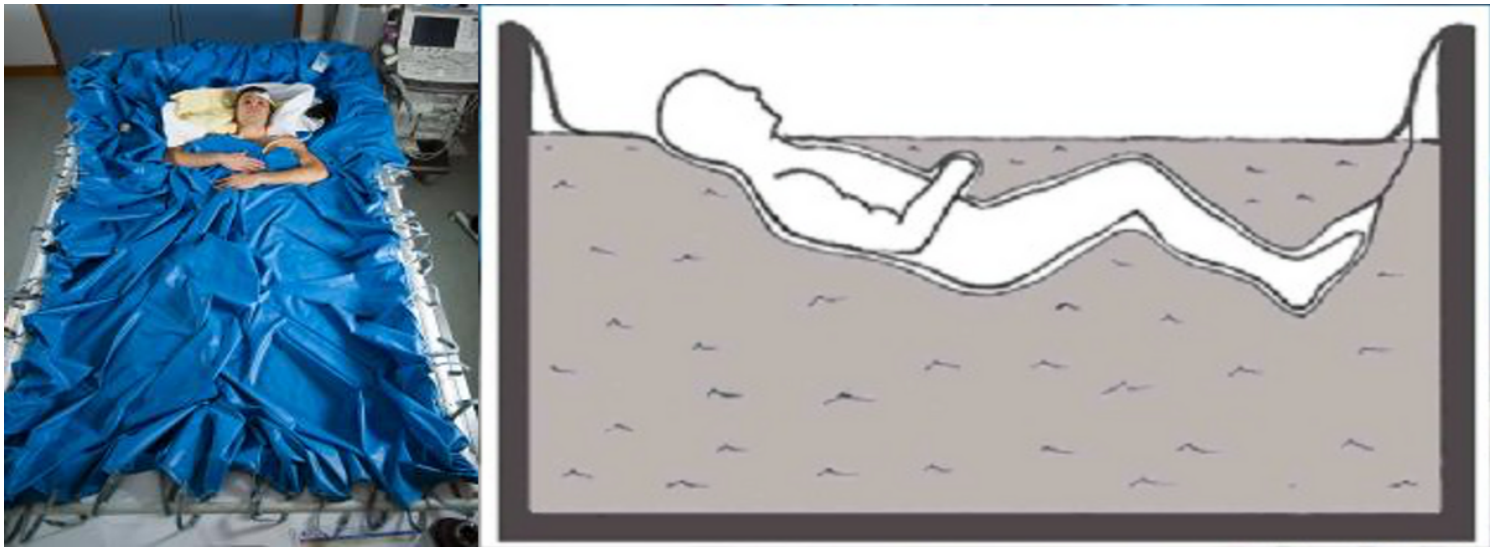

Figure 2

Illustration of dry immersion: participants are immersed in a half-seated position, up to the neck and separated from the water by a waterproof fabric (Photo MEDES/E. GRIMAULT)

