Cerebral critical closing pressure and CO₂ responses during the progression toward syncope

K. A. Zuj,1 P. Arbeille,3 J. K. Shoemaker,2 and R. L. Hughson1

1University of Waterloo, Waterloo, Ontario, Canada; 2University of Western Ontario, London, Ontario, Canada; and 3Univ. Hopital Trousseau, Tours, France

Submitted 1 October 2012; accepted in final form 22 January 2013

Zuj KA, Arbeille P, Shoemaker JK, Hughson RL. Cerebral critical closing pressure and CO₂ responses during the progression toward syncope. J Appl Physiol 114: 801–807, 2013. First published January 31, 2013; doi:10.1152/japplphysiol.01181.2012.—Syncope from sustained orthostasis results from cerebral hypoperfusion associated with reductions in arterial pressure at the level of the brain (BP MCA) and reductions in arterial CO₂ as reflected by end-tidal values (PET CO₂). It was hypothesized that reductions in PET CO₂ increase cerebrovascular tone before a drop in BP MCA that ultimately leads to syncope. Twelve men (21–42 yr of age) completed an orthostatic tolerance test consisting of head-up tilt and progressive lower body negative pressure to presyncope, before and after completing 5 days of continuous head-down bed rest (HDBR). Cerebral blood velocity (CBFV), BP MCA, and PET CO₂ were continuously recorded throughout the test. Cerebrovascular indicators, cerebrovascular resistance, critical closing pressure (CrCP), and resistance area product (RAP), were calculated. Comparing from supine baseline to 6–10 min after the start of tilt, there were reductions in CBFV, PET CO₂, BP MCA, and CrCP, an increase in RAP, and no change in cerebrovascular resistance index. Over the final 15 min before syncope in the pre-HDBR tests, CBFV and CrCP were significantly related to changes in PET CO₂ (r = 0.69 ± 0.17 and r = 0.63 ± 0.20, respectively), and BP MCA, which was not reduced until the last minute of the test, was correlated with a reduction in RAP (r = 0.91 ± 0.09). Post-HDBR, tilt tolerance was markedly reduced, and changes in CBFV were dominated by a greater reduction in BP MCA with no relationships to PET CO₂. Therefore, pre-HDBR, changes in PET CO₂, with orthostasis contributed to increases in cerebrovascular tone and reductions in CBFV during the progression toward syncope, whereas, after 5 days of HDBR, orthostatic responses were dominated by changes in BP MCA.

cerebral blood flow; head-up tilt; Doppler ultrasound; head-down bed rest

SYNCOPE OCCURRING WITH PROLONGED orthostasis results from a reduction in cerebral perfusion and is normally associated with a large, rapid reduction in arterial blood pressure at the level of the middle cerebral artery (BP MCA) (5, 8, 18, 19). A reduction in cerebral blood flow velocity (CBFV) with the progression toward syncope has been attributed by some researchers to a reduction in the partial pressure of arterial carbon dioxide (PACO₂) (5, 6, 18, 21). However, other work has suggested a poor relationship between PACO₂ and changes in CBFV, at least during the early phase of orthostasis (13, 34). Thus the mechanisms underlying reduced CBFV in the approach to syncope have not been clearly identified.

Assessment of cerebrovascular hemodynamics frequently relies on measurement of CBFV and calculation of cerebrovascular resistance or conductance, but these measures may not fully explain alterations in cerebrovascular properties. The calculations of critical closing pressure (CrCP) and resistance area product (RAP) have been used to further describe cerebral hemodynamic changes with exercise (23), alterations in end-tidal PCO₂ (PET CO₂) (1, 25, 26), and neurovascular coupling with cognitive and motor tasks (27). Carey et al. (5) showed changes in both CrCP and RAP with approaching syncope, but did not relate these changes directly to alterations in PET CO₂ or BP MCA. Further analysis of CrCP and RAP may help to better describe cerebrovascular hemodynamic responses to orthostasis, since CrCP reflects changes in cerebrovascular tone, while RAP, as an index of cerebrovascular resistance (CVRi), does not make the assumption that cerebral perfusion pressure gradient is referenced to 0 mmHg (9, 25). Recently, Panerai et al. (27) advanced the hypotheses that RAP is related to myogenic properties of the cerebrovascular system, while CrCP reflects metabolism and cerebrovascular reactivity to CO₂.

Cardiovascular deconditioning from exposure to real or simulated microgravity impairs orthostatic tolerance (2, 4, 40). The potential contributions of change in PET CO₂ and the cerebrovascular response to CO₂ have not been investigated with respect to this reduction in tolerance. Animal models provided a basis to suspect change, as the nitric oxide system that has been linked to cerebrovascular CO₂ reactivity (16, 17, 32) is downregulated with simulated microgravity (20, 29, 38). Recent observations of reduced cerebrovascular CO₂ reactivity in astronauts returning from long-duration spaceflight (41) further suggest that these factors should be investigated following head-down bed rest (HDBR). To date, no studies have been conducted examining the relationship between blood pressure, PET CO₂, cerebral blood flow, and cerebrovascular tone with the progression toward syncope before and after exposure to simulated microgravity.

The following study provided the opportunity to examine cerebrovascular responses to head-up tilt sustained to presyncope, both before and after exposure to simulated microgravity, with particular emphasis on the influences of changes in arterial pressure and PET CO₂. It was hypothesized that, with the progression toward syncope, there would be a reduction in CBFV related primarily to the change in PET CO₂ and not BP MCA and that the reduction in CBFV would be associated with an increase in CrCP, indicating increased cerebrovascular tone. Post-HDBR, it was hypothesized that these relationships with the progression toward syncope would remain consistent, but changes would occur sooner after tilt due to a reduction in orthostatic tolerance. Additionally, it was hypothesized that the initial tilt responses would show greater changes post-HDBR, also related to a reduction in orthostatic tolerance.
METHODS

Experimental protocol. Twelve healthy men (21–42 yr of age) were tested before and after 5 days of strict, continuous 6° HDBR. All experimental procedures were approved by the Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale, Midi-Pyrenees (France) and local ethics committees, including the Office of Research Ethics, University of Waterloo. The entire protocol was in accordance with the declaration of Helsinki. Each subject signed a consent form after receiving full disclosure of the experimental protocol and was aware of his right to withdraw from the study for any reason without prejudice.

All subjects completed a medical screening before entry into the study to ensure normal health with no contraindications, including, but not limited to, orthostatic intolerance, chronic back pain, and elevated blood pressure. The baseline characteristics of the subjects included body mass (75 ± 8 kg), height (178 ± 8 cm), and maximal oxygen uptake (39.3 ± 6.5 ml kg⁻¹ min⁻¹). Subjects were considered to be recreationally active; none was in athletic training.

Orthostatic tolerance, tilt testing was conducted 2 days before the start of HDBR (pre-HDBR) and as the first transition to an upright posture after the 5 days of HDBR (post-HDBR). Following instrumentation (~15 min in the supine position), baseline data were collected for 5 min in the supine position. Participants were then passively tilted to an 80° head-up position, where they were required to stand quietly for 30 min. If signs of orthostatic intolerance did not occur within this time (for 10 of 12 subjects in pre-HDBR), lower body negative pressure (LBNP) was progressively applied with step decreases of −10 mmHg every 3 min. Testing continued until one or more of the following test termination criteria were met: systolic blood pressure <70 mmHg, a sudden drop in heart rate (>15 beats/min), severe light headedness, nausea, or the request of the subject to terminate the test. No differences in responses were evident in pre-HDBR testing between subjects with presyncope before LBNP vs. with LBNP, so all results were pooled.

A standard three-lead electrocardiogram (Roxon Medi-Tech, St. Leonard, QC, Canada) was recorded. Finger photoplethysmography (Nexfin, BMEYE BV, Amsterdam, the Netherlands) was used for the continuous assessment of arterial blood pressure. Values recorded in the finger were corrected to heart level. Postprocessing of the blood pressure signal involved the correction of recorded values to a manual blood pressure measure, taken by a trained experimenter before the start of the tilt test with the subjects resting in a supine position. An additional height correction was also applied during postprocessing to determine BPMCA. Participants were equipped with a nasal cannula for monitoring of expired CO₂ (Ohmeda 5200 CO₂ Monitor, Madison, WI). Values were recorded as percent CO₂ to be later converted to values of CBFV and BPMCA being calculated (24).