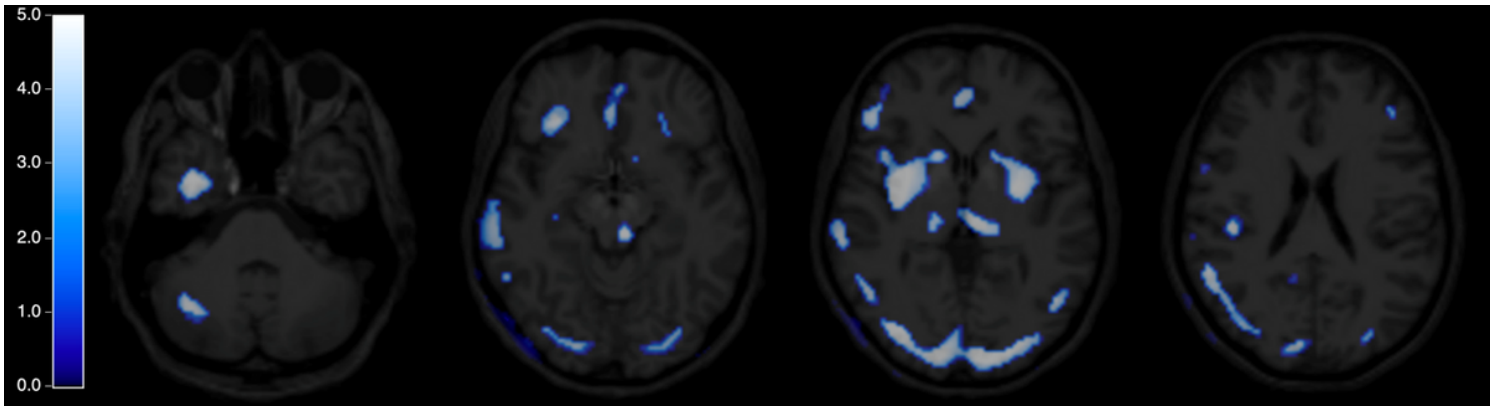

Figure 3

Statistical parametric map (tscore) of the negative variation in regional cerebral perfusion at Post-DI compared with Pre-DI, paired t test, puncorr < 0.001, extent threshold > 100 voxels.

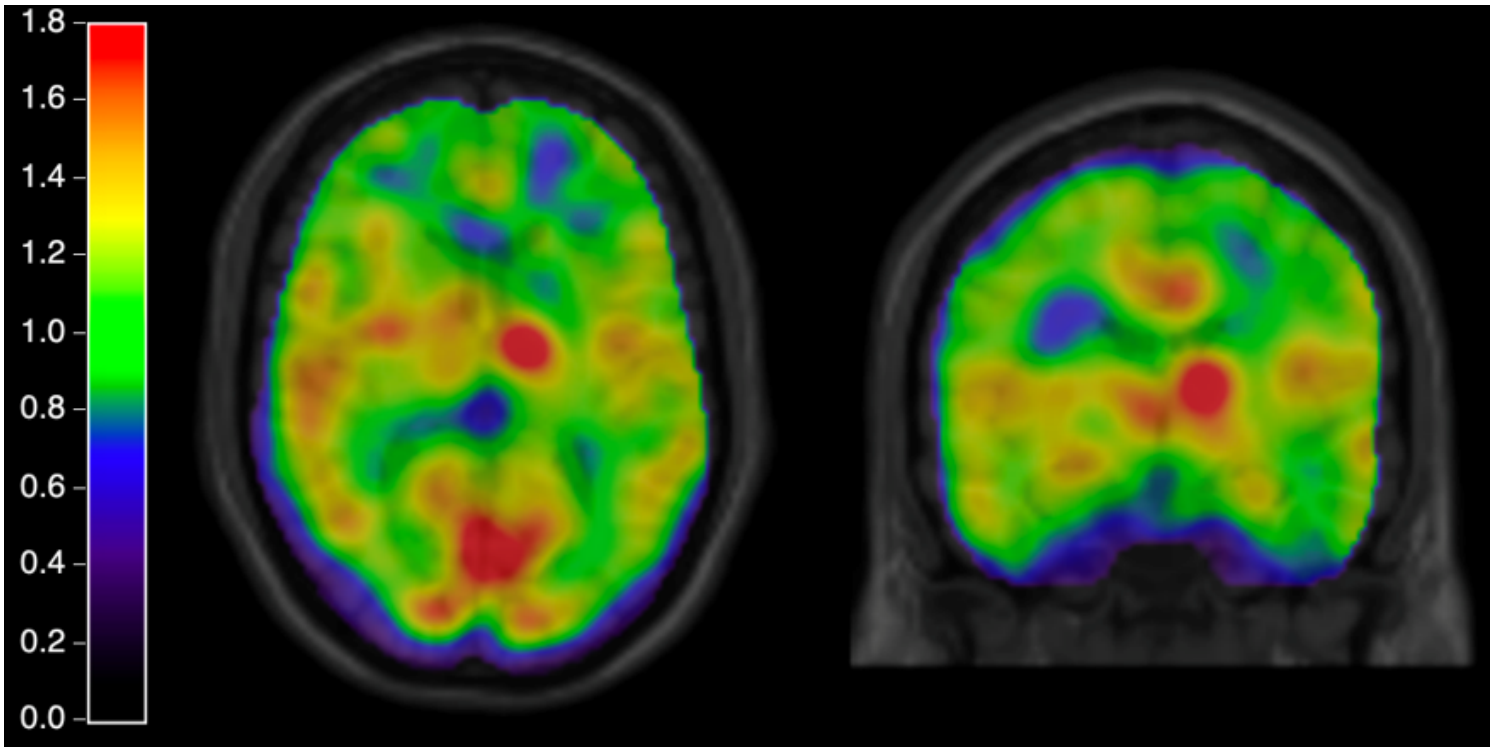

Figure 4

Hypersignal in the left thalamus in one participant at post-DI. Normalized SUVr images of the occipital cortex.