Blood flow velocity in the MCA (CBFV) was determined using a standard three-lead electrocardiogram (Roxon Medi-Tech, St. Leonard, QC, Canada) and additional height correction was also applied during postprocessing to determine BPMCA. Participants were equipped with a nasal cannula for monitoring of expired CO₂ (Ohmeda 5200 CO₂ Monitor, Madison, WI). Values were recorded as percent CO₂ to be later converted to millimeters of mercury based on ambient temperature and barometric pressure. Electrocardiogram, BPMCA, and CO₂ data were collected at 1,000 Hz using Chart software (ADInstruments, Colorado Springs, CO).

Blood flow velocity in the MCA (CBFV) was determined using transcranial Doppler ultrasound. A 2-MHz pulsed Doppler probe was placed over the right temporal window, which allowed for the insonation of the M1 segment of the right MCA and the assumption of 0° for the angle of insonation. A head band was used to hold the probe in place throughout the testing. Doppler signals were collected using CardioLab hardware (CNES device, European Space Agency) and were recorded at 100 Hz using CardioMed software (CNES-European Space Agency).

Cerebrovascular variables. Mean CBFV values were calculated from the outer envelope of the Doppler spectrum over a cardiac cycle. The Doppler recording was also assessed to determine maximum (systolic) and minimum (end diastolic) values for each cardiac cycle. An index of CVRi was calculated as CVRi = mean BP_MCA/CBFV_mean where CBFV_mean is mean CBFV.

Further analysis was conducted with the CBFV waveform for the calculation of CrCP and RAP. Following the methods described in a recent critical analysis (24), CrCP was calculated for each beat using mean (CBFV_mean and MAP_MCA) and diastolic (CBFV_diast and DBP_MCA) values for CBFV and BP_MCA. The slope of the relationship between CBFV and BP_MCA was calculated as a = (CBFV_mean - CBFV_diast)/(MAP_MCA - DBP_MCA). RAP was then calculated as RAP = 1/ta, and CrCP calculated as CrCP = MAP_MCA - (CBFV_mean*ta).

Previous studies of head-up tilt and sustained orthostasis have used a linear regression method for assessing CrCP and RAP (5, 37). However, in this study, the two-point method using mean and diastolic values of CBFV and BPMCA was chosen, as it has recently been shown to have greater repeatability and fewer instances of negative CrCP values being calculated (24).

Data analysis. This study reports on data collected during the control; however, the original design of the study included two countermeasure conditions for the same subjects with pre-HDBR data collected three times for each subject. Post-HDBR data for the countermeasure conditions were not included in the present analysis, as only a small number of individuals completed both of the protocols. Previous work has demonstrated good reproducibility of responses to combined tilt and LBNP (18), and statistical analysis of these data (not shown) showed no differences between the pre-HDBR responses defined as the difference between a 5-min average taken at supine baseline, a 5-min average taken between minutes 6 and 10 of the tilt, and the last minute of the test. Therefore, for analysis purposes, pre-HDBR was considered as the average of all three pre-HDBR data collections.

Variables were assessed beat by beat and then averaged every 10 s pre-HDBR and 5 s post-HDBR after the transition to the tilted posture. PETCO₂, values were linearly interpolated to determine values for each heartbeat. Early tilt responses were assessed as the difference between a 5-min average taken during supine rest and a 5-min average taken between minutes 6 and 10 of the tilt. For seven tests, following HDBR, the participants were unable to complete 10 min of the stand test. Therefore, tilt values were taken as the average from 5 min post-tilt, excluding the last minute of the test, which included rapid changes in measured variables.

Statistical analysis. The analysis of cardiovascular responses to tilt was performed in several ways to analyze both the transition to tilt and the period of tilt before presyncope. The early phase of tilt was analyzed by comparisons to supine baseline with two-way repeated-measures ANOVA (Systat Software, Chicago, IL). Next, responses were assessed over the final 15 min of the test pre-HDBR and, due to the shorter duration of testing post-HDBR, over the final 5 min of the test post-HDBR with one-way repeated-measures ANOVA being used to assess each minute value. Finally, linear regression analysis was used on data from each individual to explore possible interrelationships between different variables. From the data in the final 15 min of the test pre-HDBR, two distinct sections were identified based on the point at which BPMCA became different from the value at 15 min. BPMCA was not different until 1 min before syncope; therefore, regressions were performed on data between minutes 15 and 2, and minutes 2 and 0 separately to determine relationships between PetCO₂, BPMCA, CBFV, CrCP, and RAP. Post-HDBR, regressions were performed between minutes 5 and 2 and minutes 2 and 0. Data for the linear regressions are presented as the Pearson product moment correlation (r).

All values are shown as means ± SD, unless otherwise stated. Significance was set at P < 0.05 for the repeated-measures analysis of variance and P < 0.01 for linear regression analysis.

Analysis limitations. Data were available for all 12 individuals pre-HDBR; however, post-HDBR, some data were not available for the planned analyses. For the comparison of early tilt responses (average between 5 and 10 min posttilt), three individuals post-HDBR experienced syncope within 5 min of tilt; therefore, only 9 of 12 individuals were included in the repeated-measures analysis. Post-HDBR, the linear regression analysis was attempted for eight of the nine individuals who were able to complete greater than 5 min of tilt, with one individual being excluded as the test was terminated early due to a power outage.
RESULTS

Pre-HDBR, the average tilt tolerance time was 36 ± 10 min, with all but two individuals reaching the LBNP portion of the tolerance test. In comparison, tilt tolerance time was reduced post-HDBR (16 ± 15 min) with 7 of 12 individuals unable to complete 10 min of the test, and 3 of these individuals developing presyncopal symptoms within 3 min of tilt. Plasma volume, assessed by change in hemoglobin, was reduced 16 ± 6% after HDBR.

Analysis of the cerebrovascular early tilt responses, presented in Fig. 1, showed significant reductions in CBFV (A, P < 0.001), PETCO2 (B, P < 0.001), BPmca (D, P < 0.001), and CrCP (E, P < 0.001), along with an increase in RAP (F, P = 0.035) and no significant changes for CVRi (C, P = 0.366) both pre- and post-HDBR. A significant interaction was found suggesting a greater reduction in BPmca post-HDBR (P = 0.016). There was a weak trend toward a greater reduction in CBFV (P = 0.183), but no differences in early tilt responses with HDBR for any other variable assessed.

The changes in CBFV, PETCO2, and BPmca during the last 15 min before syncope pre-HDBR are presented in Fig. 2. CBFV and PETCO2 progressively decreased with significant reduction from the value at 15 min by 4 min (−10.8 ± 7.0%) and 5 min (−10.1 ± 7.2%) before syncope, respectively. Conversely, BPmca was only different 1 min before and at syncope, where BPmca was reduced by −8.0 ± 6.0 and −35.1 ± 11.8%, respectively. Similar to PETCO2, CrCP was significantly increased from the value at 15 min by 5 min before syncope (4.8 ± 4.2 mmHg), whereas RAP was only reduced at syncope (−0.5 ± 0.3 mmHg·cm⁻¹·s⁻¹). Post-HDBR, compared with the value 5 min before syncope, BPmca, PETCO2, and CBFV were all significantly reduced at syncope (P < 0.05), and there was a trend (P = 0.054) for RAP to also be reduced at syncope. No changes in CrCP were seen for the post-HDBR data.

Linear regression analysis results pre-HDBR and post-HDBR are presented in Tables 1 and 2, respectively. Pre-HDBR between 15 and 2 min before syncope, significant relationships were found between CBFV and BPmca for only 4 of 12 people, while the relationship between CBFV and PETCO2 was significant in 10 of 12 people (Table 1). This is in contrast to the last 2 min of the test, where only 3 of 12 people had significant relationships between CBFV and PETCO2, whereas 9 of 12 had CBFV significantly related to the change in BPmca (Table 1). Post-HDBR, only one individual showed a significant relationship between CBFV and PETCO2 between 5 and 2 min and over the last 2 min of the test (Table 2). However, similar to pre-HDBR, over the last 2 min of the test, significant relationships were found between CBFV and BPmca for six of the eight people (Table 2).

A visual display of the interrelationships between PETCO2, BPmca, CrCP, and RAP is shown in Fig. 3 for pre-HDBR tests (note the inverted scale for CrCP). Linear regressions were performed on data pairs for these variables between 15 and 2 min before syncope for each individual. The majority of subjects (Table 1) had significant RAP-BPmca and CrCP-PETCO2 relationships in this time interval. In contrast, there were only weak RAP-PETCO2 and CrCP-BPmca relationships (Table 1). During the last 2 min of the test, only RAP-BPmca was significant in 8 of 12 people (Table 1). In contrast to the
Table 1. Correlation coefficients for linear regression assessing the relationship between $BPMCA$ and $PETCO_2$ with $CBFV$, $CrCP$, and $RAP$ during the last 15 min before syncope pre-HDBR

<table>
<thead>
<tr>
<th></th>
<th>Time = 15 to 2 min</th>
<th></th>
<th>Time = 2 to 0 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$BPMCA$</td>
<td>$PETCO_2$</td>
<td>$BPMCA$</td>
</tr>
<tr>
<td>$CBFV$</td>
<td>0.566 ± 0.137 (4)</td>
<td>0.688 ± 0.169 (10)</td>
<td>0.881 ± 0.075 (9)</td>
</tr>
<tr>
<td>$CrCP$</td>
<td>0.525 ± 0.106 (4)</td>
<td>0.631 ± 0.196 (7)</td>
<td>0.926 ± 0.053 (2)</td>
</tr>
<tr>
<td>$RAP$</td>
<td>0.653 ± 0.141 (9)</td>
<td>0.662 ± 0.171 (6)</td>
<td>0.913 ± 0.091 (8)</td>
</tr>
</tbody>
</table>

Values are means ± SD of the Pearson product moment correlation (with the no. of individuals out of 12 with significant relationships in parentheses). HDBR, head-down bed rest; $BPMCA$, blood pressure at the level of the middle cerebral artery; $PETCO_2$, end-tidal $PCO_2$; $CBFV$, cerebral blood flow velocity; $CrCP$, critical closing pressure; RAP, resistance area product.

Table 2. Correlation coefficients for linear regression assessing the relationship between $BPMCA$ and $PETCO_2$ with $CBFV$, $CrCP$, and $RAP$ during the last 5 min before syncope post-HDBR

<table>
<thead>
<tr>
<th></th>
<th>Time = 5 to 2 min</th>
<th></th>
<th>Time = 2 to 0 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$BPMCA$</td>
<td>$PETCO_2$</td>
<td>$BPMCA$</td>
</tr>
<tr>
<td>$CBFV$</td>
<td>0.520 ± 0.099 (3)</td>
<td>0.539 (1)</td>
<td>0.680 ± 0.136 (6)</td>
</tr>
<tr>
<td>$CrCP$</td>
<td>0.503 (1)</td>
<td>NS</td>
<td>0.733 ± 0.128 (4)</td>
</tr>
<tr>
<td>$RAP$</td>
<td>0.622 ± 0.107 (8)</td>
<td>NS</td>
<td>0.803 ± 0.045 (6)</td>
</tr>
</tbody>
</table>

Values are means ± SD of the Pearson product moment correlation (with the no. of individuals out of 8 with significant relationships in parentheses). NS, no significant relationships.
further investigate relationships between variables. One set of regressions was computed for the period of constant BP_{MCA} (15 to 2 min before syncope), the second set when BP_{MCA} was changing (last 2 min of the test). These analyses confirmed distinct regions over which changes in PETCO₂ or BPMCA were changing (last 2 min of the test). These analyses confirmed BPMCA (shaded line).

**Effects of HDBR.** Contrary to the hypothesis that CBFV would show greater reductions post-HDBR, there were no differences in the early phase tilt responses compared with pre-HDBR. A greater reduction in BP_{MCA} may have contributed to the observed reduction in orthostatic tolerance. The potential mechanisms responsible for impaired orthostatic tolerance after HDBR, such as reduced plasma volume noted in this study, have been considered in other research (7, 10, 36). Previous studies investigating cerebral blood flow responses before and after exposure to real or simulated microgravity have shown varied responses. In response to LBNP or assuming a head-up posture, there have been reports of greater reductions in CBFV, potentially indicating an impaired ability to regulate cerebral blood flow (14, 40). However, other work has shown no change in the CBFV response to tilt or LBNP (2, 3) or with rapid deflation of leg cuffs (28). To date, no studies have been conducted examining the potential influences of PCO₂ and changes in cerebrovascular tone in CBFV responses to orthostasis after HDBR.

Similar to the pre-HDBR results, BP_{MCA}, CrCP, and CBFV decreased over the last 5 min before syncope, but, in contrast, there were no significant changes in CrCP. Post-HDBR, it would appear that changes in BP_{MCA} dominated the cerebrovascular responses, while the effects of PCO₂ and changes in CrCP were not evident with the shorter duration of tilt. These cerebrovascular results suggest that, although orthostatic tolerance was reduced post-HDBR, the reduction does not appear to be the result of altered cerebrovascular hemodynamics, but was related to the more rapid decline in BP_{MCA}.

**Limitations.** In the present study, PETCO₂ was measured as a surrogate for P_{ACO₂} with the progression toward syncope. The relationship between PETCO₂ and P_{ACO₂} is altered during postural transitions, but it is unknown if the relationship then remains constant with sustained orthostasis (13, 34), since

---

**Fig. 3. Interrelationships between variables for the last 15 min of the tilt tests for pre-HDBR (lines are mean for \( n = 12 \)). A: BP_{MCA} (solid line) and RAP (shaded line). B: PETCO₂ (solid line) and CrCP (shaded line, note the inverted scale).**
further changes in the ventilation-to-perfusion ratio could occur as cardiac output is reduced with prolonged tilt (12).

The use of transcranial Doppler ultrasound for the assessment of cerebral blood flow is dependent on the diameter of the insonated vessel remaining constant. Studies have shown that the diameter of the MCA remains constant with changes in PETCO2 and the application of a mild orthostatic stress (11, 35). However, it is possible that prolonged orthostatic stress and reduced PCO2 could have reduced the MCA diameter so that measurements of CBFV might underestimate the decrease in cerebral blood flow. The MCA diameter and cerebrovascular properties might also change with HDBR, as seen in animal models of hindlimb suspension (20, 39), but this seems unlikely with 5 days of HDBR in humans. Regardless, these issues raise a need for caution in interpreting the observed changes in CBFV as alterations in cerebral blood flow.

Perspectives. This study examined the cerebrovascular responses to head-up tilt, with particular emphasis on the contributions of changes in arterial blood pressure and PETCO2, both before and after exposure to 5 days of HDBR. In the early phase of head-up tilt, CBFV was reduced as a consequence of both reduced BPMCA and PETCO2. The dominant change in cerebrovascular properties during this phase was a reduction in CrCP, while RAP was slightly increased. These data contrasted with the proposal that CrCP is related to metabolic factors and should increase as PETCO2 is reduced (5, 27), but are consistent with the recent observations of Stewart et al. (37). As well, RAP is proposed to be predominantly linked to myogenic mechanisms and should have decreased, not increased, with a reduction in BPMCA (27). With sustained tilt, during the period from 15 to 2 min before the onset of syncope, the decrease in PETCO2 progressively increased CrCP though a metabolic mechanism (5, 27), decreasing CBFV with no apparent contribution from BPMCA and RAP. The progressive increase in CrCP might have contributed near syncope to a collapse of cerebral vessels throughout part of the cardiac cycle (5, 33), resulting in reduced CBFV. In the final 2 min before syncope, the rapid decline in BPMCA initiated a myogenic response seen as a reduction in RAP, but this failed to maintain CBFV. Pre-HDBR, the increase in cerebrovascular tone (CrCP), rather than an increase in RAP, contributed to the reduction in cerebral blood flow and eventual development of syncope, whereas, after 5 days of HDBR, orthostatic responses were dominated by the reduction in BPMCA and the actions of cerebrovascular autoregulation. This study revealed the complex interactions of CrCP and RAP in the regulation of CBFV during head-up tilt to presyncope. CrCP responded to myogenic stimuli in the early phase of head-up tilt, and then to metabolic stimuli (PETCO2) as tilt was sustained. In contrast, RAP did not appear to respond to metabolic stimuli and decreased in response to myogenically stimuli only near syncope and not the early period of head-up tilt. Therefore, it would appear that, with prolonged head-up tilt, metabolic factors related to changes in PETCO2 contributed to alterations in cerebrovascular tone and the development of syncope.

ACKNOWLEDGMENTS

The study was conducted at MEDES, Institute for Space Physiology and Medicine in Toulouse, France. The authors thank the MEDES staff for excellent support throughout the project and the subjects for the commitment to the study’s success.

GRANTS

This research was supported by the Canadian Space Agency (9P007-071471001/ST), the Natural Sciences and Engineering Research Council of Canada (RGPIN6473-07), and European Space Agency and Centre National d’Etudes Spatiales Grants 4800000583 and 480000557.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


